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Original Research Article

Prevalence and Molecular Characterization of *Trypanosoma* Species in Tsetse, Tabanid and Stomoxys in Bayelsa Oil Palm, Bayelsa State, Nigeria

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Abstract: Prevalence and molecular characterization of trypanosome species in flies within Bayelsa oil palm, Bayelsa State, Nigeria was investigated through molecular characterization. Fifteen (15) biconical standard traps, baited with cow urine were deployed in the study area for tsetse and tabanid trapping. A total of two hundred and two (202) flies were caught and preserved in well labelled bottles containing 99% ethanol for morphological identification. Out of the 202 caught and identified, 199 (98.5%), 2(0.99%) and 1(0.5%) were tabanid, stomoxes and tse tse flies respectively. Out of the 199 tabanid flies identified, 4(2.0%) were infected while stomoxes and tse tse flies were not infected. Molecular analysis showed that the all the four tabanids were infected with *Trypanosoma brucie brucei*. The prevalence of trypanosome was recorded to be 4/202(2%). Tabanids was the most abundant vector in the study area and it is a potential vector of trypanosomiasis as it harboured *Trypanosoma brucei brucei*.

Keywords: Molecular Characterization, Tabanids, Tse tse fly, stomoxes, Trypanosoma species.

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INTRODUCTION

Africa Animal trypanosomiasis (AAT) constraints animal industry and human wellbeing in 37 sub-sabara African countries (Mayer *et al.*, 2016). About sixty million persons are at risk in these areas for this disease called sleeping sickness, also known as trypanosomiasis (Franco *et al.*, 2017). The protozoan responsible for disease belong to the genus *Trypanosoma* and it afflicts wide range of animals badly in Nigeria, and cause great damage in animals including abortion, insufficient milk supply and death (Malele *et al.*, 2016). The disease's widespread dispersion is due to the disease's biological and mechanical transmission vectors being readily available. These vectors are tsetse flies, stomoxys and tabanids (Dial *et al.*, 2017). Animals that are infected and involved in the transmission cycle of the disease includes warm blooded animals and wild life species (Reichard, 2002). In Nigeria, *Trypanosoma brucei, T congolense, T. brucei, and T. vivax* are the three most important species which infect animal and are found where tsetse flies, stomoxys and tabanids exist (Centre for Food Security and Public Health-CFSPH, 2010) and an annual loss of seven million dollars ($7Million) resulting from trypanosomiasis infections in cattle in some states from the North in Nigeria has been reported (Mariam, 2006). The host preference of *Trypanosoma* species differs, *T. vivax* is known to infect broad range of host, which includes cattle, goat, sheep, horses and donkeys. These parasites are biologically transmitted by tsetse fly while other biting flies may transmit it mechanically (Duffy *et al.*, 2010). This poses a serious danger to animals and humans who come in contact with infected animals and flies.

The piercing flies known as tabanids are sometimes called horse flies, deer flies, and march flies. They are true flies and members of the order diptera and family tabanidae (Croof *et al.*, 2017). These flies are medically very important because, some female species feeds on human and animal blood, and transmit several infective parasites to their host during feeding (Sevidzem & Mavoungou, 2019). Tabanids have been implicated in the transmission of certain pathogenic viruses, bacteria, protozoa and helminths (Baldacchino

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et al., 2014; Keita *et al.,* 2020). Tabanid and tsetse are recorded transmiters of trypanosomes which cause sleeping sickness or trypanosomiasis in animals and humans. There is scanty information on tabanid transmission of trypanosomes to livestock and humans in Nigeria, which has been established in other parts of Africa. Therefore, this study is aimed at expanding the existing literature and to determine the prevalence of trypanosomes and its mode of transmission in Bayelsa oil palm, Bayelsa State, Nigeria.

MATERIALS AND METHODS

This study was conducted at Bayelsa Oil Palm in Yenagoa, Bayelsa State, Nigeria (Fig. 1.0). The state is geographically located within latitude 04^0 45N and 05^0 23S; longitude 05° 22W and 06° 45E. Rivers State and Delta State borders it on the east and north respectively while the Atlantic Ocean borders it on the west and south. Bayelsa Oil Palm is located in Yenegoa and lies between latitude 4°53'N and longitude 6°19'E, and covers 21,110 square kilometers. Bayelsa State is characterized by mangrove and tropical rain forest, about three quarter of the entire land is covered with water. It lies completely below sea level and amazed with meandering creeks, mangrove and swamps.

