J Vector Borne Dis 45, December 2008, pp. 292–300

Immunodiagnosis of bovine trypanosomiasis in Anambra and Imo states, Nigeria, using enzyme-linked immunosorbent assay: zoonotic implications to human health

M.C. Ezeani[a], H. Okoro[b], V.O. Anosa[c], C.C. Onyenekwe[d], S.C. Meludu[e], C.E. Dioka[f] & C.C. Azikiwe[g]

[a]*Department of Immunology, College of Health Sciences,* [b,c]*Department of Veterinary Pathology, University of Ibadan, Oyo State;* [d,e,f]*Department of Chemical Pathology,* [g]*Department of Pharmacology, Faculty of Medicine, Madona University, Elele Campus;* [a,d,e,f] *Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

Abstract

Background & objectives: The prevalence of trypanosomiasis was studied in cattle, being a major source of animal protein in Nigeria, thus, a very likely means of spread of Human African Trypanosomosis (HAT).

Methods: Enzyme-linked immunosorbent assay (ELISA) was used to diagnose bovine trypanosomiasis in 264 samples collected from adult cattle of mixed breeds, age and sex, in Anambra and Imo states, Nigeria.

Results: Out of 264 samples analysed, 21 (7.96%) were seropositive for *Trypanosoma congolense* while 20 (7.58%) were seropositive for *T. vivax* and 8 (3.03%) were seropositive for *T. brucei* infections in both the states.

Interpretation & conclusion: The predominant species was found to be *T. congolense*. Mixed infection of three species, *T. vivax, T. congolense* and *T. brucei* was found to dominate other mixed infections in both the states. ELISA detected the infection of the three species of trypanosomes in the same group of animals. The usefulness of antigen capture ELISA in the diagnosis of human or animal trypanosomiasis was established, and the possibility of the spread of HAT caused by *T. brucei gambiense* and *T.b. rhodesiense* through cattle was expressed.

Key words Bovine – diagnosis – human – implications – trypanosomiasis – zoonosis

Introduction

African animal trypanosomiasis caused by *Trypanosoma brucei, T. congolense* and *T. vivax* remains one of the major constraints to health and productivity of cattle and other domestic animals in the tsetse infested areas of tropical Africa owing to its varied clinical manifestations[1].

In Nigeria, it has continued to be a menace in the livestock industry despite the age-long attempts to control the disease[2]. In the same vein, Human African Trypanosomosis (HAT) arising from *T. brucei gambiense* and *T.b. rhodesiense*, constitute a major problem arising from the controversial role of animal reservoir hosts. Although campaigns against HAT in the 1960s brought the disease below epidemic pro-

portions, there is presently a dramatic resurgence in both forms of HAT which has been blamed on wide spread of civil disturbances and wars, declining economies, reduced health financing, dismantling of disease control programmes and animal reservoir hosts[3].

The HAT, sleeping sickness is a complex and debilitating disease of man. The disease still ravages in several parts of sub-Saharan Africa despite decades of efforts aimed at control[4]. The HAT poses as an emerging public health crisis in several countries including Nigeria[5], and a major health risk to tourists coming to tropical Africa[1]. Human animal trypanosomosis is caused by the parasitic protozoa of the Genus *Trypanosoma*[4]. World Health Organization (WHO), had commented on the complex nature of the epidemiology of the human disease arising from two species of the infecting trypanosome in various parts of Africa[4–6].

The fact that many animals had *T. brucei* infection might be of zoonotic importance, since domestic animals are known reservoirs of *T.b. gambiense* in west Africa[7]. A recent sero-prevalence study employing the standard direct sandwich technique of ELISA showed that of 320 sheep and goats, blood samples assayed in Ondo and Ekiti states, Nigeria, 47.8, 46.6 and 47.18% have had previous contact with *T. brucei, T. congolense* and *T. vivax* respectively[8]. Trypanosomiasis seems to be remerging as an important livestock disease in Nigeria, assuming major clinical importance in small ruminants and extending to previously designated tsetse free zones[9]. The reasons for this need are to be investigated in other to avoid animal human transmission.

Material & Methods

A total of 264 adult cattle of mixed breeds and sexes (male 220, female 44) from cattle markets in Awka and Orlu in Anambra and Imo states respectively were used for this study.

