

Vectors and parasitological prevalence of African Animal Trypanosomiasis (AAT) in the cattle of Djerem Division (Adamaoua –Cameroon)

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Abstract: In sub-Saharan countries, livestock production is under the biggest constraint due to African Animal Trypanosomiasis (AAT) threatening cattle of the area. In order to forward baseline information on the extent of the problem caused by AAT and to provide sustainable control strategies, a cross sectional survey of trypanosomiasis bovine disease and its vectors tsetse fly was carried out from October 2009 to January 2010 in the cattle of the Djerem division of the Adamaoua region of Cameroon. The survey of tsetse fly vectors was conducted using biconical traps and the captured insects were identified binocular optic microscope. The parasitological survey was realized through blood samples collection from cattle jugular vein and screened using Buffy coat diagnostic technique for parasite examination. In results, a total of 296 flies vectors of AAT were captured in which after identification, 4 vector species including *Glossina fuscipes fuscipes* (13.45%), *Tabanus bovinus* (15.33%), *Atylotus agrestis* (24.75%) and *Stomoxys calcitrans* (46.73%) were recorded and representing apparent densities of 0.26, 0.29, 0.49 and 0.88 fly/trap/day, respectively. The prevalence of trypanosomiasis in the Djerem division was significantly ($P<00001$) up to 19.16% with a confidence interval ranging from 15.22 to 23.61%. The black cattle and the anemic cows were the most significantly ($P<0.05$) infected with trypanosomes. The trypanosomes species diagnosed in this work included *Trypanosoma congolense* (78.26%), *T. brucei* (52.17%) and *T. vivax* (11.59%) showing that *T. congolense* is the most prevalent species in the study area. However, the present investigation highlighted the maintaining of the trypanosomose parasites in the Djerem division without reinvasion of the cleaned and buffer zones with the biological vector (*G. f. fuscipes*). Thus, intervention within the framework of keeping out tsetse flies from the zone and trypanosomiasis control programmes should be forecast.

Keywords: Trypanosomes, prevalence, vectors, bovine, Adamaoua region

Date of Submission: 24-09-2018

Date of acceptance: 31-10-2018

I. Introduction

In sub-Saharan Africa, food security is threatened because of the presence of African Animal Trypanosomiasis (AAT), which has the biggest constraints to livestock production [1]. AAT is a cattle disease complex caused by *Trypanosoma* species, biologically transmitted by tsetse flies (*Glossina* species) and mechanically by other biting flies, which infested about 10 million km² areas of sub-Saharan African countries [2]. It is an endemic animal disease that causes a drain on the financial resources of livestock farmers and the productivity of their livestock [3]. This cattle disease constitutes one of the key constraints in the progress of animal production in the area [4], and is responsible of 20% of production losses (about 1 billion US dollars yearly) in term of strength reduction and mortality of animals as well as milk, meat and calves production rate [5][6]. All these losses affect also the herd size and herd composition [7]. Besides, the burden of tsetse flies and AAT constrains a moving of herds to the free vector-disease lands and this movement reduces the availability of draft animals to plough fields and manure fertilizer for crop production [6]. In Cameroon, the bovine breeding has been intensified these last years with a national livestock estimated at 10 million heads [8]. But this intensification is accompanied by several difficulties generated by trypanosomiasis pathologies with economic and social repercussions considerable [9].

To reduce the impact of AAT, trypanocides are applied and trypanotolerant cattle breeds are introduced. To reduce the risk AAT transmission, vector tsetse fly control by diverse methods including insecticide treatment of cattle, the use of traps or targets, ground or aerial insecticides spraying, or reducing the

risk of exposure through changes in livestock management is largely practiced. However, because of the misuse of trypanocides, trypanosome species are becoming highly resistant to these common treatments [10][11]. Thus, in the past half century, through integrated control programmes managed by specialist government institutions in the Adamaoua plateau of Cameroon, the suppression of tsetse was successfully achieved, however, control efforts were not sustained since many occurrence of reinvasion were reported in some area of this region [12][13][14][15]. From the past years after control programmes till 2009, no published parasitological and entomological data exist on animal trypanosomiasis in the Djerem division part of the Adamaoua region. Nevertheless, herds from the localities are complaining for the decrease in animal production. Knowing the parasitological situation of AAT and the vectors involved in the transmission of that zoonotic disease may constitute a first step for the efficient control of the trypanosomiasis in this area. The objective of this present investigation was to determine diversity and abundance of AAT vectors and the prevalence of the *Trypanosoma* infections in cattle of the Djerem Division, locality known as a high potential cattle population production in the region.

