



## PREVALENCE AND DIVERSITY OF HEMATOPHAGOUS FLIES IN FARMING COMMUNITIES IN JOS, PLATEAU STATE, NIGERIA AND THEIR POTENTIAL ROLE IN *TRYPANOSOMA* SPECIES TRANSMISSION TO CATTLE

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### Abstract

Haematophagous flies remain significant in disease transmission, hindering agriculture and livestock development in sub-Saharan Africa. This study assessed the prevalence and diversity of these flies in agrarian communities in two Local Government Areas (LGAs) in Plateau State, Nigeria, and assessed their role in the mechanical transmission of *Trypanosoma* sp. to cattle. Between March to August 2023; Biconical and Nzi traps were used to trap hematophagous flies around farming families in twelve communities from the two study areas. Blood samples were concurrently collected from cattle from the herds in the community. Collected flies were sorted, identified morphologically and screened for the presence of *Trypanosoma* sp. DNA using PCR. The cattle blood samples were analyzed via Haematocrit Centrifugation Technique (HCT) and PCR. Overall, 776 flies were captured with higher abundance (491; 63.6%) in Jos North than (285; 36.4%) in Jos South. *Stomoxys calcitrans* dominated haematophagous flies (248; 31.7%), while *Musca domestica* was the most common non-haematophagous species (128; 14.4%). No *Glossina* spp. was caught. All the 250 cattle blood samples screened by microscopy and PCR were negative for trypanosome infection, but *Trypanosoma evansi* DNA was detected in one pool of *S. calcitrans*. The detection of *T. evansi* DNA in *S. calcitrans* underscores the potential role of this fly in the epidemiology of trypanosomes in cattle in this study area. There is the need for an elaborate study to determine the vector capacity of *S. calcitrans* to the various *Trypanosoma* spp. in Nigeria.

**Keywords:** *Stomoxys calcitrans*, PCR, Haematophagous flies; Plateau state, Trypanosomes

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### INTRODUCTION

African trypanosomosis is caused by protozoan parasites of *Trypanosoma* spp. and

is mainly transmitted by tsetse flies (Steverding, 2008). This disease is a major concern in sub-Saharan Africa, with detrimental effects on both human and animal health causing significant losses in the affected countries (Steverding, 2008). Animal African Trypanosomosis (AAT) caused by *Trypanosoma congolense*, *Trypanosoma vivax*, and *Trypanosoma brucei brucei* affects domestic animals, including cattle, goats, sheep, and pigs, with varying pathogenicity according to the host and pathogen species (Spickler, 2018, Pereira *et al.*, 2024). The common clinical signs of AAT include, fever, anaemia, loss of weight and productivity, abortion, decreased fertility, oedema, paralysis, and even death (Bekele and Begejo, 2015, Obaloto *et al.*, 2025). In draught animals, the affected animals progressively weakens and eventually becomes unfit for work, hence the name of the disease ‘N’gana which is a “Zulu” word that means “powerless/useless” (Pereira *et al.*, 2024). Hence, AAT constrains agricultural production in areas of Africa that hold the continent’s greatest potential for expanded agricultural production. *Glossina* species (tsetse flies) are the biological vectors of trypanosomes, although some hematophagous flies have been incriminated in the mechanical transmission of some *Trypanosoma* species like *T. vivax* and *T. evansi*. Therefore, the geographical range of trypanosomosis extends beyond the known tsetse belt (Osório *et al.*, 2008; Mamman *et al.*, 2021; Peter *et al.*, 2025).

Hematophagous flies especially members of the families *Tabanidae* and *Muscidae* (*Stomoxys*), play an important role in human and animal health as vectors of disease agents (Baldacchino *et al.*, 2013). They have been incriminated as mechanical vectors of *T. vivax* and *T. evansi*, extending the geographical range of these pathogens beyond the recognized tsetse belts

(Hutchinson *et al.*, 2021). Recent findings have highlighted the potential role of other hematophagous flies such as *Stomoxys* and *Tabanus* beside the *Glossinidae* in the transmission of trypanosomes and other pathogenic organisms in man and animals (Elkan *et al.*, 2009; Peter *et al.*, 2023; Kallu *et al.*, 2023)

