IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 7 Ver. III (July. 2015), PP 43-48
www.iosrjournals.org

Surveillance for Tsetse and Trypanosomosis in Bagudo Local Government Area North-Western Nigeria

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Abstract: *Tsetse-transmitted trypanosomosis is still a major impediment to livestock productivity in many parts of sub-Saharan African countries, Nigeria inclusive. However, the prevalence of the disease in Bagudo Local Government Area in Northwestern Nigeria is unknown, despite several claims, by Fulani herdsmen, of its existence in the region. Thus, a short survey was conducted to investigate the presence of Trypanosome species using microscopic examination and molecular technique by Polymerase Chain Reaction (PCR) using ITS-1 generic primers. Microscopic examination showed only 1out of 118 blood samples was infected with T. vivax, representing 0.8% prevalence. However, PCR revealed 11 samples were infected with different trypanosome species (T. vivax (10%), T. godfreyi (10%), and either of T. simiae tsavo or T. simiae or T. grayi (70%), representing 9.3% prevalence. One sample was a mixed infection of T. vivax / T. simiae tsavo or T.simiae or T. grayi / T. congolense kilifi. All the animals were anaemic with packed cell volume values of 20.3% ± 2.9 – 22.7% ± 4.4. Also, 6 tsetse flies were caught using biconical traps deployed along riverine vegetation and morphologically identified as Glossina palpalis palpalis and Glossina tachinoides. Microscopic dissection of midgut, proboscis and salivary gland detected no trypanosomes, but polymerase chain reaction detected 1 fly was infected with a T. vivax. Since Bagudo LGA is ideally suited for livestock production but has high tendency of trypanosomiasis due tsetse infestation, there is therefore a need for general surveillance of the entire northwestern Nigeria for integrated control of the disease and its vector.*

Keywords*: Microscopy, Polymerase chain reaction, Detection, Trypanosomes, Bagudo LGA.*

I. Introduction

African trypanosomes, transmitted by tsetse fly, are the causative agents of African trypanosomosis, a disease that for centuries has thwarted livestock productivity and human development in sub-Saharan Africa. The economic impacts on livestock and agriculture are colossal as tsetse fly is distributed over a wide range of habitats covering about 10 million square kilometers of potential grazing lands in 38 countries which are thus, rendered unsuitable for livestock breeding and farming (1). In Nigeria alone, some 14 million cattle, 22 million sheep and 34 million goats are at risk of infection (2).

Successful control measures against tsetse and trypanosomosis were taken during colonial administrations. Unfortunately, the success achieved was not sustained largely due to infrastructural collapse, political instability, conflicts, negligence, etc. These reasons and perhaps other factors are partly responsible for the re-appearance of tsetse fly in previously reclaimed areas in many countries, including Nigeria.

Bagudo Local Government Area (LGA), located in Kebbi State (formerly part of old Sokoto State), North-Western Nigeria, bordering Benin Republic, and drained by River Niger and some of its tributaries, is home to over a million head of cattle due to vast wetlands which provide grazing lands throughout the year. The prevalence of African animal trypanosomosis (AAT) and apparent density of tsetse flies in the area is unknown. However, there are recent claims by Fulani herdsmen of appearance of the vector at different locations in the area. Therefore, this study was carried out to investigate the presence of trypanosomal infections and tsetse fly in the area.

II. Methods

2.1) Study Site:

