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Full Length Research Paper

Phenotypic and Molecular Characterization of *Rhipicephalus (Boophilus) decoloratus* (Koch, 1844) from Cattle in Zaria, Northwestern Nigeria

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Abstract

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Purpose: Hard ticks (Acari: Ixodidae) are important ectoparasites of cattle and other domestic livestock serving as vectors of pathogenic microorganisms. This study aimed to determine the prevalence of Ixodid ticks and genetically identify *Rhipicephalus (Boophilus) decoloratus* sampled from cattle in Zaria, Kaduna State in Northwestern Nigeria. **Methods:** A total of 384 cattle were sampled from June 2018 to October 2018 to determine the prevalence and true identity of ixodid ticks of cattle in the region and 439 ticks (Ixodidae) were collected from 335 infested cattle. **Results:** Three genera of ticks comprising five species were found infesting cattle in Zaria. *Amblyomma variegatum* constitutes the highest percentage of ticks collected from the sample sites (47.8%; 210/439), followed by *Rhipicephalus (B) decoloratus* (31.0%; 136/439). *Hyalomma* species had the least percentage of infestation, with *Hyalomma truncatum* having (18.5%; 81/439) infestation. *Hyalomma impeltatum* had (1.8%; 8/439) and *Hyalomma rufipes* had (0.9%; 4/439) infestation respectively. The presence of *Rhipicephalus (B) decoloratus* was identified molecularly from all the sampling sites with accession numbers (MK501835, MK501836, and MK501837). **Conclusion:** This finding was confirmed for the first time in Zaria, Kaduna State, in Northwestern Nigeria using a molecular technique targeting the ITS-2 region of the ticks. This study confirms the presence of *Rhipicephalus (B) decoloratus* in Zaria, Kaduna State in Northwestern Nigeria in addition to other tick species. This finding implies that there may be an additional economic burden to livestock farmers due to the increased cost of tick control as well as disease management.

Keywords: Morphological, Molecular, *Rhipicephalus (B) decoloratus*, Cattle, Zaria.

INTRODUCTION

Ticks are obligate blood-sucking ectoparasites and are regarded as a major constraint to improving livestock production in sub-Saharan Africa (Muriithi, 1984). The infestations of ticks on various domestic animals, like cattle, sheep, goats, and horses have been investigated by various authors in Nigeria (Dipeolu, 1975; George *et al.*, 1990; Ahmed and George, 2002). About 850 species of ticks have been described worldwide. Ticks are important ectoparasites of cattle and other domestic species of tropical and subtropical countries, serving as vectors of pathogenic microorganisms (Muhammed *et al.,* 2008).

Ticks are classified in the Phylum Arthropoda, Class Arachnida, Subclass Acari, Order Parasitiformes, and Suborder Ixodida (Santiago *et al.,* 2009). Ticks share the

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Order Parasitiformes with the suborders *Holothyrida*, *Mesostigmata* (commonly known as mites), and *Opilioacarida* (Santiago *et al.,* 2009). *Ixodida* contains two well-established families: *Ixodidae* (hard) ticks and *Argasidae* (soft) ticks (El-Kammah *et al.,* 2001), and a third family, *Nuttalliellidae* (an ill-known monotypic family represented by *Nuttalliella namaqua*) (Santiago *et al.,* 2009). Few among the ticks of the family *Ixodidae* are of economic importance in terms of geographical spread, the effect of feeding on animals, and the disease they transmit. These include various species of *Hyalomma, Amblyomma, Boophilus,* and *Rhipicephalus* (Hoogstraal, 1985).

The African blue tick *Rhipicephalus (Boophilus) decoloratus* is a small inornate tick having a short and broad capitulum with rounded lateral margins. This species of tick is one of the most common, widespread, and frequent one-host ticks of cattle and other livestock in Africa (Walker *et al.,* 2003). The tick has a much wider host range than *Rhipicephalus (B) microplus.* Cattle are the most preferred host, however other animals like horses, donkeys, sheep, goats (Walker *et al.,* 2003), and several wild ungulates including Impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*), Eland (*Taurotra gusoryx*) and Sable (*Hippotragus niger*) are also the important host of the tick species (Spickett and Fivaz, 1992; Norval, 1994). Cattle play a very important role in serving as the only maintenance host for this tick and infestations of other hosts will only occur when a population of ticks is maintained by cattle. The preferred predilection or feeding sites of all stages on cattle are usually the back, upper legs, neck, shoulders, dewlap, and belly (Walker *et al.,* 2003).

