

Tsetse and trypanosomes relationship in Southwestern part of Burkina Faso

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Abstract

African Animal Trypanosomosis (AAT), transmitted by tsetse flies, are one of the main constraints for the development of livestock farming in Burkina Faso. The objective of this study was to understand the impact of the level of degradation of tsetse habitat on the epidemiology of AAT. Entomological and parasitological surveys were carried out in two different landscape areas: conserved (Folonzo) and fragmented (Moussodougou) in southwestern part of Burkina Faso. The results revealed that the diversity, abundance and infection rate of tsetse flies were related to the conservation status of the habitat. In Folonzo, four species of tsetse were present (84.76% (2870/3386)) versus a single species (15.24%) in Moussodougou. The tsetse infection rate was also higher in Folonzo (21% (133/633)) than in Moussodougou (7.8% (23/294)). In addition, in Folonzo, the tsetse flies were infected with all 3 species of trypanosomes. However, in Moussodougou, *G. p. gambiensis* (the only species present) was infected primarily with *T. congolense* (68.44% (13/19)). We suggest that the current fragmentation of tsetse habitats does not reduce the risk of trypanosomiasis, but leads to selection and maintaining the best trypanosome and tsetse (*G. p. gambiensis* / *T. congolense*) pair that may affect the epidemiology of AAT.

Keywords: Habitat fragmentation, Trypanosomiasis, Tsetse abundance, Infection rate, Southwest Burkina Faso.

Introduction

Human African trypanosomiasis (HAT) and Animal African trypanosomosis (AAT) are life-threatening parasitic diseases occurring in sub-Saharan Africa. These neglected tropical diseases are caused by infection with protozoan parasites of the genus *Trypanosoma* mainly transmitted by tsetse flies of the Glossina genus. The medical and economic impact of these diseases is considerable for the countries affected (1,2).

The dynamics of tsetse populations are intimately linked to ecological and environmental factors. Indeed, host availability, seasonal climatic variations (temperature and humidity) and habitat fragmentation (vegetation) due to human pressure can affect the distribution and abundance of tsetse flies (3–5); and indirectly the prevalence of trypanosome infections (6,7).

Recent observations conducted on the Comoe River at its source in the village of Moussodougou and in the protected area of Folonzo, two localities in southern Burkina Faso, revealed that the degradation of tsetse habitat observed in Moussodougou due to anthropization had a negative impact on the diversity and abundance of tsetse. In addition, the different species of tsetse (i.e. *G. tachinoides* and *G. palpalis gambiensis*, two riparian species) do not have the same preference in terms of biotope (8). Although the relationship between tsetse and environmental factors has been established in these two areas (9,10), little is known on the relation between tsetse and trypanosomes, which is important for trypanosomiasis risk overview within the two sites. This

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information will be very useful in some areas, such as Moussodougou, where agricultural and pastoral activities are widely carried out. The objective of our study is to analyze the effects of seasonal variations and habitat fragmentation on the epidemiology of TAA in southwestern Burkina Faso.

I. Materials and methods

1.1. Study areas

Entomological surveys were conducted at two locations in Southwestern Burkina Faso along the Comoé River, Moussodougou and Folonzo (figure 1). The locality of Moussodougou (~10°49'N, 04° 57'W) is characterized by a landscape highly anthropized by cultural practices and livestock breeding. The source of the Comoé River is located in this village. According to the description given in 2005 by BOUYER et al. (11), of the ecological characteristic of this locality, the river is narrow in width and the habitat of *G. p. gambiensis* is closed and dark, isolated from the neighboring savannah. These environmental characteristics are favorable to the establishment of *G. p. gambiensis*, considered as a species of springs (9,12). Currently, vegetation bordering the river is severely degraded with however, several phyto-sociological groups : riparian forest surrounded by orchards, farms and fallow lands, farms on woody parks, woody parks on fresh, and domestic fallows (8). Domestic animals (cattle, goats, sheep) which look for water and grazing, as well as breeders and farmers remained the main hosts of tsetse.

