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Short communication

Prevalence of ticks and haemoparasitic infections in dogs presented at the University of Ilorin Veterinary Teaching Hospital

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ABSTRACT

Introduction: Tick-borne haemoparasitism is an important disease of dogs in Nigeria with limited information on prevalence and distribution in Kwara State. This study was aimed at providing information on the prevalence of the disease and to determine the risk factors for infection.

Methods: Between May-August 2017, blood samples were collected from dogs presented at the University of Ilorin, Veterinary Teaching Hospital (VTH) were sampled and Wright-Giemsa stained blood smear were examined for haemoparasites using Wright-Giemsa staining. Also, retrospective data of dogs between May-August 2016 was retrieved from clinical records and analysed.

Result: A total 93 dogs were presented (48 in 2016 and 45 in 2017). More female dogs (54.2%) were presented than males in 2016; whereas in 2017, more male dogs (53.3%) were presented. Alsatians were the most common breed presented. Majority (68.9% and 64.6%) of the dogs were infested with ticks in 2017 and 2016 respectively. More cases of haemoparasites were recorded in June for 2016 (42.9%) and in May for 2017 (50%) corresponding to period when dogs were more infested with ticks. In 2016, 29.2% of dogs tested positive for haemoparasites of which 22.9% for canine babesiosis; 4.1% for canine ehrlichiosis, while 2.1% had mixed infection of *Babesia* and *Ehrlichia* parasites. In 2017, 13.3% of dogs were infected with *Babesia* parasite only. Dogs aged >12 months had higher tick infestation in 2017 and the difference was statistical significant ($p = 0.047$).

Significance: Findings from this study showed that canine babesiosis is prevalent in dogs in Ilorin. Control measures during the period of high tick population is suggested.

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Introduction

The domestic dog (*Canis lupus familiaris* or *Canis familiaris*), is a member of genus *Canis* (canines) that forms part of the wolf-like canids (Wang and Tedford, 2010). Dog is the most widely abundant carnivore (Vilà *et al.*, 1999; Thalmann *et al.*, 2013), selectively bred over other animal due to its various capabilities such as behavioural, sensory and physical attributes (Dewey and Bhagat, 2002; Perri, 2016). It is estimated that three-quarters of the world's dog population live as village or community dogs and only a few kept as pets (Coppinger and Coppinger, 2001).

In tropical and subtropical regions of the world, vector-borne diseases commonly occur in various species of animals (Irwin and Jefferies, 2004; Elelu *et al.*, 2016). Globally, dogs have been documented to be infected by different haemoparasites such as bacteria, viruses and eukaryotic parasites, which are transmitted through different arthropod vectors such as ticks and mosquitoes causing huge morbidity and mortality (Otranto *et al.*, 2009). Furthermore, the geographical expansion of tick vectors, influenced by the climatic changes and movement of humans together with their pets increase the vulnerability of dogs to infections (Otranto *et al.*, 2009; Gray *et al.*, 2009). The-

se groups of diseases cause huge economic losses to both live-stock and pet animal owners due from mortality, hospital bills and loss in productivity. There is dearth of information on the prevalence of haemoparasitic diseases of dogs in Kwara State despite the widespread reports of the disease from across Nigeria. This study therefore provides information on the prevalence of haemoparasitic infection of dogs from Ilorin, Kwara State and the associated risk factors of infection for effective control.

Materials and methods

Study area

The study was conducted at the University of Ilorin Veterinary Teaching Hospital (VTH), in Ilorin, the capital city of Kwara State, North-central Nigeria located between 8°30'N and 4°33'E (Fig. 1). Ilorin is made up of Ilorin West, Ilorin East and Ilorin South with a total land mass of about 765 km² (295 sq. mi) and an estimated population of 908,490 (Kwara, 2017).

