Man-fly contact in the Gambian trypanosomiasis focus of Nola-Bilolo (Central African Republic)

[1] J. P. Gouteux, [2] J. C. Kounda Gboumbi, [2] L. Noutoua, [3] F. D'Amico, [2] C. Bailly, [2] J. B. Roungou.

[1] Université de Pau et des Pays de l'Adour;
[2] Direction de la Médecine préventive et des Grandes Endémies;
[3] Centre ORSTOM de Montpellier

Abstract

A study using bipyramid tetse fly traps in the Nola-Bilolo sleeping sickness focus (Central African Republic) reveals ecological and behavioural differences between two vectors, *Glossina palpalis palpalis* and *G. fuscipes fuscipes*. The latter species inhabits mainly open water sites and surrounding forest, whereas *G. p. palpalis* occurs mainly in coffe plantations near villages. Consequently, the man-fly contact differs considerably according to the species. The intensity of trypanosomiasis transmission, estimated by the probable distribution of cases, showed significant positive correlation with the density of the flies. Analysis of the fly blood meals in two villages show that, unlike *G. g. palpalis*, *G. f. fuscipes* feeds on men more than on pigs. *Trypanosoma vivax* infection was observed only in *G. fuscipes fuscipes*. The differences in occupation of the environment between the two vectors must be taken in account in trapping programmes which may modify this distribution.

Introduction

Although sleeping sickness has been studied for nearly a century (Martin *et al.*, 1909), the modes of transmission of *Trypanosoma brucei gambiense* are not well understood (Noireau *et al.*, 1986a). Little is known about (i) the cycle of the parasite within the vector (Molyneux, 1980a); (ii) the vectorial ability of the various species of tsetse fly with regard to the various strains of trypanosomes; (iii) the role and the importance of the animal reservoir (Molyneux, 1980b). In addition, although the modalities of the man-vector contact are one of the fundamental epidemiological characteristics of a given focus, they are often unknown or neglected. In central Africa, where a few studies have dealt with this aspect, the thorough research carried out in the main Congolese foci by Frezil and his team (Frezil *et al.*, 1979, 1980, 1981) constitutes a interesting exception. The forest focus of Nola-Bilolo (Fig. 1) is situated in a residual pocket of *Glossina palpalis palpalis* (Robineau-Desvoidy, 1830)

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within the distribution area of *G. fuscipes* Newstead, 1911. The Nola area is the only place in Central African Republic where *G. p. palpalis* has been found (Finelle *et al.*, 1962; Gouteux, unpublished). This focus lies along the Nola-Yokadouma road in the south-western part of Central African Republic, between the Cameroon and the Congo. Documented since the beginning of the century, the focus is currently in full reviviscence, with a thousand cases detected in the last four years. In 1990–91, *G. p. palpalis* occupied most of the focus (the eastern part, towards Nola) and *G. f. fuscipes* was predominant in only a few villages in the west, towards the Cameroon border. The distribution of both species overlaps in two villages (Gouteux *et al.*, 1992). In order to determine (i) whether *G. p. palpalis* and *G. f. fuscipes* have the same contact with man, and (ii) the main sites of man-fly contact, we compared the results of a socio-medical survey and an entomological survey, both carried in the Nola-Bilolo focus between January and March 1991.

Materials and methods

Survey zone

The survey zone, which is approximately 400–500 m above sea-level, is situated in the semi-deciduous rain forest (mainly *Triplochiton scleroxylon, Terminalia superba, Mansonia altisima*). The average rainfall is about 1.600 m/year. There are two seasons: a short dry season from December to March, and a rainy season from April to November. The region is well drained by major rivers, the Mambéré and the Kadeï, which merge at the village of Nola to become the Sangha. The population directly affected by sleeping sickness is spread over 54 km in about twenty villages along the road from Nola to the Cameroon border (towards Yokadouma) and represents about 7,000 inhabitants. The main ethnic group is the M'Bimou belonging to the Bantu linguistic group. There are also some pygmies (Aka group) and a few Muslim trades people. Traditional pig-breeding (animals roan freely) was onserved in all the villages investigated. The largest herds of pigs are found at M'Poyo, Bilolo, Domissili and Ziendi (Fig. 1).

