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Full Length Research Paper

Prevalence and impact of bovine trypanosomiasis in Mayo Rey division, a Soudano-Sahelian zone of Cameroon

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For several years, pastoralists in Mayo Rey division have reported the presence of trypanosomiasis within their herds, and have used trypanocides in the complete absence of laboratory diagnosis. This present study aimed to establish the prevalence of trypanosomiasis and its impact on local cattle. A parasitological survey was carried out in 24 herds for a total of 270 cattle selected and followed-up for a period of one year. Blood samples were collected aseptically and screened using the Buffy coat technique, and the packed cell volume (PCV) was measured. The results showed that 149 animals were found infected with trypanosome at least once during the four follow-ups, corresponding to an annual prevalence and incidence rates of 55.2 and 31.9%, respectively. The risk of bovine trypanosomiasis was higher in the rainy season. Three species of trypanosome were identified: *Trypanosoma congolense, Trypanosoma brucei* and *Trypanosoma vivax*. *T. congolense* was the most abundant species. The PCV was significantly higher for mixed and single infections with *T. vivax.* Analyses have shown that cattle breed and age group affect significantly the prevalence of trypanosomiasis. The effect of trypanosomiasis on weight loss was noticeable, but significant in the rainy season only. This study has established the prevalence and the endemicity of trypanosomiasis in the Mayo Rey division; it suggests that tsetse flies and other mechanical vectors may be abundant in the zone and raise the need for entomological investigations.

Key words: Cattle, trypanosomiasis, Buffy Coat Test, packed cell volume, Soudano-Sahelian region, Cameroon.

INTRODUCTION

Cameroon is one of the largest producers of beef in the Economic Community of the Central Africa States (CEMAC) region with an estimate of ten million cattle (Minepia, 2003; Ankogui-Mpoko et al., 2010). The northern

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region of the country is one of the most important cattle breeding regions; it supplies both the local and the international market demands. In this region, pastoral activities are focused in the Mayo Rey division which concentrates 50% of all the pasture (Labonne et al., 2003). However, herds' productivity is unable to satisfy the constantly growing local demands of the population and it is known to be gravely impeded by problems related to water and feeding, breeding practices and health. Among the health issues is animal trypanosomiasis (Minepia, 2009).

Animal trypanosomiasis is a parasitic disease that causes serious economic losses to pastoralists (Winrok Inter, 1992); it manifests anemia, loss of condition, agalaxia and emaciation. Many untreated cases are fatal (Troncy et al., 1981). The prevalence of this disease in Africa overlaps with the distribution of its biological vector, the tsetse fly, endemic between latitude 15°N and 29°S (Leak, 1999). One quarter of economical losses due to animal pathologies is attributable to trypanosomiasis (De Hann and Bekure, 1991). In Cameroon, animal trypanosomiasis had been ravaging for many years following the invasion of tsetse flies that occurred in the fifties and precisely in the Adamawa region (Mamoudou et al*.,* 2009); since then many studies on the epidemiology of this disease were conducted in the region (De Wispelaere, 1994; Boutrais and Cuisance, 1995; Mamoudou et al., 2006, 2008; Mpouam et al., 2011). This region is limited northward by the north region where trypanosomiasis had been reported nearly two decades ago by Ndamkou and Chare (1995) and where little is known of the current situation of this disease. In Mayo Rey division, a Soudano-Sahelian zone in the north region, adjacent to the Vina division (Adamawa region), the presence of trypanosomiasis has been reported by pastoralists who attribute huge economic losses to this disease in the complete absence of laboratory diagnosis.

The present study was carried out to ascertain the prevalence of bovine trypanosomiasis in the Mayo Rey division in order to improve the understanding of its impact and epidemiology.

MATERIALS AND METHODS

Study area

The study was conducted in Yoko village, Mayo Rey subdivision, north region of Cameroon. Ecologically, this area is classified as a Soudano-Sahelian zone (annual rainfall variesbetween 400 mm and1200 mm). There are two main seasons: the rainy season (from early May to September) and the dry season (from October to April). Farming and animal husbandry are the main income generating activities. Animal husbandry is practiced mostly by Mbororo and Foulbe tribes for centuries (Bronsvoort et al., 2003). However, pastoral systems have remained primarily subsistence-orientated rather than capitalist-orientated. The pastoralists exploit common pool grazing resources composed mostly of *Andropogon gayanus, Brachiaria bryzantha, Loudetia togoensis* and *Pennisetum pedicellatum.* The sampling was done in four villages: Bini, Kombo, Gada Raou and Kaou (Figure 1).

Study design

Sampling procedure and data collection

A total of 24 herds of about 70 animals each were selected for the survey. Animals were sampled randomly and their number varied between 11 and 14 animals. A survey questionnaire was developed and administered to collect background data on animals (age, sex, breed and weight). Weight of the animals was estimated by the formula developped by Njoya et al. (1998). The blood samples of each of the 270 animals (identified by code) included in the survey was collected via ear vein into heparinized micro-haematocrit centrifuge capillary tubes and onto glass slides, as thick and thin blood smears. Blood samples were collected twice in the dry season (November, 2012 and March, 2013) and twice in the rainy season (May and September, 2013) on the same animals.The surface around the ear vein were sterilized with ethanol before each blood collection.

Diagnostic of trypanosome infection

Blood samples were used for paraclinical (packed-cell volume, PCV) and parasitological detection of trypanosomes by buffy-coat technique (BCT), thick and thin smears analyses (Paris et al., 1982). The capillary tubes were sealed with "cristoseal" (Hawksley) and centrifuged immediately in a micro-haematocrit centrifuge for 5 mns at 9000 rpm. After centrifugation, PCV was determined. The Buffy coat and the upper most layers of red blood cells of each specimen were extruded on to a microscope slide and examined for the presence of motile trypanosomes. Samples were examined with a phase contrast microscope with a 40x objective lens. Giemsa – stained thick and thin blood smears were examined under 100x oil immersion objective lens for trypanosome identification. Trypanosome species were identified by reference to criteria defined by Murray et al. (1977). The specificity (Sp) of BCT is unequivocal (no false positive reactions) but sensitivity (Se) is low because BCT cannot detect parasitaemia below 200 to 1000 trypanosomes/ml (Murray et al., 1977)

Data analysis

Data were processed in Microsoft excel. The prevalence and incidence rates of trypanosome infections as well as the prevalence of species of trypanosomes identified were compared by Chi-square (X^2) at a precision level of 5% using the XLSTAT software version 2014. Real prevalence was estimated with the formula below (Toma et al., 1996).

$$PR = \frac{PA + Sp - 1}{Sp + Se - 1}$$

Where PR = real prevalence; PA = apparent prevalence (test); Se = test sensitivity; and Sp = test specificity.

Prevalence referred to the proportion of the study population diagnosed with trypanosomiasis either during the dry or the rainy seasons or throughout the study period; the incidence referred to the proportion of new cases of trypanosomiasis diagnosed within the study population case at the above periods. The paired-sample

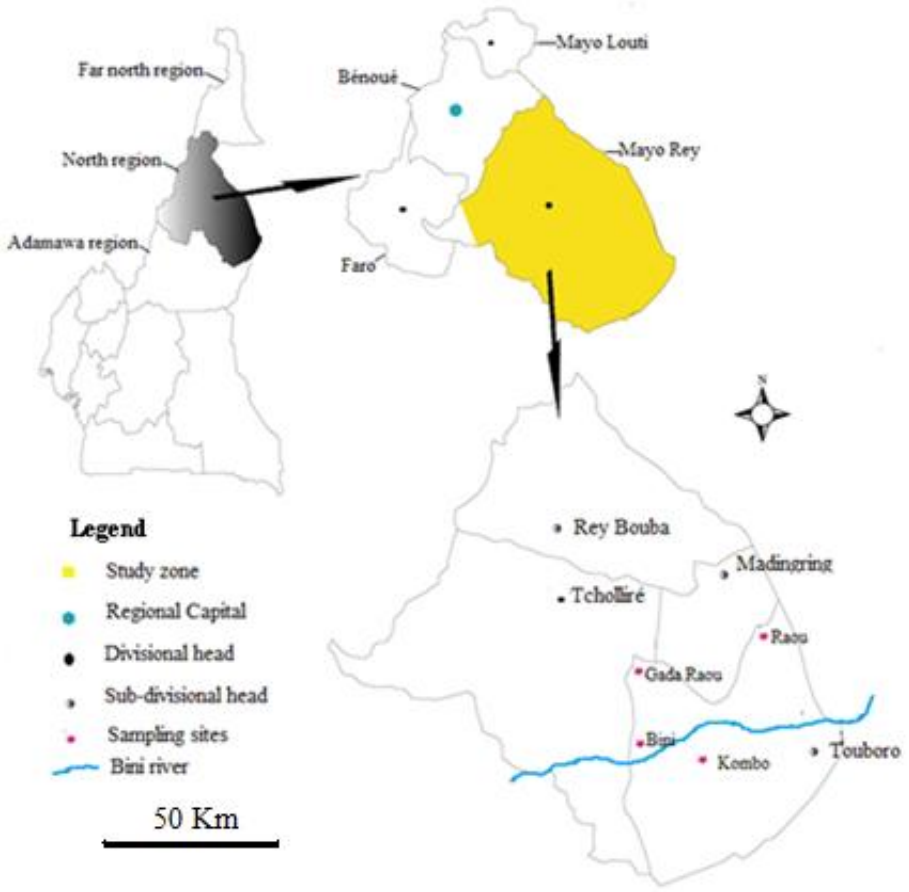

Figure 1. Study zone showing sampling sites.

T-test was used to compare means of PCV and weight. A regression model was used to assess the effect of age category (Adult: > 4 years; Juvenile: > 2 and ≤ 4 years; Young ≥ 1 and ≤ 2), sex and breed on the prevalence of trypanosome infections.

RESULTS

Prevalence and incidence of trypanosome infections

The parasitological analysis revealed that 149 animals were found infected with trypanosome at least once, corresponding to an annual prevalence of 55.2%. The majority of these animals, 59.06% (88/149) were found infected once (X^2:147.714, P<0.001), followed by 26.85% (40/149), 8.72% (13/149) and 2.01% (3/149) for animals infected respectively twice, thrice and four times during the survey. Assuming a BCT sensitivity to be between 0.6 and 0.9 and a specificity of 1, the estimates of true prevalence ranged from 61.33 to 92.00%. The prevalence varied significantly with the season; with more prevalent cases found in the rainy season (Z:-3.269; P=0.001). No significant difference was found among the prevalence of trypanosome in each village (X^2:6.317; P=0.097). The annual incidence rate was 31.90%(86/270). The incidence rate was significantly higher in the rainy season (Z: 3.340; P=0.001) and the estimates of the true monthly incidence in the rainy season is twice as high as that of the dry season (Table 1). The prevalence and incidence were similar among villages (Table 2).

Species of trypanosomes

Parasitological tests revealed the presence of three species of trypanosome: *T. congolense*, *T. brucei* and *T. vivax*. Among the 234 BCT positive, *T. congolence* was the most prevalent species (64.96%) (Figure 2) and its prevalence was significantly higher than that of the other species (X^2:266.826; P<0.001).

Effect of breed on the prevalence of trypanosome infections

All the four cattle breeds (Gudali, White Fulani, Red Fulani and Bokolodji) identified within our sample were

Table 1. Estimate of true monthly incidence of trypanosome infections in study site between November, 2012 and September, 2013.