Sampling methods

A structured questionnaire on socio-demographic data including information on location, age, sex and breed of dogs was adminis-

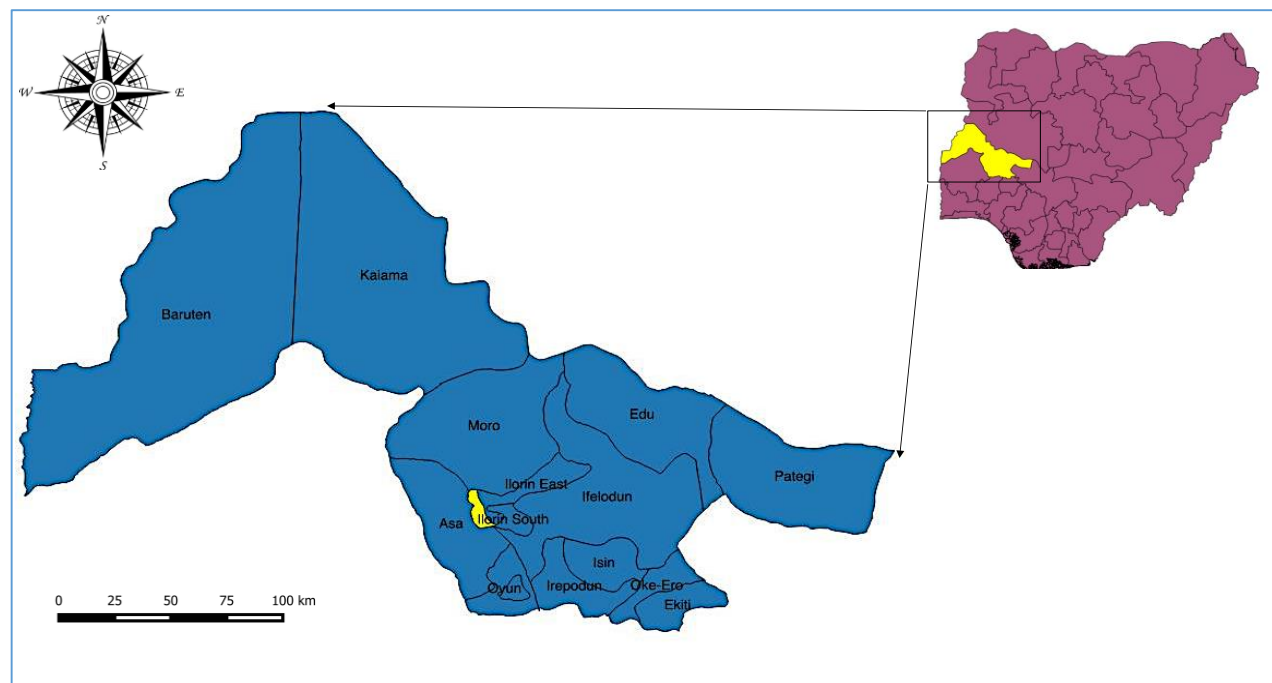


Figure 1. Map of Kwara State showing the state capital (Ilorin) shaded, where the study was carried out.

Inset is the map of Nigeria showing Kwara State. Map created using QGIS® software. Created by Nusirat Elelu, 2017.

tered to dog owners between May to August, 2017. Concurrently, laboratory techniques were carried out to detect haemo- and ecto-parasites in sampled dogs. Comparably, a retrospective analysis of similar data on sociodemographic characteristics and information on vector borne haemoparasitic cases presented between May and August 2016 was also carried out.

The data collection for this study was done within 4 months, during which dogs presented between the hours of 8:00am to 2:00pm at the VTH were thoroughly examined for ecto-parasites. Ticks were confirmed to be present by handpicking and placing them into plain sterile sample bottles. Five (5ml) of blood was collected from the cephalic vein of each dog into Ethylene diamine tetraacetic acid (EDTA) bottle. The blood was carefully mixed with the anti-coagulant by gently inverting and rotating the tube 3 to 4 times. The samples were then transported in ice-pack to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, University of Ilorin for haemoparasite screening.

Approval was sought from the Director of VTH before the commencement of the study and also verbal consent was obtained from the clients before sampling of the dogs.

