Journal of Parasitology and Vector Biology

Volume 8 Number 5, May 2016

ISSN 2141-2510

Academic Journals

ABOUT JPVB

The **Journal of Parasitology and Vector Biology (JPVB)** is published monthly (one volume per year) by Academic Journals.

Journal of Parasitology and Vector Biology (JPVB) provides rapid publication (monthly) of articles in all areas of the subject such as Parasitism, Helminthology, Cloning vector, retroviral integration, Genetic markers etc.

Contact Us

Editorial Office: jpvb@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/JPVB

Submit manuscript online http://ms.academicjournals.me/

Editors

Editorial Board

Journal of Parasitology and Vector Biology

Table of Content: Volume 8 Number 5 May 2016

ARTICLE

academicJournals

Vol. 8(5), pp. 47-54, May 2016
DOI: 10.5897/JPVB2015.0228
Article Number: 4420FBD58644
ISSN 2141-2510

http://www.academicjournals.org/JPVBB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Animal reservoirs of *Trypanosoma brucei gambiense* around the old Gboko sleeping sickness focus in Nigeria

Solomon Ngutor Karshima[1, 3]*, Idris A. Lawal[2], Shalangwa Ishaku Bata[3], Israel Joshua Barde[4], Pam Victoria Adamu[3], Abbas Salihu[3], Paman Nehemiah Dross[3] and Adebowale Obalisa[3]

[1]Department of Veterinary Public Health and Preventive Medicine, University of Jos, P. M. B 2084, Jos, Nigeria.
[2]Department of Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Animal Health, Federal College of Animal Health and Production Technology, P. M. B 001, Vom, Plateau State, Nigeria.
[4]Central Diagnostic Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Received 26 October 2015; Accepted 28 April 2016

The exact role of animal reservoirs in the epidemiology of *Trypanosoma brucei gambiense*, a parasite ravaging affected rural parts of sub-Saharan Africa, still remains unclear. However, the existence of the parasite in animals is well documented in other parts of Africa. A randomised cross sectional study was conducted in an old sleeping sickness focus in Nigeria by screening 600 cattle and 600 pigs using the card agglutination test for trypanosomosis (CATT) and identifying trypanosomes using ITS 1 and TgsGP PCRs. Data generated were analysed using the Chi square test and odds ratio at 95% confidence interval. The overall infection rates for the CATT and TgsGP-PCR were 8.9 and 0.9%, respectively which varied significantly between cattle (7.2%) and pigs (10.7%) with the CATT. The CATT based infection rates in relation to study sites, breeds and management practices varied significantly ($p < 0.05$) between 2.0 and 17.0%, while the PCR based ranged between 0 and 1.7%. Trypanosomes of animal origin identified by ITS 1 PCR were *T. brucei* (4.2%), *Trypanosoma congolense* forest (3.2%), *T. congolense* savannah (2.0%), *Trypanosoma vivax* (2.2%) and mixed infections (1.5%) in cattle as well as *T. brucei* (4.8%), *T. congolense* forest (1.8%), *T. congolense* savannah (1.0%) and mixed infections (1.2%) in pigs. *T. brucei gambiense* and other animal trypanosomes were identified among animals in the focus, indicating the existence of animal reservoirs of human infective *T. b. gambiense*. This suggests that the inclusion of reservoir control component in *T. b. gambiense* control programmes will help in the control of this parasite in this focus.

Key words: Animal reservoirs, *Trypanosoma brucei gambiense*, sleeping sickness focus, card agglutination test for trypanosomosis (CATT), ITS-PCR, TgsGP-PCR, Gboko, Nigeria.

INTRODUCTION

Trypanosoma brucei gambiense is the cause of Gambian sleeping sickness which is a chronic, debilitating,

