Molecular Screening of *Trypanosoma* spp. in *Glossina*, *Stomoxys* and Tabanids in the Moukalaba Doudou National Park (South-West, Gabon)

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Abstract: Studies on the knowledge of the pathogens responsible for trypanosomosis remain insufficient in Gabon, particularly in protected areas, such as the Moukalaba Doudou National Park (MDNP). In order to uphold ecotourism in the Moukalaba Doudou National Park (MDNP), an entomological and parasitological study was carried out during the rainy season, from February to March 2017, to identify the trypanosome species harbored by tsetse, stable fly and tabanid vectors in this protected area. Fly collection was carried out using Vavoua and Nzi traps. Trypanosome infections in the flies were detected using the polymerase chain reaction (PCR). In total, seven species of tabanids, five species of tsetse flies and two species of stable flies were collected. These vectors harboured six species of trypanosomes, namely *Trypanosoma simiae* Tsavo (48%), *T. theileri* (17%), *T. simiae* (14%), *T. brucei s.l.* (10%), *T. congolense* (9%) and *T. vivax* (2%). The trypanosome infection rate (IR) varied somewhat among sites. It was 82% in the forest, 92% in settled areas and 95% in the savanna. The high prevalence of trypanosomes in this area raises the possibility of some trypanosomosis transmission risk to humans and animals, but the risk appears to be very low given the low frequency of *T. brucei s.l.* Studies on the blood meals typing could be done to identify sources and estimate the real risk of infection.

Keywords: Biting flies, trypanosomes, PCR, National Park, Gabon.

INTRODUCTION

African trypanosomes are responsible for Human African Trypanosomiasis (HAT), also known as sleeping sickness, and African Animal trypanosomosis (AAT). These parasitic diseases are transmitted by insect vectors, namely tsetse, *Glossina* spp. [1]. Mechanical transmission is theoretically possible through other biting insects such as tabanids and *Stomoxys*, but has yet to be demonstrated [2]. According to the World Health Organization (WHO), about 65 millions of people were exposed to this disease in 2017. Moreover, because of their blood feeding trait, these vectors represent a great menace to human and animal populations. Their direct nuisance (disturbance and predation) and their role as vectors of various pathogens pose a real threat to the survival of humans and wildlife [3-6]. In addition, vector-borne diseases account for more than 17% of infectious diseases and cause more than one million deaths each year [7]. In addition, the distribution of these parasitosis is a function of the distribution of their vectors [8]. Therefore, the implementation of strategies to combat these vector-borne diseases necessarily requires a better knowledge of their bio-ecology and above all, the identification of dangerous pathogens they can transmit to humans [1,3,4].

In Gabon, studies on the knowledge of the pathogens responsible for trypanosomosis remain insufficient, particularly in protected areas, including the MDNP [8]. However, studies conducted in this Park have shown the presence of several species of insect vectors including tsetse, tabanids and *Stomoxys* [9-11]. These insects are known to be major biological and mechanical vectors of trypanosomes in several localities [2, 8-11]. According to the OMS [1], less than

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E-ISSN: 2310-0796/18

10 000 new cases of trypanosomiasis are recorded each year in many countries, including Gabon. In Central Africa, trypanosomosis risk is strongly related to the concomitant occurrence of parasite reservoirs (humans and animals) and biting flies that serve either as biological and/or mechanical vectors of HAT [12]. To analyze the risk of trypanosomosis transmission, Gouteux [12] used the infection rate approach. This is one of the indices commonly used to demonstrate the expression of contact of a biting fly with a reservoir of parasites. This index provides information on the potential transmission of the disease and allows a better understanding of the epidemiology of HAT and AAT [12]. The objective of this study was to identify the different species of trypanosomes that occur in hematophagous flies and to determine their infection rates.

