



## Current prevalence of cattle trypanosomiasis and of its vector in Alme, the infested zone of Adamawa plateau Cameroon, two decades after the tsetse eradication campaign

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### ABSTRACT

Livestock diseases especially cattle trypanosomiasis remains a challenge and a call for concern. A Cross sectional study was carried out on the entomological and parasitological prevalence of cattle trypanosomiasis, in the tsetse fly infested zone-Alme in Faro and Deo division, Adamaoua region Cameroon. The aim of this study was to determine the prevalence of bovine trypanosomiasis, apparent density and distribution of its vectors. Blood samples were randomly collected from 504 selected cattle and analyzed using the Haematocrit Centrifugation Technique (HCT); identification of different trypanosomes was achieved using the method of May-Grünwald Giemsa. An entomological survey was conducted using laveissière type blue biconical traps (n=11 traps; 9 traps in the Alme Ranch and 2 around the Faro Game Reserve). The overall prevalence of trypanosome infection in the study area was 29.4%. However, there was a statistical significant ( $P<0.05$ ) difference of trypanosome species with site. There were single as well as mixed infections. The single infections identified were: *Trypanosoma congolense* (14.09%), *Trypanosoma vivax* (6.15%) and *Trypanosoma brucei* (3.37%) and mixed infections were *Trypanosoma congolense*+*Trypanosoma vivax* (2.38%), *Trypanosoma congolense*+*Trypanosoma brucei* (0.39%), *Trypanosoma vivax*+*Trypanosoma brucei* (0.39%) and *Trypanosoma congolense*+*Trypanosoma vivax*+ *Trypanosoma brucei* (2.57%). Entomological findings indicated that *Glossina morsitans* (47.27%) was the only tsetse fly species caught in the study area with others (44.5%), *Stomoxys* (4.50%) and *Tabanus* (3.59%). The overall apparent mean tsetses and biting flies' density of 9.05 and 1.46 flies/trap were recorded respectively. Current prevalence is witnessing a decrease in this area due to improved farmer's knowledge in the usage of barriers such as trypanocides and screens in disease management. Maintenance of these barriers can bring the disease to a bay in this trypanosomiasis risk zone of the Adamawa plateau.

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**Keywords:** Cattle trypanosomiasis, prevalence, Alme and Adamawa plateau Cameroon.

### INTRODUCTION

Cattle trypanosomiasis is a disease complex caused by *Trypanosoma* spp,

biologically vectored by tsetse flies (*Glossina* spp) and mechanically by other biting flies. In the Central African Region, cattle

trypanosomiasis caused by both *Trypanosoma vivax* and *T. congolense* is prevalent in areas where rainfall is above 1000 mm (Awa et al., 2004), typical of Faro and Deo in Cameroon. The transmission of this disease in the Adamaoua region of Cameroon has been attributed to three tsetse fly species (*Glossina morsitans submorsitans*, *Glossina fuscipes fuscipes* and *Glossina tachinoides*). Generally, some tsetse foci have been currently reported in Cameroon such as: Fontem foci (Nimpaye et al., 2011) and in the Adamawa region (Achukwi et al., 2013). Parasitological studies in Faro and Deo Division of this Region by Achukwi and Musongong (2009) and Mamoudou et al. (2009) respectively have reported a remarkable increase in prevalence of the disease.

The invasion of tsetse flies in the Adamaoua plateau of Cameroon occurred around 1950. It happened that 40% of herds of cattle in the country resided in this area (Banser, 1979). The effects of this situation were catastrophic due to loss of meat and milk production, decrease fertility, increase calf mortality and decrease draught power. This further led to emigration of cattle owners from tsetse infested areas to tsetse free zones, thereby further decreasing grazing area. This situation made transhumance activities common in the area and a persistent tsetse density increase and distribution from areas that eradication was void to tsetse free zones and those which were cleared. According to Cuisance and Boutrais (1995) the gravity of the disease was so high that a district in the affected region known as Tignere showed no signs of existence of cattle. The vectorial pressure was so alarming that the Cameroon Government and other International organizations intervened. This was done by organizing biennial trypanocide drug campaigns between 1960 and 1975 in the affected region of the Adamawa plateau. Despite the relentless efforts of this concerted initiative, re-invasion of formerly cleared areas are still in a rise (Cuisance, 1991).