Rhipicephalus (B) decoloratus is widely distributed in sub-Saharan Africa. The tick occurs in parts of Namibia and Botswana, and it is also present in Mozambique, Zimbabwe, Angola, Zambia, Malawi, parts of Tanzania, Burundi, Uganda, Western Kenya, and in the wetter highlands of Ethiopia. It is also present in parts of West Africa (Mason and Norval, 1980; FAO, 1984; Matthysse and Colbo, 1987; Norval, 1994; Walker *et al.,* 2003). *Rhipicephalus* (*B*) *decoloratus* has been recorded as the second most common tick specie infesting small ruminants (Sheep) in Zaria, Northwestern Nigeria with peak population towards the end of the rainy season in September to October (George *et al.,* 1990).

Rhipicephalus (B) decoloratus is known to transmit a pathogen that causes babesiosis (*Babesia bigemina*) (Estrada-Pe˜na *et al.,* 2006). The disease-causing organism is transmitted only by the nymphal and adult stages after it has passed transovarially from one generation to the next (Walker *et al.,* 2003). Babesiosis is an important tick-borne disease of cattle (Radostits *et al.*, 2000; Futse *et al.,* 2003), it has great economic importance, simply because it affects adults more severely than young cattle, leading to direct losses through death. *Rhipicephalus (B) decoloratus* also transmits *Anaplasma marginale* and *Borrelia theileri* (Norval and Horak, 2004).

Rhipicephalus (B) decoloratus infestation causes huge economic losses in livestock production in terms of diseases, reduced productivity, and fertility, and often death (Rajput *et al.,* 2006), and continues to cripple the industry, especially in sub-Saharan Africa (Horak *et al*., 1983). The negative impact of infestation with this tick species and the diseases they transmit to animals warrant the application of appropriate tick control strategies (Bansal, 2005). The control of tick infestation, the disease they transmit, and limiting their spread is an expensive task and accurate identification of the tick vector is vital to make it effective (Lampo *et al*., 1997).

Reliable and quick means of identification of tick species are therefore important in the control of and spread of tick-borne diseases. Morphological characterization using phenotypic traits has been the traditional method of identifying ticks. However, this approach is toilsome, sometimes inaccurate, and may result in incorrect identification. Also, the differentiation of larvae and nymph of closely related species of ticks is difficult (Abdigoudarzi *et al*., 2011), and often impossible, as detailed morphological descriptions of the immature stages remain unknown for most tick species (Marrelli *et al.,* 2007). In addition, traits used to differentiate species tend to overlap between species or vary within species or according to age and size and this further weakened the morphological identification and finally, morphological identification of physically damaged ticks due to poor handling and preservation is often inaccurate. The invention of molecular techniques and the development of markers that can identify ticks have enhanced the understanding of other insect vectors and may be useful in studies of tick taxonomy and diversity (Muruthi *et al.,* 2016).

Although the presence of *Rhipicephalus (B) decoloratus* had been reported in Zaria (George, 1987; Obadiah and Shekaro, 2012), these reports were based on morphological identification and have never been verified at the molecular level. To date, there is no documented information on the molecular characterization of this tick species in the study area. Therefore, the purpose of this study was to morphologically and genetically characterize *Rhipicephalus (B) decoloratus* and confirm its presence in the study area.

MATERIALS AND METHODS

Study area

The study was carried out in Zaria, Kaduna State, located about 74km north of Kaduna, along the Kaduna-Kano highway (Mortimore, 1970), in Northwestern Nigeria, within latitudes 11° 3' N and longitudes 7° 42' E (Mamman *et al.,* 2000), with an altitude of 500-700 meters above sea level and a total area of 300km^2. The climatic characteristics are that of tropical savannah (Mortimore, 1970), with a monthly mean temperature of 25.25^{0}C

(ranging from 13.8^{0}C to 36.7^{0}C) and annual rainfall of 1092.8mm (Agbogbu *et al.,* 2006). Zaria comprises two Local Government Areas namely; Zaria and Sabon Gari. To the existing pattern of settlement, Zaria Urban is composed mainly of four districts namely; Zaria City, Tudun Wada, Sabon Gari, and Samaru (Obadiah and Shekaro, 2012).

Ethical Statement

Ethical approval was obtained from Committee on Animal Use and Care (ABUCAUC) of Ahmadu Bello University, Zaria, Nigeria with approval number: ABUCAUC/2023/112 prior to the commencement of the study. Also, verbal informed consent was sought from the Fulani herdsmen and their community leaders.

Collection of Ticks

The sampling took place over five (5) months, from June 2018 to October 2018. Ticks were collected from cattle in three selected sampling sites namely; Bomo village, Zangon Shanu, and Angwan Fulani. The sampling was done after obtaining verbal consent from the herd owners. Restraining of the cattle was done with the help of farm attendants, and the ticks were detached from the body of cattle by hand picking which were singly put in universal glass tubes and loosely plugged with cotton wool. The glass tubes were labeled indicating tick species and date of collection. The ticks were then transported to the Entomology Laboratory, Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria for identification.