Serum sample collection: Appropriately labelled plain 10 ml vacutainer tubes with monoject needles were used to collect 5–8 ml of blood from the cattle via the jugular veins. The tubes with blood were kept in ice-packs in slanting position and transported to the laboratory, where the tubes were stored at 4°C overnight to allow for adequate retraction and clotting. The samples were centrifuged at 3000 rpm for 10 min and sera dispensed in duplicate into appropriately labelled sample tubes and stored at –20°C until used analysed.

Generation of monoclonal antibodies: This was carried out as per the procedure described by Milstein and Kõhler[10], with little modifications. Succinctly, monoclonal antibodies were raised by immunising Balb/c mice with the *T. congolense, T. vivax* and *T. brucei* antigens lysate in Freud's adjuvant. The spleens were removed from the mice and the individual cells fused with constantly dividing (immortal) B-tumour cells in polyethylene glycol (PEG). The B-tumour cells were selected for a purine enzyme deficiency and often for their inability to secrete immunoglobulin[11]. The resulting cells were distributed into micro-well plates in hypoxanthine aminopterin thymidine medium which killed off the fusion partners, in that each well was left with one hybridoma cell. The immunoglobulins were prepared from the ascetic fluid purified and tested by ELISA against different trypanosome species-specific antigens, *Anaplasma marginale* antigen, *Babesia bijemina* antigen *Theeileria parva* antigen, to establish their specificity.

Generation of conjugate: Briefly, 10 mg horseradish peroxidase (HRP) type VI was dissolved in 0.2 ml of 1.25% glutaraldehyde in phosphate buffered saline (PBS) (pH 7.2) and left at room temperature overnight. About 1.0 ml of 0.1 M carbonate buffer (pH 9.2) was added and dialysed changing twice in 4 h. To this 5 mg of purified monoclonal antibodies raised against different bovine trypanosome species were added in 0.25 carbonate buffer and incubated at room

temperature overnight and 0.1 ml of 0.2M lysine was added followed by the addition of equal volume of glycerol and kept at –20°C. A chequer board titration was used to determine the dilution of the conjugates.

Sandwich antigen capture ELISA: The monoclonal antibodies raised against *T. brucei, T. congolense* and *T. vivax*, separately were used at a dilution of 1 : 500 in coating buffer to coat each well of the flat bottom micro ELISA plates (Dynateck, Virginia, U.S.A.), and kept at 4°C overnight. The monoclonal antibody IgM raised specifically against *T. brucei* and IgG raised specifically against *T. congolense* and *T. vivax* were used[11]. The plates were flipped empty and rinsed once with washing buffer and the excess buffer drained off the plates by gentle tapping of the inverted plate on a thick towel and 100 µl of diluent buffer (washing buffer) was added in each well, then sera to be tested were added in duplicate in the wells using micro-pipettes with tips as follows 5 µl in *T. brucei* plates and 10 µl in the *T. vivax* and *T. congolense* plates. The wells in row A column 1 and 2 and wells in row B column 1 and 2 contain no sera and were left as reagent controls, while wells in row G column 11 and 12 contain positive controls and row H column 11 and 12 contain negative controls. The plates were incubated at 37°C for 15 min, then flipped empty and rinsed once with washing buffer. About 100 µl of antis species monoclonal antibodies (IgM for *T. brucei* and IgG for *T. congolense* and *T. vivax*), separately raised against the species and conjugated to horseradish peroxidase and diluted at 1: 1000 in conjugate diluting buffer (phosphate buffer, 0.5% tween 80, 1% BSA) were added in each case. The plates were incubated at 37°C for 15 min, flipped empty and rinsed once. The plates were then filled up with washing buffer and soaked for 10 min and this was repeated twice. Then the substrate and chromogen solutions were added (100 µl/well). The chromogen and substrate used consisted of 300 µl of hydrogen peroxide (H_2O_2) in 30 ml of substrate buffer and 300 µl of 22-azinobis (3-ethyl) benzthiaozoline 6-sulfonic acid (A.B.T.S) in same 30 ml of substrate buffer. The plates were incubated at 37°C in dark and the change in colour was observed and absorbance was read after 30 min at 405 nm wave length using a micro ELISA reader SFC 400 (Biotek Instruments, Inc, Vermount, U.S.A.). The wells with colour change were recorded. The quantitative value of absorbance of the colour intensity was extrapolated from the standard plot, indicating the quantity of antigen in the test serum.