Parameters	Sample size	N° of BCT+	Apparent monthly incidence (%)	True monthly incidence (%)	
	-	-	-	BCT Se = 0.9	BCT Se = 0.6
November, 2012 - March, 2013 (5 months)	270	20	1.48	1.65	2.47
May, 2013 – September, 2013 (5months)	270	45	3.33	3.70	5.55

Monthly average incidence over one 5 months period: (N°BCT+/(Sample size × 5 months)) × 100; True incidence = apparent incidence/BCT sensitivity (with assumptions of two BCT sensitivity, 0.6 and 0.9, and BCT specificity = 1.0).

Table 2. Prevalence and incidence of trypanosome infection per village and season in Mayo Rey, between November 2012 and September 2013.

Season	Village	Sample size	Prevalence			Incidence		
			Infected animals (%)	X^2; P-value	2; P-value	Infected animals (%)	X^2; P-value	2; P-value
Dry season	Bini	67	15 (22.39)			4 (5.9)		
	Kombo	67	15 (22.39)			3 (4.48)		
	|Kaou	68	24 (35.29)	5.473; 0.140		6 (8.83)	2.065; 0.559	
	Gada Raou	68	24 (35.29)			7 (10.29)		
	Total	270	78 (28.89)			20 (7.40)		
					-3.269; 0.001			-3.3062 ; <0.001
RainySeason	Bini	67	24 (35.82)			10 (14.92)		
	Kombo	67	25 (37.32)			11 (16.41)		
	Kaou	68	35(51.47)	4.727; 0.234		15 (22.06)	2.149; 0.542	
	Gada Raou	68	30 (44.11)			9 (13.23)		
	Total	270	114 (42.22)			45 (16.67)		

concerned by trypanosome infections. Analysis using general linear models (GLM) showed that breed affects significantly the prevalence of trypanosomiasis infections (X^2:30.424; P<0.001). The lowest infection rate was recorded in the Gudali (Table 3).

Effect of Age group and sex on the prevalence of trypanosome infections

The prevalence of trypanosome infections varied among age groups; with a significant effect (X^2:9.260; P=0.010). The prevalence of trypanosome infection was different between male (52.83%) and female (56.71%) with no significant difference (X^2:0.014; P=0.906).

Effect of trypanosome infection on PCV

The means PCV were different between the dry (35.07%) and the rainy (35.41%) seasons and this difference was statistically significant (t:-1.999; P=0.047). This difference was even higher between the beginning and the end of both the dry and the rainy (t: 7.916; P<0.001and t:-7.310; P<0.001) seasons. The Mean PCV was statistically different among study villages; Kaou had the lowest mean of PCV in both seasons. Moreover, it was found that the PCV also varies within the age category, but the difference observed was not significant (F: 0767; P=0.466). The PCV of infected cattle was significantly lower than that of non-infected cattle in both seasons (P=0.001). Among infected cattle, the means of

Table 3. Annual prevalence of trypanosomiasis by cattle breed.

Parameters		Non infected	Infected	Prevalence (%)	Total	X^2 ; P-value
Cattle breed	Bokolodji	16	23	59	39	-
	Gudali	81	53	40	134	28.413 ; <0.0001
	Red F	9	25	74	34	-
	White F	15	48	76	63	-
Total		121	149	55	270	-

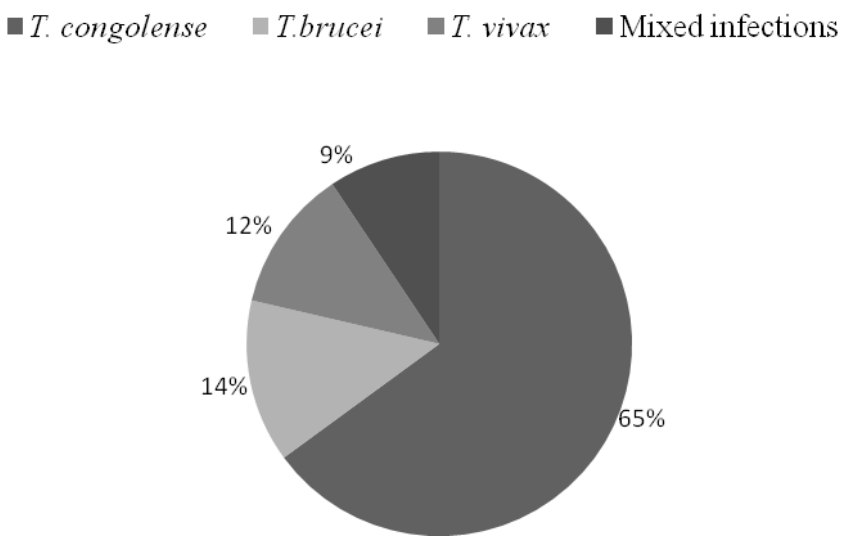

Figure 2. Prevalence of trypanosome species.

PCV varied significantly among the species of trypanosomes in the rainy season only, with the lowest mean recorded for mixed infection, followed by *T. vivax* (X^2:7,814; P=0.009).

Effect of trypanosome infection on weightgain

The overall weight gain of study population at the end of the study period was 27.67 Kg. The mean weight gain of these cattle varied significantly with the seasons; cattle gained 9.48 and 35.33 Kg respectively at the end of the dry season and of the rainy season (t:-31.30; p<0.001). The annual weight gain between non-infected and infected cattle were respectively 30.41 and 25.44; these values were significantly different (t:-3.29; P=0.001) (Figure 3). The mean weight loss among infected cattle was 11.15 kg against 8.80 Kg among non-infected ones; this difference was not significant (t: 1.53; P=0.128) at the end of dry season (Figure 4). In the rainy season cattle gain weight and a significant difference was observed between the mean weight gain of infected cattle (33.95 kg) and of non-infected cattle (39.49 kg) (t:-3.41; P = 0.001) (Figure 5).

DISCUSSION

The prevalence of trypanosomiasis among the 270 animals followed-up for one year in the Mayo Rey division was 55.2%. This prevalence was higher than all those obtained in adjacent localities of the Adamawa region; for example in Mbe and Plateau, the prevalence were respectively 15.22 and 8.12% in 2010 (Mpouam et al*.,* 2011) and 40.7% in the Faro etDeo divisions in 2010 (Tanenbe et al., 2010). The difference observed with the latter may be due to the sampling methods. The latter were transversal studies while the present study is longitudinal. Additionally, the samplings in these studies were done during the dry season only. Nevertheless, the prevalence in the Mayo Rey division (28.89%) in the dry season remains higher than that of the Vina but lower than that of the Faro et Deo divisions. This situation could be attributed to the fact that the vector control campaigns which were implemented in these zones reduced

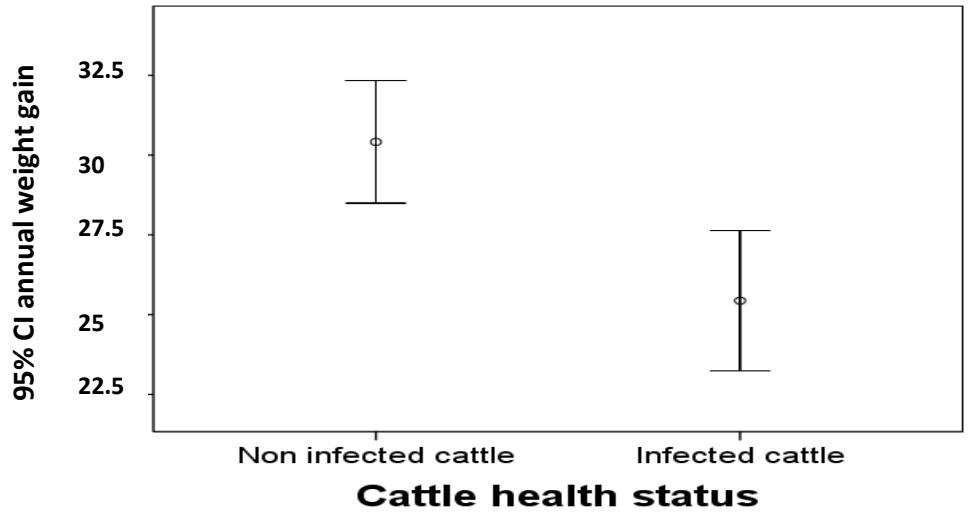

Figure 3. Effect of trypanosomiasis on cattle annual weight gain.

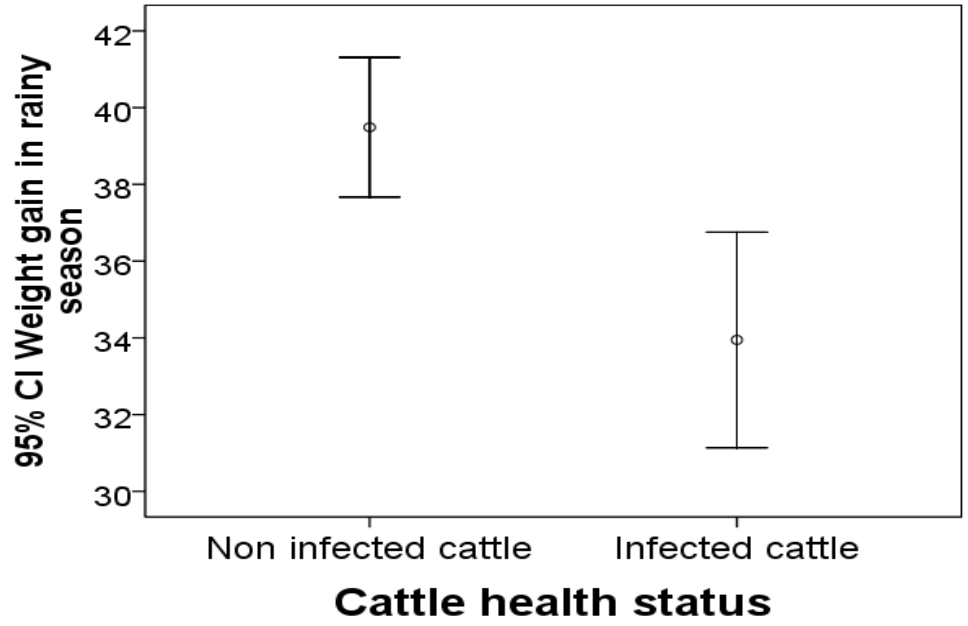

Figure 4. Effect of trypanosomiasis on cattle weight loss in the dry season.

considerably the risk of transmission of trypanosomes and the cattle breeding practices which tend to become semi intensive with limited transhumance (Mpouam et al., 2010).

