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Full Length Research Paper

Serological and parasitological prevalence of bovine trypanosomosis in small holder farms of the Vina division, Adamawa region of Cameroon

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In the Adamawa region, the number one cattle-producing area in Cameroon, there is spacity of reliable data on bovine trypanosomosis and the disease epizootiology but animal breeders attribute huge economic losses to trypanosomosis in the complete absence of laboratory diagnosis. This led to the ugly situation where most sick animals are injected with trypanocides. Blood was collected from 330 zebu cattle in small holder livestock farms in the Mbe Plain and Plateau of the Vina Division of Cameroon to assess the parasitological and seroprevalence rates of bovine trypanosomosis using the Buffy coat technique (BCT) and Enzyme linked immunosorbent assay (ELISA-antibody). The overall bovine trypanosomosis prevalence rate in both Mbe plain and the plateau was 11.5% when BCT was used and 31.2% with antibody ELISA. In the Mbe plain serological prevalence was an epizootic proportion of 51.5%, being 3.39 times higher than the prevalence rate (15.2%) using BCT. On the Plateau this ratio was 1.49 represented by a serological prevalence rate of 12.1% and a BCT prevalence rate of 8.12%. The parasitemia was relatively low (lower than 5×10^4 trypanosomes/ml) in both zones. The mean PCV of animals in Mbé Plain (26.22%) was significantly lower than that of animals on the Plateau (30.2%). In the Mbé Plain the mean PCV of ELISA-positive animals (23.71%) was very significantly ($p < 0.001$) lower than 28.93% for ELISA-negative animals. On the Plateau, ELISA- positive animals had a mean PCV of 29.76% which was not significantly different from that (30.27%) of ELISA-negative animals. The seroprevalence was significantly higher ($p < 0.001$) in older animals than younger ones in the Mbe plain but not in the plateau. Sex did not influence ($p > 0.05$) the seroprevalence and the mean PCV in both zones whereas the prevalence with the BCT was significantly higher in male cattle than in female in both Plateau and the Mbe plain ($p < 0.05$). The risk of bovine trypanosomosis was higher in the Mbe plain than in the plateau. In both study areas the intensity of animal trypanosomosis varies according to the ecological niches, suggesting that the vectors may be localized in a number of foci.

Key words: Bovine trypanosomosis, seroprevalence, packed cell volume, Vina-Cameroon,

INTRODUCTION

The Adamawa region harbours 28% of the estimated 10 million cattle of Cameroon and contributes about 38% of cattle beef production in the country (MINEPIA, 2003). Different control measures such as aerial spray, impregnated screens (Cuisance et al., 1987) and erratic topical treatment of cattle with deltamethrine (Tanyimboh, 1986) used at different periods during the last three decades have proved inadequate and not sustainable as solutions for the disease control. The majority of previously

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cleared tsetse-infested pastures have been reinfested (Cuisance, 1991). In 1994, a preliminary evaluation of the activities of the special mission for the control of vectors of trypanosomosis on Adamawa plateau revealed that the strategy was not 100% effective as various foci of *G. m. submorsitans* and *G. f. fuscipes* were identified (Cuisance and Boutrais, 1995). Since 1995 to date, extensive operations on tsetse control activities virtually came to a stand still, due mainly to limited budgetary allocations and this period has been marked by lack of entomological and parasitological information on the disease (Mamoudou et al., 2006).

The present study was to evaluate the serological and parasitological prevalence of bovine trypanosomosis in small holder cattle farms in the Mbe plain and the Adamawa plateau where extensive government-guided control operations were replaced since 1994 by individual erratic trypanosomosis vector control activities. In the Mbe plain control operations had not been undertaken for decades but during the same period the area experienced growth in cattle population due to fodder shortages in the neighbourhood.