Results

All 264 different serum samples were collected from selected local governments in Imo and Anambra states. The animals were adults and of mixed breeds and sexes and were transported to the states from northern parts of Nigeria. From each state, 132 samples were collected. Out of the 264 a total of 110 (41.66%) different serum samples were positive for *T. congolense* while 108 (40.91%) were positive for *T. vivax* and 86 (32.58%) were positive for *T. brucei* in both the states.

Out of 110 (41.66%) sera positive samples for *T. congolense*, 48 (18.18%) were positive in Anambra state whereas 62 (23.48%) were positive samples in Imo state. Out of 108 (40.91%) positive for *T. vivax* in both the states, 50 (18.94%) were detected in Anambra and 58 (21.97%) in Imo state. Out of the 86 (32.58%) sera samples positive for *T. brucei* in both the states, 33 (12.5%) were detected in Anambra and 53 (20.08%) were detected in Imo state (Table 1).

Single infections were detected for these three species in both the states as 21 (7.95%) for *T. congolense*, 20 (7.58%) for *T. vivax* and 8 (3.03%) for *T. brucei*. In Anambra state alone, single infections were 11 (4.17%) for *T. congolense*, 10 (3.79%) for *T. vivax* and 5 (1.89%) for *T. brucei*. In Imo state, single infections were 10 each for *T. congolense* and *T. vivax* and 3 (1.14%) for *T. brucei* (Table 2).

Mixed infections were also detected. This was ob-

Table 1. Prevalence of trypanosome species in sera of cattle in Anambra and Imo states

Species	Anambra	Imo	Total
No. of samples	132	132	264
T. congolense	48 (18.18)	62 (23.48)	110 (41.66)
T. vivax	50 (18.94)	58 (21.97)	108 (40.91)
T. brucei	33 (12.5)	53 (20.08)	86 (32.58)

Figures in parentheses indicate percentage.

served during the plate reading. Since the three organisms were tested for in each serum sample, in separate plates, a serum appearing positive in the three plates is an indication of mixed infection of the three organisms. Positive in only two plates indicates mixed infection of the two organisms while positive in only one plate indicates single infection. The most predominant, involved three species—*T. congolense*, *T. vivax* and *T. brucei* and this occurred in 35 (13.26%) animals in Imo and 18 (6.81%) animals in Anambra, with a total of 53 (20.08%) in the two states. This is followed by the mixture of *T. vivax* and *T. congolense* which was detected in 14 (5.30%) animals in Anambra state and 6 (2.27%) in Imo state with a total of 20 (7.58%) in both the states. Mixed infections of *T. vivax* and *T. brucei* were detected in 8 (3.03%) cattles in Anambra and 5 (1.89%) cattles in Imo states making a total of 13 (4.92%) in two states. *T. congolense* and *T. brucei* mixed infections were also detected in 3 (1.14%) animals in Anambra and 10 (3.79%) animals in Imo states making a total of 13 (4.92%) in both the states (Table 2).

The absorbance values which determine the quantitative value of each trypanosome antigen used in each state were shown in Table 3. The readings that fall between 0.05 and 0.1 showed a very high quantity of antigen, hence depicting an active infection. *T. vivax* is found to have the highest number of high absorbance value with 14, followed by *T. congolense* and *T. brucei* having four each (Table 3).

Discussion

Indications from the results show that mixed infections caused by *T. congolense*, *T. vivax* and *T. brucei*

Table 2. Prevalence of single and mixed infections of trypanosomes in sera of cattle in Anambra and Imo states, Nigeria

Species	Anambra	Imo	Total
No. of samples	132	132	264
T. congolense	11 (4.17)	10 (3.79)	21 (7.95)
T. vivax	10 (3.79)	10 (3.79)	20 (7.58)
T. brucei	5 (1.89)	3 (1.14)	8 (3.03)
T. vivax/T. brucei/T. congolense	18 (6.82)	35 (13.26)	53 (20.08)
T. vivax/T. congolense	14 (5.30)	6 (2.27)	20 (7.58)
T. vivax/T. brucei	8 (3.03)	5 (1.89)	13 (4.92)
T. congolense/T. brucei	3 (1.14)	10 (3.79)	13 (4.92)

Figures in parentheses indicate percentage.