Haemoparasites identification

Thin blood smear was prepared from each blood sample, fixed in methanol for 3-5mins and stained with Giemsa-Wright solution. The smears were examined at x100 magnification (oil immersion) for presence of parasites and identification as described by Cheesbrough (2009). At least 50 fields were searched per slide. Presence of haemoparasites was recorded and identification was carried out.

Statistical analysis

The data obtained were entered into Microsoft Excel and transferred to Open Source Epidemiologic Statistics for Public Health (OpenEpi), version 3.03a (Dean *et al.*, 2013) for analysis. Descriptive statistic including mean, range, frequencies, and percentages were carried out. Relationship between variables was further analysed using Chi-square or Fisher's exact tests. P values <0.05 were considered significant.

Results

Demographic information of dogs

The age of dogs presented at the VTH between May and August, 2016, ranged between 1 month and 69 months with a mean of 1.85 ± 0.95 months. Most of the dogs (52.1%) were puppies less than six months of age, a few (10.4%) were between the ages of 6-12 months old. More female dogs (54.2%) were presented than males (45.8%); while majority (75.0%) of the dogs were from Ilorin South LGA. Alsatian (41.7%) dogs were the most common breed with only one Terrier (2.1%) presented within 2016 period studied. The month of May recorded the least (10.4%) number of cases while June recorded the highest (35.4%) Further details of the socio-demographic characteristics are shown in Table 1. The age of dogs presented at the VTH between May and August 2017, ranged between 2 months to 120 months with a mean of 16.1 ± 19.7 months. More male dogs (53.3%) were presented than female dogs (46.7%); while majority (73.3%) of the dogs came from Ilorin South LGA. Alsatian (24.4%) dogs were the most common breed presented within the 2017 period studied. Majority (51.1%) of the cases were presented in May. Further details of the socio-demographic characteristics are shown in Table 1.

Table 1. Demographic distribution of dogs presented at the Veterinary Teaching Hospital, Ilorin, Kwara State.

	May – August 2016	May – August 2017
Categories	n (%)	n (%)
Sex		
Male	22 (45.8)	24 (53.3)
Female	26 (54.2)	21 (46.7)
LGA		
Ilorin South	36 (75.0)	33 (73.3)
Ilorin West	7 (14.6)	9 (20.0)
Ilorin East*	-	2 (4.4)
Not indicated ⁺	5 (10.4)	1 (2.2)
Breed		
Alsatian	20 (41.7)	11 (24.4)
Local breed	4 (8.3)	7 (15.6)
Lhasa Apso	5 (10.4)	2 (4.4)
Caucasian	6 (12.5)	7 (15.6)
Rottweiler	3 (6.3)	8 (17.8)
Boer bull	4 (8.3)	8 (17.8)
Terrier	1 (2.1)	1 (2.2)
Pitt-bull ^α	5 (10.4)	-
Bull mastiff ^β	-	1 (2.2)
Age (months)		
<6	25 (52.1)	10 (22.2)
6-12	5 (10.4)	17 (37.8)
>12	18 (37.5)	18 (40.0)
Month		
May	5 (10.4%)	23 (51.1%)
June	17 (35.4%)	9 (20%)
July	13 (27.1%)	9 (20%)
August	13 (27.1%)	4 (8.9%)

LGA – Local government area

*– Not recorded during the retrospective study

+ – No LGA records were provided

α – Only found during the retrospective study in 2016

β – Only found among dogs presented during in 2017

Ticks and haemoparasitic infection of dogs presented

From the VTH records, between May and August 2016, twenty-two 22/48 (45.8%) of the dogs presented were screened for haemoparasites. More than half (64.6%) of the dogs presented during the 2016 study were infested with ticks, and (22.9%) were positive for *Babesia* parasite. Two dogs (4.1%) were positive for *Ehrlichia spp*, while only one (2.1%) dog was positive with co-infection of *Babesia* and *Ehrlichia* parasite during the 2016 period (Table 2).

Similarly, during the 2017 study period, majority (68.9%) of dogs were infested with ticks. However, *Babesia spp* was the only haemoparasite present in 13.3% of the dogs presented. Neither *Ehrlichia spp* alone nor a combination of *Babesia spp* and *Ehrlichia spp* were identified during the 2017 period (Table 2).

