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Research Article

Molecular Detection of Vector-Borne Pathogens in Apparently Healthy Dogs (*Canis familiaris*) in Jos Plateau State, Nigeria

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Abstract

Dogs are recognized host and reservoir of pathogens of veterinary and public health significance. To elucidate the role of dogs in the epidemiology of some vector-borne pathogens (VBPs) in Nigeria, 204 blood samples obtained from apparently heathy dogs were screened for the presence of piroplasmids, filarioids, *Bartonella* spp. and *Trypanosoma* spp. using conventional PCR. Four VBPs; *Hepatozoon canis*, *Babesia rossi*, *Acanthocheilonema reconditum* and *Theileria* spp. were detected in the study population with an overall prevalence of (23.0%). Single and mixed infections were recorded in 27 (13.2%) and 20 (9.8%) samples, respectively. The Nigerian local breed of dogs was significantly ($\chi^2 = 6.14$, $p = 0.01$; OR = 8.3, $p = 0.01$) at risk of VBPs infection than the exotic breeds. Male dogs were 1.6 times at risk of infection with VBPs than females, just as dogs less than one year of age were twice at risk of infection than dogs older than one year, although the differences were not significant ($p > 0.05$). None of the samples was positive for the DNA of *Bartonella* spp. or *Trypanosoma* spp. The detection of the DNA of four VBPs of veterinary importance in apparently healthy dogs has implication for the control of VBPs in Nigeria. The findings from this study suggest that dogs in the study area may serve as reservoirs of VBPs and constitute risk of infection to animals and man.

Keywords: *Dog; Vector-Borne Pathogens; PCR; Public Health; Epidemiology; Nigeria*

Introduction

The domestication of dogs dates back to ~23,000 years ago, and their relationship with man is the earliest since the ancient civilization [17,25]. Since their domestication, dogs have played key roles in the human society including security, hunting, sports, shepherding, pets/companionship, etc [6,25]. In addition, dog meat is consumed in some countries including Nigeria, as a source of protein to man [8]. Dogs, especially when allowed to roam freely are exposed to a wide range of disease agents, thereby serving as reservoir of pathogens of zoonotic and veterinary importance [28]. Several reports from across the world have incriminated dogs in the epidemiology of vector-borne pathogens of zoonotic importance such as *Bartonella* spp. [7], *Dirofilaria* spp. [24], *Trypanosoma* spp. [18,29] and *Rickettsia* spp. [20], among others. Regardless of the purposes for which they are kept, dogs usually come in close contact with humans and livestock which may facilitate the transmission of a wide range of pathogens. As such, dogs are considered as an important source of emerging and re-emerging diseases to humans [20,28]. Considering the multifarious role of dogs in the socio-economic life of the inhabitants of Jos,

Plateau State, this study was conducted to determine the presence and prevalence of VBPs of veterinary and public health significance in dogs and to make appropriate recommendations for safeguarding the health of the populace.

Materials and Methods

Animal ethics

Approval for this study was granted by the Institutional Animal Use and Care Committee (IAUCC), National Veterinary Research Institute (NVRI) Vom, Nigeria, reference number- AEC/03/56/18. Permission for sampling the dogs was obtained from the officials of the dog market operators and the dog owners.

Study area

The study was conducted on dogs brought to the dog market/slaughter slab in Bukuru ((9.765°N, 8.859°E), Jos South Local Government Area (LGA), Plateau State Nigeria. Bukuru is a suburb of Jos city situated on an elevation of 1277m above sea level and has a population of 15,540 inhabitants. Being an old mining settlement, Bukuru town attracts diversity of settlers beside the indigenous Berom people. The main occupation in the study area is subsistence farming, artisans, mining, trading and civil service. Dog meat is a delicacy among the Berom people who are the predominant population of Bukuru town. Therefore, a good number of the inhabitants are engaged in dog breeding and marketing.