Table 3. Optical density range, showing prevalence of trypanosome antigen quantity among the species used in this study

O.D. range	*T. congolense*		*T. vivax*		*T. brucei*	
	Anambra	Imo	Anambra	Imo	Anambra	Imo
001–002	20	26	31	24	29	28
003–004	3	10	8	5	4	4
005–006	0	1	0	5	0	3
007–008	0	0	2	2	0	1
009–0.1	0	3	2	3	0	0
0.05–0.1	0	4	4	10	0	4

in Anambra and Imo states, were the prevalent infections in the two states. The findings depict a contrary view to that found by Dipeolu[12], that the prevalence of mixed infection caused by *T. congolense* and *T. vivax* is the most significant. It also differs from the observations of Akinboade[13] who reported that a mixed infection due to *T. congolense* and *T. brucei* was most prevalent in cattle. However, the contrary observations could be attributed to differences in diagnostic methods used. Dipeolu[12] worked on "survey of blood parasites in domestic animals" using parasitological methods. The sensitivity of these methods especially in low parasitaemic conditions is unreliable compared to the reliable and highly sensitive ELISA technique used in this study. Supportably Losos and Ikede[14] reported a mixed infection of *T. congolense*, *T. vivax* and *T. brucei* in one cattle using DNA hybridization technique[14]. This technique is also sensitive on its own although hybridisation signals failed to be obtained when samples that are to be used were kept at 4°C for up to four days where by the DNA gets degraded because of microcondensation.

The failure to detect mixed infection of the three species by the parasitological method (which is based on the mortality and the morphology of the trypanosomes), is not unusual because of the fluctuating parasitaemic behaviour of blood stream trypanosomes (*T. vivax* and *T. congolense*). This means that the trypanosome species with highest proportion are likely to be diagnosed and infection attributed to these parasites only, where as the species which are low in numbers might not be identified microscopically.

The detection of mixed infection comprising *T. congolense*, *T. vivax* and *T. brucei* in the same group of animals and the high prevalence rate of trypanosomes in this study in spite of the increased use of trypanocides have highlighted the following—the stable occurrence of drug resistant strains of trypanosomes; the utmost need for more effort towards the control of the disease; and the distribution of animal reservoir and insect vectors. All these factors act either individually or in concert to maintain the infection in domestic animals and as reservoir for HAT.

In this study, the prevalent species among the three species of trypanosomes is *T. congolense* in both the states though the difference is not significant from *T. vivax* infection in between the states. In Anambra alone, the prevalent specie is *T. congolense* while equity is the case in Imo between *T. congolense* and *T. vivax*. One can significantly say that infections due to *T. congolense* and *T. vivax* are the main constraint on

livestock development in the two states and in Nigeria as a whole considering the fact that preponderant majority of the livestocks raised for consumption in Nigeria are raised in the northern part of the country and transported to the southern part. However, in Africa, *T. congolense* infection usually manifests as a chronic form of the disease and milder than that caused by *T. vivax*[15]. The importance of this work should not be over emphasised considering lack of current research on trypanosomiasis in Nigeria. Some of the factors that affect the prevalence of trypanosomiasis in Nigeria include animal breed, type of management, season of the year and the type of vegetation[16]. It is also known that nomadism tends to expose animals to high tsetse challenge and hence trypanosome infection.

In Nigeria, cattle are considered as one of the principal livestock, and their survival and development are necessary to ameliorate the worsening situation regarding the supply of animal protein. Cattle production in the country has been restricted to the northern part because of the erroneous belief that the southern part (forest zone, including Anambra and Imo states) was highly infected by tsetse flies which transmit trypanosomiasis[17]. Afterwards the cattles are transported to the south for marketing.

Consequently, the prevalence of these parasites in cattle and other domestic animals gives room for concern, as indications have occurred that *T.b. gambiense* and *T.b. rhodesiense*, to which man is the natural host, have been detected in several animal reservoirs, using molecular techniques. HAT described as Rhodesian sleeping sickness is caused by *T.b. rhodesiense,* resulting to acute disease course leading to death of infected persons within few weeks or months[4]. In west and central Africa, HAT is caused by *T.b. gambiense* and is transmitted principally by tsetse flies, *Glossina palpalis* and *G. tachinoides* resulting to a devastating, chronic form of the disease described as Gambian sleeping sickness (Gambian trypanosomosis).

