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# Prevalence of trypanosome species in wild tsetse flies (*Glossina* spp) from Kagarko forest, North-Central Nigeria

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**ABSTRACT:** The causative agents of Human African Trypanosomiasis (HAT) are trypanosome parasites belonging to the trypanosoma brucei complex: *T. brucei gambiense* is responsible for the chronic form of the disease in West and Central Africa; *T. brucei rhodesiense* is responsible for the acute form in East and Southern Africa; While *T. brucei brucei* is used as an animal model for the HAT. Other trypanosomes responsible for the disease in animals called Animal African Trypanosomiasis (AAT) includes: Trypanosoma congolense, Trypanosoma vivax and Trypanosoma evansi. In this study, the prevalence and identity of trypanosome species in wild tsetse flies from Kagarko forest was investigated by nested PCR using ITS-1 generic and species-specific primers. Five hundred and eight-six flies were trapped using bi-conical traps and identified by standard morphological features. DNA extracted from whole flies was used for nested PCR to amplify the ITS-1 region of trypanosomal rDNA. Two tsetse fly species were identified in the Kagarko forest: *Glossina tachinoides* (480) and *Glossina palpalis palpalis* (106), representing 81.91% and 18.09% respectively. While the predominant trypanosome spp found were Trypanosoma vivax (38.2%), Trypanosoma brucei (30.9%) and Trypanosoma congolense Savanna (21.8%). These results revealed high prevalence of trypanosome infections in wild tsetse flies in Kagarko forest which has important impacts on the health and socio-economic wellbeing of the people and animals in communities around the forest.

**Keywords:** African Trypanosomiasis, infection, Kagarko forest, *Trypanosoma vivax.*

## INTRODUCTION

Tsetse fly is vector of Trypanosomes. Many of these trypanosomes are pathogens of humans and domestic animals, causing Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT), diseases with significant medical and economic impact. Human disease is caused by two subspecies of *Trypanosoma brucei*, whereas AAT is caused by at least seven species/subspecies of Trypanosoma (CFSPH, 2009). *Trypanosoma vivax* and *Trypanosoma congolense* are the main causative agents of AAT because of their prevalence throughout sub-Saharan Africa and their economic impact on livestock production (CFSPH, 2009). AAT is recognized as a major impediment to agricultural development, costing ranchers and consumers over $1.3 billion annually (Shaw *et al.,* 2014). The number of disease management methods within vertebrate hosts is limited. The emergence of resistance to trypanocidal drugs makes it difficult to maintain chemotherapy to control AAT (Delespaux, 2008). For these reasons, vector control remains a very important part of the integrated management of AAT (Holmes, 2013). Detection and identification of Trypanosomes spp. infecting the tsetse fly is fundamental to understanding the epidemiology of associated diseases and establishing efficient tsetse control programs aimed at reducing the

prevalence of AAT in a given area. This allows tsetse control programs to, for example, focus on areas of the high prevalence of the most virulent pathogens and use appropriate techniques depending on the vector species. There is little information on biodiversity and distribution of trypanosomes in Nigerian tsetse populations. The number of disease management methods within vertebrate hosts is limited. Furthermore, the emergence of resistance to trypanocidal drugs makes it difficult to maintain chemotherapy to control AAT (Delespaux, 2008). As such, further research into the biology and ecology of the tsetse fly is essential to determine the most effective way to control the disease and improve public health in Nigeria.

Eleven species of tsetse have been reported from Nigeria (Baldry and Riordan, 1967); infesting about 70% of the country's land mass (Dede *et al.,* 2005). Some of these species are major vectors of *T. vivax, T. brucei* sub spp, and *T. congolense* sub spp. (Ahmed, 2007; Madubunyi, 1987). However, most of the available data based on microscopy are believed to be much less sensitive and accurate than DNA-based detection and identification methods (Njiru *et al.,* 2005). DNA-based methods have the advantage of being more sensitive and able to identify trypanosomes at the sub-species level and detect mixed infections.

