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Tsetse flies ecology and trypanosomes circulating in the Manoka Island, Littoral-Cameroon

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Abstract
The insularity of Manoka is *a priori* favorable to trypanosomiasis eradication. Our purpose is to determine the spatio-temporal distribution, dispersion of tsetse, hosts-vectors contact areas and identifying circulating trypanosomes for efficient trypanosomiasis control. Flies were sampled using pyramidal traps during the main dry, the small and the main rainy season. They were identified, sexed, density evaluated and dissected for tenerals determination and blood meals collection. Specific PCRs were carried out for species confirmation, hosts and trypanosomes identification. *Glossina palpalis palpalis*, predominant and *Glossina caliginea* were found in Manoka. Densities were higher in mangroves and did not significantly vary between seasons. Flies were actives from 8 am to 6pm with a peak between 12 and 4 pm and a dispersal capacity up to one kilometer. Humans and wild animals were feeding hosts while *Trypanosoma congolense* and *Trypanosoma vivax* infested these flies. Mangrove seems to be the biotope to prioritize in tsetse control campaigns.

Keywords: Tsetse, ecology, formal focus, vector control, Manoka-Island

Introduction
Trypanosomiasis are parasitosis whose causative agent belongs to the genus Trypanosoma [1], and its vector, a bloodsucking dipteran of the genus Glossina Wiedemann, 1830 [2]. It affects both humans and animals in an area of more than ten million square kilometers in Africa. *Trypanosoma brucei gambiense* [1] determines the human's chronic disease form in West and Central Africa while *Trypanosoma brucei rhodesiense* [3] circulating in East and Southern Africa determines the acute form. World Health Organization (WHO) estimates that 65 million people are still at risk of trypanosomiasis with a low proportion of under medical monitoring [4]. *Trypanosoma cogonlense, Trypanosoma simae, Trypanosoma brucei brucei* affect animal whit a disease known as Nagana. At least 17,500 new cases are reported every year in meat production areas [5], generating a shortfall of nearly $ 4.5 billion each year [6, 7]. Trypanosomiasis control is based on: the correct administration of drugs to diagnosed cases and vector control, which allows interrupting transmission. This is linked to vectorial capacity of tsetse flies, their feeding hosts behaviour and ecological facies in which they evolve [8, 9]. It is therefore necessary to know the bio-ecology of vectors for more efficient vector control; and to always update these data because they evolve with environmental changes, anthropogenic pressure and climate change, which could inevitably impact the epidemiology of trypanosomiasis. Sampling and identifying tsetse flies, studying transmission parameters can provide a better understanding of trypanosomiasis epidemiology in order to identify threats for a successful vector control strategy including better planning of methods and activities that fit into local realities. In Cameroon, several foci of human african trypanosomiasis are no longer active, mainly because of integrated disease control and environmental changes. Some resurgent outbreaks a few decades ago, such as those of Bipindi, Campo, Fontem and Doumé, have been the subject of several studies [10-18]. However, in former foci, HAT remains neglected while the bio-ecological conditions prevailing may allow maintenance of host / vector / parasite system; which could, in some conditions lead to resurgence [19]. It is therefore necessary to frequently monitor these formers foci to update data on vectors, hosts, parasites and transmission parameters. In Manoka the last entomological survey took place more than 40 years ago [20]. The present article updates data on Manoka's tsetse fauna, theirs spatial distribution, dispersion, host contact areas and circulating trypanosomes for an efficient vector control in this area where it seems possible because of insularity.

Materials and Methods

Study site

This study was conducted on Manoka island (03°47'N; 09°37'E), located south of the Youpwé landing stage in Douala. It is the sixth district of the Wouri Department in the Littoral Region of Cameroon. Manoka, the largest Cameroonian island makes 15 kilometers long and 8.5 kilometers wide [20] and is home to just over 40,000 Cameroonians and Nigerians [21], fishing and selling those product as main economic activity. The climate is tropical type with two seasons: one dry from November to February and one rainy from March to October. Local rain average is 3600 mm/year and 27 °C respectively for rainfall and temperature while relative humidity average is 85% [22]. The vegetation is characteristic of those of mangrove areas, composed of shrubs of coastal forests, dominated by trees of the Rhizophoracae and Aviceniacae families. The soil is sandy and consists of quartz grains with low organic matter content [23]. The area is largely marshy, whit a hydrographic network consists of small rivers that converge towards the sea [23]. The island's wildlife consists of primates, rodents, reptiles and domestic animals are represented by pigs, sheep, goats and dogs.