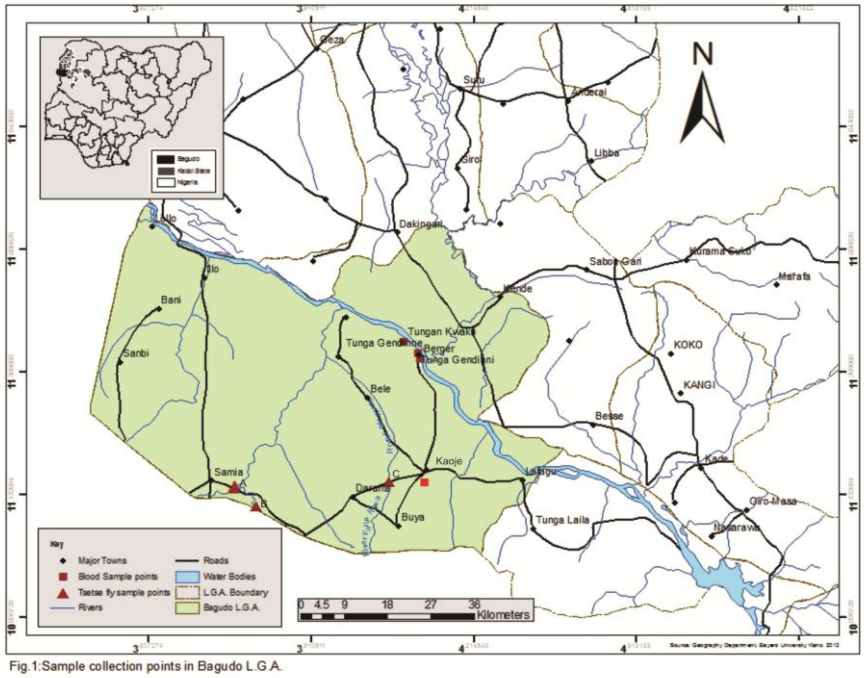

Fig.1:Sample collection points in Bagudo L.G.A.

The study was carried out in Bagudo LGA of Kebbi state, Northwestern Nigeria. The areas covered include Kaoje, Bega, Gindane Kwaku and Joga Ehbo villages in Kaoje district. Other areas sampled are Farin Ruwa and Surumpu grazing reserve, and Tungar Bature in Kaoje and Illo districts, respectively. These areas fall within guinea savannah with sparse forest vegetation mainly due to human activities. Oral consent of the cattle owners' was sought prior to sampling after the study protocol was clearly explained to them (figure 1).

2.2) Epidemiological Survey and Sampling Methods:

A representative random sample (3) of 118 cattle were selected from the above stated districts, that fall within guinea savana. From each animal, about 5ml of blood were collected from the jugular vein in a heparinised tube, and brought back to the field camp which was in the immediate vicinity. Two samples were prepared from each blood samples collected. The first was blood sample in heparinized capillary tube which was used to measure haematocrit and to detect trypanosomes by wet preparation and examination of the buffy-coat using dark-ground microscopy (4). The second samples involved careful spotting about 0.5 ml of each blood sample onto roundly–cut Whatman filter paper (150 mm) and allowed to dry separately at room temperature before preserving it in a transparent plastic container with silica gel (desiccant) and stored at 4^0C for detection of DNA by PCR.

2.3) Tsetse fly Trapping and Dissection:

Seven biconical traps were set for 72 hrs to catch tsetse flies in suitable riparian/riverine vegetation and cattle drinking/water crossing points. The traps were placed at least 100m apart and the position of each trap was recorded on a global position system monitor (e-trex). Flies were harvested every morning and later dissected in 0.9% saline solution on a glass slide under a dissecting microscope. The proboscis, salivary gland and midgut were removed separately and examined under a light microscope for presence of trypanosomes on same day of collection (5).

2.4) DNA Extraction and Amplification:

DNA was extracted from the tsetse fly and dried blood spotted on Whatman filter using AccuPrep^R Genomic DNA Extraction kit (Bioneer). The whole fly was homogenized using pestle and mortar, and transferred into a 1.5 ml tube. For dried blood, four pieces of 2 mm discs were punched out from the blood spotted region of the filter, and placed in a 1.5 ml tube. Thereafter, the manufacturer's instructions were followed for the extraction. Briefly, lysis buffer (400 µl) and Proteinase K (40 µl), were added to the tube

containing the blood spotted filter paper and incubated at 60^0C for 1 hr. The lysate was then transferred to a binding column after addition of binding buffer (200 µl), precipitated with Isopropanol (100 µl), centrifuged at 8000 rpm for 1 min, and then filtered. This was followed by double washing and finally eluted to obtain the DNA samples. The DNA samples were used immediately.

