ORIGINAL ARTICLE

WILEY

Molecular characterisation of *Rhipicephalus sanguineus* sensu lato ticks from domestic dogs in Nigeria

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Abstract

Rhipicephalus sanguineus is the most widely reported tick in the world. Molecular characterisation is important to verify its taxonomic status in the different parts of the world. In this study, we provide information on the molecular characterisation of *R. sanguineus* tick of dogs collected from Nigeria. Ticks were collected from 62 of 93 sampled dogs. The collected ticks were subjected to morphological identification with the aid of appropriate entomological keys. Deoxyribonucleic acid (DNA) was extracted from the most prevalent tick species (*R. sanguineus*) and was subjected to further molecular characterisation protocols. The partial mitochondrial 16S rRNA gene sequences (~300 bp) were obtained from representative specimens. Data were statistically analysed using the chi-square (χ^2) test. Phylogenetic analysis was performed including different lineages of *R. sanguineus* (sl) from Africa, Asia, Europe and America, and other species belonging to the *R. sanguineus* 'tropical lineage' (*R. linnaei*) as well as *Rhipicephalus turanicus* and *Ixodes ricinus*. Results of this study showed that *R. sanguineus* was the most abundant ticks of dogs with a prevalence of 61.8% (68/110; 95% CI = 52.5–70.54), followed by *Amblyomma variegatum* (20.0%) and *Haemaphysalis leachi* (18.2%). The molecular analysis shows that they are genetically different from the temperate strains but closely related to those from other West African countries. There is a need to establish the vector competence of this common Nigerian dog tick.

KEYWORDS
16S rRNA gene, brown dog tick, endemic, Kwara State, North-central

1 | INTRODUCTION

Ticks are obligate blood-sucking arthropods and they are believed to be next in importance only to mosquitoes among other arthropods as vectors of bacterial, viral and protozoan disease agents (Abdullah et al., 2016; Ola-Fadunsin et al., 2021; Smith et al., 2011).

The *Rhipicephalus sanguineus* group (Acari: Ixodidae) includes 12 tick species, namely: *R. sanguineus, R. sulcatus, R. rossicus, R. schulzei,* *R. pumilio, R. pusillus, R. turanicus, R. leporis, R. guilhoni, R. moucheti, R. bergeoni* and R. *camicasi* (Nava et al., 2015). These groups of ticks have been documented to transmit several pathogenic bacteria such as *Rickettsia massiliae* reported in the Americas and *R. rickettsia* in Mexico (Eremeeva et al., 2006). They have also been associated with protozoan diseases such as *Babesia canis, Ehrlichia canis* and *Hepatozoon canis* (Dantas-Torres & Otranto, 2015; Dantas-Torres et al., 2018; Taylor et al., 2016).

Ambiguity in taxonomy *R. sanguineus* tick has been repeatedly questioned and debated for many centuries (Dantas-Torres & Otranto, 2015; Gray et al., 2013). Several factors contribute to these existing ambiguities such as the limited description of the original species and the high level of morphological similarity among ticks within the *R. sanguineus* tick complex (Dantas-Torres & Otranto, 2015). Accurate description of species of *R. sanguineus* is important in other to determine their vector role, as their morphological identification will make this difficult (Dantas-Torres et al., 2013; Walker et al., 2000).

The brown dog tick, *Rhipicephalus sanguineus* sensu stricto (Latreille 1809) is one of the most studied tick species in the world due to its medical and veterinary importance (Dantas-Torres, 2010). Although this tick typically infests dogs, they have however been reported from other domestic animal species such as cattle, sheep and goats in different parts of the world (Hadi et al., 2016; Monfared et al., 2015; Taylor et al., 2016). The tick was originally regarded as a single taxon; however, current genetic studies suggest the existence of at least two distinct lineages based on geographical location: the 'temperate' and 'tropical' lineages (Dantas-Torres et al., 2013; Nava et al., 2012).

