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Determination of Tsetse Fly Occurrence and Their *Trypanosoma* Infection Status in Selected Communities of Enugu South L. G. A.

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Trypanosomiasis is a debilitating public health disease affecting both humans and Animals. It is transmitted mainly by tsetse flies. The abundance of this fly in an area is correlated with the disease prevalence. This study determined the occurrence and *Trypanosoma* infection status of tsetse flies in selected communities of Enugu South Local Government Area of Enugu State. The biconical trap method was used to capture tsetse flies while sections of proboscis, midgut and hindgut wet smears were made, and microscopy was performed to investigate *Trypanosoma* infection. Ninety-one flies were captured during the study, 56(61.5%) were female, while 35(38.5%) were male. All the tsetse flies caught during the study were morphologically identical to *Glosinia palpalis palpalis*. The average daily catch was 15.17±3.43. A daily mean catches of 9.17±3.14 and 5.83±0.98 were recorded for female and male tsetse flies, respectively. There was no significant difference in

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number of flies caught in both communities (P = 0.8021). More female than male flies were caught (P = 0.0012). Twenty-two (24.18%) flies were found to harbour *Trypanosoma* sp. Infection varied according to the sex of the flies (P<0.05). Female flies were more infected than males. Tsetse fly's abundance and their infection with *Trypanosoma* is a risk for inhabitants of Enugu South local government of Enugu state. Therefore, there is a need to initiate tsetse control measures in the affected areas, and it is imperative to conduct public health education intervention on the implications of the disease and the mode of disease transmission. Further studies are recommended to ascertain the cryptic species of trypanosome prevalent in the study areas using a molecular approach.

Keywords: Trypanosomiasis; proboscis microscopy; glosinia; public health.

1. INTRODUCTION

Among the debilitating neglected tropical diseases ravaging Africa is Human Africa Trypanosomiasis (HAT), which occurs in sub-Saharan Africa [1]. The disease is caused by *Trypanosoma* sp, a slender monophyletic flagellate protozoan parasite that lives and multiplies extra-cellularly in the blood and tissue fluids of the host [2]. The disease is predominately found in Africa, hence the name African trypanosomiasis. The disease in humans is called Human African trypanosomiasis (HAT), which could be described as sleeping sickness and in animals it is called African animal trypanosomiasis (AAT) [3].

Human African trypanosomiasis is classically transmitted by the bite of blood-sucking tsetse flies (Diptera, genus Glossina) [4]. Two forms of the disease exist: the slow-progressing form, caused by *Trypanosoma brucei gambiense*, which is endemic in western and central Africa; and, the faster-progressing form, caused by *Trypanosoma brucei rhodesiense*, found in eastern and southern Africa [5]. Trypanosomes are obligatory parasites in nature usually having two hosts, namely the vertebrate and invertebrate hosts. The invertebrate host is generally biting insect-tsetse fly while the vertebrate host includes humans and other wild animals [6–8]. Trypanosomiasis, and its vector as far as humans and animals in Africa are concerned, is considered a major challenge to socio-economic development in sub-Saharan Africa. It is listed sixth about the number of causes of death among Neglected Tropical Diseases (NTDs) [9–12]. The disease could affect both males and females, young and old, including livestock, inducing severe morbidity and mortality in humans and livestock [13,14].

The major vector of HAT is the tsetse fly genus *Glossina* [2,7,15]. The vector acquires the parasites during blood meal while feeding on an infected person and its subsequent transmission when feeding on a non-infected person [16,17]. The disease is common in remote or rural areas of Africa, where most of the population are farmers and movement of herds and herders is unrestricted with consequent fly-cattle-human contact [9]. The situation is made worse because early detection of infection does appear impossible. After all, individuals do not have complete access to health facilities and qualified health personnel.