All tsetse species are susceptible to trypanosomes, but susceptibility varies by species. In general, species of the Morsitan group are considered more susceptible to *trypanosomal* infection than species of the *Palpalis* group. Within each species, the ability of individual flies to trypanosomes can be influenced by a variety of factors specific to the insect host. For example, many factors have been found to influence trypanosome susceptibility, including the individual's age and sex at the time of the first infected diet (Peacock *et al*., 2012). For example, *Glossina palpalis palpalis* are more likely to be infected than *Glossina tachinoides*. Studies describing the distribution of *trypanosoma* species diversity among different soma species can provide insight into the vectoring ability of different soma species and reveal nonrandom associations between soma species and trypanosomes.

Therefore, the primary aim of the study was to identify trypanosome species obtained from the Kagarko forest using nested PCR, and the specific objectives were to:

1. determine the density of tsetse fly in the study area.
2. detect natural trypanosome infections among wild tsetse flies captured in Kagarko forest.

## MATERIALS AND METHODS

### Study area

Kagarko Forest is located in Kagarko Local Government Area. Its geographic coordinates are 9° 26' 0" North, 7° 48' 0" East. It is located in the Guinea steppe belt. Its ecological features are characterized by hills, forests, tall grass, streams, and rivers that mark the terrain.

### Sample size and sampling method

Simple random sampling was used in this study. The sample size was determined using an expected prevalence of 50%. An absolute desired precision of 5% with a 95% confidence level was used.

### Tsetse flies survey

A total of 20 biconical standard traps were used in the study area. All traps were uniformly filled with octanol (1-oct-3-nel), acetone, and urine. All odorants were placed on the ground about 30 cm away from the trap upwind. The rods of the traps were lubricated to repel fly-eating flies, mainly ants. They are allowed to remain in place for 48 hours before retrieving the traps. Trap locations were chosen to represent all types of vegetation/habitats potentially relevant to fly breeding, behavior, feeding, and other relevant aspects. After 48 hours of use, the area covered by each trap was sorted by fly species, counted, identified, and analyzed (Leak *et al.,* 1987).

### Flies collection, identification, and storage

Trapped flies were collected from the trap, and before separating them into different sexes; species of tsetse flies were recorded. Tsetse flies were identified as male or female using their morphological features. The flies were stored in absolute ethanol and transported to the laboratory where it is kept at -20°C until further analysis.

### DNA extraction and purification

The whole fly was crushed using a pestle in 5 ul Eppendorf tubes followed by DNA extraction and purification which was carried out using *AccuPrep*® Genomic DNA Extraction Kit following the manufacturer's instructions. DNA was quantified using a Nanodrop spectrophotometer and only samples with an absorbance ratio between 260 and 280 nm were used in the agarose gel electrophoretic analysis.

### Trypanosome detection and identification by PCR

The extracted DNA was subjected to a Nested PCR assay using the ITS primer (Table 1). The primers were synthesized by Bioneer Company and were provided by DNA labs, Kinkino road Unguwar Rimi GRA Kaduna. The

**Table 1.** Primer sequences used

| Parameters | Sequences | References |
|---|---|---|
| External Reverse | CTTTGCTGCTTCTT | Cox *et al.,* 2005 |
| External Forward | TGCAATTATTGGTCGCGC | Cox *et al.,* 2005 |
| Internal Forward | TAGAGGAAGCAAAAG | Cox *et al.,* 2005 |
| Internal Reverse | AAGCCAAGTCATCCATCG | Cox *et al.,* 2005 |

primers were designed to amplify the ITS region of ribosomal DNA (rDNA), a region that varies in size within trypanosome species. 10 $\mu$L of the PCR product was resolved in a 2% agarose gel at 80 volts for 45 minutes to analyze the amplicons. The gel was visualized under a UV trans-illuminator following Ethidium-bromide staining.

### Data analysis

The infectious trypanosomes were identified by comparing the molecular sizes of their DNA fragments with the documented band sizes of trypanosome species. The data was then used to calculate the infection rates and prevalence of the trypanosomes on *Glossina* spp.

## RESULTS

Table 2 shows the prevalence of two species of tsetse flies were found in Kagarko area. The two species are *Glossina tachinoides*, also known as *G. tach*, and *Glossina palpalis palpalis*, also known as *G.p. palpalis*. Of the 586 tsetse flies observed, 480 were identified as *G. tach*, making up 81.91% of the entire population. However, 106 flies were specifically identified as *G.p. palpalis*, making up 18.09% of the overall tsetse fly population. The percentages demonstrate that *G. tach* was the prevailing species in the study area, exhibiting a significantly greater prevalence in comparison to *G. p. palpalis*.

