



Original Paper

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“Silent” circulation of *Trypanosoma* spp. in Tabanids (Diptera: Tabanidae) and Cattle in a Tsetse free Range land of Ngaoundere (Adamawa-Cameroon)

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ABSTRACT

The Adamawa region falls within the tsetse belt of Cameroon but harbours isolated pockets of tsetse free range lands like Ngaoundere. There is no report on the occurrence of tsetse and bovine trypanosomiasis in Ngaoundere. To provide information on this subject, two Vavoua traps were used to trap vectors of bovine trypanosomiasis and at the same time, blood was collected from cattle. Genomic DNA was extracted from buffy coat of cattle blood (n=42) and biting flies (n=53). The nested PCR was used to screen the samples for *Trypanosoma* spp. During the 14 days of trapping in November and December 2017 in Ngaoundere, 127 flies were documented and classified under two taxa: Tabanidae and Stomoxyini. Three *Trypanosoma* spp. DNA was isolated from tabanid (18.9%) samples and identified as *T. theileri*, *T. vivax* and *T. evansi* and two of them that is *T. theileri* (4%) and *T. vivax* (3%) were also detected in cattle (7%). There was no case of trypanosome DNA isolated from all the screened Stomoxyini. This result indicates the “silent” transmission of *T. theileri* and *T. vivax* by tabanids in the absence of glossines in Ngaoundere.

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Keywords: Trypanosomes, tabanids, stomoxyini, PCR, Ngaoundere, Adamawa-Cameroon.

INTRODUCTION

Bovine trypanosomiasis is considered as one of the most important cattle diseases in Adamawa region of Cameroon (Mbahin et al., 2006). The last tsetse eradication of 1994 did not include Ngaoundere because it was considered free of tsetse and trypanosomiasis. Because of fear of the re-introduction of tsetse and trypanosomiasis in tsetse free areas by neighbouring infested areas, deltamethrin coated screen barriers were pitched at the Ngaoundere hill by the special mission for tsetse eradication to prevent the re-infestation of Ngaoundere by wild tsetse from neighbouring and tsetse-infested North region. In Cameroon, the Adamawa plateau harbours several cattle markets (Motta et al., 2017) and receives cattle from neighbouring Chad, Sudan, Central African Republic, Nigeria and Niger which are potential sources for the introduction and re-introduction of new strains of vector-borne livestock diseases. Apart from commercial cattle, cattle of transhumant pastoralists from the aforementioned neighbouring countries come in contact with sedentary cattle thereby introducing different strains of *Trypanosoma* in local cattle through bites of local vectors. The report of Sevidzem et al. (2015) revealed the occurrence of mechanical vectors (Tabanids and *Stomoxys*) of bovine trypanosomiasis in the Adamawa plateau. Such vectors have been shown to acyclically and cyclically transmit trypanosomes (Boese et al., 1987; Desquesnes et al., 2003a; Desquesnes et al., 2003b; Taioe et al., 2017; Mounioko et al., 2018). The review document of Wells (1972) highlights the importance of mechanical transmission in the epidemiology of nagana. Wells (1972) stated that transmission of nagana in the absence of glossines is caused by mechanical vectors such as tabanids, stomoxynes and hippoboscids. The study of Mamoudou et al. (2016) reported the occurrence of high tabanid densities in several ecozones of the Far North region of

Cameroon. Some authors report that *T. evansi* is geographically most widespread trypanosome (Desquesnes et al., 2013) and was originally reported in Camels in Africa (Lun and Brun, 1992). In the tsetse free Far north region of Cameroon, Suh et al. (2017) reported 0.67% and 1.46% prevalence of *T. vivax*. The continuous usage of trypanocides and insecticides in Ngaoundere is an indication of the existence of trypanosomiasis and its mechanical vectors in the apparent absence of glossines. There is need to apply sophisticated tools like PCR to identify the *Trypanosoma* spp. occurring in cattle and biting flies. Bosson-Vanga et al. (2012) found out that PCR was far more specific than microscopy in trypanosome identification. The goal of the present study is to molecularly screen cattle and flies of trypanosomes in the tsetse free range land of Ngaoundere.

MATERIALS AND METHODS

Study area

The study was conducted in a herd located 25Km from the town of Ngaoundere and falls within the following geographical limits: latitude 07° 11, 887' N and longitude 013° 34, 919' E. Ngaoundere is a major livestock production zone and is considered a tsetse free zone but hyper infested with other flies (Lendzele et al., 2019) (Figure 1). In addition to cattle, other potential hosts in the area include camels that are kept in the Bois de Mardock, a recreational center found some 15 km from the study site. The herd that was used for the study consisted of 42 Zebu Goudali cattle heads.