At Folonzo (~09° 54' N, 04° 36' W), in the Niangoloko department of the Comoé Province, the survey was conducted in the protected forest, on the shores of the same Comoé River, about 100 km downstream from Moussodougou. This locality is a protected area characterized by abundant and diversified vegetation (13) and four tsetse species have been reported to occur: *G. tachinoides* and *G. palpalis gambiensis* (riverine group), *G. morsitans submorsitans* (savannah group) and *G. medicorum* (forest group) (5,14). Several wildlife species are found in this protected area, including different species of antelope, warthogs (*Phacochoerus aethiopicus*), hippopotamuses (*Hippopotamus amphibius*), monitor lizards (*Varanus niloticus*) and other reptiles (crocodiles and snakes), which constitute the main hosts of the tsetse (5,14).

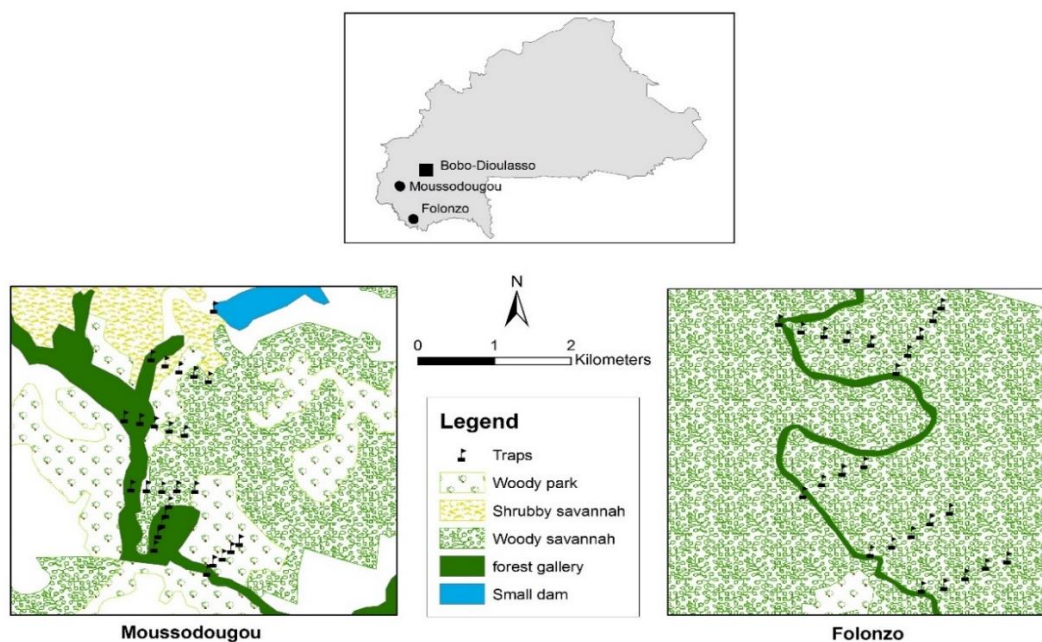


Figure 1. Localization of study sites in South Western Burkina Faso. 5 biconical traps per transect (Rayaisse et al., 2015).

1.2. Study design and sampling

Two entomological surveys were conducted in each location. The first in the dry season, in December 2011 at Folonzo and in January 2012 at Moussodougou and the second in the rainy season, respectively in May 2012 and June 2012.

