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Original Article

Prevalence and risk factors associated with *Dirofilaria immitis* infection in dogs in Makurdi, Benue State, Nigeria

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ABSTRACT

Objective: This study was designed to assess the prevalence and the associated risk factors (*e.g.*, sex, age, breed, management system and climate) of *Dirofilaria immitis* in dogs in Makurdi metropolis in Nigeria.

Materials and methods: Prevalence study of canine heartworm disease in dogs was conducted over a period of six months covering five localities of Makurdi metropolis in Benue State, Nigeria. A total of 186 blood samples were collected from apparently healthy and sick dogs, and the samples were examined for the presence of microfilaria between September 2015 and February 2016. Three methods (wet mount, Buffy coat and modified Knott's techniques) were used for the examination of the samples. The Packed Cell Volume (PCV) and complete blood count for each sample were also determined.

Results: Out of the 186 dogs, 4 (2.15%) were found to be positive for the presence of microfilaria. Out of the 4 positive cases, 3 (1.61%) were microfilaria and 1 (0.54%) was unidentified motile parasite. A total of 104 females were examined and only 1 (0.96%) was positive, while 3 (3.66%) males out of 82 examined were positive. Out of 141 older dogs examined, 4 (2.84%) were positive. Hematology of the positive dogs revealed mild anemia and moderate thrombocytopenia with Mean±SD of 34.8±15.30% and 108±60.81x10^9/L, respectively.

Conclusion: The study confirms Knott's technique to be the most sensitive in the diagnosis of dirofilariasis in dogs using parasitological techniques. The findings confirm the occurrence of *D. immitis* in dogs in Makurdi with low prevalence and that the general public are at high risk of spreading infection from the dogs. Infection is more in male and adult dogs. This work can assist in planning appropriate strategies for controlling and prevention of *D. immitis* infection in Nigeria.

KEYWORDS

Dogs, Dirofilaria, Heartworm, Mosquito, Zonoosis

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INTRODUCTION

The danger post 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).

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 years old Dorbaman in Ibadan, Nigeria. Patent infections of *D. immitis* are possible in numerous wild and companion animals. Animals and mosquitoes infected are capable of transmitting the infection to humans, but there are no reports of such infection becoming patent (Guerrero, 2012). Transmission takes place when a potential vector bites dog or other host during subsequent blood meal (CAPC, 2015). The worm goes through a total of four moults to mature into adult worm; the first two occur inside mosquito and the next two inside the final host (Dunn, 2014). Most infected dogs do not show any sign of the disease for months or years, depending on the worm burden, individual reactivity and level of exercise (Anuchai et al., 2006). 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 et al., 2015). A large number of infected dogs had abnor-malities in their hematological and biochemical profile. Clinical hematology study showed mild to moderate anemia in microfilaremic dogs (CAPC, 2015).

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 Snap^{R}, Idexx, DiroCHEK^{R}, Agen, Witness^{R} (Altas et al., 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). Currently, different test have been developed for the detection of circulating antigens such as employing lateral flow immunochromatographic technique, membrane ELISAs or conventional ELISAs (Little et al., 2014). However, potential cross-reaction with other helminths, mainly against filarial nematodes like *Dipetalonema reconditum* or *D. repens* occasionally occurs when evaluated in animals with natural or experimental infection, but modern test kits may overcome cross-reaction detected in previously developed test kits for these parasite (Manuela and Deplazes, 2012). Necropsies of heartworm infected dogs have confirmed the presence of *Wolbachia* bacteria in heartworm. All dogs had a humoral response to Walbachia surface protein (Kozek, 2004). Modified Knott's test enables scientist to clearly distinguish between *D. immitis*, *D. repens* and *Acanthocheilonema (Dipetalonema) spp*. Microfilariae of *D. repens* has conical front end and curved caudal end. *A. drancunculoides* has round front end and straight tail end. *A. reconditum* has blunt front end and small hook at rear end (Johnnes et al., 2013). *D. immitis* microfilariae has straight body and tail with tapered head (AHS, 2015). The cephalic space of *D. repens* is short and terminated by distinct pair of nuclei separate from the remaining somatic nuclei, while the cephalic space in microfilariae of *D. immitis* is longer without the distinct nuclei that separate from somatic column nuclei near the anterior end (Liotta et al., 2013).

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).

This study was designed and conducted to check for the presence, prevalence rate and some epidemiological risk

factors associated with *D. immitis* in dogs in Makurdi town.