MATERIALS AND METHODS

Study area

The Adamawa region of Cameroon is a mountainous area of 72000 km^2 which marks the boundary between the forest south and the Sahel north of Cameroon (Figure 1). The Vina division, our study area, has a surface area of about 17196 km^2 and is sub divided into two administrative and geographical units (the plateau: the districts of Ngaoundere and Belel) and the plain (district of Mbe). The plain and the plateau are separated by an escarpment. Due to repeated burning of pasture land in the region over several years the vegetation of the region is mainly wooded savannah. Small holder cattle farmers (people who have less than 300 cattle) own an estimated 40 percent of the cattle population in the Adamawa region. The rest of the cattle are kept by ranchers who stock their ranches with thousands of cattle. The main difference in the production systems between these ranches and the small holder cattle breeder farms is that the ranches have their own frequently operational trypanosomosis control systems such as dipping vats or use of the knapsack sprayer for cattle impregnation with pyrethroides and more elaborate or intensive use of trypanocides. These preventive measures are very erratically applied in the small holder cattle farms where production inputs are scarce.

The Plateau

The plateau is about 1200 m above sea level with temperature range of 22 to 28 °C and mean annual rainfall of 1800 mm. The rainy season occurs from mid March/April to October and the highest rainfall is recorded between June and September. The Dry season is the period from November to March. The Zebu gudali is the main cattle breed in the Plateau. Most of the plateau was last cleared of tsetse flies in 1994 but a number of uncleared foci remained (Boutrais and Cuisance, 1995). There is limited transhumance as a cattle management practice in the plateau and many farms tend to become semi intensive. Blood sampling was undertaken in animals from small holder cattle farms of nine villages in the plateau.

The Mbe plain

The Mbe plain lies to the north of the plateau at about 700 m above sea level and is characterised by higher temperatures (27 to 30°C) than those of the plateau. Further north of the plain (Figure 1) lies the Benuoe national reserve (park). The rainy season is only slightly shorter than that described for the plateau. There are about 5000 heads of cattle in the plain which are all in the hands of small holder cattle breeders and the transhumance livestock husbandry system is more practiced there. The plain has never received any extensive tsetse control operation of the type indicated for the plateau.

The animals

This cross-sectional study was undertaken during the months of January and February 2008 (in the middle of the dry season) in Zebus cattle. In all, samples were randomly collected in 40 herds belonging to small scale holder farmers who owned less than three hundred cattle in all their herds. The size of a herd in the region ranges from about 20 to 100 animals. Twenty three of them were in 10 villages in the Mbe plain where 158 animals were examined and 18 of the herds came from 9 villages in the plateau where 172 animals were sampled. Only animals which had not received trypanocides preventive (isomethamedium) or curative (diminazene aceturate) treatment during the last three months before the study were examined. The origin of herd, herd size, breed, age and sex of each animal sampled were recorded.

Packed cell volume, trypanosome detection and parasitaemia score

Plasma was prepared from whole blood collected from the 330 randomly chosen animals and stored frozen at -20°C until used for serological analysis. Part of the blood was put in heparinized capillary tubes and centrifuged at 12000 rpm in a Hawkley haematocrit centrifuge (Hawkley Limited, UK) for five minutes. From this preparation, the packed cell volume (PCV) was determined using a haematocrit reader. Animals that developed a PCV value less than or equal to 20% were treated with Dibenzamidine diaceturate (Merial, France). Trypanosomes in the blood samples were detected and counted by darkground/phase contrast buffy coat technique (Paris et al., 1982). Briefly, the buffy coat section was cut off by aid of a diamond cutter. The buffy coat was extruded onto a glass slide and covered with a cover slip. Species of trypanosomes namely: *T. congolense*, *T. vivax* and *T. brucei* were identified as described by Murray et al. (1983). The parasitaemia was also scored using the +1 to +6 score method described by Murray et al. (1983).

Indirect ELISA

The plasma samples were analysed by indirect ELISA at CIRDES, Burkina Faso, where the protocol for the serological detection of trypanosomes was earlier standardized (Desquesnes et al., 2001). This was a modification of the method described by Luckins (1977) based on the detection of trypanosome specific IgG.