Sleeping sickness threatens millions of people in 36 countries in sub-Saharan Africa. The current report has estimated that the population at risk is 55 million people for the period 2016 – 2020: with only three million people at moderate or higher risk [3,12,18]. According to the global burden of disease, (GBD) data from the Institute for Health Metrics and Evaluation (IHME), Human African trypanosomiasis contributed an estimated five hundred and sixty-two thousand, two hundred and sixty-two disability-adjusted life years (DALYs) to the global burden of disease [19]. The disease in livestock AAT is recognized as an important constraint in about 38 countries with a high potential of producing meat, dairy products and food crops, and most particularly denies the use of cattle for transport and traction [12]. For agricultural communities, this means that only small areas can be tilled by hand, leaving the communities vulnerable to food shortages, low natural resource utilization, poor animal health, starvation and famine [13]. The economic impacts of the disease on humans, livestock, agriculture and forestry development are colossal as the vector (tsetse flies) are distributed over a wide range of habitat covering about 10 million square kilometres of potential grazing land in 38 countries extending on both sides of the equator-a third of the landmass of Africa, which are rendered unsuitable for livestock breeding and farming, [14,20,21]. Globally about 55 million

herds of cattle, 30 million sheep and 40 million goats are at risk of infection [22].

Its burden cuts across Nigeria to other sub-regions of Africa inflicting untold hardship, particularly to people in the rural communities giving rise to extreme poverty, a decrease in the quality of life and productivity of employees, hindering physical and cognitive growth, school dropout due to lack of concentration in class, disturbances in sleep pattern, maternal and child disease and even death [10,12,16,23–25]. The infection in livestock reduces milk production by 10-40%, the number of cattle by 10-50% and agricultural production by up to 2-10%. Currently, trypanosomiasis threatens the livestock sector and agricultural Gross Domestic Product (GDP) [26]. In Nigeria, Agriculture contributes 30% of the total national GDP. It's a key activity for Nigeria's economy after petroleum oil and provides livelihood to many Nigerians, [27]. Forestry policies have been hampered (Idumah et al., 2016), owing to the close relationship of vegetation to tsetse flies' distribution and their peri-urban activities in the ecosystem [17].

1.1 Statement of Problem

There was nearly total elimination of trypanosomiasis in the 1960s by colonial control programmes, but the disease has re-emerged in epidemic proportions due to wars, population movements and the collapse of healthcare systems over the past two decades [28]. Between 2008 to 2009 Nigeria was among the 10-12 African countries reporting fewer than 100 new cases per year. Following the decline of infection rates over the years, the overwhelming success achieved in the control of the disease in the past appeared to have affected the way governments perceived the disease, attention and funding dwindled over several decades, resulting in the reduction of surveillance and vector control activities [1,29].

In Nigeria, animal husbandry or livestock business is common among urban and rural dwellers. Nigeria is reported to have 19.5 million cattle, 72.5 million goats, 41.3 million sheep, 7.1 million pigs, 28,000 camels and 974,499 donkeys, [30] and the majority of this livestock at risk of AAT because they are located in tsetse-infested regions [31]. Obeagu and Akwuke are riverine towns in Enugu state which have been reported to be endemic to Trypanosomiasis [31]. Their savannah-like vegetation, topography and River system have encouraged epidemiological activities such as cattle herding, piggery, fishing, crop cultivation etc. which increases human tsetse fly contact. The towns due to their proximity to the state capital city is also hosting many cluster piggery farms and Fulani cattle herders. The availability of herders and piggery farms within the communities influences the prevalence of tsetse flies and trypanosomiasis within and around the community. There is a paucity of information regarding the current abundance of tsetse flies and their Trypanosoma status. The information available from literature search concerning this problem is negligible yet the problems of resurgence of tsetse fauna and climate change, unstable social circumstance and disruption in disease surveillance, also make updating of this information indispensable. Therefore, this study was designed to fill this entomological and socio-economic knowledge gap by determining tsetse flies' status as well as the disease burden on the lives of people in selected communities of Enugu state. The current study therefore aims to determine tsetse fly infection with Trypanosoma sp and the socioeconomic burden of trypanosomiasis in selected communities of Enugu South L G A.

