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Prevalence and risk factors of canine dirofilariasis in Kano metropolis, Kano State Nigeria

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Abstract

Dirofilariasis is a zoonotic disease caused by *Dirofilaria immitis* causes heartworm disease and invasive human filariasis. This study determined the prevalence and risk factors for the acquisition of canine dirofilariasis in dogs in Kano metropolis, Kano state, Nigeria. Blood samples were collected from 170 dogs of 6 months and above in Kano metropolis and tested by the Modified Knott's technique for circulating microfilaria, an Enzyme-linked Immunosorbent Assay in order to detect the presence of filarial antigens of *D. immitis* as evidence of occult infection. A pre-tested, structured questionnaire was administered to each of the dog owner to obtain demographic, management and environmental information associated with each dog sampled. Test for association and hypothesized factors was determined and the its strength was tested using a binary logistic regression. Out of the 170 dogs tested, 17 (10.00%) were positive for *D. immitis* infection with the Modified Knott's technique. However, after subjecting the samples for antigen detection by ELISA tests 23 (13.53%) were positive for *D. immitis* antigen, giving a sero-prevalence of 13.50%. There was agreement between the two tests on the 17 microfilaria positive samples. Majority of the infected dogs were males (56.5%), aged 24-47 months (43.50%) and of local breed (65.2%). There was significant association ($p \leq 0.05$) between *D. immitis* infection and dogs usage, breed and dogs with stagnant water (gutters) close to their houses (OR**=** (9.714; 95% CI (1.179-80.024))**,** OR=5.33; 95% CI (1.349-21.079), OR= 5.775; 95% CI (0.697- 47.832), respectively. The study established that *D. immitis* infection is prevalent (13.53%) in household dogs indicating the possibility of zoonotic transmission in Kano metropolis. This demands for intensified vector control and good dog management practices to reduce dog to dog and dog to human transmission.

Keywords: *Dirofilaria immitis*, Dogs, ELISA, Mosquito, Modified Knott's Techniques, Kano

Introduction

Dirofilariasis is a zoonotic nematode disease of domestic and wild carnivores transmitted to man mainly by infected mosquitoes (*Aedes, Armigeres, Culex,* and *Mansonia*) (James, 2014). Dirofilariasis is distributed throughout the world and is endemic in tropical and temperate regions (McCall *et al.,* 2008). Canine dirofilariasis due to *Dirofilaria immitis* is called heartworm disease, believed to cause infection to heart of dogs (Anyanwu *et al.,* 2000; Lee *et al*., 2012).

The parasite infects other animals e.g domestic cats and wild felids, ferrets, coyotes, foxes, wolves and other wild canids (McCall *et al*., 2008).

Dogs are the definitive host of the parasite while mosquitoes such as *Aedes, Armigeres, Culex,* and *Mansonia* serve as the vectors that transmit the parasite from dog to dog and to humans (Fernando *et al.,* 2012). However, studies indicated that fleas, lice, and ticks are also recognized to act as vectors (Joseph *et al*., 2011).

The symptoms in dogs with dirofilariasis include persistent cough, difficult breathing, and poor exercise tolerance, ascites, anorexia, and weight loss (Morchon *et al*., 2009).

A clinical sign of the disease in dogs begins with pulmonary hypertension that eventually changes into a right-sided heart failure and sometime lethal obstruction of the vena cava (Atkins *et al*., 1988).

Dirofilaria *immitis* is the most common specie that causes infection to humans among the forty known species of *Dirofilaria* (Horst, 2003).

In human, the parasite infects the lungs leading to formation of granuloma within the lungs which is highly invasive for the patient during treatment (Foroulis *et al*., 2005).

Development of the *D. immitis* is favored by symbiotic relationship between the parasite and a bacterium of genus *Wolbachia Wolbachia* involved in the sexual maturation, moulting and embryogenesis of the parasite; while filariae contribute amino acids for bacterial growth and development (Foster, 2005; Fernando *et al.,* 2012; Simoncini *et al*., 2001).