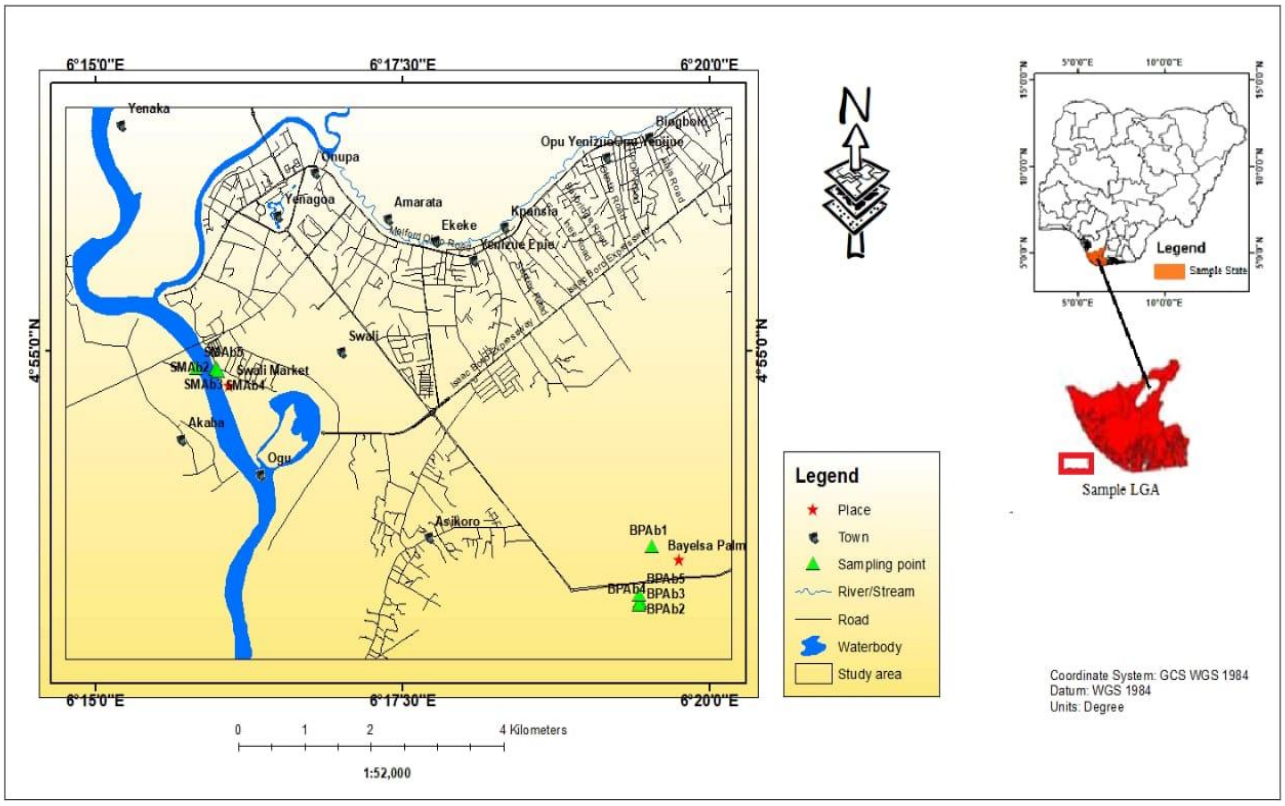

Figure 1: Map of Bayelsa State showing the study area

Collection of flies

The entomological survey was performed between March - May, 2023. During this survey, 15 biconical standard traps, baited with cow urine were deployed in the study area for tsetse and tabanid trapping. The traps were allowed to stay at the site of deployment for at least 12 hours before collection. The flies were preserved in a bottle containing 99% ethanol and transported to the molecular and biotechnology laboratory, Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) Kaduna for laboratory analysis.