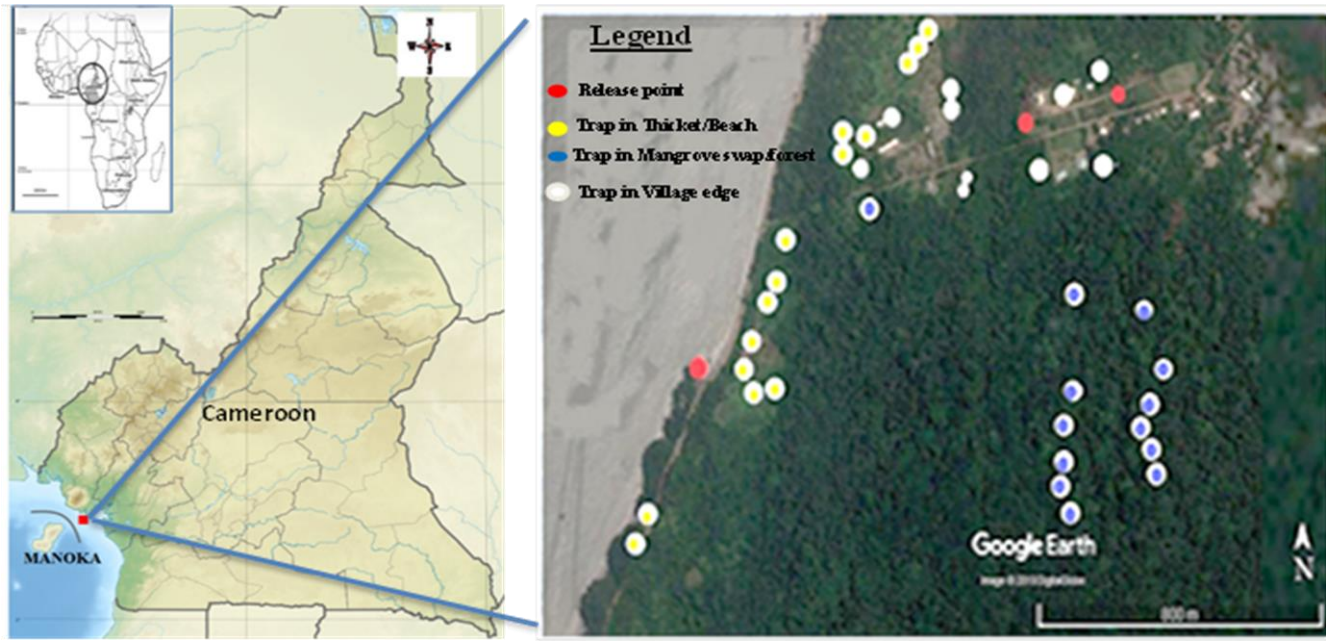

Fig 1: Numerical model of Manoka and localization of capture and release points of tsetse

Sampling of tsetse flies

Tsetse flies were sampled in biotopes that could shelter them. It was done during the low rainy season in June 2017, the high rainy season in September 2018, and the low dry season in December 2018 using the Gouteux et Lancien [24] pyramidal traps based on visual attraction principle using blue and black colors. For each survey, fifteen pyramidal traps were deployed during four successive days, ecological characteristics and geographical coordinates of every biotope and capture point were recorded. Flies were collected every two hours from 8am to 6pm. They were labeled and brought back to the field laboratory for their morphological identification, sexing [25, 26] and dissection of sips for blood meal collection and sorting of teneral. These specimens have been preserved as well as blood meals for subsequent molecular analyses.

Determination of spatial dispersion

Tsetse flies dispersive behavior was assessed using the mark-release-recapture method of Gouteux *et al.* [27]. For each session, flies were captured during the first four days, then flushed on rabbits and marked with different color of *gouache* according to Jackson [28] so that each fly could be identified by an individual number. Flies were secondary released at least one kilometer from their capture location. Three days after release, traps were removed to allow tsetse flies to disperse, and then relocated during another three days for recapture.

