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Bovine trypanosomiasis and vectors in Lom and Djerem Division of East-Cameroon

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Abstract

Bovine trypanosomiasis is a major threat to the livestock sector of Cameroon. The main objective of this study was to determine the prevalence of bovine trypanosomes and vectors in the SODEPA Ranch of Ndokayo. A cross sectional parasitological survey was carried out in the late dry season, where blood was collected through the jugular vein of 390 cattle. The trypanosome detection in cattle blood was carried out using the buffy coat technique (BCT). An entomological prospection was carried out in the late dry season and early rainy season using two unbaited trap types notably Nzi (n = 4) and Vavoua (n = 4). The overall bovine trypanosomiasis prevalence was 2.82% and species specific prevalence included: *Trypanosoma congolense* (63.6%), *Trypanosoma brucei* (18.2%) and mixed species infections (*T. congolense* + *T. brucei* and *T. brucei* + *T. vivax*) (18.2%). Sixteen (16) species of flies were identified and grouped under six genera: *Stomoxys*, *Ancala*, *Tabanus*, *Chrysops*, *Hematopota* and *Glossina*. The overall trap apparent density (ADT) was 3.92 flies/trap/day, with a higher ADT in early rainy season than in the late dry season. The highest species richness was noticed with the genus *Tabanus*, while the highest ADT was found in Stomoxyini. The Nzi trap recorded a higher mean ADT for most fly vectors than Vavoua, except for Stomoxyini that rather recorded a higher ADT with the Vavoua trap. The present findings will instruct fly control authorities of Cameroon to better manage trypanosomiasis and vectors in Ndokayo.

Keywords: *Trypanosomiasis, Buffy coat, Prevalence, Vectors, Abundance, Season, Ndokayo*

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1. Introduction

Cattle rearing is a lucrative activity that contributes to the considerable increase in the Gross Domestic Product (GDP) of several countries in the world and particularly sub-Saharan Africa. In tropical Africa, livestock play an important role not only as a source of income, food and fertilizer, but also as a source of livelihood, food security and health for the poor (FAO, 2015). In Cameroon, livestock provides about 30% of income to the rural population. It accounts for about 20% of agricultural GDP or 5 to 8% of total GDP, equivalent to 398.4 billion FCFA (INS, 2015). Cattle rearing in the Eastern region are concentrated in the Lom and Djerem Division. However, this activity is subject to several constraints that hinder its development (Report DDEPIA Lom and Djerem, 2016). Some of these difficulties include: strong agricultural and human pressure and overgrazing linked to the influx of Central African cattle herds to the zone. These constraints are worsened by the lack of information on the epidemiological situation of many diseases and their vectors, affecting livestock in the region and the unavailability of control measures for these diseases (Report DDEPIA Lom and Djerem, 2016).

Despite the direct undesirable effects (pain, allergies, weight loss, etc.) caused by biting stomoxyini and tabanid insects, their high disease transmission potential makes them serious pests of man, domestic and wild animals (Baldacchino *et al*., 2013 and 2014). Several studies have revealed the importance of tabanids in harboring and transmitting zoonotic haemoparasites through molecular evidence (Bitome *et al*., 2017; Moetio *et al*., 2017; and Mounioko *et al*., 2018). In Baghdad, *Stomoxys calcitrans* have been shown to harbor important haemoparasites including *Trypanosoma spp.* by microscopy (Hadi and Amery, 2012).

