

THE PREVALENCE OF TRYPANOSOMA SPECIES IN CATTLE IN CALABAR METROPOLIS OF CROSS RIVER STATE, NIGERIA.

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ABSTRACT

This study was conducted to determine the prevalence of *Trypanosoma species* in cattle because of the increasing movement of the animals within the metropolis. Blood specimens were collected from 1200 cattle during slaughter at 4 abattoirs in Calabar Metropolis from February to May, 2016. Dry universal containers were positioned in the stream of blood oozing from the cut neck of each cow. From these containers, 4 millilitres of blood were aspirated into an ethylene diamine tetra-acetic acid (EDTA) bottle using a 5 millilitres syringe. The blood specimens were processed for microscopy at the Parasitology Laboratory of the University of Calabar Teaching Hospital. Wet preparation of each specimen was done and examined for motile haemoparasites. Also, triple centrifugation was carried out on 2 millilitres of each blood specimen at increasing speeds of 1000, 1500 and 3000 revolutions per minute for 5 minutes. At each centrifugation process, the resulting plasma was harvested

and spun again. Finally, the resulting Buffy coat layer and deposit were re-suspended and used to prepare smears on clean microscope slides. After drying in air, the blood smears were stained with 3% Giemsa solution for 30 minutes. Upon microscopy, 570 stained blood specimens were positive for *Trypanosoma species*, which morphologically resembled *Trypanosoma b. brucei* by their long free flagellum. The overall prevalence of trypanosomes among the cattle was 47.5%. Out of the total number of infected cattle, 330 were male while 240 were female. The results show prevalence rates of 27.5% and 20% among male and female cattle, respectively. There was no statistically significant difference in the prevalence of trypanosomes among the male and female cattle ($X^2 = 9.41$; $p > 0.05$). But the findings suggest that male cattle tend to forage more into the bush and are, thus, more likely to be exposed to bites of infected *Glossina* flies than their female counterparts. The high prevalence of trypanosomes in cattle, as shown in this study, may have a major epidemiologic significance, considering the increasing rate of open grazing by cattle within residential areas in major cities all over Nigeria. However, it is important to point out major drawbacks of this study to include: (1) due to limited resources and the short duration of the study, the trypanosomes could not be identified at the molecular level, in order to establish their pathogenic potential to human; (2) blood specimens were not collected from the cattle rearers for parasitological examination. These shortcomings shall form the basis of future investigations on this subject in the same study area.

Keywords: Blood, Cattle, *Glossina*, Prevalence, Trypanosoma.

INTRODUCTION

Trypanosomes are protozoan parasites which inhabit blood and tissues of wild and domestic animals, including human beings. They cause various forms of trypanosomiasis in human beings and livestock. Human African Trypanosomiasis (HAT), caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*, is classified among the Neglected Tropical Diseases (1). The parasites classified under the Genus *Trypanosoma* were first reported by Gruby in 1843. The first human infection with trypanosome was reported by Forde in 1901 in The Gambia and the parasite caused recurrent fevers in the patient (2). Later, the same parasite was reported in trouts and, in 1902, Dutton coined the name *Trypanosoma gambiense* for it (3).

There are many species and sub-species of trypanosoma. Those with health and socio-economic importance to man are broadly classified into two groups, viz., *Trypanosoma brucei* and *T. cruzi*. *Trypanosoma brucei* is a complex group with three important human parasites, namely: *T. brucei brucei*, *T. b. gambiense* and *T. b. rhodesiense*. These parasites are the cause of Human African Trypanosomiasis (HAT) or sleeping sickness in most of Central, Eastern and Western Africa. It is estimated that about 60 million people are at risk of infection with HAT in 36 countries of Africa (4). African Animal Trypanosomiasis (AAT) is found mainly in those regions of Africa where its biological vector, the tse tse fly, exists. The most important vectors of AAT are *Trypanosoma congolense*, *T. vivax* and *T. brucei*. Apart from cattle, other domesticated animals can be infected e.g. water buffalo, sheep, goats, camels, horses, donkeys, pigs, dogs, cats, etc. (5).

Trypanosoma cruzi causes South American Trypanosomiasis or Chagas' disease. The disease is named after the Brazilian Carlos Chagas who discovered it in 1909 (6). About 7 million people worldwide are estimated to be infected with *T. cruzi*, mostly in Latin America (7). Various species of *Glossina* are intermediate hosts of *T. brucei* group of parasites. The tse-tse flies, as they are commonly called, are important in the salivarian (i.e. from the mid-gut to the fore-gut and up to the salivary glands) life cycle of the parasites. The flies bite game animals such as antelopes, zebras, as well as pigs. These animals are the natural

reservoirs of trypanosomes. Infective forms of trypanosomes called meta-cyclic trypomastigotes are transmitted to human beings by infected tsetse flies during haematophagy.