Remedial bi-aerial spraying campaigns took place in the nineties and were reinforced with the use of traps, targets and screens in the infested zone, serving as re-invasion barriers in the prevention of tsetse flies from reaching the non infested zone such as the plateau. These aerial spraying activities were later on replaced by insecticide treatment of cattle; three pyrethroids were used during these campaigns: Deltamethrin ULV® (3.2 g/l), Fendena® (Alphacypermethrin: 6 g/l) and Solfac® (Cyfluthrin: 7.5 g/l). The only organochlorous compound used was Thiodan ULV® (Endosulfan: 25g /l). Preliminary studies reported the survival of some pockets of *Glossina morsitans submorsitans* and *Glossina fuscipes fuscipes* (Cuisance and Boutrais, 1995). Studies carried out ten years after the 1994 eradication campaign reported that prevalence of bovine trypanosomiasis was in a rise in formerly cleared areas (Mbahin et al., 2008). Wild life in natural Game Reserves serve as an efficient re-invasion barrier of the disease in the area during transhumance. In Africa, despite the struggle in the fight against cattle trypanosomiasis and its vectors, there still exist few examples of successful tsetse elimination projects (Unguju Island and Okovango Delta) (Geerts, 2011). Many large scale eradication campaigns for instance the tsetse eradication campaign in the Adamaoua region of the country failed Mamoudou et al. (2009). Few epidemiological surveys have been carried out on this important disease of cattle in this region. The present study aimed at determining the current trends in prevalence of cattle trypanosomiasis, its vector density and distribution in the tsetse infested area of Faro and Deo division twenty years after the 1994 eradication campaign.

## MATERIALS AND METHODS

### Description of the study area

The study was carried out in Alme at the beginning of the rainy season (second month of rains). This zone is located between Latitude 7° North and Longitude 12° East (Figure 1). This site is best described as a

livestock/wildlife agro-ecological zone, with livestock activities being the main stay of economic activity of the indigenes of this area. In addition, there are no fishing activities, because water bodies are still suffering from pollution caused by excessive use of insecticides used during the eradication campaign. Wild animals such as: Antelopes, hyenas, monkeys, lions, giraffes and bush pigs are usually seen roaming around the area. This site had more than 20 sedentary herds at the time of the study.

#### **Ethical considerations**

An approval of the protocol was made by the Faculty of Science of the University of Dschang via the Department of Animal Biology. A verbal consent was sought from cattle owners and herders before physical examination and blood collection.

#### **Study animals**

Livestock present in this area included: sheep, goats, donkey and cattle. Transhumance is the order of the season involving herds from neighboring Nigeria; in search of rich pasture land, thereby increasing cattle population in this area. Cattle breeds found in this area also include: Gudali (*Bos taurus taurus*), White Fulani (*Bos indicus*), Red Fulani and the Brahman/Bokolo. These animals were usually allowed to fodder freely in a traditional husbandry style with communal herding. Treatment of the animals during the study was prohibited but envisaged after the study. All the animals detected with trypanosomes were appropriately treated intra-muscularly (IM) with trypanocides such as Veriben B12® (Ceva, France) at curative dose of 7 mg/kg body weight. Pour-on product known as Vectochlor® and Cypermethrin, Chlorpyrifos, Piperonyl butoxide and Citronel were also applied on these sick animals immediately after the study.