Standard PCR amplifications and electrophoresis were carried out using the generic primers (external primers: OSK1425 reverse and OSK1426 forward; internal primers: OSK1424 reverse and OSK1423 forward) designed and used by Adams et al, (6) to amplify the ITS-1 region of rRNA. Nested PCR involving two consecutive rounds of standard PCR was used; the first with external primers and the second with internal primers. The PCR amplified products (15 µl) were resolved on a 1.5% agarose gel stained with 0.5 µg/ml ethidium bromide, and photographed under ultraviolet illumination and interpreted according to Adams et al,(6). .

III. Results

3.1) Parasitological and Vector Morphology

Out of one hundred and eighteen cattle that were bled only one was parasitologically positive, representing a prevalence of 0.8%. All animals sampled were anaemic with PCV values ranging between 20.3% ± 2.9 - 22.7% ± 4.4 (Table 1).

Table 1: Blood sampling locations and PCV

Location	Number of samples	Average PCV (%)
Kaoje	19	22.7 ± 4.4
Bega village	35	20.3 ± 2.9
Joga Ehbo	11	22.1 ± 3.1
Gindane Kwaku	52	21.1 ± 3.2

Six non-teneral tsetse flies were caught (one alive and five dead). The flies were identified morphologically to be G. p. palpalis and G. tachinoides according to the described methods (7, 8). Dissection and light microscopy of the tsetse fly caught alive showed no infection. Other three biting flies caught were identified as Tabanus, Stomoxys, and Haematopota (Table 2).

Table 2: Tsetse sampling locations and catch

Location	G.p.palpalis	G.tachinoides	Other biting flies
Farin Ruwa	0	0	0
Surumpu	0	0	1
Tungar Bature	3	3	6

Note: The numeral indicates the number of flies caught at each location

3.2) PCR Results:

The overall prevalence obtained on the use of PCR method was 9.3% while that of tsetse fly was 16.7% infection rate. In total, ten blood samples were infected with T. vivax, T. godfreyi and either T. simiae or T. simiae tsavo or T grayi, while one sample was mixed infection of T. vivax/T. simiae tsavo or T. simiae or T. grayi/T. congolense kilifi, while the tsetse fly was infected with T. vivax.

Molecular detection revealed different trypanosome species as shown in Table 3. Each species produced a single band of about 190 – 560 bp in length, which is in agreement with previous studies (6). However, some bands seems to have the same ITS-1 region size of 380 bp length which can be either T. simiae tsavo, or T. simiae, or T. grayi.

Table 3: Blood samples collection sites, PCR amplification band sizes, and Predicted Trypanosome species.

Sample Collection Sites	No of Samples	No of Infected Samples	ITS-1 band size obtained (bp)	Predicted Trypanosome spp
Kaoje	20	6	190, 380, 560[x]	T. vivax/T. simiae or T. simiae tsavo or T. grayi/T. congolense kilifi
			380	T. simiae or T. simiae tsavo or T. grayi
			380	T. simiae or T. simiae tsavo or T. grayi
			380	T. simiae or T. simiae tsavo or T. grayi
			380	T. simiae or T. simiae tsavo or T. grayi
			380	T. simiae or T. simiae tsavo or T. grayi
Bega	35	4	240	T. godfreyi
			380	T. simiae or T. simiae tsavo or T. grayi
			380	T. simiae or T. simiae tsavo or T. grayiT.
			380	simiae or T. simiae tsavo or T. grayi, T. vivax
Joga Ehbo	11	0		
Gindane Kwakwu	52	1	190	
Total	118	11		

