Distribution of tsetse flies and its *Trypanosoma* species infection in Old Oyo National Park, Nigeria

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Abstract

Tsetse fly infestation in national parks is a major health risk to both the wildlife and tourists coming to sub-Saharan Africa. However, information on distribution and diversity of tsetse flies and trypanosome infection rate in Protected Areas like Old Oyo National Park in south-west Nigeria is largely unknown. Thus, the study evaluates distribution and diversity of tsetse flies in Magurba Range of Old Oyo National Park. Twelve Nzi traps were set at 50 m equidistance to capture *Glossina* species for a period of six months between February and August, 2019, considering both the altitudinal and ecological significance. A total of 136 tsetse flies belonging to four species; *G. palpalis*, *G. tachinoides*, *G. morsitans* and *G. fusca*; were captured. More *Glossina* species were captured during dry season 77.9% (70.0-84.6) than the wet season 22.1% (15.4-30.0). There was significant difference (p = 0.0001; x^2 = 84.9; *OR* = 12.5) between the proportion of *Glossina* species captured at the riverine areas (106; 77.9%) and the woodland/forest areas (30; 22.1%). *Glossina* captured at ground level and 30 cm above ground were 71 (52.2 %) and 65 (47.8%) respectively. The overall prevalence of trypanosome infection (2.94%) was observed for *Glossina* spp. The presence of infected *Glossina* spp. indicated an urgent need to establish a concise strategic vector control in National Parks, in order to reduce the risk of transmission to both wildlife and humans in the area. The park is frequently visited by tourists, rangers, researchers and students for educational purposes.

Keywords: *Glossina* spp.; *Trypanosoma* spp.; vector distribution; Old Oyo National Park.

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Introduction

Tsetse flies (*Glossina* species) are biological vectors of trypanosomes and haematophagous dipteran flies that inhabit much of tropical Africa (Farrar *et al* 2013; Odeniran *et al* 2020). They occupy an area over 10 million km^2, often referred to as tsetse belt (Bouyer *et al* 2018). In Nigeria, tsetse flies infest 75% of the total land mass covering the five agro-ecological zones. Out of the thirty-one species and sub-species described in Africa, Nigeria harbours eleven of these species and subspecies (Goodings and Krafsur, 2005; PAAT, 2008; Odeniran *et al* 2018a). They all have prominent economic impact in sub-Saharan Africa as the biological vectors of trypanosomes, which cause human sleeping sickness and animal trypanosomiasis (Farrar *et al* 2013; Odeniran and Ademola, 2018a).

Trypanosomes are protozoans of the genus *Trypanosoma* which multiply in the body fluids especially blood of the vertebrate hosts (man, domestic and wild animals) and live in the salivary glands or digestive tract of the invertebrate hosts, which are basically tsetse flies and other biting insects (Bouyer *et al* 2012). Different species of trypanosomes infect different hosts range (Odeniran *et al* 2019). Important species of trypanosomes that infect both vertebrate and invertebrate hosts include *T. congolense*, *T. vivax*, *T. brucei*, *T. grayi*, *T. godfreyi*, *T. simiae*, etc. (Isaac *et al* 2016; Odeniran *et al* 2019; Weber *et al* 2019).

Wildlife is an important natural resource, that plays a pivotal role in supporting livelihoods of communities in sub-Saharan Africa, and it is a major component of ecotourism (Odeniran *et al* 2018b). National parks provide ideal habitat for the flies and the pathogenic trypanosome, consequently the wild animal in the park and domestic livestock grazing around the periphery of parks are constantly exposed to the risk of trypanosomiasis. The occurrence of tsetse flies in national parks and game reserves where fauna and flora species are protected has been reported (Ajibade and Agbede, 2008; Okoh *et al* 2011; OIE, 213; Isaac *et al* 2016; Shaida *et al* 2018; Wama *et al* 2018).

Factors affecting the health and productivity of wild animals severely constrain the development and stability

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of the ecosystem. Trypanosomiasis transmitted by tsetse flies has been reported as important causes of morbidity and occasional mortality of wild animals (OIE, 2013). Wild animals majorly serve as reservoir hosts, while some species of tsetse fly have been associated with the presence of certain species of wildlife. Also, the presence of pathogenic trypanosomes in tsetse flies could predispose staff, tourists and researchers frequenting the parks to trypanosomiasis.