*Corresponding author. E-mail: torkarshima@yahoo.co.uk, +2347031092062

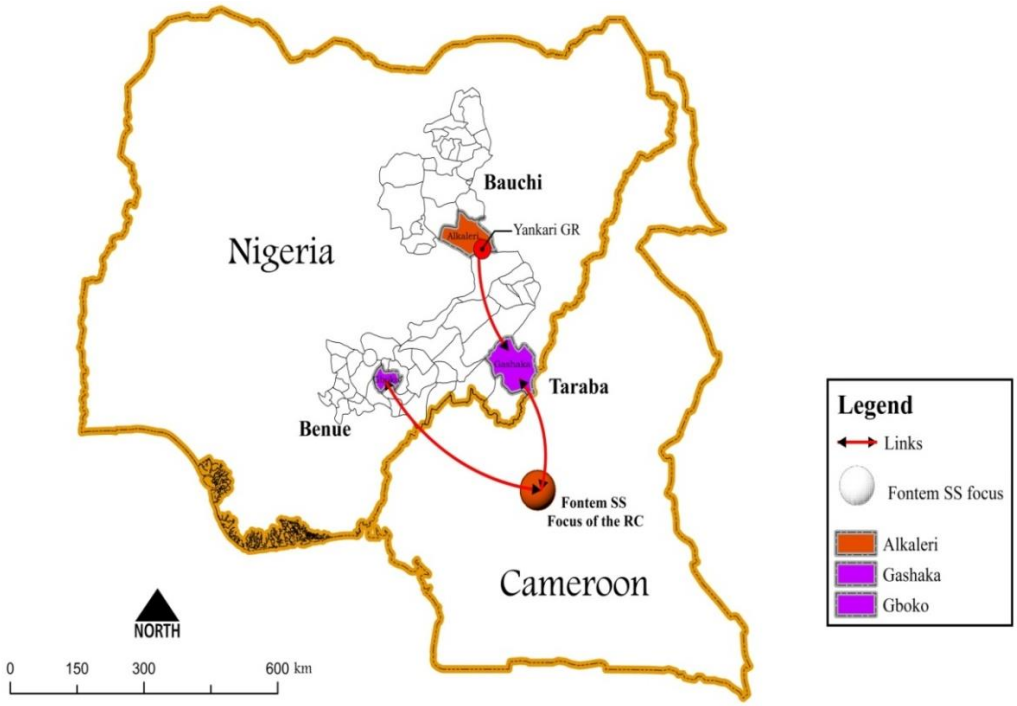


Figure 1. Links between the study sites and possible sources of *T. b. gambiense* infections. Game reserve (GR), Republic of Cameroon (RC), sleeping sickness (SS).

complex and fatal parasitic zoonosis ravaging affected rural parts of sub-Saharan Africa. The disease transmitted by tsetse flies is widespread, posing serious public health problems in the region and to visiting tourists (Conway-Klaaseen et al., 2002; Jelinek et al., 2002). Sleeping sickness is seriously neglected in the areas of drug development and sustainable control programmes.

Trypanosoma brucei gambiense and *T. b. rhodesiense* are both sub-species of *T. brucei* which are infective to man. In natural conditions, transmission of the parasite is cyclical through bites of infected tsetse flies including *Glossina palpalis, Glossina tachinoides* and *Glossina fuscipes*. These vectors are especially common at watering places like rivers or lakes where people frequently visit to collect water and do their washings, and animals visit to drink water (Service, 1980). The parasite is divided into two sub-types: type 1, causes a more chronic disease and represents over 90% of all cases of sleeping sickness (Cordon-Obras et al., 2010; Wombou et al., 2011), type 2, has not been isolated for ages, while *T. b. rhodesiense* is said to be associated with an acute like disease and represents the remaining 10% of the disease (Hide et al., 1990). Though, fewer than 10,000 cases are documented yearly from 2009 due to under reporting, the pathogen still ranks ninth of the 25 human infectious diseases in Africa based on its socio-economic impact (WHO, 2012).

Studies in animals revealed the involvement of numerous wildlife species such as primates, reptiles, antelopes and wild bovids (Mbaya et al., 2009; Njiokou et al., 2010), as well as domesticated animals such as pigs, goats, sheep and cattle (Nkinin et al., 2002; Simo et al., 2006) as suitable reservoirs for *T. brucei gambiense* and consequently their roles in the transmission of the disease.

In Nigeria, no attention has been given to the animal reservoirs of *T. b. gambiense*. However, human infection in the Gboko endemic area of Benue State was first reported in 1974, and since then no successful attempt has been made to control the disease in the affected region (Aiyedun and Amodu, 1974). This study conducted an active screening of *T. b. gambiense* to ascertain the possible role of animal reservoirs in the epidemiology of the parasite in the old Gboko sleeping sickness focus in Nigeria and also characterized isolates using TgsGP polymerase chain reaction.

MATERIALS AND METHODS

Study area

This study was carried out around the old Gboko sleeping sickness focus of Benue State which is located in the North-central zone of Nigeria between longitudes 7° 47′ and 10° 00′ East and latitudes 60 25′ and 8° 8′ North. It shares boundaries with five other states, namely: Nassarawa (north), Taraba (east), Cross River (south), Enugu (south-west) and Kogi (west) and with the Republic of Cameroon to the south-east (Figure 1). The major occupation in this region is agriculture particularly crop and livestock farming as well as fishing.

Study design

We conducted a randomised cross sectional study around the old Gboko sleeping sickness focus in Nigeria. Six Local Government Areas (LGAs) namely; Gashaka, Gboko, Ibi, Karim Lamido, Ukum and Vandikya were randomly selected around the focus using simple balloting. Three villages from each of the LGAs were randomly selected. Herds of animals were considered as clusters from which animals were sampled using stratified sampling technique. A total of fifteen cattle herds and fifteen piggeries were selected using simple balloting. From each selected herd, 30 or 50% of animals were sampled from herds with sizes of ≥50 or <50 animals, respectively. The selected animals were stratified based on breed, sex and age groups for the purpose of this study as follows: cattle ≤ 1year as young, > 1 year as adult; pigs ≤ 6 months as young, > 6 months as adult.

Blood sampling and the card agglutination test for *T. b. gambiense*

Two milliliters of blood was aseptically collected from cattle via the jugular vein using 5 ml syringe and 18 G needles and from pigs via the ear vein using 5 ml syringe and 21 G needles. These samples were immediately transferred into clean labelled sample bottles containing ethylene diamine tetra-acetic acid (EDTA) at 1 mg/ ml and mixed gently until the blood was properly mixed with the anticoagulant. Samples were then subjected to the card agglutination test for *T. b. gambiense* (CATT) within one hour of collection as described by Magnus et al. (1978). This test is based on the detection of *T. b. gambiense* specific Li Tat 1.3 antibodies using a purified *T. b. gambiense* variable surface antigen.