DNA Extraction

The method of Asghar *et al.* (2015) was adopted in the extraction of the gene. The flies were properly identified by an entomologist using standard morphological keys (Baldacchino *et al.,* 20014) with the help of tripod magnification glass lens. They were air dried at room temperature and the wings removed. The insects were crushed with a mortar and pestle to powder form, and sample homogenized. About 25-50mg of the harmonized sample was placed in a clean 1.5ul tube and 200ul of TL buffer added. 20ul of proteinase k and 10ul of Rnase A were added and mixed by vortexing and incubated at 60^0C for 1 hour. 200ul of GB buffer was added and mixed by vortexing, 400ul absolute ethanol was added mixed wall by pipetting carefully transferred the lysate into the upper reservoir of the binding column tube, the tube was closed and centrifuged at 8,000 rpm for 1 min, the solution was discarded from the collecting tube and

reused the collection tube, 500ul of WA1 buffer was added, closed the tube and centrifuged at 8,000 rpm for 1 min, the solution was discarded from the collection tube and re-used the collection tube, 500ul of WA2 buffer was added, closed the tube and centrifuged at 8,000 rmp for 1 min, the process was repeated once more at 13,000 rpm for 1 min to completely remove ethanol. The binding column tube was transferred to a new 1.5ul tube for elution 50ul of EA buffer was added onto binding column and waited for at least 1 min RT (15-25%) centrifuged at 8,000 rpm for 1 min to elute. DNA strand recovered from the top by Pasteur pipette and washed by 70% ethanol and centrifuge at 13,000 rpm for 5 min and eluted I sterile analytic graded H_2O (Nancy et al., 2010).

PCR amplification

The method of Ouedraogo et al. (2018) was adopted in the amplification of the gene. The samples were amplified with 1ul of forward and reverse primer. 5ul of DNA template was added and 13ul nuclear free H_2O was added, the tube was shaken to mixed the solution and transferred to thermal cycler or PCR machine and the following conditions were programmed. 95°c for 15 mins. Followed by 35 cycles of 94°C for 30_5, 48^0C for 40_s and 72^0C for 45_s and final extension at 72^0C for 10 minutes.

Gel electrophoresis

The technique used by Sambrook *et al.* (1989) was applied in the electrophoresis. 1% agarose gel was prepared by dissolving lg of agarose powder in 100ul of TBE buffer, the mixture was heated in microwave ovum to dissolve, 5ul of Ethidium bromide was added and stirred to mix, the mixture was poured into a gel casting tray and allowed to polymerise, the gel was then transferred into gel running tank and electric current was connected to run at 100 volts for 1 hour. The result was visualized using gel documentation system.

Identification of trypanosome species

The following primer sequence was used for this reaction

Kin 1 GCGTTCAAAGATTGGCA.

Kin2 CGCCCGAAAGTTCACC.

The above primer was able to amplify the kinetoplasm of almost all the pathogenic African trypanosome species with varying band sizes. Therefore, it serves as a generic primer for detecting trypanosome species.

RESULTS

The study revealed the presence of 202 flies belonging to three genera. The genera were *Glossina*, *Stomoxys* and *Tabanus.* Out of the 202 flies trapped and identified, 199 (98.5%), 2(0.99%) and 1(0.5%) were tabanid, stomoxes and tse tse flies respectively. Out of the 199 tabanid flies identified, 4(2.0%) were infected while stomoxes and tse tse flies were not infected (Table 1.0). Molecular analysis showed that all the four tabanids were infected with *Trypanosoma brucie brucei.* The prevalence of trypanosome was recorded to be 4/202(2.0%). The primer used in this study was able to successfully amplified the 177bp region of the gene of *Trypanosoma brucei brucei* (Plate 1.0) based on the PCR amplification, four tabanids were infected with *T. brucei* species (Plate 2.0).

Table 1: Abundance and Prevalence of Trypanosoma sp in Tabanid, stomoxes and tsetse flies

Species of Insect	No. of insects trapped	No. infected (%)
Tabanus sp. (Tabanids)	199	4(2.0)
Stomoxys sp. (Stomoxis)	2	0(0)
Glossina sp. (tsetse fly)	1	0(0)
Total	**202**	**4(1.98)**

Table 2: PCR amplification for identification of species

Target gene	Sequence	SIZE(bp)	Ref:
KIN 1	GCGTTCAAAGATTGGCA	177	Njiru *et al.*, 2008
KIN2	CGCCCCAAAGTTCACC	177	