The death of adult filarial worms occasioned by treatment or natural cause results inflammatory lung response in dogs infected with *D. immitis;* this is attributed to the release of *Wolbachia* antigens after disintegration of the worms (McCall *et al*., 2008). Antibiotic treatments targeting *Wolbachia* have shown to reduce filarial induced pathology (Fernando *et al.,* 2012).

Genchi *et al.* (2001), reviewed reports of the presence of *D. immitis* in dogs in Morocco, Tunisia, Nigeria, Egypt, Tanzania, Kenya, Mozambique, Malawi, Senegal, Angola, and Gabon. The review also described the disease in both dogs and cats in Sierra Leone and South Africa. There are few reports on the prevalence of canine dirofilariasis in Nigeria where 3.36% was reported in Nsukka, Enugu State (Chukwuebuka *et al.,* 2015) and 12.7% in Zaria, Kaduna State (Anyanwu *et al.,* 1996).

The aim of the study was to determine the prevalence and risk factors of canine dirofilariasis in Kano metropolis, Kano State, Nigeria.

Materials and Methods

Study area

The study location is Kano metropolis, Kano State, Nigeria . It is situated between latitudes 11° 25' N to 12°47' N and longitudes 8° 22' E to 8°39' E east and 472m above sea level. Kano metropolis is bordered by Madobi and Tofa Local Government Areas (LGAs) to the South West, Gezawa LGA to the East, Dawakin Kudu LGA to the South East, and Minjibir LGA on the North East. The study area covered eight (8) out of 44 LGAs in the state. These include Dala, Fagge, Gwale, Kano Municipal, Nassarawa, Tarauni and parts of Ungogo and Kumbotso local governments. Kano is the most populated city in Nigeria after Lagos. It has a population of 2,826,307 people (Bello *et al.,* 2014). Kano is referred to as the center of commerce in the country. This is based on the fact that marketing and trading have been the dominant, long flourishing economic activities of the populace of the metropolitan Kano (Bello *et al.,* 2014).

Study design

A cross sectional study was carried out to determine the prevalence of canine dirofilariasis in dogs within Kano metropolis. Dogs and dog owners within Kano metropolis were the target population, while the list of clients that attended or were being served by Veterinary Clinics constituted the sampling frame with individual clients or households that had dogs attending or were served by the Veterinary Clinics in Kano metropolis constituted the sampling units.

Study animals

A total of 170 male and female dogs (local, cross and exotic breeds) were included in this study.

Blood collection

Each dog brought for sampling was properly restrained and 5ml of whole blood was aseptically drawn from the cephalic vein of the dog by a trained Veterinary Surgeon using 5ml sterile syringe and needle. Aspirated blood sample was transferred into a sample bottle containing anticoagulant (EDTA) and processed.

Inclusion criteria

All dogs that were taken to or were being offered services by government or private Veterinary Clinics in Kano metropolis, dogs whose owners had consented, dogs that could be accessed, restrained and dogs that were at least six (6) months of age

Exclusion criteria

All dogs that were not receiving veterinary care**,** dog owners' disapproval**,** dogs that could not be restrained or captured for sample collection and dogs younger than six (6) months were excluded from the study considering the life cycle of *D. immitis*

Administration of questionnaires

A pretested, structured questionnaire was administered to the owners of dogs at the time of sample collection to obtain information on dogs' demographic, management and other practices as related to the acquisition of the infection in dogs. This information was used for identifying the risk factors of` the infection in dogs.

Detection of D. immitis infection in blood using modified Knott's technique

From every blood sample collected; One milliliter (1 ml) was taken and mixed with nine ml of 2% formalin; leading to the disintegration of erythrocytes and fixing of the leukocytes and the microfilariae. Three minutes later, the mixture was centrifuged for five minutes at 3000 revolution per minutes and the supernatant was discarded. Thirty-five microliters (35 µl) of the sediment was smeared on glass slide, the slide was then stained with methylene blue solution prepared at two percent for about three minutes and examined to determine the presence of *D. immitis* microfilariae, under X10 and X40 magnification (Newton & Wright, 1956).

