**Phenotypic and Genotypic Identification of Ticks infesting dogs (*Canis familiaris*) in Abuja, Guinea Savannah region of Nigeria**

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**Abstract**

Hard ticks are not only haematophagus ectoparasites of vertebrates, but vectors of several micro-organisms that cause severe diseases around the world. In Sub-Saharan Africa, particularly Nigeria, tick infestation is major setback to improvement of livestock and companion animals due to misdiagnosis. The combination of morphological and molecular approach have been documented as a reliable and most appropriate procedure for accurate identification of tick species. Therefore, this study aimed to identify ticks that infest dogs across the six Area Councils of Federal Capital Territory (FCT), Abuja-Nigeria. Ticks (n=2041) were collected from dogs sampled (n=480) for a period of one year and identified morphologically. Genomic DNA was extracted from representative identified tick specimen (n=30) and were amplified in Polymerase Chain Reaction (PCR) using genus specific primers targeting 18S rRNA gene. The products were electrophoresed on 1.5% agarose gel, purified and sequenced bidirectionally. The nucleotide sequences were analysed in BLASTn in NCBI data base. A phylogenetic tree was constructed using Neighbour-Joining Algorithms. The identification by morphology showed that ticks were belonging to 2 Genera: *Rhipicephalus* and *Amblyomm*a. The most abundant genus was *Rhipicephalus* (99.9%), and then *Amblyomma* (0.1%). DNA amplification showed bands size of 173 bp and the BLASTn search analysis revealed 99-100% identity with *R. sanguineus* in the GenBank. The phylogenetic analysis showed that strains of the *R. sanguineus* were similar to those previously reported in Israel and Cuba. In conclusion, *Rhipicephalus sanguineus* was the most abundant tick found among dogs screened in Abuja, Nigeria, irrespective of their use. This is the first molecular identification of dog ticks in the FCT and may have epidemiological significance in the control of ticks and tick-borne disease in the region.

Keyword: Dog, Tick, *Rhipicephalus*,Identification, FCT-Nigeria

**1. Introduction**

Ticks (Family: Ixodidae) are ubiquitous blood-feeding ectoparasites of humans and animals, which are found in different climates and host species (Dantas-Torres et al., 2012). Documented evidence shows that they are representing the most diverse group, occurring in tropical, temperate and even arctic regions (Estrada-Peña et al., 2012). Their medical and veterinary importance are due to their capacity to transmit infectious agents to humans and animals, which may cause a diverse range of disease conditions, commonly referred to as tick-borne diseases (TBDs). Other reports have demonstrated that factors such as climate change, deforestation, changes in land use, urbanization, increased trade and travel affect animal host populations worldwide (Dantas-Torres and Otranto, 2015), which is in consistent with the hypothesis that vector-borne diseases will continue to expand their infection foci due to climate change (Balogun et al., 2016). Consequently, the ability of ticks to survive under different climatic conditions and ecological niches have contributed immensely to their cosmopolitan distribution and making their control a serious challenge to animal owners and veterinarians (Cafarchia et al., 2015). The brown dog tick (*Rhipicephalus sanguineus*) has been reported as the most cosmopolitan species among the families, transmitting a wide range of pathogens to dogs and other animals including humans (Okoli et al., 2006; Fourie et al., 2013), such as Ehrlichia canis, Babesia vogeli and Hepatozoon canis in dogs and Rickettsia conorii and Rickettsia rickettsii in humans ([Dantas-Torres](https://www.ncbi.nlm.nih.gov/pubmed/?term=Dantas-Torres%20F%5BAuthor%5D&cauthor=true&cauthor_uid=25651851) et al*.,* 2012). Worthy of note, that several TBDs of companion animals are zoonotic (Cito et al., 2015) and may place human owners at risk of infection (Rijks et al., 2015) underscoring the need for continued investigation on them as an effort towards One-Health approach to curbing the diseases (Balogun et al., 2016). Identification of ticks based on morphological features has been the standard used in the most developing countries and have given rise to problems of misdiagnoses of both the ticks and the diseases they transmit (Muruthi et al., 2016). Among the drawbacks are the facts that morphological identification may incorrectly applicable to damaged ticks, as well as in some phenotypic characteristics that may vary with size and age which reportedly could represent polymorphism in a single population (Rumer et al., 2011).