Plate 1.0: Region of the gene of trypanosoma brucei brucei

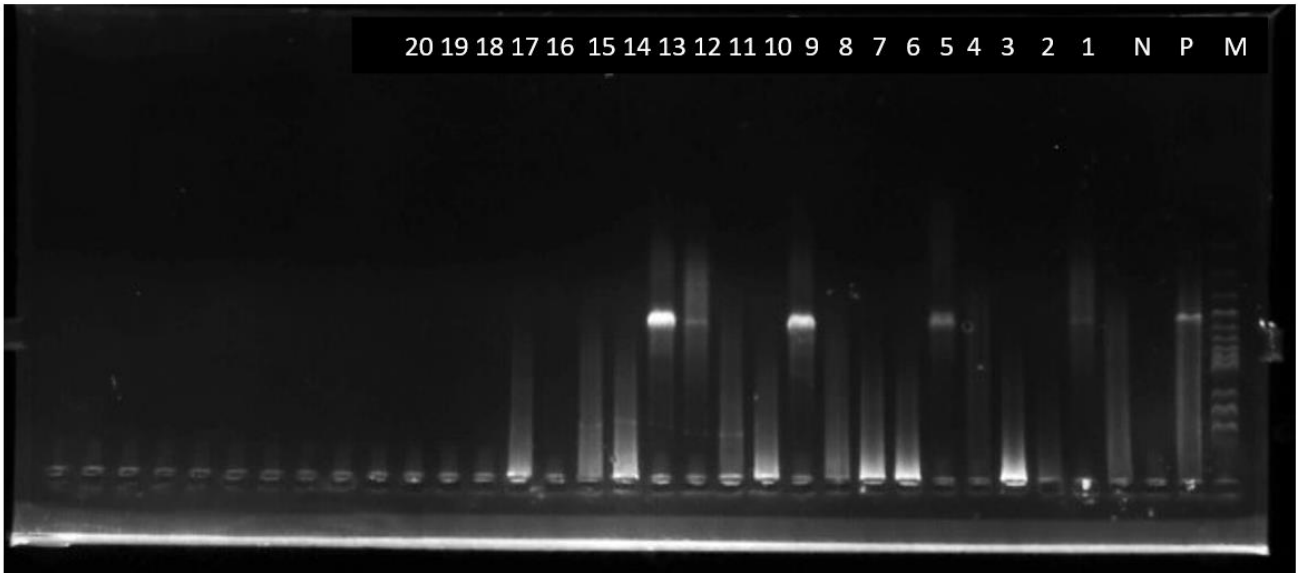

Plate 1. Gel electrophoresis image of DNA amplification using kin primer
M. 100bp plus DNA Ladder
P. positive control
N. Negative Control
Lane. 1 – 20 are the positive and negative samples.
Expected band size 177bp.