The biological transmission of both Human African Trypanosomiasis (HAT) and African Animal Trypanosomiasis (AAT) is possible by tsetse flies (Bruce 1895), but in the apparent tsetse free pasture regions, mechanical vectors play an important role in transmission (Duke, 1919). Suh *et al*. (2017) reported that in the absence of tsetse flies in the Far North region of Cameroon, cattle were still detected with *Trypanosoma vivax*. Tabanids and stomoxyines have been experimentally shown to transmit both African and South American *Trypanosoma* spp. (Baldacchino *et al*., 2013 and 2014). In Cameroon, little interest is focused on the role of mechanical vectors in the transmission of trypanosomiasis. The preliminary work of Sevidzem *et al*. (2016) in the Sahel savanna of North Cameroon led to the identification of four *Stomoxys* species, *Stomoxys niger niger*, *Stomoxys calcitrans*, *Stomoxys niger bilineatus* and *Stomoxys sitiens*. In another report in the same region, six species belonging to the family Tabanidae were identified: *Tabanus gratus*, *Tabanus taeniola*, *Tabanus par*, *Tabanus sufis*, *Tabanus biguttatus* and *Chrysops distinctipennis* (Lendzele *et al*., 2017).

The survey of Eteme *et al*. (2017) in the Bétaré Oya zone of East Cameroon was mainly entomological and focused on vectors of bovine trypanosomiasis and did not include a parasitological survey. To the best of our knowledge, no study on bovine trypanosomiasis have so far been carried in East Cameroon. In order to contribute to a better understanding of the current situation of bovine trypanosomiasis, parasitological and entomological studies were carried out in the Ndokayo ranch of SODEPA to determine the prevalence of bovine trypanosomiasis as well as its vectors species composition and abundance.

2. Materials and methods

2.1. Study area

The study was carried out in the SODEPA ranch in Ndokayo. It is located along the N'Gaoundéré-Bertoua motorable highway, beside the Garga-Sarali village, 30 km from Ndokayo. A non-tarred road of 15 km leads to the Ranch camp. Figure 1 shows the location of the SODEPA ranch. The study sites were located between latitude 05021'051''N and longitude 014010' 319''E and elevated at an altitude of 890 m.

2.2. Parasitological study

A cross-sectional parasitological survey was conducted during the dry season in February 2017. The sample size of cattle in the study area was determined using the formula already published by Thrusfield (2005).

$$n = \frac{Z_\alpha^2 \times [P(1-P)]}{\alpha^2}$$

Z_α : Current area $(1-\alpha)$ of the normal curve (Z_α), 1.96 when the confidence interval is accepted at 95%.

P : Prevalence of a disease, P= 50%.

α : Precision which is stated at 5%.

n : Sample size.

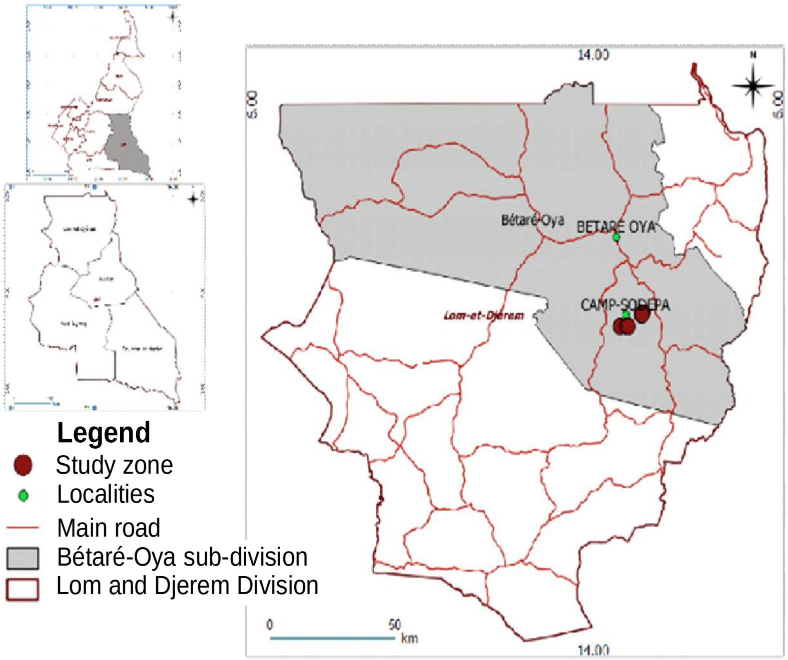

Figure 1: Location of the study area

Using the above formula, we obtained 384 animals from the four sites and the number was increased to 390 in order to eliminate sampling errors.