Entomological prospection

An entomological prospection was carried out for 14 days using Vavoua (n=2) traps. Traps were emptied every 24 hours throughout the prospection days. Traps were placed in potential vector breeding

environments, located in cattle pen (open grass savanna) and gallery forest.

Blood collection and buffy coat preparation

Blood was collected through the jugular vein-puncture of the 42 sampled Zebu Goudali cattle, using a vacutainer tube containing ethylenediamine tetraacetic acid (EDTA) as anticoagulant. To prepare the buffy coat, micro-capillary tubes were dipped in the whole blood and centrifuged at 12,000 revolutions per minute for 5 minutes using a micro-haematocrit centrifuge (Hawksley, UK). The capillary tubes were cut with a diamond micro-haematocrit tube cutter, few millimetres below the buffy coat. Buffy coat samples were stored at -20 °C prior to DNA extraction.

Fly preparation

Trapped flies were individually placed in eppendorf tubes containing 1ml of 70% alcohol and stored at -20 °C prior to DNA extraction.

PCR amplification and Gel electrophoresis of amplicons

Genomic DNA from the buffy coat and flies were extracted using the Wizard Genomic DNA Purification Kit (Promega, Germany) according to the manufacturer's instructions, and then stored at -20 °C. Generic primers were used in a nested PCR targeting kinetoplastid ITS-1 as described by Ngomtcho et al. (2017). Briefly, the first 25 µl volume reaction contained 2 µM of each outer primers (ITS1-Out Forward: CTT TGC TGC GTT CTT and ITS1-Out Reverse: TGC AAT TAT TGG TCG CGC) with product size between 180 to 640 bp, 0.2 mM dNTP mix, 0.5 U DreamTaq DNA polymerase (Thermo Scientific, Dreieich, Germany), 1× DreamTaq buffer, and 1 µl of extracted DNA isolates. Water and genomic DNA of *T. grayi* were added as negative and positive controls respectively.

PCR amplification was carried out as follows: initial denaturation step at 95 °C for 60 s, followed by 30 amplification cycles with 94 °C for 60 s, 52 °C for 60 s, 72 °C for 30 s, and final extension at 72 °C for 5 minutes. After that, the second PCR reaction was carried out with 1 µl of first 1:80 diluted PCR template under the same cycling conditions as described above, except an annealing temperature of 54 °C, and using the inner primer pairs (ITS1-Inner Forward: TAG AGG AAG CAA AAG, ITS1-Inner Reverse : AAG CCA AGT CAT CCA TCG). 20 µl of the resultant amplicons were visualized in Midori green-stained 1.5% agarose gel.

Statistical analysis

The trap index of apparent abundance (ADT) was determined following the already published formula by Sevidzem et al., (2015) as follows:

$$\text{ADT} = \frac{\text{Number of flies}}{\text{Number of traps} \times \text{Number of trapping days}}$$

The prevalence of trypanosomes in cattle and flies was determined as follows:

$$\text{Prevalence in cattle} = \frac{\text{Number of positive cattle}}{\text{Total number of cattle screened}} \times 100$$

$$\text{Prevalence in flies} = \frac{\text{Number of positive flies}}{\text{Total number of flies screened}} \times 100$$

The non-parametric Kruskal Wallis rank sum test of the R-statistical software 3.3.2 (R Core Team, 2016) was used to compare the ADTs of the haematophagous flies caught. The significant level of the statistical test was set at $p < 0.05$.