Isolation and purification of trypanosomes

The bloodstream form trypanosomes isolated from cattle and pigs were concentrated by centrifugation and separated from host blood using a DEAE 52 column (Whatman, Maidstone, Kent, UK) as described by Lanham and Godfrey (1970). These parasites were then stored at -20°C until needed for DNA extraction.

Extraction of DNA from purified trypanosomes

Trypanosome DNA extraction was done with GeneJET genomic DNA extraction kit (Thermo Scientific, Germany) using the method described by Oury et al. (1997). The extracted DNA was stored at -20°C until needed for PCR at the Biotechnology Laboratory of the Ahmadu Bello University, Zaria, Nigeria.

PCR detection of trypanosomes

Internal transcribed spacer 1 polymerase chain reaction (ITS 1-PCR) was used to identify *Trypanosoma* species using primer sets with forward sequence 5^{l}–CCGGAAGTTCACCGATATTG-3^{l} and reverse sequence 5^{l}-TTGCTGCGTTCTTCAACGAA-3^{l} (Inqaba Biotec, South Africa) targeting the variable length ITS 1 regions of trypanosome ribosomal DNA to identify trypanosomes species and sub-species with variable band sizes for different species and sub-species (Njiru et al., 2004). PCR was carried out in a final volume of 11 µl containing 5 µl phusion flash high fidelity PCR master mix (phusion flash II DNA polymerase, dNTPs, $MgCl_2$), 1 µl each of forward and reverse primers, 1 µl of extracted DNA and 3 µl of PCR grade water. PCR conditions were as follows: an initial denaturation step for 10 s at 98°C to activate the phusion flash II DNA polymerase, four cycles of amplification with 1 s denaturation at 98°C, 5 s hybridization at 58°C and 15 s elongation steps at 72°C; eight cycles of amplification with 1 s denaturation at 98°C, 5 s hybridization at 56°C and 15 s elongation steps at 72°C; 23 cycles of amplification with 1 s denaturation at 98°C, 5 s hybridization at 54°C and 15 s elongation steps at 72°C; and a final extension step of 5 min at 72°C as described by the manufacturer.

Detection of *Trypanosoma gambiense* specific glycoprotein (TgsGP)

The TgsGP primer set designed by Radwanska et al. (2002) with forward sequence 5^{l}-GCTGCTGTGTTCGGAGAGC-3^{1} and reverse sequence 5^{l}-GCCATCGTGCTTGCCGCTC-3^{1} (Inqaba Biotec, South Africa) was used to characterize the *T. brucei* isolates identified using the ITS-PCR as *T. b. gambiense* type 1 by amplifying the TgsGP gene yielding band sizes at 308 bp. Cycling conditions for TgsGP PCR were: denaturation at 98°C for 10 s to activate the phusion flash II DNA polymerase, followed by 40 cycles at 98°C for 1 s, annealing at 63°C for 30 s, 30 s elongation at 72°C and a final extension at 72°C for 5 min. All the amplified products were analyzed by electrophoresis in a 2% agarose gel with 100 bp molecular makers and UV illumination after ethidium bromide staining. All amplifications were carried out using a Gene Amp PCR system 9700 (Applied Biosystems, UK).

Data analysis

All data obtained during the study were analyzed using Graph-Pad Prism 4.0. Infection rates of *T. b. gambiense* were calculated by dividing the number of infected animals by the total number of animals examined and expressed as percentages. This was done for different variables such as animal species, study sites, sex, age, breeds and management practices. The Chi square (X^2) test and odds ratio were used where appropriate to compare the infection rates based on different variables and values of $p < 0.05$ were considered significant.

RESULTS

A total of 1200 animals including cattle (600) and pigs (600) were subjected to the CATT, ITS 1 and TgsGP-PCRs revealing overall infection rates of 8.9 and 0.9%, respectively. A significantly higher ($p = 0.03$) *T. b. gambiense* infection rate was observed in pigs (10.7%) than cattle (7.2%) using the CATT as shown in Table 1. However, very few sub-type 1 cases were detected using TgsGP-PCR and infection rate was statistically insignificant with this method (Table 1). CATT-based infection rate varied significantly ($p < 0.001$) between villages with the highest prevalence in Gboko (17.0%) and the lowest in Ibi (4.0%). The variations between the PCR-based infection rates across villages were statistically insignificant ranging between 0 and 2.0% (Table 2).

Age and sex based *T. b. gambiense* infection rates using the CATT showed no significant variations ranging between 7.0 and 10.2% (Table 3). PCR-based infection rates in relation to both age and sex ranged between 0.4 and 1.2% with no significant variation (Table 3). There were significant variations between the CATT-based infection rates recorded across cattle breeds ($p = 0.0023$),

Table 1. PCR and CATT based infection rates of *T. b. gambiense* in relation to animal species.

Animal species	Number examined	CATT positive (%)	TgsGP PCR positive (%)
Cattle	600	43 (7.2)	3 (0.5)
Pigs	600	64 (10.7)	8 (1.3)
Total	1200	107 (8.9)	11 (0.9)
X^2	-	4.525	2.294
P-value	-	0.0334	0.1299
Odds ratio	-	0.6465	0.3719

Table 2. PCR and CATT based infection rates of *T. b. gambiense* in relation to study sites.