The difference observed with the Faro et Deo divisions may be due to the fact that herds of this division frequently cross the buffer zone to graze in the tsetseinfested zone, thus increasing their risk of trypanosome infection (Tanenbe et al., 2010). The difference of prevalence between seasons in Mayo Rey could be due to the direct and indirect impact of climate (temperature and rainfall) variations on the abundance of the vectors. The increase in the abundance of biting flies during the rainy season may have increased the risk of flies-animals contacts and of trypanosome transmission (Tchouomene-Labou et al., 2013). BCT incidences revealed infections in the dry season as well as in rainy season. The true monthly incidence rate of trypanosome

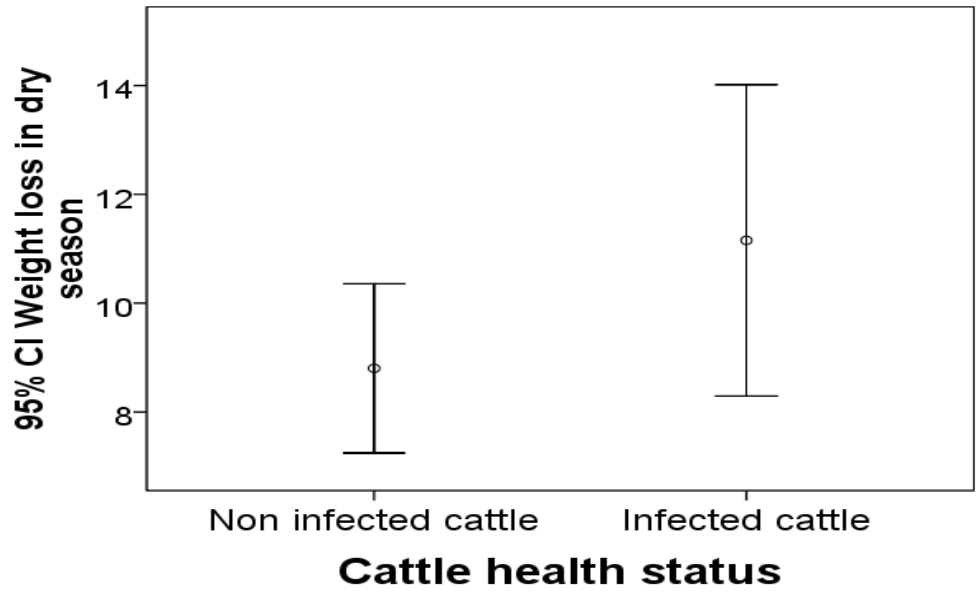

Figure 5. Effect of trypanosomiasis on cattle weight gain in rainy season.

infections shows that the risk for new infections is twice as high in the rainy season.

In this study, three species of trypanosome were identified and among them, *T. congolense* was the most prevalent one. This result is similar to that obtained by Tanenbe et al. (2010). The high predominance of this species may imply the existence of tsetse, the biological vector, and contacts with animals (Hoare, 1972). The risk of infection increased with age. This result corroborates with the findings of Desquesnes et al. (1999) in Burkina Faso and Mahama et al. (2004) in Ghana. The effect of age is most likely due to the longer period of exposure to the risk of infection in adults. The sex prevalence rates revealed a slightly higher percentage among the females. Onyiah (1997), Quadeer et al. (2008) and Sam-Wobo et al. (2010), in Nigeria observed no statistically significant difference in the prevalence rates of cattle by sex. The tsetse flies or other biting flies do not have any criteria to discriminate between male and female when they require their blood meal.

Cattle breed influenced significantly the prevalence of trypanosome infections. The risk of infections is increased in three cattle breeds: Red Fulani, White Fulani and the Bokolodji (mixed). It suggests that these breeds are more susceptible to trypanosomes. Contrary to the latter, the Gudali breed showed the lowest infections rate, suggesting a better adaptability in the study zone. Similar results were found by Sam-Wobo et al. (2010) who compared the white Fulani and the Gudali in Nigeria.

The impact of trypanosome infection on cattle body weight was tracked in this study. This impact varies normally with the climatic conditions as they influence the availability of fodder. The effects of trypanosome infections on cattle weight gain were noticeable but not significant in both seasons. The non significance of the weight loss in cattle according to their health status could be attributed to a practice common among pastoralist of the north of Cameroon; pastoralists often give additional food to cattle with clinical signs of diseases or other drugs in order to facilitate their recovery (Healy et al., 2013). The significant difference in weight gain observed in the rainy season between the infected and the non infected cattle may be due to the higher abundance of biting flies at this period; their buzz and visual harassment and their painful bites disturb grazing thus reducing cattle weight gain (Foil and Hogsette, 1994; Hollander and Wright, 1980) most importantly among infected animals. The presence of tsetse flies had been reported in this zone many decades ago (Rageau and Adam, 1953). The development of these flies may have been maintained along the Benoué River and facilitated by the importance of transhumance in the zone.

The level of anemia through PCV is one of the reliable indicators of trypanosomes in cattle (Rowlands et al., 2001). In this study, the means of PCV were significantly higher in non-infected cattle. Similar results were found by Quadeer et al. (2008), Sam-Wobo et al. (2010) in the Nigeria and Desquesnes and Dia (2003) in Burkina Fasso. The PCV values equally varied with the species of trypanosome and these values were the lowest with *T. vivax* (in a single infection); this indicates the pathogenic effect of this species.

Conclusion

The present study has confirmed the presence of bovine trypanosomiasis in a Soudano-Sahelian zone in Cameroon with an annual prevalence which is remarkably high (55.2%). The disease is endemic with a higher risk of infection for cattle in the rainy season. The age group and cattle breed affect the prevalence. The predominance of *T. congolense* mainly transmitted by tsetse flies suggests that the study zone is infested by the biological vector of the disease. The findings call for further entomological investigations on the species of biting flies present in the zone and their distribution necessary for control interventions.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Review

A survey of common gut helminth of goats slaughtered at Ankpa abattoir, Kogi State, Nigeria

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The survey of common gut helminth of goat in Ankpa Local Government Area (L.G.A), Kogi State was carried out between August, 2013 and November, 2013. Faecal samples of 248 goats were collected from Ankpa abattoir, and screened using floatation and sedimentation methods in the Biological Sciences Laboratory, Kogi State University, Anyigba. The result revealed that out of 62 samples of adult males examined, 47 were positive with a total of 76% nematode parasite while 15 faecal samples were not infected. Out of 62 adult females examined, 49 were positive with a total of 79% nematode parasites. There is no significant difference between the rates of infection in male and female adults (P > 0.05). For 62 young male examined, 53 were positive with a total of 85% while for 62 young female examined, 51 were positive with a total of 82%. The data analysis between male and female young goats showed no significant difference (P > 0.05). Results revealed that most of the goats were infected with nematode's eggs/larvae (85%) of *Strongyloides* species, *Oesophagostomum* species, *Trichostrongylus* species, *Haemonchus* species, *Ostertagia* species, *Bunostomum* species, *Gongylonema* species, *Giageria* species, *Ascaris* species and *Trichuris* species followed by cestode's eggs (14%) of *Avitellina* species, *Taenia* species and Trematode's eggs (1%) of *Schistosoma bovis*. The minor helminth like trematodes and cestodes were least manifested in the faecal samples analyzed which may be as a result of seasonal infestation and/or the system of management used (semi-intensive management system) in Ankpa. The whole outcome of the work revealed that goats slaughtered at Ankpa abattoir are not free from infection. Hence there is need for effective system of management and treatment before consumption.

Key words: Helminths, infection, public health importance and goats.

INTRODUCTION

Helminthiasis is one of the most important causes of mortality and morbidity in tropical and sub-tropical regions of the developing world, especially where adequate water and sanitations are lacking (De Silva et al., 2003; Amadi and Uttah, 2010). In Nigeria, an important killer disease of small ruminants and high morbidity in man is caused by nematodes, trematodes and cestodes (Larson, 1999; Debela, 2002). The most pathogenic helminthes of goats commonly encountered in Nigeria includes *Haemochus contortus, Strongyloides papillosus, Trichostrongylus*

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columbriforms, Oesophagostonum columbianum, Fasciola species and *Moniezia benedeni* (Aliu et al., 2001; Van wyk et al., 2004). In the southern part of Nigeria, Srongyloidosis is a constant feature of gastro-intestinal parasitism especially during the rainy season (Van wyk and Bath, 2002; Okoli et al., 2006). Goats are often the main supply of diary meat in Ankpa Local Government Area, Kogi State of Nigeria particularly the indigenous breed of West African Dwarf (Oni, 2002).

In most part of the world, goats are kept mainly for meat, milk and leather (Peacock, 1996; Abubakar, 2002). In Northern part of Nigeria, the skin of goat is used as raw material in leather industries for manufacturing shoes, bags, belts etc. Goats although representing an important source of animal protein in Ankpa, seem to have benefited little from the veterinary care and production improvement. Goats are also hampered by infections and parasitic diseases coupled with inadequate management (Tembely et al., 1997; Torina et al., 2004; Dauda, 2004). The most important cestode parasites of small ruminant both in terms of public health and veterinary medicine belong to the family Taeniidae. These include cystic or larval stages of *Echinococcus granulosus, Taenia hydatigena, Taenia ovis* and *Taenia multiceps* (Urquhart et al., 1996). All trematode species that are parasitic in small ruminants belong to the sub class *Digenea* and the most important species in Africa are Liber flukes, *Fasciola hepatica, Fasciola gigantic, Dicrocoelium* species and rumen flukes (paraphistomes) *paramphistomum* species (Anon, 1994; Hansen and Perry, 1994). The Nemathelminthes (nematodes) include several super families of veterinary importance; these are Trichostrongyloidea, Stronglyloidea, metastrongyloidea, Ancytostomatoidea, Rhabditoidea, Trichuroidea, Filarioidea, Oxyruoidea, Anscaridoidea and spiruroidea (Githigia et al., 2001; Anon, 1994; Hansen and Perry, 1994).

The most common gut helminthes of goats are usually acquired by the ingestion of the infected eggs or larvae or by its penetration through the skin (Githigia et al., 2001; Nwoke et al., 2013). Gut nematodes of goats are round worms living in the abomasums, small intestine and large intestine of goats. Infection usually occurs primarily through contaminated feed and water, enhanced by poor hygiene (Gatongi, 1996; Zajac 2006). Most goats infected have been shown to be asymptomatic or produce only mild symptoms, as a result they are often over looked till serious complication or chronic clinical symptoms occurs (Kassi, 1999; Zajac, 2006). Some of the symptoms associated with gut heliminth parasites are anaemia, diarrhoea, loss of weight, oedaema, recumbency, destruction of liver parenchyma dead liver tissue and general condemnation of the liver of slaughtered animals, spleenomegaly, unthriftiness, emaciation and even death of the animal (Perry and Randolph, 1999; Abubakar, 2002). In poorly managed system of goat keeping, or where infection is massive, these parasites enhance all other ailments both intestinal and others making them acute and lethal (Mondal et al., 2000). Gut worms in goat cause economic and nutritional hardship in poor farming communities and livestock operations, which are meaningless without sound knowledge of the animal care, prevention and eradication of diseases.

Majority of the animals do have faecal worm parasites egg count of below 500 eggs/grain feaces. A high proportion of small ruminants shed stongyle eggs during the post parturient period. Fakae (1990) studied the epidemiology of helminthosis in small ruminants under the traditional system in eastern Nigeria. The epidemiology of helminth infections in West African dwarf goats under the traditional husbandry system is prevailing in the derived savanna area of eastern Nigeria. The result of study revealed that infections were due to *Haemonchus, Trichostrongylus* spp., Metacestodes of *T. hydatigena, O. columbianum*, *Strongyloides* sp, *Moniezia expanza*, etc. and mixed infections were most prevalent.

The endemicity of parasitic gastroenteritis in the area was indicated by the high prevalence of the helminthes irrespective of the season of the year.

MATERIALS AND METHODS

Study area

Ankpa town is under Ankpa Local Government Area in Kogi State, Nigeria. Its headquarters are in the town of Ankpa on the A233 highway in the West of the area. It has an area of 1, 200km^2 and a population of 267, 353 at the 2006 census. The area is characterized by two seasons, the dry season and rainy season. The population of Ankpa is growing rapidly as a result of the presence of the Kogi State College of Education, and also as a result of the establishment of a private college of education. It has enhanced social amenities and its food demand has increased enormously which includes goat meat, hence there is need for increased production of goats in the area. Ankpa is a centre of commerce in Igala land, thus, it is said to be located at the centre of Igala towns and villages. It has a large market which draws people from far and near to trade. Goats are among the commonest goods found in Ankpa main market. Majority of the Ankpa residents are farmers and traders with animal rearing which serves as income supplement. It can multiply very fast and it also serves as source of income to the farmers.