Morphological identification

The ticks collected were examined morphologically. Ticks were first washed twice with sterile water to remove excess particulate contamination from animal skin and then rinsed once in 70% ethanol. Each tick was placed in a petri dish using a pair of forceps and examined using a stereo microscope at magnifications of x40, x80, and x100. Where possible, the ticks were identified up to the species level using both taxonomic descriptions (Walker *et al.,* 2003) and morphological keys (Hoogstraal, 1956; Hoogstraal and Kaiser, 1959; Madder 2012a, b). The identified ticks were preserved in sterile vials containing 70% alcohol until further processing at DNA Laboratory Kaduna.

Molecular identification of *Rhipicephalus (Boophilus) decoloratus*

The morphologically identified *Rhipicephalus (B) decoloratus* ticks from the three (3) selected farms were confirmed at the molecular level. Molecular analysis was carried out on a subset of 3 *Rhipicephalus (B) decoloratus* ticks from each of the three farms at the DNA Laboratory Kaduna, Nigeria. DNA from nine (9) morphologically identified female *Rhipicephalus (B) decoloratus*, 3 from each of the three farms, was extracted using *AccuPrep*®Genomic DNA Extraction Kit following the manufacturer´s protocol (DNA extraction protocol of BIONEER). These samples were amplified by polymerase chain reaction (PCR) using the primers Boophits2 F 5′-GCC-GTC-GAC-TCG-TTT-TGA- 3′ and Boophits2 R 5′–TCC-GAA-CAG-TTG-CGT-GAT-AAA-3′. The PCR program consisted of an initial denaturation at 94 °C for 4 min followed by 40 cycles of 92 °C for 30 s, annealing at 58 °C for 45 s, extension at 72 °C for 60 s with a final extension at 72 °C for 8 min according to the protocol of Lempereur *et al.,* (2010), to obtain an expected amplicon size of 821bp of the ITS-2 gene. A non-template control containing all the reaction mixtures except DNA was included in the PCR run to detect the presence of contaminations. Positive control DNA of *Rhipicephalus (B) decoloratus* was not available; hence it was not included in the PCR run.

Gel electrophoresis

The amplified products were analyzed by electrophoresis on 1.5% Agarose gel by aliquoting 4*µl* of PCR products and 100bp plus DNA ladder and run on the gel stained with Ethidium bromide for 40 min at 100 volts following the protocol of DNA Lab. The gel was prepared by dissolving 3g of Agarose in TAE (Tris Acetate EDTA). The solution was heated in a microwave until the agarose was completely dissolved. It was allowed to cool in a water bath. The gel casting tray was prepared by sealing the ends of the gel chamber with tape and the appropriate number of combs were placed in the gel tray. 5*µl* of Ethidium bromide was added to the cooled gel and poured into the gel tray. It was allowed to cool for 15-30 min at room temperature. The combs were removed TAE buffer (Tris-acetate-EDTA) was poured into the chamber to cover the gel. DNA and 100bp plus ladder were loaded onto the gel. Electrophoresis was done at 100V for 40 min. The DNA bands were visualized using a UV light box.

Sequencing and analysis

Purified DNA products were sequenced at DNA Laboratory Kaduna, Kaduna State Nigeria. The purified PCR amplicons were precipitated and sequenced using dye terminator cycle sequencing with a quick start kit (Applied Biosystems, Foster City, CA, USA), with the forward and reverse amplification PCR primers. The sequence chromatogram for the forward and reverse were viewed and manually edited in Finch TV before they were aligned to generate a single sequence product for each of the samples. BLAST (Basic Local Alignment Search Tool)

for the aligned sequences was carried out for sequence identity and similarity in the GenBank database (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*). ITS-2 sequences from the GenBank with 98 percent or closest similarity to the sample sequence were considered (Muruthi, 2011).

Phylogenetic analysis of *Rhipicephalus (B) decoloratus*

The phylogenetic tree was constructed using the Molecular Evolutionary Genetic Analysis (MEGA 7.0) software program (Kumar *et al.,* 2016) to determine the evolutionary relationship of *Rhipicephalus (B) decoloratus* in this study with the reference sequences of *Rhipicephalus spp.* in the GenBank. The ITS-2 gene sequences of *Rhipicephalus (B) decoloratus* in this study were first imported into a notepad page of a computer as well as the sequences of other *Rhipicephalus (B) spp.* from the GenBank and saved in FASTA format. Multiple sequence alignments were carried out using Clustal W for pair-wise comparison and the aligned sequences were edited manually and saved under the same session. The evolutionary distances were computed using the Maximum Composite Likelihood method and Neighbor-joining (NJ) algorithm was used to construct a phylogenetic tree (Saitou and Nei, 1987). A bootstrap of 500 replicates was statistically used to evaluate the branching of the phylogenetic tree (Felsenstein, 1985).