Detection of D. immitis antigen in dogs using ELISA test

All the samples collected were examined for presence of antigens of *D. immitis* using ELISA kit (FilarCheck®, Agrolabo S.p.A. CO. Italy). Serum was used for the detection of the antigens. The serum was harvested after the blood sample was allowed to clot. The clot was removed by centrifugation and the resulting supernatant (serum) was removed using a Pasteur pipette.

The test was conducted according to the manufacturer's instructions.

Data analysis

The prevalence of *D. immitis* infection was calculated and expressed as percentage (%).

Test for association between the infection and demographic, management and environmental factors was determined using chi-square (χ^2) and Fisher's Exact Test. Strength of association was tested using a binary logistic regression to calculate odds ratio at 95 percent confidence interval for assessment of risk factors and $p \leq 0.05$ was considered significant.

Results

Out of the 170 dogs tested, 17 (10.00%) were positive for *D. immitis* infection with the Modified Knott's technique. However, after subjecting the samples for antigen detection by ELISA tests 23 (13.53%) were positive for *D. immitis* antigen, giving a sero-prevalence of 13.53% (Table 1). Six samples that were tested negative from the Modified Knott's techniques showed evidence of infection with the ELISA technique. All the 17 parasitological positive samples were also positive with the ELISA test.

Of the 170 dogs investigated in this study, 91 (53.5%) were males and 79 (46.5%) were females. Male dogs were more infected, 13 (56.5%) than female dogs 10 (43.5%) from the ELISA result as shown in the Table 2. But this difference was not statistically significant (p =0.757)

Table 1: Prevalence of *D. immitis* infection in dogs by Modified Knott's test and ELISA test

Technique	No. Tested	Positive	Percent
Modified Knott's technique	170	17	10.00
ELISA Kit (FilarCheck®)	170	23	13.53

Table 2: Sex-based Prevalence of *D. immitis* infection in dogs in Kano Metropolis using ELISA

Sex	No. Examined		
		No. Positive	Percent
Male	91	13	56.52
Female	79	10	43.48
Total	170	23	100.0

Chi Square = 0.096; df=1, p value = 0.757

Dogs within the range of 6-23 months had two samples (8.7%) positive for *D. immitis* infection. Ten dogs (43.5%) were found positive within the range of 24-47months age group, eight (34.8%) and three dogs (13.0%) were positive dogs in the 48-71months and 72-95months respectively (Table 3). The distribution of infection as determined within the various age groups was not statistically significant.

The sero-prevalence of *D. immitis* was 65.2% in local dog breeds (mongrel dogs), 17.4% in cross and exotic breeds respectively. Table 4 shows the number of positive cases with respect to breed distribution. There was significant association between the breed and occurrence of *D. immitis* infection (p = 0.05)

Dogs that are used for guarding had the highest rate of infection (56.5%). The lowest prevalence of 4.3% was observed in hunting dogs; 7 (30.4%) and 2 (8.7) had infections in the dogs used as pet and herding dogs respectively

(Table 5). Significant difference was observed among the dogs in relation to their uses (p = 0.040). Dog with gutters within or near their houses had the highest prevalence rate of infection; 11 (47.8%) among the total dogs examined. Those close to open dumps and stagnant water bodies had prevalence of 8.7% and 4.3% each. Up to 13.0% and 26.2% prevalence was recorded in other mosquito breeding areas and those with no stagnant water bodies respectively (Table 6).

Significant difference was observed (p = 0.022). Variable outcomes of risk factors analysis such as age, sex, breed, dog usage, presence of water bodies near or close to the house, etc. were summarized and presented in Table 7.