Production of different trypanosome antigens and sensitization of ELISA microplates

The trypanosomes were inoculated in previously irradiated mice and there after blood collected from the mice was collected in haperinzed tubes at the peak trypanosome parasitaemia. Blood

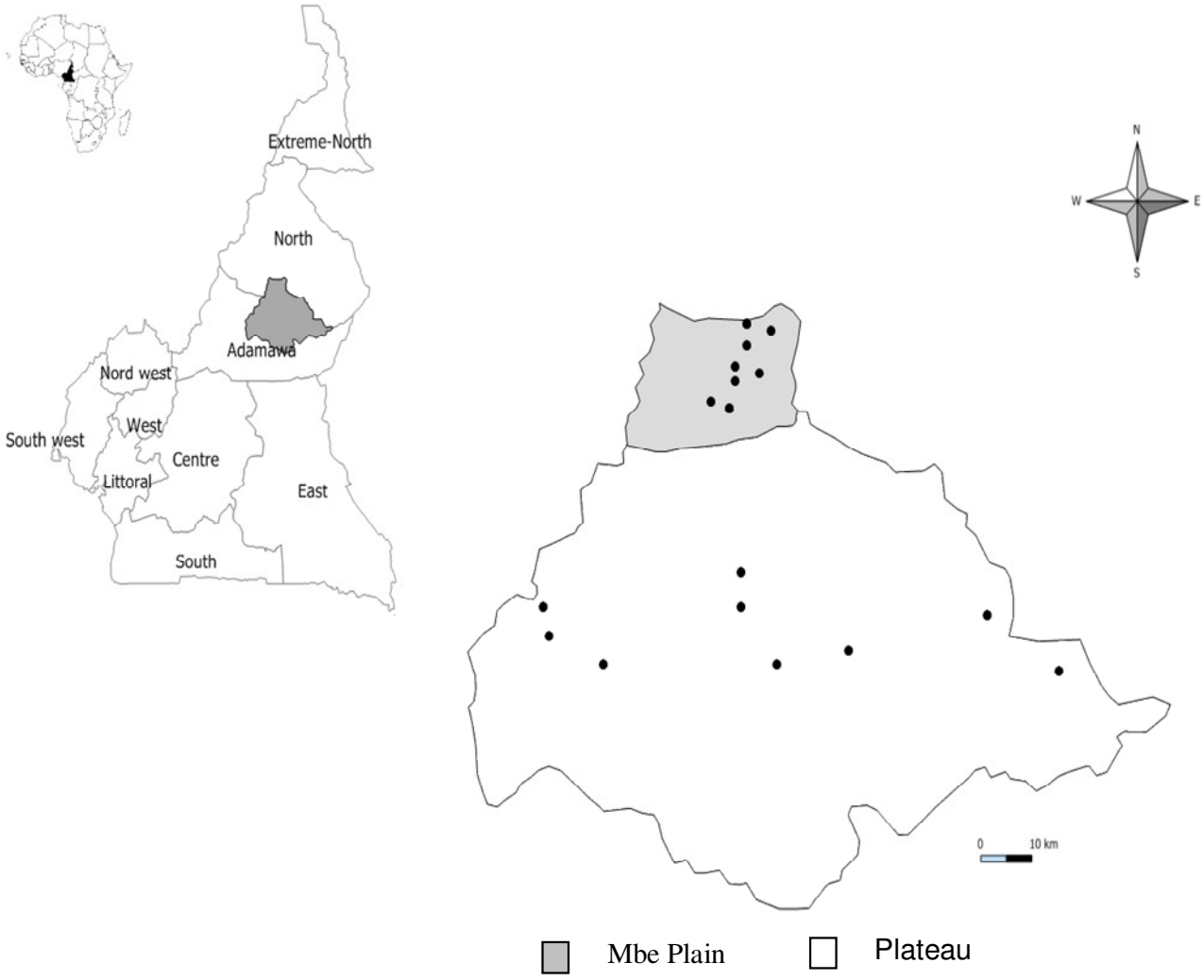

Figure 1. Map of Cameroon showing the study zone and the areas where sampling was undertaken in the Vina.

cells were separated from trypanosomes through a DEAE-cellulose colon (Lanham et Godfrey, 1970). Soluble antigen was extracted from the parasite by sonication and ultracentrifugation. 100 µl of a 5 µg/ml solution in Phosphate Buffered Saline containing 0.5 µg of protein, pH 7.2 of each antigen as indicated: *T. vivax* (Zaria 81/Y486/699, Guidot et Roelants, 1982), *T. congolense type savanna* (IL1392, Guidot and Roelants, 1982) and *T. brucei* (Cross, 1977) was used to coat the plates at 37°C incubation for two hours. Rinsing of the plates was undertaken with wash PBS containing 0.1% Tween 20 (PBST). The free binding sites were blocked with PBST containing 2% casein incubated at 37ºC for 1 h.