RESULTS

Prevalence of tick species on cattle collected from the sample sites in Zaria

The sampling took place for five (5) months, from June 2018 to October 2018. During this period, a total of 384 cattle were sampled and 439 ticks were collected from 335 infested cattle. All the ticks collected were correctly identified morphologically and classified into three genera namely; *Amblyomma, Rhipicephalus,* and *Hyalomma*. Five species of ticks were correctly identified from all three sample sites. In the genus *Amblyomma,* only one species was identified as *Amblyomma variegatum.* In the genus *Rhipicephalus,* only one species was identified in the subgenus *Boophilus* as *Rhipicephalus (B) decoloratus* while in the genus *Hyalomma* three species were identified as *Hyalomma rufipes, Hyalomma impeltatum,* and *Hyalomma truncatum. Amblyomma variegatum* constituted the highest percentage of sampled ticks (47.8%; 210/439), followed by *Rhipicephalus (B) decoloratus* (31.0%; 136/439) and *Hyalomma* species (21.2%; 93/439) (Table I).

Fig 3: Visualization of the polymerase reaction product of ITS 2 amplicons of *Rhipicephalus (Boophilus) decoloratus* ticks from Zaria ran on Agarose gel (1.5%). 100 bp plus DNA ladder at the left (L). Lanes: 1-3 represent PCR products of samples from Bomo village; Lanes 4-6 represent PCR products of samples from Zangon Shanu; Lanes 7-9 represent PCR products of samples from Angwan Fulani; -VE is the Negative control.

Morphological identification of *Rhipicephalus (B) decoloratus* was confirmed by the use of PCR and sequencing in three of the sampled sites. DNA from morphologically identified *Rhipicephalus (B) decoloratus* ticks from Bomo village, Zangon Shanu, and Angwan Fulani were successfully amplified at an expected amplicon length of 821bp and sequenced. The sequence chromatogram was viewed in Finch TV and manually edited before they were aligned to give a single sequence product for each of the samples. BLAST search for the edited sequences was done in GenBank (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*) and the sequences with the highest percentage similarity were considered the closest matches to the sequences obtained in this study. The study sequences were assigned names with the highest species from the GenBank.

All the sequences of *Rhipicephalus (B) decoloratus* from each of Bomo village, Zangon Shanu, and Angwan Fulani showed 94–96% homology with *Rhipicephalus (B) decoloratus* GenBank deposited sequences. Sequence from Bomo village had 94% identity to *Rhipicephalus (B) decoloratus* (accession number U97716) deposited in the GenBank. Sequence from Zangon Shanu had 96% identity to *Rhipicephalus (B) decoloratus* deposited sequence. And the third sequence from Angwan Fulani had 96% identity to *Rhipicephalus (B) decoloratus* sequence deposited in the GenBank. *Rhipicephalus (B) decoloratus* ITS-2 sequences generated in this study have been deposited in GenBank and were assigned accession numbers: MK501835, MK501836, and MK501837. All the sequences from this study aligned well with the reference sequences of *Rhipicephalus (B) decoloratus* in the GenBank (Fig 2).

Fig 4: Phylogenetic tree of *Rhipicephalus (B) decoloratus* ticks: Sequence from the study and reference sequence obtained from the GenBank were aligned using multiple alignment programs Clustal W. The optimal tree with the sum of branch length = 16.73489183 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted using MEGA 7 software. The study sequences (MK501835, MK501836, and MK501837) are in bold.

DISCUSSION

The studies carried out in the selected sites within Zaria, show that the ticks infesting cattle in Zaria belong to three

Table 1: Prevalence of tick infestation in relation to location.