In Nigeria, diagnosis of trypanosomosis mainly depends on parasitological and immunological methods (Jun Li *et al.*, 2007). Parasitological techniques have significant limitations exemplified by inability to detect trypanosomes when present at low levels in the blood or other organs of infected animals (OIE, 2013) as well as their inability to differentiate between the members of the Trypanozoon group (Feng-Jun *et al.*, 2007). The prevalence of Trypanosomosis has been extensively studied using parasitological and immunological methods in Nigeria (Chibuogwu *et al.*, 2023; Chukwudi *et al.*, 2025). The polymerase chain reaction (PCR) has been employed for the diagnosis of trypanosomes with high efficiency, although, technical requirement and cost have precluded its widespread use (Delespau *et al.*, 2008, Nakayima *et al.*, 2012). There are few studies on AAT in cattle from different parts of Nigeria using the PCR method, reporting prevalence of 33-77 %, (Danyayam and Abdullahi, 2025), suggesting widespread AAT across the ecological zones of Nigeria. The aim of this study is to determine the prevalence and diversity of Hematophagous flies in farming communities in Jos, Plateau State, Nigeria and their Potential role in *Trypanosoma* Species Transmission to Cattle.

## MATERIALS AND METHODS

### Study Area

The study was carried out in 12 communities located in two LGAs-Jos South

and Jos North in Plateau State, Nigeria. Plateau state is located in the North Central Nigeria within the Guinea Savannah vegetation. It lies within latitudes 8°22'N and 10°24'N and longitudes 8°32'E and 10°38'E. The total land is 26,899 square kilometers. The climate within the state is influenced by its relief and its position on the path of the Inter-Tropical Convergence Zone (ITCZ). The high altitude (1,829meters) of the Jos Plateau has so much moderated its temperature making it like that of temperate climate, with an average minimum temperature, of between 18°C and an average maximum of 22°C (Hudu and Ibrahim, 2021). The mean annual rainfall varies from 131.75cm to 146cm. The highest rainfall is usually recorded in the month of July. The yearly average relative humidity is 54%, varying by only 2% during the rainy months.

### **Ethical Approval**

The study protocol was approved by the Institutional Animal Use and Care Committee (AUCC), National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria with the certification ID: NVRI AEC REF No: ACE/02/88/20.

### **Study Population and sample size**

A total of 250 cattle, consisting of 125 from each of the two LGAs were included in the study. Cattle of all breeds, age and any sex from different farms that had not received trypanocide for a while were included in the study. The desired sample size was determined by considering 20% of the expected prevalence by Peter *et al.*, 2023 with 95% confidence level and 5% precision. Using the formula:  $N = 1.96^2 P_{exp} (1 - P_{exp}) / D^2$  (Thrusfield 1995), a minimum of 246 samples were needed. To take the precision to be near accurate, the sample size was adjusted to 250.

### **Collection of Blood Samples**

Animals selected for sampling were properly restrained and approximately 5 mL of venous blood was obtained from the jugular vein. The blood samples were aliquot into pre-labeled ethylene diamine tetra acetic acid (EDTA) tubes. The samples were transported in a container with ice pack, to Parasitology Laboratory of the National Veterinary Research Institute Vom, for analysis.

### **Trapping of hematophagous flies using Biconical and Nzi Traps**

Biconical and Nzi traps were set at about 100 meters apart at strategic places especially river side, forested areas and herders resting place (Dede *et al.*, 2005). Three traps were deployed per community. The traps were examined 48 hourly for 20weeks, and flies trapped were carefully collected into labeled universal containers with 70% ethanol. The traps were then moved to other communities after 72 hours. This was repeatedly done for 5months. All trapped flies were taken to the Entomology Laboratory at the National Veterinary Research Institute, Vom for identification. The sorting and preliminary identification of caught flies were made based on size, color and morphology (Pollock, 1992; Potts, 1973).