MATERIAL AND METHODS

Study Area

This study was conducted in the MDNP. It is located in the Nyanga province, southwest of Gabon (Figure **1**). This Park covers almost 5028 km^2 and harbors several types of habitats, the most important of which are wetlands, savannas, forests, rocky areas and caves [13]. The climate of this region is of the equatorial type and is characterized by a dry season that lasts for four to five months (May to September) and a rainy season of seven to eight months (October to April or May) and is practically uninterrupted [14, 15]. The floristic landscape of the region includes many species that are not seen or rarely found elsewhere in Gabon [13]. As for wildlife, it is represented by several species of large mammals including buffalos (*Syncerus caffer nanus*), elephants (*Loxodonta africana cyclotis*), chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla*) and duikers (*Cephalophus sp*.).

Trapping of the Hematophagous Flies

Trapping was carried out from February to March 2017 (Great rainy season) using Vavoua traps [16] and Nzi traps [17]. These two types of traps were placed in three different habitats characteristic of MDNP, namely forest, savanna and more settled environments. Thus, the trapping design consisted of setting up 30 traps in the three habitats, with 10 traps per biotope (*i.e.* 5 Vavoua and 5 Nzi). Traps were set about 500 meters apart at points previously identified along a transect corresponding to an anthropogenic gradient from the forest to the settled environment (Doussala village) through the savanna. Traps were activated in the

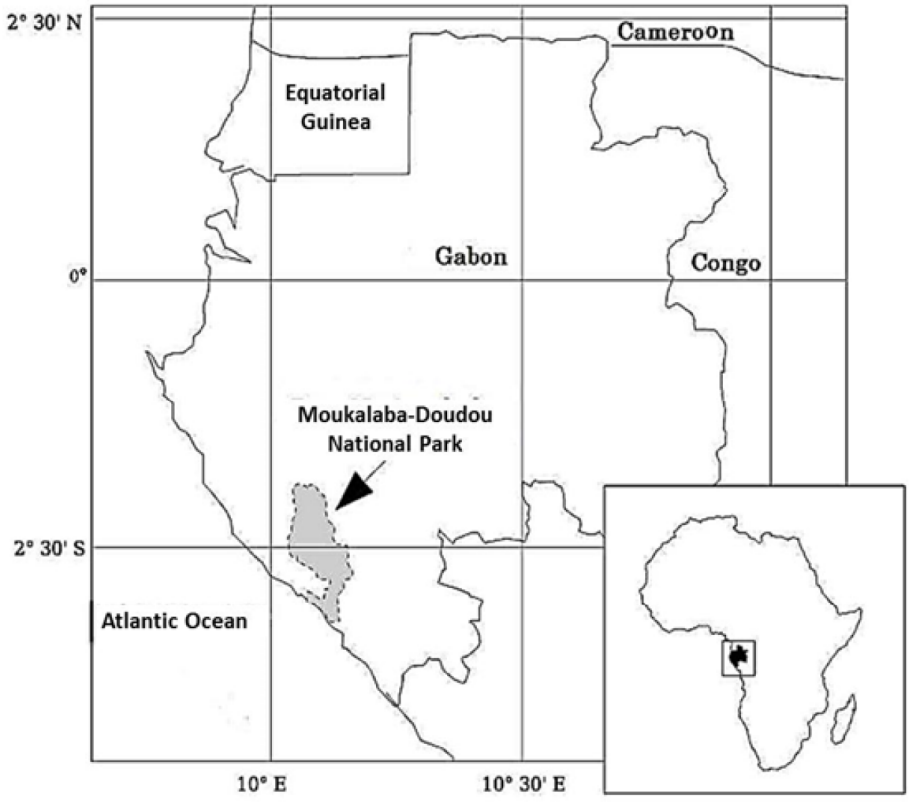

Figure 1: Location of the study area [54].

morning (7:00 AM) and collection was made in the evening (6:00 PM). The collected flies were brought to the laboratory for identification.

Identification of the Biting Fly Species

In the laboratory, flies were sorted into *Glossina*, *Stomoxys* and Tabanids. Subsequently, they were identified under a binocular microscope using identification keys published by Oldroyd [18-21], Zumpt [22], Surcouf and Ricardo [23], Garros *et al*. [24], Matyas [25] and tsetse identification software [26]. Finally, individual flies belonging to each of the three groups of hematophagous flies were counted.