Dog sampling

Dogs that were brought to the market for sale or slaughter were included in the study. Approximately 5 mL of blood was collected from the cephalic vein of dogs slaughtered in the dog slaughter slab from April to August 2021. Blood samples were preserved in ethylene diamine tetra-acetic acid (EDTA) tubes at -20°C in the Molecular Biology Laboratory, Parasitology Division, NVRI Vom until analysis. Data on age, breed and sex of dogs were obtained at the time of sampling. None of the dogs sampled was neutered and the minimal age of dogs included in this study was six months.

DNA extraction

Genomic DNA was extracted from the EDTA preserved blood samples using the Quick-DNA ™ Miniprep Plus Kit (Zymo Research, USA) according to the manufacturers protocol. DNA was eluted in 80 µL elution buffer and preserved at -20°C until analysis.

Conventional PCR for the amplification of vector-borne pathogens in dogs

A conventional PCR targeting various genes/loci for the detection of the DNA of piroplasmids (*Hepatozoon* spp., *Babesia* spp., *Theileria* spp), filarioids, *Bartonella* spp. and *Trypanosoma* spp. was conducted using the primers and reaction conditions outlined in table 1. The reaction was conducted in a final volume of 25 µL consisting of 12.5 µL of 2X Master Mix with standard buffer (New England Biolabs Inc.), 0.5 µL of each primer (10 mM), 5 µL of template DNA and 6.5 µL of PCR grade water (Solis BioDyne, Estonia). Positive control DNAs corresponding to each VBP being tested and a non-template control (NTC) containing all the reaction mix except DNA template was included in each PCR run. The PCR products were electrophoresed and checked for appropriate size bands on a 1.5% agarose gel stained with SafeView™ Classic (abm, Canada) in a TAE buffer in comparison to a 100 bp molecular ladder under a blue light trans illuminator (Cleaver Scientific, UK). Positive amplicons were Sanger sequenced at a commercial facility (Inqaba Biotech West Africa Ltd, Ibadan, Nigeria) using the PCR primers.

Organism	Primer code	Primer sequences (5'-3')	Target gene	Annealing Temp.(°C)	No. of cycles	Amplicon size (bp)	References
Piroplasmida	HeppiroF	CCAGCAGCCGCGGTAATTC	*18S rRNA*	64	35	356	30
	HeppiroR	CTTTCGCAGTAGTTYGTCTTTAACAAATC T					
Hepatozoon spp	HepF	ATACATGAGCAAAATCTCAAC	*18S rRNA*	57	35	666	11
	HepR	CTTATTATTCCATGCTGCAG					
Babesia spp	PiroA	AATACCCAATCCTGACACAGGG	*18S rRNA*	64	40	408	22
	PiroB	TTAAATACGAATGCCCCCAAC					
Dirofilaria spp	DIDRF1	AGTGCG AATTGCAGACGCATTGAG	*ITS-2*	60	35	490-650	27
	DIDRR1	AGCGGGTAATCACGACTGAGTTGA					
Dirofilaria imitis	DIITSF	CATCAGGTGATGATGTGATGAT	*ITS-2*	63	30	302	19
	DIITSR	TTGATTGGATTTTAACGTATCATTT					
Bartonella spp	CshF	GCGAATGAAGCGTGCCTAAA	*gltA*	51	35	350	4
	1137n	AATGCAAAAAGAACAGTAAACA					
Trypanosoma spp	TITS1F	CCGGAAGTTCACCGATATTG	*ITS-1*	60	40	250-710	21
	TITS1R	TTGCTGCGTTCTTCAACGAA					

Table 1: *Oligonucleotide primers used for the PCR amplifications of vector-borne pathogens in dogs in Nigeria. Citrate synthesis gene (gltA), Internal transcribe spacer region (ITS), bp = Base Pairs.*

Statistical analysis

The prevalence of VBPs in dogs was calculated as percentage of samples positive over the total number of dogs examined. The association between VBPs and the variables: Age (≤ 1year or > 1year), breed (local or exotic), PCV (≤ 25% or > 25%) and the sex of the dogs were analyzed. Univariate analysis was performed for each risk factor using the Chi-square test. The risk of VBPs infections was also analyzed using the Fisher's exact test. The analysis was performed using the R Statistical Software [26]. The level of significance was set at $p \leq 0.05$.