### **Parasitological Analysis**

Each blood sample was screened for trypanosomes by microscopy according to the Standard Trypanosome Detection Methods (STDM). Specifically, the following procedures; Giemsa stain thin smear, Hematocrit Centrifugation Technique (HCT) and Buffy Coat Technique were employed according to established procedures (Murray and McIntyre 1977; Ayana *et al.*, 2015; OIE, 2021). Processed samples were evaluated using a binocular light microscope at various magnifications depending on the procedure and the results were recorded.

### **DNA extraction and molecular detection of trypanosomes in cattle blood and hematophagous flies**

Genomic DNA was extracted from 100  $\mu$ L of blood samples using the QIAmp®DNA mini kit according to manufacturer's specification. The eluted DNA was stored at -20°C in the parasitology division in National Veterinary Research Institute in Vom, until analysis. The hematophagous flies were pooled according to species and study area and processed for DNA extraction. The flies were first removed from the ethanol and subjected to three steps of washing in sterile Phosphate Buffered Saline (PBS). In total seven pools consisting of *Stomoxys* spp (n=4), *Tabanus* sp. (n=2) and *Haematopota* sp (n=2) were processed for DNA extraction. Briefly, each pool of flies was macerated and homogenized using a plastic pestle in a 2 mL Eppendorf tube containing 100  $\mu$ L of sterile PBS. The homogenates were spun in at 11000 rpm for 1 minute. The supernatants were harvested and subjected to DNA extraction using the QIAamp® DNA mini kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. Genomic DNA was eluted in 100  $\mu$ L of elution buffer and stored at -20°C until analysis. The screening of the DNA samples from cattle blood and hematophagous flies for the presence of *Trypanosoma* spp. DNA was conducted using published primers (*ITS1CF*: CCG-GAA-GTT-CAC-CGA-TAT-TG and *ITS1BR*: TTG-CTG-CGT-TCT-TCA-ACG-AA) to amplify a 250-710 bp of *Trypanosoma* species. The reaction mix was performed in a total volume of 25  $\mu$ L that contained 3 $\mu$ L of genomic DNA template, 0.25 $\mu$ L of 20 $\mu$ M of each primer, 12.5 $\mu$ L of one Taq® Quick-Load® 2xMaster Mix with Standard Buffer (New England Bio Labs) and the volume was made up with 9.0  $\mu$ Nuclease Free Water (Promega®). The amplification was

conducted on Applied Biosystem®9700 PCR thermocycler. The cycling profile was, initial denaturation at 94°C for 5 minutes which was followed by 4 cycles of denaturation at 93°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 1 minute. This was followed by 8 cycles of denaturation at 94°C for 30 seconds, and annealing at 56°C for 30 seconds, extended at 72°C for 1 minute, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, extended at 72°C for 1 minute. A final extension at 72°C for 5 minutes was observed and the reaction was held at 4°C for  $\infty$  until ready for electrophoresis. The PCR product was evaluated via electrophoresis using 1.5% agarose gel stained with ethidium bromide and compared to a 100 bp DNA ladder. The image was documented using a Gel Documentation System (SynGene® Tokyo Japan).

### **Data Analysis**

All analyses were performed using SPSS version 25. Data obtained from this study was expressed as frequencies, percentages and tables. Chi-square was used to test for association between occurrences of trypanosomes between the locations. The prevalence of the flies was determined by dividing the number flies caught by traps from or in the district by the total number of flies caught in the study, multiplied by 100. All the analyses were conducted at 95% confidence interval and P values  $\leq 0.05$  was considered significant.

## **RESULTS**

### **Prevalence and diversity of flies in the study area**

A total of 497 flies were caught from different wards in Jos north, with Kunga wards having the highest prevalence of 235(47.9%) followed by Yan shanu 75(15.1), Yan Kaji 52(10.5), Jarawa 51(10.3), Maza

29(5.8%), Babale 28(5.6) with the least prevalence in Naraguta wards A and B having 13(2.6) and 14(2.8) respectively. *Stomoxys* species were the dominating flies having a total prevalence of 223(44.9%), followed by *Musca domestica* 148(29.8%), *Tabanus* spp 65(13.1%), Bot fly 48(9.7%) and *Anthomyia* 11(2.2%) the least was Rubber fly with prevalence of 1(0.2%) (Table 1). A total of 285 flies were caught from Jos South study

area, with Vwang ward having the highest occurrence of flies; 110 (38%), followed by Kuru 86(30.2%), Du 65(22.8) and Gyel 24(8.4%). *Musca domestica* was the dominating flies in the study area accounting for 228(80.0%) followed by *Stomoxys* spp 31(10.9), Botfly 19(6.7%) and *Sarcophaga* sp. 6(2.1%) the least was *Hematopota* sp. 1(0.4%) (Table 1).