Identification of *Trypanosoma* spp.

All captured flies (n = 2270) were used for molecular studies using the PCR technique. Flies were previously crushed in batches of 2 to 10 specimens according to the species and the habitat using polycarbonate tubes (OPS Diagnostics LLC, USA) containing a steel ball and 600 µl of phosphate buffered saline (PBS) 1X (GibcoTM, Canada). They were crushed at 1500 rpm for 2 min using a Geno/Grinder 2000 ball mill (Corporate Headquarters, Ukraine). Extraction of the total DNA was performed using 10 mg of fly suspension using the PureLink Genomic kit according to the manufacturer's instructions. The DNA was stored at -80°C until analysis. Thus, *Trypanosoma* spp. identification was performed using a nested PCR using the ITS primers [27] targeting the partial 18S, ITS1, 5.8S, ITS2 and partial 28S regions. In the initial PCR, the intergenic sequence primers (ITS1 and ITS2) were used to amplify the partial intergenic sequence region of the ribosomal DNA (rDNA) of the trypanosomes [28]. The amplification of the DNA was carried out in a final reaction volume of 25 µl, containing 2.5 µl of 10X PCR buffer (Platinum® Taq DNA Polymerase, Invitrogen); 2 µL of dNTPs (10 µM each), 2 µL of $MgCl_2$ (50 mM), 0.24 µL of each primer (ITS1 and ITS2, 10 µM each), 0.3 µL of platinum Taq enzyme (Platinum® Taq DNA Polymerase, Invitrogen), 12.72 µL of RNase-free water (Invitrogen) and 5 µl of DNA. Moreover, during the second PCR, the primers of the intergenic sequence (ITS3 and ITS4) were used. Amplification was carried out in a final volume of 25 µL containing 2.5 µl of 10X PCR buffer (Platinum® Taq DNA Polymerase, Invitrogen), 2 µl of dNTPs (10 µM each), 2 µl of $MgCl_2$ (50 mM), 0.24 µL of each primer (ITS3 and ITS4, 10 µM each), 0.3 µL of platinum Taq enzyme (Platinum® Taq DNA polymerase, Invitrogen), 16.72 µL of RNase-free water (Invitrogen) and 1 µL of initial PCR product. The amplification conditions for both PCRs consisted of 7 min pre-denaturation at 95° C followed by 35 cycles of 1 min at 94° C, 1 min at 56° C, and 2 min at 72° C and a final elongation at 72° C for 10 min. The amplification products were visualized by electrophoresis using 2% agarose gel.

Statistical Analyses

The infection rate (IR) was calculated according to the following formula:

$$IR = \frac{\text{Number of infected fly groups}}{\text{Number of screened fly groups}} \times 100$$

In addition, to better understand the distribution of the different species of biting flies caught in the three biotopes surveyed, one-way ANOVA and the Kruskal-Wallis statistical tests were carried out using the R software (version 3.2.2) with a significance level of 5%.

RESULTS

Abundance and Distribution of Biting Flies

A total of 2270 biting flies was captured in this study. These flies were heterogeneously distributed among the three habitats. In fact, maximum catches were observed in the forest with 885 individuals, *i.e.* 39% of the total number of catches, followed by the settled area with 852 individuals *i.e.* 38% of the total catches. Savanna was the least abundant site with 533 individuals (23%). However, the one-way ANOVA statistical test showed that there was no statistically significant difference with respect to the distribution of these flies according to the three habitats (p=0.15). Thus, the 2270 captured biting flies were divided into 191 pools (with 2 to 10 specimens per pool), including 74 pools in the forest, 74 pools in the settled environment and 43 pools in the savanna. The majority of the pools were obtained in the forest and in the settled environment (39%). The minimum pool was obtained in savanna with 22%.