In previous report, there was a recent case of Human African Trypanosomiasis in a patient from the United Kingdom who lived greater part of active years in endemic focus of the disease in Nigeria (Luintel *et al* 2016). Okoh *et al* (2012) reported two species of *Glossina* (*G. palpalis palpalis and G. tachinoides*) in Kamuku National Park. However, information on distribution and diversity of tsetse flies and trypanosome infection in protected area like Old Oyo National Park in south-west Nigeria is rare. Since, effective Human and Animal Trypanosomiasis (HAT) and animal disease control measures rely on the determination of both tsetse and vertebrate reservoir infection rates (Cox *et al* 2005). This study seeks to provide health-based information for tourists and adequate information for future researchers and game wardens in terms of health management of the wildlife and possible re-emergence of diseases in the park.

Materials and methods

Study area

Old Oyo National Park (OONP) is one of the seven national parks in Nigeria established under the Decree 36 of 1991 (NNPS, 2004). The landmass is an estimated 2,512 km^2 in Oyo State, south-west Nigeria. The co-ordinates are between Latitude 8° 15′ and 9° 00′N and Longitude 3° 35′ and 4° 42′E (Oladeji *et al* 2012). It is surrounded by eleven Local Government Areas namely, Oyo West, Iseyin, Ifedapo, Irepo, Oorelope, Orire, Atiba, Itesiwaju, Olorunsogo, Saki West and Saki East of which all in Oyo State except Ifedapo which is in Kwara State (NNPS, 2004). The national park consists of lowland plains at heights of 330 and 508 m above the sea level. The southern part is drained by the Owu, Owe and Ogun Rivers, while the northern sector is drained by the Tessi River.

The annual rainfall in the park ranges between 900 and 1,300 mm and the mean annual temperature is between 25 and 29ÚC. It has two distinct seasons, wet season from May to October and dry season from November to April (NNPS, 2004). The vegetation of OONP has been classified as southern Guinea Savannah. The park has five ranges which are Oyo Ile, Iyemoso, Marguba, Tede and Sepeteri (Figure 1).

Marguba Range is the largest of the five ranges and located at the centre of the park (NNPS, 2004). It has Oyo Ile and Sepeteri Ranges in the north, Tede Range and Iyemoso Range in the south. It represents the core zone of the park where the tourist activities are mostly profound because game viewing and bird watching are common. Marguba Range also provide perennial water source for the animal especially at Ibuya Pool which serves as major water holes for the animals during dry season. Several wildlife species have been reported from the study-area (Afolayan, 1997; Alarape, 2002; Oladeji *et al* 2012).

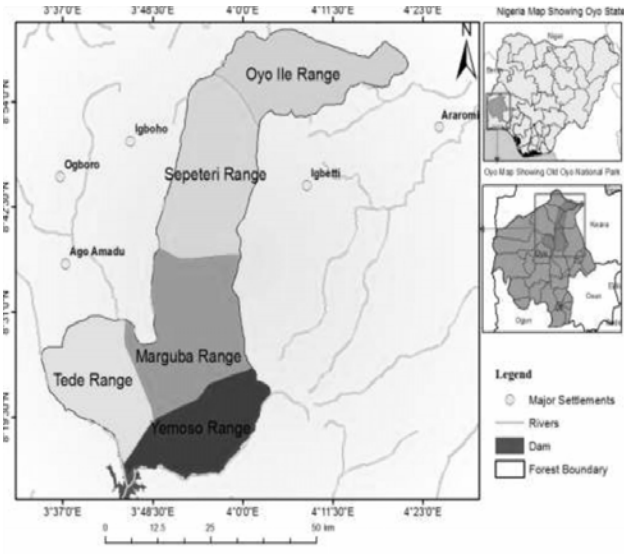

Figure 1. Map of Old Oyo National Park showing location of the five ranges.
Source: NNPS, 2004.

Sampling techniques

Twelve Nzi traps were deployed for this study. Six traps were set at different water holes along River Ogun. (Three Nzi traps each deployed were at ground level and at 30 cm above the ground, respectively; all at 50 m equidistance). In addition, two traps were set at three different woodlands (Anogeisus, Afzelia and Detarium woodlands), totaling three traps each at ground level and at 30 cm above ground, respectively. A total of twenty-four sampling days were used for this study, in other words, four days/month of collection for six months. Fly collection was done at 12 hours interval to prevent mortality and desiccation of flies. Trap positions were geo-referenced using a GPS device (GPSMAP® 60CSx Garmin) (Table 1).