Table 2. Ticks and haemoparasites in dogs presented at the Veterinary Teaching Hospital, Ilorin, Kwara State.

		May – August, 2016	May – August, 2017
Categories		n (%)	n (%)
Ticks	Present	31 (64.6)	31 (68.9)
	Absent	17 (35.4)	14 (31.1)
	Total	48 (100)	45 (100)
Haemoparasites			
<i>Babesia spp</i>	Positive	11 (22.9)	6 (13.3)
<i>Ehrlichia spp</i>	Positive	2 (4.1)	0 (0.0)
<i>Babesia+Ehr-lichia</i>	Positive	1 (2.1)	0 (0.0)

Factors associated with tick infestation and occurrence of babesiosis

Age of dogs was not significantly associated ($P = 0.239$) with tick infestation in 2016. However, in 2017, dogs older than 12 months were more infested ($P = 0.047$) with ticks than dogs in other age groups (Table 3). There was no significant association between the sex of the dogs presented with babesiosis in 2016 and 2017 (Table 4). Also, in 2016 there was no significant association ($P = 0.507$) between breed of dogs and babesiosis. However, in 2017 there was a significant association ($P = 0.004$) between the breed of dogs sampled and babesiosis (Table 5).

Discussion

This study showed that more female dogs were presented in 2016 compared to 2017, where the number of male dogs was higher. Canine babesiosis was the most prevalent tick-borne disease although with low prevalence rates of 22.9% and 13.3% obtained in 2016 and 2017 respectively. In contrast, higher prevalence were reported in past studies carried out from other parts of Nigeria that utilised similar microscopic methods. For instance, 59.3% prevalence for *Babesia spp* was reported in dogs attending ECWA Veterinary clinic in Jos, Nigeria (Ifeoma, 2013). Another study utilizing molecular methods reported a prevalence of 53% due to *B. rossi* among dogs in Plateau State, Nigeria (Adamu *et al.*, 2014). However, lower prevalence of canine babesiosis (6.6%) similar to those obtained in this study were reported from four states in Nigeria using sensitive molecular techniques (Kamani *et al.*, 2013). Interestingly, another past study in Maiduguri also reported a very low prevalence of 2.8% for both *Babesia* and *Ehrlichia spp* (Adamu *et al.*, 2012). The variable prevalence rates reported from different parts of the country might be as a result of different ecology which affects the population of tick vectors and hence the presence of the haemoparasites they transmit. It might also be as a result of low parasitaemia, which might be missed by microscopic examination of blood smear, although absence of parasites in blood smear is not an absence of infection.

There was no significant association between the sex of the dogs presented in this study with presence of babesiosis in both 2016 and 2017. However, a study carried out in South Africa, showed

Table 3. Association between tick infestation and age of dogs presented within 2016 and 2017 at the Veterinary Teaching Hospital, Ilorin, Kwara State

Year	Age (months)	Ticks present (n)	Ticks absent (n)	χ^2	df	p-value
2016	<6	13	12	2.855	2	0.240
	6-12	4	1			
	>12	14	5			
	Total	31	18			
2017	<6	8	2	6.108	2	0.047*
	6-12	8	9			
	>12	15	3			
	Total	31	14			

χ^2 – Chi square; df – degree of freedom; * - significant at $p < 0.05$

Table 4. Comparative association between sex of dogs and babesiosis studied within 2016 and 2017 at the Veterinary Teaching Hospital, Ilorin, Kwara State

Year	Sex	<i>Babesia</i> positive (n)	<i>Babesia</i> negative (n)	χ^2	df	p-value
2016	Male	7	4	1.173	1	0.280
	Female	4	6			
	Total	11	10			
2017	Male	3	21	0.031	1	0.860
	Female	3	18			
	Total	6	39			