Tsetse sampling was performed as described by RAYAISSE et al. (8). This method consisted in deploying a total of 25 biconical traps (5 transects x 5 traps) per locality and per season, during 5 days. Five transects were set in each locality (Folonzo and Moussodougou), starting from the river bank to the Savannah. Distance between two adjacent transects was approximately 1 km. Each transect was composed of 5 biconical traps (15). Adjacent traps were distanced 150 -200 m from each other. This capture design for tsetse sampling with the transects was done for 5 days with daily collection of cages. Captured flies were identified and separated by species and sex. When collected after 24 hours, tsetse that were still alive were identified and dissected for research of trypanosomes in the proboscis, salivary glands and midgut, using a microscope. These three organs of each positive tsetse were then collected and stored individually in eppendorf tubes (0.5ml) containing sterile distilled water and kept at +4°C in the field (16,17), for the molecular identification of trypanosome species in laboratory.

1.3. Identification of trypanosomes by PCR

This has been done as described in DJOHAN et al.(17). After trypanosomes DNA extraction from different organs of infected tsetse flies, standard polymerase chain reaction (PCR) was performed using specific primers for *T. congolense* Savannah Type (18), *T. congolense* Forest Type (19), *T. vivax* West Africa (19) and *T. brucei s.l.* (18) (table I). This diagnosis was made using specific satellite sequences of trypanosome taxonomic groups. DNA samples were amplified in 25 µl reaction blend containing: 10 mM Tris-HCl pH 8.3, 50 mM KCl, 200 mM each of four deoxynucleoside triphosphates (dNTPs), 1 µM of each primer and 0.5 units of Taq DNA polymerase. This blend was placed in a thermocycler with the PCR conditions comprising an initial denaturation step at 94°C for 3 min, then 40 cycles of 94°C for 30s, 55° C for 30 s, and 72° C for 1 min. The elongation step was continued at 72° C for 5 min. Five (5) microliters (µl) of each amplified sample was resolved by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide and photographed under ultraviolet light. A positive (with the reference DNA) and a negative control (without DNA and with only distilled water) were added to each reaction series.

Table I. Primers used for detection of trypanosomes and expected band sizes by PCR

Target	Sequences	Trypanosomes	Size (bp)
TCS1	5'-CGAGCGAGAACGGGCAC-3'	<i>T. congolense</i> Savannah Type	321
TCS2	5'-GGGACAAACAAATCCCGC-3'		
TCF1	5'-GGACACGCCAGAAGGTACTT-3'	<i>T. congolense</i> Forest Type	350
TCF2	5'-GTTCTCGACCAAATCCAAC-3'		
TVW1	5'-CTGAGTGCTCCATGTGCCAC-3'	<i>T. vivax</i> West Africa	150
TVW2	5'-CCACCAGAACACCAACCTGA-3'		
NRP1	5'-CGAATGAATATTAACAATGCGCAG-3'	<i>T. brucei s.l.</i>	173
NRP2	5'-AGAACCATTATTAGCTTTGTTGC-3'		

1.4. Data analysis

Analyses were performed using the R software version 4.0.2 (R Core Team, 2020). Tsetse infection rate was analyzed using generalized linear mixed models with a binomial distribution

(20). The position of the trap was considered as a random effect that may induce a spatial variation in the infection. The tsetse species, sex, season, biotope and locality were used as fixed effects. To compare the best interaction between trypanosomes and tsetse species, a generalized linear mixed model with Poisson distribution fitted by maximum likelihood (21) was applied. The package “lme4” for R (22) was used to fit generalized linear mixed binomial models with the glmer function. The MuMin package was used for the implementation of the AICc (23), in order to select the best model.

II. Results

2.1. Entomological survey in Folonzo and Moussodougou

A total of 3386 tsetse was captured in the two localities. The proportions of tsetse captured by locality were 84.76% (n = 2870) and 15.24% (n = 516) in Folonzo and Moussodougou respectively, (table II). Apparent density of tsetse (ADT) was significantly higher at Folonzo (11.48 tsetse / trap / day) compared to Moussodougou (2.06 tsetse / trap / day); (p < 0.001). However, when comparing between the two localities ADT of *G. p. gambiense* which was the single species captured in Moussodougou, Univariate analysis revealed high concentration of this species at Moussodougou (p < 0.05). Regardless of the locality, the DAP was not statistically affected by the season, with 7.47 tsetse / trap / day in wet season vs 6.08 tsetse / trap / day in dry season.