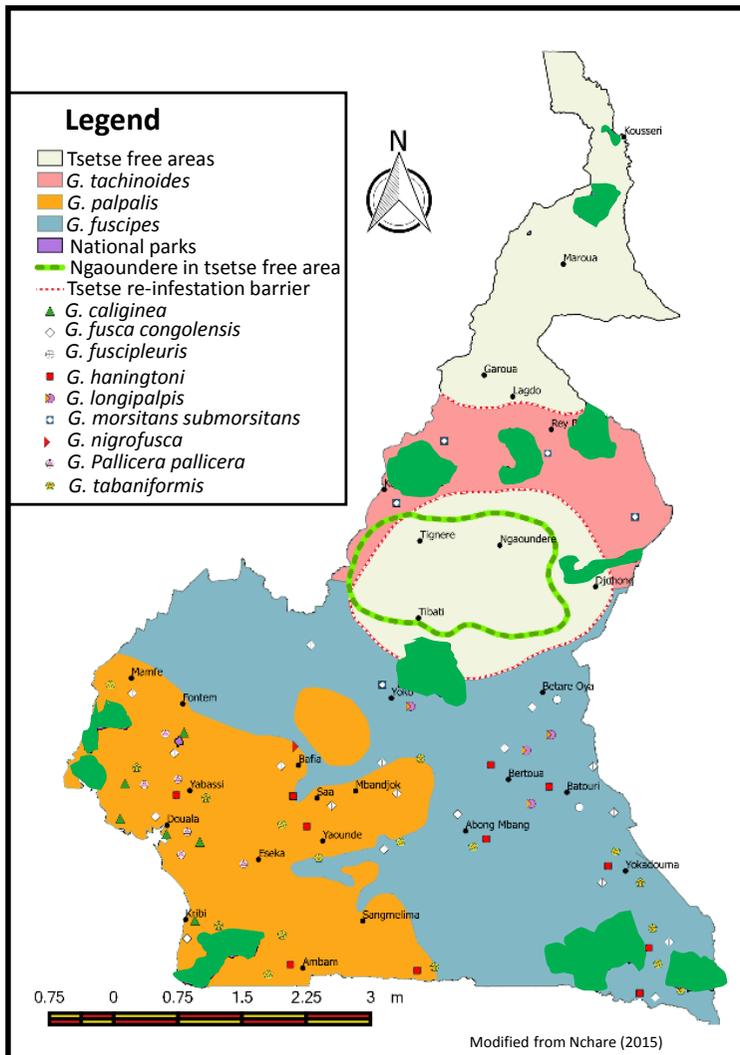


Figure 1: Tsetse fly distribution map of Cameroon with Ngaoundere as tsetse free area. Tsetse data used in this map was provided by the special mission for tsetse eradication (MSEG), Cameroon.

RESULTS

Entomological findings

Entomological prospection resulted in 127 flies grouped under two families notably Tabanidae and Stomoxyini. For Tabanidae, two genera were identified notably *Chrysops* (deer flies), *Tabanus* (horse flies) and *Haematopota* (clegs) with *Chrysops* highly represented. For the stomoxyini, only one genus was identified as *Stomoxys*. At the species level, the ADTs of the members of the aforementioned genera were recorded, 21 *C.*

longicornis (1.2 flies/trap and day), 10 *H. decora* (0.6 flies/trap and day), 9 *T. taeniola* (0.5 flies /trap and day), 6 *T. gratus* (0.3 flies/trap and day), 52 *S. niger niger* (3.0 flies/trap and days), 14 *S. omega* (0.8 flies/trap and days), 8 *S. niger bilineatus* (0.4 flies per trap and day) and 7 *S. inornatus* (0.2 flies per trap and day) (Table 1). There was no statistically significant difference ($P > 0.05$) in the ADTs of biting flies caught.

Molecularly identified trypanosomes from cattle

By comparing the product size from the present gel electrophoresis with the reference product sizes in literature (Ngomtcho et al., 2017), *Trypanosoma theileri* (4%) and *T. vivax* (3%) were detected in the cattle sample with an overall molecular prevalence of 7% (Figure 2).

Molecularly identified trypanosomes from haematophagous flies

Among the 127 haematophagous flies caught, 53 of them were screened, 10 were positive for trypanosomes, resulting in an overall fly infection rate of 18.9%. There was one mix infection of *C. longicornis* with *T. evansi* and *T. theileri*. It was noticed that *Haematopota decora* harbored all the *Trypanosoma spp.* identified in the present study. *C. longicornis*, *T. taeniola* and *T. gratus* harbored only *T. theileri* (Table 1). All the *Stomoxys spp* screened were negative for trypanosomes.

Table 1: Flies caught/screened and the *Trypanosoma spp.* they harbor.

Species	Number	screened	ADT	nPCR	nPCR (%)	<i>Trypanosoma spp.</i>		
						<i>T. theileri</i>	<i>T. vivax</i>	<i>T. evansi</i>
<i>C. longicornis</i>	21	21	1.2	3	14.3	2	1	1
<i>H. decora</i>	10	9	0.6	5	55.6	5	0	0
<i>T. taeniola</i>	9	7	0.5	1	14.3	1	0	0
<i>T. gratus</i>	6	4	0.3	1	25.0	1	0	0
<i>S. n. niger</i>	52	8	3.0	0	0.0	0	0	0
<i>S. omega</i>	14	2	0.8	0	0.0	0	0	0
<i>S. n. bilineatus</i>	8	1	0.4	0	0.0	0	0	0
<i>S. inornatus</i>	7	1	0.2	0	0.0	0	0	0
Total	127	53	7.0	10		9	1	1