2.3. Animal sampling and parasitological screening of cattle blood

Random sampling was carried out and for this reason, the ranch was divided into four sites, an average of four herds per site was considered, depending on the herder's availability. In each herd, an average of 24 animals was sampled. The age of the animals ranged from six months to 12 years old. The animals that lastly received trypanocidal treatment three months before sampling were excluded. Parasitological screening was carried out using the buffy coat technique (BCT) (Murray and McIntyre, 1977). Blood was collected from each randomly selected animal through veino-puncture into EDTA tubes, transferred into micro-haematocrit tubes and centrifuged at 3000 rpm for 5 min. The micro-haematocrit tubes were cut few mm into the whole blood column using a diamond micro-haematocrit tube cutter. The buffy coat was observed microscopically to look for trypanosomes.

2.4. Entomological prospection

An entomological study consisted of the trapping and identification of haematophagous flies. This work was carried out in two phases: at the end of the dry season (end of February) and beginning of the rainy season (mid-May). The Nzi (n = 4) and Vavoua (n = 4) traps were used. Traps were pitched in four sites (Minali, Oudou, Camp Génerale and Gabong) of the Ranch and two trap types were placed in each of the four sites. The traps were emptied after 24 h for two weeks successively in the two seasons. The captured flies were put into 5 ml tubes containing 15% ethanol and 5% glycerin solution (Mamoudou *et al*., 2016). The identification of glossines was carried out using the CIRAD identification disc, which considers the morphological features of the species. The identification of Stomoxyini was carried out using the morphological key of Zumpt (1973) and that of tabanids was made using the morphological key of Oldryod (1854).

2.5. Data analysis

The apparent density per trap and day (ADT) was calculated as shown:

$$ADT = N/(D \times T))$$

where *ADT* is the trap apparent density; *N* is the number of species captured; *D* trapping days and *T*, number of traps.

The statistical analyzes were carried out using two software packages: STAT GRAPHIC version 15.1.0.2 and SPSS version 20. The chi-square test (χ^2) was used to compare the influence of site on trypanosome prevalence. The student *t*-test was used to compare the mean of fly catches with site, trap type and season. The significance level was stated at 0.05.

3. Results

3.1. The species composition of the trypanosomes detected

The overall bovine trypanosomiasis prevalence was 2.82%. Parasitological examination revealed three different species: *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax*. The distribution of these species showed that two out of 11 cases had mixed species infections. Cases of mixed species infections (*T. congolense* + *T. brucei* and *T. brucei* + *T. vivax*) accounted for 18.2% of the infections. *T. congolense* was the predominant species with seven out of 11 cases (63.6%). Also, two *T. brucei* infections (18.2%) were identified (Figure 2).