#### **Cattle sampling**

A total of 19 (n=27) sedentary herds in Alme were sampled. A random sampling approach was used, based on logical reasoning by considering criteria such as sex, age and size of herd. An estimated trypanosomiasis prevalence of 23% was considered in the computation of sample size. The precision level of 5% and a confidence interval of 95% were adopted. The formula of Thrusfield (2005) was used in the calculation as shown below:

$$N = \frac{T^2 \times P_{exp} [1 - P_{exp}]}{L^2}$$

Where;

**N:** The sample size to be determined;

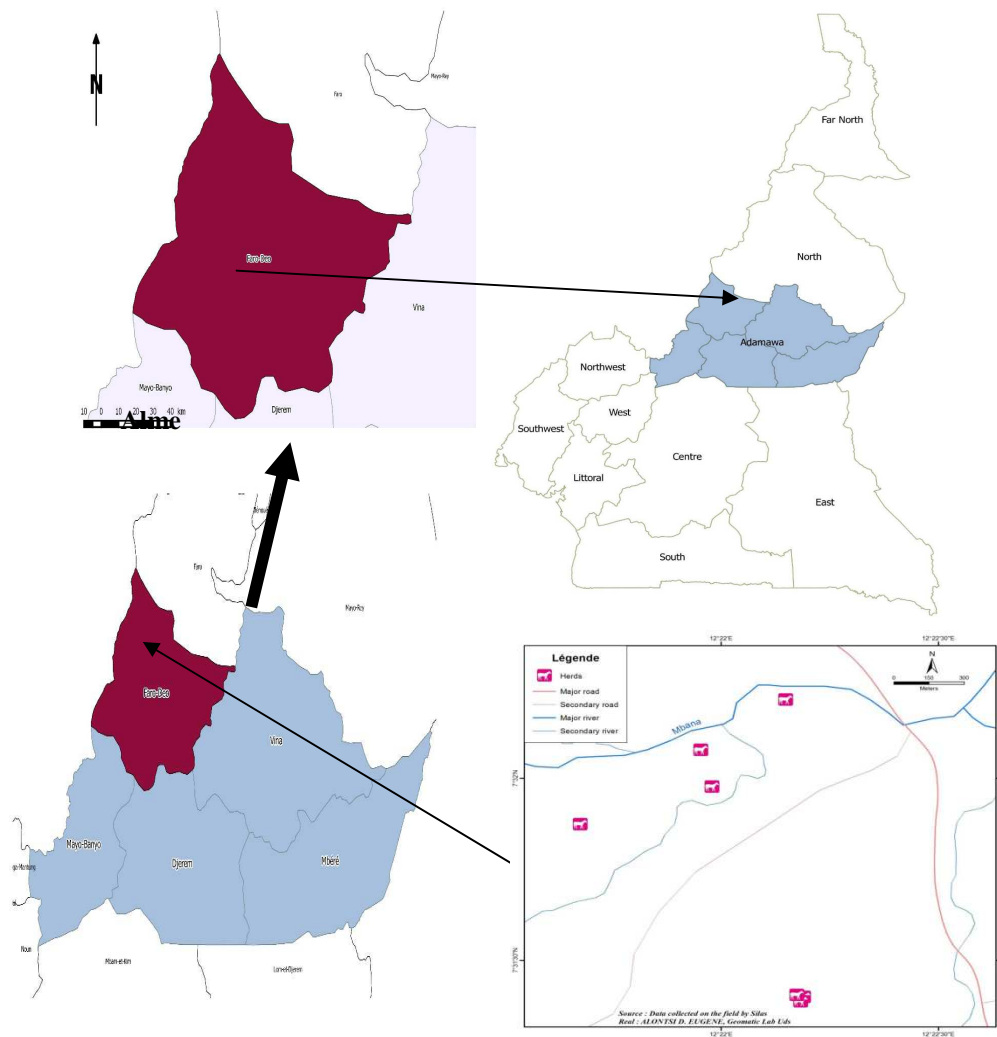
**T:** Student's t-value at 95% confidence level;