In Nigeria, many tick species such as *R. sanguineus, R.* (*Boophilus*) *decolaratus* and *A. variegatum* have been reported to infest domestic animals and pets (Ogo et al., 2012). These aforementioned species do not only infest dogs (Konto et al.*,* 2014), but are also associated with transmission of babesiosis, anaplasmosis, erhlichiosis and rickettsiosis (Natala et al., 2009). Recent findings have shown that occurrence of tick-borne diseases is dependent on available tick species infesting animal host in the region (Obeta, et al., 2020). Worthy of note, it has been reported that cases of canine babesiosis is on the rise in FCT Abuja with as high as 12.5% prevalence (Obeta et al., 2020) hence, the need to establish an accurate identity of the vector(s). Therefore, accurate and prompt identification of tick species in a given animal host and geographical location is important for effective management, monitoring and control of ticks and TBDs. This study was conceived with an aim to determine the identity of the most common tick species infesting dogs in the study area.

**2. Materials and methods**

**2.1 Study area**

This study was carried out in Abuja, the Federal Capital Territory (FCT), Nigeria comprising of six Area Councils namely; Abaji, Bwari, Gwagwalada, Kuje, Kwali, and Municipal (**Fig. 1a**) It has a Guinea Savannah type of vegetation; with annual rain-fall of 1100 to 1600 mm. There are two major seasons in each year: dry season (November - April) and rainy season (May – October). The maximum temperature is 37 °C and minimum, 30 °C (Balogun, 2001). The city lies between latitude 8°35’ and 9° 25’ north of the Equator and longitude 6° 45‟ and 7° 45‟ east of the Greenwich Meridian with a land area of about 8000 square kilometers. Opara et al. (2017), reported that the ecological environments favours the survival and multiplication of ticks. The FCT is bordered to the East by Nasarawa State; to the West by Niger State; to the North by Kaduna State and to the South by Kogi State **(Fig. 1b)**

**Fig. 1a**

## https://www.researchgate.net/profile/Yusuf_Oladimeji/publication/319310675/figure/fig2/AS:697609643630592@1543334508507/Map-of-Abuja-FCT-showing-the-study-area_W640.jpg

## Map of the Federal capital Territory showing the six Area Municipal Councils

## <https://images.app.goo.gl/q9sr3v38n18wCAVn6>

## Fig. 1b



Map of Nigeria showing Abuja (study area} and some major cities

Source: Nigerian Ministry of Environment. Available via license: [CC BY-NC-ND 4.0](https://creativecommons.org/licenses/by-nc-nd/4.0/)

**2.2 Study design, tick collection and identification**

This was a cross-sectional study in which all ticks samples were collected for one year. Dogs sampled were those presented to Government and some private veterinary clinics as well as those from randomly selected households within the study area. Ticks were removed from the body of dogs (n=480), with the aid of a straight blunt-tipped forceps with attention to predilection sites, for approximately 5-10 minutes per animal. Ticks from each animal were placed separately into a vial containing 70% ethanol mixed with 5% glycerol and transported to the Entomology Laboratory, Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. All ticks were identified to species level using morphological features under stereomicroscope as described by Walker et al. (2014). The morphological characteristics used for identification of *Rhipicephalus* includes; hexagonal shaped basis capituli, hypostome, palp, cervical groove, coxa, genital aperture and anal pore.Males were identified by their medium size (2-3.2mm), presence of scutum covering entire dorsal surface; regular rows of large punctuation, while females were identified by the presence of adanal plate, genital aperture, wide posterior, scutum covering 1/3 of dorsal surface and punctuation on the conscutum. The nymphs were identified by the absence of anal plates and genital aperture, while the presence of 3 pairs of legs was used for identification of the larvae**.** The *Amblyomma* was identified by its large-mouth parts, presence of ornamented scutum, posteriomedian stripe, legs with pale rings, genital aperture and anus. To confirm the morphological identification and validate it genetically, genomic DNA was extracted from individual representative tick of *Rhipicephalus* sp. (n=30) using Qiagen DNeasy Blood and Tissue Kit, (Hilden Germany), following the manufacturer’s instructions. The quality and quantity of the extracted DNA were measured using Nanodrop ND 1000 spectrophotometer (Waltham, MA, United States). The DNA was amplified by polymerase chain reaction (PCR) using genus specific primers (5'-GTGAAACTGCGAATGGGCTCA-3' and 5'-GTGAAACTGCGAATGGGCTCA-3') targeting partial region of the 18S rRNA gene of *Rhipicephalus* sp. PCR reaction was performed in a final volume of 20μL, containing 100 ng of the test gDNA, 2.5 μM of each primer, 1× of PCR buffer (including 15 mM MgCl2), 200 μM of dNTPs, 1.0 U of PfuUltra II Fusion HS DNA Polymerase (Agilent technologies, Santa Clara, CA, USA) and Nuclease-free water was used to complete the total volume of the reaction. Thermocycling conditions were initial denaturation at 94 °C for 5 min, 40 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, extension at 72 °C for 10 sec, and final extension at 72 °C for 10 min. The PCR products were electrophoresed on 1.5% Agarose gel plus SYBR Green (Roche Diagnostic, Mannheim, Germany) for 1 hour at 80 volts. The products were purified using MagExtractor-PCR & Gel Clean up kit (Osaka, Japan) following the manufacturer’s instructions. The Molecular size of the amplified fragment was estimated by comparison with mobility patterns of fragments in 1 kb DNA ladder.