Molecular identification of tsetse, their hosts and hosted trypanosomes

Prior to molecular analysis, DNA was extracted from whole tsetse and from blood meals with Cetyl Trimethyl Ammonium Bromide (CTAB, 5%) buffer according to Navajas *et al.* [29] method. Tsetse species identity were confirmed by diagnostic PCR according to Dyer *et al.* [30] while trypanosomes was identified by nested-PCR according to the Desquesnes *et al.*, [31] protocol. Feeding hosts where identified by heteroduplex PCR as described by Boakye *et al.* [32], improved by Njiokou *et al.* [33]

Data analysis

Data were entered into a 2010 Excel® matrix and analyzed by PAST software. The exact Fisher probability and the Ordered Analysis of Variance (ANOVA) were used to compare proportions. Differences were considered significant for values of $P \leq 0.05$. Apparent density of tsetse per day (ADP) was evaluated according to the relationship: DAP=C/PJ where C is the number of tsetse caught, P number of traps installed in the biotope and J number of caught days. Population size was estimated using the Lincoln index (N) according to the relationship: N M (n+l)/r+l where M is the number of tsetse tagged and released, n the total number of tsetse tagged and r the number of tsetse tagged and recaptured. Distance covered by the tsetse between release and recapture point was obtained by applying the Pythagorean theorem from geographical coordinates. Human/tsetse contact index (P) of

Laveissière *et al*. [34] was evaluated using the relationship: P = k x n x n x C0.23/P J (1. 23) where K is a constant equal to 632, n the number of human blood meals collected, C the number of dissected flies, P the number of traps used and J the number of caught days. The transmission risk index (r) of Laveissière et Grébaut, [35] was assessed by the relationship: r = k (T + 1) x n2 x C0.46/PJ 3.69 where k is a constant equal to 632, T i the apparent density of general tsetse, n the number of meals of human blood collected, C the number of dissected tsetse, P the number of traps installed and J is the number of caught days.

Results

Composition of tsetse fauna in Manoka island

A total of 799 tsetse flies were captured during our survey. *Glossina palpalis palpalis* (96.3%, N = 770) and *Glossina caliginea* (3.6%, N = 29) belonging to the subgenus Nemorhina composed the tsetse fauna of the island whit a significantly more abundance of *G p. palpalis* (P = 0.0004333) was found. Teneral flies accounted for 22.8% (N = 24). The sex ratio was 2.12 and varied significantly in favor of females (P = 0.002803) which accounted for 67.9% (N = 543) of the sample and males 32.04% (N = 256).

Spatio-temporal distribution of tsetse flies

The overall apparent density of tsetse on Manoka Island was 3.80 F/T/D (Table 1). These ADP was significantly different between biotopes (p= 0.02) but not between seasons (p = 0.3996) (Table 1).

Table 1: Apparent density of tsetse flies according to biotopes and seasons M/V= Mangrove/Forest; VE= Village edge; T/B= Thicket/Beach;

Session	June			September			December		
Facies	M/F	**VE**	**T/B**	M/F	**VE**	**T/B**	**M/F**	**VE**	**T/B**
Glossina p. palpalis	2.95	2.25	1.3	2.49	0.66	1	2.7	1.1	1.89
Glossina caliginea	0.2	-	-	-	0.02	0.1	0.04	0.08	1.7
Total	2.39			3.8			5.08		
	3.8								

Dispersion of tsetse flies

For this study, 344 tsetse flies were tagged and released. Of these, 15 have been recaptured, with a recapture rate of 4.36% (Table 2) and an estimated tsetse population of 17,200 on the island. The greatest distance traveled reached the kilometer achieved by a male. The forest with 60% (N = 9) of recaptured flies appears to be the most attractive facies.

Table 2: Frequency of recaptured tsetse according to biotopes and seasons. NFL= Number of flies released; NFR= Number of flies recapture; M/V= Mangrove/Forest; VE= Village edge; T/B= Thicket/Beach;

Session	September			December			Total
Biotopes	**M/F**	**T/B**	**VE**	**M/F**	**T/B**	**VE**	
NFL	167			177			344
NFR	3 (20.50%)	1 (6.00%)	1 (6.00%)	6 (41.00%)	1 (6.00%)	3 (20.50%)	15 (100%)

Daily activity cycle of tsetse flies

Figure 2 shows the tsetse flies activity cycle on Manoka Island. This activity was uni modal and flies were active from 8 am and 6 pm with the most important peak of activity observed between 12 and 4 pm. The mangrove/forest in which a peak was observed at 12pm is the biotope where the highest activity was recorded.