**P<sub>exp</sub>:** Expected prevalence of trypanosomiasis in the study area; and

**L:** Accepted absolute error/level of precision (L<sup>2</sup>=0.0025).

#### **Physical examination of cattle**

Pre-diagnostic examination of cattle was made for the following parameters: Body Condition (BC), weight and age. To begin with, BCS is an easy and economical way to evaluate the body fat percentage of cattle. The commonly used Whitemann scale (Herd and Sprott, 1986) of 1 to 9, with 1 representing emaciation and extremes (9), an obese case was adopted. This semi-quantitative examination is characteristic of picture charts for easy identification with specific anatomical landmarks such as: Spinous processes, the transverse processes of the back bone, the rib region and the edge of loin were emphasized. These features enabled us to group the animals into "Poor", "Medium" and "Good" body conditions. The weight of an animal is an important vital sign of the health status of the animal and was realized with the use of a measuring tape used for the estimation of weight of cattle and pigs. The age parameter was determined using the modified horn-ring method, which considers



**Figure 1:** Map of Cameroon showing Faro and Deo in Adamawa indicating Alme (study area).

horn growth and horn ring development as parameters (Grace et al., 1993) with 99.38% accuracy. This enabled us to group the animals in the following age cohorts: 1.5–2.5, >2.5–4.5 and >4.5 years.

**Parasitological diagnosis**

An 18 gauge syringe was inserted into the distended jugular vein; blood was drawn directly into 4ml vacutainer tube containing

di-sodium salt of ethylene diamine tetra-acetate (EDTA –K3) as anticoagulant. The vacutainer tubes were labeled and stored in an ice-packed cool box awaiting examination within 2 hours of collection. Micro-haematocrit capillary tubes were filled with blood samples to ¾ of their height. These tubes were then sealed with a “Cristalseal” and then centrifuged at 12000rpm for 4 minutes, using a Hawksley haematocrit

centrifuge. A portable diesel generator of 6.5 kV was used as power source in the field. The packed cell volume (PCV) of each animal was estimated soon after centrifugation using the Timer and Micro-capillary reader type 316-1(Poland). Animals with PCV reading below 24% were considered anaemic. The tubes were cut 1 mm below the buffy-coat and the buffy-coat zone expressed onto a slide, covered with a cover slip and observed under the microscope for motile trypanosomes (MOARD, 2005). Further confirmation of positive samples and detail morphological examination was done using the May-Grunwald Giemsa thin smear staining technique. Species and subspecies of trypanosomes namely: *T. congolense*, *T. vivax*, *T. brucei brucei* were identified.

#### Entomological surveillance

A 300m<sup>2</sup>-grid survey was conducted using blue biconical tsetse traps (n= 11), pitched in two sites: Alme ranch (n=9) and Faro Game Reserve (n=2) where the animals usually grazed and had water. Apparent density of flies is relative to the type of sampling gear used and is expressed as the average number of flies caught per trap. Traps were baited with a mixture of 3 weeks cow urine and acetone as odor attractants (Ayele et al., 2012). The position and direction of these traps were altered and site selection was based on suitable tsetse habitats. The flies were collected and stored in 70% ethanol for identification in the laboratory using a stereomicroscope. The species of tsetse fly were identified based on their morphological characteristics. Other biting flies were also separated morphologically by considering features such as: size, color and wing variation structures at the genus level (Fisher and Say, 1989).

#### Statistical analysis

Data collected was first entered into Microsoft Excel work sheet of Windows Version 2007, and analysed using SPSS software package of Version 19.0. The

prevalence of trypanosomiasis was expressed as percentage, with 95% confidence level by dividing the total number of animals positive for trypanosomiasis to the number of animals examined. Chi-square test was applied to compare the infection rate with regards to various parameters considered as risk factors (age, sex and BCS). Student t-test was also used to compare the mean PCV and weight of parasitaemic and aparasitaemic animals.