Abundance of Biting Fly Groups

In terms of taxonomy, tsetse flies were the most abundant group with 1600 specimens, of which 580 were in forest, 650 in the settled area and 370 in savanna (Figure **2**). Tabanids were averagely represented with 210 individuals, 130 in the forest, 20 in the savanna and 60 in the anthropized environment. *Stomoxys* were the least abundant group with 100, 30

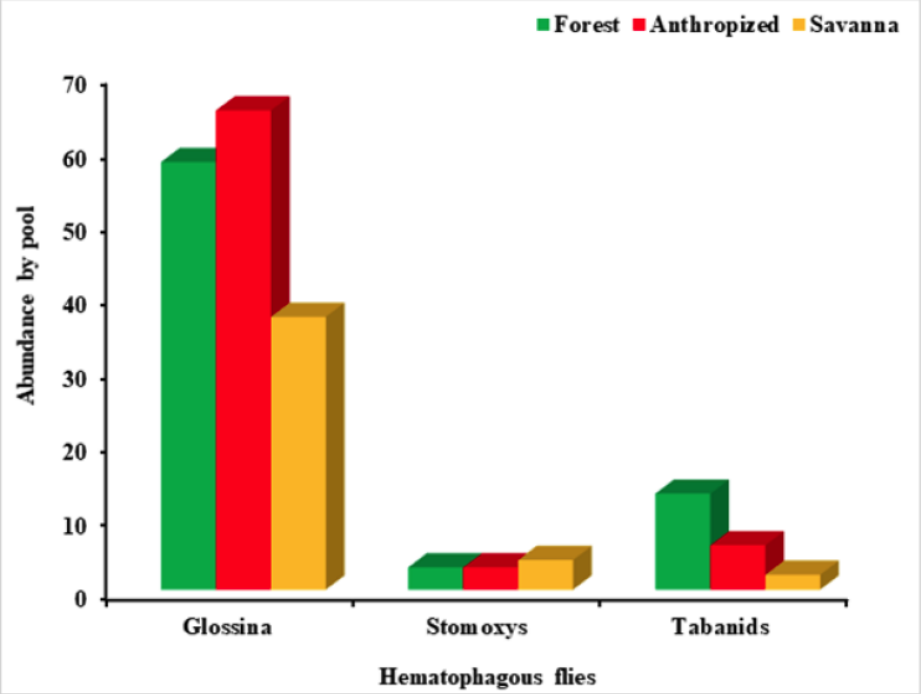

Figure 2: *Glossina, Stomoxys* and Tabanids abundance by pool in relation to prospected sites.

in forest, 30 in the anthropized biotopes and 40 in the savanna. Also, the one-way ANOVA showed that there was a significant difference in the distribution of abundance by taxonomic groups (*Glossina*, *Stomoxys* and Tabanids) (p = 0.0008).

Species Composition of the Hematophagous Flies

For this study, 191 biting flies were identified and divided into 14 species including 7 species of tabanids, 5 species of tsetse flies and 2 species of *Stomoxys*.

Tabanids were the most diversified taxonomic group with seven species including *Tabanus disjunctus*, *Tabanus ruficrus*, *Tabanus taeniola*, *Tabanus sp.*, *Tabanus par*, *Chrysops longicornis* and *Haematopota pluvialis*. However, the distribution of these species varied according to the prospected sites. In fact, in the forest, six species were identified, namely *T. disjunctus* (38%), *T. taeniola* (23%), *T. ruficrus* (15%), *Tabanus sp* (8%), *T. par* (8%) and *C. longicornis* (8%). In the settled environment, 5 species of tabanids were collected including *T. taeniola* (33%), *T. disjunctus* (17%), *T. ruficrus* (17%), *C. longicornis* (17%) and *H. pluvialis* (17%). The savanna site was the least diversified biotope with 2 species: *T. disjunctus* (50%) and *T. ruficrus* (50%). However, the non-parametric Kruskal-Wallis test showed that there was no statistical significant difference in the distribution of tabanids within the three prospected habitats (p=0.30).