Study sites	Number examined	CATT positive (%)	TgsGP PCR positive (%)
Gashaka	200	22 (11.0)	3 (1.5)
Gboko	200	34 (17.0)	4 (2.0)
Ibi	200	8 (4.0)	0 (0.0)
Karim Lamido	200	13 (6.5)	3 (1.5)
Ukum	200	9 (4.5)	0 (0.0)
Vandikyaa	200	21 (10.5)	1 (0.5)
Total	**1200**	**107 (8.9)**	**11 (0.9)**
X^2	-	29.97	8.166
P-value	-	<0.0001	0.1473

Table 3. PCR and CATT based infection rates of *T. b. gambiense* in relation to age and sex.

Variable	Number examined	CATT positive (%)	TgsGP PCR positive (%)
AGE			
Young	471	33 (7.0)	2 (0.4)
Adult	729	74 (10.2)	9 (1.2)
Total	**1200**	**107 (8.9)**	**11 (0.9)**
X^2		3.484	2.067
P-value		0.0620	0.1506
Odds ratio		0.6669	0.3412
SEX			
Female	668	65 (9.7)	7 (1.1)
Male	532	42 (7.9)	4 (0.8)
Total	**1200**	**107 (8.9)**	**11 (0.9)**
X^2		1.229	0.2857
P-value		0.2676	0.5930
Odds ratio		1.258	1.398

pig breeds (p = 0.003) and management practices (p = 0.0117) with prevalence rates ranging between 2.0 and 14.3% with the White Fulani cattle, indigenous pigs and extensively managed pigs revealing highest infection rates (Table 4). PCR-base infection rates in relation to breeds and management practices ranged between 0 and 1.7% with no statistical association (Table 4).

The prevalence of *T. brucei* species by ITS 1 (including *T. b. gambiense*) was 4.2% in cattle and 4.8% in pigs. Majority of *T. brucei* infections were in pigs (4.8%) while majority of the *T. congolense* infections were in cattle (5.2%).

Other species and sub-species identified by ITS 1-PCR were *T. vivax*, *T. congolense* forest and *T. congolense*

Table 4. PCR and CATT based infection rates of *T. b. gambiense* in relation to breeds and management practices.

Variable	Number examined	CATT positive (%)	TgsGP PCR positive (%)
BREED			
Cattle			
Bokoloji	200	9 (4.5)	1 (0.5)
Muturu	100	2 (2.0)	0 (0.0)
White Fulani	300	32 (10.7)	2 (0.7)
Total	**600**	**43 (7.2)**	**3 (0.5)**
X^2		12.14	1.283
P-value		0.0023	0.5266
Pigs			
Indigenous	300	43 (14.3)	5 (1.7)
Mixed breed	150	15 (10.0)	2 (1.3)
Exotic	150	6 (4.0)	1 (0.7)
Total	**600**	**64 (10.7)**	**8 (1.3)**
X^2		11.30	0.7601
P-value		0.0035	0.6838
Management			
Extensive	787	82 (10.4)	10 (1.3)
Intensive	413	25 (6.1)	1 (0.2)
Total	1200	107 (8.9)	11 (0.9)
X^2	-	6.357	3.155
P-value	-	0.0117	0.0757
Odds ratio	-	1.805	5.302

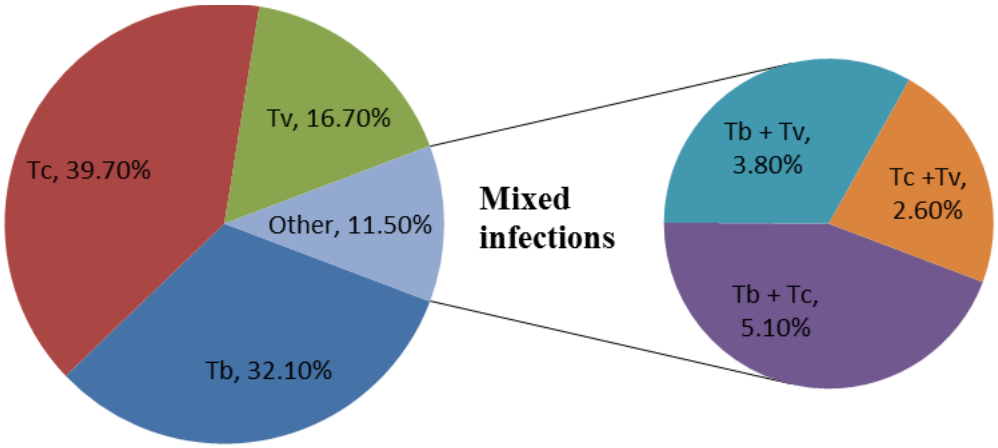


Figure 2. Distribution of *Trypanosoma* species of animal origin among the 78 positive cattle. [Tb (*T. brucei*), Tc (*T. congolense*), Tv (*T. vivax*), Tb + Tc (*T. brucei* and *T. congolense* mixed infections), Tb + Tv (*T. brucei* and *T. vivax* mixed infections), Tc + Tv (*T. congolense* and *T. vivax* mixed infections)].

savannah (Figures 2 and 3).