χ^2 – Chi square; df – degree of freedom; * - significant at $p < 0.05$

that male dogs (both intact and neutered) had higher odds of being diagnosed with babesiosis (Mellanby *et al.*, 2011). Furthermore, only dogs sampled in 2017 in this present study showed statistically significant association between breed of dogs and *Babesia* infections ($p = 0.004$) unlike in 2016, where there was no difference based on breeds of dogs. The difference observed in 2017 in agreement with a past study which stated that sex, tick infestation and breeds have significant influence on the prevalence of canine babesiosis (Daniel *et al.*, 2016). The variations in level of significance between the 2016 and 2017 in the present study might warrant a more extensive sampling of dogs to determine the association between dog phenotypic and physiologic characteristics and haemoparasitic infection.

Ehrlichia spp was identified among two dogs singly, while another dog had mixed infection of both *Ehrlichia* and *Babesia* spp in 2016. Absence of ehrlichiosis during the 2017 study could be attributed to the fact that it is difficult to detect an *Ehrlichia* morula in a blood smear (Mylonakis *et al.*, 2003). A more sensitive diagnostic method might detect more positive cases (Daramola *et al.*, 2017). Furthermore, previous studies had shown that all breeds of dogs are prone to ehrlichiosis, however, German Shepherd dogs and Siberian Huskies are more predisposed to develop more severe clinical signs of ehrlichiosis and therefore, have worse prognosis (Harrus *et al.*, 1997). In the present study, data analysed from dogs presented in 2016, showed that only female dogs had ehrlichiosis. However, no sex predisposition has so far been reported with the acquisition of canine ehrlichiosis even though some studies have found higher sero-positivity in males,

which may be explained by a higher exposure to vectors than female due to behavioural characteristics (Costa *et al.*, 2007). Further investigations are needed to determine breed and age predispositions to ehrlichiosis in Kwara State.

The proportion of dogs infested with ticks during the 2016 and 2017 periods of study were 64.6% and 68.9% respectively which were higher than the 47% prevalence reported in Maiduguri amongst slaughtered dogs (Adamu *et al.*, 2012). Results from this study (2016 records) showed higher tick infestation (29.2%) in dogs older than one year compared to dogs in other age groups. This might also be the reason why more adult dogs 6/11 (54.5%) were positive for babesiosis within the 2016 study period. Similarly, during the 2017 study period, a higher proportion (33.3%) of dogs older than 12 months were infested with ticks compared to dogs less than 12 months of age and the difference was statistically significant. This result is in contrast with a past study from Nigeria that reported dogs aged between 6-12 months were more susceptible to tick infestation (Konto *et al.*, 2014). The reason for the higher tick infestation among older dogs in this study might be due to older dogs being more mobile and likely to come more in contact with tick infested areas.

The consistently higher prevalence of ticks in dogs presented in 2016 and in 2017 indicates that not much has been done to reduce the abundance of these vectors which are the major source of infection for dogs. Routine tick control effort should be encouraged to reduce the burden. A major limitation of this study, is the lack of morphological and molecular identification of the infecting tick species. It is therefore recommended that specific studies

Table 5. Comparative association between breed of dogs and babesiosis studied within 2016 and 2017 at the Veterinary Teaching Hospital, Ilorin, Kwara State

Year	Breeds	<i>Babesia</i> +ve (n)	<i>Babesia</i> -ve (n)	χ^2	df	p-value
2016	Alsatian	4	6	6.284	7	0.507
	Boerboel	0	2			
	Caucasian	3	0			
	Local breed	1	1			
	Lhasa Apso	1	0			
	Rottweiler	1	1			
	Pitt bull	0	0			
	Terrier	1	0			
	Total	11	10			
2017	Alsatian	1	10	20.880	7	0.004*
	Boerboel	2	6			
	Bull mastiff	1	0			
	Caucasian	0	7			
	Local breed	0	7			
	Lhasa Apso	2	0			
	Rottweiler	0	8			
	Terrier	0	1			
	Total	6	39			

χ^2 : Chi-square; df: degree of freedom; *: significant at $p < 0.05$; +ve : positive; -ve: negative

to identify the species of ticks infesting dogs within Ilorin metropolis should be carried out using both morphological and sensitive molecular methods. This could help identify the possible parasite transmitting vectors prevalent in Ilorin as well as those of possible zoonotic importance.

