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Keywords: morphological, molecular, hard ticks, herds, cattle, zaria.

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Morphological and Molecular Identification of Hard Ticks (Acari: Ixodida) Infesting Herds of Cattle in Zaria, Northwestern Nigeria

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Abstract- Ticks are important vector arthropods of human and animal pathogens. Information on tick vectors as well as their distribution in Zaria and environs is very scanty. The study was aimed at identifying the various tick species infesting cattle in Zaria using a molecular technique. Ticks were collected from two hundred cattle (n=200) of which 193 (96.5%) were tick infested. The individual ticks were washed, crushed and incubated at 56°C overnight. The extracted DNA of each sample was extracted and amplified targeting the 16S rRNA portion. Ampl icons were sequenced using a capillary sequencer (ABI PRISMR 3130x1 Genetic Analyzer, Applied Biosystems). All sequences were subjected to a Basic Local Alignment Search Tool (BLAST) to determine their identities and assess their homologues and similarities to those in the Gen Bank. Morphological and genetic data of individual specimens gathered in this study provide relevant information for future studies on tick population dynamics in the Zaria. The outcome of this study suggests that Amblyomma variegatum 82 (7.6%), Rhipicephalus (Boophilus) decoloratus 158 (14.7%), Rhipicephalus (Boophilus) microplus 55 (5.1%), Rhipicephalus simus Group 699 (62.3%), Rhipicephalus sanguineus 14 (1.3%), and Hyalomma dromedarii96 (8.9%) are present on cattle in Zaria, northwestern Nigeria and pose a high risk of pathogens transmission and hence will contribute adversely in productivity losses to livestock owners.

Keywords: morphological, molecular, hard ticks, herds, cattle, zaria.

I. Introduction

Ticks are destructive blood sucking ectoparasites of livestock and wild animal species causing huge economic losses, thus creating food insecurity (Natala et al., 2009; Habeeb, 2010), with an estimated global cost of control and productivity losses of 7 billion US-Dollar annually (Nchu et al., 2012). Their effects are diverse, including reduced growth, milk production, paralysis/toxicosis, and transmission of tick-borne pathogens that reduce production or cause mortality, extensive damage to body surfaces exposing animals to secondary attacks from other parasites and microbial infections (Walker et al., 2003). Certain factors such as globalization and increased international trade, urbanization, climate change and increased travel and mobility of livestock have resulted in rapid extension of the zoo-geographical range for many tick species (Shaw et al., 2001; Kamani et al., 2010).

Morphological features are commonly used for the gross and microscopic identification of tick specimens; however, this method is not effective for damaged specimens, engorged female ticks, and the immature stages (Obadiah and Shekaro, 2012). To comprehend the epidemiology of tick-borne pathogens and develop effective strategies for controlling the diseases, accurate identification of the vector is very vital. Molecular analytical tools have proven valuable and complementary for overcoming this ineffectiveness associated with morphological identification of ticks and have been used to identify and differentiate tick species (Mohammed et al., 2016; Ogo et al., 2017).

There are currently little studies on the prevalence and epidemiology of ticks commonly affecting cattle production in Zaria, despite the fact that it is endowed with favorable weather condition suitable for the proliferation and multiplication of ticks as well as serving as a focal point of cattle concentration in the Northwestern Nigeria. Therefore, this necessitates the need for this study on the distribution of tick species, their morphological features as well as their genomic make up affecting cattle in Zaria, Kaduna State, Nigeria.

II. Materials and Methods

Study area: Zaria comprises of two Local Government Areas namely; Zaria and Sabon Gari. It is located between latitude 11°07' N and longitude 7°44' E within the Northern guinea savanna zone. By the existing pattern of settlement; it is made up of a natural and stable ecosystem in the Northern Guinea Savannah zone, with a discontinuous layer of sparsely distributed short trees followed by relatively continuous layers of tall, medium and short grasses (Jatau et al., 2012; Obadiah and Shekaro, 2012). Zaria is an old commercial, administrative and academic town in Northern Nigeria. The mean annual rainfall in the area is 1100 mm lasting from May to October (816 mm/month).

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Mean daily temperatures during the wet season are 25oC and mean relative humidity of 72%. The dry season lasts from November to April, the mean daily temperature ranging from 14 to 36oC and the relative humidity of 20-30% (Natala et al., 2009).