Plasma testing

After washing 100 µl of plasma diluted 1:50 with PBST containing 2% creme free milk (Protifar) was applied in each well and incubated at 37° C for 30 min.

After three washings 100 µl of the conjugate bovine anti- IgG – peroxidase diluted 1:10 000 was applied. Another washing was undertaken and 100 µl of the substrate made up of ABTS (2.2-azino-di [3-ethylbenzthiazoline sulfonate] and hydrogen peroxide was added in obscurity and after bench incubation, the optical density results were read off using an MCC/340-405 nm spectrophotometer.

Data analysis

The optical densities (OD) obtained were expressed as percentage of relative positivity (PRP) with respect to standard positive and negative samples (Wright et al., 1993) for *T. congolense*, *T. vivax* and *T. brucei* with a cut off positivity rate of 20% (Desquesnes et al., 2001).

$$PRP\ (\%) = \frac{\text{Mean OD (sample)} - \text{Mean OD (Test negative sample)}}{\text{Mean OD (test positive sample)} - \text{Mean OD (test negative sample)}} \times 100$$

Comparison of PRP was undertaken according to (Desquesnes et al., 2001) so that the maximum score was used to determine the species of parasite infecting the animal. Thus using the PRP and the maximum score, the serological prevalence rates for each trypanosome species was determined. The means of packed cell

Table 1. Trypanosome serological and parasitological prevalence.

Trypanosome species	Parasitological (BCT)				Serological (indirect-ELISA)							
	Plain (N=158)		Plateau (N=172)		Plain (N=158)				Plateau (N=172)			
					PRP		MS		PRP		MS	
	n	Pre (%)	n	Pre (%)	n	Pre (%)	n	Pre (%)	n	Pre (%)	N	Pre (%)
T.*c*	9	5.7	3	1.74	65	41.1	34	21.2	9	5.2	4	2.3
T.*v*	3	1.9	5	2.9	68	43	48	30.3	19	11	17	9.8
T.*b*	8	5.1	4	2.32	14	8.8	0	0	4	2.3	0	0
T.*v*+T.*c*	0	0	0	0	38	24	0	0	5	2.9	0	0
T.*c*+T.*b*	3	1.9	2	1.16	0	0	0	0	0	0	0	0
T.*v*+T.*b*	1	0.6	0	0	1	0.6	0	0	0	0	0	0
T.*v*+T.*c*+T.*b*	0	0	0	0	13	8.2	0	0	4	2.3	0	0
Global prevalence	24	15.2	14	8.12			82	51.5			21	12.1

T.*c*: Trypanosoma *congolense*, T*v*: trypanosoma *vivax*, T*b* : trypanosoma *brucei* sp, BCT =buffy coat technique, N : number of animals in zone, n: number of infections due to trypanosome species, pre: prevalence, PRP : percentage relative positivity, MS: maximum score.

volume (PCV), were derived from the data collected and the statistical test ANOVA was undertaken using the statacorp T×: stata corporation (2001) software release 6.0. The parasite prevalence rates were calculated and graphs drawn using excel.

Prevalence and parasitaemia

The overall bovine trypanosomosis prevalence rate in both Mbe plain and the plateau was 11.5% when BCT was used and 31.2% with antibody ELISA. The parasitological and serological prevalence varied within the same zone and these variations were greater in the Mbe plain than in the plateau. The trypanosomosis prevalence rate using BCT in the Mbe plain was15.2% while that of the plateau was 8.12% (Table 1). Trypanosomosis seroprevalence rate was 51.5% for the Mbe plain while that for the Plateau was 12.1% (Table 1). Based on the technique of Paris et al. (1982) and that described by Murray et al. (1983), the parasitaemia score more frequently observed in the two zones were 10^2 - 10^3 trypanosomes /ml, 10^3 - 10^4 trypanosomes/ml and $5 \times 10^3 – 5 \times 10^4$ trypanosomes/ml. Of these, the parasitaemia score of 10^3 - 10^4 trypanosomes /ml was detected more frequently (Figure 2).