II. Materials and methods

2.1 Study area

The study was carried out from October 2009 to January 2010 in the Djerem division, area about 13 284 Km² located in the southern part of Adamaoua region of Cameroon and situated between latitude 6° and 7° north, 12° and 13° East with an altitude of 1100 m. It is limited from north with the Faro & Déo and Vina divisions, in the west with Mayo Banyo division, the east by the Mbéré and Lom & Djerem in East, and Mbam & Kim division in the south. The climate is soudano-guinean with two seasons: a raining season from April to October with an annual rainfall of 1600 mm in average and a dry season from November to March. The vegetation covering the Djerem division is a shrubby, grassy savannah and marked out with forest galleries [16][17]. The hydrographic system is dominated by the Djerem river and its tributaries, maintained by Mbakaou dam. Animal breeding and fishery constitute a significant activity, and the cattle population is estimated about 100,000 heads for a population of 100,000 people [8]. From south to north of Djerem division after tsetse eradication campaigns in 1994, the territory was divided in three zones, which constitute areas studied in this present investigation (Figure 1):

- The first zone situated in the south side of the Division and includes Mbitom and Mbakaou localities known as the infested zones.
- The second zone located in the front line of Adamaoua region and considered as postmark zone including of Ngaoundal and Danfilé localities.
- The third zone was the *Glossina* cleaned up (treated area) covering Laidé Ng-Gouda and Béka Gotto localities.

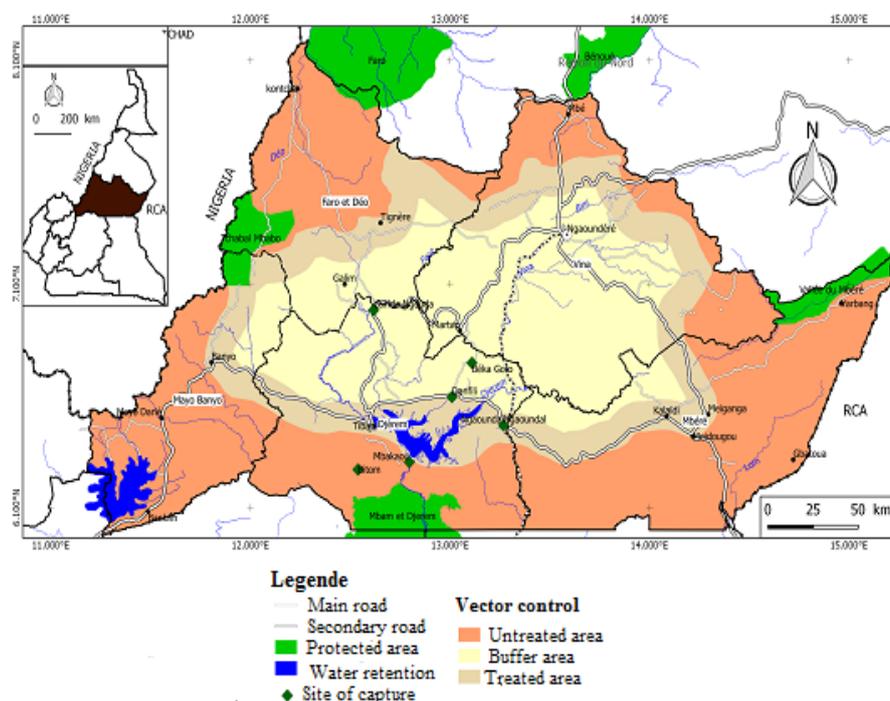


Figure 1. Map of the Adamaoua region showing the study area

2.2 Entomological monitoring of tsetse flies

The potential vectors were collected using 9 biconical traps [18] and geographically referenced 100 m interval [19]. Insects were collected from the traps every day for 5 consecutive days [18]. Insects collected were identified under the optic microscope following the identification keys described by CIRDES [20]. The different apparent densities (APD) were calculated using following formula:

$$APD = \frac{\text{number of insects captured}}{\text{number of traps} \times \text{number of days}}$$

2.3 Parasitological survey

For the parasitological prevalence determination, 360 blood samples were collected in 24 identified herds distributed in three zones. From each cattle randomly selected, 5 ml of blood was collected from the jugular vein using 10 ml syringe and immediately transferred into bijoux bottles containing EDTA, stored in a flask containing icepacks. Buffy Coat Technique method was used to process the blood. Parasitological assays were conducted in the field within less than four hours after sampling using buffy coat method. The diagnostic technique used was a field examination of a stained thin blood film of buffy coat for the observation *Trypanosoma* parasites.