**2.3 Sequencing and sequence analysis**

To determine the species of the tick, larger segment (~860 bp) of the 18S rRNA gene was amplified and sequenced bidirectionally using the ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, CA, USA). All the reagents were kept on ice and used in the following order: dH2O (0 – 0.95 μL), DNA template (0.5 –10.0 μL), primers (2 μL), and DTCS Quick start master mix (8.0 μL). The following conditions were used for sequencing: 96°C for 20 s, 50°C for 20 s (30 cycles), and 60°C for 4 min. The sequence data of forward and reverse were aligned using the Plasmid editor ApEsoftware to obtain a consensus sequence for each isolate. Sequences data obtained were assembled and edited using the Bio Edit version7.0 (Hall, 1999). Homologous sequences were analysed using Basic Local Alignment Search Tool (BLAST) package (Altschul et al. 1990) in comparison with sequences available in the Genbank database (<https://www.ncbi.nlm.nih.gov/BLAST>). The nucleotide sequence data of the isolates from this study were deposited in the GenBank database with Accession Numbers KY799078 - KY799086. To construct a phylogenetic tree, Neighbour-Joining (NJ) Algorithm was performed in MEGA v7.0 (Kumar et al., 2016) in order to determine the evolutionary relationship of the tick isolates. The genetic distances between pairs of sequences were calculated using Kimura’s two parameter model (Tamura et al., 2013), A bootstrap of 1000 replicates was statistically used to evaluate the branching of the phylogenetic tree, and bootstrap value of 50% was considered significant and an evidence of phylogenetic grouping. The tree was rooted to the *Amblyomma* *variegatum*.

**2.4 Data analysis**

Data obtained were expressed as percentages and presented in forms of tables, charts and odd ratio. A Statistical Package for Social Science (SPSS, Chicago, Ill USA, and Version 20), was used. The percentage of nucleotide variation among sequences of the species was determined by pair-wise comparison, using Neighbour-Joining (NJ) Algorithm in MEGA v7.0 software (Kumar et al., 2016). For all comparisons, a p value < 0.05 was statistically significant.