Laboratory description and analysis

All trapped-flies were counted, sorted and identified using conventional identification keys (Oldyrod, 1967). The tsetse flies were identified to species level using earlier described identification techniques (FAO, 2008). The hypopygium, superior and inferior claspers were the major identification organs for sexing the flies. Morphology of tsetse flies such as length of the fly, width of head, length of head, length of thorax, length of abdomen and length of wing were measured using graph paper. Tsetse flies were dissected under the stereo-microscope after washing in buffer solution (g/l NaCl 8.0, Na_2HPO_4 1.15, KCl 0.2, KH_2PO_4 0.2, pH 7.3) (Sigma-Aldrich, Germany). The

Table 1: Geo-reference of trap locations at Old Oyo National Park.

Trap No	Latitude	Longitude	Altitude	Ecological zone
1	8°45′55.9′′N	3°77′10.4′′E	327 m	Anogeissus Woodland.
2	8°45′31.7′′N	3°77′32.2′′E	329 m	Anogeissus Woodland.
3	8°45′19.2′′N	3°77′31.6′′E	326 m	Deuterum Woodland.
4	8°45′11.9′′N	3°77′44.4′′E	327 m	Deuterum Woodland.
5	8°45′55.7′′N	3°77′24.7′′E	330 m	Afzelia Woodland.
6	8°45′52.3′′N	3°77′20.1′′E	325 m	Afzelia Woodland (Rangers' quarters).
7	8°45′18.7′′N	3°77′66.7′′E	309 m	Riparian forest (Waterhohe).
8	8°45′25.7′′N	3°77′68.5′′E	307 m	Riparian forest (Waterhohe).
9	8°45′31.6′′N	3°77′67.8′′E	310 m	Riparian forest (Waterhohe).
10	8°45′28.5′′N	3°77′66.1′′E	314 m	Riparian forest (Waterhohe).
11	8°45′31.7′′N	3°77′64.5′′E	310 m	Riparian forest (Waterhohe).
12	8°45′55.4′′N	3°77′73.7′′E	302 m	Riparian forest (Waterhohe).

proboscis, salivary glands and midgut were harvested separately. Contents of proboscis, salivary glands and midgut were stained with Giemsa on microscope slides and viewed for the presence of trypanosomes under the light microscope (Kashima *et al* 2012).

Identification of Trypanosoma species

Flies were placed in phosphate buffer solution and then pinned to an extraction box. The mid-gut, salivary glands and mouth-parts of the flies were extracted and smeared on the microscope slide. The smears were fixed with absolute methanol and stained with 10% Giemsa's stain at pH 7.2 for 15 minutes. The stained slides were then washed gently using distilled water and air-dried at room temperature. The slides were examined under microscope (Olympus®) with an oil immersion eyepiece of 100× magnification and parasitaemia determined as described by Gibson (2003). Morphological characterisation was based on position of the kinetoplast, undulating membrane, nucleus and length of the flagellum.

Statistical analysis

Data obtained were analysed using descriptive statistics (abundance and percentage of each species of flies, their sex and physiological state). Analysis of Variance (ANOVA) was used to determine significant differences between species abundance, trypanosome infected flies and ecological zones using GraphPad prism Version 5.

Results

Abundance of Glossina species trapped in Marguba Range of OONP

A total of 136 *Glossina* belonging to four species were captured between February and August, 2019. Monthly catches and distribution showed that abundance was highest in February with 42.7% (34.2-51.4), and least in August 1.5% (0.2-5.2) (Figure 2). *G. palpalis* represents 62.5% (53.8-70.7) of the total flies captured while *G. tachnoides*, *G. morsitans* and *G. fusca* have 22.8 (16.0-30.8), 5.9 (2.6-11.3) and 8.8% (4.6-14.9), respectively. More tsetse flies were captured during dry season 77.9% (70.0-84.6) compared to wet season 22.1% (15.4-30.0). Out of the 136 tsetse flies captured, 70 (51.5%) were female while 66 (48.5%) were male ($p = 0.628$).

The unfed *Glossina* species 107 (78.6%) captured during the study were more in abundance when compared ($p = 0.0001$; $x^2 = 89.5$; $OR = 13.6$) to those that were fed 29 (21.4%). Based on fly maturity, most were non-teneral 114 (83.8%) compared to the teneral flies 22 (26.2%) shown in Table 2.

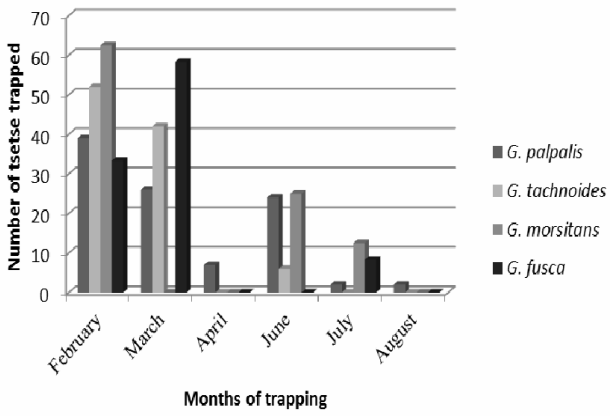

Figure 2. Monthly catches of *Glossina* species in Marguba Range of Old Oyo National Park.