Similarly, in this study, five species of tsetse flies were identified. They were heterogeneously distributed in the surveyed habitats. In fact, in the forest, five species were observed, namely: *Glossina nashi* (47%), *G. frezili* (29%), *G. f. fuscipes* (19%) and *G. p. palpalis* (3%) and *Glossina* spp. (2%). In the savanna, five species were caught including *G. f. fuscipes*, *G. p. palpalis*, *G. f. fuscipes*, *G. frezili* and *Glossina* spp. *G. f. fuscipes* (65%) was the most abundant species, followed by *G. p. palpalis* and *G. nashi* with relative proportions of 14% and 11% respectively. However, *G. frezili* (8%) and *Glossina* spp. *(*3%) were uncommon. In the settled environment, 3 species were collected, *G. f. fuscipes*, *G. p. palpalis* and *G. frezili*. *G. f. fuscipes* (83%) was the most abundant species unlike *G. p. palpalis* (15%) and *G. frezili* (2%) that were scanty. However, the one-way ANOVA parametric test showed that there was no statistical significant difference in mean tsetse distribution across the prospected habitats (p = 0.47).

Finally, *Stomoxys* constituted the least diversified taxonomic group with only two species identified, namely *Stomoxys n. niger* and *S. omega*. The distribution of these two species varied according to the study site. Indeed, *S. n. niger* was more abundant in anthropized and savanna with 67% and 50% respectively. In contrast, in the forest, *S. n. niger* (33%) was poorly represented. As regards *S. omega*, it was strongly represented in the forest and savanna, with

67% and 50% respectively. In the settled environment, this species was poorly represented.

Trypanosoma spp. Composition in the Screened Flies

The results of the molecular screening of the hematophagous flies from the three study sites revealed the presence of six species of Trypanosomes. These species were *Trypanosoma simiae* Tsavo (48%), *T. theileri* (17%), *T. simiae* (14%), *T. brucei s.l.* (10%), *T. congolense* (9%) and *T. vivax* (2%).

Infection Rate of Biting Flies in the Study Sites

The overall IR from pools of biting flies in the different study sites was 89%. However, this rate and the species richness of the parasites varied according to the surveyed sites. In fact, in the forest, the IR was 82% for six species of trypanosomes. In the settled environment, it was 92% for five *Trypanosoma* spp. In the savanna, the IR was 95% for five *Trypanosoma* spp.

Trypanosome Infection Rates in the Biting Flies Collected from the Savanna

The IR of the identified biting flies groups varied according to the fly vector species and the prospected sites. Indeed, in the savanna, five species of trypanosomes were identified in tsetse flies, namely *T. simiae* Tsavo, *T. simiae*, *T. theileri*, *T. congolense* and *T. brucei s.l.* All these trypanosomes were found in *G. f. fuscipes* while *G. p. palpalis* harboured three species of trypanosomes, especially *T. simiae* Tsavo, *T. theileri* and *T. brucei s.l. G. nashi*, *G. frezili* and *Glossina* spp. harboured only *T. simiae* Tsavo at different rates (Table **1**). *Trypanosoma simae* Tsavo was the most common trypanosome species in the screened flies. In *Stomoxys*, *S. n. niger* and *S. omega* only harboured *T. simiae* Tsavo, with infection rate of 100% for each of them. In tabanids, *T. disjunctus* and *T. ruficrus* harboured *T. simiae* Tsavo and *T. simiae*, with IR of 100% for each.