For many years, pigs have been identified as animal reservoir hosts for *T.b. gambiense* and recently has been associated with the persistence and epidemics of sleeping sickness in Uganda[18], Equatorial Guinea and Cameroon[19]. It has also been associated with maintenance of old sleeping sickness endemic foci in Nigeria[20]. Use of standard procedures such as, resistance to human serum based on the blood incubation infectivity test[21] and molecular characterisation of *T. brucei* stocks from man and animals by isoenzyme electrophoresis[22] and polymerase chain reaction based methods have led to the identification of more mammalian hosts as reservoirs for *T.b. gambiense*. Such hosts include dogs, sheep, cattle and a range of game animals. An estimated 60 million people are believed to be at risk while about 300,000 new cases are reported each year. Higher number of cases are likely to occur in several countries in view of the current upsurge in both forms of sleeping sickness[23]. Going by WHO statistics of 1998, Gambian trypanosomosis has a wider geographical spread than Rhodesian disease with about 77.8% of HAT endemic countries suffering from *T.b. gambiense*[4]. In Nigeria, although the exact sleeping sickness situation is not well-known, there is increase in number of volunteer cases presented for treatment each year[24]. Apart from the old Gboko endemic focus remaining active, outbreak of sleeping sickness in the new Abraka focus presently constitute a major health risk[24], resulting to several deaths. During an outbreak, out of 3,583 volunteers from 24 communities scattered around this focus, 359 were seropositive and 104 parasitologically positive for *T.b. gambiense*[25].

Identification of serum-resistance-association (SRA) gene in eight *Trypanozoon* isolates resistant to human serum has been reported by[26] while such genes were absent in isolates sensitive to human serum. Although this technique identified human-infective trypanosomes in cattle as reservoirs in the sleeping sickness endemic foci in Uganda arising from *T.b. rhodesiense,* it may similarly be used for identification of animal reservoir hosts in *T.b. gambiense* endemic

areas[27] using PCR methods reported 8% *T.b. gambiense* infection rate in wild animals in the Bipindi sleeping sickness focus of Cameroon which was believed to be responsible for resurgence and perpetuation of the disease in the area. High prevalence of *T.b. gambiense* was observed in rodents (*Atherurus africanus* and *Cricetomys gambianus*), monkeys (*Cercopithecus* and *Cercocebus*) and ungulates (*Cephalophus* spp). Two small carnivores (*Genetta servalina* and *Nandinia binotala*) also harboured trypanosomes infective to man.

Similarly dogs, pigs, wild animals and bovids were identified as reservoir hosts in Liberia, Cote d'Ivoire and Burkina Faso based on isoenzyme electrophoresis, resistance to human serum and DNA analysis. The capacity of *T.b. gambiense* to proliferate and persist in alternative hosts in the absence of symptoms with prolonged maintenance of infectivity to the vectors makes it maintain a wide range of reservoir hosts which may differ from one endemic area to another. Adaptation of PCR technique for identification of *T.b. gambiense* and blood meals of both animal and human origins in tsetse flies[5], has paved way for determination of the roles of animal reservoir hosts in epidemics of Gambian sleeping sickness. The reports have shown involvement of *Glossina palpalis* in cyclical transmission of sleeping sickness from pigs to man in Cote d'Ivoire[28]. Pig-tsetse-human, cattle-tsetse-human and sheep-tsetse-human transmission cycles have similarly been reported in three endemic foci in south-eastern Uganda[5].