The specific composition showed that the four reported species were captured in Folonzo. *G. tachinoides* was predominant (77.88%; ADT = 17.89 tsetse/trap/day (maximum daily catch= 199 flies)); *G. submorsitans morsitans* (15.68%; ADT= 3.60 tsetse/trap/day (15); *G. palpalis gambiensis* (5.12%; ADT= 1.17 tsetse/trap/day (13)), and *G. medicorum* (1.32%; ADT= 0.30 tsetse/trap/day (5)). In Moussodougou, *G. palpalis gambiensis* was the predominant species (ADT=2.06 tsetse/trap/day (75 flies).

Table II. Epidemiological characteristics of AAT in two sites with increasing levels of habitat fragmentation in Comoé River

Site		Folonzo					Moussodougou		
Environment	Degree of fragmentation	low					high		
Tsetse species		ADT	11.48***					2.06	
	Species	Gpg	Gms	Gt	Gmed	Total	Gpg Total		
	Abundance of tsetse	Rainy season	71	256	13 49	9	1685	182	
		Dry season	76	194	88 6	29	1185	334	
Trypanosome species		ADTi	8.23**					3.41	
	Infection rates (percentages)	<i>T. brucei sl.</i>	0					15.78 (3/19)	
		<i>T. congolense</i> Forest type	15.69 (8/51)					0	
		<i>T. congolense</i> Savannah type	47.05 (24/51)					68.5 (13/19)	
		<i>T. vivax</i>	37.24 (19/51)					15.78 (3/19)	
		Total infection (%)	72.85 (51/70)					27.15 (19/70)	
Wild fauna	Density	Present					absent		

Gmed = *Glossina medicorum*; Gms= *Glossina morsitans submorsitans*; Gpg= *Glossina palpalis gambiensis*; Gt= *Glossina tachinoides*. ADT, apparent density of tsetse (number of flies captured per trap per day); “-“notavailable. ADTi, apparent density of infecting tsetse flies (per trap per day).

2.2. Prevalence of trypanosomes in tsetse under microscopic examination

A total of 633 and 294 tsetse flies were dissected respectively at Folonzo and Moussodougou. Microscopic examination of organs showed that 21% (133/633) and 7.8% (23/294) of the tsetse dissected were positive for trypanosomes at Folonzo and Moussodougou respectively.

The infection rate was significantly high in the dry season at 10.25% (95/927) compared to the wet season at 6.6% (61/927) ($p < 0.008$). When comparing the two localities, there were more infected tsetse in Folonzo (21% (133/633)) than in Moussodougou (7.8% (23/294)), ($p < 0.023$). Tsetse infections rates were not significantly different according to the biotope (forest and savanna), $p > 0.308$; and no significant difference was observed between males and females ($p > 0.815$) (table III).

Table III. Fixed effects of the infection in tsetse model depending on species, sex, season, biotope and locality.

Parameters	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-1.40557	0.28507	-4.931	8.2e-07 ***
Species [<i>G. p. gambiensis</i>]	-0.02536	0.48009	-0.053	0.95787
Species [<i>G. tachinoides</i>]	0.23223	0.29332	0.792	0.42852
Sex [Male]	0.04699	0.20098	0.234	0.81512
Season [wet]	-0.51153	0.1947	-2.627	0.00861 **
Biotope [savannah]	0.33538	0.32953	1.018	0.3088
Locality [Moussodougou]	-1.0465	0.46283	-2.261	0.02375 *

Significance codes : 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1. *G. medicorum* was not used in this analysis because of its very low density compared to other tsetse species. *G. m. submorsitans*, female, dry season, gallery and Folonzo were considered respectively as reference of species, sex, season, biotope and locality.

