

Amblyomma Variegatum as Bioindicators for the Presence Of Trypanosoma Congolense In Semi-Extensively Managed White Fulani Cattle In Kaduna Metropolis, Kaduna State, Nigeria

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Abstract: The presence of trypanosome parasites in ixodidae ticks of cattle naturally infected with trypanosomiasis in semi-extensively managed herd of white Fulani cattle was studied. The ticks were naturally attached to the cattle. Each of the cattle harbours about 120 ticks with one tick marked for this study on each of the following regions; sternal region, peri-anal region and under the tail. Three ticks from each of the eleven cattle were examined. *Amblyomma variegatum* was the only specie of ticks found. On the first day of the parasitological analysis, 14 (42.4%) of the ticks were found to be positive for *Trypanosoma congolense* (the only specie found). The *Amblyomma variegatum* ticks which infest these cattle are of veterinary importance, because they may be mechanical vectors of the economically important blood protozoan parasites. These ticks can also be used as a source of monitoring and thus enabling strategic planning, more effective in combating the diseases caused by the haemo-parasites.

Keywords: *Amblyomma variegatum*, Ixodidae, peri-anal, sternal and trypanosomiasis,

I. Introduction

Trypanosomiasis is a debilitating protozoan disease caused by parasites classified in the Phylum Sarcomastigophora, Order Kinetoplastida, family Trypanosomatidae and of the Genus *Trypanosoma* (1). The trypanosome species of veterinary and medical importance had been described (2, 1).

Animal trypanosomiasis, an important protozoan disease caused by the genus *Trypanosoma* is transmitted through bites by different species of *Glossina* and mechanically by a number of biting flies such as *Tabanus* and *Stomoxys* sp. (4). It is characterized by intermittent fever, parasitaemia, anaemia, lymphadenopathy, jaundice, progressive emaciation, weakness, and reduced productivity. It is a disease complex caused by one or more pathogenic species of trypanosomes such as *Trypanosoma vivax*, *Trypanosoma congolense*, and *Trypanosoma brucei brucei*.

In Africa, tsetse transmitted trypanosomiasis is endemic in human and livestock populations distributed in an estimated 10 million km² of land space (19), corresponding to the geographical distribution of the *Glossina* vector. This covers the tropical area extending from latitude 15⁰N to 30⁰S of the equator (11, 20). It is estimated that at least 50 million people as well as 30% of cattle (19, 22) are at risk to African trypanosomiasis. Africa, as estimated by FAO, loses over 3 million cattle and other domestic livestock through deaths caused by trypanosomiasis every year (10).

Trypanosomes replicate in haematophagous tsetse flies, primarily *Glossina morsitans*, *G. palpalis*, and *G. fusca*. When an infected fly bites an animal, the parasites are transmitted in the saliva. Trypanosomes can also be spread by fomites and mechanical vectors: including surgical instruments, needles, syringes, and biting flies (3). Mechanical transmission of pathogens by biting insects is a non-specific phenomenon in which pathogens are transmitted from the blood of an infected host to another host during interrupted feeding of the insects (8).

In mechanical transmission, vector mouthparts serve as contaminated hypodermic needles since there is no replication or development of the microparasite in the vector; the vector does not serve as an alternate host as in the case of biologically transmitted diseases (6). For infectious organisms to be mechanically transmitted by arthropods efficiently, they must be abundant in circulating blood or cutaneous tissues and able to survive external exposure. Diseases that are mechanically transmitted by arthropods generally have other transmission mechanisms as well (7).

The economic impact of tick infestations is enormous worldwide. In 1984, the United Nation Food and Agriculture Organization (10) estimated the global cost of Ixodidae tick infestation to be US\$7.0 billion annually. Apart from trypanosomiasis and other disease agents, ticks also affect the production of animals; they are obligatory blood-sucking arachnid arthropods infecting mammals, birds, reptiles and amphibians. They are vectors of disease agents causing anaemia, dermatitis, paralysis, otocariasis, as well as loss of production (14). The present study aimed to provide preliminary data checking for the presence of Trypanosome specie in engorged ticks from cattle in a natural setting.