MATERIALS AND METHODS

Study area: The study was conducted in Makurdi, the capital of Benue State located in the middle belt region of Nigeria (**Figure 1**). Makurdi is located between longitude $8^0$35'E and $8^0$41'E and latitude $7^0$45'N and $9^0$52'N (NMA, 2011). It is intersected by the River Benue which is the major source of water with other networks of streams, standing pools, over filled and blocked drainages, over-grown bushes and fields, even around residential homes and offices (Manyi et al., 2014). These provide suitable breeding sites for mosquitoes throughout the wet and dry seasons. There is also a characteristic high temperature in Makurdi, (30-39^{0}C), which helps in the speedy development and hatching of mosquito eggs (Manyi et al., 2014). It is suspected that temperature may have an impact on the transmission of vector diseases in Makurdi throughout the year, (Manyi et al., 2014).

Samples collection: A total of 186 blood samples were randomly collected from household mixed breed of dogs of both sexes from September 2015 to February 2016 in different areas (North Bank, Wurukum, Highlevel, Wadata and Gyado Villa) of Makurdi metropolis and from dogs brought to Veterinary Teaching Hospital, University of Agriculture Makurdi. Each dog was registered and the details such as sex, age (usually above 6 months old), breeds, use of the dog and management system (indoor/outdoor at night) were recorded. The dogs were then restraint and about 4 mL of blood was taken through the cephalic vein using 5 mL syringe and 21 gauge needle. Two (2) mL of the blood sample was then transferred into EDTA sample bottle and the remaining blood been emptied into a non EDTA (plain) sample container for serum.

Ethical approval: All the dogs were handled according to the international guiding principles for Biomedical Research with Animals (CIOMS, 1985) and under the guidance of ethical committee of college of Veterinary Medicine, Federal University of Agriculture, Makurdi, Nigeria.

Sample transportation: All samples were labelled appropriately and transferred into a flask containing ice pack which were then transported by road immediately to Department of Veterinary Parasitology and Entomology laboratory of College of Veterinary Medicine, University of Agriculture, Makurdi.

Modified Knott's Test: The samples were analysed according to (Knott, 1939; Ciocan et al., 2010; Bohadori et al., 2011). One mL of blood was taken from the EDTA sample then 9 mL of 2% buffered formalin was added and the solution obtained was centrifuged for 5 min at 2,000 rpm. The supernatant was decanted from the centrifuge tube and the sediment was mixed with equal parts of methylene blue dye (0.1%). The coloured sediment was spread on a slide, covered with cover slip and examined under a microscope using X10 and X40 objectives.

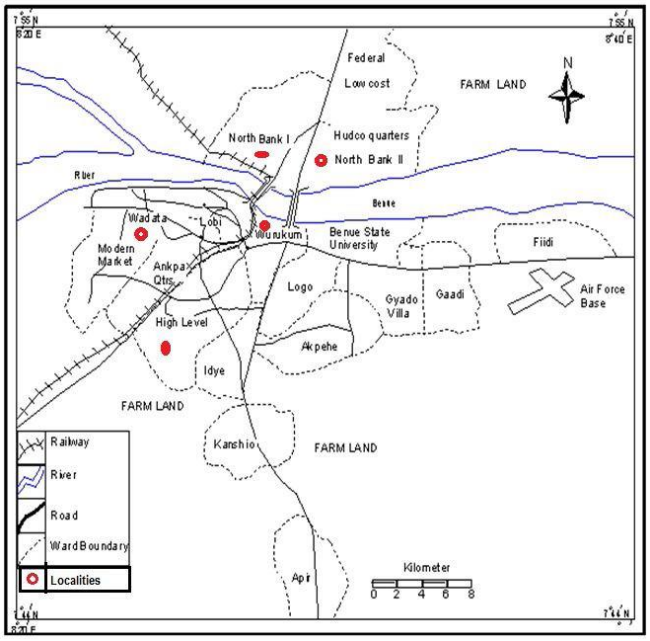

Figure 1: Map of Makurdi Showing the Study Localities (Ministry of Lands and Survey Makurdi, 2011).

Buffy Coat Technique: One end of the capillary tube was placed on blood sample and allowed to fill to about three-quarter by capillary action and then was sealed by plastasin at the other end. It was placed in the hematocrit centrifuge machine and was centrifuged for 5 min at 12,000 rpm. After the centrifugation, the packed cell volume (PCV) was read and then capillary tube was placed on a clean microscopic slide and covered with a drop of distilled water and examined microscopically at the buffy coat area using x10 magnifications to detect motile parasites (trypanosomes and microfilariae) as described by Cheesbrough, (2010).

Wet Mount: One drop of fresh blood was placed on a clean microscope slide and then covered with cover slip and examined microscopically for detection of motile parasites at x10 and x 40 objectives.

Thin Blood Smear: Thin blood smear was prepared and later examined under microscope with oil immersion (x100) objective for hemoparasites and differential cell count, as described by Cheesbrough, (2010).