Influence of age on trypanosomosis prevalence and packed cell volume of animals

Trypanosomosis seroprevalence and packed cell volume varied amongst the age groups but the differences were not significant ($p = 0.51 > 0.05$ and $p = 0.48 > 0.05$ respectively) (Figure 3).

Relationship between packed cell volume and trypanosome infection with respect to study area

The mean PCV of animals in the plain (26.22) was lower than the normal value (30%) according to (Hoste et al., 1982) while that of the plateau (30.2%) was within the normal range. In the Mbe plain, the mean PCV of seronegative animals was very significantly higher ($p < 0.001$) than that of seropositive animals but this was not the case using the buffy coat technique (BCT) in the detection of trypanosome parasites. In the Plateau, there was no significant difference ($p > 0.05$) between these two groups even though there was a significant difference ($p < 0.05$) between parasitologically positive and negative animals (Table 2). The mean PCV of animals infected with *T. congolense* was lower than that of animals infected by the other two trypanosome species in the two study sites; however this difference was not significant (data not shown).

Effect of sex of animal on the packed cell volume and trypanosomosis prevalence

With the BCT, the prevalence was significantly higher in male cattle than in female in both the Plateau and the Mbe plain ($p < 0.05$). Trypanosomosis seroprevalence was lower in male cattle than females with a general trend of higher mean PCV in males than females in both seropositive and seronegative animals but these differences were not significant in the two study areas. (Table 3).

DISCUSSION

The parasitological prevalence of trypanosomosis in the present study (8.1%) in the Vina plateau was higher than that reported by Mamoudou et al. (2006) for Faro et Deo plateau (1.8%) both in the

