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Original Article

Variations of *Glossina sp.* and trypanosome species frequency within different habitats in a sleeping sickness focus, Gabon

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Abstract

Introduction: Knowledge of the infectious status of the Glossina is an indicator of risk of resurgence of Human African Trypanosomiasis (HAT). Environmental conditions have an impact on the density and diversity of both vector and Trypanosoma. The aim of the study was to determine the diversity and the infection rate of Glossina as well as the diversity of trypanosome species within habitats of an old HAT focus, in Gabon.

Methodology: *Glossina* were captured in September 2012 in three ecological sites. Vavoua traps were installed for twelve days. All captured flies were identified. Glossina were selected for trypanosome identification by Polymerase Chain Reaction.

Results: 1178 *Glossina* were captured: 55.8% in degraded forest, 28.9% in flood area and 15.4% in secondary forest. *Glossina fusca congolensis* (37%) and *G.palpalis palpalis* (36.4%) were the most abundant vector species. *G. fusca congolensis* was predominant in secondary forest and in flood area, while in degraded forest, it was *G.palpalis palpalis*. *Trypanosoma* infection rate was 30.7%, 42% in secondary forest, 32% in degraded forest and 18% in flood area. *Trypanosoma congolense savannah* was the main species detected (18.7%) followed by *T.brucei brucei* (10.7%) and *T. brucei gambiense* (4%). *T. congolense savannah* type was predominant in the secondary forest and in degraded forest (66.7% versus 55.5%).

Conclusion: *Glossina* density and trypanosome infection rate varied according to the habitat within HAT focus. The density of tsetse was the highest in degraded forest while the infection rate was highest in secondary forest. Continuous disease surveillance and control measures are needed.

Key words: *Glossina*; Trypanosome; habitat; historic focus; HAT; Gabon.

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Introduction

Human African Trypanosomiasis (HAT) or sleeping sickness is a disease of public health importance that occurs only in sub-Saharan Africa. It is on track of elimination, with a significant reduction of the number of new cases over the last 15 years. In 2016, there were about 2, 200 new cases of HAT [1]. However, the risk of a resurgence may persist in old foci. Indeed, disease transmission requires the simultaneous presence of three components: the parasite, trypanosome (*Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense*), the vector, tsetse fly (*Glossina spp.*) and the host (*human or animal*) which may be located in an appropriate habitat. Population of vectors and hosts as well their biodiversity are in fact modulated by local environmental conditions that can substantially affect their dynamics and influence the disease transmission patterns and impact. Environmental changes due to human activities, such as land use leading to deforestation and erosion may affect interactions between the host, the parasite and the vector [2]. In Malawi, a change in *Glossina* distribution has been observed, following an expansion of human population and concomitant destruction of the vegetation; tsetse flies were mostly located in areas without important vegetation disturbance [2,3]. Thus, host availability for *Glossina* and habitat suitability comprising an appropriate vegetation cover are essential to provide adequate breeding grounds and shelter under adverse climatic conditions [3,4].

Sleeping sickness is often present in areas where *Glossina* are abundant [5]. In Central African regions, *Glossina* lives in vegetation close to water sources, such

as forests, forests gallery, secondary forest, degraded forests, flood area, river banks and lakes, swamps and mangroves, coffee or cocoa plantations, and can even adapt to environmental changes (mainly the species of *G. palpalis*), surviving in peri-urban areas of medium and large towns and in areas of intensive agriculture [6]. However, the spatial dynamics of the vector and the associated vector infections may vary between these different types of habitat encountered in foci and depending on the amount of favorable resources present.

In Gabon, historical foci of HAT are known and are distributed in almost the whole country. The average number of new cases reported annually since 2001 is less than fifty [7]. New cases are mainly notified in two provinces: Estuaire and Ogooué-maritime, which are regularly screened by the national HAT control programme (PNLTHA reports) [8,9]. Little information is available about the current situation of HAT in other foci, although infected tsetse flies have been recently detected and *Trypanosoma* species identified in the old focus of the Ogooué Ivindo [10]. In Lambaréné, in the Moyen Ogooué province located in the centre of Gabon, in an old focus of HAT, an entomological survey showed a high abundance and diversity of *Glossina* species [11]. In this focus, different types of habitats are encountered: secondary forest, degraded forest and flood area. However, the infectious status of the vectors is not known, although the potential risk of HAT resurgence exists in this area and may vary depending of the type of habitat.