Collection procedure

Faeces were collected from the rectum of the goats that are brought for slaughter. The faeces were put in a separate polythene bags which were masked with a tape. The number and sex of the goat is then noted on the polythene bag. The samples were then immediately taken to the laboratory for examination. The faeces are then examined for the presence of parasite eggs and oocysts. The method employed is the simple flotation method (Perry and Randolph, 1999). Simple flotation method is based on the observation that helminth eggs will float to the surface of the floatation medium which has a higher specific gravity than the eggs.

Table 1. Shows the frequency of gut helminth parasites of goats slaughtered in Ankpa Ankpa Local Government Area (L.G.A).

Samples	No. examined	No. infected	Percentage
Adults	136	96	38.7
Young	112	87	35.1
Total	248	183	73.8

Table 2. The sex distribution of gut helminth parasites of goats slaughtered in Ankpa L.G.A.

	Adult goats			Young goats		
Sex	**No. examined**	**No. infected**	**% infected**	**No. examined**	**No. infected**	**% infected**
Male	60	45	34.4	55	38	32.48
Female	71	51	38.9	62	49	41.9
Total	131	96	73.3	117	87	74.4

And the other technique employed if the sedimentation method for the detection of trematode eggs, the modified method of Dennis, Stone and Swanson as cited by Abubakar (2006).

Examination procedure of faeces

Simple floatation

About 2 to 3 g of the faeces is poured into a centrifuge tube. And $ZnSO_4$ sucrose (floatation medium) is added to the faeces to almost 2/3 of its volume. A glass/rubber rod is then used to break the faeces which in most cases are in pellets. After a homogenous mixture is obtained, it is then sieved through a sieve placed on a test tube. The coarse debris is then trapped on the mesh. The floatation media is then added to the filtrate and filled to the brim, until a convex meniscus is formed on the test tube. A cover slip is placed on it and left for about 5 min. After about 5 min, the cover slip is then pulled gently from the test tube and placed on a slide, which is now ready for viewing. Two cover slips are laced per slide for examination under microscope (×10 and ×40) objectives.

Sedimentation method

About 4 g of the faeces is thoroughly broken and mixed in a centrifuge tube using a rod by adding appropriate quantity of distilled water. The mixture is sifted into a specimen bottle. Distilled water is then added to the filtrate and filled to the brim. This is then left for about 10 min. After 10 min, the supernatant is decanted and the sediment is ready for examination. A Pasteur pipette is used to collect the sediment and place on slide. It is then examined under the low power objective of the compound microscope.

Identification

Each individual species of the helminth parasite is recognizable by the characteristics of its eggs, shapes, sizes, colour, nature of shell (thickness), stage in development appearance of embryo if present and using parasitological atlas. But in a situation where the examination of the collected faeces is not possible, 10% formaline is added immediately to hinder further development of the eggs and kept in a cupboard or the sample can be placed in a refrigerator overnight and examined later.

RESULTS

The study revealed an overall prevalence of gut helminth infections of goats slaughtered at Ankpa abattoir, 73.8% (Table 1). Of the population sampled (N = 248; 136 adult goats and 112 young goats), the adult goats accounted for 38.7% and young goats 35.1% (Table 1). A total of 96 adult goats and 87 young goats were positive for parasites. The percentages of infections were high for both adults and young goats (Table 1). Statistical analysis showed no significant difference between the infection rates in both sexes ($P > 0.05$). The results of Table 2 showed the sex distribution of gut helminth parasites of goats slaughtered in Ankpa L.G.A. out of total population sampled (N = 248; 131 adult goats and 117 young goats), the infected adult male and female goats accounted for 34.4 and 38.9%, respectively. While young male and female goats accounted for 32.5 and 41.9%, respectively (Table 2). A total of 60 and 71 adult male and female goats were examined respectively. While young male and female goats accounted for 62 and 55 respectively. The results of presented study also showed that the infected young male and female goats accounted for 38 and 49 respectively been positive for the parasites. Table 3 showed the frequency distribution of nematodes, cestodes and trematodes eggs/ova and adult worms in the faecal samples of goats slaughtered in Ankpa L.G.A. The proportions of nematodes eggs/ova

Table 3. The frequency of nematodes, cestodes and trematodes eggs/ova and adult worms in the faecal samples of goats slaughtered in Ankpa L.G.A.

Parameters	Species	No. of eggs/ova	No. of adult worms
Helminthes	*Ascaris sp.*	35	19
	Trichuris sp.	3	12
	Strongyloides sp.	87	39
	Trichostrongylus sp	40	17
Nematodes	*Oesophagustomum sp*	17	24
	Haemonchus sp	12	7
	Bunostomium sp	22	3
	Gaigeria sp	14	21
	Gongylonema sp	18	23
	Ostertagia sp	19 = 267 (85.9%)	4 = 169 (85.4%)
Cestodes	*Avitellina sp*	17	8
	Taenia sp	24 = 41(13.2%)	18 = 26 (13.1%)
Trematodes	*Schistosoma sp*	3 = 3 (1.0%)	3 = 3 (1.5%)
Total	-	311	198

and adult worms recovered accounted for 267 (85.9%) and 169 (85.4%) respectively. These were *Ascaris sp., Trichuris sp., Strongyloides sp., Trichostrongylus sp., Oesophagustomum sp, Haemonchus sp., Bunostomium sp., Gaigeria sp, Gongylonema sp.* and *Ostertagia sp.,* The cestodes showed that eggs/ova and adult worms recovered accounted for 41 (13.2%) and 26 (13.1%) respectively. While least proportion of trematode eggs/ova and adult worms were recovered which accounted for 3 (1.0%) and 3 (1.5%) respectively. The cestodes were *Avitellina* sp., *Taenia* sp and trematode was only *Schistosoma* species.

DISCUSSION

Gut helminthes represent a major public health problem in rural communities which Ankpa is among. The research made it obvious that there are helminthes parasites in the goats of the sampled area, Ankpa, Kogi State. In this study, the frequency distribution of nematodes, cestodes and trematodes eggs/ova and adult worms in the faecal samples of goats slaughtered in Ankpa L.G.A. The proportions of nematodes eggs/ova and adult worms recovered showed high prevalence pattern of helminthiasis. This can be attributed to the ubiquitous nature of egg distributions and hence very high prevalence in the area. The percentages of infections were high for both adults and young goats. The high prevalence of soil transmitted helminthiases in the area is influenced by a multifactorial system, which comprises hosts, parasite and environmental effects (Okoli et al., 2006). Githigia et al. (2001) attributed several factors that is, warmer and wetter grazing seasons, the greater time animals spend on pasture, ineffective deworming practices or the development of anti-helminthic resistance in this parasite. Although, statistical analysis showed no significant difference between the infection rates in both sexes ($P > 0.05$); the study also revealed that adult animals were carrying heavy worm burden than the young ones. This might be as a result of intermittent relaxation of immunity at post periparturient periods as suggested by Urquhart (1996).

The result also showed that cestodes and trematodes were not common in Ankpa goat and if they do, they occur in mild form which may not be harmful to the host as a single infection. They may occur as multiple infections in combination with the infested worms. From these, it shows that Ankpa and its environment are quite endemic to helminthic infections (of which nematodes are the commonest). In fact, the parasites encountered are pathogenic routine that should have been solved by deworming. The high prevalence of gut transmitted helminthiases in Ankpa and is comparable with previous reports in Northern and Southern Nigeria (Urquhart et al., 1996; Larson, 1999; Aliu et al., 2001; Githigia et al., 2001; Abubakar, U. 2002; Oni, 2002; Okoli et al., 2006). Goats are often the main supply of diary meat in Ankpa L.G.A, Kogi State of Nigeria particularly the indigenous the breed, West African Dwarf (Oni, 2002). Goats although representing an important source of animal protein in Ankpa, seem to have benefited little from the veterinary

care and production improvement. Goats are also hampered by infections and parasitic diseases coupled with inadequate management (Doma et al., 1999; Dauda, 2004). The development of the variable eggs of parasitic helminthes are influenced by climatic factors such as sunlight, temperature, rainfall, humidity and soil moisture within the feacal pallets herbage (Jacquiet et al., 1992). Most parasitic goats which may appear to be healthy can have high worm lodges when examined (Urquhart et al., 1996; Nginyi et al., 2001). The prevalence pattern of helminthiasis in the study shows that, management plays an important role as well as climatic factor in the occurrence of helminthiasis of goats. Under semi-intensive management system in which little or no veterinary action such as deworming and improper feeding, the goats are prone to helminthiasis.

Conclusion

The incidence of helminthes parasites in the faecal samples of goats in Ankpa as examined can be due to poor management since the study was carried out using goats kept under semi-intensive system of management with little or no routine deworming, frequent cleaning, (removal of their droppings) and bedding from their pens which may contribute to helminthiasis. They may account for the ubiquitous nature of egg distributions and hence very high prevalence in the area and its environs.

Conflicts of interest

Authors have none to declare.

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Full Length Research Paper

Culicid forms distribution and breeding sites in Nsukka ecological zone of South Eastern Nigeria

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The culicid forms distribution and breeding sites in Nsukka ecological zone of south eastern Nigeria were investigated in four hundred and fifty eight (458) oviposition sites between October and December, 2010. Soup ladle dipper method was used in larvae/pupae collection while the environmental parameters were determined *in situ* using field meter. Anova were used to investigate the association between the physicochemical variables, the relative abundance and distribution of the mosquito larvae. Of the 458 containers sampled, automobile tyre had container index% of 59.62 while the least was 20.95%, recorded in clay pots. Four thousand, four hundred and twenty five species of culicid distributed in four genera (*Aedes, Anopheles, Culex* and *Toxorhynchites*) were identified. *Aedes aegypti* recorded 562 (12.70%) of the total collection in the automobile tyre. Also in the discarded cans, *A. aegypti* ranked second highest. The *Culex quinquefasciatus* had a prevalence of 302 (6.82%) in the automobile tyre while *Aedes albopictus and Toxorhynchites* 248 (5.60) and 242 (5.47) were recorded in the clay pots respectively. Also, Table 3 shows that a total of 458 (40.17) containers were sampled out of which 184 (38.14%) contained mosquito larvae. A total of 204 (20.95%) clay containers were sampled during the course of the studies. Water drums recorded 36 (30.55%) and a total of 104 (59.62) were observed in automobile tyres. Out of the 184 (40.17%) containers that held mosquito larvae, the highest container type that held mosquito larvae was automobile tyre with a total of 62 (59.62%). Among the oviposition sites, clay pots recorded highest dept of 18.50 cm and this was closely followed by buckets with 17.28cm while cans was the least with 5.2cm. The more important vectors of mosquito-borne diseases are those which show a close association with man and prefer him to other animals as source of food. These include *A. aegypti, A. albopictus, Culex fatigans* and *Agkistrodon bilineatus taylori.*

Key words: Culicid, breeding sites, ecological zone, Nigeria.

INTRODUCTION

The disease transmission and biting nuisance problems arising from the occurrence and interaction of mosquitoes with humans appear to have defied several scientific advances and health services instituted to combat them. Part of the problems militating against effective and sustained control of mosquitoes and the diseases

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transmitted by them is the overt advantages available to mosquitoes to breed in diverse aquatic media that are naturally occurring or the creation of human activities (Adebote et al., 2008). *Aedes aegypti* species is known to be the most thoroughly domesticated of any of the mosquitoes and apparently greatly prefers the blood of man to that of other animals (King et al., 1944).