Results

A total of 204 dogs were included in this study. Data was available for 200 of the dogs while four had no data available. The majority of the dogs, 194 (96.5%) were of the local breed, most of them 130 (65%) being males, between six months and one-year-old (n = 115; 57.5%) (Table 1).

The DNA of some vector-borne pathogens were detected in 47 (23.0%) of the dogs examined in this study. Single infections were recorded in 27 (13.2%) samples, while mixed infections were detected in 20 (9.8%) of the samples. Single infection due to *H. canis* was the most prevalent, 6.9%, followed by *B. rossi* (2.9%) and *Theileria* spp (1.9%). The DNA of *Acanthocheilonema reconditum* was detected in three samples (1.5%). *Hepatozoon canis* was found in mixed infection with all the other three VBPs detected in this study. None of the samples yielded positive amplification of the DNA of *Bartonella* spp. or *Trypanosoma* spp. (Table 2).

The PCV of most of the infected dogs were > 25%. Two dogs with *H. canis* infection, and one other with dual infection due to *H. canis* and *Theileria* spp. had PCV ≤ 25% (Table 2).

Pathogen	Characteristics of positive dogs								Numbers positive (%)
	Age (years)		Sex		Breed		PCV (%)		
	≤ 1	> 1	F	M	Lc	Ex	≤ 25	> 25	
Single infections									
Hepatozoon canis	12	2	3	11	11	3	2	12	14 (6.9)
Babesia rossi	4	2	3	3	6	0	0	6	6 (2.9)
Theileria spp.	1	3	2	2	4	0	0	4	4 (1.9)
Acanthocheilonema reconditum	1	2	0	3	3	0	0	3	3 (1.5)
Bartonella spp.	0	0	0	0	0	0	0	0	0
Trypanosoma spp.	0	0	0	0	0	0	0	0	0
Subtotal 1	18	9	8	19	24	3	2	25	27 (13.2)
Mixed infections									
H. canis + *B. rossi*	2	0	0	2	2	0	0	2	2 (1.0)
B. rossi + *Theileria* spp.	1	1	2	0	2	0	0	2	2 (1.0)
H. canis + *Theileria* spp.	10	1	3	8	9	2	1	10	11 (5.4)
H. canis + *B. rossi* + *Theileria* spp.	2	0	0	2	2	0	0	2	2 (1.0)
H. canis + *A. reconditum*	0	2	0	2	2	0	0	2	2 (1.0)
A. reconditum + *Theileria* spp.	0	1	0	1	1	0	0	1	1 (0.5)
Subtotal 2	15	5	5	15	18	2	1	19	20 (9.8)
Overall	33	14	13	34	42	5	3	44	47 (23.0)
χ^2	3.41		1.06		6.14		3.45		
P	0.07		0.30		0.013*		0.06		
Odds Ratio	2.0		1.6		8.3		10.2		
P	0.06		0.29		0.01*		0.04*		
95% CI	0.97 - 4.46		0.72 - 3.48		1.30 - 89.8		0.79 - 545.6		

Table 2: *Prevalence of vector-borne pathogens in dogs in Jos, Plateau State, Nigeria.*

*Values with * indicate significance difference.*

F = Female, M = Male, Lc = Local Breed, Ex = Exotic Breed, PCV = Packed Cell Volume, χ^2 = Chi Square, CI = Confidence Interval.

Male dogs were 1.6 times at risk of infection with VBPs than females, just as dogs less than one year of age were twice at risk of infection than dogs older than one year, although the differences were not significant ($p>0.05$) in both cases (Table 2). However, the Nigerian local breed of dogs was significantly ($\chi^2 = 6.14$, $p = 0.01$; OR = 8.3, $p = 0.01$) at risk of VBPs infection than the exotic breeds (Table 2).

The nucleotide sequences obtained in this study have been deposited in the GenBank database under the following accession numbers: ON155604-ON155606; ON556414-ON556418.