The aim of our study was to determine the diversity and the infection rate of tsetse flies as well as the diversity of trypanosome species within the old HAT focus of Lambarene in Gabon according to the type of habitat (secondary forest, degraded forests, and flood area).

Methodology

Study site, capture method and species identification

Tsetse flies were collected in September 2012 during the rainy season, in a rural area located at 80 km south of Lambaréné capital city (0°42'05'' S-10°14'04''E), of the Moyen Ogooué Province, in Gabon. Fifteen Vavoua traps were used during twelve consecutive days to capture the flies in different ecological areas; i) secondary forests which correspond to forests regenerated after the destruction of primary forest by human activities ii) degraded forests which are regenerated forests after the destruction of primary forests under the effect of natural phenomena; and iii) flood area which is a delimited geographical location that has been covered by water during a flood [12,13]. The geographical coordinates of each trap were recorded using a Global Positioning System (GPS). Each trap was activated before 7 am and withdrawn after 5 pm. Tsetse flies were identified morphologically, counted and classified using entomological keys [4]. Collected flies were preserved in alcohol 90°C and stored at room temperature for further analysis.

Sample preparation and DNA extraction

As previously reported, a dissection was done [10]. Tsetse flies were dried at room temperature, and cut transversally with a sterilized blade between the abdomen and thorax [11]. The abdomen of each fly was collected in a 1.5 mL Eppendorf tube, homogenized with a pipette tip in 100 µL of sterile water and then centrifuged at 10,000 rpm for 5 minutes. Then, 100 µL of 1% Chelex (Bio-Rad, Marnes la Coquette, France) was added to each tube and mixed by pulse using a vortex [14]. The homogenized mixture was incubated at 56°C for 1 hour and at 95°C for 30 minutes. After centrifugation at 13,000 g for 10 minutes, the supernatant containing nucleic acids extract was collected and stored at -20°C for further molecular analyses.

Molecular characterization

Trypanosome identification was performed using polymerase chain reaction [10]. Three pairs of specific primers were used to detect *Trypanosoma congolense forest type* (TCF 1/2), *T. congolense savannah* (TCS 1/2) and *T. brucei* sl (TBR 1/2). Each amplification reaction was performed in a final volume of 25 µl containing 10 pmol of each primer, 1 unit of Taq (*Thermus aquaticus*) DNA polymerase, 1X Taq Buffer (Thermo Fisher Scientific, Scotland, UK) with KCl, 1.8 mM of $MgCl_2$ and 200µM of each desoxynucleotide. After the amplification, the PCR products were analyzed on 1.5 % agarose gel which was subsequently stained with ethidium bromide and visualized under UV light [10].

Data analysis

Data were recorded on Excel spreadsheets and analyzed using Vassar Stats: Website for Statistical Computation (Vassarstats.net, Vassar College, Poughkeepsie, New York, USA). The Z-test was used to compare infection frequency according to sites. The sex-ratio (Number of males/ Number of females) and the apparent density of tsetse flies per trap and per day (ADT) to assess the relative abundance of tsetse flies at

each trapping site, were calculated: ADT = C/TD (where C is the number of tsetse flies caught, T the number of traps deployed and D the number of days of trapping). Infection rate was expressed as percentage: number of infected flies/the total number of dissected flies. The diversity of tsetse species in the different biotopes was assessed using The Margalef Diversity Index: D = (S-1) / log N, (where "S" is the number of species and "N "is the total number of individuals harvested) [15].

Results

Entomological surveys

During the entomological surveys, 1178 tsetse flies were caught in the 15 traps: 657 (55.8%) in the degraded forest, 340 (28, 9%) in the flood area and 181 (15.4%) in the secondary forest. Tsetse flies apparent density per trap was 6.05 flies/trap/day. It was highest in the degraded forest (10.9 flies/trap/day), while in the flood area, flies density was 5 flies/trap/day and in the secondary forest, 3 flies/trap/day (p = 0.0001). The Margalef index was 1.06 in the degraded forest; 1.32 in the secondary forest and 1.18 in the flood area. The sex ratio was 0.75. Female tsetse flies were significantly more frequent than males within forest areas, while in flood plains males outnumbered (p = 0.04) (Table 1).