Concerning the infection in the different organs, at Folonzo, the infection rate was at 45.11% (60/133) from proboscis similarly to that of midgut, 39.85% (53/133). In Moussodougou however, midgut infections were significantly higher, compared to proboscis; 91.30% (21/23) vs 8.70% (2/23) respectively. Some tsetse were found positive at both proboscis and midgut, 15.03% (20/133) in Folonzo, and no salivary gland was found positive in both localities, (table IV).

Table IV. Percentage of trypanosome positive organs under microscopic examination

Locality	organs	Tsetse species				Total
		Gt	Gpg	Gms	Gmed	
Folonzo	proboscis	4.42	0.15	4.26	0.63	45.11 (60/133)
	midgut	4.9	0.79	2.05	0.63	39.85 (53/133)
	salivary glands	-	-	-	-	-
	proboscis + midgut	1.26	0.15	1.1	0.63	15.03 (20/133)
Moussodougou	proboscis	-	8.7	-	-	8.70 (2/23)
	midgut	-	91.3	-	-	91.30 (21/23)
	salivary glands	-	-	-	-	-
	proboscis + midgut	-	-	-	-	-

Gmed = *Glossina medicorum* ; Gms= *Glossina morsitans submorsitans*; Gpg = *Glossina palpalis gambiensis*; Gt = *Glossina tachinoides*.

2.3. Identification of trypanosomes in *Glossina* spp by PCR

From the 155 tsetse flies analyzed by PCR in the two localities, 70 tsetse (45.16%) were infected with a trypanosome species. The molecular characterization results showed three trypanosome species, *T. vivax* (16.77 % (26/155)), *T. congolense* (Savannah type at 23.87 % and Forest type at 9.03 %) and *T. brucei l.s.* (5.16 %). Results revealed also mixed infections at 9.67 % (15/155). The mixed infections were constituted by 12 cases in Folonzo with three trypanosome species, *T. vivax-T. congolense* Savannah type, *T. congolense* Forest and 3 cases in Moussodougou with *T. brucei l.s.-T. congolense* Savannah type. The results of parasitological method and PCR assay, showed a concordance rate of 45.16% (70/155) (table V). More than half of the samples were not positive by PCR analysis.

Table V. Concordance rate of infection between microscopic and PCR analysis by organ type in both localities

locality	organs	number of infections on microscopic examination	number of infections to PCR analysis	concordance rate (%)
Folonzo	salivary glands	-	-	-
	midgut	63	19	30.15
	proboscis	70	32	45.71
Total Folonzo		133	51	38.34
Moussodougou	salivary glands	-	-	-
	midgut	20	17	80.95
	proboscis	2	2	100
Total Moussodougou		22	19	82.6
Total		155	70	45.16

2.4. Infection rates patterns by tsetse species, locality and season

In Moussodougou, where the only tsetse species captured was *G. p. gambiensis*, the infection rate was 68.44%, higher than the maximum value of 50% value recorded for *G. m. submorsitans* in Folonzo. However, in this locality of Folonzo, although *G. tachinoides* was the predominant species, the risk of parasite transmission from this species was relatively low ($p > 0.028$, table VI). Overall, the model analysis revealed a significant effect of locality on the infection rate (table VI). Data also revealed seasonal fluctuation of *T. vivax* which was more pronounced in Moussodougou ($p < 0.008$) (table VI).

Table VI. Fixed effects of the trypanosome infection rates model depending on tsetse species, season and locality.