Discussion

The DNA of four pathogens of veterinary importance were detected in apparently healthy dogs examined in this study. Although, the pathogen diversity was similar to reports from previous studies in Nigeria, the overall prevalence of 23.0% in this study was lower than the 85.4% previously reported [1,10]. This is not surprising, but worrisome since the dogs examined in this study were apparently healthy and can unsuspectedly serve as source of infection to animals and man. *Hepatozoon canis* was the most prevalent pathogen detected in dogs in this study both as single and mixed infection with other pathogens. Previous molecular studies have reported *H. canis* in dogs in Nigeria [10,15]. Dogs infected with *H. canis* may either present as mild subclinical infection or a severe and potentially lethal disease which is characterized by pyrexia, anemia, emaciation and weakness [3]. Most of the dogs examined in this study were apparently healthy, suggesting the subclinical form of hepatozoonosis in the positive individuals. *Babesia rossi* was the second most prevalent pathogen detected in dogs in this study. The prevalence of 2.9% reported in this study is lower than 6.6 - 85.4% prevalence reported in previous studies in Nigeria [1,10,15]. *Babesia rossi* infection usually affect dogs of all age groups and is considered the most pathogenic to dogs among the large canine Babesidae [12]. Paradoxically, none of the six dogs harboring *B. rossi* infection in this study had PCV below 25%, suggesting a kind of endemic stability. Near equal number of dogs were harboring single or mixed VBPs infections i.e. 18 verse 15. This suggests that the dogs were exposed to continues and diverse vector challenges. The DNA of *Theileria* spp. was detected in four dogs as a single infection and in 15 dogs as mixed infections. Previous molecular studies in Nigeria have reported the DNA of *Theileria* spp. in dogs, though the veterinary and public health significance of this parasite has not been fully elucidated [1,15]. More studies to determine the vector(s) and pathogenicity of *Theileria* spp. in dogs are needed to elucidate the veterinary and public health perspective of this pathogen.

The DNA of *Acanthocheilonema reconditum* was detected in three (1.5%) of the dogs examined in this study. This prevalence was higher than the 0.5% reported in a previous study, using a highly sensitive and specific High Resolution Melt Real Time PCR in Nigeria [13], but lower than results obtained by classical diagnostic methods [2,31]. *A. reconditum* are mainly located in the subcutaneous tissues of dogs and was initially reported as being non-pathogenic to dogs, but there are reported cases of pruritic dermatosis and focal alopecia in dogs. Interestingly, the nucleotide sequences obtained in this study had less than 96% identity to sequences of *A. reconditum* in the GenBank. A similar report of low (< 96%) sequence identity to sequences in GenBank was observed with the sequence obtained by the HRM RTPCR in our previous study [13]. This may suggest the existence of a new species or variant of *A. reconditum* circulating in the dog population in Nigeria. On the other hand, the DNA of *Dirofilaria imitis* was not detected in any of the samples in this study using species-specific primers, despite previous reports of 0.5 - 3.4% using classical methods [2,31]. Therefore, in order to elucidate the true status of canine filarioids in Nigeria, future studies should screen samples from different cohorts of dogs as well as potential vector(s) across the agro-ecological zones of Nigeria using classical and molecular methods.

None of the samples examined in this study was positive for *Trypanosoma* spp. Unlike the zero prevalence reported in this study, 8.4% (20 out of 205) of dogs examined by PCR in Zambia were positive for trypanosomes including the zoonotic *Trypanosoma brucei rhodesiense* [18]. However, there were reports of serological and microscopic detection of *Trypanosoma* spp in dogs in Nigeria using the classical methods [9,29]. Ecological factors, methods of diagnosis and the uses of the dogs included in these studies may account for the differences in the results. Similarly, we did not detect the DNA of *Bartonella* spp. in dogs in this study. Dogs have been incriminated as