II. Materials And Methods

2.1 Study Area

The study was conducted in Unguwan Rimi area situated at latitude 10°32'16.41"N and longitude 7°28'19.99"E in Kaduna state, north-western Nigeria.

2.2 Experimental Animals (Ticks)

The Ticks were attached naturally to the cattle, with each animal harbouring about 120 ticks at different metamorphological stages. The preferred sites of attachment for ticks among cattle examined are dorsum, tail, under the tail, peri-anal region, inner thigh, scrotum for the males, legs, abdomen, vulva and udder in the females, sternal region, dewlap, and ears. Two (2) ticks were carefully hand-picked from each of the cattle. They were taken to the veterinary entomology laboratory of Ahmadu Bello University Zaria, for identification. Three (3) engorged adult ticks (1 each from sternal region, peri-anal region and under the tail) were marked on each of the animals for this study, which makes a total of 33 ticks. The ticks were labelled as follows, sternal region as A, peri-anal region as B and under the tail as C.

2.3 Ticks Identification (External Morphology)

All ticks were stored in 70° ethanol. Once in the laboratory, all collected ticks were counted and identified to the genus and species level using a stereomicroscope (up to 100 × magnifications) and following the morphological keys in (21). These features were presented as documented in the text by (21). The live ticks were placed in a petri dish containing 10% alcohol using forceps and they were examined with low-power dissecting microscope (Nikon, Made in Japan). The identification was done using the key of morphological characters as described by (21).

The external morphology of the four genuses of ticks were examined and identified based on the descriptive terminology of (21). The body had four pairs of legs which were segmented (coxa, trochanter, femur, genu, tibia and tarsus) and terminated in a pair of claws. The ticks were observed to have the capitulum (where the head and mouthparts are located) exposed and easily visible from the top. The upper side of their body bears a distinctly sclerotized shield or scutum. This structure covered most of the upper body surface in the male tick, but was restricted to a much smaller area (immediately behind the capitulum) in the female.

The engorged female had her abdomen increased to many times its normal dimensions and the scutum appeared to be extremely small in relation to the body size. Male ticks were not so large when engorged like the female. *Amblyomma variegatum* was the only specie of ticks found.

2.4 Parasitological Analysis

Each of the 3 marked ticks was pricked using 18G sterile needle, blood was collected with a heparinized capillary tube by applying mild pressure on the abdominal region of the tick and blood allowed to rise freely into the tube up to 3/4th of the length and sealed with crystal seal. The collected blood was then centrifuged for about 5 minutes with 10,000 rpm (revolutions per minute).

Parasitological examination was done at the field using the haematocrit centrifugation technique HCT (13) and Buffy coat method BCM (16). The packed cell volume of each animal was also determined (as haematological index for anaemic conditions) through capillary centrifugation of blood with centrifuge and haematocrit reader. Trypanosome parasites were identified based on their motility using BCM and morphological features. Samples that appear negative under microscopic examination were subjected to molecular analysis.

2.5 Molecular Analysis

In furtherance to the field analysis, two aliquots of each blood sample were transferred in nunc^R cryotubes and taken to DNA LABS Limited Kaduna for molecular analysis. Genomic DNA for the screening of trypanosomes was extracted from the blood samples using the salt out method. The technique in brief is stated below: 500 µl of each blood sample were washed four times. Then, 500 µl of the washing buffer, 100µl of cell lysis buffer, 10 µl of SDS 10% and 10 µl of Proteinase K (concentration 10 mg/ml) were added to pellet and incubated at 56°C for 30 minutes. Afterwards, DNA was precipitated using ethanol 70%. Pellets were air-dried

for about 4 hours, and DNA was re-suspended in 75 ml of Tris-EDTA (TE) buffer and stored at -20°C until it use for Polymerase Chain Reaction (PCR).