Four *Glossina* species were identified. *Glossina fusca congolense* (37%; n = 436) and *Glossina palpalis palpalis* (36.4%; n = 429) were the most abundant, followed by *Glossina nashi* (16.7%; n = 197) and *Glossina frezili* (9.8%; n = 116). All species were found in the different areas (Table 1). According to the study sites, *Glossina palpalis palpalis* was predominant (44.3%) in the degraded forest, whereas it was *G. fusca congolense* subspecies in the secondary forest (47%) and in the flood zone (33.3%).

Trypanosoma *infection rate and* Trypanosoma *species identification*

For molecular analysis, 150 *G. palpalis palpalis* were randomly selected, 50 per site. *Trypanosoma* DNA was detected in 30.7% (46/150) of the flies. The infection rate was higher in flies collected in the secondary forest (42%; n = 21), as compared to the degraded forest (32%; n = 16) and flood zone (18%; n = 9) (p = 0.002). Three mixed infection containing *Trypanosoma congolense savannah* and *Trypanosoma brucei brucei* were found. *Trypanosoma congolense savannah type* (n = 28; 18.7%) and *Trypanosoma brucei brucei* (n = 16; 10.7%) were the most commonly identified species (Table 1). *Trypanosoma brucei gambiense* was found in 6 mid-guts of *Glossina palpalis palpalis*. No tsetse flies were infected by *Trypanosoma congolense forest type*. *T. congolense savannah* type was predominant in the secondary forest and in degraded forest, while in flood area, the most frequent *Trypanosoma* species detected were *T. brucei brucei* and *T. congolense savannah* (Table 1).

The frequency of *Trypanosoma* infection was 35.6% (32/90) in females and 23.3% (14/60) in males (p = 0.1). In degraded forest, males were more commonly infected by *Trypanosoma* than females (40% *vs* 2%), but in the secondary forest almost all infected *Glossina* (n = 20) were female. In flood area, no tsetse male was infected.

Discussion

In the present study, an important population of tsetse flies was captured. Flies density and trypanosome infection varied according to the habitat, with highest tsetse densities in degraded forest area, and highest infection rates in secondary forest. Tsetse flies, major vector of human and animal African Trypanosomiasis, require specific climatic conditions (temperature and

Table 1. Tsetse diversity and *trypanosoma* infection rate according to environmental condition in old *trypanosoma* focus in Moyen Ogooue, Gabon.

	Secondary forest	Degraded forest	Flood area	P-value	Total
***Glossina* species**					
G. palpalis palpalis, n (%)	66 (36.5)	291 (44.3)	72 (21.2)	0.0001	429 (36.4)
G. fusca congolense, n (%)	85 (47)	238 (36.2)	113 (33.3)	0.007	436 (37.0)
G. nashi, n (%)	25 (13.8)	80 (12,2)	92 (27.1)	0.0001	197 (16.7)
G. frezili, n (%)	5 (2.7)	48 (7.3)	63 (18.6)	0.0001	116 (9.8)
Total	181	657	340		1178
***Trypanosoma* species**					
T. congolense savannah, n (%)	14 (66.7)	10 (55.5)	3 (42.9)	0.003	27 (58.7)
T. brucei brucei, n (%)	4 (19.0	6 (33.3)	3 (42.9)	0.008	13 (28.3)
T. brucei gambiense, n (%)	3 (14.3)	2 (11.2)	1 (14.2)	0.6	6 (13)
Total	21	18	7		46

F: Female; M: Male; G: *Glossina;* T: *Trypanosoma.*

humidity) for their survival, reproduction and propagation [16].

A previous study reported slightly lower numbers in the secondary forest of the Ivindo National Park (NPI) in Gabon using the same methodology [11]. The density of tsetse flies was found to vary according to the type of habitat, being higher in the degraded forest compared to the flood area and secondary forest. In all these sites, the sample collection was performed during the same period and using an identical methodology. The significantly higher ADT observed in the degraded forest might be due to the presence of vertebrate hosts [17]. Indeed, the distribution of mammals, the type of soil, human activities, the breeding sites of tsetse flies as well as temperature, humidity, sunshine influence the abundance of tsetse flies in a given biotope [18,19]. These factors may vary within each site that could explain the different abundance of the tsetse flies between degraded forest, flood area and secondary forest.