Mosquitoes prefer an environment with certain resources (food, shelter, breeding sites; favourable temperature and suitable humidity) in sufficient amount at an appropriate time for survival and development (Adeleke et al., 2008). The policy, in the 1970s, of clearing forest in Enugu region of Nigeria for planting of the valuable timber trees, teaks and gmelina, increased the people's exposure to the bites of forest mosquitoes. They also noted that the situation was aggravated as a result of rot holes which developed in the tree stumps left behind after clearing operations which later became filled with water and formed ideal larval habitat for *Aedes africanus* – a sylvatic yellow fever vector (Onyido et al., 2009). According to Anosike et al. (2007), in their aquatic stages different species of mosquitoes may occupy the same habitat, and also form part of a single guild. The recent increase in ecological and environmental modification due to agricultural activities and urbanization has been observed to contribute to the breeding of various mosquito species (Amusan, 2005; Anizoba and Obudulu, 2006).

Mosquitoes develop in a wide range of aquatic larval habitats and in all climates from the Arctic to the Tropics (Edington and Gilles, 1978). There have been no known information on the culicid forms distribution and breeding sites in Nsukka ecological zone of south eastern Nigeria hence this study is concerned with exploring how different types of containers may act on the mosquito proliferation and how the knowledge on these mechanisms may help in establishing better policies to control the dengue vector. Accordingly, this study focused on surveying several containers considering their type of use, volume, factors, and materials as potential factors that could facilitate culicid forms distribution.

MATERIALS AND METHODS

Sample collection

Outdoor water holding containers within 270 dwellings (sites) in these ecological zones were examined. The containers holding water found in these sites were sampled. Five scoops in each of the water holding containers were obtained with a plastic soup ladle dipper for containers holding water of more than 250 ml while in containers holding below 250 ml of water was poured directly into plastic beakers. Specimens in recipients like barrels, drums and tanks were collected with the aid of plastic bowls and buckets of known volumes. The water collected was then poured into a white plastic bowl and carefully observed for the presence of mosquitos' larvae. Culicine larvae collected were concentrated in a sieve and carefully picked with dropping pipette into labeled small cups and covered with mosquito net. Specimens in recipients like reservoir and fish pond were collected by the sweep net method, as proposed by Tun-lin et al., 1995). These were transported to the post graduate research laboratory of the department of Zoology University of Nigeria where they were reared with a diet of baker yeast until they emerged to adults. Other invertebrates collected were preserved in labeled collecting bottles containing 70% alcohol.

Species identification

Mosquitoes collected were identified to species level and counted with the aid of binocular microscope in the Entomological laboratory of the Department of Zoology, University of Nigeria, Nsukka using pictorial keys of Gillett (1972) for the culicines.

Determination of physicochemical parameters of water in the container

Depths of water in containers were obtained by lowering a metre rule to the bottom of the container at three locations and the mean depths recorded. The surface areas of water in the containers were determined from length and width measurements with a metre rule. The pH, electrical conductivity, total dissolved solids and temperature of water in each container was determined by means of a HANNA HI 991300 pH/EC/TDS/Temp meter. Relative humidity, temperature and rainfall data covering the study area during the course of the research was obtained from Centre for Basic Space Science in the University of Nigeria, Nsukka.

Experimental design

The breeding sites were classified according to the guidelines of Medronho et al. (2009).

1. Volume.
2. Construction material.

Concerning volume, the recipients were classified as:

1. Very small-under 250 ml.
2. Small from 250 to 1,000 ml.
3. Medium-from 1,000 to 25,000 ml.
4. Large-from 25,000 to 1,000,000 ml
5. Very large-above 1,000,000 ml.

According to material, the recipients were classified as:

1. Automobile tyre.
2. Clay pots.
3. Water drums.
4. Buckets.
5. Bottles.
6. Cans.

Statistical analysis

Simpson's index was used in determining the relative abundance of each species of mosquito encountered in each site and during the entire course of the study using the formula given:

$$D = \frac{\sum n\,(n-1)}{N\,(N-1)}$$

n = the total number of organisms of a particular species
N= the total number of organisms of all species.
D = the diversity of species.

Table 1. Range (mean±SE) of physic chemical parameters of water in oviposition sites supportive of mosquito species around Nsukka, Nigeria.

	Physical parameters		Chemical parameters		
Oviposition site type	**Depth (cm)**	**Temperature (°C)**	**pH**	**Total dissolved solids (ppm)**	**Electrical conductivity**
Automobile tyre	4.50-17 (10.30±1.30)	25.8-29.4	6.86-8.74	0-193	0-376
Clay pots	4.20-30 (18.50±60)	20.2-25.2	6.23-7.53	10-120	26-170
Water drums	15-20 (17.28±2.01)	21.4-24.0	6.30-6.85	10-180	22-230
Buckets	9-18 (17.28±1.15)	24.2-29.2	5.82-7.25	15-115	25-165
Bottles	4.50-15 (6.2±1.42)	25.6-30.0	6.54-7.80	10-120	22-230
Cans	0.5-10 (5.2)	25.0-30.3	5.87-8.10	10-184	28-165

Table 2. Monthly relative abundance of larval mosquito species from oviposition sites around Nsukka area (September-December 2010).

Season/Month	*A. aegypti* No. (%)	*A.albopictus* No. (%)	*A. gambiae* No. (%)	*A. funestus* No. (%)	*Culer nebulosus* No. (%)	*C. Tigripes* No. (%)	*C.quinquefascitus* No. (%)	*Toxorhynchites viridibasis* No. (%)	Total No. (%)
Wet season									
September	535 (43.3)	324 (44.5)	372 (49.3)	103 (53.6)	46 (41.8)	66 (35.9)	167 (45.8)	82 (39.0)	1695 (44.9)
October	700 (56.7)	404 (55.5)	383 (50.7)	89 (46.4)	64 (58.2)	118 (64.1)	198 (54.2)	128 (61.0)	2084 (55.1)
Sub total	1235 (32.7)	738 (19.3)	755 (20.0)	192 (5.1)	110 (2.9)	184 (4.9)	365 (9.6)	210 (5.5)	3779
Dry season									
November	148 (75.5)	81 (58.3)	89 (88.1)	24 (75.0)	12 (92.3)	15 (55.6)	43 (58.1)	37 (57.8)	449 (69.5)
December	48 (24.5)	58 (41.7)	12 (11.9)	8 (25.0)	1 (7.7)	12 (44.4)	31 (41.9)	27 (42.2)	197 (30.5)
Sub total	196 (30.3)	139 (21.5)	101 (15.6)	32 (5.0)	13 (2.0)	27 (4.2)	74 (11.5)	64 (9.9)	646
Total	1431 (32.3)	867 (19.6)	856 (19.3)	224 (5.1)	123 (2.8)	211 (4.8)	439 (9.9)	274 (6.2)	4425

Simpson's index of diversity = 1- D.

Statistical package for social science (SPSS) was used to check the correlation amongst the physicochemical parameters of water in containers, and abundance of species of mosquito breeding therein. GENSTAT was used to test for One-way analysis of variance (ANOVA) test for significant differences in the relative abundance of mosquitoes amongst containers; using least significant difference to separate means that differ significantly. While a two way analysis of variance was used to test the interaction of mosquito species and containers.

RESULTS

Table 1 shows ranges of physic chemical parameters of water in oviposition sites supportive of mosquito species around Nsukka, Nigeria. Among the oviposition sites, clay pots recorded highest dept of 18.50 cm and this was closely followed by buckets with 17.28cm while cans was the least with 5.2cm.Temperature recorded highest in cans with 30.3°C as against 24.0°C that was observed in water drums. pH was highest in automobile tyre with 8.74 while buckets recorded the lowest of 7.25. Also, Total dissolved solids (TDS) was highest in automobile tyre with 193 ppm as against the lowest value recorded in buckets with 115ppm. The electrical conductivity was highest in automobile tyre with 376 while buckets registered the lowest value of 165.

Table 2 shows monthly relative abundance of larval mosquito species from oviposition sites around Nsukka area between September to December, 2010. Among the total number (4425) of larval mosquito species recorded, the *A. aegypti* was most abundant with (143) 32.7% in wet season when compare with (196) 30.3% abundance recorded in dry season. The *C. tigipes* larval species was least abundant among the mosquito species observed with 184 (4.9%) recorded in wet season as against 27(4.2) available in the dry season. Also, *Aedes albopictus* was second abundant mosquito species with 738 (19,3%) in the wet season as against 139 (21.5%) in the dry season. *Toxohynchies viridibas* recorded 210 (5.5%) in wet season as against 64(9.9%) that was observed in dry season.

Table 3. Artificial oviposition site preferences of mosquito species around Nsukka area, Nigeria (September to December, 2010).

Oviposition site type	No. examined	No. positive for larvae	Container index %	Total larvae collected (%)	Mosquito species	Species occurrences No. (%)
Automobile tyres	104	62	59.62	1252 (28.30)	*A. Aegypti*	562 (12.70)
					A. albopictus	329 (7.44)
					C. nebulosus	59 (1.33)
					C. quimquefasciatus	302 (6.82)
Clay pots	204	44	20.95	864 (19.53)	*A. albopictus*	248 (5.60)
					A. Gambiae	242 (5.47)
					A. Funestus	132 (2.98)
					Toxorhynchites viridibasis	242 (5.47)
Discarded buckets	60	20	46.66	709 (16.02)	*A. Aegypti*	234 (5.29)
					A. Albbopictus	156 (3.53)
					C. tigripes	211 (4.77)
					C. quinquefasciatus	108 (2.44)
Discarded cans	40	32	80.00	720 (16.27)	*A. Aegypti*	333 (7.53)
					A. albopictus	134 (3.03)
					A. Gambiae	224 (5.06)
					C. Quinquefasciatus	29 (0.66)
Water drums	36	11	30.55	513 (11.59)	*A. aegypti*	174 (3.93)
					A. Funestus	92 (2.08)
					A. Gambiae	247 (5.58)
Broken bottles	8	7	87.50	367 (8.29)	*A. aegypti*	128 (2.89)
					A. Gambiae	143 (3.23)
					C. Nebulous	64 (1.45)
					Toxorhynchites viridibasis	32 (0.72)
Total	458	184	40.17	4425	-	4425

Table 3 displayed artificial oviposition site preferences of mosquito species around Nsukka area , Nigeria (September to December 2010). A total of 4,425 mosquito species were collected and *A. aegypti* recorded 562 (12.70%) of the total collection in the automobile tyre. Also in the discarded cans, *A. aegypti* ranked second highest. The *Culex quimquefasciatus* had a prevalence of 302 (6.82) % in the automobile tyre while *A. albopictus and T. virdibasis* 248 (5.60) and 242 (5.47) were recorded in the clay pots respectively. Also, Table 3 shows that a total of 458 (40.17) containers were sampled out of which 184 (38.14%) contained mosquito larvae. A total of 204 (20.95%) clay containers were sampled during the course of the studies. Water drums recorded 36 (30.55%) and a total of 104 (59.62) were observed in automobile tyres. Out of the 184 (40.17%) containers that held mosquito larvae, the highest container type that held mosquito larvae was automobile tyre with a total of 62 (59.62%).