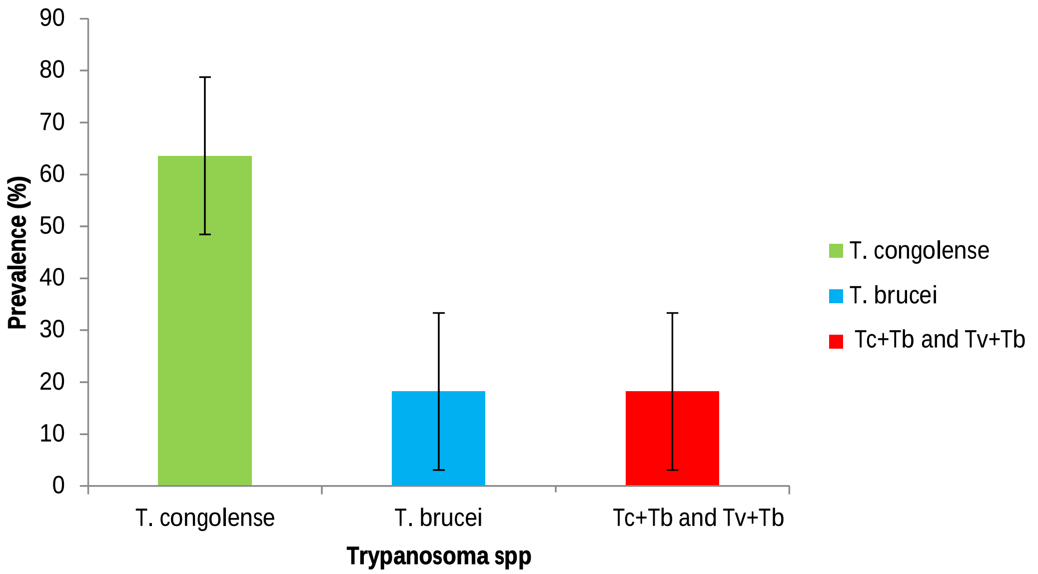

Figure 2: Species specific prevalence of bovine trypanosomiasis

3.2. Parasitological prevalence with respect to site

Prevalence of bovine trypanosomes with sites was between 0 and 1.28%. The highest prevalence was obtained in Gabong (1.28%) and Camp Général (1.28%). The Chi-Square test indicated that there was no statistical significant difference between sites (Table 1).

Table 1: Prevalence of bovine trypanosomiasis with respect to sites

Site	Number	Positive	Prevalence (%)	*p*-Value
Oudou	65	0	0	
Gabong	112	5	1.28	
Camp Général	136	5	1.28	
Minali	77	1	0.26	
Total	**390**	**11**	**2.82**	**0.25**

3.3. Species composition of fly-vectors in the study sites

The entomofauna of the SODEPA ranch of Ndokayo is species-rich with upto 16 species grouped under three families, Muscidae, Tabanidae and Glossinidae. Generally, the Stomoxyini group was most frequent and consisted of four species while, the genus *Tabanus* recorded the highest species richness (six species). Individuals of the genera *Ancala* and *Haematopota* were rare. Only two species of the genera *Chrysops* and *Glossina* were encountered (Figure 3).