### Infection rate

The study examined Kagarko's trypanosome diversity. Four trypanosome species were found. With 38.2% of samples, *Trypanosoma vivax*, the most common. Over one-third of samples tested positive for this trypanosome species. *Trypanosoma brucei* was found in 30.9% of samples, indicating a large presence in the study area. *Trypanosoma congolense* Savanna was found in 21.8% of samples, while forest types was found in 9.1%. Interestingly, no Kagarko samples contained *Trypanosoma simiae or grayi*. This suggests that these species were absent or below the diagnostic methods' detection limits. Kagarko has a variety of trypanosome species, with *Trypanosoma vivax* and *brucei brucei* being the most common (Table 3 and Figure 1).

**Table 2.** Shows the species of tsetse fly circulating the Kagarko

| Species of flies | No of flies | Prevalence (%) |
|---|---|---|
| *G. tach* | 480 | 81.91 |
| *G.p. palpalis* | 106 | 18.09 |
| Total | 586 | |

T=11.88, p=0.001.

## DISCUSSION

An entomological survey conducted in the study area identified two species of tsetse flies, including *Glossina palpalis palpalis*, and *Glossina tachinoides.* These tsetse fly species belong to the family *Glossinidae*, class *Insecta*, order *Diptera*, and genus *Glossina*. Their distribution in the study locations aligns with the findings of previous studies by Abubakar *et al.* (2016) and Shaida *et al.* (2018) who found similar species of tsetse flies in the study area.
Specifically, *Glossina tachinoides* were observed in the vicinity of water tributaries in Kagarko, as reported by Shaida *et al.* (2018). On the other hand, *Glossina palpalis palpalis* were exclusively caught in the dense vegetation Kagarko, specifically in closed canopy areas. The diversity of tsetse fly species in Kagarko is notably influenced by human activities. Land use changes, such as deforestation and agricultural practices, are prevalent in these regions and have significantly modified land cover, thereby impacting the suitability of habitats for these insects. The high process of deforestation in Kagarko has resulted in the depletion of appropriate vegetation and microclimates, thereby reducing the suitability of the area for tsetse flies. Moreover, the agricultural methods employed in Kagarko such as land clearance and pesticide usage, have adversely impacted the species diversity in those regions (Shaida *et al.,* 2018). In addition to environmental factors, the availability of a suitable food source is critical for the survival of tsetse flies which is very limited in Kagarko, this has affected the density of tsetse population.

The high prevalence of *Trypanosoma vivax* (38.2%) observed in this study is consistent with findings from other

**Table 3.** Species of trypanosome circulating in the study areas.

| Location | *TCF* n(%) | *TCS* n(%) | *Tb* n(%) | *Tv* n(%) | *Ts* n(%) | *T. grayi* n(%) | Total |
|---|---|---|---|---|---|---|---|
| Kagarko | 9.1(10) | 21.8(24) | 30.9(34) | 38.2(42) | 0 | 0 | 110 |

TCF= *Trypanosoma congolense* forest, TCS= *Trypanosoma congolense* Savanna, T. b *Trypanosoma brucei brucei*, *T.v = Trypanosoma vivax*, *T.s= Trypanosoma simiea, T. grayi.= Trypanosoma grayi.*