Study design: Ticks were collected from two (2) areas; Tofu district from Sabon Gari Local Government Area and Majeru district in Zaria Local Government Area. In each district, 10 herds were selected, and all visible adult ticks were collected from 10 randomly selected cattle varying in age and sex, all belonging to the indigenous (Bos indicus) white Fulani breed from 10 herds in each sampling area. Age of the animals were estimated on the basis of the dentition score method developed for zebu cattle under a low plane of nutrition (Kikule, 1953) and on information provided by their owners.



Source: Adopted and modified map from https://www.google.com/maps

Figure 1: Map of study areas/locations

Tick collection: The collection was performed using blunt steel forceps, by thorough examination of the entire body surface of two hundred cattle (n=200). Ticks were collected from different parts of the body including the neck/dewlap, eyes, ear, udder and external genitalia, inner thighs (ventrum), under the tail/perineum and legs/interdigital spaces by using forceps and hand gloves. Ticks from each animal were stored separately in vials containing 70% ethanol, labelled with information on the host (i.e., sample number, age, and sex), village, and date of sampling as well as the site (s) collected.

Ticks identification (morphological): The experiment was carried out in the Research and Teaching laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. All collected ticks were

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counted and identified to the genus and species level using a stereomicroscope (up to 100× magnification) and following the morphological key described by Walker et al., 2003. For those belonging to the genus Rhipicephalus, keys by (Walker et al., 2000; Madder et al., 2012) were also used.

Molecular identification: Generally, three ticks each from the same species were examined in one pooled sample. First, each tick was washed three times in sterile phosphate-buffered saline and then stored at −20°C. The frozen ticks were mechanically crushed using pestle and mortar and transferred into 1.5 ml Eppendorftube. Digestion with proteinase K (200 µg/ml; Roche, Mannheim, Germany) was performed by incubation at 56°C overnight.

DNA was isolated by phenol-chloroform extraction and followed by ethanol precipitation (Jain et al., 2017). DNA pellets was washed twice with ice-cold 70% ethanol, air dried, and re-suspended in 100 µl elution buffer. The efficiency of the nucleic acid extraction was evaluated by electrophoresis in 1.5% (w/v) agarose gels containing ethidium bromide and visualized under ultraviolet light. The concentration of each DNA sample was determined in a Nano Drop 2000c spectrophotometer (Thermo Scientific, San Jose, CA, USA) for quality and quantity. The DNA was then stored in -200C until further use.

PCR amplification: Tick species identification was carried out using universal primers TQ16S+1F and TQ16S-2R targeting the 323bp fragment of 16S rRNA portion of the DNA (Halos et al., 2004) with the following nucleotide sequences (5'-3') CTGCTCAATGAT TTTTTTAAATTGCTGTG and ACGCTGTTATCCCTAGAG as the forward and reverse primers respectively. Five µl DNA extract from each tick sample was amplified in a 24 µl reaction mixture containing; 13.65 µl nuclease free water, 2.5 µl of 10X PCR buffer, 1 µl of 50mM MgCl2, 0.5 µl of 10mM Deoxynucleoside Triphosphate (dNTP), 0.25 µl of DMSO, 1 µl each of the forward and reverse primers (Halos et al,. 2004), and 0.1 µl of Taq polymerase following conditions by the manufacturer. Amplicons were visualized with ethidium bromide after electrophoresis in 1.5% agarose gels. Bands were detected from the gel by viewing and capturing under UV light with the aid of a Gel documentation system. Expected amplicon size was 323bp.

Amplicon sequencing: Prior to sequencing, the PCR product was purified by loading it into 1% agarose gel. Gel was run in the electrophoresis tank till bands were neatly separated. Bands of interest were excised using a scapel blade under illumination for accuracy, with eye goggles worn to protect the eyes from the UV rays. Excised bands were kept in a 1.5 ml Eppendorf tubes. Sanger method was used to sequence the purified products. Amplicons were sequenced using a capillary sequencer (ABI PRISMR 3130x1 Genetic Analyzer, Applied Biosystems).

Phylogenetic analysis: BLAST search for previously reported sequences that are identical to the sequences in this study was done using the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST). Phylogenetic tree was constructed using the Molecular Evolutionary Genetic Analysis (MEGA 7.0) software program (Kumar et al., 2016). 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).

Submission to Gen Bank for Ascension Numbers: The sequences were assembled in notepad using Bankit method and then the corrected ticks' sequences were submitted in Gen Bank to obtain their ascension numbers.