Presently to the best of our knowledge, there are no molecular data to support the morphological claims of these tick species in Nigeria. The present work was performed to enlarge our knowledge on the most reported dog tick by providing information on the molecular studies of *R. sanguineus* tick of dogs collected from Nigeria. This was compared to how these ticks differ from those reported from other parts of the world in order to verify their taxonomic status.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in Ilorin, the administrative capital of Kwara State, Nigeria. Kwara State is located in North-central Nigeria, with savannah vegetation, with a typically tropical climate of between 1000 and 1500 mm annual rainfall and a mean temperature above 30°C. The State sits on a geographical coordinate of latitude 8°30'N and longitude 5°00'E and covers an area of 35,705 km^2 (13,947.27 sq. miles) (Elelu et al., 2016; Ola-Fadunsin et al., 2020).

2.2 | Tick collection and identification

A total of 110 ticks were collected from 62 of the 93 dogs that were sampled at the University of Ilorin, Veterinary Teaching Hospital (VTH), Kwara State, between May and August, 2017. Dogs were thoroughly examined for ectoparasites. Ticks were gently collected with the use of forceps and placed in labeled plain sterile sample bottles containing 70% alcohol. Morphological identification was carried out under a stereo microscope using morphological keys as described by Walker et al. (2014). Images of *R. sanguineus* were captured using Nikon 5MZ745T microscope attached with a Motic digital camera.

2.3 | Deoxyribonucleic acid (DNA) extraction and PCR

Genomic DNA was extracted from *R. sanguineus* ticks using a commercially available DNA extraction kit (DNeasy Blood & Tissue Kit, Qiagen), in accordance with the manufacturer's instructions. The tick identities were confirmed by using the polymerase chain reaction (PCR) protocols to amplify the 16S mitochondrial rRNA gene (~300 bp) from individual tick DNA as previously described (Mangold et al., 1998). Each PCR reaction consisted of 25 μl mix consisting of 2 μl tick genomic DNA and 23 μl of PCR mix containing 2.5 mM MgCl$_2$, 10× buffer, 2 mM of each dNTP, 5 mM of each primer and 1.75 U of Taq polymerase (Invitrogen). Amplified products were examined on 1.5% agarose gels stained with Syber safe (Invitrogen) and visualised on a DocIT gel documentation system (BioRad).

Forty-one representative amplified products were randomly selected for purification and sent to DBS genomics (Durham University) for sequencing in both directions using the same primer set. The consensus nucleotide sequences (gotten from the 41 sequences) reported in this study have been deposited in the GenBank database with Accession Numbers: MT568621 and MT568622.

2.4 | Statistical and phylogenetic analyses

The Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, Illinois) was used for the statistical analysis. Prevalence and the corresponding 95% confidence interval (CI) were used to measure the level of tick infestations. The Chi-square (χ^2) test was used to evaluate the level of each tick species infestation on dogs. Statistical significance was set at $p < 0.05$.

The mitochondrial 16S rRNA gene sequences were aligned using ClustalW program and compared with those available in the GenBank using the BLASTn tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis was performed including different lineages of *R. sanguineus* (sl) from Africa, Asia, Europe and America, and other species belonging to the *R. sanguineus* 'tropical lineage' (*R. linnaei*) as well as *Rhipicephalus turanicus* and *Ixodes ricinus*. The phylogenetic tree was constructed by the Maximum Likelihood method, 1000 replicates bootstrap using the Mega X software (https://www.megasoftware.net/).

3 | RESULTS

Of the total 93 dogs sampled, 62 were infested with ticks representing 66.67% (95% CI = 56.63–75.68) of the sampled population.

Three different species of ticks were identified, with *Rhipicephalus sanguineus* being the most prevalent (68/110; 61.82%; 95% CI = 52.48–70.54), followed by *Amblyomma variegatum* (22/110; 20.00%; 95% CI = 13.32–28.25). *Haemaphysalis leachi* was the least prevalent. There

TABLE 1 Prevalence of tick species infesting dogs in Kwara State, Nigeria

Ticks species	Number recovered	Prevalence (%)	95% CI
Amblyomma variegatum	22	20.00	13.32–28.25
Haemaphysalis leachi	20	18.18	11.80–26.22
Rhipicephalus sanguineus	68	61.82	52.48–70.54

$\chi^2 = 60.33$, df $= 2$, $p < 0.0001$.