reservoirs of *Bartonella* spp. with zoonotic potential in some countries. A study in Peru targeting the 16S-23S ribosomal RNA (rRNA) intergenic transcribed spacer (*ITS*) of *Bartonella* species reported the detection of the DNA of *Bartonella rochalimae* and *B. vinsonii berkhoffii* genotype III in 10% of 205 dogs [7]. However, it was the citrate synthase gene (*gltA*) of *Bartonella* that was the target for amplification in this study. This genus specific *gltA* primers used are reputed to be sensitive for the amplification of the members of the *Bartonella* genus [4]. Previous studies in Nigeria targeting the *gltA* gene have detected the DNA of various *Bartonella* spp. in rodents and bats [14,16] suggesting that animals in Nigeria are infected with *Bartonella* spp. Similar to the results from this study, zero prevalence of *Bartonella* spp. by PCR have been reported in studies from some countries [5,23]. A large scale study targeting different genes/fragments may elucidate the role of domestic dogs in the epidemiology of bartonellosis in Nigeria.

In addition to the globally recognized role of dogs as hosts or reservoirs of emerging and re-emerging zoonotic agents, they play an important role in the socio-economic life of Nigerians. Dog breeding and marketing provide employment to many people and bring them in close contact regularly. This practice may favor the transfer of pathogens or vectors with public health consequence. Hence, the need to routinely examined the presence and prevalence of VBPs in dog populations in order to inform and educate the populace of possible untoward effects.

Conclusion

In conclusion, the finding of four pathogens of veterinary importance in apparently healthy dogs in this study suggests their widespread distribution in the study area. This finding necessitates the implementation of appropriate control measures in order to safeguard human and animal health. Although the DNA of *Bartonella* spp. and *Trypanosoma* spp. were not detected in dogs in this study, caution should be exercised in interpreting the results. A large scale study targeting multiple genes/loci is needed to elucidate the role of dogs in the epidemiology of some VBPs of veterinary and public health importance in Nigeria.

Bibliography

1. Adamu M., *et al*. "Occurrence of tick-transmitted pathogens in dogs in Jos, Plateau State, Nigeria". *Parasite Vectors* 7 (2014): 119.
2. Anyanwu IN., *et al*. "Canine filariasis in Zaria, Nigeria". *African Journal of Medicine and Medical Science* 25 (1996): 323-326.
3. Baneth G and Weigler B. "Retrospective case-control study of hepatozoonosis in dogs in Israel". *Journal of Veterinary Internal Medicine* 11 (1997): 365-370.
4. Birtles RJ and Raoult D. "Comparison of partial citrate synthase gene (gltA) sequences for phylogenetic analysis of Bartonella species". *International Journal Systematic Bacteriology* 46 (1996): 891-897.
5. Clarke LL., *et al*. "Prevalence of select vector-borne disease agents in owned dogs of Ghana". *Journal of South African Veterinary Association* 85 (2014): 996.
6. Dantas-Torres F. "Canine vector-borne diseases in Brazil". *Parasite and Vectors* 1 (2008): 25.
7. Diniz PPVP., *et al*. "Infection of domestic dogs in Peru by zoonotic *Bartonella* species: A Cross sectional prevalence study of 219 asymptomatic dogs". *PLoS Neglected Tropical Disease* 7.9 (2013): e2393.
8. Ekanem EE., *et al*. "Stray dog trade fuelled by dog meat consumption as a risk factor for rabies infection in Calabar, southern Nigeria". *African Health Science* 13.4 (2013): 1170-1173.
9. Ezeh IO., *et al*. "Berenil- Resistant *Trypanosoma brucei brucei* infection in a dog in Nsukka area of Enugu state, Nigeria". *Nigerian Veterinary Journal* 29 (2009): 34-42.

10. Happi AN., *et al*. "Detection and identification of blood-borne infections in dogs in Nigeria using light microscopy and the polymerase chain reaction". *Veterinary Parasitology Regional Studies Reports* 11 (2018): 55-60.

11. Inokuma H., *et al*. "Analysis of the 18S rRNA gene sequence of a Hepatozoon detected in two Japanese dogs". *Veterinary Parasitology* 106 (2002): 265-271.