Complete blood count: Automated cells analyser (BC-2800 Vet Auto Hematology Analyzer) was used for the full blood count according to Mindray Shenzhen operator's Manual (2011). Complete blood count was performed by an automated Hemo-analyzer that counts the numbers and types of different cells within the blood (**Table 4**). It aspirates a very small quantity of the blood sample through the narrow tubing. Within this tubing, there are sensors that count the number of cells going through it, and it can identify the type of cell; this is called flow-cytometry. The results then appear on the screen under the inputted sample's information.

Statistical analysis: Data were analysed using Graph pad Prism version 5.03 statistical software and Microsoft Excel 2007 version and value of $P<0.05$ was considered significant.

RESULTS

Out of the total of 186 dogs screened, 3 (1.61%) were found positive for microfilaria and 1 (0.5%) was positive of motile parasite using Modified Knott's technique and Buffy coat method, respectively (**Table 1** and **Figure 2**). Among the 186 samples, 97.85% (n=182) were negative in all the three methods (Modified Knott's, Buffy coat technique and Wet mount) used. None was positive using Wet mount. A total of 104 females were examined and only 1 (0.96%) was positive while 3 (3.66%) males out of 82 were positive (**Table 2**). Out of the total dogs examined, 45 (24.19%) were between ages of 7 months to 1 year (*i.e.*, ≤1-year), while 141 (75.81%) were above 1-year. Two (2) out of the 4 positive dogs were 5 years old while the other two (2) were 4 years old. The four positive dogs were out door dogs (**Table 3**).

Assessment of hematological parameters demonstrated mild anemia from PCV (%) with Mean±SD of 34.8±15.30 and thrombocytopenia, platelets count ($x10^9$/L) with Mean±SD of 108±60.81. Other hemo-parameters were within normal range.

DISCUSSION

The findings from this study revealed the occurrence of this serious zoonotic filarial parasit in Makurdi metropolis. Currently, the prevalence of this serious public health important worm is yet to be ascertained in Africa due to paucity of epidemiological studies, 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 prevalence rate of 2.15% obtained in this study suggests low occurrence of the parasite (*D. immitis*) in the study area as compared to what was obtained in other places but is of great public health concern. Anyanwu et al. (2000) reported a prevalence rate of 12.7% from Zaria, Nigeria, 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). The disease is gradually spreading to most of the areas considered before now to be free-zones because of the global climatic changes. This is in agreement with Genchi et al. (2014) report of increasing cases of infections from non-endemic areas. However, the low prevalence rate obtained from this study may be due to low sensitivity of the methods used.

Table 1: Number of positive cases through different diagnostic techniques for heartworm infection of dogs in Makurdi Metropolis.

	Wet mount	Buffy coat tech	Modified Knott's tech
Positive	0	1	3
Negative	186	185	183
Total	186	186	186
Prevalence (%)	0	0.54	1.61

x^2=1.58, df=1, P value=0.2081(>0.05), OR=3.91

Table 2: Number of positive and negative cases in relation to sex.

	Female	Male	Total
Positive	1	3	4
Negative	103	79	182
Total	104	82	186

x^2=1.58, df=1, P value=0.2081(>0.05), OR=3.91

Table 3: Number of positive and negative cases in relation to age.

	≤ 1year	>1 year	Total
Positive	0	4	4
Negative	45	141	186
Total	45	141	186

X^2=1.30, df=1, P value=0.25 (>0.05), OR=0.33

It is possible to detect more positive cases with some of the modern techniques that are not readily available in the developing countries like Nigeria. Based on previous reports (Knonefeld et al., 2014; Montoya-Alonso et al., 2015), the study area have a suitable climatic conditions for high incidence of the disease. The outcome of this study also agrees with the previous reports (AHS, 2015), that Knott's technique remains the most effective method in the diagnosis of the disease than other parasitological methods such as Buffy coat, wet mount, thick and thin blood smears. This is probably because Knott's test, concentrates the microfilariae from a larger portion of blood through centrifugation. Although, some serological test like commercially available ELISA kits for antigen or antibody detection have been reported to be

Table 4: Hemogram of both positive and negative cases of dogs.