DISCUSSION

The zoonotic nature of *T. b. gambiense* had remained doubtful for over a century until recent reports on its detection from different animal hosts (Njiokou et al., 2010; Njitchouang et al., 2010). The present study revealed an overall animal CATT infection rate of 8.9% which is lower than earlier findings of 34.3% (Simo et al., 2006), 22.7% (Njiokou et al., 2010) and 64.0%

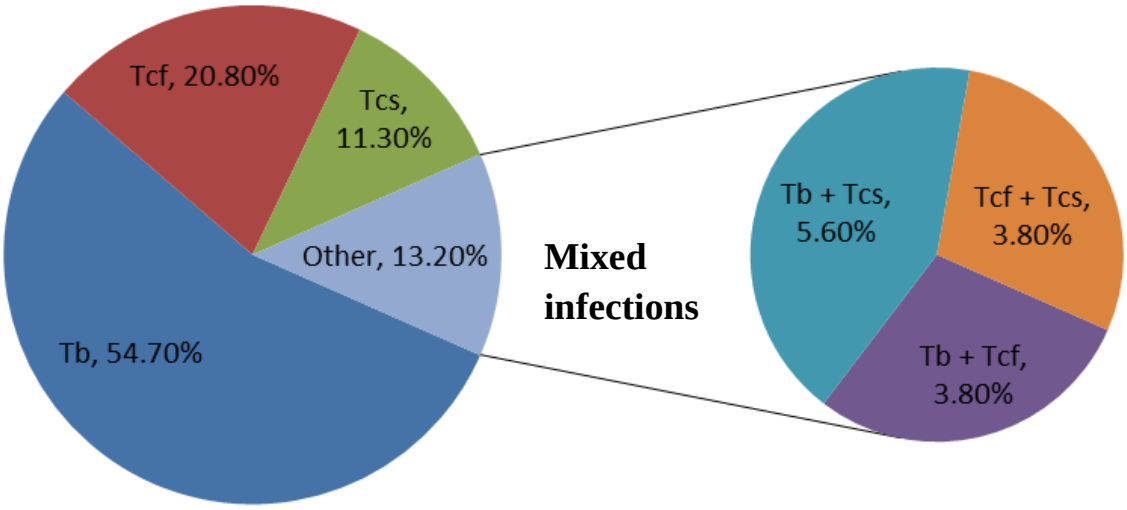


Figure 3. Distribution of *Trypanosoma* species of animal origin among the 53 positive pigs. [Tb (*T. brucei*), Tcf (*T. congolense forest*), Tcs (*T. congolense savannah*), Tb + Tcf (*T. brucei* and *T. congolense forest* mixed infections), Tb + Tcs (*T. brucei* and *T. congolense savannah* mixed infections), Tcf + Tcs (*T. congolense forest* and *T. congolense savannah* mixed infections)].

(Njitchouang et al., 2010) from different human African trypanosomosis (HAT) foci in the Republic of Cameroon using the same technique. A PCR-based overall infection rate (0.9%) lower than that of earlier findings: 3.08% (Njiokou et al., 2010), 9.82% (Njitchounang et al., 2010) and 14.8% (Simo et al., 2006) was also observed using the same PCR. The possible explanations for these variations may include differences in the sensitivity and specificity of the diagnostic tests used by the various studies, vector densities and the vectoral capacities of the *Glossina* species infesting the different study areas.

The variations between the prevalence rates recorded by the two diagnostic techniques used may not be unconnected with the high specificity of polymerase chain reaction, absence of *T. b. gambiense* DNA due to inactive infections and possibilities of false positive results arising from CATT screening (Magnus et al., 1978). It is important to mention that majority of samples that were positive for the CATT were infected with other trypanosomes like *T. vivax* and *T. congolense* which are also shown to cause false positivity (Magnus et al., 1978).

Pigs were earlier said to be capable of acting as reservoirs of *T. b. gambiense* in Benue State (Stephen, 1986). The present finding may therefore be a confirmation to this speculation. The infection rate observed in pigs in both Ukum and Vandikya may not be unconnected with the free movement of livestock within the State. Local and International trade (particularly with the neighbouring Cameroon Republic) of pigs which is a delicacy in the state may be an additional factor. The role of trade pigs in the spread of human serum resistant *Trypanosoma* species was also reported earlier (Onah and Ebenebe, 2003).

It was not unexpected to have observed the highest infection rate in Gboko LGA probably due to the old HAT focus in the area and the negligence in the area of disease control (Aiyedun and Amodu, 1974). The high density of tsetse flies and wildlife species in the Gashaka-Gumti game reserve may be a possible explanation for the higher prevalence recorded in Gashaka LGA. Adult animals are known to release higher concentrations of insect attractants such as phenols in their urine (Kremar et al., 2006) as well as acetone and octenol in their breath (Kremar, 2007). These substances attract tsetse flies to the animals resulting in more frequent bites and subsequent infection. Tsetse flies are also known to be more attracted to larger animals than smaller ones (Torr et al., 2007). These may be possible reasons for the slight difference in the prevalence between adult and young animals.

Though, it was statistically insignificant, the infection rate in relation to sex was higher in females than males in accordance with earlier reports by Shah et al. (2004) in the Republic of Cameroon and Karshima et al. (2012) in Taraba State, Nigeria. This may be attributed to stress associated with hormonal imbalances during pregnancy and lactation. Also, because of the chronic nature of *T. b. gambiense* infection, the chances of detection in females might be higher because they are kept longer in herds for the purpose of breeding, thus allowing the chronic infection to manifest.