Key: [x] is mixed infection

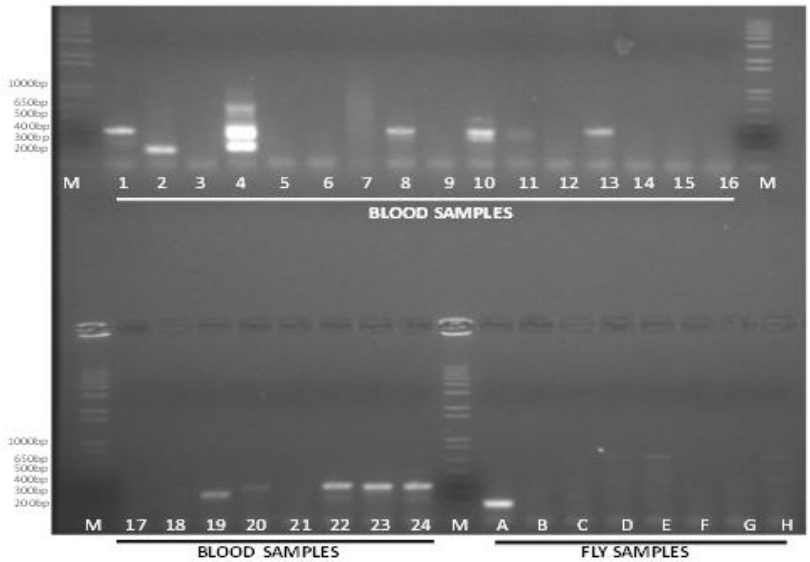

Figure 2: Trypanosomes detection by Nested PCR

Lane 1, 8, 10, 13, 22, 23 and 24 were T.b.brucei (300 bp DNA size). Lane 2 is T.vivax (200 bp DNA size). Lane 19 is T.simiae (260 bp DNA size). Lane 20 is T.simiae tsavo (280 bp DNA size). Lane 4 is a mixed infection of T.vivax (200 bp DNA size), T.b.brucei (300 bp DNA size) and T.congolense savannah (600 bp DNA size). A – E tsetse flies; F – H other biting flies; M DNA ladder.

IV. Discussion

The present study has demonstrated the presence of trypanosomosis and its vector tsetse fly in Bagudo LGA. This is the first of such report in the area as previous studies were essentially carried out in other parts of old Sokoto State which mainly paid more attention to animals brought to abattoirs for slaughter rather than animals in the field and the vector, tsetse fly.

Different trypanosome species were detected in the study area; T. vivax, T. congolense kilifi,

T. godfreyi and either of T. simiae or T. simiae tsavo or T. grayi with the later responsible for 70% of the infections. This indicates high diversity of trypanosomal infections in the area. Previous studies in neighboring areas have mostly reported T. vivax and T. evansi as the predominant trypanosome species causing infection in cattle, goats and camels. Studies by Fajinmi et al, (9) reported infection due to T. vivax was 66.7% in nine positive cases out of five hundred cattle blood samples collected in Sokoto central abattoir. The appearance of new trypanosome species as seen here could be due to invasion and international border crossing into the area by wild animals and/or Fulani herdsmen with their cattle and other domestic animals. In fact, at Kaoje village, the team met a Fulani settlement whose inhabitants came from Burkina Faso few years ago. Similarly, there is neither documented nor any previous case of T. godfreyi, T. simiae or T. simiae tsavo in the area. Thus, this study may represent the first report of these species in Bagudo LGA. These three species are predominantly found in suids, with T. simiae tsavo, first detected in tsetse fly in Tsavo National Park and later confirmed as a sub group of T. simiae been found to only experimentally infect pigs and warthogs (10). In Bagudo LGA, pigs are rare due to cultural and religious reasons but nevertheless, warthogs were said to prosper in the area in the past but their population have reduced due to human activities. The suids-associated trypanosomes (T. godfreyi, T. simiae, T. simiae tsavo and newly discovered T. godfreyi-like) are likely to be found together in mixed infections, although this may not necessary be so as reported previously (6). In present study, a mixed infection of T. vivax/T. simiae tsavo or T. simiae or T. grayi/T. congolense kilifi was detected. Mixed infections of different trypanosome species have been extensively reported (6, 11). The possible explanations for the mixed infection may be that tsetse flies feed on same-kind or different-kind of suids, in addition to also taking blood meal from another completely different animal species such as cattle. Put together, the findings of diverse trypanosomal infections in cattle, and infected tsetse fly, may be an indication of an active on-going disease transmission in the area that requires an extensive study in order to fully understand the transmission mechanisms and develop integrated control and intervention measures.