CI = confidence interval; Df = degree of freedom.

FIGURE 1 Dorsal view of female *Rhipicephalus sanguineus* (brown dog tick) collected from domestic dogs in Kwara State, Nigeria
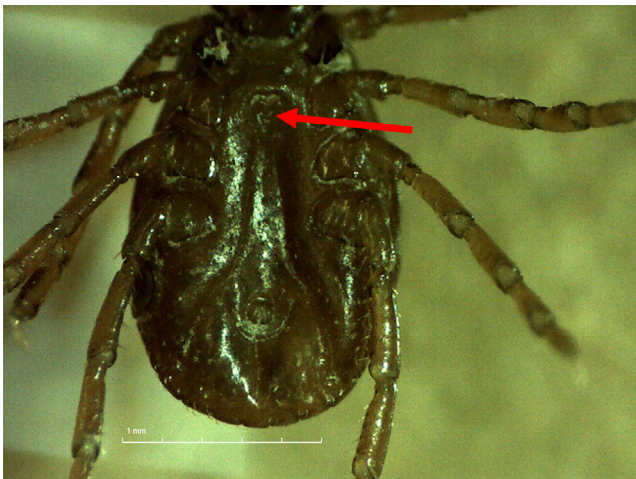
FIGURE 2 Ventral view of female *Rhipicephalus sanguineus* tick collected from domestic dogs in Kwara State Nigeria. The focus is on the genital opening (shown with an arrow) of the female *Rhipicephalus sanguineus* tick

was a statistical difference ($p < 0.0001$; $\chi^2 = 60.33$) in the prevalence of the different species of ticks (Table 1).

Morphologically, the *Rhipicephalus sanguineus* ticks identified were large, dark coloured and females have a u-shaped genital opening (Figures 1 and 2).

The nucleotide sequence from Nigerian *Rhipicephalus sanguineus* (sl) ticks from this present study have been deposited in the GenBank with Accession Numbers: MT568621 and MT568622. BLAST search of the partial sequence of the 16S mitochondrial RNA from this study compared to those available in the GenBank showed high identity (99%) to sequence of *R. sanguineus* isolated from Cote d'Ivoire (Accession no: KX793744) and those from Argentina (Accession no: KR909459).

The maximum likelihood tree constructed based on 16S mitochondrial rRNA gene sequences showed that the species of the *R. sanguineus* ticks in this study clustered alongside the 'tropical lineage' from the tropical areas of America, China, Thailand and Sub-Saharan Africa. Although the *R. sanguineus* group from Nigeria in the present study were more closely related to those from Africa (Ghana and Cote d'Ivoire), they formed a distinct sub-group under the tropical lineage with a 68% bootstrap value. *Rhipicephalus sanguineus* tick from Israel, and South Africa, and *R. turanicus* from Afghanistan formed separate groups away from the tropical group. *Rhipicephalus sanguineus* ticks from temperate parts of America and Europe clustered in the 'temperate lineage' (Figure 3).

4 | DISCUSSION

The brown dog tick, *R. sanguineus*, is the most reported tick in Nigeria and globally (Agu et al., 2020; Konto et al., 2014; Walker et al., 2014). They also differ in their vector competence for pathogen transmission thus, it is very important to correctly identify *R. sanguineus* tick species in a geographical location. Past studies carried out on brown dog ticks from Nigeria utilised morphological methods of identification, hence so far there are currently no molecular data on these important group of ticks from Nigerian dogs. This study appears to be the first molecular analysis based on the 16S mitochondrial rRNA of *R. sanguineus* collected from Nigerian domestic dog.