The Bokoloji and Muturu cattle breeds are known to be trypano-tolerant (Murray et al., 1983). This resistance to *Trypanosoma* species may explain the lower prevalence recorded in these two breeds. The higher prevalence observed among the White Fulani may be attributable to their trypanosusceptibility and perhaps due to their higher

representation in the sampling. Of the three cattle breeds studied, the White Fulani are usually raised using the nomadic system of management. This may be another possible explanation for the higher prevalence recorded by this group.

Extensively managed animals usually visit swampy areas and streams in search of drinking water thereby being exposed to the risk of bites by riverine species of tsetse flies. This may be a probable explanation for the higher prevalence observed among extensively managed animals. However, teneral tsetse flies that breed and live around human settlements may be responsible for the low prevalence observed among intensively managed animals.

Though this study targeted animal reservoirs of *T. b. gambiense*, the authors also considered the distribution of *Trypanosoma* species of animal origin in the focus to estimate possible risk of atypical *Trypanosoma* infections in human as reported elsewhere (Truc et al., 2007; 2013; Deborggraeve et al., 2008). The species of trypanosomes identified in this study were earlier reported by other workers (Yanan et al., 2007; Fasanmi et al., 2014) indicating that they are endemic in Nigeria. Mixed infections with similar *Trypanosoma* species as those reported by this study have also been documented in Nigeria (Karshima and Bobbo, 2011; Majekodunmi et al., 2013). These mixed infections might be due to bites from tsetse flies carrying more than one *Trypanosoma* infections or successive bites from different tsetse flies infected with different *Trypanosoma* species.

One of the major objectives of this study was to characterize *T. brucei* isolated from serologically positive cattle and pigs. This was necessary because of the roles of domestic and wild animals in the epidemiology of *T. b. gambiense* as earlier reported (Abenga and Lawal, 2005). It has been established that the TgsGP gene is present in all group 1 *T. b. gambiense* isolates examined to date but not in *T. b. brucei*, *T. b. rhodesiense* or group 2 *T. b. gambiense* (Capewell et al., 2013). This study confirmed only group 1 *T. b. gambiense* isolates in cattle and pigs in the study area, which was not surprising since it has been shown to be associated with over 90% of all sleeping sickness cases with the remaining assigned to *T. b. rhodesiense* since type 2 has not been isolated for ages (Cordon-Obras et al., 2010; Wombou et al., 2011).

The clinical significance of detecting only one species of human infective trypanosome in the study area is that, the risk of treatment failure associated with mixed infections of *T. b. rhodesiense*, groups 1 and 2 *T. b. gambiense* which responds differently to sleeping sickness chemotherapy (Brun et al., 2001) will be overcome.

The study identified cattle and pigs as domesticated animal reservoirs of group 1 *T. b. gambiense* in this region. The epidemiological significance of this finding is that at least cattle and swine populations may play a role in the maintenance and possible resurgence of the disease in this region.

Conclusion

The study identified cattle and pigs as domesticated animal reservoirs of group 1 *T. b. gambiense* in this region. Other trypanosomes of animal origin identified were *T. brucei*, *T. vivax*, *T. congolense forest* and *T. congolense savannah.* The implication of this finding is that, these reservoir animals might be traded for breeding purposes thereby initiating and spreading new sleeping sickness foci in non-infected areas. It is therefore very pertinent to include reservoir control component in *T. b. gambiense* control programmes to curtail the maintenance and resurgence of this parasite by animal reservoirs.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to the Director of the Biotechnology Centre, Ahmadu Bello University Zaria, Nigeria, Prof. Kabir Junaidu and all other staff of the centre for allowing them use of their facilities.

REFERENCES

Abenga JN, Lawal AI (2005). Implicating roles of animal reservoir hosts in the resurgence of Gambian trypanosomosis (Sleeping Sickness). Afr. J. Biotechnol. 4(2):134-137.

Aiyedun BA, Amodu AA (1974). Human sleeping sickness in the Gboko endemic areas in Nigeria. Acta Trop. 33(1):88-95.

Brun R, Schumacher R, Schmid C, Kunz C, Burri C (2001). The phenomenon of treatment failures in human African typanosomiasis. Trop. Med. Int. Health 6:906-914.

Capewell P, Cooper A, Duffy CW, Tait A, Turner CM (2013). Human and animal trypanosomes in Coˆte d'Ivoire from a single breeding population. *PLoS ONE* doi:10.1371/journal.pone.0067852.

Conway-Klaaseen JM, Wyrick-Glatzel JM, Neyrinck N, Belair PA (2002). Sleeping Sicknees in young American tourist. Lab. Med. 33:783-788.

Cordon-Obras C, Garcıa-Estebanez C, Ndong-Mabale N, Abaga S, Ndongo-Asumu P (2010). Screening of *Trypanosoma brucei gambiense* in domestic livestock and tsetse flies from an insular endemic focus (Luba, Equatorial Guinea). PLoS Neg. Trop. Dis. 4(6):e704.

Deborggraeve S, Koffi M, Jamonneau V, Bonsu FA, Queyson R (2008). Molecular analysis of archived blood slides reveals an atypical human *Trypanosoma* infection. Diagn. Microbiol. Infect. Dis. 61:428-433.

Fasanmi OG, Okoroafor UP, Nwufoh, OC, Bukola-Oladele OM, Ajibola ES (2014). Survey for *Trypanosoma* species in cattle from three farms in Iddo Local Government Area, Oyo State. Sok. J. Vet. Sci. 12(1):57-61.