**Table 1. Prevalence, diversity and distribution of flies in the study areas**

| Wards                 | Total (%)       | Species of Fly (%)               |                                  |                              |                                 |                                 |                             |
|-----------------------|-----------------|----------------------------------|----------------------------------|------------------------------|---------------------------------|---------------------------------|-----------------------------|
| JOS NORTH             |                 | <i>Stomoxys</i><br><i>spp</i>    | <i>Musca</i><br><i>domestica</i> | <i>Tabanus</i><br><i>spp</i> | <i>Botfly spp</i>               | <i>Anthomyia</i><br><i>spp</i>  | <i>Rubber</i><br><i>fly</i> |
| Naraguta<br>"Ward A"  | 13(2.6)         | 4(30.8)                          | 8(61.5)                          | 0(0)                         | 0(0)                            | 1(7.7)                          | 0(0)                        |
| Yanshanu<br>(1 and 2) | 75(15.1)        | 10(13.33)                        | 19(25.33)                        | 23(30.7)                     | 23(30.7)                        | 0(0)                            | 0(0)                        |
| Naraguta<br>"Ward B"  | 14(2.8)         | 7(50)                            | 2(14.3)                          | 0(0)                         | 0(0)                            | 5(35.7)                         | 0(0)                        |
| Jarawa                | 51(10.3)        | 36(70.6)                         | 0(0)                             | 0(0)                         | 12(23.5)                        | 3(5.9)                          | 0(0)                        |
| Maza                  | 29(5.8)         | 10(34.5)                         | 4(13.8)                          | 13(0)                        | 0(0)                            | 0(0)                            | 1(3.5)                      |
| Yan Kaji              | 52(10.5)        | 13(25)                           | 8(15.4)                          | 24(46.2)                     | 7(13.5)                         | 0(0)                            | 0(0)                        |
| Babale                | 28(5.6)         | 8(28.6)                          | 14(50)                           | 1(3.6)                       | 3(10.7)                         | 2(7.14)                         | 0(0)                        |
| Kunga                 | 235(47.9)       | 135(57.44)                       | 93(39.6)                         | 1(3.6)                       | 3(1.3)                          | 0(0.0)                          | 0(0)                        |
| <b>Total</b>          | <b>491(100)</b> | <b>223(44.9)</b>                 | <b>148(29.8)</b>                 | <b>65(13.1)</b>              | <b>48(9.7)</b>                  | <b>11(2.2)</b>                  | <b>1(0.2)</b>               |
| JOS SOUTH             |                 | <i>Musca</i><br><i>domestica</i> | <i>Stomoxys</i><br><i>Spp</i>    | <i>Botfly spp</i>            | <i>Sarcophaga</i><br><i>spp</i> | <i>Hematopota</i><br><i>spp</i> |                             |
| Vwang                 | 110(38.6)       | 84(76.4)                         | 10(9.1)                          | 14(12.7)                     | 1(0.91)                         | 1(0.91)                         |                             |
| Kuru                  | 86(30.2)        | 80(93.02)                        | 6(7.0)                           | 0(0)                         | 0(0)                            | 0(0)                            |                             |
| Du                    | 65(22.8)        | 51(78.5)                         | 14(21.5)                         | 0(0)                         | 0(0)                            | 0(0)                            |                             |
| Gyel                  | 24(8.4)         | 13(54.2)                         | 1(4.2)                           | 5(20.8)                      | 5(20.8)                         | 0(0)                            |                             |
| <b>Total</b>          | <b>285(100)</b> | <b>228(80)</b>                   | <b>31(10.9)</b>                  | <b>19(6.7)</b>               | <b>6(2.1)</b>                   | <b>1(0.4)</b>                   |                             |