Table 3: Seropositive cases of *D.immitis* by age group of dogs

Age group	No. Examined	Percentage positive	
		Positive	Percent
6-23	28	2	8.7
24-47	57	10	43.5
48-71	48	8	34.8
72-95	37	3	13.0
Total	170	23	100

Fishers Exact=2.857, df=3, p value=0.419

Table 4: Prevalence of *D. immitis* according to dogs' breeds

Breed	No. Examined	Percentage positive	
		Positive	Percent
Exotic	10	4	17.4
Cross	35	4	17.4
Local	125	15	65.2
Total	170	23	100

Fishers Exact=5.253, df =2, p value= 0.052

Table 5: Seroprevalence of *D. immitis* based on purpose of keeping dogs

Dog use	No. examined	Percentage positive	
		Positive	Percent (%)
Guarding	65	13	56.5
Herding	4	2	8.7
Hunting	25	1	4.3
Pet and others	76	7	30.4
Total	170	23	100

Fishers Exact=9.488, df=4, p value= 0.040 (p <0.05)

Discussion

Several studies on filarial worms have been done in Kano metropolis and its environs, more especially on *Wuchereria bancrofti, Brugia malayi and B. timori* the causative agents of lymphatic filariasis (Anosike *et al*., 2005). To the best of our knowledge, this is the first-time heartworm (*D. immitis)* has been described and reported among dogs in the Kano metropolitan, northern- western Nigeria.

This study utilized two reliable test techniques for identifying *D. immitis* infection in dogs globally (concentration and antigen test)

Table 6: Seroprevalence of *D. immitis* based in the presence of water bodies and vector breeding sites

Water type	No. examined	Percentage positive	
		Positive	Percent
Gutters	51	11	47.8
Stagnant water	22	1	4.3
Open dumps	29	2	8.7
Other breed area	32	3	13.4
No stagnant water	36	6	26.2
Total	170	23	100

Fishers Exact=5.494, df=4, p value= 0.022

Table 7: Analysis of risk factors associated with *Dirofilaria immitis* in dogs

Variables	Options	Total (%)	OR	(95% CI)	p- value
Sex of the dog	Male	91 (53.5)	1 (Ref.)		
	Female	79 (46.5)	0.870	0.359-2.109	0.757
Breed type	Exotic	10 (5.9)	5.33	*1.349-21.079	0.017
	Cross	35 (20.6)	1.032	0.320-3.311	0.958
	Local	125 (73.5)	1 (Ref.)		
Age	6-23mn	28 (16.5)	1 (Ref)		
	24-47mn	57 (33.5)	2.276	(0.563-13.590)	0.210
	48-71mm	48 (28.2)	2.600	(0.561- 13.22	0.249
	72-95mn	37 (21.8)	1.147	(0.178-7.373)	0.885
Present water body					
near or close to the house	No water bodies	36 (21%)	4.200	0.470-37.499	0.199
	Other breed areas	32 (18.8)	2.172	0.211-22.368	0.514
	Gutters	51 (30.0)	5.775	0.697-47.832	0.104
	Open dumps	29 (17.1)	1.556	0.132-18.340	0.726
	Stagnant water	22 (12.9)	1 (Ref.)		
Dog usage by owner	Guarding	65 (38.2)	2.429	0.905-6.518	
	Herding	4 (2.4)	9.714	*1.179-80.024	
	Hunting	25 (14.7)	0.389	0.045-3.319	
	Pet	75 (44.1)	1 (Ref)		

* Signifies significance

and employed them in order to achieve precision in estimating the prevalence of the parasite (*D. immitis*) in the study area. The prevalence rates of *D. immitis* were 10.00% and 13.5% respectively by Modified Knott's technique and by antigen test. The variation observed in prevalence rates in this study could be attributed to the ability of serological technique to detect the antigen of the infecting parasite in dogs (Hoover *et al.,* 1996). Antigen tests by monoclonal antibodies allow the detection of infections; that could not to be detected by Modified Knott's test. All samples that were tested positive by Modified Knott's method were positive by serology in addition to 6 not detected by the former.