Glossina captured at the riverine areas 106 (77.9%) was more in abundance when compared ($p = 0.0001$; $x^2 = 84.9$; $OR = 12.5$) to those from woodland/forest areas 30 (22.1%). No significant difference ($p = 0.417$; $x^2 = 0.53$; $OR = 1.2$) was observed between the number captured at the ground level (52.2%) and those captured at 30 cm above (51.8%) the ground level (Table 3).

Prevalence of Trypanosoma infection in wild-trapped Glossina species at Old Oyo National Park

All the 136 *Glossina* species captured were dissected for the presence of trypanosomes. Four *Glossina* species

Table 2: Characterization of tsetse flies in Marguba range, Old Oyo National Park.

Dipteran species	Sex		Season		Physiological status		Maturity	
	Male	Female	Wet	Dry	Unfed	Fed	Teneral	Non-teneral
Glossina palpalis	41	44	24	61	67	18	10	75
G. tachnoides	17	14	2	29	25	6	5	26
G. morsitans	4	4	3	5	6	2	3	5
G. fusca	4	8	1	11	9	3	4	8
Total	**66**	**70**	**30**	**106**	**107**	**29**	**22**	**114**

Table 3: Ecological preference of tsetse flies in Marguba Range, Old Oyo National Park.

Species	Ecological effect		Altitudinal effect	
	River course	woodland/forest	Ground level	30cm above ground level
Glossina palpalis	78(91.8)	7(8.2)	45(52.9)	40(47.1)
G. tachnoides	25(80.7)	6(19.3)	18(58.1)	13(41.9)
G. morsitans	1(12.5)	7(87.5)	5(62.5)	3(37.5)
G. fusca	2(16.7)	10(83.3)	3(25.0)	9(75.0)
Total	**106(77.9)**	**30(22.1)**	**71(52.2)**	**65(47.8)**

(2.94%) were infected with trypanosomes. The distribution of trypanosomes showed that three (2.20%) and one (0.74%) were *T. vivax* and *T. brucei brucei*, respectively. Out of the 85 *G. palpalis* examined, two (2.35%) were infected with trypanosomes, which were identified as *T. vivax*. While one each of 31 *G. tachnoides* (3.2%) and 12 *G. fusca* (8.3%) were infected with *T. vivax* and *T. b. brucei* respectively. However, none of the eight *G. morsitans* dissected was infected.

Out of the 70 female *Glossina* species examined, three (2.2%) were infected; two *G. palpalis* were infected with *T. vivax*, while one *G. fusca* was infected with *T. b. brucei*. Out of 66 male *Glossina* species examined, only one *G. tachinoides* was infected with trypanosomes. The incriminating trypanosome species found in the *G. tachinoides* was *T. vivax* (Table 4). The analysis of variance test showed that there was no significant difference (p=0.6993) between *Glossina* species abundance and trypanosome infection rate.

Table 4: Prevalence of *Trypanosoma* spp. infection in *Glossina species* captured in Marguba Range, Old Oyo National Park.

Glossina spp	Number examined	Percent (%)	*Trypanosoma* species		Prevalence (%)
			T.b. (%)	T.v. (%)	
G. palpalis	85	62.5	0(0.0)	2(2.35)	2(2.35)
Male	41	48.2	0(0.0)	0(0.0)	0(0.0)
Female	44	51.8	2(0.0)	2(4.5)	2(4.5)
G. tachnoides	31	22.5	0(0.0)	1(3.2)	1(3.2)
Male	17	54.8	0(0.0)	1(5.9)	1(5.9)
Female	14	44.2	0(0.0)	0(0.0)	0(0.0)
G. morsitans	8	5.9	0(0.0)	0(0.0)	0(0.0)
Male	4	50	0(0.0)	0(0.0)	0(0.0)
Female	4	50	0(0.0)	0(0.0)	0(0.0)
G. fusca	12	8.8	1(8.3)	0(0.0)	1(8.3)
Male	4	33.3	0(0.0)	0(0.0)	0(0.0)
Female	8	66.7	1(12.5)	0(0.0)	1(12.5)
Total	**136**	**100**	**1(0.74)**	**3(2.20)**	**4(2.94)**

T.b. (*Trypanosome brucei brucei*).
T.v. (*Trypanosome vivax*).