Location	*A. variegatum*	*Rh. (B) decoloratus*	*Hy. rufipes*	*Hy. Impeltatum*	*Hy. truncatum*	*Total (%)*
Bomo	86 (48.86)[a]	52 (29.55)[a]	1 (0.57)[a]	3 (1.70)[a]	34 (19.32)[a]	176 (40.1)[a]
Zangon Shanu	78 (46.70)[b]	58 (34.73)[a]	3 (1.80)[a]	1 (0.60)[a]	27 (16.17)[b]	167 (38.0)[a]
Angwan Fulani	46 (47.92)[c]	26 (27.08)[b]	0 (0.00)[a]	4 (4.17)[b]	20 (20.83)[c]	96 (21.9)[b]
Total	210 (47.8)	136 (31.0)	4 (0.9)	8 (1.8)	81 (18.5)	439 (100)

Values in the same column with different superscripts differ significantly at P<0.05

Morphological features of *Rhipicephalus (B.) decoloratus*

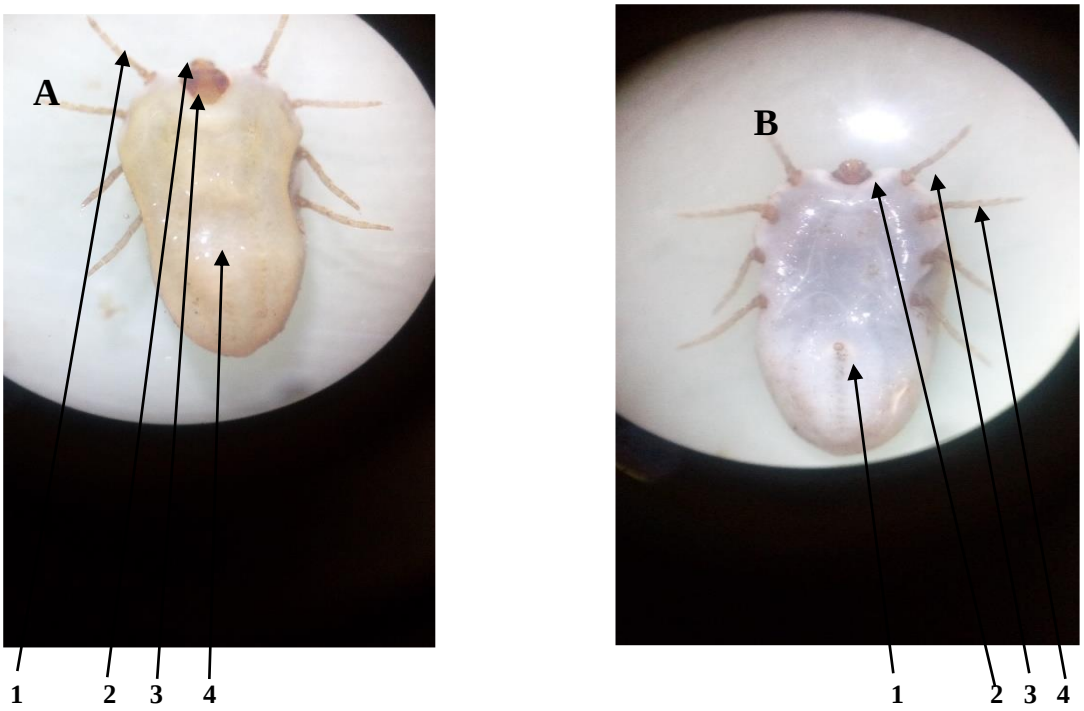


Fig 1: *Rhipicephalus (B) decoloratus (female) at X80;* ***a.*** *Dorsal view; (**1**) Yellowish slender legs with pale rings;* ***(2)*** *Small eyes;* ***(3)*** *Two distinct grooves divide the scutum into a central yellow area and two lateral areas that are reddish-brown; the scutum of females has numerous fine hairs.* ***(4)*** *The engorged female is blue, frequently with a constriction in its middle and a soft integument:* ***b.*** *Ventral region; (**1**) U-shaped genital aperture* ***(2)*** *The basis capituli is hexagonal, the mouthparts are short and the dentition on the hypostome is arranged in two columns each of which consist of three denticles (3/3 dentition);* ***(3)*** *Coxae 1 spurs are distinct;* ***(4)*** *Coxae 2 and 3 spurs are present.*

genera namely; *Amblyomma, Rhipicephalus* (subgenus *Boophilus* included), and *Hyalomma.* Five different species of ticks from the three genera were identified from the survey carried out, these include; *Amblyomma variegatum, Rhipicephalus (B) decoloratus*, *Hyalomma rufipes, Hyalomma impeltatum,* and *Hyalomma truncatum.* Infestation percentage by these tick genera was variable in degrees but observed in all the different sample sites. *Rhipicephalus (B) decoloratus* accounted for 31.0% of the total number of ticks collected representing the second most prevalent tick species in the region. This finding is consistent with the reports of George *et al.* (1990), Obadiah and Shekaro (2012), Aminu (2015), and Obadiah *et al.* (2017). The number of male ticks recovered from all the sampling sites is less compared to the female and immature (developmental) stages. This observation differs