Even though the role of animal reservoir hosts in the transmission of *T.b. gambiense* have been controversial current biotechnologies that differentiate between *T.b. brucei*, *T.b. gambiense* and *T.b. rhodesiense* in the tsetse vector and animals confirm the true zoonosis of Gambian trypanosomosis. Going by such findings maintenance of animal-tsetse-animal *T.b. gambiense* transmission cycle is not unlikely. The zoonotic nature of the disease seems to be further enhanced by the feeding pattern of *G. palpalis* group which apart from feeding on man, feeds on domestic pigs, wild ruminants and lizards[29]. Animal reservoir hosts for *T.b. gambiense* has been identified as one of the principal factors associated with the persistence of Gambian trypanosomosis in endemic areas in spite of chemotherapeutic campaigns[27]. For example, the presence of pigs in Mbini focus of Equatorial Guinea is believed to be responsible for the persistence of infection despite several years of treatment[30]. This is supported by the ability of *G. palpalis* group to cyclically transmit *T.b. gambiense* from unlimited number of animal reservoir hosts, to man[28,5] and the difficulty associated with the control of trypanosomosis in game animals. Based on observations using human serum resistance, isoenzyme electrophoresis and DNA-test for molecular characterisation of *trypanozoans* leading to more definitive identification of *T.b. gambiense* in pigs and other domestic and wild animals, the role of animals as reservoir hosts for Gambian sleeping sickness and resurgence of the disease can not be questioned any longer. Going by the feeding habit of *G. palpalis* group on unlimited range of animal hosts, and the ability of *T.b. gambiense* to perpetuate in such hosts without clinical symptoms supports the fact that animal reservoirs indeed constitute important complications militating against eradication of Gambian sleeping sickness in sub-Saharan Africa. An integrated approach to the control of human and animal trypanosomosis is essential in the control of the current upsurge in human trypanosomosis and in limiting the present economic impact on Africa and tourism potentials of sleeping sickness endemic countries.

References

1. Ouma JO, Mwangi JM, Mdachi RE, Murilla GA. Evaluation of indirect enzyme-linked immunosorbent assay (ELISA) systems for the serodiagnosis of bovine trypanosomosis in disease endemic areas of Kenya. *S World J* 2007; *2*(1): 11–13.

2. Okoli IC. Incidence and modulating effects of environmental factors on trypanosomosis, peste des petit ruminants (PPR) and bronchopneumonia of West African dwarf

goats in Imo state, Nigeria. *Livestock Res Rural Dev* 2003; *15*(9): 3.