2.4 Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) Version 16.0 software. The proportions were statistically compared using the Chi-square test. Graphs were designed using Microsoft Excel software.

III. Results

3.1 Entomological survey of the trypanosomiasis vectors

During the study period, a total of 296 flies vectors of African Animal Trypanosomiasis were captured. After insect vectors captured and identified, 4 species were recorded including *Glossina fuscipes fuscipes*, *Tabanus bovinus*, *Atylotus agrestis* and *Stomoxys calcitrans* (Table 1). The proportion of the mechanical vector *S. calcitrans* (46.73%) was high compared to the proportion of the biological vector *G. f. fuscipes* (13.45%) and others mechanical vectors *T. bovinus* (15.33%) and *A. agrestis* (24.75%). The apparent densities recorded were 0.26, 0.29, 0.49 and 0.88 fly/trap/day for *G. f. fuscipes*, *T. bovinus*, *A. agrestis* and *S. calcitrans*, respectively. The mechanical vector *T. bovinus* was present in the all localities studied while the biological vector *G. f. fuscipes* was identified only in the untreated localities Mbakaou and Mbitom. The mechanical vector species *A. agrestis* and *S. calcitrans* were found in all localities except the treated area Beka Gotto and the number of *S. calcitrans* vector species captured in the existing localities was high compared to other vector species. Globally, apparent density was high in the untreated areas including Mbitom and Mbakaou with APD values of 2.58 and 1.72 fly/trap/day, respectively compared to the cleaned zones. The apparent density was also significant in Ngaoundal (APD=2.19), showing the maintaining of AAT vectors in that area.

Table 1. Apparent density of fly species captured in the localities of the Djerem division. APD = Apparent density (flies/trap/day), T = total.

| Localities | Total flies caught | | tse-tse flies | | Other biting flies | | | | | |
|-------------|--------------------|------|-----------------------|------|--------------------|------|----------------------|------|-------------------|------|
| | | | <i>G. f. fuscipes</i> | | <i>A. agrestis</i> | | <i>S. calcitrans</i> | | <i>T. bovinus</i> | |
| | T | APD | T | APD | T | APD | T | APD | T | APD |
| Beka Gotto | 10 | 0.22 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.22 |
| Danfili | 21 | 0.47 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0.47 |
| Laïdé Gouda | 31 | 0.69 | 0 | 0 | 11 | 0.24 | 9 | 0.20 | 11 | 0.24 |
| Mbakaou | 62 | 1.38 | 13 | 0.29 | 8 | 0.18 | 24 | 0.53 | 17 | 0.38 |
| Mbitom | 93 | 2.07 | 26 | 0.58 | 14 | 0.31 | 42 | 0.93 | 11 | 0.24 |
| Ngaoundal | 79 | 1.76 | 0 | 0.00 | 11 | 0.24 | 58 | 1.29 | 10 | 0.22 |
| Total | 296 | 6.58 | 44 | 0.98 | 39 | 0.87 | 133 | 2.96 | 80 | 1.78 |

3.2. Parasitological prevalence of trypanosomiasis

The overall trypanosomiasis prevalence in the Djerem division was significantly ($P < 0.0001$) up to 19.16% with a confidence interval ranging from 15.22 to 23.61%. Within the Djerem division studied, the prevalence of the trypanosomiasis infection decreased significantly ($P < 0.05$) from in the treated zone compared

to the infested. The prevalence of 26.66% was recorded in the cattle living in the infested zone while prevalence of 17.50% and 12.50% were registered in the cattle of buffer and treated zones, respectively.