Trypanosome Infection Rates in the Biting Flies Collected from the Forest

In the forest, six *Trypanosoma* spp. were identified, including *T. simiae* Tsavo, *T. simiae*, *T. theileri*, *T. congolense*, *T. brucei s.l.* and *T. vivax* (Table **2**). The distribution of these trypanosomes and IRs varied according to the species of flies caught. Indeed, for tsetse flies, *G. nashi* and *G. frezili* harboured six species of trypanosomes, with variable IRs (Table **2**). *Glossina f. fuscipes* harboured *T. simiae* Tsavo and *T. theileri* while *G. p. palpalis* harboured *T. theileri* and *T. simiae* at varying IRs. *Glossina* spp. harboured only *T. simiae* Tsavo. *Trypanosoma simiae* Tsavo and *T. theileri* were the most abundant trypanosome species in these insect populations. Three *Trypanosoma* spp. were identified in *Stomoxys* namely *T. simiae* Tsavo, *T. simiae* and *T. congolense*. *Stomoxys omega* harboured *T. simiae* (50%) and *T. congolense* (50%). *Stomoxys n. niger* harboured only *T. simiae* Tsavo (Table **2**).

Four Trypanosoma spp. were identified in tabanids including T. simiae Tsavo, T. congolense, T. simae and T. theileri. Tabanus ruficrus harboured T. simiae Tsavo

Table 1: Trypanosome Infection Rate of Flies Caught in the Savanna

Flies species	Number of pool screened	*T. simiae* Tsavo	*T. simiae*	*T. theileri*	*T. congolense*	*T. brucei s.l.*	*Uninfected*
Glossinidae							
Glossina f. fuscipes	24	15 (63%)	7 (29%)	1 (4%)	1 (4%)	5 (21%)	1 (4%)
Glossina frezili	3	2 (67%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)
Glossina p. palpalis	5	4 (80%)	0 (0%)	1 (20%)	0 (0%)	3 (60%)	0 (0%)
Glossina spp.	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Glossina nashi	4	4 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stomoxyinae							
Stomoxys n. niger	2	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stomoxys omega	2	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tabanidae							
Tabanus disjunctus	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tabanus ruficrus	1	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 2: Trypanosome Infection Rate of Flies Caught in the Forest

Flies species	Number of pool screened	*T. simiae* Tsavo	*T. simiae*	*T. theileri*	*T. congolense*	*T. brucei s.l.*	*T. vivax*	*Uninfected*
Glossinidae								
Glossina f. fuscipes	11	6 (55%)	0 (0%)	4 (36%)	0 (0%)	0 (0%)	0 (0%)	1 (9%)
Glossina frezili	17	6 (35%)	2 (12%)	6 (35%)	5 (29%)	1 (6%)	1 (6%)	1 (6%)
Glossina p. palpalis	2	0 (0%)	2 (100%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Glossina spp.	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Glossina nashi	27	9 (33%)	4 (15%)	7 (26%)	6 (22%)	2 (7%)	2 (7%)	5 (19%)
Stomoxyinae								
Stomoxys n. niger	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stomoxys omega	2	0 (0%)	1 (50%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Tabanidae								
Tabanus disjunctus	5	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	4 (80%)
Tabanus ruficrus	2	1 (50%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Tabanus spp.	1	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tabanus taeniola	3	0 (0%)	0 (0%)	2 (67%)	0 (0%)	0 (0%)	0 (0%)	1 (33%)
Tabanus par	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Chrysops longicornis	1	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

(50%) and T. congolense (50%). Trypanosoma theileri was identified in T. disjunctus, T. taeniola and Tabanus spp. with IRs of 20%, 67% and 100% respectively. Trypanosoma simiae was only identified in Chrysops longicornis.

Trypanosome Infection Rates in the Biting Flies Collected from the Settled Environment

In the settled environment, six *Trypanosoma* spp. including *T. simiae* Tsavo, *T. simiae*, *T. theileri*, *T. congolense*, *T. brucei s.l.* and *T. vivax* were identified with variable IRs depending on the fly species. Indeed, in tsetse flies, *G. f. fuscipes* harboured all the six species of trypanosomes at varying IRs (Table **3**). *Glossina p. palpalis* was infected with *T. simiae* Tsavo, *T. theileri* and *T. brucei s.l.* while *G. frezili* only harboured *T. simiae*. However, *T. simiae* Tsavo was the most abundant trypanosome species in these flies followed by *T. simiae* and *T. theileri* with IRs of 63%, 17% and 15% respectively. Other trypanosomes including *T. congolense* and *T. vivax* were very little identified (Table **3**).