nPCR: nested polymerase chain reaction, ADT: trap index of apparent abundance

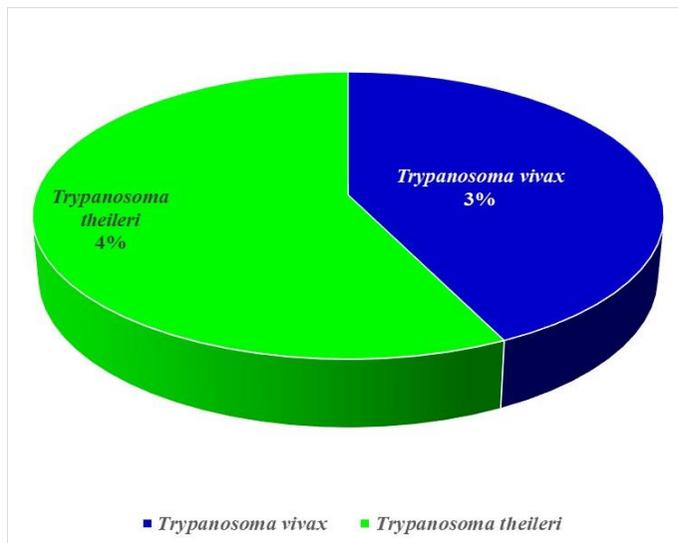


Figure 2: *Trypanosoma spp.* from screened cattle.

DISCUSSION

The occurrence of tabanids and stomoxiines, mechanical vectors of bovine trypanosomiasis in the pasture area of Ngaoundere is an indication of the risk of transmission of the disease in the absence of glossines (Lendzele et al., 2019a; Sevidzem et al., 2019a; 2019b, 2019c). In the Far north region of Cameroon, there are no tsetse flies but the area is heavily infested by tabanids and stomoxiines. Suh et al. (2017) reported the occurrence of *T. vivax* in cattle from the Far North region of Cameroon, indicating the important role of mechanical vectors in transmission. The present study identified one species of the genus *Chrysops* (*Chrysops longicornis*), one species of the genus *Haematopota* (*Haematopota decora*), two species of *Tabanus* (*Tabanus taeniola* and *Tabanus gratus*) and four species of *Stomoxys* (*S. n. niger*, *S. omega*, *S. n. bilineatus* and *S. inornatus*). *C. longicornis* was infected with all the screened *Trypanosoma* spp. (*T. theileri*, *T. vivax* and *T. evansi*), but the positive cases of *H. decora*, *T. taeniola* and *T. gratus* were only from *T. theileri*. From the screening results, *H. decora* was frequently contaminated with *T. theileri* as compared to any other infected fly. These findings were not surprising as Taioe et al. (2017) and Mounioko et al. (2018) already reported that some members of the genera *Tabanus*, *Haematopota*, *Philoliche*, *Atylotus* and *Ancala* were positive for trypanosomes. Similarly, Boese et al. (1987) reported the cyclical transmission of *T. theileri* in *Haematopota* sp. The overall molecular prevalence of trypanosomes in cattle was 7% and was caused by *T. theileri* and *T. vivax*. These trypanosomes have been reported to be transmitted cyclically and acyclically (Boese et al., 1987; Desquesnes et al., 2003). There was no *T. evansi* detected in cattle samples of the sampled animals. This can be explained by the fact that during the sampling, wild animal host like monkeys were common in the area as well as animals from neighbouring Dawa Ranch and Centre Zootechnique that were not sampled. The absence of positive cases of trypanosomes in *Stomoxys* could be explained by the low number of samples screened in the present

study and not their incapacity to be contaminated with trypanosomes. *Stomoxys* spp. have been shown to be implicated in the acyclical transmission of *T. evansi* and *T. congolense* (Sumba et al., 1997). The presence of camels in Ngaoundere might be a source of contamination of local cattle breeds and flies with *T. evansi* since camels are reservoir host of this species (Lun and Brun, 1992).

Conclusion

In a herd located in the tsetse free belt and trypanosomosis hypo-endemic rangeland of Ngaoundere, two *Trypanosoma* spp. (*T. theileri* and *T. vivax*) circulates 'silently' in cattle and tabanids. *Trypanosoma evansi* is reported for the first time in tabanids caught from Ngaoundere. Therefore, the role of Tabanidae and other mechanical vectors in the epizootiology of bovine trypanosomosis in tsetse free areas should not be neglected if control measures are to be taken

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

SA and SSL conceived the idea for the work and wrote the proposal; SSL, SA, AMNN, AM, AP, JFM and AZ assisted in the technical aspect of this study and in editing the manuscript. Both parties gave final approval to the manuscript.

ACKNOWLEDGEMENTS

We are grateful to the special mission for tsetse eradication for providing the traps needed for this survey. Authors thank the Baden Wurttemberg Stipendium Scholarship program (BWS-REK) for the financial support given to the second author (SSL) to conduct the molecular analysis. We thank Leif for assisting in the field survey. We equally thank Dr Renz for permitting us to work with the COBE project cattle herd.

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