Socio-medical methods

An epidemiological survey to determine the sites of probable infection with trypanosomes was carried out simultaneously with a screening campaign. The survey involved 142 patients, 72 were diagnosed through routine examination in 1990 in the Nola Hospital (passive screening), and 70 were detected by the mobile medical team during this survey (active screening). The survey involved finding these subjects in the field, then interviewing them about their work, movements, successive residences, contact with tsetse flies (site, time, activities).
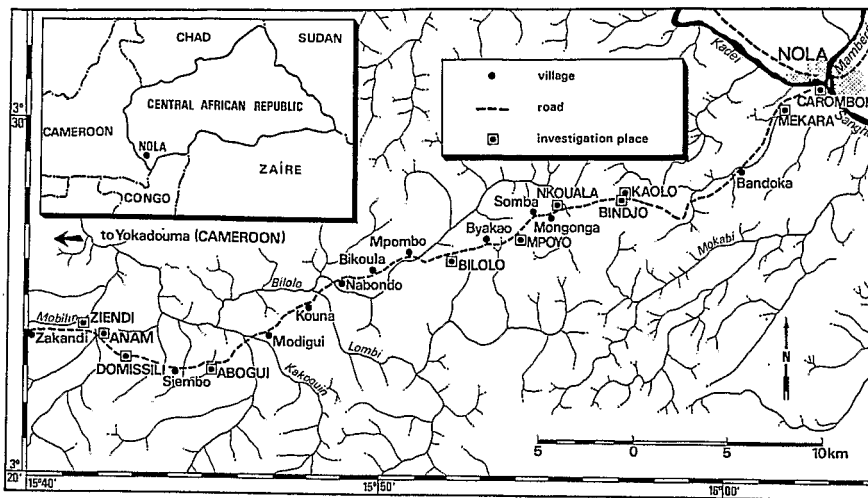
Fig. 1 The Nola-Bilolo focus.

Villages	PDC (a)		ADT (b)					
	1	2	mean	max 1	max 2	coffee	retting	bathing
Crambois*	0.3	5.7	0.21	0.65	2	0.10	0.34	0.35
Mékara*	4.7	6.7	0.18	1.21	3	0.02	0.41	0.33
Binjo-Kaolo*	4.7	5.0	0.41	1.19	2	1.12	0.15	0.55
Nkouala*	1.3	3.7	0.93	1.50	3	1.23	0.69	1.42
M'Poyo	18.3	7.0	10.66	27.55	29	24.78	4.00	2.44
Bilolo*	4.7	7.7	4.58	10.80	14	8.13	1.00	–
Abogui*	1.0	5.3	0.66	1.06	3	0.78	0.60	0.60
Domisili***	4.3	5.3	0.78	1.06	5	0.71	0.35	1.06
Anam***	1.0	2.0	0.38	1.20	2	0.65	1.20	0.30
Ziendi**	6.7	3.0	5.04	18.54	36	1.08	4.39	18.54

Table 1 Comparison of the probable distribution of cases (PDC) with the apparent density per trap (ADT) of *G. palpalis palpalis**, G. fuscipes fuscipes*** or both species captured together***. The ADTs are expressed as the number of flies per trap per day.

(a) The PDCs were èstimated during a survey on actively (PDC1) or passively (PDC2) screened patients (see text).
(b) ADT calculated for all the catches in a given village (mean), for the catches at the best site (max1), for the best daily catches (max2), in the coffee plantations (coffee), in the casava retting sites (retting) and in the bathing areas (bathing)

When more than one site could be suspected for each patient, each site was considered as presenting an equivalent probability of infection (1/2 for two sites, 1/3 for three). By grouping these sites by village it was possible to draw a map of the distribution of cases according to the probable site of infection (Gouteux *et al.*, 1993). These "probable distributions of cases" (PDC) may be considered as an estimate of the intensity of epidemiological significant man-fly contact (i.e. the number of infective tsetse fly bites) in the various sites. The PDC1 were determined from the 70 actively screened patients, the PDC2 from the 72 passively screened patients. The PDC3 as the PDC1 but without the village of Ziendi, where *G. f. fuscipes* is the only species present. Thus, the PDC3 concern only the zone of *G. p. palpalis*.