For *Trypanosoma cruzi*, reduviid bugs are the intermediate hosts. They pick up the parasites during blood meals from infected persons and transmit them to others via faecal contamination. *Trypanosoma cruzi* undergoes a stercorarian life cycle (i.e. from the mid-gut to the hind-gut and down to the rectum) in the reduviid bugs.

Human African Trypanosomiasis (HAT), caused by *T. gambiense*, runs a chronic course with an inevitable death, if not treated within several months or years, depending on the host's susceptibility. Diseases caused by *T. rhodesiense* run a more rapid course, with an imminent death, within days or weeks of infection, if not treated. Thus, somnambulism (i.e. tendency to sleep), usually noticed in *gambiense* infections, is not usually noticed in *T. rhodesiense* infections, since the patient usually dies before characteristic nervous disorders pathognomonic of *T. gambiense* disease becomes manifest. All forms of African trypanosomiasis in humans are characterized by fever, headache, generalized pains, lymphadenopathy, anaemia and emaciation. Finally, coma and death may ensue as a result of a combination of malnutrition, pneumonia, heart failure, or a severe fall (7).

The most convincing method of laboratory diagnosis of human African trypanosomiasis is the microscopic detection of trypanosomes in clinical specimens such as blood, bone marrow, synovial fluid or cerebrospinal fluid (8). Serologic diagnosis is also possible by the use of card agglutination trypanosomiasis test (CATT) to detect antibodies against trypanosomes in whole blood or serum (8). Originally, arsenic compounds were used in the treatment of African trypanosomiasis. But the drugs are fraught with drawbacks, such as eye damage and parasite resistance (9). Other drugs such as suramin, pentamidine and Berenil have proven efficacious in early cases of human African trypanosomiasis (10).

The epidemiology and control of human African trypanosomiasis depend largely on the presence of reservoir hosts (e.g. cattle and wild game) and suitable arthropod vectors, *Glossina species* (11). A successful programme to control the spread of human African trypanosomiasis must involve restriction in the movement of cattle and related species. Breeding and grazing of these animals must be done at locations which are sited far from residential areas. Also, brush removal from trees is an inexpensive method of curtailing the advancement of tsetse flies towards residential areas.

MATERIALS AND METHODS

The study area

This study was carried out in Calabar Metropolis, which comprises Calabar Municipality and Calabar South Local Government Area. Calabar is the capital of Cross River State, located in Southern Nigeria.

Collection and processing of specimens

Collection of blood samples from slaughtered animals was done between February and May 2016. Blood was collected from cattle at the point of slaughtering the animals at Bogobiri, Ikot Eneobong, Nasarawa abattoirs in Calabar Municipality and Ibesikpo abattoirs in Calabar South Local Government Area. A dry universal bottle was used to collect an aliquot of the oozing blood. From the universal container, 4 millilitres of blood were placed in an ethylene diamine tetra-acetic acid (EDTA) container, using a sterile syringe and needle. The blood sample was swirled gently to mix it properly in the container. The blood specimens were processed for microscopy at the Parasitology Laboratory of the University of Calabar Teaching Hospital. Wet preparation was done by placing a drop of blood on a clean glass slide, covering with a cover slip and examining with x 10 and x 40 objective lenses for motile haemoparasites (12).

For triple centrifugation, 2 millilitres of anticoagulated blood was centrifuged at 1500 revolutions per minute for 10 minutes. The Buffy coat layer with admixed plasma was transferred into another tube and spun at 2000 revolutions per volume for 15 minutes. With the sediment, smears were made on clean slides, allowed to dry in air and stained with 3 per cent Giemsa solution for 30 minutes (13). All stained smears were examined using x 100 objective lens with immersion oil applied.

Entomological Survey

Tsetse flies are larviparous and they deposit their larvae on the soil under brush (14). The egg is retained in the fly's uterus and the extra-uterine life of the single larva is so brief as to be insignificant. Consequently, entomological samples for tsetse flies are usually

confined to puparia and, very rarely, adult flies may be caught in fly traps (15). For this study, entomological survey was carried out in the surrounding bushes (where cattle were kept before slaughter at Ikot Eneobong and Nasarawa) for tsetse fly puparia according to the method described by Gordon and Lavoipierre (15).