The variation in host density is an important driver of heterogeneity in tsetse population dynamics. Indeed, the number of tsetse caught is correlated with the number of hosts in the field [20]. The lowest density of tsetse flies captured in the secondary forest may be due to lower wild host densities resulting from human activities [20]. Environmental modifications can have a direct or indirect impact on the biodiversity of vectors and hosts (humans and animals). The flies density obtained during this study is similar to that obtained in the forest areas of West Africa [21].

Tsetse (genus *Glossina*) consists of more than 30 species or sub-species which occupy a wide range of habitats in sub-Saharan Africa. In the focus of Lambaréné, *G. fusca* were predominant, representing the two third of the captured flies. Nevertheless, their distribution was different depending on the habitat; tsetse flies of the *fusca* group were more frequent in the secondary forest and in the flood area. It has been reported that abundance of *fusca* flies is observed in non-anthropized environments [22]. This species is mainly found in forest area and its frequency drops severely in areas where human activities are deployed as observed in the present study. Indeed, there is an inverse relationship between the density of the human population and that of the *fusca* group underlying the importance of wild animals which are preferential hosts for *fusca* flies [17,22].

In contrast, *G. palpalis palpalis*, the main vector of Human African Trypanosomiasis, is a species found in different type of vegetation. In the present study, the predominance of this species in the degraded forest, may be related to the presence of appropriate hosts. According to the type of habitat, it has been reported that host densities may drop below those required for sustaining tsetse populations, as illustrated by a difference of the flies' density between protected areas and farmland, for example [20].

The overall tsetse infection rate for trypanosome was about 31% and can be considered as high. This frequency is similar to previously reported data from Makokou in an old HAT focus, but is higher to that recorded, in the active focus of sleeping sickness of Komo-Mondah in Gabon [8].

The different trypanosome species identified in this study are similar to those reported in tsetse flies in other African regions. In secondary forest and degraded forest, most trypanosoma infections were due to *T. congolense* as found in most of southern Africa countries [23,24]. In this study, the tsetse fly infection rate was higher in secondary forest than in the other sites although the fly apparent density and *Glossina palpalis palpalis* prevalence were highest in the degraded forest.

Considering the environmental features, secondary and degraded forests resulting from human activities are characterized by small trees and herbs that attract many hosts as well as by relatively high temperatures and a better sunshine [25]. Sy (2011) demonstrated that flies kept at a higher temperature had a significantly higher mature infection rate than flies kept under normal breeding conditions [26,27]. Moreover, it has been shown that the trypanosoma infection rate in *Glossina* increases significantly with fragmentation [26]. Indeed, increased fragmentation generally rises the temperature at which tsetse flies are exposed, this is in favor of a relation between maximum daily temperature and overall tsetse infection rates as observed in secondary forests [27].

The present findings also showed that trypanosome prevalence is different between male and female flies. These results are similar to those found elsewhere [9,28]. The infection was due to three species and subspecies: *T. congolense savannah* predominated, followed by *T. brucei brucei* and *T. brucei gambiense*. The high frequency of *T. congolense savannah* type in the forest areas (secondary or degraded forest) suggests that this species can adapt to this environment. These findings are similar to those obtained in the Campo in Equatorial Guinea, in Malanga in Democratic Republic of Congo and in Ogooue Ivindo in Gabon [10,24,29,30]. *T. brucei gambiense* frequency did not differ according to the environmental conditions, while

T. brucei brucei frequency was high in degraded forest and flood area.

Conclusion

This study shows a variation of the abundance, the diversity and the infectious status of tsetse flies according to the type of habitat within a HAT foci constituted of degraded, secondary forests and flood zone. Infection rate was the lowest in degraded forest and flood zone, suggesting that they are the less appropriate environments for parasite transmission.

Despite the reduced number of human cases, infection risk is still high in the area, and constant surveys, disease surveillance and control measures need to be considered to further contribute to the elimination of HAT.

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