2. MATERIALS AND METHODS

2.1 Study Area

The study took place in the Akwuke and Ugwuaji communities of Enugu South Local Government Area, Enugu State, Nigeria. Enugu South is among the seventeen Local Government Areas (LGA) of Enugu State, located at coordinates 6°24′N 7°30′E, and covering an area of approximately 67 square kilometers. The area experiences an average temperature of 27°C and a relative humidity of 69%. The region distinctly experiences two main seasons: the dry season from November to March and the rainy season from April to October. According to the 2006 Nigeria National Census report, Enugu South has a population of 198,723 individuals. The majority of the population is comprised of farmers, with others working as civil servants, traders, and artisans.

The two selected study communities; Akwuke and Ugwuaji are semi-urban few kilometers from the Enugu capital city. Both communities are riverine, each with a river that transverses the community. The choice of these communities was based on previous reports [16,25].

The Akwuke community is richly endowed with the Nyaba River, a vital water source for its

inhabitants and a thriving hub for herders, piggery, vegetable, and fish farming along its shores. Moreover, a section of the Nyaba River boasts an expansive sandy beach, drawing numerous tourists to its scenic beauty. The area is predominantly adorned with guinea forest trees and experiences a dominant tropical hinterland climate. With its lateritic soil and an average temperature of 26°C, the region enjoys ample surface water throughout the year, albeit experiencing temporary flooding during the rainy season. The annual average rainfall ranges from 1730 to 2500 mm.

Ugwuaji is located at latitudes 6°19'N and 6°51'N and longitudes 7°42'E and 7°51'E with enormous natural resources while residents are predominantly farmers, civil servants, herders, businessmen and women. Common rivers, such as Ine and Afa, serve the community for domestic water supply, washing, bathing, piggery, cattle rearing and fish farming. Both communities have a history of Human African Trypanosomiasis [31] hence they were selected for the current study.

2.2 Entomological Study

The study was conducted simultaneously in both communities in January 2024. Both communities were selected based on herders/cattle presence and many piggery farm clusters/settlements within the communities. Tsetse flies were captured using traps. Biconical traps were positioned at sites near various piggery farms in the selected villages in the Akwuke community (Umugwunta, Amagu, Akwuke Uwani, Umuatugbu) and Ugwuaji community (Ishiagu, Umuaro, Amauzam Umuneugwu). Each village had four traps, making a total of 8 traps. The grass around each trap was trimmed, and the traps were baited with cattle or pig urine and faeces to attract tsetse flies. The traps were checked and emptied every 24 hours for 6 days in each village. The number of tsetse flies captured in each trap was recorded and then totalled for each community. The captured tsetse flies were sexed, and their species were identified according to the method of Leak *et al* (2008). Specimens were sent to the Nigeria Institute for Trypanosomiasis Research (NITR) in Kaduna state for confirmation by a qualified entomologist. The captured tsetse flies were temporarily preserved in normal saline before dissection. They were washed twice in distilled water, dried on filter paper, and dissected on the same day of capture. Dissections were done using disposable glass slides, Dissected Salivary gland, midgut and guts were smeared in glass slides containing a drop of sterile PBS covered with a slip and observed under a light microscope while morphological identification of *Trypanosoma* species was done using Giemsa stained film at a magnification of x40. All tsetse fly specimens with trypanosome parasites were carefully documented.

2.3 Data Analysis

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0. The total occurrence of Tsetse flies and infection with Trypanosoma species were expressed as percentages. The Kruskal-Walis H test was used to compare tsetse fly occurrence and Trypanosoma infection across different communities while numerical data were presented as mean ± standard deviation. The level of significance was set at $P<0.05$.

3. RESULTS

Tsetse fly prevalence and their *Trypanosoma* infection status was investigated in Akwuke and Ugwuaji communities and the results are shown in tables. All the tsetse flies caught during the study were morphologically identical to *Glosinia palpalis palpalis* Robineau-Desvoidy, 1830 (Diptera: Glossinidae).