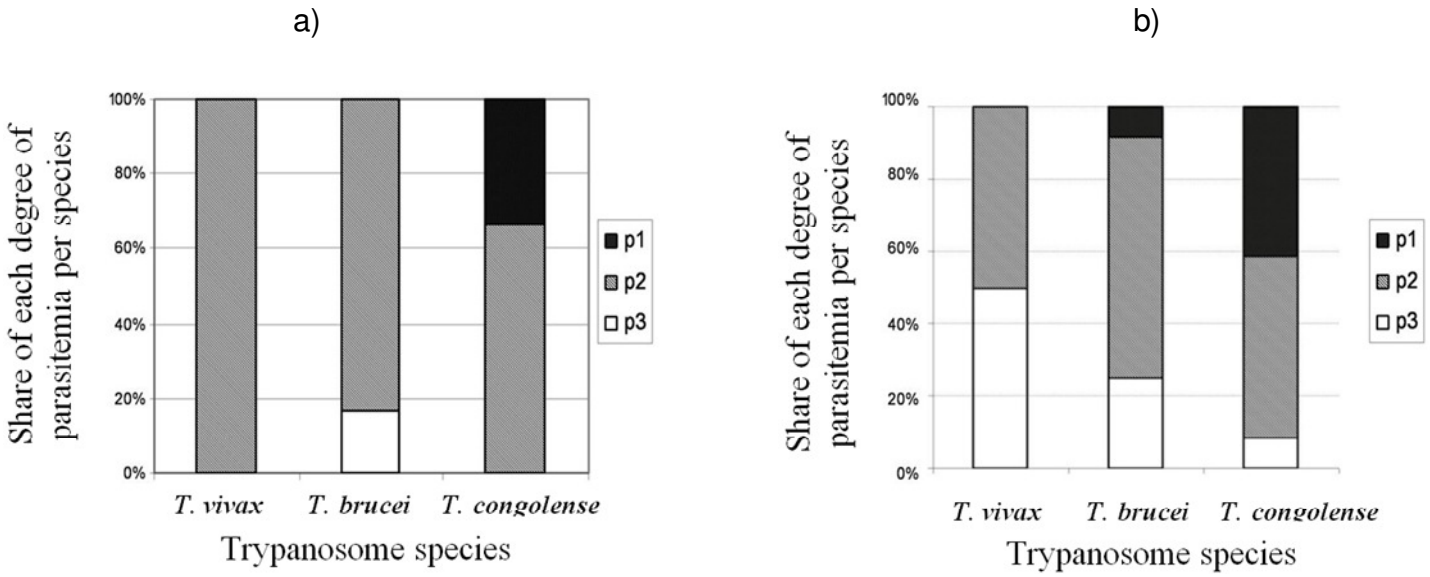

Figure 2. Proportion of parasitaemia estimated by BCT with respect to each trypanosome species (p3 = 10^2 -10^3 trypanosomes/ml, p2 = 10^3 - 10^4 trypanosomes/ml, p1 = 5.10^3– 5.10^4 trypanosomes/ml) in the plateau (a) and Mbe plain (b).

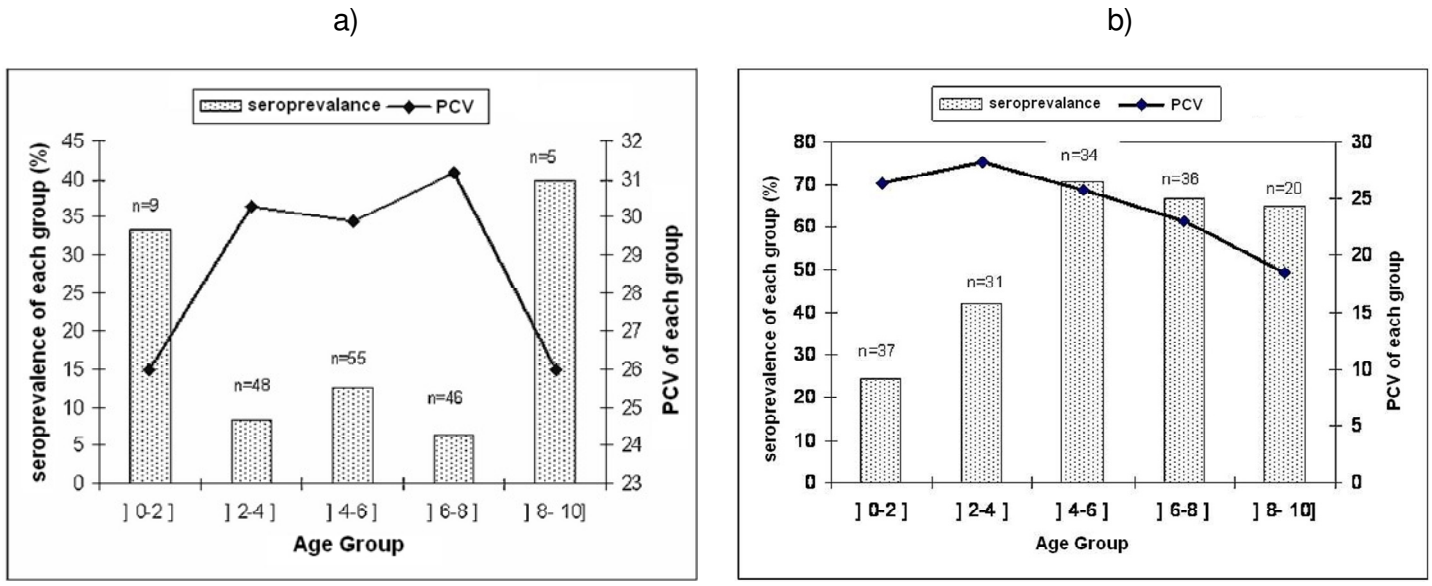

Figure 3. Packed cell volume and seroprevalence with respect to age group (years) of animal in the plateau (a,p>0.05) and Mbe plain (b,p<0.001) of the Vina Division.