The prevalence rate (13.50%) of this study was higher than the prevalence rates of 3.36% and 2.15%, reported in the studies conducted in Nsukka (Chukwuebuka *et al.,* 2015), and Makurdi (Christopher & Abel, 2016) in Nigeria respectively. This variation could be attributed to the higher sensitivity and specificity of methods employed in this study compared to the methods used in those studies (concentration). The high prevalence rate in this study could also be attributed to the climatic factors of the study area, because Kano city bids the ideal condition (> 26.1°C) for the mosquito development and the larvae of *D. immitis* and availability of water bodies. But in contrast the prevalence is lower than that reported in many states of Turkey; these include 46.2% for the town of Van (Agaoglu *et al*., 2000) and 27.46% in Kirikkale (Yildiz *et al*., 2008). Likewise, in Italy, prevalence reached 50% along Po river valley (Genchi *et al.,* 2001); 24.46% in Algiers (Ben-Mahdi & Madani, 2008) and16.01% in city of Ahvaz republic of Iran (Ranjbar-Bahadori*,* 2011). This indicates that the disease is widespread in areas of different climatic conditions and may also indicate or suggest that there are other dipteran vectors responsible for the transmission

Genchi *et al.* (2014) reported increasing cases of *D. immitis* infections from non-endemic areas; across the world; suggesting probable explanation of the disease geographical change

Male dogs were more infected in this study than female dogs, although there was no significant difference between the either group and the generally higher infection rate in male can be linked to the fact that male individuals are often kept outdoors for their use as guard and hunting dogs (Song *et al*., 2003).

Studies indicated that the mean sero-prevalence of *D. immitis* infection in dogs increased with age, meaning the older the age, the higher the sero-prevalence (Razi Jalali *et al.*, 2010); this statement was attributed to longer exposure to the vectors (mosquitos). The finding of this study was contrary, because, the

results showed that dogs in the age group of 24-47 months have higher rate of infections compared with their counterparts in 48-71 and 72-95 months respectively. No significant difference was observed among the dog's age groups and is in line with findings of Ben-Mahdi & Madani (2008).

Prevalence of heartworm by breeds was 65.2% in local dogs, 17.4% in cross and exotic breeds respectively. This means the prevalence rate was higher in local breed. The high prevalence rate of heartworm infection in local dogs could be attributed to lack of adequate veterinary supervision and heartworm chemoprophylaxis. In addition, based on demographic data of the owners obtained majority of the local dog owners had not attended higher institutions of learning and this perhaps may limit their knowledge to some degree on canine dirofilariasis.

For the exotic breeds in this Study, the remarkable percentage obtained could be attributed to interstate and intercontinental movement of dogs or could be attributed to trips to or from the endemic regions. Although by intuition, exotic breeds in Nigeria are expected to be less affected with heartworm disease due to availability of proper management approaches in place and care they do normally receive from their owners.

In conclusion, this study established the occurrence of heartworm disease (*D. immitis)* in the dog population in Kano metropolis using Modified Knott's test with a prevalence of 10.00%. The presence of the parasite was also detected by antigen test with higher prevalence of 13.50% indicating the presence of infections that were not detectable microscopically. Therefore, the overall prevalence of heartworm disease in Kano metropolis, Kano state Nigeria in this study was 13.50%.

Infection is more likely in male 13(56.5%) than female 10(43.5%) dogs. Therefore, the general public is at risk of acquiring infection from dogs since the conditions suitable for mosquito-vector survival and human-mosquito contact exist. This study, to the best of our knowledge is the first report of *D. immitis* infection in dogs in Kano State, Nigeria.

From the results of this study, it is recommended that: The presence of *D. immitis* in Kano metropolis demands for the implementation of a prophylactic plans in canine populations.

Campaigns to improve knowledge by Kano state government on emerging, re-emerging and neglected zoonosis disease that are mosquito-borne among dog owners should be carried out.

Using the One Health approach an integrated vector-borne disease control strategy targeted at reducing vector population and reducing human vector contact should be instituted.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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