Data Analysis: Data obtained were organized, edited and analyzed using statistical package for social sciences (SPSS) Version 20. Results generated from the investigation were expressed using descriptive statistics (mean ± standard error of mean, percentage, and graph).

III. Results

Tick species collected: In this study, out of 200 cattle screened 193 (96.5%) were tick infested. A total of 1074 ticks were collected and of three genera Amblyomma, Hyalomma, Rhipicephalus and including the sub-genus Rhipicephalus (Boophilus). Six species of ticks were identified: Amblyomma variegatum, Rhipicephalus (Boophilus) decoloratus, Rhipicephalus (Boophilus) microplus, Rhipicephalus simus Group, Rhipicephalus sanguineus, and Hyalomma impeltatum. The frequency of occurrence in the study showed that Rhipicephalus simus Group669 (62.3 %) was the commonest ticks observed in cattle in all the herds followed by Rhipicephalus (Boophilus) decoloratus, 158 (14.7%), Hyalomma impeltatum 96 (8.9 %), Amblyomma variegatum 82 (7.6%), Rhipicephalus (Boophilus) microplus 55 (5.1%) and Rhipicephalus sanguineus 14 (1.3 %) respectively. A relatively high number (n = 699) of adult Rhipicephalus simus Group ticks were collected from cattle of all age groups.

Tick distribution in relation to predilection sites: The distribution described in percentage (%) of tick infestation in different body parts of cattle examined revealed that; tail (46.5%), ventrum (17.9%), neck/dewlap (14.1%), udder/scrotum (9.5%), prepuce/perineum (8.2%), head (1.9%), legs/interdigital space (1.1%) and dorsum (0.8%). It showed that the tail region/area (46.5%) is the most infested followed by the ventrum (17.9%), the neck (14.1%), while the dorsum (0.8%) is the least infested.

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Molecular identification of tick species: DNA was isolated from five (5) tick samples (as in materials and methods), three (3) from each species of ticks. Following amplification of the 16S rRNA and gel electrophoresis of the PCR products, all the five (5) tick species were positive and yielded products of approximately 323 bp. (see plate 1.0).

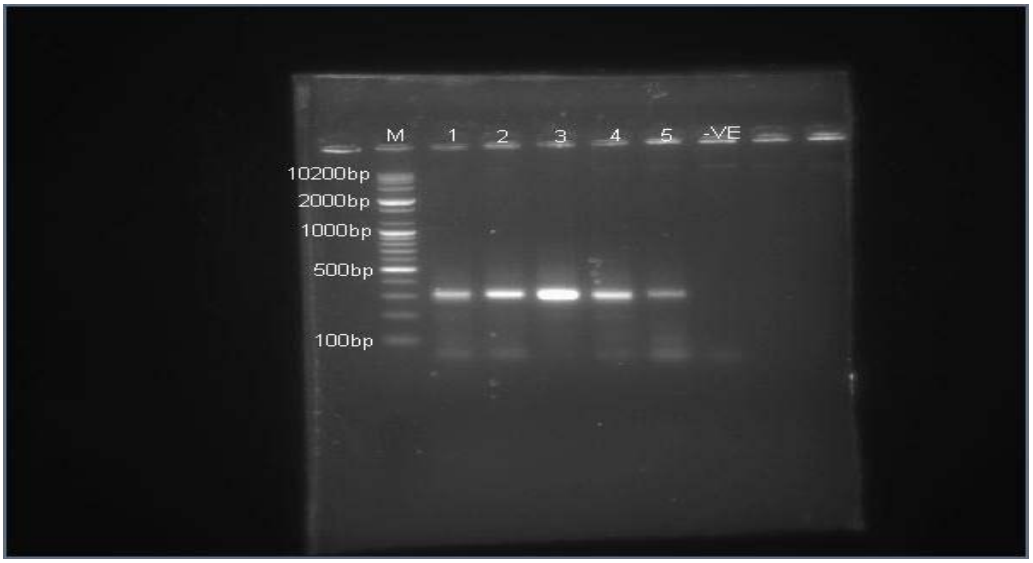

Figure 1: Gel image for amplified DNA of ticks

Figure 1.0: DNA extractions from five different tick species. Lanes: M; 100 bp ladder, 1. Amblyomma variegatum, 2. Rhipicephalus decoloratus, 3. Rhipicephalus microplus, 4. Hyalomma impeltatum 5. Rhipicephalus simus Group 6. Non-template control (NTC). Tick 16S rRNA gene, 1.5% agarose gel run for 35 mins at 65 V.