## RESULTS

### Parasitological findings

Cattle trypanosomes were detected in 148 cattle out of 504, with an overall prevalence of 29.4%. The trypanosome species were identified in thin blood films with single trypanosome infection (n=3) and mixed trypanosome infections (n=4) as shown: *T. congolense* (14.09%), *T. vivax* (6.15%) and *T. brucei* (3.37%); *T. congolense* + *T. vivax* (2.38%), *T. congolense* + *T. brucei* (0.39%), *T. vivax* + *T. brucei* (0.39%) and *T. congolense* + *T. vivax* + *T. brucei* (2.57%). Infections with different trypanosomiasis causing parasites was statistically significant with site ( $\chi^2=6.454$ ;  $P<0.05$ ) (Table 1).

### Host health and related parameters

The parameters of cattle assumed as risk factors were analyzed against trypanosome prevalence such as: sex, age and body condition. Among these risk factors, only body condition showed a significant difference in disease distribution ( $P<0.05$ ) (Table 4). Based on sex groups, male cattle (91/504, 18.1%) had a slightly higher prevalence rate as compared to female cattle (57/504, 11.3%). However, statistically, there was no significant difference ( $\chi^2=3.648$ ,  $P>0.05$ ) observed between the two sex groups (Table 4). The age category parameter was also considered and compared with prevalence. It was noticed that animals

with ages 1.5-2.5 years (7.5%), >2.5-4.5 years (13.9%) and >4.5 years (7.9%). This result revealed that animals aged < 2 years had the least infection rate as compared to animals >2 years old even though there was a difference in the rate of infection in the different age groups; ironically it did not result in a significant statistical difference ( $\chi^2=2.081$ ,  $P>0.05$ ) (Table 4).

Also, of the 504 animals sampled, 15.3%, 10.5% and 3.6% prevalence of cattle trypanosomiasis was observed in “Poor”, “Medium” and “Good” conditioned animals respectively. From these findings, it can be deduced that cattle infected with trypanosomes have a poor condition than non-infected animals. Interestingly, there was a significant difference ( $\chi^2=70.943$ ,  $P<0.05$ ) on prevalence of trypanosomiasis among animals of different body conditions (Table 4). Mean weight of aparasitaemic animals was

282.28±62.26sd and that of parasitaemic animals was 265.41±95.36sd, which was statistically significant ( $P<0.05$ ) (Table 3). Based on hematological findings, infected cattle had a lower mean PCV (27.64%) as compared to non-infected counterpart (32.57%) with a significant difference ( $P<0.05$ ) (Table 2).

**Entomological findings**

A total of 110 tsetse flies and other biting flies were caught in the two trapping sites. Out of this total, 52 (47.27%) belonged to tsetse of genus *Glossina* and species *Glossina morsitans*, Others 49 (44.5%), *Stomoxys* 5 (4.55%) and *Tabanus* 4 (3.59%). The overall apparent density was 4.59F/T. A scanty catch was registered in the Alme Ranch (1.30F/T) as compared to Faro Game Reserve (7.88F/T) (Table 5).

**Table 1:** Prevalence of trypanosome infection and species of trypanosomes with site.

Site	Trypanosome infections %				x <sup>2</sup>	P-value
	<i>T. congolense</i>	<i>T. vivax</i>	<i>T. brucei</i>	Mixed Infections		
Ranch	56(18.6%)*	23(7.6%)	12(4.0%)	7(2.3%)		
LH	42(8.3%)	35(17.5%)	22(4.4%)	22(10.8%)		
Total	98(26.9%)	58(25.1%)	34(8.4%)	29(13.1%)	6.454	P<0.05

$\chi^2$ -Chi-square test, LH-Local Herds,\*number in bracket indicate the relative proportion of *T. congolense*, *T. brucei* and *T. vivax* infection at each site.

**Table 2:** Comparison of mean PCV of parasitaemic and aparasitaemic animals.

Condition	Number examined	Mean PCV	t-test	P-value
Parasitaemic	148	27.64±5.62		
Aparasitaemic	356	32.57±4.76	10.038	P<0.05

PCV- Packed cell volume; Mean ± standard deviation.