Data analysis

Data obtained in the study were subjected to statistical analysis for test of statistical significance, using the Chi square test.

RESULTS

Blood samples were obtained from 1200 cattle from 4 abattoirs during the study. For the purpose of uniformity, blood samples were obtained from 300 cattle from each abattoir. At Bogobiri abattoir, none of the 300 cattle harboured trypanosomes. At Nasarawa abattoir, 180 cattle were positive for *Trypanosoma species*, with a prevalence of 60% for the presence of trypanosomes among the cattle. At Ikot Eneobong abattoir, *Trypanosoma species* were detected in 270 cattle. This result shows a prevalence rate of 90% for trypanosomes in cattle slaughtered in that area. At Ibesikpo abattoir; 120 cattle harboured trypanosomes with a prevalence rate of 40%. Overall, a total of 570 cattle slaughtered from 4 abattoirs harboured *Trypanosoma species*. This result showed a prevalence rate of 47.5% for the presence of trypanosomes in cattle slaughtered in Calabar within the period of this study, as shown in Table 1.

Table 2 shows the occurrence of *Trypanosoma species* in cattle according to gender. A total of 936 cattle slaughtered were males. Out of this number, 330 male cattle harboured trypanosomes. This result showed that 27.5% of the male cattle harboured trypanosomes. A

total of 264 cattle were female, out of which 240 were positive for trypanosomes. The result showed that 20.0% of the female cattle were positive for trypanosomes.

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TABLE 1 The prevalence of *Trypanosoma species* in cattle slaughtered at 4 abattoirs in Calabar Metropolis

Abattoir	Number examined	Number positive	(%) age Positive
Bogobiri	300	0	0
Nasarawa	300	180	60
Ikot Eneobong	300	270	90
Ibesikpo	300	120	40
TOTAL	1200	570	47.5

TABLE 2 The prevalence of Trypanosomes in cattle according to gender

Gender	Number examined	Number positive	(%)age Positive
Male	936	330	27.5
Female	264	240	20.0
TOTAL	1,200	570	47.5



Fig.1. Trypomastigotes of *Trypanosoma species* detected in the blood specimens of slaughtered cattle.

DISCUSSION

The results of this study show an overall prevalence of 47.5% for *Trypanosoma species* in cattle slaughtered in Calabar Metropolis within the period of study. This prevalence rate is higher than 27% prevalence rate generally recorded for Nigeria due to non-tsetse flies transmission (16). The trypanosomes detected in this study generally possessed a long free flagellum; and this characteristic is typical of *Trypanosoma congolense*, which is usually very common in cattle. However, no molecular technique was carried out for definitive identification of the species of trypanosomes detected. The prevalence of *Trypanosoma species* among cattle according to abattoir showed that Nasarawa, Ikot Enebong and Ibesikpo abattoirs recorded 60%, 90% and 40% prevalence rates, respectively, for trypanosomes in the slaughtered cattle. As the cattle are usually brought in from Northern States to Southern parts of Nigeria, it was not possible to trace the original source of infection of the cattle with trypanosomes. Moreover, entomological surveys did not reveal larvae, pupae or adult stages of *Glossina species* in the vegetation and bushes surrounding the abattoirs. This finding is not new or surprising, as there have been pockets of tsetse surveys in Northern and Southern parts of Nigeria without trapping the adult flies or finding their larvae or puparia (17, 18, 19, 20). No Trypanosome was detected among cattle slaughtered at Bogobiri abattoir. This may be due to limited exposure of the cattle to bites of tsetse flies. Due to the presence of surrounding bush behind the Akim Army Barracks, cattle are usually kept in small herds and transported to Bogobiri abattoir for slaughter as they reach maturity. So, by not roaming into forests, these cattle were not so much exposed to bites by tsetse flies.

In conclusion, this study has established the presence of *Trypanosoma species* in cattle in Calabar Metropolis. Another study may be necessary to confirm the species of these trypanosomes using molecular technique(s). It is, hereby, recommended that movements of cattle for the purpose of grazing should be restricted to circumscribed locations which are well separated from residential quarters. Also, professional advice from Medical Entomologists, Public Health Officers and Environmental Protection Officers is required in the design of Calabar city. With a careful town planning, strategies shall be put in place to reduce the distribution of animal trypanosomiasis and other arthropod-borne infectious diseases within the city.

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