Gen Bank Ascension Numbers: In this study, we molecularly established the status of Amblyomma variegatum (MN044771), Rhipicephalus (Boophilus) decoloratus (MN044772), Rhipicephalus (Boophilus) microplus (MN044773), Hyalomma dromedarii (MN044774) and Rhipicephalus simus Group (MN044775) speciesticks from Zaria, Kaduna State, Nigeria, based on the partial sequence of 16S rRNA gene.

Phylogenetic tree for ticks collected on cattle: The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 2.46834081 is shown. (Next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 14763 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

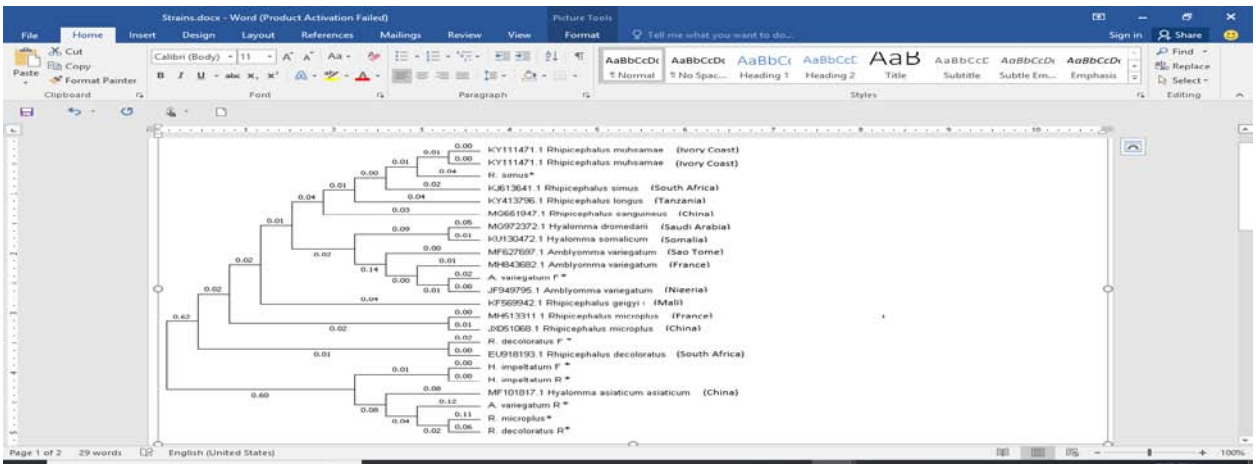

Figure 2: Phylogenetic tree for ticks. (Note: * indicate the isolates analyzed in this work)

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IV. Discussion

Of the three taxa currently ascribed to the 'simus Group', only Rhipicephalus muhsamae (Morel and Vassiliades, 1965) was expected to be present in West Africa. However, in addition to the Group-specific punctuation pattern visible on the males' conscutum, a number of morphological features (e.g., female genital aperture and shape of adanal plates) of the specimens collected in this study appeared closely related to the East African taxon Rhipicephalus praetextatus (Unsworth, 1952). It was assumed these were the same specimens retrieved in the 1950s from several localities in central and northern Nigeria, identified as Rhipicephalus simus muhsamae 3.83% and 4.0% respectively (Mohammed, 1977; Lorusso et al., 2013). Usually found in regions with a savanna climate, the distribution of Rhipicephalus simus (or Rhipicephalus. simus simus) is believed to be restricted to southern Africa (Pegram et al., 1981), where the adults preferentially parasitize cattle, never reaching high loads (Lorusso et al., 2013). These findings are in contrast to studies carried out by Joseph et al., (2014) were he recorded Rhipicephalus (Boophilus) microplus (49.2%), Amblyomma variegatum (40.89%), Rhipicephalus (Boophilus) annulatus (7.39%) and Amblyomma maculatum (2.46%) respectively. Biu et al., (2012) recorded tick species infesting ruminants in University of Maiduguri, Nigeria and reported an overall high prevalence of 64% with 39 (68.01%) for cattle followed by 13 (20.13%) for sheep and 12 (18.75%) for goat. In their study, Rhipicephalus (Boophilus) species was observed to be most predominant with a prevalence of 56.1% followed by Hyalomma species (43.9%). Olabode et al., (2010) in a study of occurrence, species composition and economic impact of tick in Buruku market Jos-Plateau, Nigeria, observed that 12.5% of cattle were infested by ticks of which Rhipicephalus (Boophilus) spp were most prevalent with 7.5% followed by Amblyomma spp (4.5%) and Hyalomma spp (3.0%). Obadiah and Shekaro (2012) reported four species of ticks from Zaria, Nigeria and showed that Rhipicephalus (Boophilus) decoloratus was predominant with prevalence rate of (22.5%) followed by Amblyomma variegatum (17.7%), Hyalomma spp (6.7%) and Rhipicephalus sanguineus (3.3%). The differences in the results of the present and earlier studies might be due to variation in the geographical locations, climatic conditions of the experimental areas, region and method of study as well as sample size selection.