From Table 1, a total of 91 flies were caught at the end of the study with mean daily catch of 15.17±3.43. Out of the total number, 35 (38.5%) were male while 56 (61.5%) were female flies. A daily mean catches of 9.17±3.14 and 5.83±0.98 were recorded for female and male tsetse flies respectively. There was no significant difference in number of flies caught in both communities ($P = 0.8021$). More female than male flies were caught ($P = 0.0012$).

In Akwuke community, Umugwunta village recorded the highest number of tsetse-flies 14(31.82%) while Amagu, Uwani and Umuatugbu villages had 12(27.27%), 10(22.72%), 8(18.18%) tsetse flies respectively. The daily mean capture of 2.33±0.82, 2.0±1.10, 1.17±1.03 and 1.33±1.37 were observed for Umugwunta, Amagu, Uwani and Umuatugbu villages respectively (Table 2). There was a significant difference between captured male and female tsetse ($P<0.05$), with more female tsetse flies caught.

Table 1. Overall tsetse fly catch in both Communities

	Akwuke community			Ugwuaji community			Total		
Days	**M**	**F**	**Subtotal (%) n=44**	**M**	**F**	**Subtotal (%) n=47**	**M**	**F**	**Total (%) n=91**
1	2	6	8 (18.18)	4	4	8 (17.02)	6 (17.14)	10 (28.57)	16(17.58)
2	4	5	9 (20.45)	2	2	4 (8.51)	6 (17.14)	7 (20)	13(14.29)
3	1	4	5 (11.36)	6	3	9 (19.15)	7 (20)	7 (20)	14(15.38)
4	3	6	9 (20.45)	3	4	7 (14.89)	6 (17.14)	10 (28.57)	16(17.58)
5	2	3	5 (11.36)	2	4	5 (10.63)	4 (11.42)	7 (20)	11(12.09)
6	3	5	8 (18.18)	3	10	13 (27.66)	6 (17.14)	15 (42.86)	21(23.08)
Total	**15 (34.1)**	**29 (65.9)**	**44 (48.4)**	**20 (42.6)**	**27(54.5)**	**47 (51.7)**	**35 (38.5)**	**56 (61.5)**	**91**
Mean± SD	2.5±1.05	4.83±1.16	7.33±1.86	3.33±1.51	4.5±2.81	7.83±3.20	5.83±0.98	9.17±3.14	15.17±3.43

Table 2. Daily Tsetse fly catch per village in Akwuke Community

Day	**Umugwunta**			**Amagu**			**Uwani**			**Umuatugbu**			**Community Total**		
	M	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**G.Total (%)**
1	1	2	3	1	2	3	0	1	1	0	1	1	2	6	8(18.18)
2	2	0	2	1	1	2	0	2	2	1	2	3	4	5	9(20.45)
3	1	2	3	0	2	2	0	0	0	0	0	0	1	4	5(11.36)
4	0	3	3	2	1	3	1	2	3	0	0	0	3	6	9(20.45)
5	1	1	2	0	0	0	1	1	2	0	1	1	2	3	5(11.36)
6	0	1	1	1	1	2	1	1	2	1	2	3	3	5	8(18.18)
Total	5	9	14(31.82)	5	7	12(27.27)	3	7	10(22.72)	2	6	8(18.18)	15(34.09)	29(65.91)	44(48.4)
Dailymean±SD	**0.83± 0.75**	**1.5± 1.05**	**2.33± 0.82**	**0.83± 0.75**	**1.17± 0.75**	**2.0± 1.10**	**0.5± 0.55**	**1.17± 0.75**	**1.67± 1.03**	**0.33± 0.52**	**2.0± 0.89**	**1.33 ±1.37**	**2.5± 1.05**	**4.83 ±1.17**	**7.33 ±1.86**