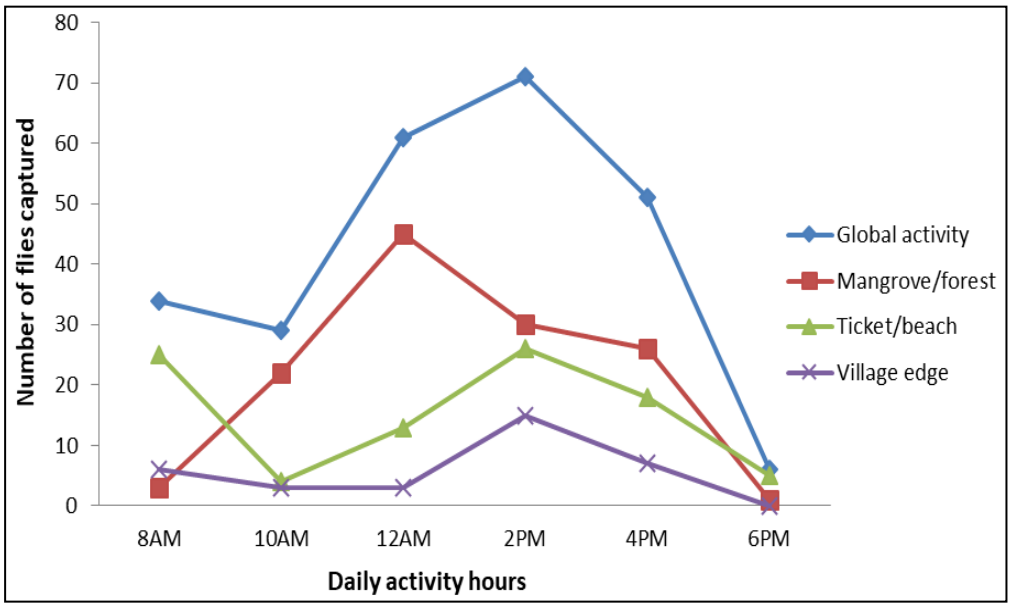

Fig 2: Daily activity of tsetse flies in biotopes in Manoka

Tsetse feeding hosts and transmission risk index

Eight blood meals were collected and analyzed. Only two of them were successfully identified, they were of human origin and came from tsetse caught in the mangrove. The overall transmission risk index of Laveissière *et al*. and the hosts/vector contact index were respectively 0.2 and 27.58 at Manoka. In mangrove/forest, the only biotope where these index could be calculated we found 7.58 and 82.25 respectively. It was not possible to determine the values of these index at the edge of the village and the thicket / beach faults of human blood meal identified.

Table 3: Transmission risk index on Manoka M/V= Mangrove/Forest; VE= Village edge; T/B= Thicket/Beach; C: number of dissected tsetse flies; p: number of traps used; j: number of days of capture; P: Man / tsetse contact; n: number of meals of human blood taken; r: risk transmission index; T: apparent density of teneral flies.

Transmission parameters	Biotopes		
	M/V	VE	T/B
C	63	45	26
ADP	3.5	2.25	1.62
T	0.6	0.45	0.18
N	2	0	0
n/C (%)	3.17	-	-
P	82.28	-	-
R	7.58	-	-

Trypanosomes circulating in Manoka

After PCR amplification for trypanosomes identification five (5) flies belonging to *G. palpalis palpalis* specie were found infested. The overall infestation rate was 4.76%. Two trypanosomes species belonging to two subgenera have been identified. It was *Trypanosoma congolense* (Nannomonas) with a prevalence of 0.95% and *Trypanosoma vivax* (Duttonela) with a prevalence of 3.80%. Parasites identified were from the forest 60% and from the thicket/beach 40%.