The distribution (%) of tick infestation in different body parts of cattle examined revealed that tail (46.5%), ventrum (17.9%) and neck (14.1%) were the most tick – infested sites in the body of examined animals and the dorsum (0.8%) was the least part infested. This further confirms that ticks prefer to attach and feed on some parts of the body of animals. This finding is not in agreement with the work by Jajere et al., (2014) that recorded prevalence in predilection site as follows; udder and external genitalia (83.4%), inner thighs (79%), under the tail/perineum (69.8%), eyes (26.3%), neck and dewlap (14.6%) and ears (12.2%) respectively. Joseph et al., (2014) in his work suggested that ticks are widely distributed in different parts of the host body such as armpit, inner thigh, penis, udder, mammary gland, scrotum and vulva, of which inner thigh (26.66%) was most infected, while vulva (10.80%) was the least infected animal body part. These findings could be attributed to the fact that external genitals, perineum and inguinal/groin region of the body are highly supplied with blood and ticks usually prefer thinner and short hair skin for infestation. This helps in easy penetration of mouthparts of ticks into richly vascular area for feeding (Sajid, 2007). The higher number of Rhipicephalus simus Group (46.5%) recorded in the present study could be mainly attributed to the practice of hand-picking of ticks by the Fulanis, carried out up to three times a week during the wet season (Pullan et al., 1980; Lorusso et al., 2013). This control method mainly targets the most conspicuous Amblyomma adults, regarded as 'koti' (i.e., 'dangerous ticks' in Fulfulde language), by the local herdsmen, as opposed to the smaller Rhipicephalus and boophilid ticks that are consciously left attached, as they are believed to be 'miri' (i.e., 'less harmful') (Bayer and Maina, 1984; Lorusso et al., 2013).

The sequence of the amplified 16S rRNA gene fragment of Amblyomma variegatum was 99% identical from specimen from Nigeria JF949795.1 from ticks collected in Jos (Ogoh et al., 2012). And it shows 100% similarity from the sequence obtained from Sao Tome (MF627697.1) and 99% identical to MH843682.1 from France. The amplified 16S rRNA gene fragment of Hyalomma impeltatum revealed 92% identical to Hyalomma asiaticum asiaticum MF101817.1 from China. The sequence of the amplified 16S rRNA gene fragment of Rhipicephalus simus Group was 99% identical from specimen from Rhipicephalus muhsamae (KY111471.1) from Ivory Coast, and 98% similarity with Rhipicephalus simus (KJ613641.1) from South Africa. Also, the sequence of the amplified 16S rRNA gene fragment of Rhipicephalus (Boophilus) decoloratus was 98% identical from specimen from Rhipicephalus (Boophilus) decoloratus (EU918193.1) from South Africa. The sequence of the amplified 16S rRNA gene fragment of Rhipicephalus (Boophilus) microplus was 100% identical from specimen from Rhipicephalus (Boophilus) microplus (MH513311.1) from France and 99% identical from Rhipicephalus (Boophilus) microplus (JX051068.1) from China.

V. Conclusion

The demonstration of the presence of high number of Rhipicephalus simus Group (62.3%) from this

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study poses a great danger to the environment as well as the cattle owners because of its affinity to rodent hosts and its ability to transmit anaplasmosis to cattle and humans. The presence of Rhipicephalus (Boophilus) microplus (5.1%) from this study is alarming because of their high fecundity which enable them to spread and establish fast in the environment. Also, Rhipicephalus (Boophilus) microplus are known with their acaricide resistance and hence lead to environment contamination due to abuse of acaricides by the pastoralists which leaves residues in the environment that may be toxic to other organisms. This makes transmission of ticks and tick-borne pathogens very easy and a serious problem to combat in Nigeria today.

VI. Conflicts of Interest

The authors unanimously confirm that there are no known conflicts of interest associated with this publication.

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