Chi-square test =, DF=1, *p*-value= 0.05

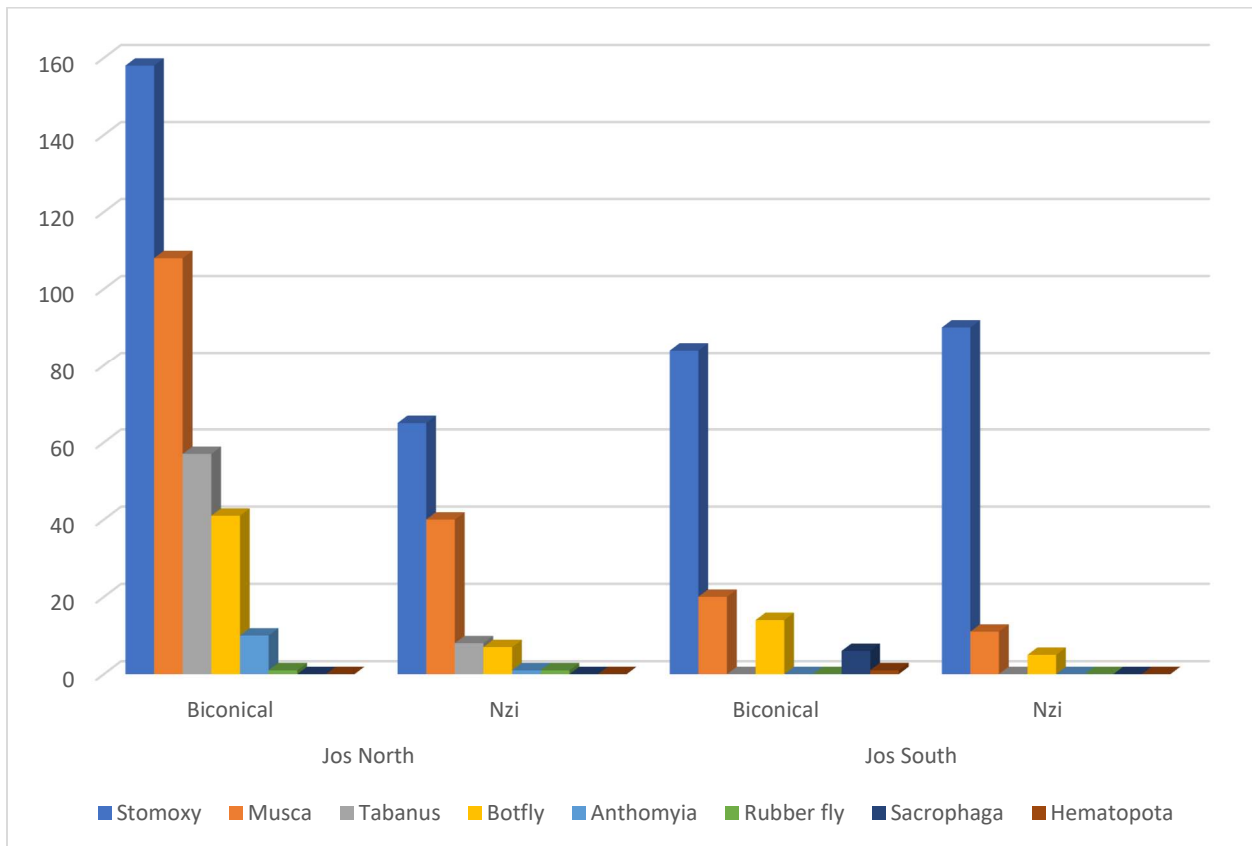
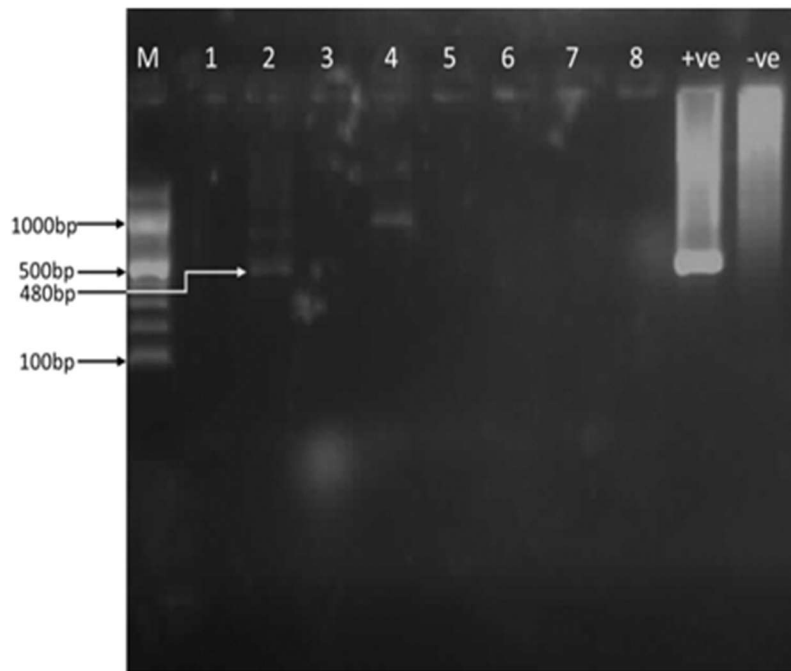