In line with our findings, the brown dog tick, *R. sanguineus*, has been reported to be the most prevalent tick species of dogs in Nigeria (Adetayo et al., 2021; Akande et al., 2018; Kamani et al., 2018; Shitta et al., 2018) and other parts of the world including Algeria (Kebbi et al., 2019) Egypt (Abdullah et al., 2016), Great Britain (Smith et al., 2011), Italy (Maurelli et al., 2018), Indonesia (Hadi et al., 2016) and China (Zhang et al., 2017). The 61.8% prevalence of *R. sanguineus* we observed in this study is within the prevalence documented by previous researchers on the abundance of *R. sanguineus* within and outside of Nigeria (Abdullah et al., 2016; Akande et al., 2018; Maurelli et al., 2018; Shitta et al., 2018). *Amblyomma variegatum* and *Haemaphysalis*
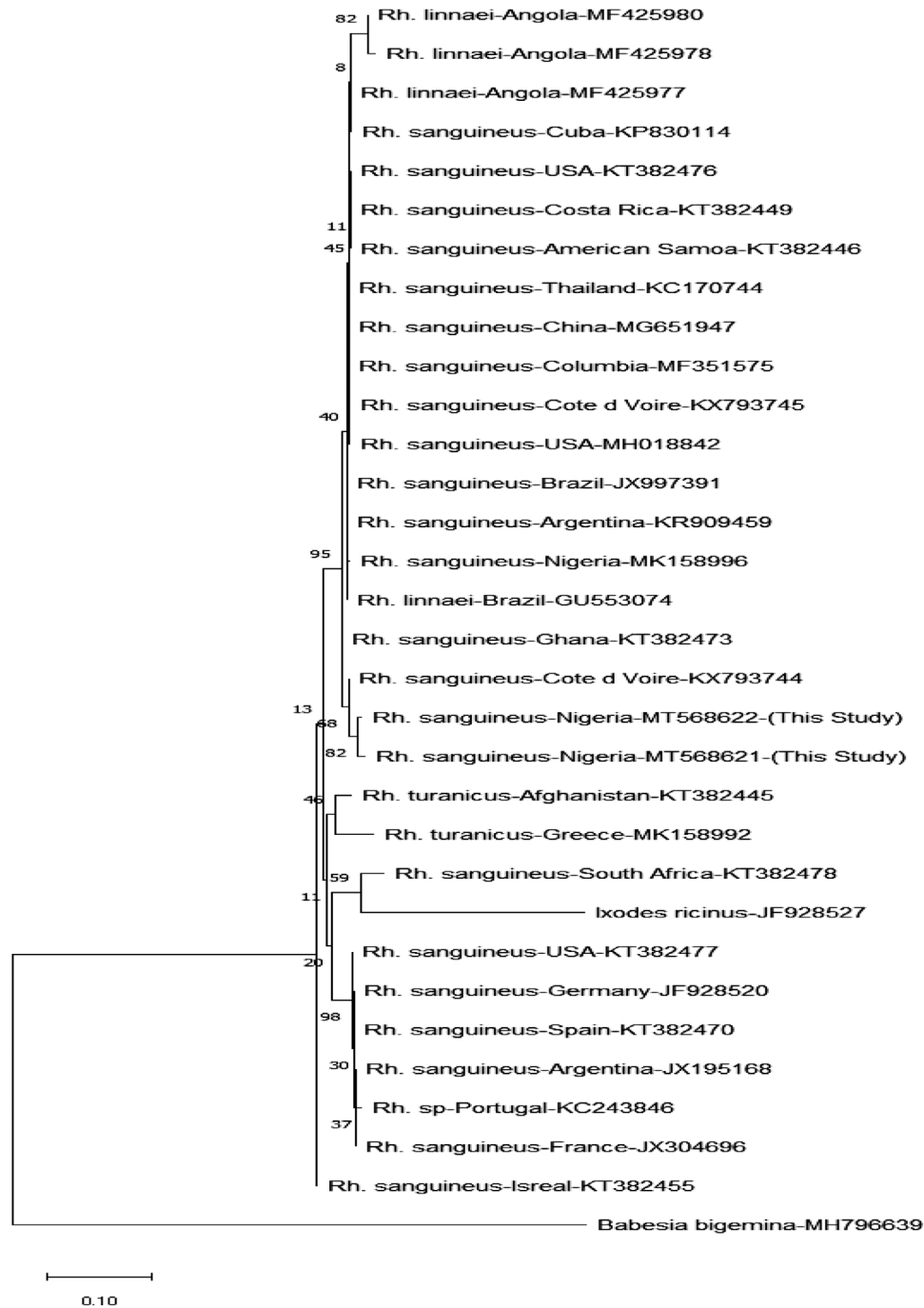
FIGURE 3　Molecular Phylogenetic analysis by Maximum Likelihood method of *Rhipicephalus sanguineus* based on the 450 bp 16S mitochondrial RNA partial gene sequence of known *Rhipicephalus* species taken from the NCBI database and sequence amplified from *R. sanguineus* tick collected from Nigerian dogs