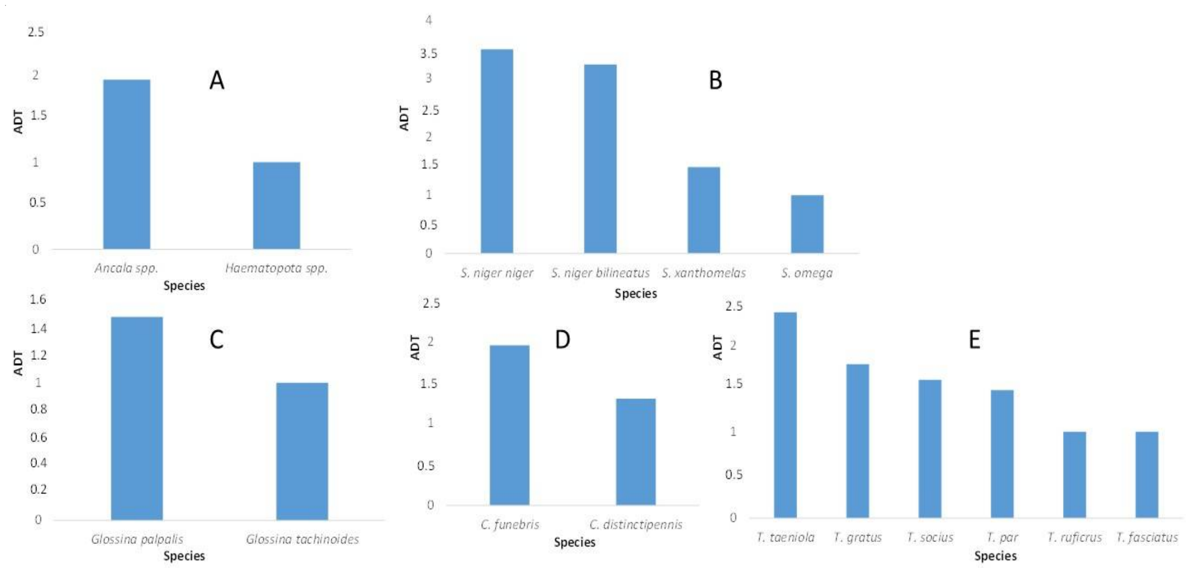

Figure 3: The mean abundance of each species

3.4. The mean abundance with trap type

The mean abundance of the different genera of flies with trap type showed that the Nzi trap recorded higher mean ADT for most fly groups except for the genus *Stomoxys* that rather recorded higher mean ADT with the Vavoua trap with a statistically significant difference ($p < 0.05$) (Table 2).

Table 2: Mean catches at genus level with trap type

Genus	Nzi (mean ADT ± SE)	Vavoua (mean ADT ± SE)	*p*-Value
Glossina	0.05 ± 0.47	0.00±0.00	0.00
Tabanus	0.41 ± 1.97	0.25 ± 1.39	2.68
Chrysops	0.14 ± 0.68	0.01 ± 0.12	0.09
Ancala	0.11 ± 0.49	0.00 ± 0.00	0.00
Haematopota	0.01 ± 0.12	0.00 ± 0.00	0.00
Stomoxys	0.86 ± 2.91	5.99 ± 24.36	0.00

3.5. The apparent density of vectors in the sampled sites

The ADT of fly-vectors in the study area was 3.92 flies / trap / day (f / t / d) and was genera dependent: 13.71 *Stomoxys* / trap / day, 1.35 *Tabanus* / trap / day, 0.30 *Chrysops* / trap / day, 0.22 *Ancala* / trap / day, 0.11 *Glossina* / trap / day and 0.03 *Haematopota* / trap / day. Most of the species caught showed no significant difference between the capture sites, except *Chrysops funebris*, which showed a significant difference between the Oudou and Gabong sites ($p < 0.05$) (Table 3). Minali was the site where a scanty fly-catch was observed.

Based on fly-catches with season, only *T. gratus, C. funebris and C. distinctipennis* did not show statistically significant difference ($p > 0.05$) with season, but the others recorded a statistically significant difference ($p < 0.05$) with season (Table 3). Generally, higher fly ADT was noticed in the late dry season than in the early rainy season (Table 4).