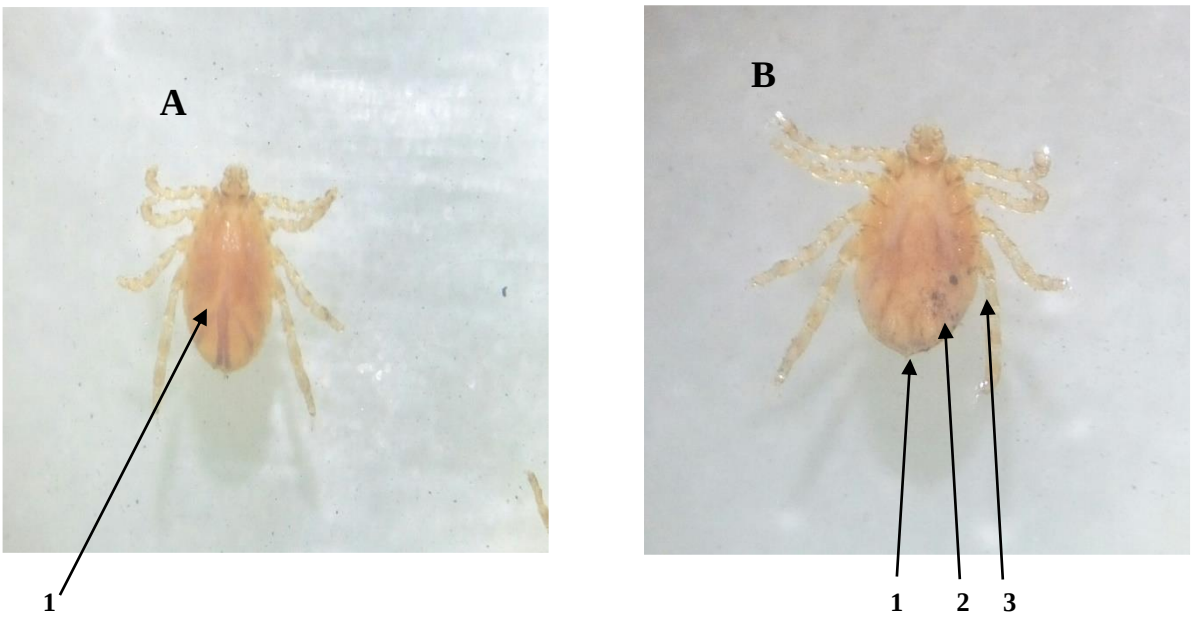


Fig 2: Rhipicephalus (B) decoloratus (male) at X80; a. Dorsal view; (1) The conscutum of the male is yellowish and often so poorly sclerotized that the outlines of the gut can be seen through it. There are numerous fine hairs on the conscutum of males. b. Ventral view; (1) Narrow caudal appendage in males; (2) Adanal plate with a long, narrow posteriorly directed internal spur and a shorter external spur; (3) Accessory adanal plate with distinct spurs. The tips of ventral plates (adanal and accessory adanal plate) are visible dorsally where they protrude beyond the posterior margin of the conscutum.