DISCUSSION

The species of mosquitoes encountered in this study compares favorably with the five species recorded in Nsukka (Agwu, 2005) and also with those recorded in Benin (Wagbatsoma and Ogbeide, 1995). The species also compares favorably with other works carried out in the south-eastern Nigeria. Which include the five species encountered by Onyido et al. (2009) in Awka and that of Anosike et al, (2007) in Imo

State. In the South- west, these *spp* encountered here were also compares favorably with the nine *spp* recorded in Abeokuta (Adeleke and Adeyi, 2008). This study is also not far from the report in Zaria (Adebote et al., 2008). The species recorded do not also differ much from what was recorded in Santo Domingo city (Carlos et al., 2003). The slight difference might be due to different microhabitat sampled and the distribution of the species and also due to the fact that different species of mosquitoes show individual ecological preference for breeding habitat (Service, 2004).

Agwu (2005), reported that temperature and relative humidity are factors that determine the preference of many mosquito species, and that low relative humidity is known to cause death of mosquitoes through desiccation and also at very low temperatures, mosquito development is slow, while at high temperatures, mosquito development is rapid but producing sterile adults. Death also occurs at temperature above 35°C. Adebote et al. (2008) also stressed on aquatic microhabitat drying out due to cessation of rainfall. This may be the reason why the number of mosquitoes encountered in this study decreased as one moves from the rainy to the dry season (that is from October to December). Water storage mode, the level of sanitation, frequent use of water from containers may determine the availability of containers holding water around dwellings thus this could be the reason why there was difference in the level of sites being positive in a given micro-ecological habitat. In October, *A. aegypti* was relatively more abundance (0.75) than all the other mosquitoes *species* encountered in Nsukka, and this is in agreement with the findings of Agwu (2005). The least encountered was *Toxoryhnchites sp and* this might be due to its prolonged life cycle. *Toxoryhnchites sp* spends longer time in developing from egg to adult than the other mosquito species. In November, *C. fatigans* was relatively more abundant than the other species encountered. *C. fatigans* are known to be most widely distributed mosquito in the world.

It is found on every continent except in Antarctica. This species of mosquito is known to breed in foul water like in septic tanks, soakage pits, cess-pit etc in urban environment. This mosquito is generally known to be more opportunistic in the selection of oviposition sites, and have an ubiquitous distribution of immature in various geographical areas and water body types. This may be the reason why this mosquito was more abundant, and also the period in which the study was carried out coincided with the dry season and according to Russell et al. (2001) the eggs of *A. aegypti* when embryonated, the eggs can survive up to one year until they are flooded and hatched and the completion of the immature stages depend on continued presence of water in the container and on the water temperature. This may account for the decrease in the prevalence of *A. aegypti* in the dry season since water tend to dry out of container as it was reported by Carlos et al. (2003) that mosquitoes species decrease due to seasonal changes. *A. albopictus,* native to the tropical and sub tropical area of South-east Asia was reported to have entered the USA in shipments of scrap tyres from Northern Asia.

According to Anosike et al. (2007), the route of importation of this mosquito into Nigeria is not clear but Agwu (2005), reported that the importation of used tyres from Asia might be the possible route. The impact of this mosquito is very unhealthy, is a fast colonizer, catholic in its feeding habit and is capable of breeding in both domestic and forest environments (Anosike et al., 2007). The relative abundance of this mosquito species might be affected due to the fact that its most preferred habitat (vulcanized rubber) did not hold water during the dry season and thus didn't support its' productivity as reported by Anosike et al. (2007). *A. Taylori,* reported by Gillett (1972), as a tree breeder was found in containers during this study, This might be due to ecological changes which include deforestation, land excavation and landscaping (Amusan, 2005; Anizoba and Obudulu, 2006; Onyido, 2009). This may also be the reason why it was the least encountered since it may be trying to adapt to container life. In Nsukka ecological zone, among the seven physicochemical parameters monitored, only total dissolve solid (ppm) and electrical conductivity correlated significantly ($p<0.01$) with the prevalence of *A. aegypti*. The correlation of *A. aegypti* with high total dissolve solids shows that it has preference for such habitat. The abundance of *A. albopictus* correlated significantly ($p<0.01$) with volume of water, and this might be that this species of mosquito has preference for containers having high volume of water. Though depth, water temperature, pH, total dissolve solid (ppm) and electrical conductivity did not correlate with its abundance.

Studies on physicochemical parameters carried out by Umar and Don-pedro (2008) reported that the survival of *A. aegypti* occurred mainly between pH 6.5 and 8.0, and that high mortality resulted above this level. Clark et al. (2004) reported that pH value of 4.0 has no significant effect on the growth and development of *A. aegypti*. Carpenter (1982), reported that larvae mortality was not significant at pH 6 to 8 and that pH 4.5, 10.0, and 10.5 had no significant difference on *C. fatigans.* According to Dario and Nicolas (2002), preimaginal stages of mosquito develop in artificial containers of small volume, such as flask, bottles and flower vases. This might be the reason why volume of water had no effect on most of the mosquito species except *e. albopictus* in Nsukka ecological zone. The fact that only a few *species* abundance was affected by some physicochemical parameters is in line with the report of Adebote et al. (2008) in which no physicochemical parameter correlated with larvae abundance in two of the sites he sampled in Zaria and while other species abundance correlated significantly with the physicochemical parameters in the third site. Mosquitoes utilize a great variety of water

sources for breeding. These include ground pools, water in artificial containers, water holding tree, leaf axils, rock pools, gutters and septic tanks etc (Kittayapong and Strickman, 1993; Wagbatsoma and Ogbeide, 1995; Dario and Nicolas, 2002; Amusan, 2005; Adebote et al., 2008; Adeleke et al., 2008; Onyido et al., 2009).

Mosquitoes are reported to share habitat with each other or other invertebrate. Seng and Jute (1994) reported that the larvae of *A. aegypti* shared habitat with *A. albopictus* in urban houses while Adebote et al. (2008a), reported the sharing of habitat by different mosquitoes species. This was also reported by Agwu (2005). In this study there were also cases of different mosquito *species* co- existing together and also with other invertebrate which inhabit containers. Such included unidentified dragon fly nymph, unidentified chironomid larvae, unidentified earth worm, unidentified snail and unidentified water (pond) skater. The containers in which these organisms existed were examined and the level of container not holding mosquito larva might be due to the predatory nature of some of the organism that exist in the container thus feeding on the larvae of mosquitoes. According to Hammon (1984), the larvae of *Toxorhynchites sp* are predaceous to all instars of mosquito larvae. The report that this *species* of mosquito being low in abundance from its record against most mosquitoes as reported by Hammon (1984), Agwu (2005) and Anosike et al. (2007) was also encountered in this study. Though it is a mosquito *spp*, it got its name from the nature of its proboscis which means arrow (toxo) and snout (rhynch). According to Wikipedia (2010), it is known as mosquito hawk or mosquito eater. It is the largest known *species* of mosquitoes, but in spite of their alarming appearance, they are among the few kinds of mosquitoes that do not suck blood. Rather, the adults subsist on whatever carbohydrate- rich materials might be available. The larvae prey on the larvae of other mosquitoes and similar nektonic prey. In order for this mosquito *spp* to be use as an effective mosquito predator, then its limitations (low prevalence and long pupa phase) have to be improved on.

Among the mosquitoes *species* found only *Toxorhynchites spp* has not been incriminated to transmit disease since the adults don't feed on blood. This poses a serious public health implication. Also, in case of possible out breaks of infectious diseases which these mosquitoes are known to transmit like yellow fever, dengue, Chikungiya *Wuchereria bancrofti,* filarial worm etc, like the case of outbreak of yellow fever in Uganda after forty years which claimed 45 lives in December, 2010 was as a result of the prevalence of the vector in such area. The fact that these mosquitoes are collected in artificial containers around living houses is a sign that if, eventually, any infected person is around the area when this mosquitoes are abundant then there might be a sporadic man- to- man transmission as many will be exposed to it. The more important vectors of mosquito-borne diseases are those which show a close association with man and prefer him to other animals as source of food. These include *A. aegypti, A. albopictus, C. fatigans, C. trigripes* and *A. taylori.*

Conflicts of interest

Authors have none to declare.

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Effects of treatment with fraction IV extract of *Ximenia americana* on the survival rate, packed cell volume and total plasma proteins of *Trypanosoma congolense* infected mice

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The effect of treatment with fraction IV extract of *Ximenia americana* on the survival rate, packed cell volume and total plasma proteins of *Trypanosoma congolense* infected mice was investigated. Following infection with 10^4 *T. congolense*, the mice were treated with 25 mg/kg body weight fraction IV extract and 20 mg/kg body weight Diminazene aceturate, respectively, while a group was left untreated. Parasitaemia, packed cell volume, total plasma proteins and survival rate was determined. The results showed fraction IV portion of *X. americana* significantly ($P < 0.05$) enhanced the survival rate, packed cell volume of the treated animals, and also reduced the levels of parasite and total plasma proteins compared to the infected untreated groups. Fraction IV portion of *X. americana* could act as an adjunct therapeutic agent in the treatment of trypanosomosis.

Key words: *Ximenia americana*, *Trypanosoma congolense*, survival rate, total plasma proteins.

INTRODUCTION

African trypanosomes are protozoan parasites that cause sleeping sickness in humans and nagana in domesticated animals. It remains one of the most neglected human disease in Africa (Maudlin et al., 2004) and is of major animal health and economic impact in sub-saharan Africa (Mbuthia et al., 2011). The control of human and animal trypanosomosis is based on a limited number of compounds many of which are chemically related and have been used for more than 60 years (Leach and Roberts, 1981; Gutteridge, 1985; Kinabo, 1993; Anene et al., 2001; Maudlin et al., 2004). The repeated use of trypanocidal drugs in control of animal trypanosomosis has led to the development of drug resistant trypanosome population (Geerts and Holmes, 1998; Geerts et al., 2001; De Koning, 2001). Renewed interest in traditional pharmacopeias is increasing worldwide most especially among African people who are becoming reliant on herbal medicines for their health care needs. This is because medicinal plants are more accessible and affordable (Mander, 1998). Traditional knowledge of plants could help researchers target plants that are medicinally useful (Cox and Balick, 1994).

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Ximenia americana is a medicinal plant that has been reported to have shown several biological activities such as antimicrobial, antifungal, anticancer, antineoplastic, antitrypanosomal, antirheumatic, antioxidant, analgesic, moluscicide, pesticidal and also having hepatic and heamatological effects (Asres et al., 2001; Ogunleye et al., 2003; Elnima, 2003; Geyid et al., 2005; Voss et al., 2006; James et al., 2007; Maikai et al., 2009; Siddaiah et al., 2009; Soro et al., 2009; Maikai et al., 2010). The plant has been reported to contain biologically active compounds such as saponins, flavonoids, tannins, terpenoids, sterols, quinones, alkaloids, cyanogenetic glycosides, cardiac glycosides, saponosides, isoprenoids, fatty acids, triterpenes, sesquiternes and carbohydrates in the form of sugars and soluble starch (Ogunleye et al., 2003; Geyid et al., 2005; James et al., 2007; Araújo et al., 2008, 2009; Soro et al., 2009; Maikai et al., 2009; Maikai et al., 2010).

Therefore, the aim this study was to investigate if fraction IV extracts of *X. Americana* could extend the survival rate and reduce parasitaemia, total plasma proteins and modulate packed cell volume of *Trypanosoma congolense* infected mice.

MATERIALS AND METHODS

Plant sample

The bark of *X. americana* was collected from Afaka village 35 km to Kaduna (11°10' N, 7°38' E) and taken to the Department of Biological Sciences, Ahmadu Bello University Zaria for identification and confirmation with the voucher No. 1612. The voucher specimen (No. 1612) was deposited in the herbarium. The stem bark was dried at room temperature before crushing it into powder, then stored in air tight container and kept at 4°C until needed.