Table 3. Daily Tsetse fly catch per village in Ugwuaji Community

	Ishiagu			Umuaro			Amauzam			Umuneugwu			Community Total		
	M	**F**	**Total(%)**	**M**	**F**	**Total(%)**	**M**	**F**	**Total(%)**	**M**	**F**	**Total(%)**	**M**	**F**	**Total(%)**
DAY															
1	0	0	0	3	1	4	0	1	1	1	2	1	4	4	**8 (17.02)**
2	1	1	2	0	0	0	1	1	2	0	0	3	2	2	**4 (8.51)**
3	2	1	3	1	1	2	1	1	2	2	0	2	6	3	**9 (19.15)**
4	1	0	1	2	1	3	0	2	2	0	1	0	3	4	**7 (14.89)**
5	1	2	3	0	0	0	1	1	2	0	1	1	2	4	**5 (10.63)**
6	1	3	1	0	1	1	1	3	4	1	2	3	3	10	**13 (27.66)**
Total	4	7	11(23.4)	6	4	10(21.3)	4	9	13(27.66)	4	6	10(21.3)	20(42.55)	27(57.44)	**47**
Daily mean±SD	**0.67± 0.63**	**1.17± 1.17**	**1.83± 1.21**	**1.0± 1.26**	**0.67± 0.52**	**1.67± 1.63**	**0.67± 0.52**	**1.5± 0.82**	**2.17± 0.98**	**0.67± 0.82**	**1.0± 0.89**	**1.67± 1.21**	**3.33± 1.51**	**4.5± 2.81**	**7.83± 3.20**

Table 4. *Trypanosoma* sp infection status of Tsetse flies in both study communities

	Akwuke				Ugwuaji						
Day	**Flies caught**	**Infected flies**			**Flies caught**	**Infected Flies**					
		M	**F**	**Total %**		**M**	**F**	**Total (%)**	**M**	**F**	**Total (%)**
1	8	0	1	1(12.5)	8	1	2	3(37.5)	1	3	4(25)
2	9	1	2	3(33.33)	4	1	0	1(25)	2	2	4(30.77)
3	5	1	0	1(20)	9	2	2	4(44.44)	3	2	5(35.71)
4	9	0	1	1(11.1)	7	1	2	3(42.85)	1	3	4(25)
5	5	0	1	1(20)	5	0	0	0(0)	0	1	1(9.09)
6	8	1	1	2(12.5)	13	1	1	2(15.38)	2	2	4(19.05)
	44	3(6.82%)	6(13.63%)	9(20.45)	47	6(12.77)	7(14.89)	13(27.66)	9(25.71)	13(23.2)	22(24.18)

The village prevalence of the flies in the Ugwuaji community indicated that 13 (27.66%) tsetse flies were caught in Amauzam village, while Ishiagu, Umuaro and Umuneugwu recorded 11(23.4%), 10(21.3), and 10(21.3) respectively, (Table 3). Daily mean catches of 2.17□0.98, 1.83□1.21, 1.67□1.63, and 1.67□1.21 were recorded for Amauzam, Ishiagu, Umuaro and Umuneugwu villages respectively. More female tsetse were caught than males (P<0.05) (Table 3).

The Trypanosoma species encountered in this study was Trypanosoma brucei gambiense. Among 91 flies caught, 22 (24.18%) of them were positive for Trypanosoma species. According to sex, 9 (9.89%) male and 13 (14.29%) female tsetse flies were positive for Trypanosoma. The difference between infection in male and female flies was significant (P= 0.02101). Ugwuaji community recorded 13(27.66%) Trypanosoma infected tsetse, while 9 (20.45) infected flies were recorded in the Akwuke community. Trypanosoma species infected female flies in Akwuke were more (13.63%) than the infected male flies (6.82%). Similarly, there were more Trypanosoma-infected female flies (14.89%) in Ugwuaji than the infected male flies (12.77%). There was also a significant difference between Trypanosoma infection in both sexes (P=0.05) (Table 4).