Parameters	Estimate	Std. Error	z value	Pr (> z)
Overall infection rate under microscopic examination				
Intercept	0.1101	0.6064	0.182	0.85597
species[T.Gms]	-0.3145	0.6713	-0.468	0.63949
species[T.Gpg]	-0.1512	0.9758	-0.155	0.87683
species[T.Gt]	-1.4449	0.6579	-2.196	0.02807 *
Sex [T.male]	0.4559	0.3939	1.157	0.24713
season[T.wet]	0.4675	0.3947	1.184	0.23626
locality[T.Mouso]	1.7765	1.1039	1.609	0.10756
season[T.wet]:locality[T.Mouso]	-3.1558	1.1262	-2.802	0.00508 **
T. congolense Savannah type infection rates				
(Intercept)	-1.2212	0.2568	-4.756	0.0001 ***
season[T.wet]	-0.8357	0.4404	-1.897	0.0578 .
locality[T.Mouso]	1.7416	0.4818	3.615	0.0003 ***
T. vivax infection rates				
(Intercept)	-2.0986	0.4055	-5.176	0.0001 ***
season[T.wet]	1.2552	0.4733	2.652	0.008 **
locality[T.Mouso]	-0.3286	0.7583 '1	-0.433	0.665

Significance codes : 0 '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 ' '1.

III. Discussion

Our results confirmed abundance and diversity variation of tsetse and trypanosomes according to the stage of conservation of tsetse habitat. Although the two study sites are on the Comoe River, the riparian vegetation differed according to the location. Indeed, the riparian forest shifts from a Guinean type upstream, suitable to *G. p. gambiensis* (Moussodougou) to a Sudanese type downstream, more suitable to *G. tachinoides*, as already described along the Mouhoun river, the main river of Burkina Faso (11).

In Folonzo, the preserved habitat of tsetse and the availability of some wild hosts resulted in the abundance and diversity of tsetse, while the poor vegetation in Moussodougou and the absence of wild game in Moussodougou may certainly be the reason why the tsetse diversity and density are low (5,13,24).

The negative impact of vegetation degradation on tsetse abundance and diversity was observed in Tanzania (25) and in Ivory Coast (17).

The diversity of tsetse (four species occurring) and the abundance of hosts may favor the heterogeneity of circulating trypanosomes species (three species identified by PCR) in Folonzo. This correlation between tsetse diversity and trypanosomes was already observed in some protected areas (17,25).

These environmental factors may be the cause of these high infection rates observed under microscope in tsetse in this locality, compared to Moussodougou.

Trypanosome's identification using PCR generally gives higher precision of infection detection and trypanosome species identification than direct observation under microscopy. Indeed, direct

observation under microscopy is not very sensitive and one may miss some infections, more particularly in the midgut (26). In our study however, that was not the case and even some organs found positive with the direct examination were negative with the PCR. Similar observations were made by MALELE et al. (27). This poor rate of concordance between direct examination and PCR (only 45.16%) may be due to several factors: (a) there may be an inhibition of the amplification reaction of some samples; (b) the heterogeneity of trypanosomes species or strains (reptiles trypanosomes for instance) circulating within the area for which we have no primer for the PCR (28,29). Indeed, the primers we used only allow detection of pathogenic trypanosomes known for cattle and small ruminant, i.e. *T. vivax*, *T. congolense* and *T. brucei s.l.* Similar situation was observed in Guinea, where trypanosomes were firmly seen in tsetse by direct examination, but none of them was identified with the PCR (30). In addition, one may expect more concordance between microscopy and PCR method for proboscis (26).

In Moussodougou, where vegetation is severely degraded (because of agricultural and pastoral activities), the situation is different. Only one tsetse species (*G. p. gambiensis*) still occurs (8) and infected mainly by *T. congolense* Savannah type. The impact of vegetation fragmentation and anthropization on the abundance and diversity of tsetse and trypanosomes confirms what was reported earlier (13,17,31,32). According to SALEKWA et al. (33), the abundance of tsetse flies and their infection with trypanosomes are directly linked to anthropological activities. In addition, the authors argue that habitat degradation by anthropogenic activities could reduce the risk of AAT, what may especially be the case in Moussodougou. The ADTi calculated on the two study sites confirm that the trypanosomian risk is higher in Folonzo than in Moussodougou.