Adamawa region. This marked difference is surprising as Faro et Deo division is known to be one of the main niches of tsetse flies in the Adamawa plateau. The observed difference might have been due to the type of animals sampled in the study of Mamoudou et al. (2006). The high statistical differences in both parasitological and seroprevalence between the plateau trypanosome infection rates and the plain of Mbe suggest that the risk of getting infected is higher for animals grazing in the plain than those in the plateau. Previous large scale antivectorial campaigns that lasted until the mid nineties and ongoing farmer-run anti trypanosomosis management strategies involving topical application of pyrethroids on cattle as live moving baits and the use of trypanocides on a more frequent basis on the plateau than in the plain could account for the differences

Table 2. Relationship between infection and PCV in the Adamawa Plateau and Mbe plain.

Technique	Plain		Plateau	
	PCV-P	PCV-N	PCV-P	PCV-N
BCT	25.33 (6.71) [a]	26.38 (7.11) [a]	27.07 (4.62) [a]	30.48 (4.84) [b]
Indirect-ELISA	23.71 (7.1) [i]	28.93 (5.8) [j]	29.76 (5.3) [a]	30.27 (4.9) [a]
Mean PCV	26.22 (7.02) [i]		30.2 (4.91) [j]	

Figures with the same superscript are not significantly different ($p > 0.05$) while those with different superscripts are significantly different ($p < 0.05$) ; the superscripts (i, j): imply that the difference is highly significant ($p < 0.001$). figures in brackets ( ) indicate standard error ; pcv (p, n) : packed cell volume of animals detected positive (p) or negative (n) for indicated test. bct = buffy coat.

observed. The differences in trypanosome infection rates observed even at village level, especially in the plain could be due to the fact that some animal herds are more frequently grazed and watered in tsetse infested areas than others.

Diagnosis by serology showed that *T. vivax* was the predominant trypanosome species infecting the animals and this was closely followed by *T. congolense* and *T. brucei*, in that order but this order was not the same when the BCT was used. The high frequency of low or undetectable parasitaemia in the two zones may be responsible for this difference (ratio of 3.39 and 1.49 for the Mbe plain and Plateau respectively) between the two tests since the sensitivity of the BCT depends on the parasitaemia (OIE, 2005). These differences may also be due to the low trypanosome species specificity of the ELISA test used as earlier reported by Desquesnes (1997a and 1997b). The predominance of *T.vivax* over the other species and its frequent association with *T. congolense* as observed in this study, has also been reported in Burkina Faso (Bengaly et al., 1998). In the present study the higher seroprevalence of *T.vivax* compared to that of *T. congolense* suggests an important contact of animals with tsetse flies which are known to be efficient vectors of *T. vivax* (Moloo et Kutuza, 1988) or other biting flies such as tabanids which serve as mechanical vectors (Bengaly et al., 1998).

The very low parasitaemia observed in the two zones, and especially in the Mbe plain where trypanosomosis prevalence was higher, may be due to frequent use of trypanocides (OIE 2005) in the production system and/or the fact that the infections became chronic in most cases with resultant un detectable parasitaemia. In the Mbe plain 83.3% of the high parasitaemia (5×10^3 to 5×10^4 tryps/ml) were due to *T. congolense*. In the Plateau, *T. congolense* was responsible for 100% of these parasitaemia scores. This observation and the lower packed cell volume of animals infected with *T. congolense* could be due to the higher pathogenicity of *T. congolense* with respect to the other two species (OIE 2005). The observation in the Mbe plain that the mean PCV of parasitologically positive animals was not significantly different from that of negative animals could equally be due to the low sensitivity of the parasitological test or it may be that most animals there have had treatment doses of trypanocides following previous trypanosome infections. This is supported by the highly significant difference ($p < 0,001$) observed between mean PCV of seropositive animals and those that were seronegative. This suggests that the antibodies were in most cases elicited by active trypanosome infections that lowered the PCV in seropositive animals. However this explanation does not take into account infected animals treated with trypanocides more than one month before this study; although recently treated animals were exempted from the sampling. One limitation of tests like ELISA used in this study is their inability to distinguish antibodies due to active infection from those of cleared or past infections. Trypanosomosis antibodies could still be detected during two to three months on the average after treatment with trypanocides or after cure of infection (Desquesnes et al., 1999). In past infections therefore, there will be no significant difference between PCV values of seropositive and sero negative animals (Bengaly et al., 2001). The situation in the plateau was contrastingly different; showing a significantly higher BCT for positive than negative animals. Meanwhile this difference was not significant for mean PCV of seropositive and seronegative animals. This implies that in this zone, the antibodies found in certain seropositive and BCT negative animals were mostly due to past infections as previously suggested by Bengaly et al., (2001) and that highly reduced PCV values occur when trypanosome parasites were detectable in blood. It is worth noting that the 'normal' value described by Hoste et al. (1982) may be relative to the study area and therefore not applicable in the context of the present study because animals with detectable high parasitaemia and PCV above 35% were found during the present study.