Hide G, Cattand P, Leray D, Barry JD, Tait A (1990). The identification of *Tryponasoma brucei* subspecies using repetitive DNA sequence. Mol. Biochem. Parasitol. 39:213-226.

Jelinek T, Bisoffi BL, Van-Theil P, Bronne U, Defery A, Gunderson SG, McWhinney P, Ripamonti D (2002). Cluster of African trypanosomiasis in travellers to Tanzania national park. Emerg. Infect. Dis. 8:634-635.

Karshima NS, Ajogi I, Lawal AI, Mohammed G, Okubanjo OO (2012). Detection of *Trypanosoma brucei gambiense* specific Li Tat 1.3 antibodies in humans and cattle in Taraba State, North-Eastern Nigeria. J. Vet. Adv. 2(12):580-585.

Karshima SN, Bobbo AA (2011). Prevalence of bovine trypanosomosis in Taraba State, North-Eastern Nigeria. Trop. Vet. 29(4):44-50.

Kremar S (2007). Responses of tabanidae (Diptera) to canopy traps baited with 4-methyl phenol, 3-isopropylphenol and naphthalene. J. Vect. Ecol. 32:188-192.

Kremar S, Mikuska A, Merdic C (2006). Responses of tabanidae (Diptera) to different natural attractants. J. Vect. Eco. 31:262-265.

Lanham SM, Godfrey DG (1970). Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Exp. Parasitol. 28:521-534.

Magnus E, Vervoot T, Van-Meirvenne N (1978). A card-agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T. b. gambiense* trypanosomiasis. Ann. Soc. Belg. Med. Trop. 58:169-176.

Majekodunmi AO, Fajinmi A, Dongkum C, Picozzi K, Thrusfield MV, Welburn SC (2013). A longitudinal survey of African animal trypanosomiasis in domestic cattle on the Jos Plateau, Nigeria: prevalence, distribution and risk factors. Parasitol. Vect. 6:239.

Mbaya AW, Aliyu MM, Nwosu CO, Ibrahim U (2009). Effect of DL-α-Difluoromethylornithine on biochemical changes in Baboons (*Papio anubis*) experimentally infected with *Trypanosoma brucei gambiense*. Nig. Vet. J. 31(1):34-44.

Murray M, Trail JCM, Turner DA, Wissocq Y (1983). Livestock productivity and trypanotolerance. Network Training Manual. ILCA, Addis Abba. pp. 45-75.

Njiokou F, Nimpaye H, Simo G, Njitchouang GR, Asonganyi T, Cuny G, Herder S (2010). Domestic animals as potential reservoir hosts of *Trypanosoma brucei gambiense* in Sleeping Sickness foci in Cameroon. Parasite 17:61-66.

Njiru ZK, Constantine CC, Guya S, Crowther J, Kiragu JM, Thompson RCA, Davila AMR (2005). The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. Parasitol. Res. 95:186-192.

Njitchouang GR, Njiokou F, Djeunga HCN, Fewou PM, Asonganyi GC, Simo G (2010). Analysis of the domestic animal reservoir at a micro-geographical scale, the Fontem Sleeping Sickness focus (South-West Cameroon). J. Cell Anim. Biol. 4(5):73-80.

Nkinin SW, Njiokou F, Penchenier L, Grebaut P, Simo G, Herder S (2002). Characterization of *T. brucei* sl by isoenzymes in domestic pigs from the Fontem Sleeping Sickness focus of Cameroon. Acta Trop. 81:225-232.

Onah DN, Ebenebe OO (2003). Isolation of human serum resistant *Trypanosoma brucei* from naturally infected pigs in NsuKka area of Enugu. Nig. Vet. J. 24(1):37-43.

Oury B, Dutrait N, Bastrenta B, Tibayrene M (1997). *Trypanosoma cruzi*: use of a synapomorphic RAPD fragment as a species-specific DNA probe. J. Parasitol. 83:52-57.

Radwanska M, Claes F, Magez S, Magnus E, Perez-morga D, Pays E, Büscher P (2002). Novel primer sequences for Polymerase Chain Reaction–based detection of *Trypanosoma brucei gambiense*. Am. J. Trop. Med. Hyg. 67:289-295.

Service MW (1980). A guide to medical entomology, third edition, published by Blackwell scientific publishers. pp. 95-101.

Shah SR, Phulan MS, Memon MA, Rind R, Bhatti A (2004). Trypanosome infections in camels. Pak. Vet. J. 24(4):209-210.

Simo G, Asonganyi T, Nkinin SW, Njiokou F, Herder S (2006). High prevalence of *Trypanosoma brucei gambiense* group 1 in pigs from the Fontem Sleeping Sickness focus in Cameroon. Vet. Parasitol. 139(3):57-66.

Stephen LE (1986). *Trypanosomiasis:.* Pergamon, Oxford. Vet. Perspect. pp. 480-487.

Torr SJ, Prior A, Wilson AJ, Schofield S (2007). Is there safety in numbers? The effect of cattle herding on biting risk from tsetse flies. Med. Vet. Entomol. 21:301-311.