Fig. 1. Prevalence and diversity of Flies trapped in the study area based on the type of Trap

Using the biconical traps, a total of 375 flies were caught in Jos North with *Stomoxys* spp accounting for 158(42.1%) of the catches followed by *Musca domestica* 108(28.8%), *Tabanus* spp 57(15.2), Botfly 41(10.9%) and *Anthomyia* spp 10(2.7), the least is the Rubber fly 1(0.3%). The corresponding figures for Nzi trap are 65, 40, 8, 7, and 1 each for *Stomoxys*, *Musca*, *Tabanus*, Botfly, *Anthomyia* and Rubber fly, respectively (Fig. 1). In Jos South, a total of 125 flies were caught using the Biconical trap. The flies caught include; *Stomoxys* spp 84(65.2%) followed by *Musca domestica* 20(16.0%), Botfly 14(11.2%) and

*Sarcophaga* spp 6(4.8%), the least is the *Hematopota* 1(0.8%). A total number of 160 flies were caught using the Nzi traps with 90 *Stomoxys* spp as the dominant catch (Fig.1).

The molecular detection of *Trypanosoma* spp. DNA in this study showed that one sample from a pool of *Stomoxys* spp out of the seven samples obtained from the pool of hematophagous flies was positive. This sample was amplified at 480 bp which is consistent with the expected size of *Trypanosoma evansi* (Fig.2). Meanwhile, the entire DNA from the 250 cattle screened for *Trypanosoma* spp. DNA by PCR was negative.



**Fig 2. Identification of species-specific *Trypanosoma evansi* in *Stomoxys* spp on a 1.5% Agarose Gel.** Lane M 100bp DNA Marker, Lane Marked Positive; is for Positive Control and Sensitive at 480bp for *Trypanosoma evansi* while Lane 2 is Positive, Lanes 1, 3, 4, 5, 6, 7, and 8 are Negative. Lane Marked -ve, is a Negative Control.(bp= base pair (starting from 100 bp to 1000 bp),M,+ve= Positive control well, Numbers 1,2,3,4,5,6,7,8 wells )

## DISCUSSION

The role of hematophagous flies in the epidemiology of livestock diseases is increasingly becoming important. They have been incriminated or highly suspected in the mechanical transmission of viral, bacterial and protozoan diseases to man and animals (Desquesnes and Dia 2004). Several studies have attested to their role in the mechanical transmission of *Trypanosoma* species especially *T. vivax* and *T. evansi* to cattle and camels, respectively across different settings globally (Baldacchino *et al.*, 2013; Desquesnes and Dia 2004). Thus, in addition to acting as nuisance to animals, they negatively impact on cattle production. In