**3. Results and Discussion**

Four hundred and eighty dogs were screened for ticks and 53% were found with tick infestation of various degree. Overall, a total of 2041 ticks were collected from the six Area Councils with no significance difference between the total number of ticks collected from each Council (P<0.05) perhaps, depicting similarity in the geo-climatic condition within the studied area. Although two genera of ticks were identified namely, *Rhipicephalus* and *Amblyomma* by morphology. The *Rhipicephalus* sp. commonly known as ‘brown dog tick’ or ‘kennel tick’ (Horak et al., 2018) accounted for 2039 (99.9%) of the ticks recovered, while only 2 (0.1%) were *Amblyomma* sp. also known as ‘tropical bont tick’ (Horak et al., 2018). These two genera have been reported as the most abundant tick species of domestic animals and pets in Nigeria (Ogo et al., 2012). The reason could be due to their short three-host life cycle that tends to prevent desiccation and long diapause situations (Solomon and Kaaya 1998). Overall, the findings in the present study are similar to the reports of Opara et al. (2017), who earlier documented a prevalence of 89% for *R. sanguineus* tick species on local dogs sampled in Gwagwalada Area Council. They attributed this high prevalence to the availability of vegetation cover which provides favorable environment for the propagation and survival of ticks. Our reports corroborates with the findings of Amuta et al*.*, (2010), with 82% prevalence of tick infestation among dogs examined in Makurdi metropolis, Benue state, North Central, Nigeria. Also in agreement with our findings on *Amblyomma* sp. Amuta et al. (2010) encountered only a single *A. variegatum* on a dog examined during the study. This finding also supports recent reports (Abalaka et al., 2018; Horak et al., 2018) that *R. sanguineus* and the pathogens they transmit can be present wherever domestic dogs are kept under relatively restricted conditions. Konto et al. (2014) reported four genera of ticks; *Boophilus* (88%), *Rhipicephalus* (10.8%), *Hyalomma* (0.9%) and *Amblyomma* (0.3%) infesting stray dogs examined in Maiduguri metropolis. Their report of 0.3% *Amblyomma* agrees with our finding, but differ because we did not find *Boophilus* and *Hyalomma* species in the dogs examined in Abuja. In the present work, the two male *A. variegatum* encountered were collected from a local dog frequently used for hunting in Gwagwalada Area Council. This finding might probably be an accidental infestation as a consequence of close association that exists between hunting dogs and ruminants (Horak et al., 2018) in the area. Studies have shown that larval, nymphal and adult stages of the *Amblyomma* ticks prefer members of the family Bovidae as hosts (Guglielmone et al., 2014). The *A. variegatum* are spreading to different parts of the world through migratory birds (Ogo et al., 2012), however transport of live cattle appears to be the major factor driving the expansion of the species’ range (Burridge, 2011). Nevertheless, the presence *Amblyomma* sp. is of significance as it has been documented to be vectors of some pathogens which are detrimental to the development of livestock in Nigeria and other African countries. For example, *A. variegatum* transmits *E. ruminantium*, the causative agent of heart water in domestic and some species of wild ruminants; *Anaplasma bovis*, the causative organism of benign bovine anaplasmosis; *Theileria mutans* and *Theileria velifera*, the causative organisms of benign bovine theilerioses. *A. variegatum* also transmits *Rickettsia africae*, the causative organism of African tick-bite fever in humans. Acute bovine dermatophilosis (*Dermatophilus congolensis*) is often associated with infestations of adult *A. variegatum* (Horak et al., 2018). *A. variegatum* are reported as known vectors of *Ehrlichia canis*, the causative organism of canine ehrlichiosis or tropical pancytopaenia in dogs; and transmits *Rickettsia conorii*, the causative organism of tick-bite fever in humans and other tick-borne infections around the world, a majority of which are suspected to be of zoonotic potential. These genera are three-host tick of which adult, nymphal and larval stages feed on dogs. Several reports suggest *R. sanguineus* as vector of *B. canis vogeli* in Nigeria (Kamani et al., 2013), the causative organism of a mild form of canine babesiosis (Adamu et al., 2014).

The sex of *Rhipicephalus* sp collected from dogs examined in the FCT is presented [**table 1**].

**Table 1:** Distribution of *Rhipicephalus* ticks collected from dogs examined in Abuja, FCT.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Area Council** | **Male** | **Female** | **Total** | **(%) of ticks collected** |  |
| Abaji | 155 | 128 | 283 | 13.8 |  |
| AMAC | 159 | 138 | 297 | 14.5 |  |
| Bwari | 192 | 168 | 360 | 17.6 |  |
| Gwagwalada | 220 | 185 | 405 | 19.8 |  |
| Kuje | 186 | 168 | 354 | 17.3 |  |
| Kwali | 183 | 157 | 340 | 16.6 |  |
| **Total** | **1,095** | **944** | **2,039** | **99.9** |  |

Of the 2039 *Rhipicephalus* collected, 1095 (53.7%) were males ticks while 944 (46.3%) were female. These reports agree with (Soulsby, 1982) who said that more male ticks than female are usually encountered on the body of animals on examinations, as female ticks drop off the host after fully engorged with blood meal.