Table 3: Vectors apparent densities with sampled sites

Species captured	Camp général	Oudou	Gabong	Minali	*p*-Value
Glossina palpalis	0.08 ± 0.50	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Glossina tachinoïdes	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.17	0.00 ± 0.00	0.00
Total *Glossina* spp.	**0.08 ± 0.50**	**0.00 ± 0.00**	**0.03 ± 0.17**	**0.00 ± 0.00**	
Tabanus par	0.06 ± 0.23	0.03 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.29
Tabanus taeniola	0.36 ± 1.05	0.22 ± 0.89	0.00 ± 0.00	0.08 ± 0.37	0.13
Tabanus gratus	0.06 ± 0.23	0.11 ± 0.52	0.00 ± 0.00	0.00 ± 0.00	0.25
Tabanus fasciatus	0.03 ± 0.17	0.03 ± 0.17	0.03 ± 0.17	0.06 ± 0.23	0.89
Tabanus socius	0.08 ± 0.37	0.06 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.29
Tabanus ruficrus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.17	0.00
Total *Tabanus*	**0.59 ± 2.05**	**0.45 ± 2.08**	**0.03 ± 0.17**	**0.17 ± 0.77**	
Chrysops funebris	0.00 ± 0.00	0.19 ± 0.58	0.03 ± 0.17	0.00 ± 0.00	0.01
Chrysops distinctipennis	0.00 ± 0.00	0.05 ± 0.33	0.00 ± 0.00	0.03 ± 0.17	0.57
Total *Chrysops*	**0.00 ± 0.00**	**0.24 ± 0.91**	**0.03 ± 0.17**	**0.03 ± 0.17**	
Ancala spp	0.03 ± 0.17	0.19 ± 0.67	0.00 ± 0.00	0.00 ± 0.00	0.10
Total *Ancala*	**0.03 ± 0.17**	**0.19 ± 0.67**	**0.00 ± 0.00**	**0.00 ± 0.00**	
Hematopota negripennis	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.17	0.00 ± 0.00	0.00
Total *Haematopota*	**0.00 ± 0.00**	**0.00 ± 0.00**	**0.03 ± 0.17**	**0.00 ± 0.00**	
Stomoxys niger niger	4.53 ± 16.15	3.14 ± 10.26	0.89 ± 2.17	0.25 ± 0.91	0.33
Stomoxys niger bilineatus	1.75 ± 8.04	2.28 ± 1.68	0.72 ± 2.57	0.03 ± 0.17	0.54
Stomoxys xanthomelas	0.00 ± 0.00	0.06 ± 0.23	0.00 ± 0.00	0.03 ± 0.17	0.29
Stomoxys omega	0.03 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Total *Stomoxys*	**6.31 ± 24.36**	**5.48 ± 12.17**	**1.61 ± 4.74**	**0.31 ± 1.25**	

Table 4: Fly-vector apparent densities with seasons

Species captured	Late dry season	Early rainy season	*p*-Value
Glossina palpalis	0.04 ± 0.35	0.00 ± 0.00	0.00
Glossina tachinoïdes	0.00 ± 0.00	0.01 ± 0.12	0.00
Total *Glossina* spp.	**0.04 ± 0.35**	**0.01 ± 0.12**	
Tabanus par	0.00 ± 0.00	0.04 ± 0.20	0.00
Tabanus taeniola	0.33 ± 0.99	0.00 ± 0.00	0.00
Tabanus gratus	0.03 ± 0.16	0.05 ± 0.37	0.99

Table 4 (Cont.)

Species captured	Late dry season	Early rainy season	*p*-Value
Tabanus fasciatus	0.07 ± 0.25	0.00 ± 0.00	0.00
Tabanus sucius	0.07 ± 0.35	0.00 ± 0.00	0.00
Tabanus ruficrus	0.00 ± 0.00	0.01 ± 0.12	0.00
Total *Tabanus* spp.	**0.50 ± 1.75**	**0.10 ± 0.69**	
Chrysops funebris	0.03 ± 0.16	0.08 ± 0.40	0.39
Chrysops distinctipennis	0.03 ± 0.23	0.01 ± 0.12	0.99
Total *Chrysops* spp.	**0.06 ± 0.39**	**0.09 ± 0.52**	
Ancala spp	0.00 ± 0.00	0.11 ± 0.49	0.00
Total *Ancala* spp.	**0.00 ± 0.00**	**0.11 ± 0.49**	
Hematopota negripennis	0.01 ± 0.12	0.00 ± 0.00	0.00
Total *negripennis* spp.	**0.01 ± 0.12**	**0.00 ± 0.00**	