3. Rahman AHA. *Report on tsetse and trypanosomiasis activities in Sudan.* Pt 3. *Tsetse Trypanosomiasis Infor Qtl* 2002; *25:* 77–78.
4. Control and surveillance of African trypanosomiasis 1998. *WHO Tech Rep Ser No*. 881; p. 1–123.
5. Waiswa C, Olaho-Mukani W, Katunguka-Rwakishaya E. Domestic animals as reservoirs for sleeping sickness in three endemic foci in southeastern Uganda. *Ann Trop Med Parasitol* 2003; *67:* 149–55.
6. Jelinek T, Bisoffi Z, Bonazzi L, Van Theil P, Bronne U, De Frey A, Gundersen SG, McWhinney P, Ripamonti D. Cluster of African trypanosomiasis in travellers to Tanzania national parks. *Emerg Infect Dis* 2002; *8:* 634–5.
7. Osho IB, Ogunsusi RA, Ogbedengbe JD. Sero-prevalence of trypanosomiasis of small ruminants in Ondo and Ekiti states of Nigeria. *Proceedings of 27th Annual Conference.* Akure, Nigeria: Nigeria Society for Animal Production 2002; p. 54–6.
8. Kalejaiye JO, Omotosho SO. Occurrence of animal trypanosomiasis in Gonawuri district of Riyom LGA of plateau state, Nigeria. Book of abstract. *37th Annual Conference.* Uyo: Nigeria Veterinary Medicine Association 2000; p. 13
9. Joshua RA, Magaji Y, Kayit YS. Isolation of human serum resistant *Trypanozoon* from cattle in Nigeria. *Tropenmedizin Parasitol* 1983; *13:* 201–2.
10. Milstein C, Kõhler. Monoclonal antibodies and cell surface antigens. *Human genetics, possibilities and realities. Excerpta Medica* 1979; *66:* 251.
11. Nantulya VM, Musoke AJ, Rurangiwa FR, Minja S, Saigar N. Monoclonal antibodies that distinguish *T. congolense*, *T. vivax* and *T. brucei*. *Parasite Immunol* 1987; *9:* 421.
12. Dipeolu OO. Survey of blood parasites in domestic animals in Nigeria. *Bull Anim Health Production Afr* 1975; *23:* 155–64.
13. Akinboade AO. The prevalence of Babesiosis in Nigeria trade cattle. Ph.D thesis 1982.
14. Losos GJ, Ikede BO. Review of pathology of disease in domestic and laboratory animals caused by *T. vivax, T. brucei, T. rhodesiense* and *T. gambiense. Vet Pathol* 1972; *9*(Suppl)*:* 1–17.
15. Anosa VO. Mammalian blood cells in health and in trypanosomiasis. *Trop Vet* 1983; *4:* 177–99.
16. Abenga JN, Enwezor FNC, Lawani FAG, Ezebuiro C, Sule J, David KM. Prevalence of trypanosomosis in trade cattle at slaughter in Kaduna, Nigeria. *Nigerian J Parasitol* 2000; *23:* 107–10.
17. Davies H. *Tsetse flies in Nigeria*. Ibadan: Oxford University Press 1977; p. 1–86.
18. Magona JW, Kakaire DW, Mayende JSP. Prevalence and distribution of animal trypanosomosis on Buvuma Islands in Lake Victoria, Uganda. *Springer Links Netherland* 1999; *31*(2): 83–7.
19. Nkinin SW, Njiokou F, Penchenier L, Grebaut P, Simo G, Herder S. Characterization of *Trypanosoma brucei* s.l. subspecies by isoenzymes in domestic pigs from the Fontem sleeping sickness focus of Cameroon. *Acta Trop* 2002; *81*(3): 225–32.
20. Onah DN, Ebenebe OO. Isolation of a human serum-resistant *Trypanosoma brucei* from a naturally infected pig in the Nsukka area of Enugu State. *Nig Vet J* 2003; *24*: 37–45.
21. Rickman LR, Robson J. The blood incubation infectivity test: a simple test which may distinguish *Trypanosoma brucei* from *T. rhodesiense. Bull WHO* 1970; *42:* 650–1.
22. Gibson W, Mihlitz D, Lanham SM, Godfrey DG. The identification of *T. gambiense* in Liberian pigs and dogs by isoenzyme and by resistance to human plasma. *Tropen Med Parasitol* 1978; *29:* 335–45.
23. WHO sleeping sickness treatment and drug resistance network. *Tsetse Trypanosomiasis Infor Qtl* 2000; *23*(1): 4.
24. Airauhi L, Unuigbe EI, Airauchi OD. Human sleeping sickness (SS) in Nigeria: knowledge, attitude and beliefs in a focus in the Abraka belt, Delta of Nigeria. *Afr J Clin Exp Microbiol* 2001; *2*(2): 6.
25. Edeghere H, Elhassan E, Ukah JCA, Sanda S, Ikenga M, Audu G. The scourge of human African trypanosomiasis in Abraka: possible control strategies. *Nig Soc Parasitol Abst* 1998; No. 46: 43.
26. Welburn SC, Picozzi K, Fevre EM, Coleman PG, Odiit M, Carrington M, Maudlin I. Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-association (SRA) gene. *Lancet* 2001; *9298:* 2017–9.
27. Simo G, Njiokou F, Nkinin S, Mgbedie M, Laveissiere C, Herder S. Etude de la prevalence des infections a trypanosomes chez les animaux sauvages du foyer de la maladie du sommeil de Bipindi, Cameroun. *Bull Liaison Docum l'OCEAC* 2000; *33*(2): 8–15.
28. Sane B, Laveissiene C, Meda HA. Repartition spatiale et

preferences trophiques de *Glossina palpalis* dans le foyer forestier De Zoukougbeu (Cote d'Ivoire)—Implications epidemiologiques. *Parasite* 2000; *7*(3): 241–4.

29. Spath J. Feeding Patterns of three sympatric tsetse species (*Glossina* spp) in the preforest zone of Cote d I'voire. *Acta Trop* 2000; *75*(1): 109–18.

30. Simarro PO, Franco JR, Ndongo P. Qu'est-ce qui ne marche pas dans le controle de la maladie du sommeil dans le foyer de Mbini. Organization of African Unity/Scientific. *Tech Res Comm Pub* 2001; *120:* 55–60.

Corresponding author: Dr Michael C. Ezeani, Department of Immunology, College of Health Sciences, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
E-mail: mikezeani@yahoo.com

Received: 14 March 2008 *Accepted in revised form:* 14 September 2008