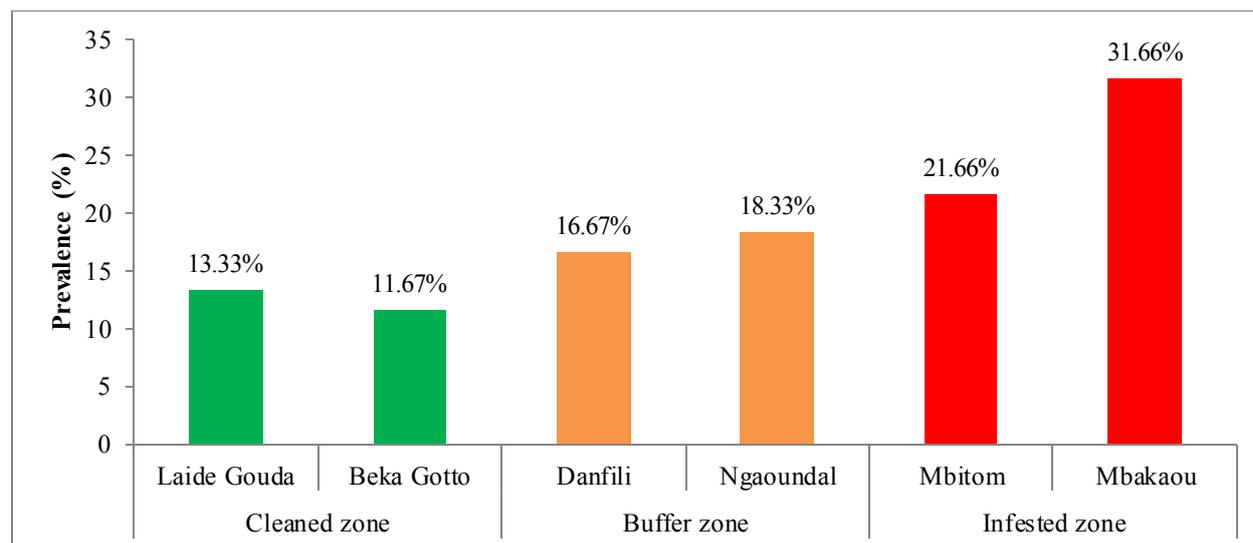


Figure 2. Parasitological prevalence of AAT in the zones and localities studied

Table 3 presents the risk factors including age, sex, breeding, color and PCV in relationship with the prevalence of trypanosomosis in cattle of the Djerem division.

In this present work, cows aged between 2-5 old and greater or equal to 5 years old representing a trypanosomosis prevalence of 19.6% and 20%, respectively were most affected compared to the young animals aged less or equal to 2 years old.

Regarding the sex of the animals, there was not significant ($P > 0.857$) relationship between prevalence in both sex male (20%) and female (19%). The difference of trypanosomosis prevalence between different cattle breeding in the Djerem division was not significantly ($P > 0.419$) even if Akou breeding with high prevalence of 25% compared to other breedings.

Considering the color of the cattle, black and white colors trypanosomosis prevalence of 34.6 and 26.6%, respectively were significantly ($P < 0.015$) the most affected to the parasite species compared to the other bovine colors.

The prevalence of the cattle in the Djerem division was also significantly ($P < 0.0001$) related to the packed cell volume of the blood samples examined. The majority of cows diagnosed and confirmed as positive to trypanosomes representing 88.5% were anemic compared to non-anemic animal representing 13.8%.

Table 3. Risk factors with the prevalence of Trypanosomosis

| Variable | | No. Examined | No. Positive | Prevalence (%) | Chi-square test |
|----------|-------------------|--------------|--------------|----------------|---------------------------------------|
| Age | ≤ 2 | 43 | 6 | 14 | $\chi^2 = 0.863$; df=2 ; P=0.650 |
| | [2 - 5] | 112 | 22 | 19.6 | |
| | > 5 | 205 | 41 | 20 | |
| Sex | Female | 300 | 57 | 19 | $\chi^2 = 0.032$; df=1 ; P=0.857 |
| | Male | 60 | 12 | 20 | |
| Breeding | Akou | 112 | 28 | 25 | $\chi^2 = 3.906$; df=4 ; P=0.419 |
| | Backale | 13 | 2 | 15.4 | |
| | Djafoun | 174 | 28 | 16.1 | |
| | Djaoun | 1 | 0 | 0 | |
| | Goudali | 60 | 11 | 18.3 | |
| Colour | White | 109 | 29 | 26.6 | $\chi^2 = 12.38$; df=4 ; P=0.015 |
| | White-Black | 1 | 0 | 0 | |
| | White-Red | 27 | 3 | 11.1 | |
| | Black | 26 | 9 | 34.6 | |
| | Red | 197 | 28 | 14.2 | |
| PCV | Anemic PCV<25 | 26 | 23 | 88.5 | $\chi^2 = 86.85$; df=1 ; P<0.0001 |
| | Non-anemic PCV≥25 | 334 | 46 | 13.8 | |

PCV: Packed cell volume; No: Number of cow; χ^2 : Chi-square; df: degree of freedom; P: Level of significance. In the Djerem division, three trypanosomes species were identified in the 69 cattle blood samples diagnosed as positive to trypanosomes. These trypanosomes species include *Trypanosoma congolense*, *T. brucei* and *T. vivax*. The prevalence of *T. congolense* (78.26%) was high compared to *T. vivax* (52.17%) and *T. brucei* (11.59%) (Figure 3).