Nigeria, cattle continue to constitute an important part of the lives and livelihood of subsistence farmers, both as source of low-cost economic investment and a source of animal protein (Obaloto *et al.*, 2025). However, complex interactions between vectors such as hematophagous flies and disease causing organisms results to severe diseases than can cause a setback to their productivity with socio-economic consequences to the livestock owners (Peter *et al.*, 2025). In this study, the survey of hematophagous flies showed that prevalence and widespread distribution and diversity of hematophagous flies belonging to different taxonomic groups around livestock farming

communities in Plateau state. Our findings are similar to reports from other parts of Nigeria (Onyiah, 1997; Peter *et al.*, 2025). *Stomoxys* spp was the dominant hematophagous fly species caught in study area. This finding aligns with the report from a similar geographic area—Assop Falls, Plateau state (Abba, 2011)—but contrasts with the results from Kaduna state, where *Glossina palpalis* was the sole species trapped (Igube *et al.*, 2021). Differences in ecological factors might account for these results. The role of *Stomoxys calcitrans* as mechanical vector of trypanosomes has been reported (Desquesnes & Dia 2004). The detection of *T. evansi* DNA in a pool of *S. calcitrans* in this study corroborates the possible role of this fly in the epidemiology of Trypanosomiasis in the study area. Although *Tabanus* sp. flies have also been incriminated as mechanical vectors of trypanosomes, the non-detection of trypanosome DNA in this fly species may be due to the small sample size analyzed in this study. Further studies are required to exhaustively confirm these observations.

The presence of non-hematophagous flies like *M. domestica* in the study area is of great concern. These flies are efficient mechanical transmitters of important human and animal diseases such as corynebacterium, pseudo-tuberculosis and causative agent of mastitis in animals. Similarly, the presence of myiasis causing *Sarcophaga* species is of medical health importance in both human and animals (Addo, 1983).

Two types of fly traps, the Biconical and NZI traps were used in this study. This was to accommodate for preferential attraction and ensure that all the fly taxonomic groups in the study were trapped. Previous studies have reported difference in preference of certain flies for different traps as well as variation in trap efficiencies (Dankwa *et al.*, 2000; Dede *et al.*, 2005; Dandam, 2017). Our results showed that the Biconical trap

outperformed the Nzi trap in both the number and diversity of fly catches. Thus, individuals conducting fly trapping studies should consider the use of the Biconical trap as the preferred option.

Although, trypanosomosis in small and large ruminants have been reported in Jos and its environs (Idehen *et al.*, 2018; Majekodunmi *et al.*, 2013, Peter *et al.*, 2023), none of the 250 blood samples examined trypanosome infection using both the standard Trypanosome Detection Method and PCR was positive. The result indicates that there was no trypanosome infection in the cattle at the time of sampling. Similar reports of zero prevalence of trypanosome infection were reported in a study conducted in Keffi, Nasarawa State (Idahor *et al.*, 2019). Furthermore, results obtained from the dissection and microscopic examination of *Glossina* sp. collected from Bassa LGA Plateau state showed that all the flies were negative for trypanosomes thus suggesting a low prevalence of trypanosomes in ruminants in that area (Idehen *et al.*, 2018). These observations were attributed to possible changes in vector dynamics or ecosystem or anthropological changes (Dede *et al.*, 2007). Although we did not assess the breed of cattle sampled, another consideration for the absence of infection may also relate to the reported resistance of some indigenous cattle breeds to trypanosomes infections (Kalu, 1995; Tekeet, M.I *et al.*, 2013). There are reports of trypano tolerance involving N'dama and Muturu breeds of cattle which may form part of the cattle sampled in this study. More studies are required to determine the extent of the involvement of hematophagous flies in the epidemiology of trypanosomes infection in cattle in plateau state, Nigeria.

## CONCLUSION

Although no trypanosome infection was detected in cattle across the studied

agrarian communities in Jos North and Jos South, and no tsetse flies (*Glossina* spp.) were captured, the detection of *Trypanosoma evansi* DNA in *Stomoxys* spp is a significant finding. This suggests that mechanical transmission by *Stomoxys* flies may play a previously unrecognized role in the epidemiology of animal trypanosomosis in the absence of the biological vector. The presence of this fly species in high abundance highlights its potential contribution to disease persistence and underscores the complexity of trypanosome transmission dynamics in the study area. An elaborate, longitudinal study is urgently needed to evaluate the vector capacity of *Stomoxys* flies for various *Trypanosoma* species across different ecological zones in Nigeria.

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