More awareness should be created so as to educate dog breeders and pet owners as regards the need for efficient control of ticks in the environment and on the body of their animals by treatments with ecto-parasiticides that repel and kill ticks thereby reducing the risk of disease transmission. These campaigns should also include periods of the year that strategic tick control should be instituted. Vaccines against babesiosis have been developed to induce partial protection against the disease by reducing severity duration of clinical disease (Solano-Gallego and Baneth 2011). However, as with vaccination, most current prophylactic measures are considered insufficient for complete protection (Bourdoiseau, 2006). A recent study showed that an attenuated *E. canis* strain may serve as an effective future vaccine for canine ehrlichiosis, but currently, no commercial vaccines are available to protect against infections with *E. canis* although potential agents have been tested (Rudoler *et. al.*, 2012).

Conclusions

The results of this study show that ticks and tick-borne haemo-parasitic diseases are prevalent in dogs presented at the University of Ilorin VTH. With the veterinary importance of ticks and

their pathogen, as well as the recent emergence of tick-borne diseases as zoonoses, there is the need for a continuous and concerted effort towards combating this menace especially in dogs that have close contact with human due to their domestic nature.

Conflicts of interest

We declare no conflict of interest.

References

- Adamu, M., Troskie, M., Oshadu, D.O., Malatji, D.P., Penzhorn, B.L. and Matjila, P.T. (2014). Occurrence of tick-transmitted pathogens in dogs in Jos, Plateau State, Nigeria. *Parasites and Vectors*, 7(1): 119.
- Adamu, N.B., Adamu, J.Y. and Salisu, L. (2012). Prevalence of ecto-, endo- and haemoparasites in slaughtered dogs in Maiduguri, Nigeria. *Revue de Médecine Vétérinaire*, 163(4): 178-182.
- Bourdoiseau, G. (2006). Canine babesiosis in France. *Veterinary Parasitology*, 138(1-2): 118-125.
- Cheesbrough, M. (2009). District laboratory practice in tropical countries. Part 1, 2nd Edition. Cambridge University Press, New York, USA.
- Coppinger, R. and Coppinger, L. (2001). *Dogs: A startling new understanding of canine origin, behavior & evolution*. Scribner 1st edition, 352pp.
- Costa, J.R., Rembeck, L.M., Ribeiro, K., Beelitz, M.F.B., Pfister, P. and Passos, L.M.F. (2007). Sero-prevalence and risk indicators for canine ehrlichiosis in three rural areas of Brazil. *The Veterinary Journal*, 174(3): 673-676.
- Daniel, L.N., Kujul, N.B., Kemza, S.Y. and Ibukunoluwa, M.R. (2016). Retrospective study of the risk factors associated with canine babesiosis diagnosed at Veterinary Clinic Federal College of Animal Health and Production Technology, Vom, North-central Nigeria, 1999-2006. *International Journal of Science and Applied Research*, 1(1): 86-93.
- Daramola, O.O., Takeet, M.I., Oyewusi, I.K., Oyekunle, M.A. and Talabi, A.O. (2017). Detection and molecular characterization of *Ehrlichia canis* in naturally infected dogs in Abeokuta, South Western Nigeria. *Acta Veterinaria Hungarica*, 66 (1): 85-93.
- Dean, A.G., Sullivan, K.M. and Soe, M.M. 2013. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. www.OpenEpi.com, updated 2013/04/06, accessed 2018/02/21.
- Dewey, T. and Bhagat, S. 2002. *Canis lupus familiaris* Archived 23 October 2014 at the Wayback Machine. Animal Diversity Web. Retrieved 6 January 2009.
- Elelu, N., Ferrolho, J., Couto, J., Domingos, A. and Eisler, M.C. (2016). Molecular diagnosis of the tick-borne pathogen *Anaplasma marginale* in cattle blood samples from Nigeria using qPCR. *Experimental and Applied Acarology*, 70 (4): 501-510.
- Gray, J.S., Dautel, H., Estrada-Peña, A., Kahl O. and Lindgren, E. (2009). Effects of climate change on ticks and tick-borne diseases in Europe. *Interdisciplinary Perspectives on Infectious Diseases*, Article ID 593232: 1-12.