leachi have been reported to infest dogs in Nigeria (Akande et al., 2018; Konto et al., 2014; Shitta et al., 2018).

Findings from the molecular analysis based on the 16S mitochondrial rRNA of *R. sanguineus* collected from Nigerian domestic dog shows that they are genetically different from the temperate strains, but closely related to these from other West African Countries. Although this is expected, phylogenetic inference shows that they cluster as a separate sub-group under the *R. sanguineus* ticks within the tropi-

cal lineage. Additional phylogenetic comparison of *R. sanguineus* tick from domestic dogs from Nigeria within the members of the *R. sanguineus* tick complex shows close clustering with those reported as *R. sanguineus* senso lato from Cote d'Ivoire. Furthermore, two distinct clusters were formed within the complex mimicking their geographical location. Those from Brazil and Africa clustered together, with *R. linnaei* from Angola forming a distinct sub-group, while the European members of the tick complex clustered separately. There is a need to

determine the various haplotypes of the tropical lineage of the *R. sanguineus* ticks from various parts of Nigeria as well as to determine their vector competence for veterinary and medical significance.

Previous studies have indicated that *R. sanguineus* sensu lato is present in areas with an annual mean temperature greater than 20°C, whereas the temperate lineage occurs in areas with an annual mean temperature less than 20°C (Zemtsova et al., 2016). This supports the presence of *R. sanguineus* (sl) in this study location, where the mean daily temperature exceeds 20°C for most part of the year.

In conclusion, this study has for the first time confirmed the identity of brown dog ticks from Nigeria and provided valuable information on the molecular characterisation of *R. sanguineus* ticks collected from domestic dogs in Nigeria. The study shows they were still the most widely reported tick from domestic dogs and this may present significant public health risk because dogs are the most widely kept species of animal by man. There is the need to establish the vector competence of these common Nigerian dog ticks and also to emphasise the potential public health risk to dog owners.

CONFLICT OF INTEREST

The authors declare that they have no financial or personal conflict that may have inappropriately influenced them in writing this article.

AUTHOR CONTRIBUTIONS

N. Elelu: Conceptualisation (lead), funding acquisition (lead), investigation (lead), methodology (equal), writing – original draft preparation (equal), writing – review & editing (equal). **A.A. Bankole**: Funding acquisition (supporting), data curation (supporting), investigation (supporting), methodology (supporting). **H.P. Daphne**: Conceptualisation (supporting), data curation (supporting), investigation (supporting), methodology (supporting). **M. Rabiu**: Investigation (supporting), writing – review & editing (supporting). **S.D. Ola-Fadunsin**: Data curation (equal), formal analysis (equal), writing – review & editing (equal). **H.M. Ambali**: Investigation (supporting). **S.J. Cutler**: Resources (supporting), supervision (lead), writing – review & editing (supporting).

ACKNOWLEDGEMENTS

ETHICS STATEMENT

This study protocol was approved by the Research and Ethical Committee of the Faculty of Veterinary Medicine, University of Ilorin, Nigeria.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.655

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