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Serological and Parasitological Survey of Canine *Dirofilaria immitis* Infection in Maiduguri, Borno state, Northeastern Nigeria

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ABSTRACT

Heartworm disease is of considerable economic importance affecting canine populations around the world which has zoonotic implications. This study was carried out to survey the presence of *Dirofilaria immitis* using parasitological (Buffy coat, wet mount, modified Knott's test) and serological (ELISA) technique. A total of 250 blood samples were collected from dogs in the study area. A total of 12(4.8%) of dogs were positive for the infection in the study area, 8(3.2%) of males were positive while 4(1.6%) of females were positive. According to age, 2(0.8%) of young were positive while 10(4.0%) of adults were also positive of the infection. However there was no significant difference (P>0.05) between the age and sex of the dogs infected. This study revealed the presence of *D. immitis* infection in the study area with ELISA being the most sensitive technique in the diagnosis of the infection based on the techniques employed in the study.

1. INTRODUCTION

Dirofilaria immitis is a parasitic nematode that occurs in canine and feline cardiopulmonary system and it also the causal agent of human pulmonary (Dirofilariosis) (Vieira et al., 2014). Heartworm disease is a non-contagious parasitic disease, caused by a filarial nematode *D. immitis* which is one of the most pathogenic nematode parasites of dogs (Anyanwu et al., 2000). Dogs are considered the definitive host for the infection; however it may infect more than 30 animal species such as coyotes, foxes, wolves, ferrets and other wild canids. This parasite is of zoonotic importance as humans have been known to be infected (Morchon et al., 2012). Over 70 species of mosquitoes serve as an intermediate host; Aedes, Anopheles and Culex are the genera acting as vectors (Mc Call et al., 2008). The infection has a worldwide distribution and is widely distributed in America, Asia, Africa and Mediterranean countries (Atlas et al., 2013). Transmission of Dirofilariosis occurs during blood meal when a potential vector bites a dog or another host (Anyanwu et al., 2000). A total of four mounting process occurs before the larvae mature into adult worm, the first two mounting process occurs inside the mosquitoes to and the last two inside the final host. The adult worms dwell in the right ventricle and pulmonary artery, but occasionally could be found in the epidural space, brain, anterior chamber of the eye, lungs or systematic arterial system (Mc Call et al., 2008). The infection poses a significant health risk to dogs because of the relatively large size (25 – 35cm length) of the female worms and also the number of worms present after a long inoculation causes numerous cardiovascular problems such as pulmonary embolism, pulmonary infarctions, emboli migration, chronic circulatory disorder and this finally results to congestive heart failure and finally death (Montoya et al., 2016).

The various techniques used in the diagnosis of the infection involves techniques such as wet mount, modified knott's technique, buffy coat concentration technique, micro-filarial density test, X-ray and ultrasound are used. Diagnosis of Dirofilariasis infection also involves the use of serological and Molecular test. These techniques detect circulating antigens or microfilariae are released by mature adult females few months after infection (Atlas et al., 2013). Commercial serological kits available include DiroCHECK, Agen, and Witness (Obaje et al., 2016). However confirmatory and reliable diagnosis of the infection is dependent on serology and molecular test. Sometimes in circulating blood of heartworm infected dogs microfilariae are absent and such condition is termed occult infection (Borthakur et al., 2015).

Unfortunately, the current distribution of canine Dirofilariosis in Africa is still not well known due to lack of information on the epidemiology of the disease, lack of details regarding the assays of the methods used and also due to large number of variety of filarial species in the entire continent. It is also worthwhile to mention that spread of this dreadful disease to new areas has been possible due to extensive movement of dogs across countries and continents, together with the availability of vectors and favourable climatic conditions (Schrey et al., 1998). Therefore, this present study was designed to fill in the information gap in Maiduguri, North Eastern Nigeria where no current information exists about the prevalence and risk factors of the infection

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Maiduguri the capital and largest urban city of Borno State, North-eastern part of Nigeria. Maiduguri is located between latitudes 11^0 and 14^0N and longitudes 10^0 and 14^0E with a population density of 1,738 people per square kilometre. The temperature ranges from 35 – 40^0C for most part of the year, with a mean annual rainfall of about 647mm (BMLS, 2007).

2.2 Animals

Two hundred and fifty dogs (135 male and 115 female) in Maiduguri were examined for the infection between December 2017 and August 2018. The age of the animals was estimated by the annual layers of teeth and bone (Otaishi et al., 1985).

2.3 Blood Collection

Animals were properly restrained prior to collection of blood sample via the cephalic venipuncture of each dog aseptically. About 5mls of blood was collected, one half of blood was collected in tube containing EDTA as an anticoagulant, with the other half put into a plain tube without anticoagulant. These samples were stored in an ice pack box and were later processed in the laboratory.

2.3 Detection of *D.immitis* Infection

2.3.1 Wet Mount

A drop of fresh blood was placed on a clean microscope slide using on applicator stick and a wet smear was done according to standard procedures as described by Byeon et al. (2007)

2.3.2 Buffy Coat Technique

Using capillary action, one end of the capillary tube was filled to about three – quarter with blood and then sealed with plasticine at the other end, using the haematocrit centrifuge machine. The blood was centrifuged for five minutes at 12,000rpm. The Packed Cell Volume (PCV) was read immediately after the centrifugation and the capillary tube was placed on a clean microscope slide, covered with a drop of distilled water and examined microscopically at the buffy coat area at 10x magnifications to detect mobile parasites (Microfilariae) as described by Cheesbrough (2010).