- Harrus, S., Kass, P.H., Klement, E. and Waner, T. (1997). Canine monocytic ehrlichiosis: A retrospective study of 100 cases, and an epidemiological investigation of prognostic indicators for the disease. *The Veterinary Record*, 141(14): 360-363.
- Ifeoma, N. (2013). The prevalence of haemoparasitic infection in dogs attending ECWA Veterinary Clinic, Bukuru, Jos South Local Government Area, Plateau State. *Advances in Microbiology*, 3 (3): 302-308.
- Irwin, P.J. and Jefferies, R. (2004). Arthropod-transmitted diseases of companion animals in Southeast Asia. *Trends in Parasitology*, 20(1): 27-34.
- Kamani, J., Baneth, G., Mumcuoglu, K.Y., Waziri, N.E., Eyal, O. Guthmann, Y. and Harrus, S. (2013). Molecular detection and characterization of tick-borne pathogens in dogs and ticks from Nigeria. *PLoS Neglected Tropical Diseases*, 7(3): e2108.
- Kwara State. City Population. Available at <http://www.citypopulation.de/php/nigeria-admin.php?adminId=NGA024> Accessed on May 21, 2017.
- Konto, M., Biu, A.A., Ahmed, M.I. and Charles S. (2014). Prevalence and seasonal abundance of ticks on dogs and the role of *Rhipicephalus sanguineus* in transmitting *Babesia* species in Maiduguri, North-Eastern Nigeria. *Veterinary World*, 7(3): 119-124.
- Mellanby, R.J., Handel, I.G., Clements, D.N., Bronsvort, B.M., Lengeling, A. and Schoeman, J.P. (2011). Breed and sex risk factors for canine babesiosis in South Africa. *Journal of Veterinary Internal Medicine*, 25(5): 1186-1189.
- Mylonakis, M.E., Koutinas, A.F., Billinis, C., Leontides, L.S., Kontos, V., Papadopoulos, O. and Fytianou, A. (2003). Evaluation of cytology in the diagnosis of acute canine monocytic ehrlichiosis (*Ehrlichia canis*): A comparison between five methods. *Veterinary Microbiology*, 91(2-3): 197-204.
- Otranto, D., Dantas-Torres, F. and Breitschwerdt, E.B. (2009). Managing canine vector-borne diseases of zoonotic concern: part one. *Trends Parasitology*, 25: 157–163.
- Perri, A. (2016). A wolf in dog's clothing: Initial dog domestication and Pleistocene wolf variation. *Journal of Archaeological Science*, 68: 1-4.
- Rudoler, N., Baneth, G., Eyal, O., van Straten, M. and Harrus, S. (2012). Evaluation of an attenuated strain of *Ehrlichia canis* as a vaccine for canine monocytic ehrlichiosis. *Vaccine*, 31(1): 226-233.
- Solano-Gallego, L. and Baneth, G. (2011). Babesiosis in dogs and cats—expanding parasitological and clinical spectra. *Veterinary Parasitology*, 181(1): 48-60.
- Thalmann, O., Shapiro, B., Cui, P., Schuenemann, V.J., Sawyer, S.K., Greenfield, D.L. and Napiwala, H. (2013). Complete mitochondrial genomes of ancient canids suggest a European origin of domestic dogs. *Science*, 342(6160): 871-874.
- Vilà, C., Amorim, I.R., Leonard, J.A., Posada, D., Castroviejo, J., Petrucci-Fonseca, F. and Wayne, R.K. (1999). Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Molecular Ecology*, 8(12): 2089-2103.
- Wang, X. and Tedford, R. H. (2010). *Dogs: their fossil relatives and evolutionary history*. Columbia University Press, 232 pp.