The ear had the highest (45.9%) tick attachment while the scrotum and the mammary region had the least (2.0%) respectively [**Fig.2**].

**Fig. 2**

**Tick attachment sites on dogs examined in Abuja, FCT**

In the present study, the ear was the most preferred attachment sites of ticks when compared to other sites of attachment. This report is partially in agreement with the findings of Konto et al. (2014) who reported that perineum and the ear were the most preferred predilection sites for ticks on dogs. Our findings differ from that of Amuta et al. (2010) that reported the paws as the most preferred sites for the brown dog ticks and attributed it to the sites being less accessible as compared to neck and head. The preferred attachment sites in the ear as demonstrated in this study could be attributed to the differences in the thickness of body skin, accessibility to blood vessels, variations of micro habitant or temperature (Konto et al., 2014). Other sites of attachment reported in our study include the neck, face, inter-digital spaces, thoracic region, perineum, inguinal region, abdominal wall, mammary gland and scrotal sac. Horak et al. (2018) also reported similar parts of the body as preferred attachment sites for ticks on dogs.

As previously reported, controversy exist with *R. sanguineus* complex due to the morphological similarity among the members of the group around the world (Chitimia-Dobler et al., 2017). The *R. sanguineus* is one of a group of five morphologically similar species, three of which are, *R. sanguineus, R. sulcatus* and *R. turanicus* or *R. afranicus*, as newly proposed (Walker et al., 2014). Recent studies have observed major differences in the ecology, vector competence, crossbreeding, and other biological attributes within the *R. sanguineus* (Labruna et al., 2017). Due to the wide-ranging confusion associated with their nomenclature and identification, a description according to their three divergent genetic lineages namely, tropical, temperate, and south-eastern rather than species identification has been proposed (Chitimia-Dobler et al., 2017).

In the present study, the genus specific primers successfully amplified DNA of the isolates at the expected band size of approximately 173 bp [**Fig. 3**].

## Fig. 3

## C:\Users\DR. SYLVESTER OBETA\Desktop\Labeled PCR result\3.tif

## Agarose gel electrophoersis for *R.* *sanguineus* 18S rRNA geneLane 1- Molecular marker, Lane 2: negative control, Lanes 3 –25 Isolates randomly selected from the six Area Councils of the FCT.

The sequences from 18 ticks showed 100% identity and from 2 ticks showed 99% identity, with previous data base 18S rRNA gene sequences of *R. sanguineus* with accession numbers KP830113, KF958430, and KF958451. *R. sanguineus*.

From the phylogeny analysis, the branch length represents evolutionary changes that have taken place over time and the amount of genetic change is represented by a scale of 0.1. The number of substitution which is related to the clustering together of the taxa as a bootstrap test, is represented by a percentage value; that is, the number of substitution per 100 nucleotide sites and is shown above in the branches. There were two clusters of tick isolates from this; *R. sanguineus:* KY799078, KY799080, KY799081, KY799083 – KY799086 (FCT) belong to the same clustered with *R. sanguineus* KP830113 and KF958451 from Cuba and Israel respectively. However, the FCT isolates KY799079 and KY799082 belong to another cluster. The clustering pattern observed from FCT isolates could be a reflection of different sequence lengths. The second clusters were *R. sanguineus* KP830113 and KF958451 from Cuba and Israel respectively [**Fig. 4**].

## Fig. 4

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Phylogenetic tree of partial nucleotide sequences (604-nt) 18S rRNA of *R. sanguinues.*

Numbers on the nodes indicate bootstrap support from 100 replications. Only bootstrap values >40 are shown. The scale bar represents the number of substitutions per nucleotide. The tree was rooted to the genus *A. variegatum*.

Molecular analyses were also done in order to provide sequence information for those tick species in the study area that were not yet available in GenBank.

In conclusion, this study affirms *Rhipicephalus sanguineus* as the most prevalent tick species found feeding on dogs sampled in the FCT, Abuja. The phylogenetic analysis revealed similarity in the evolutionary relationship of some FCT isolates with those isolates reported from Israel and Cuba. The present findings may be of significant in understanding the epidemiology of the ticks and tick-borne diseases in the region.

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