2.3.3 Modified Knott's Test

To one ml of blood taken from sample bottles containing EDTA, 9ml of 2% buffered formalin was added. The solution from the mixture was centrifuged for 5minutes at 2,000rpm according to (Ciocon et al., 2010). The supernatant was decanted from the centrifuge tube and the sediment was mixed with methylene blue dye (0.1%). The coloured sediment was spread on a slide, covered with cover slip and examined under a microscope at 10x and 40x magnifications.

2.3.4. ELISA Technique

Circulating *Dirofilaria immitis* antigens was detected by use of a commercial antigen enzyme linked immunosorbent assay (ELISA) kit (Demeditec Diagnostics GmbH, Kiel, Germany). The ELISA Kit was used according to the manufacturer's instruction. Demeditec canine heartworm antigen test kit is an enzyme immune assay designed to detect the presence of circulating antigen in serum or plasma.

2.5 Statistical Analysis.

Chi-square test was used in determining statistical significance. Significance was accepted at ($P < 0.05$).

3. RESULTS

3.1 Prevalence of *Dirofilaria immitis* Infection of Dogs in Maiduguri based on Sex.

The prevalence of (D. immitis) infection according to sex is presented in Table 1, Out 135 male dogs sampled, 8(3.2%) of males were infected while 4(1.6) were positive of the infection out of 115 females sampled for the study.

3.2 Prevalence of *Dirofilaria immitis* Infection of Dogs in Maiduguri based on Age.

The prevalence of *D. immitis* infection of Dogs in Maiduguri based on Age is presented in Table 2. Out of the 250 dogs sample, 95 were young with 2(0.8) being positive of the infection while 10 out the 155 adults were positive for the infection.

Table1: Prevalence of *Dirofilaria immitis* Infection of Dogs in Maiduguri based on Sex.

Sex	Number Examined	Number Infected (%)
Male	135	8(3.2)[a]
Female	115	4(1.6)[a]
Total	250	12(4.8)

a = Number with same superscript in 3rd column did not differ significantly ($p < 0.05$)

Table 2: Prevalence of *Dirofilaria immitis* Infection of Dogs in Maiduguri based on Age.

Sex	Number Examined	Number Infected (%)
Young (<1year)	95	2(0.8)[a]
Adult(>1year)	155	10(4.0)[a]
Total	250	12(4.8)

a = Number with same superscript in 3rd column did not differ significantly ($p < 0.05$)

4. DISCUSSION

The findings from this study revealed an overall seroprevalence of 12(4.8%) of *Dirofilaria immitis* infection with the parasitic techniques (buffy coat concentration technique and modified knots test) revealed no positive sample. The seroprevalence of 4.8% was obtained in Maiduguri, Borno state, Northeastern Nigeria. This result presents the first evidence of the occurrence of *D. immitis* infection in dog's population in Maiduguri. The prevalence even though low is of serious public health importance owing to its zoonotic implication. The prevalence rate (4.8%) obtained in this study is lower than prevalence rate of 12.7%, 24.46%, reported by Anyanwu et al, (2000) in Zaria, Nigeria and Ben-Mahdi *et al*, (2009) in Algiers, Algeria. However, it was higher than the prevalence rates reported by Ugochukwu *et al*, (2016) who reported prevalences of 3.36% in Nsukka, south eastern Nigeria. The low prevalence rate recorded in this study could be attributed to the time of the year, which was in dry season when the bulk of work was done. Excessive use of some drugs such as ivermectin, which is effective at killing immature adult heartworms, therefore with the common use of macrocylic lactones may lead to samples appearing negative (Obaje et al., 2016)). It could also be as a result of the use of serological techniques (ELISA) which is more sensitive than the most commonly employed parasitic techniques. It is important to note that only the serological technique was able to detect positive samples in the study, with the parasitic techniques yielding no positive result. This agrees with previous work done by Atkins, (2015) who reported that serological test like commercially available ELISA Kits for antigen or antibody detection have been reported to be the most sensitive and test specific method for the diagnosis of *Dirofilaria immitis* in dogs and cats, but it is expensive, requires expertise and not readily available in developing countries like Nigeria. The presence of antibodies

only indicates that an infection occurred and does not provide a guarantee that it still exists, while a positive antigen test result is indicative of an active adult infection (Mc Call et al., 2008). Positive antigen test has shown to be highly specific, but sensitivity may decline in dogs with worm burdens of two female heartworms or fewer, although it is more closely related to the actual weight of worm present (Cardoso et al., 2010). It is also possible to have low prevalence rate from occult infection when some serological tests are used (Genchi et al, 2014). The term occult infection denotes amicrofilaraemic infections leading to false negative results. This could arise as a result of low parasite burdens, prepatent infection by immature worms, geriatric female worm, infection by only male worms or the host is under microfilaricidal therapy (Borthakur et al., 2015).

The result of the present study revealed that there are no statistical significant association between sex disposition and age with the seroprevalence of *D. immitis* infection in the study area within the sampled period. However higher prevalence rate recorded in male and adult dogs may likely be attributed to the exploratory life style of male dogs, length of exposure to infected mosquitoes (Rhee et al., 1998). This is in consonance with the report of Viera et al. (2014) and Obaje et al. (2016) who reported similar results in Central Portugal and Markudi in Nigeria. Thus older dogs will have longer time of exposure and have more opportunities to be infected with heartworm, so also the long incubation period of the worm.

5. CONCLUSION

Based on the result of this study, *Dirofiloria immitis* infection of dogs exists in Maiduguri Metropolis, North-eastern Nigeria. ELISA technique was the most sensitive technique in diagnosis of this infection. The general public in Maiduguri are highly exposed to the great danger posed by this serious zoonotic disease.

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