Extraction of plant material

Two hundred (200 gm) grams of the stem bark powder was weighed into a thimble and then transferred into a Soxhlet extractor and extracted sequentially with petroleum ether, methanol and water. The extracts were individually collected after each extraction and concentrated using a rotary evaporator (Buchi, Switzerland) at 50°C under reduced pressure and then dried. The solvent free extracts were then weighed and stored in brown bottles at 4°C until use. The aqueous extract was used for column chromatography after determining activity against trypanosomes (Maikai et al., 2008).

Partial purification of aqueous crude extracts (Column chromatography)

The aqueous crude extract of stem bark of *X. americana* was partially purified using column chromatography. Briefly, slurry was prepared by shaking 120 g of silica gel (Qualikems, 60 to 120 mesh powder) with 200 ml of water and methanol in the ratio of (1:1) and then packed in a column (1.5 × 30) at a flow rate of 0.2 ml /min. The column was loaded with 20 ml of the aqueous extract that had been previously adsorbed from distilled water on 4 g of the silica gel, and then eluted with four solvent mixtures (ethyl acetate/methanol 19:1 (Fraction I); benzene/methanol 19:1 (Fraction II); acetic acid/ methanol 1:1 (Fraction III); water/methanol 1:1 (Fraction IV) in order of increasing polarity. The eluents (Fraction I, II, III and IV) were collected in separate beakers and dried at 50°C using a water bath. The fractions were tested for antitrypanosomal activity and fraction IV which had the highest *in vitro* activity (values not shown). The dried fractions were kept at 4°C for further experiments.

Experimental animals

A total of forty three healthy white Swiss albino mice of 6 to 8 weeks old and weighing between 28 to 32 g were obtained from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria. The animals were housed in clean plastic cages in a 12 h light/dark cycle, and fed with diet made from chick grower's mash (Pfizer) and mixed with groundnut cake and flour. Water was given *ad libitum* and the cages were cleaned every week, throughout the duration of the work. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO (1998). The animal Laboratory care was strictly followed (CCAC, 1993). The rodent protocols and procedures used in this study were approved by the animal welfare committee of the college.

Trypanosome (Parasite)

T. congolense NITR stabilate 212 (Federe strain) was obtained from the Nigerian Institute of Trypanosomosis Research, Vom Plateau State Nigeria and passaged into rat which was subsequently maintained by passages in mice.

Trypanocidal drug

Diminal® (Diminazene aceturate) (Eagle chemical company limited, Ikeja Lagos Nigeria) a commercial drug for the treatment of animal trypanosomosis was used as a standard reference and as positive control. A concentration of 20 mg/kg body weight dose was administered to control animals according to manufacturer's recommendation.

Experimental design

Forty (40) experimental mice were randomly grouped into four groups (I to IV) of ten animals each. Group I was uninfected and untreated served as control. Group II to IV were inoculated with 10^4 trypanosomes. When parasites were detected, group II and IV were treated with a single dose of 20 mg/kg body weight of Diminal® (Diminazene aceturate) and 25 mg/kg body weight of fraction IV portions of *X. americana* (for five days) intraperitoneally respectively, while group III were left untreated. Parasitaemia, Packed cell volume (PCV), survival rate and total plasma proteins were determined. For the determination of PCV, blood was collected in heparinized capillary tubes from their tail at the end of treatment in tubes which were sealed at one end with Crystaseal® and centrifuged at 2000 g for 5 min in micro-haematocrit centrifuge. The PCV was then read using a Hawskley haematocrit reader (Barbara, 1980). Total plasma proteins concentation was determined using the Goldberg refractometer method (Schalm et. al., 1978).

Parasitaemia

Parasitaemia was monitored in the blood obtained from the tail pre-sterilized with alcohol. Detection of parasites was by wet film and

buffy coat methods. The number of parasites was determined microscopically at ×400 magnification using an Olympus Microscope (Japan). The method of Herbert and Lumsden (1976) was used to determine parasitaemia. Briefly, the method involves microscopic counting of parasites per field of the blood. Logarithm values of these counts were obtained and matched with the table of Herbert and Lumsden (1976), which was then converted to antilog to provide absolute number of trypanosomes per ml of blood. Negative samples were further examined by the more sensitive buffy coat technique as described by (Murray et al., 1977).

Toxicity studies

A total of three (3) mice were administered with 25 mg/kg body weight each of fraction IV extract of *X. americana* to check for acute toxicity. General toxicity signs were evaluated by observing the clinical signs. If none of the mice died then the extract was considered not acutely toxic.

Statistical analysis

Data obtained were expressed as mean ± Standard deviation and subjected to one-way analysis of variance (ANOVA). The log rank test was used to examine the null hypothesis that the survival curves were identical. A p-value of < 0.05 was considered to be statistically significant.

RESULTS

Effect of fraction IV on parasite population

Parasites were detected after three days post infection in the infected groups (Figure 1). There was a progressive rise in parasite population in group III (infected, untreated) peaking at day 14 with subsequent death (Figure 1). A significant ($P < 0.05$) reduction in parasite population was recorded in group II and group IV treated with 20 mg/kg body weight Diminazene aceturate and 25 mg/kg body weight fraction IV respectively.

Effect of treatment on packed cell volume

There was a decrease in packed cell volume for all the infected animals which coincided with the appearance of the parasites day 7 to 10 post infection (Figure 2) compared to group I (uninfected, untreated). Treatment with 25 mg/kg body weight fraction IV (group IV) and 20 mg /kg body weight Diminazene aceturate (group II) improved the value of the packed cell volume significantly compared to group III (infected untreated) (Figure 2). However, the PCV values did not achieve the pre-infection values.

Effect of fraction IV on survival rate

The effect of treatment with fraction IV portion of *X. americana* on the survival rate of mice experimentally infected with *T. congolense* is shown in Figure 3. The infected group treated with fraction IV (Group IV) and Diminazene aceturate (Group II) showed significant ($P < 0.05$) survival rates respectively when compared to the infected untreated group (Group III). However, treatment with Diminazene aceturate had a longer survival rate than fraction IV treated group.

Effect of fraction IV on total plasma proteins

The effect of treatment on total plasma proteins is shown in Figure 4. There was a steady rise in total plasma proteins for (group III) infected group (Figure 4), which peaked with the population of parasites day 14 post infection (dpi). Treatment with 25 mg/kg b.w. fraction IV extract of *X. americana* and 20 mg/kg b.w. Diminazene aceturate was significantly ($P < 0.05$) reduce than the value of total plasma proteins by day 14 post treatment compared to the group infected and untreated (group III) which steadily increased till the animals died.

Toxicity effect

The animals did not show any clinical signs of toxicity in the form of anorexia, dehydration, starry hair coat, depression, difficulty in respiration, coma or death as a result of administering 25 mg/kg b.w. of the extract. Thus, suggesting the marginal safety of the extract.

DISCUSSION

X. americana has been previously reported to have *in vitro* anti trypanosomal activity (Maikai et al., 2008, 2010), experimental infection of mice with blood stream trypanosomes results in pathological conditions which could result in the death of the animals if not treated. This study earlier reported that *X. americana* had no toxic effect in mice (Maikai et al., 2008). The study toxicity result of fraction IV extract did not show any clinical signs of toxicity, which was considered marginally safe as the extract was tested at a therapeutic dosage of 25 mg/kg b.w. The mice infected with *T. congolense* showed a decrease in PCV which was associated with the first wave of parasitaemia in the blood. The result corroborates with the report of Duncan et al. (1994) who reported that infection with *T. congolense* results in a significant reduction in the PCV. PCV reduction and anemia are the common cardinal features in the pathogenesis of trypanosome infection contributing to the morbidity and mortality thus curtailing longevity (Kagira et al., 2006; Karori et al., 2008). Treatment of the infected mice with fraction IV and Diminazene aceturate however, modulated the values of the PCV of the animals which corroborates earlier report of Mbaya et al. (2010) that

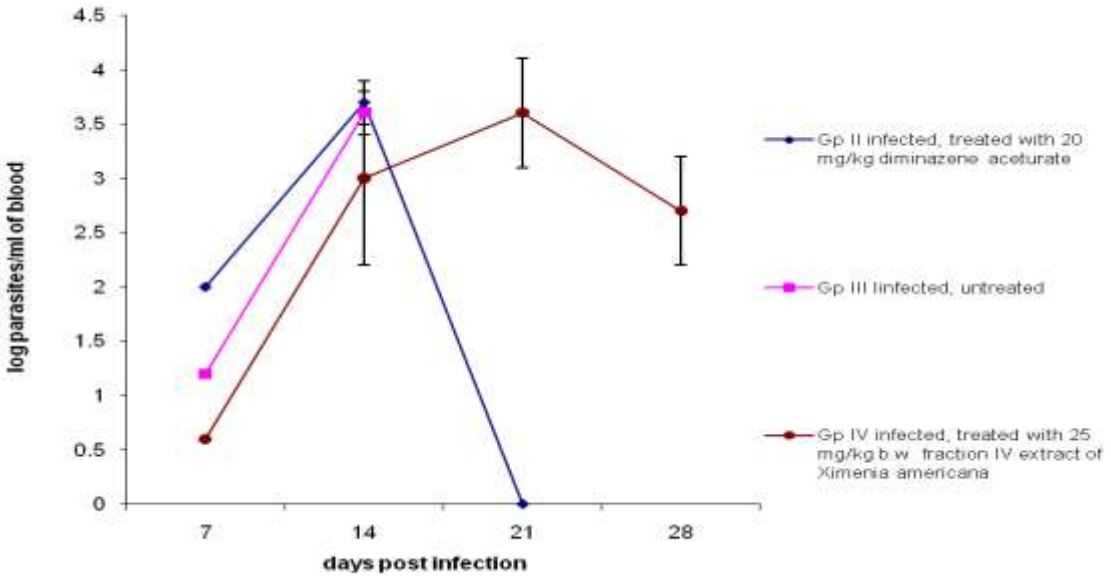

Figure 1. Effect of treatment on parasite population of mice experimentally infected with *T. congolense* (geometric mean and standard error of parasitaemia).