The microscopic method detected only one sample (0.8%) to be infected. However, the molecular detection method by PCR using ITS-1 generic primers detected infection in eleven samples (9.8%). This has once again highlighted the inadequacy, ineffectiveness and unreliability of the traditional methods – dissection and microscopy – of trypanosomes detection. The PCR based method was able to discriminate three different trypanosome species in a single mixed infection with three clear bands of different sizes. While the ITS-1 generic primers were able to detect and identify different trypanosome species as seen here and also reported by others (12, 6, 13), it is still not able to discriminate species that have very close or same ITS-1 region size length. In addition, amplification of unwanted DNA fragment is likely and thus, giving rise to false positive results. It is therefore necessary to further use species-specific primers, fluorescent fragment length barcording (FFLB) or sequencing for absolute discrimination between trypanosome species having same or close ITS-1 region size. It is noteworthy, however, that the traditional microscopic parasite detection methods are still important and remain of primary use especially in dealing with large number of samples in the field.

Most of the cattle appeared anaemic with low PCV values. Anaemia is a cardinal symptom of animal trypanosomosis resulting from hemodilution, erythrophagocytosis (14), the activity of haemolysins phospholipase A_2 (15) and sialidase (16), and oxidative stress (17). Poor nutrition could be another factor as the cattle have just come out from the long dry season.

Although the number of tsetse flies caught was low, it is still important considering this is the first of such report in the area and the fact that the study was conducted at the beginning of rainy season which is characterized with high tsetse fly dispersal (18). Direct correlation exists between trypanosomosis prevalence in dry and wet seasons and tsetse populations and distribution in Nigeria. The dry season is associated with low humidity and high temperatures, a condition that reduces fecundity and increases mortality in the tsetse species Glossina tachinoides and Glossina palpalis palpalis. It also limits tsetse dispersal within the environment and reduces transmission of AAT; by contrast, climatic conditions in the wet season support increased tsetse populations, greater tsetse dispersal and increased transmission of AAT (19). Another possible reason for low number of tsetse flies caught could be due to the fact that the study area is interspersed with crop farms which

limit tsetse population (20). Notably, two different species of tsetse fly caught in the area - G. p. palpalis and G. tachinoides - are both riverine species and members of the palpalis group. The occurrence of one to three different species in a particular area is found in over 86% of the tsetse belt (20) and the study area falls within the G. p. palpalis and G. tachinoides distribution limits in Nigeria. The presence of these two species should necessitate action because of the species adaptability to anthropogenic vegetation changes (22) and the role in transmission of human infective trypanosomes in Nigeria (23). These two aspects coupled with infection rate of 16.7% call for an extensive, seasonal and long term study of the entire area.

Usually, blood and tsetse fly samples for DNA extraction and molecular analysis are preserved using ethanol, FTA cards or RNA later solution, respectively. In the present work, it was demonstrated that tsetse and blood dried on Whatman filter paper could be well preserved in silica gel for up to 4 months. This could therefore be an alternative reliable, cheaper and easier method.

V. Conclusion

The fact that the infection rate in this area could be higher when other more sensitive diagnostic techniques are used and seasonal variations are considered raises the alarm that a concerted and consistent war should be waged against the disease and the vector. Therefore, there is a need to embark on trypanosomosis surveillance and control program for this area and others with similar vegetation of the country.

VI. Acknowledgement

The authors express their profound gratitude to Prof M. Mamman, Director-General/CEO of NITR, and Hajj Hudu Osue, Secretary Research Committee, for sponsorship. Sincere appreciation to Dr Peter Dede, Project Officer PATTEC Nigeria, for providing the team with biconical traps, GPS device and trypanocides. The team is also very grateful to the Chairman of Bagudo LGA and the field guides, for logistic support and guidance. The team also thanks the staff of DNALab Kaduna for technical assistance, and Mr A. Zaki and Mr S. Babangida for logistics.

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