Table 3: Trypanosome Infection Rate of Flies Caught in the Settled Environment

Flies species	Number of pool screened	*T. simiae* Tsavo	*T. simiae*	*T. theileri*	*T. congolense*	*T. brucei s.l.*	*T. vivax*	*Uninfected*
Glossinidae								
Glossina f. fuscipes	54	34 (63%)	9 (17%)	8 (15%)	2 (4%)	6 (11%)	1 (2%)	4 (7%)
Glossina frezili	1	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Glossina p. palpalis	10	7 (70%)	0 (0%)	3 (30%)	0 (0%)	4 (40%)	0 (0%)	0 (0%)
Stomoxyinae								
Stomoxys n. niger	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stomoxys omega	2	0 (0%)	1 (50%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)
Tabanidae								
Tabanus disjunctus	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Tabanus ruficrus	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Haematopota pluvialis	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Tabanus taeniola	2	1 (50%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Chrysops longicornis	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

For *Stomoxys*, *S. omega* was the species that harboured most of the trypanosomes. On the one hand, it was infected by *T. simiae* and *T. congolense* with IRs of the order of 50%. On the other hand, *S. n. niger* harboured only *T. simiae* Tsavo. In Tabanids, two trypanosome species were observed namely *T. simiae* Tsavo and *T. theileri*. *T. simiae* Tsavo was identified in *T. taeniola*, *T. ruficrus* and *C. longicornis* with IRs of 50%, 100% and 100% respectively. *T. theileri* was the only trypanosome species found in *T. taeniola* with an IR of 50%.

DISCUSSION

The present study represents preliminary findings on the infection of tsetse flies (*Glossina*), *Stomoxys* and Tabanids by trypanosomes in the MDNP. However, these results cannot be considered exhaustive as the study was conducted in the rainy season and for a relatively short period. In addition, the combination of Vavoua and Nzi traps used in this study maximized the capture of biting flies in the protected sites. However, the combination of other trap (tetra large and small, pyramid, Malaise, etc.) and the addition of attractants such as octenol alone or in combination with phenols [17, 29] might have improved the number of flies caught. This study also revealed the presence of six species of trypanosomes that will possibly circulate between the vertebrate fauna and the insect vectors (tsetse flies, *Stomoxys* and Tabanids) in the MDNP. The PCR technique used in this study for the detection and identification of trypanosomes has a very high sensitivity and specificity [27, 30]. Indeed, this technique makes it possible to detect the specific DNA of trypanosomes that is harboured by hematophagous flies. In addition, previous studies conducted by various authors have demonstrated, using the PCR technique, the presence of trypanosomes particularly in hematophagous Diptera, after the dissection of target organs under the microscope [31, 32]. The abundance and distribution of biting flies varied according to the sampled sites. These results could be related to the differences in landscape of the prospected environments that can generate specific microhabitats that are more or less favorable to the development of each of the fly-groups identified [33]. These results are similar to those obtained by Dibakou *et al*. [9], Mounioko *et al*. [10], Doumba *et al*. [34], Mounioko *et al*. [2] and Zinga-Koumba *et al*. [35]. These authors observed the influence of landscape differences on the distribution of hematophagous flies. High catches of tsetse flies and tabanids have been made in the forest and in the village, unlike the savanna where they have been poorly obtained. As for *Stomoxys*, they were more numerous in forest and savanna. It seemed that in the MDNP, the forest constituted one of the most favorable environments for the development of these threegroups of biting insects. This distribution could therefore be due to the stability of the forest environment (omnipresence of humidity, more or less constant temperature, presence of rivers, *etc*.) and the abundance of large vertebrate fauna which served as host for the hematophagous flies in this biotope [36]. On the other hand, in the other two environments, there was an erratic and temporal presence of vertebrate fauna, hence their low numbers in these biotopes. The tsetse flies caught in this study possessed anthropophilic, zoophilic and ubiquitous feeding traits [37]. They are involved in the transmission of HAT and AAT [38, 39]. Indeed, *G. p. palpalis* is known to be a good vector of trypanosomes of the subgenus *Trypanozoon* [40]. On the other hand, *G. f. fuscipes* constitutes a major vector of HAT [41]. In addition, *G. nashi* and *G. frezili* are involved in the transmission of AAT.