Selected hemoparameters	Positive (n = 4)		Negative (n = 182)		Reference value	
	Mean±SD	Range	Mean±SD	Range	Mean	Normal range
PCV (%)	34.8±15.30	12-45	38.9±8.96	14-57	45	35-55
RBC ($x10^{12}$/L)	5.5±2.20	2.22-7.01	5.81±1.47	2.10-10.35	6.5	5.0-8.0
WBC ($x10^9$/L)	14.7±5.13	10.1-19.4	16.03±9.61	5.0-89.1	11.5	6.0-17.0
Neutrophils($x10^9$/L)	11.3±3.96	7.37-15.59	11.7±7.51	2.71-65.58	7.0	3-11.5
Lymphocytes($x10^9$/L)	1.95±0.86	1.04-3.02	2.9±1.90	0.70-10.61	2.8	1.0-4.8
Eosinophils($x10^9$/L)	0.81±0.32	0.42-1.16	0.75±0.69	0-5.44	0.55	0.10-1.25
Monocytes($x10^9$/L)	0.59±0.36	0.31-1.13	0.61±0.39	0-2.19	0.75	0.15-1.35
platelets($x10^9$/L)	108±60.81	65-151	214±183	13-578	300	200-500

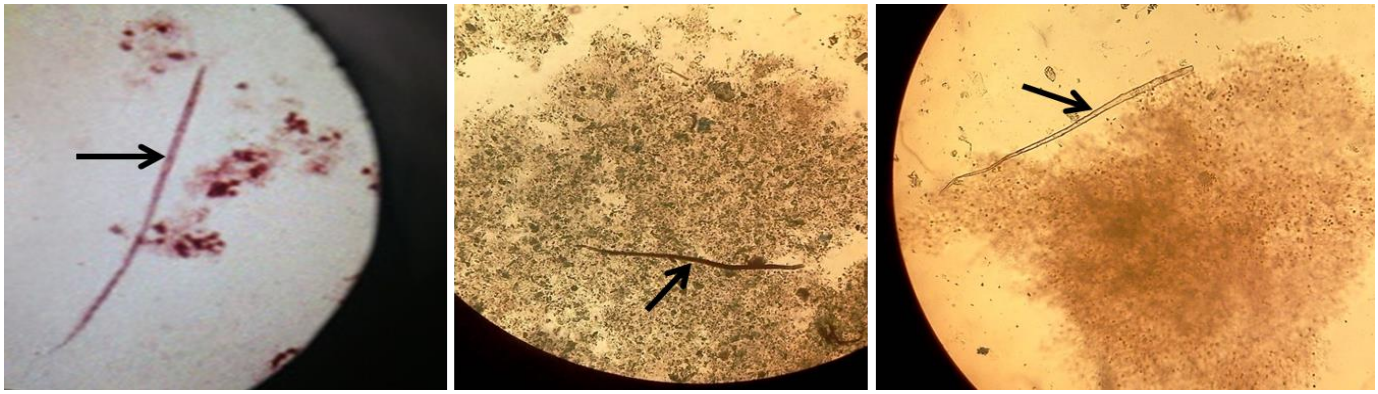

Figure 2: Microfilariae observed under microscope.

the most sensitive and test specific method in dogs and cats (Atkins, 2015), but it is expensive, requires expertise and not readily available in Nigeria. False negative and false positive cases can interfere with prevalence rate of this infection in dogs, since detection of microfilariae in infected dogs may show false negative if the heartworms are not fully mature, or if there's only male worms present, or if the females are not laying microfilariae at the time of the test as reported by Genchi et al. (2014). serological test like commercially available ELISA kits for antigen or antibody detection have been reported to be the most sensitive and test specific method in dogs and cats (Atkins, 2015), but it is expensive, requires expertise and not readily available in Nigeria. False negative and false positive cases can interfere with prevalence rate of this infection in dogs, since detection of microfilariae in infected dogs may show false negative if the heartworms are not fully mature, or if there's only male worms present, or if the females are not laying microfilariae at the time of the test, as reported by Genchi et al. (2014).

Some drugs such as Ivermectin is a good microfilariocidal drug and also appears to be effective at killing immature adult heartworms, therefore, dogs that are commonly dewormed with macrocyclic lactones may likely appear microfilaria negative (Blagburn et al., 2013; CAPC, 2015). It is also possible to have low prevalence rate from occult infection when some serological tests are used (Genchi et al., 2014). The higher incidence of the dirofilariasis in male dogs (3.66%) and older dogs (2.84%) agrees with previous report by CAPC (2015), who also reported higher rate of infection in male, older and exposed dogs. These are likely due to the exploratory life style of male, greater exposure time of adult dogs and the long incubation period of the worm. Statistically, there were no significant differences between sex and age groups in this study which also agrees with previous report by Vieira et al. (2014). The higher prevalence rate (4.04%) in outdoors system of dog management agrees with Guerrero (2012). The low level of anemia and the thrombocytopenia observed in positive cases agrees with CAPC (2015).

CONCLUSION

Dirofilaria immitis of dogs exist in Makurdi metropolis but in low prevalence rate based on the methods used in this study and modified Knott technique is the most sensitive technique in the diagnosis of infections in dogs than other parasitological methods. The general public in Makurdi are highly exposed to great danger pose by this serious zoonotic worm. Finally, I advise that more surveillance studies of this important zoonotic parasite be conducted in different parts of Nigeria for proper planning for effective control and preventive measures.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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