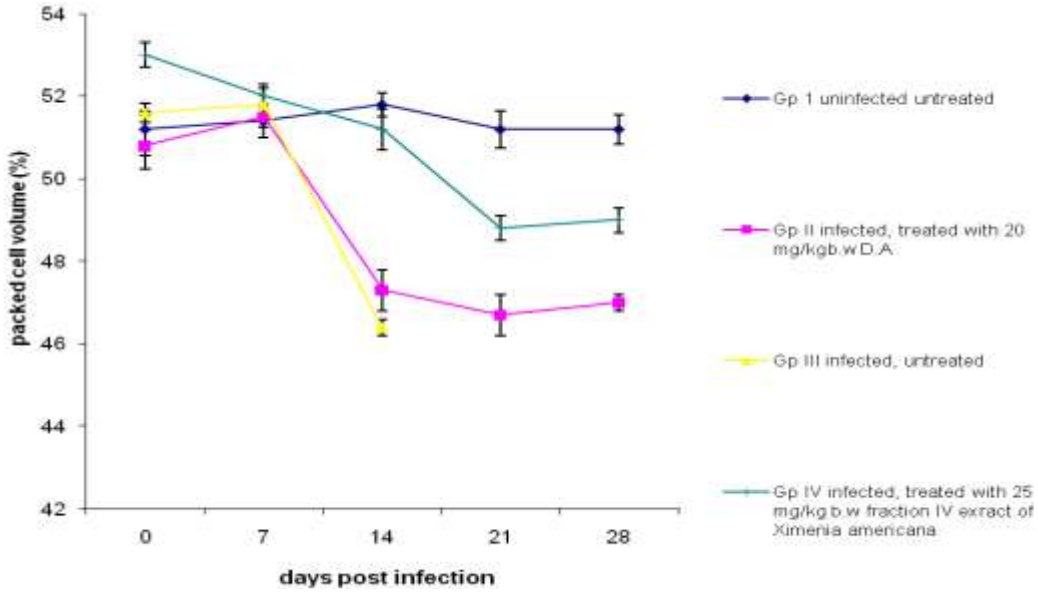

Figure 2. Effect of treatment on packed cell volume of mice experimentally infected with *T. congolense* (geometric mean and standard error of packed cell volume).

that mice infected with *T. brucei* when treated with *Azadirachta indica* had modulated values of PVC. Treatment with fraction IV portion of *X. americana* was not able to eliminate the parasites however, reduced the level of parasitaemia and thus extended the survival rate of the infected animals compared to the untreated group. The result of this study coincides with the study of Chen et al. (2004) and Mbuthia et al. (2011) who reported prolongation of survival rate in mice treated with tea extracts. They attributed it to the ability of tea flavonoids to counter the trypanosomosis induced inflammatory reaction and aiding antioxidant defense system. It has been reported that severity of trypanosomosis influences the total proteins levels of infected animals (Anosa, 1988; Ogunsanmi and Taiwo, 2001). The total plasma proteins were significantly ($P < 0.05$) higher in the infected

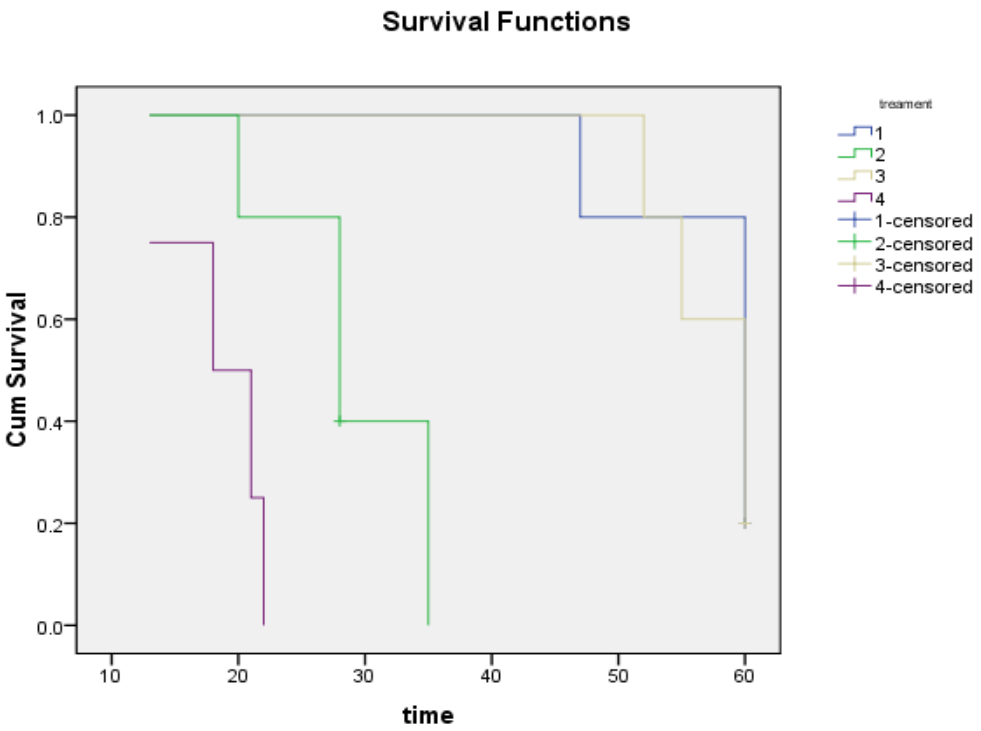

Figure 3. Kaplan meier survival curves comparing survival rates in *T. congolense* infected mice treated with fraction IV extract of *X. americana*. Key: 1 = uninfected untreated control: 2 = treated with 25 mg/kg b.wt fraction IV: 3 = treated with 20 mg/kg b.wt diminazene aceturate: 4 = infected untreated.

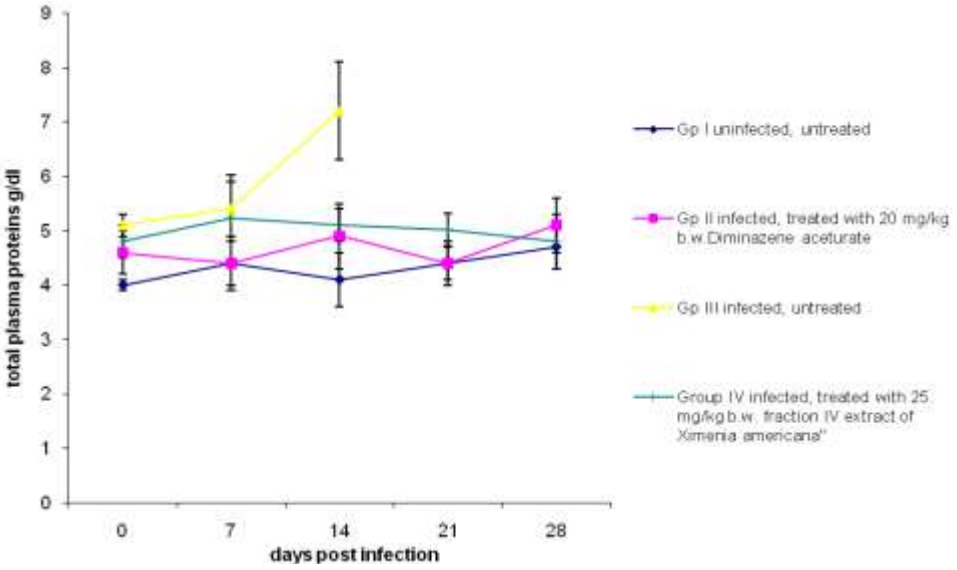

Figure 4. Effect of treatment on total plasma proteins of mice experimentally infected with *T. congolense* (geometric mean and standard error of Total plasma protein).

untreated groups when compared to the uninfected controls. The results of this study are similar with the results of Ogunsanmi and Taiwo (2001), who reported significant increases in total plasma proteins in *T.*

congolense infected sheep and goats.

Orhue et al. (2005), also reported significant increases in total plasma proteins and globulin in *T. brucei* infected rabbits. The increases in serum total plasma proteins could be due to increase release of tissue specific enzymes, and other intracellular proteins secondary to parasite induced cell membrane disruption, or increase in proteins might be due to increase in circulating antibodies as a result of the infection. It is also likely that the increase in total plasma proteins may be due to increase in mass of parasite proteins as a result of growing infection or possibly increases in parasite derived intracellular enzymes and proteins as the parasites are lysed by the host immune system. Treatment with fraction IV portion of *X. americana* led to reduction in the levels of total plasma proteins of infected animals when compared to the infected untreated group. The mechanism of action is unclear at this stage of investigation. Fraction IV extracts could be composed of a mixture of complex substances; it is not possible at this stage to identify the compounds responsible for the observed activity, but one is tempted to speculate that the antioxidants might play a protective role in countering the radicals generated as a result of the infection.

Conclusion

The study revealed that fraction IV extract of *X. americana* modulated the PCV value, prolonged the survival rate and reduced the total plasma levels of the treated animals. The results are promising, while research is ongoing to identify the type of compound responsible.

Conflicts of interest

Authors have none to declare.

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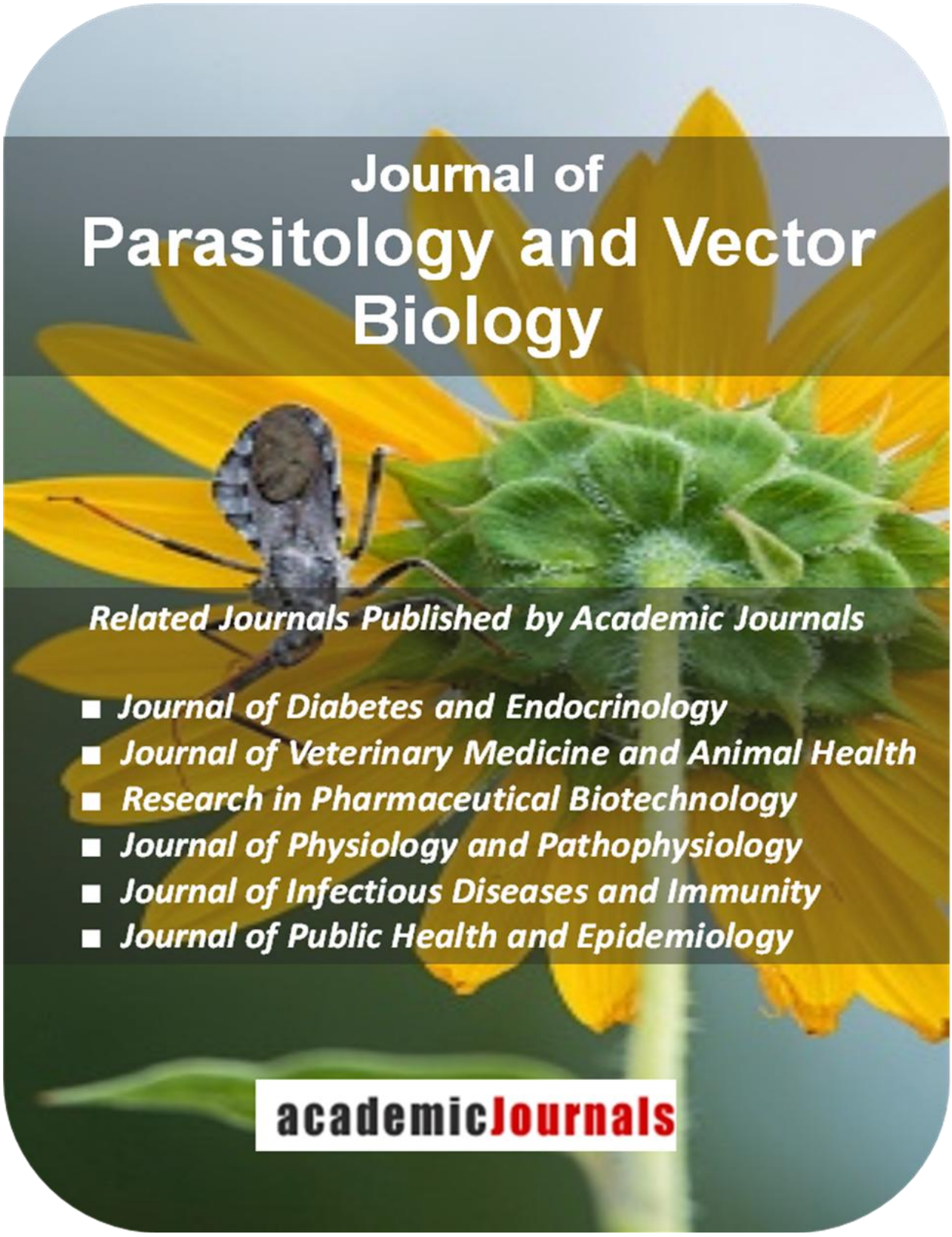
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