Entomological survey

After interviewing the patients, the interviewer carried out the entomological survey at their places of work or leisure. Fifty-eight bipyramid tsetse fly traps (Gouteux, 1991) were used. In each village, the various settings in which the patients had activities were monitored by continuous trapping for 10 to 25 days, using 4–14 traps per village. The traps were examined at least once a week. The surveyed biotopes were coffee plantations, casava retting sites, washing or bathing areas. The total number of captured flies per village or per biotope divided by the number of traps and the number of trapping days gives the Apparent Density per Trap (mean ADT). A further two parameters were introduced to caracterize each village: the mean catch during the whole trapping period at the most pro-

ductive site (maximum ADT1), the best daily catch (maximum ADT2). The infection rates of the flies were determined according to the localisation of the trypanosomes (proboscis, salivary gland, gut). This somewhat imprecise method (see Gouteux *et al.*, 1987), is used here only in order to compare the fly samples. The physiological age of the female flies was also determined. The origin of the tsetse blood meals was determined at the Institut für Veterinärmedizin des Bundesgesundheitsamtes (Professor C. Staak, Berlin, Germany), by a previously described method (Staak *et al.*, 1981).

Results and interpretation

Distribution of the vectors

The distribution of the densities of the vectors varied considerably according to the biotope (Table 1). The highest apparent densities of *G. f. fuscipes* (at Ziendi) were observed at the bathing areas. This species was practically absent from the village and the coffee plantations. On the other hand, *G. p. palpalis* showed the highest densities in the coffee plantations (M'Poyo and Bilolo) and was never very abundant in the backwaters.

Table 2 Value of the Coefficient of determination r^2. Regression between the probable distribution of cases (PDC) 1, 2, and 3 (see text) and the apparent densities per trap (ADT) of the tsetse flies (see Table 1).

	mean	max 1	max 2	ADT coffee	retting	bathing
PDC 1	0.83	0.78	0.59	0.82	0.51	0.16
PDC 2	0.11	0.05	0.00	0.24	0.02	0.14
PDC 3	0.84	0.86	0.85	0.88	0.77	0.66

Table 3 Comparison of the origin of the blood meals of *G. p. palpalis* and *G. f. fuscipes* sampling in the villages of M'Poyo and Ziendi respectively.

Tsetse	Man	Pig	Other	N
G. p. palpalis	24 %	70 %	6 %	66
G. f. fuscipes	42 %	33 %	25 %	12

N: total of blood meals identified. The column "other" includes a dog, a bird, a rodent and a monitor lizard for *G. p. palpalis* and two dogs and a cat for *G. f. fuscipes*

Table 4 Trypanosome infection rates observed in *G. p. palpalis* and *G. f. fuscipes*.

Tsetse (Villages)	*brucei*	*congolense*	*Trypanosoma* *congolense* or *brucei*	*vivax*	*grayi* like	N
G. p. palpalis (M'Poyo)	0	0	1	0	0	128
(Abogui)	0	2	3	0	3	37
G. f. fuscipes (Ziendi)	1	3	2	5	3	69

N: total of non-teneral *Glossina* dissected

Table 5 Comparison of the physiological age group of the females *G. p. palpalis*, sampled in M'Poyo and Abogui.

Villages	Tenerales	Nullipares	Young Parous	Old Parous	N
M'Poyo	3.7 %	8.4 %	51.4 %	36.4 %	107
Abogui	6.1 %	12.1 %	36.4 %	45.5 %	33

N: total of females dissected

Comparison of the distribution of the PDC and the ADT

The probable distribution of cases and the average apparent densities of the flies show a clar linear relationship (p < 5 %, Table 1). The value of r^2 (coefficient of determination, Table 2) shows that the correlation of the mean ADT or the maximum ADT 1 or 2 is much higher with the PDC1 (p = 0.0003 – 0.0008 – 0.0095, respectively) than with the PDC2 (p = 0.3414 – 0.5249 – 0.9035 respectively). The PDC1 (both vectors together), show a better corelation with the mean ADT than with the maximum ADT's (Table 2). Among the various biotopes, only the coffee plantations presented ADT which are highly correlated with PDC (p = 0.0004) compared with the retting sites (p = 0.0193). There is not a significant correlation with the bathing sites (p = 0.5341). The correlation between the PDC3 and ADT's (*G. p. palpalis*) is even better (Table 2). The coffee plantations were clearly distinguished. The densities of *G. p. palpalis* in this biotope may account for 88 % of the probable distribution of cases.