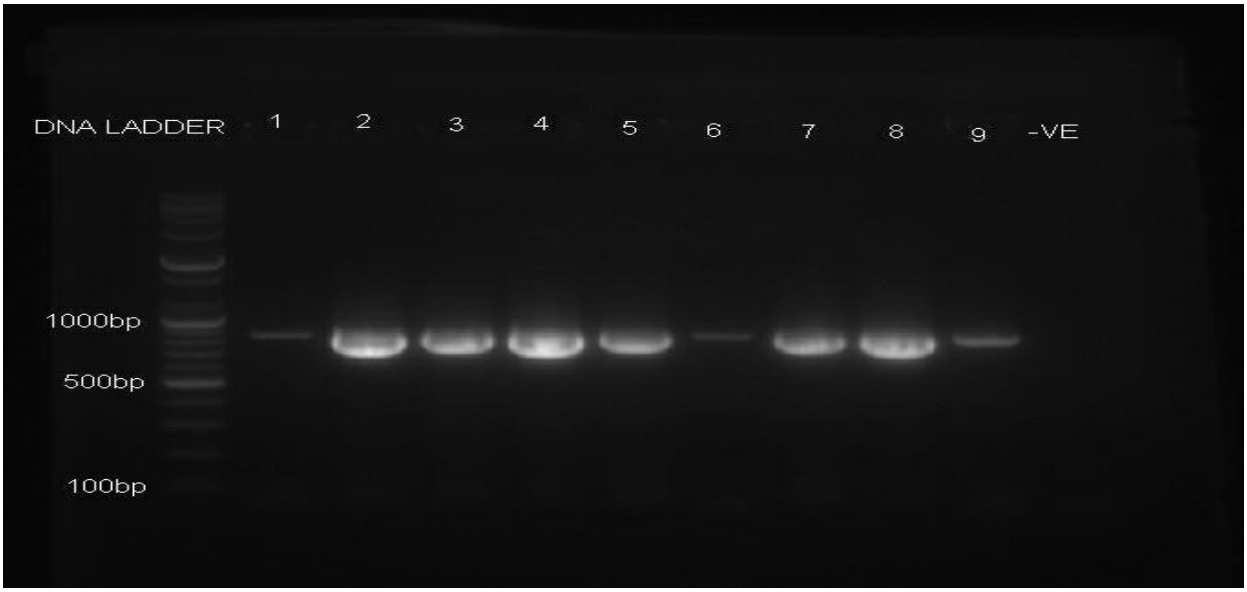
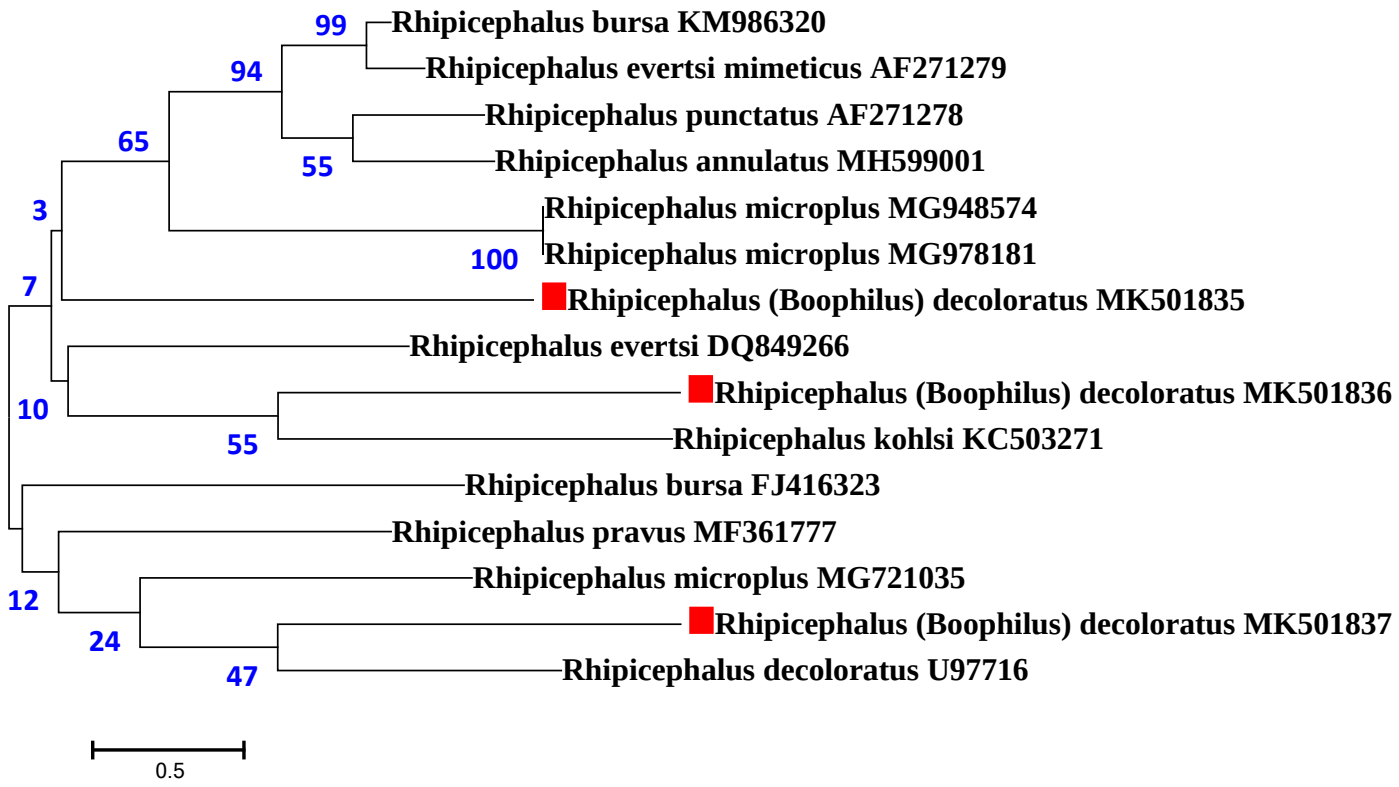


Fig 3:

from the general trend in the other tick species collected from all the sampling sites where male ticks usually predominate compared to the female and immature stages. The low number of males of *Rhipicephalus (B) decoloratus* ticks collected could be because they are very small in size and very difficult to collect from the body of



the host animal (George *et al.,* 1990). No other species of the subgenus *Boophilus* was identified from all the sampling sites. The study serves as the first confirmation of *Rhipicephalus (B) decoloratus* at the molecular level in Zaria. Although there were earlier reports on the presence of this tick species in Zaria (George *et al.,* 1990) and other parts of the country (Dipeolu 1975c), these reports were based on morphological identification.