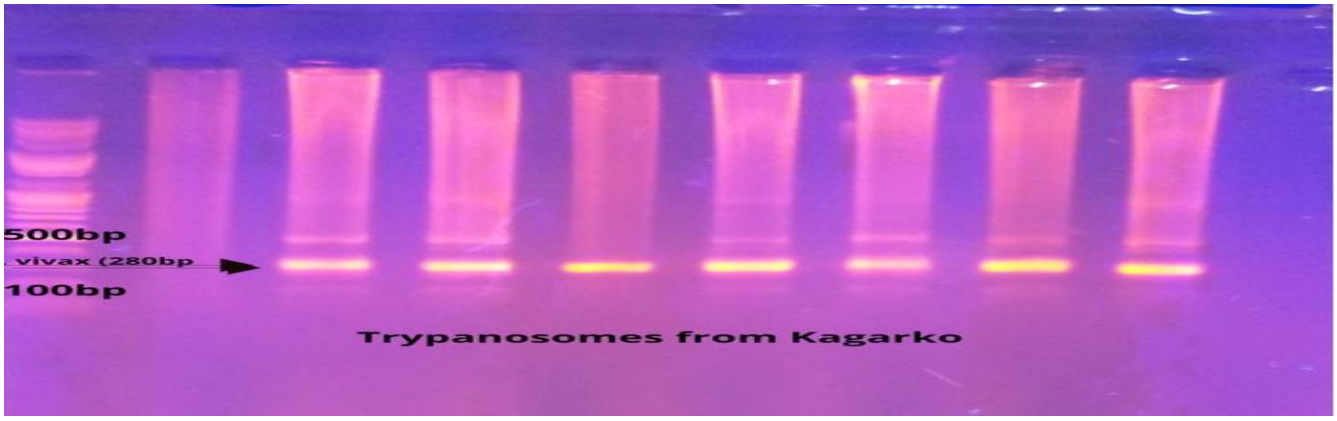


**Figure 1.** Agarose gel illustrating the DNA profile resulting from the amplification of a DNA fragment of the tsetse fly from Kagarko showing *Trypanosoma vivax* and *T. congolense* forest.

studies in Southern Kaduna by Ahmad (2007). For instance, a study by Dede *et al*. (2005) on current tsetse and trypanosomiasis situation on Jos Plateau reported *T. vivax* as the most prevalent trypanosome species, accounting for 54.5% of infections. Similarly, in East Africa, *T. vivax* was the most common species, detected in 45.6% of cattle (Shaw *et al*., 2014). These studies relatively find high prevalence of variant types of trypanosomes (30.9%) and is comparable to the findings from other regions. Dede *et al*. (2005) found *T. brucei brucei* in 28.6% of cattle in on the Plateau in North Central Nigeria, while Njiru *et al*. (2005) reported a prevalence of 23.5% in Kenya. The combined prevalence of *Trypanosoma congolense* savanna (21.8%) and forest types (9.1%) in this study is within the range reported in other studies. For instance, Fetene *et al.* (2021) reported a 19.2% prevalence of *T. congolense* Savannah in Burkina Faso, while Kalayou *et al.,* (2021) reported a 24.7% prevalence in Kenya. The absence of *Trypanosoma simiae* and *Trypanosoma grayi* in this study is not unusual, as these species are less commonly reported in livestock compared to other trypanosome species. However, some studies, in Yankari and Kainji documented the presence of *T. simiae* in cattle (Shaida *et al.,* 2018). Overall, the findings from this study are largely consistent with the trypanosome species distribution patterns observed in Nigeria. The high prevalence of *T. vivax* and *T. brucei brucei*, along with the presence of *T. congolense* Savannah, is a common trend in many areas affected by animal trypanosomiasis. However, the specific prevalence rates can vary due to factors such as vector abundance, host susceptibility, and environmental conditions specific to each region.

The presence of notable incidence of trypanosome infection in tsetse flies in Kagarko is of particular concern due to its implications for the potential transmission of trypanosomiasis to both humans and livestock in these areas. This observation underscores the necessity for enhanced vector control initiatives and surveillance endeavours in these areas to mitigate the impact of the disease.

## Conclusion

The study highlights the significance of understanding the distribution and abundance of both tsetse fly vectors and trypanosome species in Kagarko. The high incidence of *G. palpalis palpalis*, along with the elevated prevalence of *T. vivax* and *T. brucei brucei*, suggests a substantial likelihood of animal trypanosomiasis transmission in Kagarko. These findings have consequences for the creation and execution of efficient control strategies. To decrease the transmission of trypanosomes to livestock, it may be necessary to implement specific control measures that target the tsetse fly population, such as trapping or applying insecticides. In addition, it is imperative to regularly monitor and surveil trypanosome infections in livestock to monitor changes in prevalence and inform appropriate treatment and management protocols. In summary, the study offers important knowledge about the spread of animal trypanosomiasis in Kagarko and

highlights the importance of using comprehensive strategies to manage both the vector and the parasitic disease.

## Recommendation

For an effective trypanosomiasis control program, surveillance studies should be conducted to determine the prevalence of trypanosomiasis in livestock in the area. In addition, entomological surveys should be conducted at different times of the year to understand seasonal variations in vectors and the associated risk of trypanosomiasis.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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