Age significantly influence the serological status of animals in the Mbe plain ($p < 0.001$); the younger animals having lower seroprevalence than the older. But this was not the case in the plateau. This variation could be due to the different serological prevalences detected in the two zones. Increased serological trypanosomosis prevalence with increasing age has previously been demonstrated in Burkina Faso by Desquesnes et al. (1999) and in Ghana (Mahama et al., 2004). This situation could be attributed

Table 3. Effect of sex of animal on the packed cell volume and trypanosomosis prevalence.

Analytical variable	Plain			Plateau		
	Male	Female	St	Male	Female	St
Number of animals	50	108		46	126	
BCT (%)	0.48	0	s	0.186	0.05	s
Seroprevalence (%)	40	52,78	ns	12,50	9,3	ns
PCV-ELISA+	22.7±7.5	25±5.8	ns	27.5±6.3	31.3±7.1	ns
PCV-ELISA-	29.2±5.7	28.6±5.5	ns	29.6±4,5	30±5.8	ns
Mean PCV	27.6±6.3	25.6±7.3	ns	29.4±4,8	30.1±5.9	ns

PCV-ELISA+ : Packed cell volume (PCV) of ELISA positive or PCV-ELISA – packed cell volume of ELISA negative animals. st: significance of statistic test, s: significant ($p < 0.05$), ns: not significant ($p > 0.05$), bct=buffy coat technique.

to the fact that older animals had more chances of being bitten by the vectors of the disease (Torr et al., 2007). The decline of PCV with increasing age in this trypanosomosis endemic area could therefore be associated with the higher risk of having been infected with trypanosomosis.

In the present study, the trypanosomosis prevalence with the BCT was significantly higher in male cattle than in female in both Plateau and the Mbe plain ($p < 0, 05$) whereas the seroprevalence of the male animals was not significantly higher than that of the females; implying that although the vector challenge risk might be similar, farmers might be treating and taking good care of their female animals than male as they are more concerned with milk production and its shortage in case of disease occurrence. The results with BCT from this study are contrary to those reported in the North region of Cameroon involving trypanotolerant Doayo cattle by Achukwi and Musongong (2009) using the BCT for trypanosomosis diagnosis in a similar production system. However, lower trypanosomosis prevalence rates have been reported for young and female animals than males (Trail et al., 1994; Van den Bossche et al., 2000; Rowlands et al., 2001). These contrasting observations could be due to the highly significant age differences found in the sample of the present study. The males in the Mbe plain were on the greater part composed of young animals (mean age of 3.21±1.73 years while the mean age for females was 6±2,56years. In the Plateau the age differences were 3, 5±1, 23 years and 6±1, 7 years respectively for males and female animals. In general, very old males are rare in the production systems of the Adamawa region since they constitute the main source of income for any given breeder.

The combination of BCT and ELISA techniques in this study has helped to reveal that the risk of trypanosomosis in small holder cattle breeding farms in the two study areas is quite high and tends to explains why farmers spend more on trypanocides. In the case of the Mbe plain, it is logical that herdsmen in this production system consider everyone in two animals to have trypanosomosis. Extensive longitudinal entomological studies on vector density and trypanosomosis prevalence rates should be undertaken in other parts of the region to provide further epidemiological information which is required for the putting in place of efficient control strategies.

Conclusion

The present study provides useful baseline epizootiological data that could be exploited during the planning of future trypanosomosis control operations in the Adamawa plateau and the Mbe plain of Cameroon. The findings call for the institution of efficient long-term control of animal movement from the Mbé Plain to the Plateau by the veterinary services.

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