Comparison of the origin of blood meals in G. p. palpalis and G. f. fuscipes

The results of the analysis of the blood meals also revealed marked differences between these two species ($X^2 = 7.95$, p = 0.0185, Table 3), although the pig herd was comparable in both villages. At Ziendi, *G. f. fuscipes* was more anthropophilic than was *G. p. palpalis* at M'Poyo. It was found that *G. p. palpalis* had taken a

relatively high proportion of mixed pig-man blood meals (8 meals out of 58,14 %). The pig blood meals probably originated from domestig pigs. However, of the total 46 pig blood meals, 5 (11 %) were identified as originating from the warthog (*Potamochoerus porcus pictus*). It is a generally accepted fact that *Glossina* are more likely to become infective if they ingest the parasite at their first meal. Given the physiological age of the female flies it was possible to identify the origin of the first blood meals. For *G. p. palpalis* two first meals were taken on man and four on pigs. The three first meals identified in *G. f. fuscipes* were all taken on man.

Comparison of the trypanosome infection rates of G. p. palpalis and G. f. fuscipes

The overall infection rate was 22 % for *G. p. palpalis* at Abogui and 20 % for *G. f. fuscipes* at Ziendi. *G. p. palpalis* was most free of infection at M'Poyo (Table 4). The difference between M'Poyo and Abogui is not acounted for the age of the tsetse flies in each sample. Indeed, there was no significant difference in the composition by age-group in these two villages ($X^2_{3ddl} = 2.42$, Table 5). At Ziendi and Abogui, the reptilian trypanosomes (*T. sp. cf. grayi*), identifiable by their appearance and movement, were found in both fly species in comparable proportions (4–8 %). Similar results were found for *T. congolese* (intestinal infections and proboscis) and for the undetermined group *T. congolense/T. brucei* (intestinal infections alone). On the other hand, only *G. f. fuscipes* was infected with *T. vivax* (infection of the proboscis alone) which represented 36 % of the infections in

this fly species. *G. f. fuscipes* was also the only species found with infection in the salivary glands, probably with *T. brucei gambiense*, given the rarity of *T. b. brucei* in forest areas.

Discussion

Tsetse challenge and trypanosomiasis risk factors in the Nola focus

Significance of fly densities. The PDC1 were estimated from patients screened at the same time as the entomological survey, and the PDC2 from the patients screened passively at hospital in the previous year. The infection in the passively-screened cases was on average much older than that in the actively screened cases (Gouteux et al., 1993). The correlation observed between the ADP and PDC1 is notably better than that observed with PDC2. This supports the idea that there may be a direct link between the probable sites of infection of the cases and the densities of the vector at a given time. Such correlations can be found from data in publications and led Rogers (1985) to suggest that the *Glossina* density was the principal risk factor for the transmission of trypanosomiasis.

This may however be a very crude approximation of the reality. This correlation may be a statistical artefact and may mask a more complex situation. Indeed, there are only few points on the map which give a statistically good correlation (for example the villages of Bilolo and M'Poyo where high fly densities are associated with high prevalence). But in the mass of points with low statistical significance this relationship is nonexistent. At Mékara, Bindjo-Kaolo and Domissili, relatively high transmission is associated with low *Glossina* densities. This is accounted for the complexity of the sleeping sickness transmission process wich simultaneously involves other factors concerning the vector: i) the structure of the fly populations, ii) the intensity of the contact with man, iii) the epidemiological role of non-human hosts.

i) Populations and sub-populations of the vectors. Samples of a tsetse fly population at a given time show various patterns of sex-ratio and of structure by age-group according to their location in the environment (Gouteux, 1987). These different sub-populations which share the ecosystem do not have the same ability to sustain the whole transmission cycle. This was observed in the Vavoua focus in the Côte d'Ivoire, where transmission occurs mainly in the coffee plantations, whereas the sedentary people in the villages are less affected (Stanghellini and Duvallet, 1981). The fly sub-populations in the plantations (where distribution by age group and sex ratio were balanced) and in the periphery of the villages (where there was a higher proportion of older females) do not therefore present the same epidemiological significance (Challier and Gouteux, 1981). In the Nola-Bilolo focus this comparison was not possible since villages and coffee plantatons overlap.