Discussion

This study revealed the presence of *Glossina caliginea* and *Glossina palpalis palpalis* in Manoka as that carried out by Eouzan et Ferrara [20]. Their maintenance could be explained by favourable ecological conditions, including relatively high humidity, vegetation cover and hosts presence, . Mangroves may favoured developpement and local distribution t of *G. caliginea* [26, 36] while *G. p. palpalis* adapts well and rapidly in various biotopes [16, 37]. Sea may acts as a barrier for migrations and establishment of other species on this island. *G. p. palpalis* (96.3%, N = 770) was significantly more abundant than *G. caliginea* (3.6%, N = 29). This predominance may be due to its less ecological conditions requirement, its ability to live in anthropogenic environments and its food eclecticism [38]; unlike *G. caliginea* [17], which big game scarcity could explain this proportion decrease [39, 40]. (3.9% VS 30% obtained in 1978). Indeed, most ecologically demanding tsetse species densities decrease with time, habitat degradation, temperature [13]. Apparent densities did not significantly vary depending on the season when it is known that it depends on its factors vegetation and feeding hosts presence [20, 41]. Relative humidity low variation between seasons in Manoka would largely explain this non-significant variation between seasons. Mangrove had the highest densities. In this biotope, wild animals, and vegetation cover would be favourable to tsetse flies breeding [42, 43, 44] different of strong winds of beach, low attendance of hosts or anthropoization effect observed in thicket/beach and village edge.

We fund an unimodal daily activity; were from 8am to 6pm with a peak of activity between 12 and 4pm. Tsetse flies are diurnal [20, 51] and the relatively high and permanent humidity on the island is conducive to day activity with a peak that corresponded to that of sunshine. These results corroborate authors who have shown brightness effect (warms hours) on catch abundance [45, 46]. Tsetse dispersion depends on many factors.The most described is season while many remains unknown. [47]. Although they have shown the greatest distances travelled were those of females [50, 51, 52], a male travelled the greatest distance was recorded during rainy season in September. This confirms Nash and Page [48]; Cuisance *et al*. [49] observations, who noted that tsetse disperses the furthest during high rainy season. Mangrove/forest vegetation and ecological characteristic allows return of more release flies to this biotope [49].

Only 25% of the blood meals collected and analyzed were successfully identified.. Two human blood meals have been identified in *G. p. palpalis*; this eclectic subspecies also heavily feed on humans even when its wilds hosts animals are present [53, 54]. Host-tsetse contact index and transmission risk index values have been determined only in forest/mangrove, but it is possible to observe these indexes in all this site. Moreover, inhabitants activities in mangroves / forest (logging, hunting and fishing), at beaches (fishing, swimming and passage) and village edge (fields, passage) reflect human/ tsetse contact and therefore a transmission risk. Sleeping sickness transmission risk is even greater when human presence in tsetse distribution area is sustainable [55]. This behavior could lead to dangerous epidemiological situations, given the often-reported low circulation of *T. b. gambiense.*

Analysis revealed for the first time *Trypanosoma. congolense* and *Trypanosoma vivax* among tsetse flies in Manoka. It is This presence could be explained by common and ubiquitous distribution [56, 58]. Their presence has often been reported in several areas in Cameroon [59, 53, 60]. The prevalence of *T. congolense* was 1.09% and 3 times lower than that of *T. vivax*. Indeed, *T. vivax* has a lower pathogenicity than *T. congolence* [61]; this allows a greater survival of its hosts [62], increases the chances of encounter between its hosts and tsetse beetles. We recorded no *Trypanosoma brucei gambiense* infestation. However, our sampling does not allow us to conclude on the absence of this species in the area; its complex development life cycle means that their prevalence in vectors rarely higher than 1 per 1000, even in transmission areas [63, 64].

Conclusion

This study conducted in Manoka island in Wouri former HAT focus shows that ecological conditions involves in this locality are still favourable to the maintain of trypanosomiasis host/vector/parasite system. Two species of the genus Glossina were found on the island. They were infested by *T. vivax* and *T. congolense.* Thet had humans and wild animals as feeding hosts. *Glossina palpalis palpalis* and *Glossina caliginea* were actives from 8am to 6pm especially in mangrove/forest which seems to be the most dangerous biotope of the site. These data should bring us to a more important and global study whit aim to better understand the epidemiological situation of HAT in this former focus where resurgence could be possible.

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