**Table 3:** Comparison of weight of parasitaemic and aparasitaemic animals.

Condition	Number examined	Mean weight	t—test	P—value
Parasitaemic	148	265.41±95.36		
Aparasitaemic	356	282.28±61.26	2.841	P<0.05

Level of significance stated at P<0.05.

**Table 4:** Comparison of prevalence with related host parameters.

Parameters	Number examined	Number Positive	Prevalence %	x <sup>2</sup>	P—value
<b>Sex groups</b>					
Male	341	91	18.1		
Female	163	57	11.3	3.648	P>0.05
<b>Age groups</b>					
1.5–2.5	109	38	7.5		
>2.5–4.5	255	70	13.9		
>4.5	140	40	7.9	2.081	P>0.05
<b>Body conditions</b>					
Poor	131	77	15.3		
Medium	281	53	10.5		
Good	90	18	3.6	70.943	P<0.05

**Table 5:** Fly distribution and densities in two trapping sites in the study area.

Trapping sites	No.s	%(Total)	Spp	F/T	Stomoxys % (F/T)	Tabanus % (F/T)	Others % (F/T)	Total
FGR	2	34.5(38)*	Gm	19	2.7 (1.5)	1.8 (1.0)	18.2 (10)	63(31.5)
Alme Ranch	9	12.7(14)	Gm	1.6	1.8 (0.2)	1.8 (0.2)	26.4 (3.2)	47(5.2)
Total	11	47.3(52)	—	20.6	4.5 (1.7)	3.6 (1.2)	44.5(13.2)	110(6.7)

FGR Faro Game Reserve, F/T Fly per Trap, \*numbers in bracket show the distribution of flies in each trapping site.

## DISCUSSION

Despite the enormous efforts of the Cameroon Government and other International bodies in the fight against tsetse flies and the diseases they vector, the disease still continues to be a call for concern in the infested area-Alme of the Faro and Deo division. This is indicated by the overall prevalence of 29.4% which is far higher than 15.2% recorded by Mpouam et al. (2011) in

the Vina division but lower than 37.7% reported by Mamoudou et al. (2009) in the same study site of the Adamawa region. This decrease over the years from 37.7-29.4% in this site is due to improved farmer's knowledge of the usage of trypanocides and screens in the control of disease and vectors respectively.

Parasitism resulting in 3 single infections and 4 mixed infections is

characteristic of infections in this area. This result was similar to that of Shimelis et al. (2011). The four mix infections for the different trypanosomes was greater than two mix infections reported by Mbahin et al. (2008) in the same study area. High prevalence was recorded with *T. congolense* (14.09%), followed by *T. vivax* (6.15%). Higher mix-infection prevalence was recorded with triple parasitism with three different trypanosomes: *T. congolense* + *T. vivax* + *T. brucei* (2.57%). The high prevalence with triple trypanosomes could be explained by the fact that the animals and the disease have co-existed for several decades and the probability of each of the trypanosome species, occurring in the same host is true in such a vector dense environment. The dominance of *T. congolense* in the two sampled sites is in consonance with previous report of Bocoum et al. (2013). Similarly, this species identified has been reported to be the most dominant in cattle of Cameroon (Mpouam et al., 2011). There was a positive correlation between prevalence of different trypanosome species and apparent density of vectors, this type of relation is similar to that of Soud (2008). It revealed that cattle of Local herders with high prevalence frequent vector risk zones such as Faro Game Reserve, as compared to animals of the Ranch with low prevalence which are controlled from such risk zones.

Based on results of infection rate with sex, it was evident that there was no statistical significant difference ( $\chi^2=3.65$ ,  $P>0.05$ ). This same result was reported by Achukwi and Musongong (2009) in the same study area and Eyasu and Ahmed (2013) in Ethiopia. This is due to the fact that both male and female animals have equal chances of coming in contact with the fly and eventually with the diseases they transmit (Girma et al., 2014). High prevalence was recorded with animals aged >2.5-4.5 as compared to those below this cohort of 1.5-2.5 years, which did not show any statistical significant difference ( $\chi^2=2.08$ ,

$P>0.05$ ). This finding agrees with that of Fentahun and Tekeba (2013). However, the difference observed could be due to the fact that adult animals travel long distances for grazing as well as browse into tsetse infested areas. In addition, tsetse flies are attracted significantly to the odour of larger animals and those which show less defensive behavior according to Torr et al. (2006). The difference in infection rate between Body condition was statistically significant ( $\chi^2=70.94$ ,  $P<0.05$ ).