In this study, all the *Rhipicephalus (B) decoloratus* collected from cattle in all the sample areas were identified both morphologically and genetically. However, some of the sampled tick species could not be identified due to lost body parts and physical damage making it difficult to be morphologically identified. Abdigoudarzi *et al.* (2011) noted that morphological changes that occur on the body's surface usually gives misleading result during morphological identification. In their studies, they observed that some of the damaged ticks could not be easily identified morphologically due to decomposition but by applying phylogenetic analysis, they were able to identify the tick species correctly.

In recent times, molecular work on tick species had used the ITS 2 as a marker in identifying ticks, especially in differentiating closely related genera (Hillis and Dixon, 1991; Rumer *et al.,* 2011). Lu *et al.* (2014), made similar observations and showed that ITS 2 has the highest inter-specific divergence and a low intra-specific variation when compared to other markers and therefore, concluded that ITS 2 may be the most useful DNA marker for discriminating tick species. Amplification of the entire ITS 2 regions has been done by many researchers and mixed results were observed including Zahler *et al.* (1995) and Murell *et al.* (2001) who attributed the unsatisfactory results to the specific nature of the ITS 2 region in ticks and some repeated fragments in the region. In their study Abdigourdarzi *et al*. (2011) noted that partial amplification of the region is good in discriminating different species of hard ticks; however, the authors asserted that it is important to have a complete sequence of ITS 2 regions to be able to resolve issues related to partial sequences that were seen in their study.

In this research, the ITS-2 gene was used to identify *Rhipicephalus (B) decoloratus* at an expected amplicon size of 821bp, and the study sequences obtained were (MK501835, MK501836, and MK501837). The result is similar to the findings by Lempereur *et al.* (2010), who reported similar amplicon sizes for *Rhipicephalus (B) decoloratus*. Sequences of *Rhipicephalus (B) decoloratus* ticks obtained in this study were compared and identified with the sequences from the Genbank, and *Rhipicephalus (B) decoloratus* from Zangon Shanu and Angwan Fulani both had a percentage similarity of 96% to the reference sequence of *Rhipicephalus decoloratus* (U97716) deposited in the GenBank. This suggested that the

Rhipicephalus (B) decoloratus from the two sample sites could be regarded according to genotypic identification given that in the phylogenetic tree, the two species of *Rhipicephalus (B) decoloratus* fell in their respective monophyletic group, clustering together with a high bootstrap of 55%. The low percentage similarity value of 94% shown by *Rhipicephalus (B) decoloratus* from Bomo village to the reference *Rhipicephalus decoloratus* (U97716) deposited in the GenBank could be due to diverse evolution as a result of geographical separation (Taberlet *et al.,* 1997). The difference could be attributed to single nucleotide substitution between both sequences due to individual variation as a result of their wide range of distribution; and this observation is in agreement with other studies which showed that sequence divergence may be due to phylogeographical units in a given species (Avise and Walker, 1999). The sequence of *Rhipicephalus (B) decoloratus* from Bomo village clustered together with the reference with a bootstrap value of 65%. Such similarity value could be associated with the cryptic hybridization factor which according to Rees *et al*. (2003) results in nucleotide substitution.

CONCLUSION

This is the first study that confirms the presence of *Rhipicephalus (B) decoloratus* in Zaria by both morphological and molecular methods. The study also provides insight into the prevalence, and invasive nature of this tick species, and the usefulness of the ITS 2 gene for the identification of the *Rhipicephalus (B) decoloratus* tick.

Acknowledgements

The authors acknowledged the cooperation of the cattle owners, district heads, and contact persons for giving us attention and access to their animals. No funding received from government, organization or agency in the conduct of this study.

Conflicts of interest

The authors declare that there is no conflict of interest.

Contribution

Concept – R.D, R.M; Design – R.D; Supervision – G.B.D.J, L.I.A; Resources – R.D; Materials – R.D, R.M; Data Collection and/or Processing – R.D, R.M; Analysis and/or Interpretation – R.D; Literature Search – R.D, R.M; Writing Manuscript – R.M; Critical Review – R.M.

Ethical Approval Statement

All applicable international, national, and/or institutional guidelines for the collection of tick samples from the cattle were correctly followed. Informed verbal consent was sought from the district heads and cattle owners prior to the commencement of this study.

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