Some of the tabanid species (*T. par, T. taeniola*) identified in the study are mechanical and biological vectors of several pathogens (viruses, bacteria, protozoa and helminths). Indeed, studies conducted by Kasbari [42] showed that tabanids were mechanical vectors of *T. evansi* and *T. vivax*. Similarly, for *Stomoxys*, the mechanical transmission of several trypanosome species including *T. vivax*, *T. congolense* and *T. evansi* are related to *S. n. niger* and *S. omega* [4].

Trypanosome species that are pathogenic to humans and animals are cyclically transmitted by the tsetse fly and acyclically by other biting flies including *Stomoxys* and tabanids which are known mechanical vectors of the disease [43]. Thus, trypanosome infection of biting flies in MDNP may suggest the endemicity of trypanosomiasis in this protected area with wild animals as reservoir hosts [44]. In fact, six *Trypanosoma* spp. were harboured by tsetse flies, Tabanids and *Stomoxys* in the study. Numerous studies conducted by several authors in Africa have shown that blood-sucking insects, including *Stomoxys*, tabanids and tsetse flies take their blood meals from various vertebrate hosts such as the golden cats (*Caracal aurata*), moustac monkeys (*Cercopithecus cephus*), crowned guenon (*Cercopithecus monapogonias*), pigs (*Sus scrofa domestica*), giant pangolins (*Manis gigantea*), mongoose (*Bdeogale nigripes*) and man (*Homo sapiens*) [4, 37]. These

vertebrate hosts are reservoirs of parasites including trypanosomes [4, 37, 45, 46]. However, the MDNP is very rich in animal species that are blood hosts of hematophagous flies. Finally, one of the major consequences of the feeding behavior of these insect vectors is probably the exchange of pathogens between humans, hematophagous flies and wild fauna [47, 48]. The presence of *T. brucei s.l.* in some tsetse fly species including *G. f. fuscipes* could constitute a potential health risk for the human population present in the study area. In addition, the identification of *T. simiae*, *T. congolense* and *T. vivax* demonstrates that these insects feed on several vertebrate hosts [43, 49, 50]. In addition, tsetse elimination may not be sufficient to eradicate trypanosomosis in areas where mechanical transmission could be enhanced by Tabanids and *Stomoxys* [51]. Therefore, the origin of these infections requires the analysis of blood meals to identify potential reservoirs of these pathogens [52, 53].

CONCLUSION

The present study identified six species of trypanosomes including *T. simiae* Tsavo, *T. theileri*, *T. simiae*, *T. brucei s.l.*, *T. congolense* and *T. vivax* that are silently circulating in the Moukalaba Doudou National Park. The transmission of these *Trypanosoma* spp. is ensured by several species of tsetse flies, Tabanids and *Stomoxys* that live in sympatry in this study area. Thus, the presence of these hematophagous flies infected with trypanosomes testifies the existence of trypanosomosis transmission risk and suggests more investigations in order to put in place effective control strategies against the disease and its insect vectors.

ACKNOWLEDGEMENTS

This work was realized thanks to the institutional, financial and logistic support of the Agence Universitaire de la Francophonie (AUF), the Institut de Recherche en Ecologie Tropicale (IRET-CENAREST), the Université des Sciences et Techniques de Masuku (USTM), the Laboratoire d'Ecologie Vectorielle (LEV-IRET) and the Centre International de Recherches Médicales de Franceville (CIRMF). We sincerely thank Mr. Logno Nzaba Roye for his invaluable technical assistance in the field.

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Received on 01-08-2018 Accepted on 07-09-2018 Published on 12-11-2018

DOI: http://dx.doi.org/10.12970/2310-0796.2018.06.10