ii) The variations in intensity of the man-vector contact. The intensity of contact with man is often inversely proportional to the density of the vector, as ob-served by Nash (1944) and Morris (1952). Similar observations have been made more recently in the Congo in Lombo and Youlonkoyi (Niari focus, Gouteux and Noireau, 1985, unpublished). In this focus, as in the foci of Vavoua and Bouafle (Côte d'Ivoire), high *Glosina* densities were always associated with pig-breeding in the village. Fly densities seem to be positively correlated with the size of the pig herd in the village (Baldry, 1980; Gouteux et al., 1986; Rogers, 1988). Flies feed preferentially on pig rather than on men. In the Côte d'Ivoire, the proportion of blood meals taken on pigs reach 72 % to 98 % for *G. p. palpalis* in villages with high pig densities in forest areas (Gouteux et al., 1982) and savanna areas (Dagnogo et al., 1985). However, the low proportion of meals taken on man can be compensated by high fly densities. The difficulty is to assess whether a high density with a low proportion of human blood meals or a low density with a high proportion of human blood meals represents the highest epidemiological risk. This question is complicated by the intervention of other epidemiological factors, notably the structural patterns of the fly subpopulations (see above) and the possibility of pigs being reservoirs of *T. gambiense*. In the Nola-Bilolo focus the situation is complicated further by the competition between *G. p. palpalis* and *G. f. fuscipes*, which annihilate each other in the contact zone (see Gouteux, 1992 for comment). Indeed, in the village of Domissili where both species were captured together, the densities were much less than expected given the size of the pig herd in this village.

iii) Non-human hosts of Glossina. The role of pigs as "animal reservoirs" of epidemiological significance was first suggested by Greggio (1917), emphasized by Van Hoof et al. (1937) and generalized by Baldry (1980). The natural infection of pigs with human trypanosomes has been demonstrated in West Africa (Mehlitz et al., 1982; Zillman et al., 1984) and in Central Africa (Noireau et al., 1989; Kageruka, 1989). In the Congo, the low prevalence of *T. gambiense* in pigs and in other domestic animals suggests that the animal reservoir does not act as an amplifier during epidemic periods, but may play an epidemiological role during inter-epidemic periods (Noireau et al., 1986b). During this time, the reservoir can maintain the low level of endemicity in persistent foci, as suggested by Baldry (1980) and Molyneux (1983). In the Nola-Bilolo focus the numerous man-pig mixed meals in *G. p. palpalis* at M'Poyo and the fact that *G. p. palpalis* and *G. f. fuscipes* can feed on dogs (1.5 % and 16.7 % of the total blood meals, respectively) suggest a possible role for an animal reservoir. The difference observed here for the choice of hosts between *G. p. palpalis* and *G. f. fuscipes* may correspond to the differences observed in the ecodistribution rather than intrinsic feeding preferences. Indeed, both species present opportunistic feeding behaviour with regard to hosts (Weitz, 1963). On the other hand, the pigs wander about the coffee plantations with a high density of *G. p. palpalis* whereas the bathing sites are frequented by man and mainly *G. f. fuscipes*.

A dynamic balance between G. p. palpalis and G. f. fuscipes

Geographical modifications in the line of contact between *G. p. palpalis* and *G. f. quanzensis* Pirez,

1948 near Brazzaville (Congo) was reported and discussed recently (Gouteux, 1992). Any change in the environment is likely to modify the balance between these two closely related species. In the above example, the progression of *G. p. palpalis* can be accounted for by the gradual anthropisation of the Brazzaville area, to the disadvantage *G. f. quanzensis*. In the Nola-Bilolo focus, vector control by trapping was launched in March 1992. About 400 bipyramid traps were placed in the outskirts of the villages and in the coffee plantations. This campaign was thus essentially aimed at *G. p. palpalis*. If the balance between these species is modified, it is most likely that the *G. p. palpalis* pocket will regress and that the zone will be invaded by *G. f. fuscipes*. Local transmission of the disease will occur via *G. f. fuscipes* in the west of the focus, notably at Ziendi. This implies that the results of this campaign may have undesired epidemiological consequences. It will be imperative to monitor the entomological situation.

Conclusion

The man-vector contact thus seems very different for these two species. These differences are related to the two opposing strategies regarding the ocupation of the environment. In the Nola region, *G. p. palpalis* is a peridomestic species, completely dependent upon man, pig-breeding and agriculture (coffee plantations). *G. f. fuscipes* is the predominant species elsewhere, including in the forest around the villages. Its penetration into the villages could be via the water, as observed in Ziendi. This species is probably eliminating *G. p. palpalis* in certain areas, especially in the western part of the focus. The differences observed in the man-*G. p. palpalis* and man-*G. f. fuscipes* contact must be taken into consideration in trapping programmes. Indeed, such control programmes might merely favour *G. f. fuscipes* to the detriment of *G. p. palpalis*. It is thus necessary to keep track of any modifications in the distribution of these two vectors in the future.

Acknowledgements

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J. P. Gouteux

Place jean Sénac
F-32170 Miélan
France