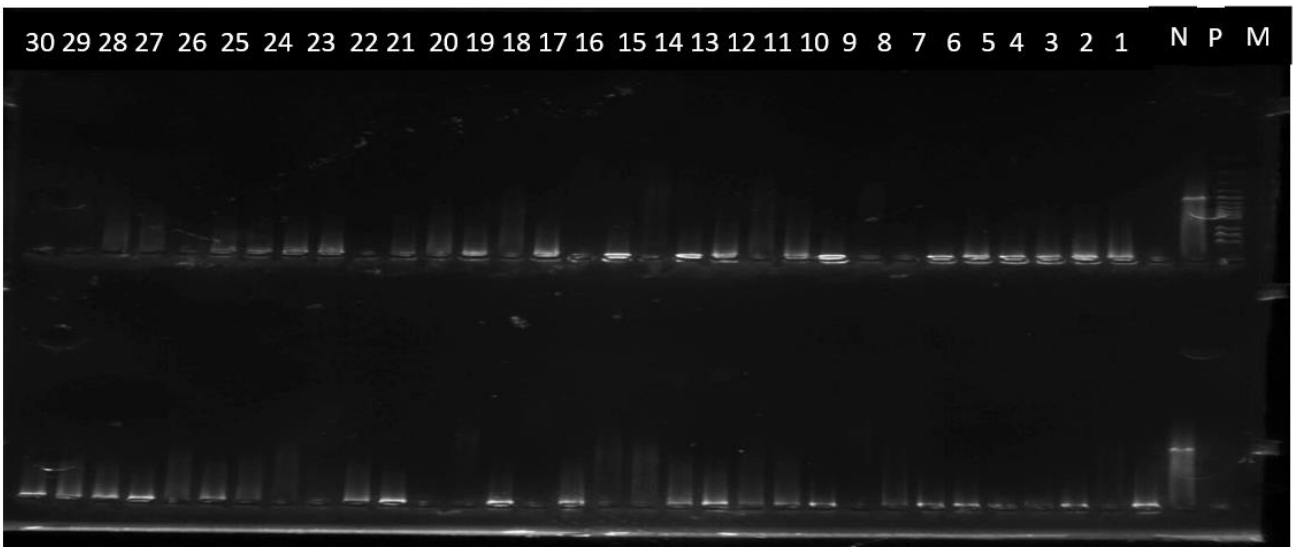

Plate 2. Gel electrophoresis image of DNA amplification using kin primer
M. 100bp plus DNA Ladder
P. positive control
N. Negative Control
Lane. 21 – 80 are the negative samples.
Expected band size 177bp.

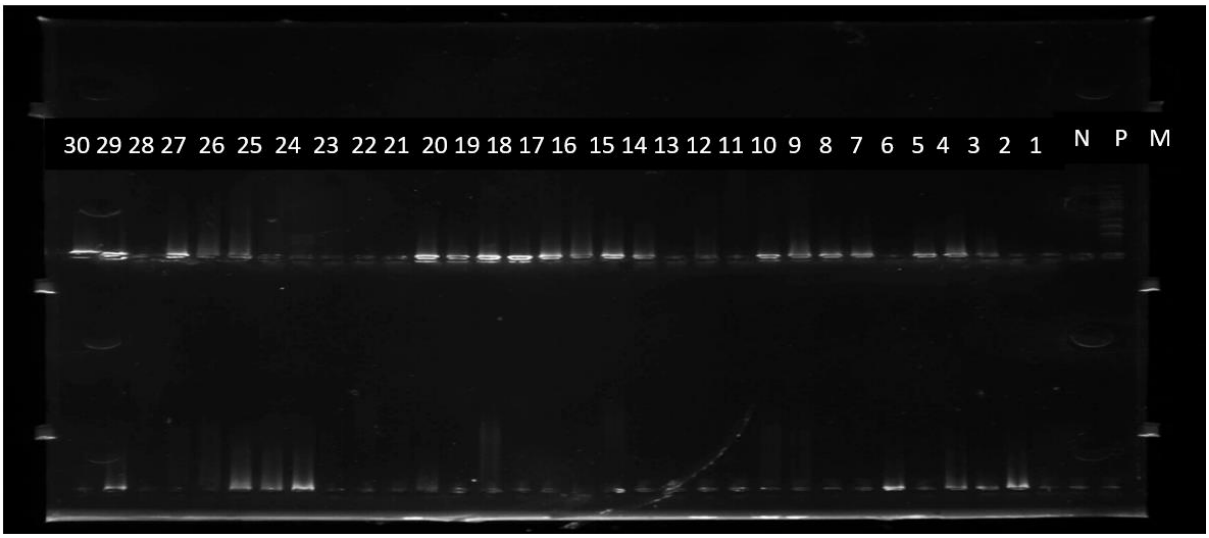

Plate 3. Gel electrophoresis image of DNA amplification using kin primer
M. 100bp plus DNA Ladder
P. positive control
N. Negative Control
Lane. 81 – 140 are the negative samples.
Expected band size 177bp.