4. DISCUSSION

Tsetse flies are epidemiologically important in the transmission of HAT and AAT [17] therefore, insight into their prevalence and *Trypanosoma* status is crucial to understanding the risk of trypanosomiasis in any locality. However, there is no information on the tsetse-trypanosome infection status in Enugu South local government Area of Enugu State. Hence, tsetse occurrence and their trypanosome status are therefore poorly understood. The result obtained in the current study showed that a total of 91 tsetse flies were captured, indicating a low occurrence of Tsetse flies. The result is in line with previous reports [32] but in contrast, [33] All the tsetse flies captured were morphologically identical to *Glosinia palpalis palpalis* Robineau-Desvoidy, 1830 (Diptera: Glossinidae). This finding collaborates with previous reports [16,31,34] confirming the occurrence of the tsetse fly species in many parts of Enugu state to be *Glosinia palpalis palpalis* Robineau-Desvoidy, 1830 (Diptera: Glossinidae). The role of *Glosinia palpalis palpalis* in the transmission of trypanosomiasis has been documented severally [7,17,31,35,36] as the important vector of HAT, especially in the West Africa sub-region. The occurrence and abundance of *Glossina* sp, as recorded in this study, may not be unrelated to the presence of herders and their cattle, piggery farms and forest ecosystem within the studied locations. Unlike other dipterans, both male and female tsetse feed on vertebrate blood for survival and reproduction. The farm animals provided a blood source to the flies while the forest/bushes gave the tsetse both breeding and resting habitat. The presence of tsetse flies in any tropical forest area is an indication of the possible occurrence of trypanosome infection. Enugu is home to Fulani herders who migrated from the Northern part of Nigeria to the south in search of pastures thereby moving possibly infected herds from the north to the south.

The significant variations (P= 0.1012) in the daily catch of tsetse flies, along with the overall daily low numbers, may be attributed to unstable weather conditions. The observed increase in female catches (61.5%) could result from ongoing fragmentation of their natural habitat due to farming activities. Male tsetse flies have been noted to be particularly sensitive to suitable habitat disturbances [37,38]. Additionally, male tsetse engages in many energy-driven activities, such as migration in search of mates. This behaviour is influenced by the fact that male tsetse flies generally copulate only once and have shorter lifespans compared to females. Such lifespan differences are common among insects and are genetically determined [39–41].

Fly abundance was higher in villages close to the community river system (Umugwunta in Akwuke and Amauzam in Ugwuaji). Vegetation and the presence of water may have influenced the variation in daily average catch per village in both studied communities. This observation aligns with Isaac *et al.* (2017), who, in their study, reported that the highest number of flies was collected along waterways (p<0.0001) while the number of flies caught along river banks during the dry seasons was significantly higher than the number collected in the wet seasons (p<0.0001) The *G. palpalis* group distribution is strictly influenced by riverine vegetation and permanent pools of water [32,42].

Also, our study found that 22 (24.18%) of the 91 captured tsetse were positive for *Trypanosoma* species. This is a potential risk factor for inhabitants of the studied locations, considering the vectorial capacity of tsetse flies. This find is

similar to previous studies that observed that tsetse fly infection rates with *Trypanosoma vivax* and *T. brucei* spp were higher in West African countries, whereas tsetse infection with *T. congolense* and *T. simiae*, *T. simiae* (tsavo) and *T. godfreyi* were higher in east, central and south African countries [43,44]. The presence of infected flies is a confirmation of trypanosomiasis in Enugu, Nigeria. The occurrence of tsetse flies infected with trypanosome species could indicate possible cases of trypanosomiasis in people or animals within that study area. This finding collaborates previous reports in Enugu [45] and Gboko [46,47] which detected *Trypanosoma* infections in patients in their separate studies.

5. CONCLUSION AND RECOMMENDATION

The current study indicates a low occurrence of tsetse flies, and all the tsetse flies captured were morphologically identical to *Glosinia palpalis palpalis* Robineau-Desvoidy, 1830 (Diptera: Glossinidae). A higher number of female than male flies were caught, though no significant difference ($P<0.05$) in the number of flies caught between the two studied communities. Twenty-two (24.18%) of the 91 captured tsetse were positive for *Trypanosoma* species, a potential danger for inhabitants of the studied locations as this can drive up the transmission level.

Therefore, it is recommended that vector control and management, sensitization and public health education should be scaled up in Enugu South Local Government Area of Enugu state.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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