4. Discussion

The overall parasitological prevalence of bovine trypanosomes was 2.82%. This prevalence was lower than that reported by Mamoudou *et al*. (2016) in the Mezam division. This low prevalence is probably due to the good cattle management program of the Ranch. Furthermore, the method of diagnosis used could also influence the results and the use of other methods such as serology (indirect ELISA) and PCR, could have been more sensitive than the parasitological method for trypanosomes detection (Clarisse, 2009; Mpouam *et al*., 2011; and Talaki, 2014). The Gabong and Camp Général sites had higher prevalences. These sites are furthest away from the center of the Ranch and human activities here are reduced. Tsetse flies tend to concentrate in such environments which are less encroached by humans. The animals in these sites are at higher risk of trypanosome infection. Identification of trypanosome species showed that *T. congolense* (63.6%) was the most predominant species. This indicates the important role of tsetse fly (biological vector of bovine trypanosomosis) in its transmission in the Ranch. These results are similar to those of Tanenbe *et al*. (2010) in the Faro and Deo and Vina division and that of Mamoudou *et al*. (2015) in the Mayo-Rey division in North Cameroon. From these studies it was noticed that in the presence of tsetse flies in pasture land, *T. congolense* was the dorminant species identified in cattle.

We trapped and identified six genera: *Stomoxys*, *Tabanus*, *Chrysops*, *Ancala*, *Glossina* and *Hematopota*. The genus *Stomoxys* was the most abundant. East Cameroon is rich in wildlife (warthog, monkey, antelope) (Pouïra, 2011) and their presence could justify the abundance of mechanical vectors within the Ranch because wild animals are a major food source of these vectors (PATTEC, 2005). This result corroborate with that of Sevidzem *et al*. (2015) in the Faro and Deo division where they found that tsetse flies, tabanids and stomoxes were dense around the Faro game reserve than in the villages of Alme. Most of the species caught showed significant difference in densities with sites except *Chrysops funebris*, *T. gratus* and *C. distinctipennis*. This indicate that the environmental conditions such as climate, host availability, breeding points and low predator numbers, favored the development and survival of these species in the four protected sites. The highest species richness noticed with the genus *Tabanus* was not astonishing as this has been reported by other authors (Ahmed *et al*., 2005; Sieumeni *et al*., 2019; and Sevidzem *et al*., 2019a). This was not surprising because the genus *Tabanus* is among the genera of the family Tabanidae with the highest number of species (Oldroyd, 1954) and adapts to the environmental conditions of the Afro-tropical region. The highest ADT noticed with Stomoxyini has been reported by several authors (Hiol *et al*., 2019; Sevidzem and Mavoungou, 2019; and Sevidzem *et al*., 2019a). Individuals of the tribe Stomoxyini have been reported to adapt well in tropical environments (Zumpt *et al*., 1973) and, their high numbers can be due to their high resilience and ability to breed in several substrates especially in human encroached environments (Mavoungou *et al*., 2017). Fly trapping success and species diversity depends on the type of trap used, because each trap type has its characteristics especially size, color; movement, odor and shape that permits it to attract particular fly groups. In the case of tabanids, stomoxynes and glossines, blue-black cloth traps, such as Nzi, Vavoua and Biconical traps have been used to trap them. The present survey showed that glossines and tabanids were highly caught by the Nzi trap, while highest stomoxyini abundance was noticed with the Vavoua trap. According to the report of Sieumeni *et al*. (2019), the Nzi trap was more efficient for trapping glossines and tabnids. Sevidzem *et al*. (2019b) reported that the Vavoua trap was most efficient in trapping Stomoxyini than Nzi and Biconical. The trap type influence on the catches of the different insect vector groups could be related to the differences in their shapes and sizes.

5. Conclusion

Animals of the SODEPA Ranch in Ndokayo are infected (2.82%) with the following species of trypanosomes: *T. congolense*, *T. vivax* and *T. brucei*. Tsetse flies as well as other biting-fly counterparts (tabanids and Stomoxyini) occur in this pasture area of the Ranch with *Stomoxys niger niger* being the most dorminant species. The current preventive and curative prophylaxis programs put in place by the administration of the Ranch needs to be coupled with a good production system as well as an anti-vectorial system in-order to minimize the risk of constant reintroduction of bovine trypanosomiasis and its vectors into the Ranch by the neighboring Central African Republic herds during transhumance.

Conflicts of interest

None is declared by authors.

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