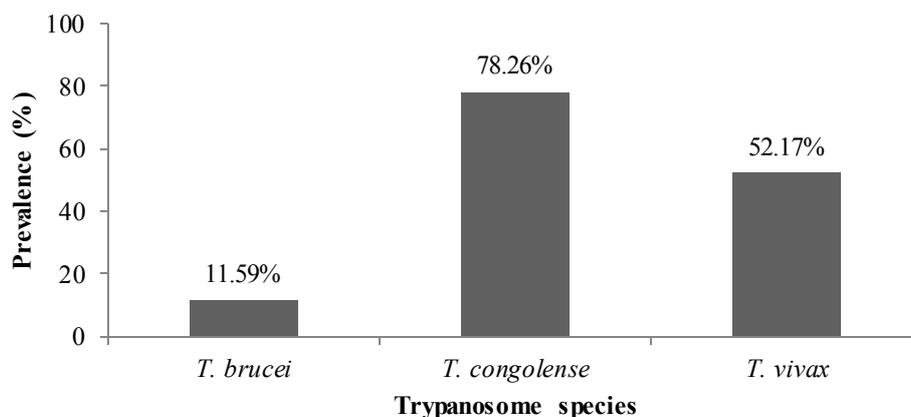


Figure 3. Prevalence of trypanosome species of the Djerem division cattles

In the present investigation, the type of bovines infection significantly ($\chi^2=360$; $df=6$; $P<0.0001$) varied among the infected animals (table 2). Within the 69 cattles diagnosed as positive to trypanosomes, 43 (62.32%) cows were single infected while 23 (33.33%) and 3 (4.35%) cows were double and triple infected with Trypanosome species, respectively. In the single infection, *T. congolense* (43.5%) was the most encountered while in the multiple infections, *T. congolense* combined to *T. vivax* representing (29%) were most found in one bovine.

Table 3. Distribution of trypanosomes diagnosed in the cattle of Djerem Division

| Infection type | Trypanosomes | No. positive | Prevalence (%) | χ^2 | P-value |
|--------------------|--------------------|--------------|----------------|----------|----------|
| Single infection | <i>T.b</i> | 2 | 2.9 | 360.0 | <0.00001 |
| | <i>T.c</i> | 30 | 43.5 | | |
| | <i>T.v</i> | 11 | 15.9 | | |
| Multiple infection | <i>T.c-T.b</i> | 1 | 1.4 | | |
| | <i>T.c-T.v</i> | 20 | 29 | | |
| | <i>T.v-T.b</i> | 2 | 2.9 | | |
| | <i>T.c-T.v-T.b</i> | 3 | 4.3 | | |
| Total | | 69 | 100 | | |

T.b: trypanosoma brucei; *T.c:* Trypanosoma congolense; *T.v:* Trypanosoma vivax

IV. Discussion

The entomological and parasitological survey of trypanosomosis in the Djerem division revealed the reinfection of the zones with trypanosomes and its vectors. Indeed, in the past half century, through integrated control programmes managed by specialist government institutions in the Adamaoua plateau of Cameroon, the suppression of tsetse was successfully achieved in the Djerem division. But, the present investigation showed the presence of tsetse flies and other mechanical vectors in the area; leading to the maintaining of the pathogenic trypanosomes in the cattle of the zones. However, ten years after the tsetse eradication campaign in the Adamaoua plateau of Cameroon, many occurrence of reinvasion were previously reported in other zones of this region [13][15] [21][22]. In this present study, Djerem division was infested with only one biological vector fly species *Glossina fuscipes fuscipes*, and others flies species including *Tabanus bovinus*, *Atylotus agrestis* and *Stomoxys calcitrans* playing role as mechanical vector of trypanosome parasites. Similar findings was reported from the Faro & Deo division belonging to the same region indicating *Glossina morsitans* (47.27%) as a only biological vector tsetse fly caught associated to the mechanical vectors including *Stomoxys* (4.50%) and *Tabanus* (3.59%) fly species [15][21]. Indeed, trypanosomes parasites appeared for the first time in the Adamaoua plateau within 1950 years through the invasion of Djerem division *Glossina morsitans submorsitans*, *G. morsitans* and *G. tachinoides* tsetse fly species [23][24][25]. Nevertheless, Tongue et al. [22] identified several biological vectors such as *G. f. fuscipes*, *G. m. submorsitans* and *G. fusca congolense* fly species from the entomological survey conducted in Dodeo basin, an area in the Adamawa region, northern- Cameroon. Variation of *Glossina* species within the zones could be linked to the structure and landscape able to create