High prevalence was observed in Poor (15%) than Medium (10.5%) and Good (3.6%) body conditions. This agree with the previous report of Ali and Bitew (2011) which revealed that good condition animals usually record least prevalence than other conditions, this is partly due to weight loss caused by trypanosomiasis since emaciation is characteristic sign of trypanosomiasis (FAO, 2002).

Haematocrit results of parasitaemic and aparasitaemic animals showed significant difference. It can be deduced from this finding that the presence of trypanosomes in blood led to a significant reduction in mean haematocrit of the animals. Moreover, in a zone where other anemia-causing factors such as tick infestation, helminthiosis and haemoparasitosis prevail, PCV might not be the sole indicator of bovine trypanosomiasis in such an area (Ayele et al., 2012).

The distribution of vectors in this area showed that *Glossina morsitans* (47.27%) was the most dominant vector in the area, followed by other flies (44.5%), *Stomoxys* (4.55%) and *Tabanus* (4.59%). The overall apparent density in this area was 4.9F/T which is superior to the index of apparent abundance (IAA) of Mamoudou et al. (2009). The apparent density of *G.morsitans* (10.30F/T) obtained was higher than the apparent density for this species reported by Achukwi et al. (2013) as 9.6 and 7.4F/T in Nguemfiti and Kontcha respectively. The differences observed in the tsetse catch with high density in the Faro Game Reserve (7.88F/T) than in the Ranch (1.30F/T) agrees with other preliminary findings such as those of



Mamoudou et al. (2009). The Faro Game Reserve harbors rich vector niches which are important in the epizootiology of the disease in the area. Transhumance herds present in the Alme Ranch help in reduction of tsetse population during their exodus to other areas.

### Conclusion

Before indulging in a control campaign, epidemiological surveys need to be undertaken using appropriate diagnostic methods to determine the extent of the problem (Luckins, 1988). The present study reveals that overall parasitological prevalence was 29.4%. Parasitological surveillance resulted in the identification of the following trypanosome species: Single infections (n=3)- *T. congolense* (14.09%), *T. vivax* (6.15%) and *T. brucei* (3.37%); Mixed infections (n=4): *T. congolense* + *T. vivax* (2.38%), *T. congolense* + *T. brucei* (0.39%), *T. vivax* + *T. brucei* (0.39%) and *T. congolense* + *T.*

*vivax* + *T. brucei* (2.57%). Infection with various trypanosome species did not differ among sites.

Infections with bovine trypanosome species negatively affected mean PCV, weight and body condition. This indicated that infections with trypanosomes led to anemia (low PCV), loss of body condition and production in this livestock/wild life agro-ecozone of the Faro and Deo division. However, risk factors such as PCV, body condition and weight can be used as clinical symptoms and signs for bovine trypanosomiasis. The only tsetse identified was *Glossina morsitans*, which recorded the highest density and distribution, followed by others then *Stomoxys* and *Tabanus*. These were the common vectors of trypanosomiasis identified in this area.

Despite the fact that Alme was cleared of the disease and its vectors, it still remains a trypanosomiasis risk zone, due to its location that is, adjacent to Faro and Gashaka forest

Reserves which are reservoirs to potential vectors. It shows that the tsetse eradication had a positive impact on the disease but was not sustainable due to lack of barriers to the vector and disease in the area. It was also noticed that as years go by, farmer's knowledge of the usage of trypanocides and screens as potential barriers to the disease have improved. To improve livestock production and health, urgent measures are needed to put the vector and disease to a bay in Alme.

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