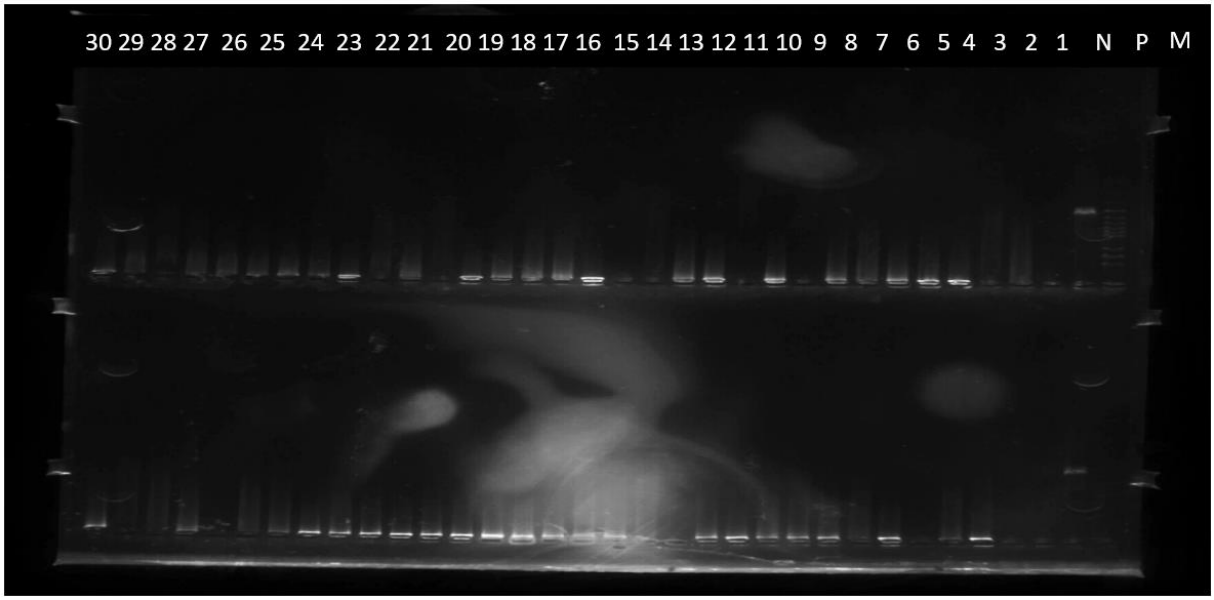

Plate 4. Gel electrophoresis image of DNA amplification using kin primer
M. 100bp plus DNA Ladder
P. positive control
N. Negative Control
Lane. 141 – 200 are the negative samples.
Expected band size 177bp.

DISCUSSION

Biting flies belonging to three genera were encountered in this study. The genera were *Tabanus, stomoxys* and *Glossina*. *Tabanus* species was the most abundant species. Similar results were recorded in Southern Kaduna and Kagaro Local Government Area, Kaduna by Ahmed *et al.* (2005) and Igube *et al.* (2021) respectively. The high abundance of biting flies in this study may be attributed to the vegetation type, regular presence of cattle in the plantation for grazing which provide regular blood meal and proximity to the river which provides natural breeding sites for the flies (Karshima *et al.,* 2011). The high prevalence of Tabanids observed in the study is in agreement with the report of Karshima *et al.* (2011). The researchers recorded a high prevalence of tabanids in three Local Government Areas of Taraba State. Our biconical traps caught more tabanids than other insects, this may be because of the colour of the traps (blue) and baiting of

traps with cow urine which serve as attractants (Alpha *et al.*, 2021). The *Glossina palpalis* identified in this study has been reported in other parts of Nigeria (Abubakar *et al.*, 2015; Kenechukwu *et al.*, 2017) and other countries in West Africa (Diarra *et al.*, 2019; Kallu *et al.*, 2023). The high humidity in the area, availability of river and the dense vegetation surrounding the oil palm plantation could also explain the reason for the presence of *Glossina palpalis* in the study area. The vector is a known riverine species (Lydie *et al.*, 2017; Urquhart *et al.*, 2003). The differences in the density of *Glossina palpalis* and *Tabanus* sp. recorded in this study and other studies could be as a result of differences in bio-geographical location and humidity levels in the study areas. The primer used in this study was able to amplified the targeted gene (kinetoplasm) of African Trypanosome species with the expected band size of 177bp on the DNA ladder. This is consistent with earlier report by Njiru *et al.* (2008), in which kin 1 and kin2 DNA were used consistently and found situated in the sub unit ribosomal RNA (Njiru *et al*, 2008). The presence of trypanosome in tabanids learns credence to the records of Keita *et al.* (2020) and Baldacchino *et al.* (2014) that tabanids are potential vector of trypanosomiasis and related diseases in West Africa.

CONCLUSION

The result obtained in this study revealed that tabanids was the most abundant vector in the study area and it is a potential vector of trypanosomiasis as it harboured *Trypanosoma brucei brucei.*

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

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