Discussion

This study revealed variation in distribution and diversity of *Glossina* species which are of medical, veterinary and economic importance. The presence of four *Glossina* species observed in the OONP may be due to the abundance of wildlife species (Okoh *et al* 2011; Wama *et al* 2015). The higher number of female flies caught compared to males may be due to blood meal necessity for ova development for most female flies. This corroborates the report of Wama *et al* (2015) that female tsetse flies were

more abundant in Ghasaka-Gumti National Park than male tsetse flies. However, it is in contrary to the report of Okoh *et al* (2011) that more male tsetse flies were captured more than females in Kamuku National Park.

The abundance of *Glossina* species captured in the dry season could be associated with the concentration of wildlife in Marguba Range, along the river course, while during the wet-season, wild animals were observed to be evenly dispersed across the green vegetation, limiting concentrated feeding of the flies. Thus, catches per fly per trap were drastically reduced in the wet-season. This finding correlates with earlier report of Okoh *et al* (2011), in which most catches were observed in dry season. On the contrary, Odeniran *et al* (2019) reported highest catches and activities of flies to be more prominent in the wet season, although; the study was conducted in cattle settlements. Hence, the aggregation of animals, vegetation type, environmental condition could contribute to the abundance and species diversity of tsetse flies (Odeniran *et al* 2020). The percentage of unfed and teneral flies were higher compared to the fed flies, which could mean that the trapping period coincides with the breeding season of tsetse flies. Poaching activities around the park could have impacted the abundance of wild animal population from which tsetse flies take their blood meal.

This study revealed that significant difference (p<0.0001) exists between *Glossina* species captured at various biotopes. More tsetse flies were captured at river course (animal waterholes) than woodland/forest area. The affinity of tsetse flies to inhabit cool area and also availability of their host along the water course typifies the abundance of the riverine species. This is in line with the study of Wama *et al* (2011) that shaded trees along the riverine area helped to maintain suitable microclimate for tsetse flies as well as habitat for their vertebrate hosts. *G. palpalis* and *G. tachnoides* which were predominant and abundant along riverine area in this study has been reported by several authors as *Glossina* of riverine species while *G. morsitans* and *G. fusca* are said to be savannah and forest species, respectively (Leak *et al* 2012).

The vertical distribution of tsetse flies in the park also revealed that more flies were captured at the ground level than at 30 cm above the ground. Though, there is no significant difference (p = 0.417) between the catches at ground level than at 30 cm above. Three of the four species of *Glossina* captured were more at low altitude (*G. palpalis*, *G. tachnoides* and *G. morsitans*) than high altitude (*G. fusca*). This corroborates reports that tsetse flies move at different altitude and height (Mavoungou *et al* 2012; Odeniran and Ademola, 2018b). Ecology of tsetse flies had been studied in the past (Isaac *et al* 2011; Wama *et al.,* 2018; Odeniran *et al* 2019), the current finding confirmed ecological preferences for many species, some being adapted to wood/forest habitats, whereas other species are more confined to the riverine. Most of the *Glossina* species tend to avoid open grasslands with discontinuous distribution determined by environmental criteria that include abiotic factors.

The findings of this study revealed two species of trypanosome – *T. brucei* and *T. vivax* in the study-area. This showed that there was an active transmission of these trypanosomes in the study-area. The prevalence was very low compared to earlier reports in some national parks in Nigeria (Okoh *et al* 2011; Isaac *et al* 2016). However, previous study conducted in OONP, reported the presence of *T. grayi*, *T. congolense* and *T. vivax* from captured tsetse flies (Weber *et al* 2019). The diagnostic techniques, wildlife component and seasonal changes could be responsible for the observed differences.

Conclusion

This study provides evidence of extensive distribution of *Glossina* spp. in the Marguba Range, OONP, with population dynamics which might be attributed to seasonal variation, availability of host and natural vegetation. It also revealed the presence of four *Glossina* species of major economic importance. Differences in *Glossina* distribution, abundance and ecology imply that different control strategies may be needed for the control of the species in the study area. The role that game reserves and other protected areas may play in sustaining tsetse populations, as well as the circulation of trypanosomes in game animals and humans and the potential dispersal of both the vector and pathogen from these areas need to be emphasised. The presence of infected tsetse flies could have serious effect on tourism and wildlife sustainability. Therefore, control efforts to sustain ecological stability and ecosystem need to be explored with concise economic guidelines (Shaw, 2013).

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