particular microhabitat favorable for insects. The combination of several factors including climate, vegetable density and the presence of hosts for blood feeding may explain also this variation [26][27].

The parasitological survey of trypanosomosis in cattle of the Djerem Division conducted to the overall prevalence was 19.16%. A survey carried out in 2005 by Mamoudou et al. [21], 10 years back after the tsetse fly eradication campaign in the Adamaoua plateau of Cameroon showed the reinfection of the region with a trypanosomosis incidence ranging from 3.7 to 20% in the valley and from 1.8 to 13.4% in the buffer zone. Compared to the prevalence recorded in this present study, a high prevalence of 37.7 and 29.4% were previously registered respectively in 2009 and 2014-2015 in the Faro & Deo division, Adamaoua region of Cameroon [13][15]. In the Vina Division of the same region, Mpouam et al. [28] reported a prevalence of 15.2%, lower than the one obtained in the present study. From Nigeria, a high prevalence of bovine trypanosomiasis up to 46.8% was reported across the Jos Plateau in which *Trypanosoma brucei brucei*, *T. congolense* savannah and *T. vivax* species were diagnosed in the cattle [29]. From Ethiopia, trypanosomosis prevalence of 5.6% was reported by Aki et al. [30] in which *T. vivax* (63.6%) and *T. congolense* (36.4%) were diagnosed as bovine trypanosome species. Variation in the trypanosomiasis prevalence could be due to the improved knowledge of the farmers on trypanosomosis and tsetse fly control using screen traps and trypanocides in the different area infested. The diagnosis method of the blood sample which is not entirely efficient or reliable may also influence the findings.

In this study, three *Trypanosoma* species in single or multiple infections were diagnosed in the bovines' blood samples with the predominance of *T. congolense* species. In the Faro & Deo division of the Adamaoua region, *T. congolense* species was the most found in the cattle blood samples [21]. Study conducted by Abdullahi et al. [31] showed also the domination *T. congolense* (78.57%) trypanosome species encountered compared to the total trypanosome infection. *T. congolense* was also the most prevalent species in the cattle of in Bokkos LGA of Plateau State, Nigeria [32]. In contrary, *T. vivax* accounts for most of the infection in cattle of north of Cameroon, Sokoto abattoir of Nigeria and Ethiopia [15][30][33]. Indeed, in areas where rainfall is above 1000 mm in the Central African Region, cattle trypanosomiasis caused by both *T. vivax* and *T. congolense* is prevalent [34]. But, experimental studies have demonstrated the possibility of mechanical transmission of these two parasites (*T. congolense* and *T. vivax*) species by tabanids and *Stomoxys* in areas not infested by tsetse flies [35][36], explaining their high prevalence in the bovine blood samples.

V. Conclusion

From this present investigation, four potential vectors species of AAT including *Glossina fuscipes fuscipes* (13.45%), *Tabanus bovinus* (15.33%), *Atylotus agrestis* (24.75%) and *Stomoxys calcitrans* (46.73%) were identified and representing respectively, apparent densities of 0.26, 0.29, 0.49 and 0.88 flies/trap/day. Mechanical vectors (*T. bovinus*, *A. agrestis* and *S. calcitrans*) are overall, the most abundant in more than 50% of the investigated areas. The overall prevalence of trypanosomiasis in the Djerem division was significantly ($P < 0.0001$) up to 19.16%. The black cattle and the anemic cows were the most significantly ($P < 0.05$) infected with trypanosomes. The cattle of the study area was infected three trypanosomes species including *Trypanosoma congolense* (78.26%), *T. brucei* (52.17%) and *T. vivax* (11.59%); showing *T. congolense* as the most prevalent species in the study area. Thus, the prevalence rate of *Trypanosoma* spp in Djerem division remains important in relation to the abundance of vectors and control strategies is needed. Further longitudinal studies of risk assessment of AAT should be conducted to define a more appropriate control plan.

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