Amplification of trypanosome DNA was conducted using species-specific primer pairs for *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* sl. The amplifications were conducted in a total volume of 50 µl containing 5 µl of PCR buffer 10× (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 3 mM MgCl₂), 15 picomoles of each primer, 200 µM of each of the four deoxynucleotide-triphosphate (dNTP), one unit of Taq DNA polymerase (Appligene-Oncor, USA), sterile water and 5 µl DNA extract. Amplification involved pre-denaturation at 94°C for 3 mins followed by 30 cycles of denaturation at 94°C for 1 min, hybridization of primers at 60°C and elongation at 72°C for 1 minute, then final elongation at 72°C for 15 min. PCR products were separated by electrophoreses on a 2% (w/v) agarose gel for 30 min at 100 V. The gel was stained in an ethidium bromide solution for 10 min and visualized under UV light.

III Results

Trypanosome parasites were detected in the engorged adult *Amblyomma variegatum* observed. Of the 33 samples examined, 14 samples were found to be positive with *Trypanosoma congolense* which represents a prevalence rate of 42.4%. At least one (1) tick was positive on each of the 7 cattle that were positive with Trypanosome parasite. On the first day of parasitaemic analysis, it was discovered that 10 (30.3%) samples were microscopically positive for *Trypanosoma congolense* and 23 (69.7%) of the samples were negative. However, when subjected to PCR, 4 (12.1%) more samples were found to be positive with the same specie of trypanosome parasite.

The table below shows the status of each marked tick in terms of trypanosome parasite

Cattle no.	tag	Sex	Labelled Ticks		
			A	B	C
1		F	-ve	+ve(PCR)	+ve
2		F	-ve	-ve	-ve
3		F	+ve	+ve	-ve
4		F	+ve (PCR)	-ve	+ve
5		F	+ve	+ve	+ve
6		F	-ve	-ve	+ve (PCR)
7		F	+ve (PCR)	+ve	-ve
8		F	-ve	-ve	-ve
9		F	-ve	-ve	-ve
10		F	-ve	-ve	-ve
11		M	-ve	+ve	+ve

Key: A= Ticks in the sternal region, B= peri-anal region, C= under the tail, F= Female, M= Male, -ve = Negative for trypanosomes, +ve = positive for trypanosomes and PCR = Polymerase Chain Reaction.

IV Discussion

In this study, all the cattle were found infested by ticks with a low mean count of 120 ticks. *Amblyomma variegatum* was the only specie found. The report of the infestation of cattle by a single species of tick is rare. *Amblyomma variegatum* has already been found as the dominating species of ticks among others species poorly represented in Ngaoundéré (9); in Mali, the dominance of this species was reported by (18, 15). To explain the exclusive prevalence of *A. variegatum* in this area we suggest the following reason: The repetitive application of acaricide on cattle at a short interval period among pastoralists, in association with hand picking of ticks, observed in the study area, may have particularly reduced the chance to find other species. This last point underlines, however, the risk for the development of resistance to Cypermethrin 10% which will results in increase of tick-borne diseases and, the likelihood of an effective rise of cost-benefits ratio as mentioned by (15).

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The dominance of *T. congolense* among trypanosome species is in accordance with (17) who carried out the same study in northern Cameroon; it suggests that the transmission of trypanosomiasis is determined by the biological fly, tsetse fly. This fly has been reported in the region of Kachia (where these animals were brought from) by (2).

Interestingly, there are no documented works carried out in Nigeria in this aspect using such methodology of blood collection from the *Amblyomma variegatum* ticks.

V Conclusion

Amblyomma variegatum is the predominant specie of ticks found around the sub-saharan Africa, apart from its associated diseases, the tick may also serve as mechanical vector and bio-indicators for trypanosome parasites as determined from this study.

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