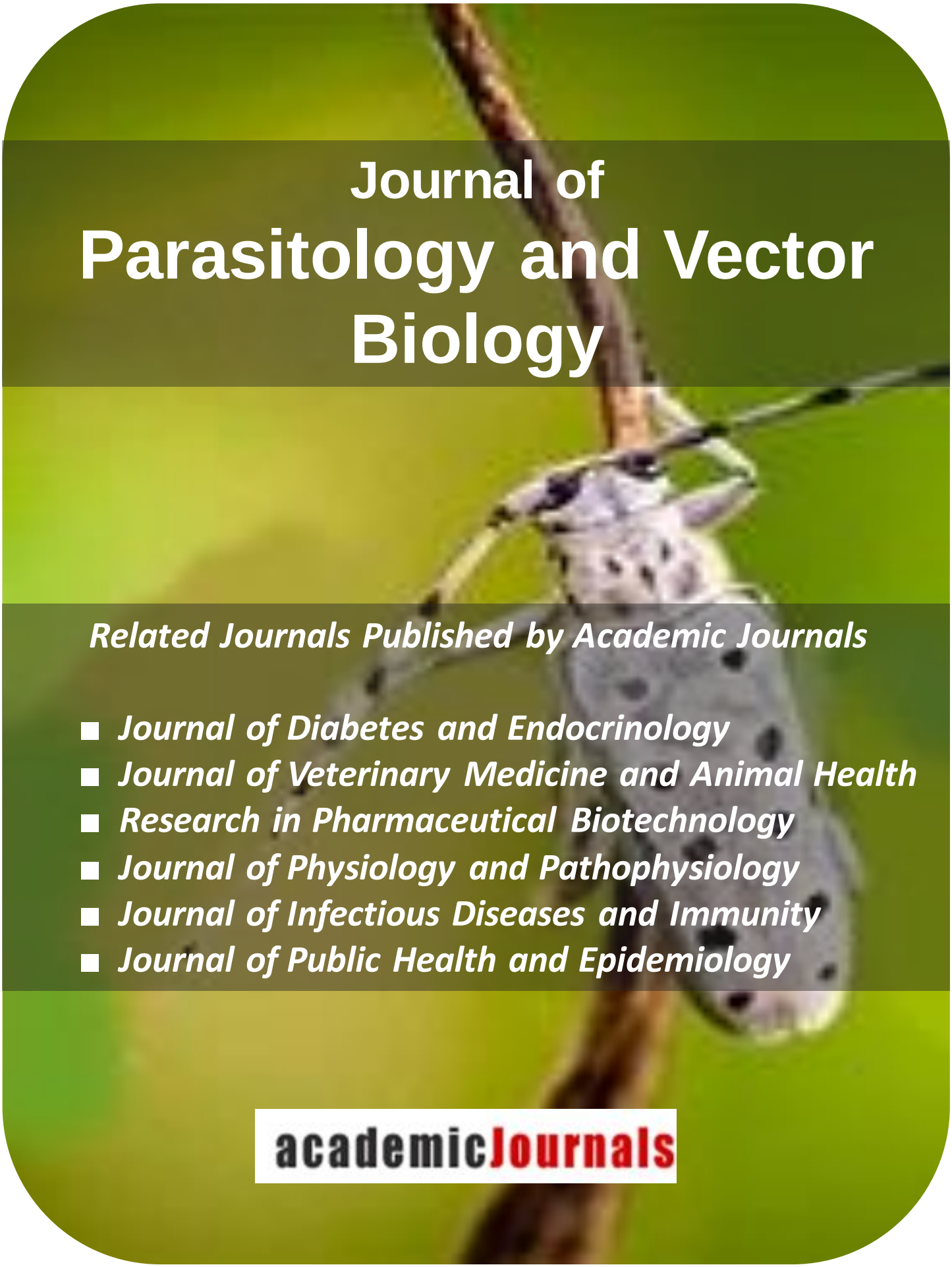
Truc P, Buscher P, Desquenes M (2013). Atypical human infections by animal trypanosomes. PLoS Negl. Trop. Dis. 7(9):e2258.

Truc P, Gibson W, Herder S (2007). Genetic characterization of *Trypanosoma evansi* isolated from a patient in India. Infect. Genet. Evol. 7:305-307.

Wombou TCM, Solano P, Bengaly Z, Jamonneau V, Bucheton B (2011). Experimental evaluation of xenodiagnosis to detect trypanosomes at low parasitaemia levels in infected hosts. Parasite 18:295-302.

World Health Organization (WHO) (2012). Report of a WHO Meeting on Elimination of African Trypanosomiasis (*Trypanosoma brucei gambiense*) Geneva, 3-5 December 2012. Geneva, Switzerland: World Health Organization.

Yanan EG, Evuti MA, Shamaki BU, Sugun MY, Dongkun C, Ogunsan EA (2007). Epidemiology of small ruminant trypanosomosis in some communities in Jos East, Plateau State: A guinea Savana Zone, North Central Nigeria. J. Protozool. Res. 17:44-47.



Journal of Parasitology and Vector Biology

Related Journals Published by Academic Journals

- *Journal of Diabetes and Endocrinology*
- *Journal of Veterinary Medicine and Animal Health*
- *Research in Pharmaceutical Biotechnology*
- *Journal of Physiology and Pathophysiology*
- *Journal of Infectious Diseases and Immunity*
- *Journal of Public Health and Epidemiology*

academicJournals