12. Jacobson LS. "The South African form of severe and complicated canine babesiosis: clinical advances 1994-2004". *Veterinary Parasitology* 138 (2006): 126-139.

13. Kamani J., *et al*. "Molecular detection of filarioid worms in dogs in Nigeria, West Africa". *Veterinarski Arhive* 89.6 (2019): 821-830.

14. Kamani J., *et al*. "Bartonella species in bats (Chiroptera) and bat flies (Nycteribiidae) from Nigeria, West Africa". *Vector-Borne Zoonotic Diseases* 14.9 (2014): 625-632.

15. Kamani J., *et al*. "Molecular detection and characterization of tick-borne pathogens in dogs and ticks from Nigeria". *PLoS Neglected Tropical Diseases* 7.3 (2013a): e2108.

16. Kamani J., *et al*. "Prevalence and diversity of *Bartonella* species in commensal rodents and ectoparasites from Nigeria, West Africa". *PLoS Neglected Tropical Diseases* 7 (2013b): e2108.

17. Larson G., *et al*. "Rethinking dog domestication by integrating genetics, archeology, and Biogeography". *Proceedings National Academy of Science U.S.A* 109 (2012): 8878-888.

18. Lisulo M., *et al*. "Determination of the prevalence of African trypanosome species in indigenous dogs of Mambwe district, eastern Zambia, by loop mediated isothermal amplification". *Parasite and Vectors* 7 (2014): 19.

19. Mar PH., *et al*. "Specific polymerase chain reaction for differential diagnosis of *Dirofilaria immitis* and *Dipetalonema reconditum* using primers derived from internal transcribed spacer region 2 (ITS2)". *Veterinary Parasitology* 106 (2002): 243-252.

20. Ng-Nguyen D., *et al*. "Domestic dogs are mammalian reservoirs for the emerging zoonosis fea-borne spotted fever, caused by *Rickettsia felis*". *Scientific Reports* 10 (2020): 4151.

21. Njiru Z., *et al*. "The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes". *Parasitology Research* 95.3 (2005): 186-192.

22. Olmeda AS., *et al*. "A subtropical case of human babesiosis". *Acta Tropica* 67 (1997): 229-234.

23. Otranto D., *et al*. "Feline and canine leishmaniosis and other vector-borne diseases in the Aeolian Islands: pathogen and vector circulation in a confined environment". *Veterinary Parasitology* 236 (2017): 144-151.

24. Otranto D., *et al*. "Vector borne helminths of dogs and humans in Europe". *Parasite and Vectors* 6 (2013): 16.

25. Perri AR., *et al*. "Dog domestication and the dual dispersal of people and dogs into the Americas". *Proceedings of the National Academy of Sciences of the United States of America* 118 (2021): 6e2010083118.

26. R Development Core Team. "A Language and Environment for Statistical Computing, R". Foundation for Statistical Computing, Vienna, Austria (2009).

27. Rishniw M., *et al*. "Discrimination between six species of canine microfilariae by a single polymerase chain reaction". *Veterinary Parasitology* 135 (2006): 303-314.

28. Salb AL., *et al*. "Dogs as sources and sentinels of parasites in humans and wildlife, Northern Canada". *Emerging Infectious Diseases* 14 (2008): 60-63.

29. Samdi SM., *et al*. "Trypanosomiasis in hunting dogs in Kaduna, north central Nigeria: implications on the disease in humans". *Journal of Biomedical Investigations* 4 (2006): 15-18.

30. Tabar MD., *et al*. "Vector-borne infections in cats: molecular study in Barcelona area (Spain)". *Veterinary Parasitology* 151.2-4 (2008): 332-336.

31. Ugochukwu CII., *et al*. "Incidence of *Dirofilaria immitis* in dogs presented at University of Nigeria, Nsukka Veterinary Teaching Hospital using wet smear and buffy coat techniques". *Asian Pacific Journal of Tropical Diseases* 6.8 (2016): 627-630.

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