However, in our study the context is particular in Moussodougou, which challenges this assertion above. Indeed, *G. p. gambiensis*, a main vector of the AAT belonging to the Palpalis group, is known to adapt to habitat fragmentation and climate change ((34,35). This capacity of adaptation of *G. p. gambiensis* and its survival in the fragmented habitat have a considerable impact on the epidemiology of AAT. In addition, it is known that habitat fragmentation has an impact on the trypanosomes species composition and in Moussodougou, and the consequence would be the maintenance of *T. congolense* Savannah type and the disappearance of others (36). In these particular conditions, it was expected that *T. congolense* would be progressively replaced by *T. vivax* (given that the species is present and has a low prevalence) as suggested by GUERRINI et BOUYER (31), but that is not the case, with consideration to the high predominance of *T. congolense* (87.5% of infections).

Environmental changes have contributed to the selection of the tsetse-trypanosome interaction (*G. p. gambiensis*-*T. congolense* savannah type). *T. congolense* Savannah type was the main trypanosome species circulating in livestock in Moussodougou (7). It is likely that we have a particular cycle in Moussodougou, due to the presence of many cattle that constitute the main blood meal source of *G. p. gambiensis*, thus explaining its high infection rate by *T. congolense*. The capacity of *G. p. gambiensis* to be highly infected by *T. congolense* is evidenced in this study, while it has been usually considered as a "bad" vector of *T. congolense* in the literature, with some exceptions (37). A possible additional factor could be thermal stress due to environmental degradation, that may increase the vectorial competence of *G. p. gambiensis*, as found with *G. m. morsitans* in a fragmented habitat in Zambia ((38). This shift of competence may be due to the disappearance of *G. m. submorsitans* that was described as the best vector of *T. congolense* in the past (37); and this tsetse species disappearance is certainly linked to that of wild game caused by human settlement (4,39).

All these factors give particular importance to the epidemiology of animal trypanosomosis in the locality of Moussodougou. As a matter of fact, DICKO et al. (7) had identified this locality as high-risk area of TAA in the trypanosomian risk spatialization model.

Prevalence of infections also varied between seasons with a high infection rate in the dry season. Our results confirm previous findings by OWAGA (40). Indeed, in dry season, tsetse reduce their dispersal area to benefit from the humid microclimate of their habitat (gallery forest). Likewise, cattle are also more concentrated on the river banks at this hot period, to benefit of the particular good conditions. This proximity between tsetse and cattle will probably increase the risk of transmission of trypanosomes to cattle. Moreover, high temperatures in the dry season could contribute to the maturation of trypanosomes in tsetse (6). Studies in Tanzania showed a strong positive relationship between the prevalence of trypanosomes and temperature (41). Contradictory results have been observed in Côte d'Ivoire, where there was no seasonal difference (42), which may be explained by a more homogeneous habitat.

Determination of preferential blood sources for the different species of tsetse flies from both studied areas (what was unfortunately not done during our study) would provide relevant data to understand the adaptability of *G. p. gambiensis* and its survival in the fragmented habitat. Indeed, and as mentioned, there is still a diversity of wild game in the protected area that may serve as blood meal sources, depending on the trophic preference of occurring tsetse species. So, for *G. palpalis gambiensis* (if we want to make a direct comparison with the same species occurring in the fragmented area), it would mainly be monitor lizard, and other reptiles inhabiting the forest gallery (43).

In the fragmented area at the opposite, cattle are the main potential blood meal source, and the blood meal analysis would probably reveal a great proportion of meal taken from cattle (44), what would be an indication of the great capacity of this species to adapt, since its preferred host are reptiles.

Our study shows that fragmentation of tsetse habitats caused by anthropogenic activities does not reduce the risk of TAA. This degradation leads to the selection and maintenance of trypanosome and tsetse couple, as shown here by *T. congolense* Savannah type and *G. p. gambiensis*. Fragmented areas constitute a potential risk of TAA that must be taken into account in the planning for control of vector and disease in South-West of Burkina Faso.

Conflict of interest

The authors have no conflicts of interest to report.

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