PREVALENCE OF SAVANNAH-TYPE *TRYPANOSOMA CONGOLENSE* IN CATTLE IN SOUTH-WESTERN NIGERIA

***Okwelum, N., Onagbesan, O. M., Shittu, O. O., Famakinde, S. A., Bemji, M. N., and Osinowo, O. A.**
Institute of Food Security, Environmental Resources and Agricultural Research, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. PMB 2240
***Corresponding Author**, E-mail: drjhephzi@yahoo.com okwelumngozi2017@gmail.com*

ABSTRACT
In Nigeria, cattle have remained a major source of meat, milk, hide and cash income. However, trypanosomosis is one of the factors militating against cattle productivity causing severe losses. Trypanosomes are transmitted by tsetse flies and the southwest is tsetse infested zone and so, endemic for trypanosomosis. This study therefore, was carried out to investigate prevalence of Trypanosoma congolense "savannah-type" infection and the effect of the infection on haematological parameters. Blood sample was collected from 180 cattle, DNA was extracted and PCR technique was used for prevalence study. Haematological analysis was carried out using Auto-haemo-analyser. Data generated were analysed in a Generalized Linear Model (GLM) using SAS statistical package. The results revealed a prevalence of Trypanosoma congolense "savannah-type" species as 93.89%. There were significant ($p<0.05$) differences between haematological parameters of infected and un-infected cattle as evident in muscle wastage and leukocytosis. The study concluded that the prevalence of trypanosome infection has both epidemiological and economic importance.
Key words: Blood picture; disease surveillance; Nigerian cattle, PCR; trypanosome

INTRODUCTION

In Nigeria, cattle have remained a major source of sustainable animal protein and cash income. Thus, cattle are a means of poverty alleviation and food security. Trypanosomosis is one of the factors militating against cattle productivity. The morphological detection of trypanosomes of cattle through the microscopic screening of blood of infected cattle using wet mounts and blood smears as diagnostic techniques is the common practice but has a very low sensitivity as compared to the molecular tool (PCR) and also, not specific (Okwelum *et al*., 2019).

There are estimated 18.4 million heads of cattle in Nigeria. These cattle are predominantly managed in large herds (99%) by small holder farmers and transhumant pastoralist. Most indigenous breeds of cattle are dual purpose (milk and meat) as reported by Gridded Livestock of the World 3.0 (FAO, 2021) and Federal Ministry of Agriculture and Rural Development (2017). Trypanosomosis as a protozoan disease has severe effect on cattle industry in Nigeria causing significant losses ranging from a decrease in meat and milk production to death. Since the trypanotolerant breed (Muturu) is less than 9% of the national cattle head therefore, the trypanosusceptible breeds accounts for over 90% of the Nigerian cattle population (Adeniran *et al*., 2018) making trypanosomosis a very important disease in cattle production.

Trypanosomosis is caused by several parasites of the genus *Trypanosoma* (Adeniran *et al*., 2018) and mechanical transmission may exist but transmission is mainly through the bite of tsetse flies. The disease is characterised by anaemia, pyrexia, emaciation, parasitaemia, generalized lymphadenopathy, splenogaly, hepatomegaly and depression. Diagnosis of trypanosome infection has involved the use of parasitological methods. The Polymerase Chain Reaction (PCR) is the most sensitive diagnostic method in trypanosomosis (Okwelum *et al*., 2019). The aim of this study was to detect the molecular prevalence of *Trypanosoma congolense* "savannah-type" in Nigerian indigenous cattle reared in the southwestern Nigeria.

Statistical analysis: Data generated was analysed using SAS 9.1 Statistical Package in a Generalized Linear Model (GLM) also, means were separated for haematological parameters for infected and un-infected cattle using student t-test.

MATERIALS AND METHODS

Sampling sites and sampling: The study was carried in Federal University of Agriculture, Abeokuta, Ogun State in South-west Nigeria, situated at $7^0$9'39" N, $3^0$20'54" W and it is 76m above sea level and falls within the rainforest vegetation zone of south-western Nigeria (Google Earth, 2006). A total of 180 cattle were sampled. The weights of the cattle were determined using a weigh-band. About 5mL of blood was collected using EDTA tubes using vacutainer kits from each cattle.

Analysis for prevalence of trypanosome infection using (PCR) technique: Blood collected was used for haematological analysis and PCR diagnostic technique was used for the detection of *Trypanosoma congolense* "savannah-type" infection using the species-specific primers for the Variant Surface Glycoprotein (VSG) gene for the parasite. The primers used for this analysis was specie specific, while the PCR conditions for the amplification of the gene is shown in Table 1. Genomic DNA was extracted from the whole blood.

Analysis of haematological parameters: Blood samples were analysed using auto-haemo – analyzer for haematology. The Machine model is BC2800VET.

DNA extraction: The DNA was extracted from blood using Norgen's Blood Genomic DNA Isolation kit by NORGEN BIOTEK CORPORATION adopting the manufacturer's protocol at the Biotechnology Centre, Federal University of Agriculture, Abeokuta (FUNAAB).

PCR protocol, Agarose gel preparation, electrophoresis and visualisation of PCR products: The PCR was carried out using a thermocycler following the Laboratory protocol and PCR conditions for the gene. The Reference Primers used for *Trypanosoma congolense* "savannah-type" is as stated; 5' – CGA GAACGG GCA CTT TGC GA-3' (TCS-F) and 5' GGA CAA AGA AAT CCC GCA CA-3' (TCS-R), 316 bp (Masiga *et al*., 1992). PCR conditions as shown in Table 1. A 1% (w/v) agarose was prepared. Then the dyed PCR products were loaded after the size marker GENEMate Quanti-Marker 100 bp DNA ladder (BioExpress, Kaysville, UT, USA) was loaded. The detection of the amplified fragments was done under UV light using a transilluminator, while the presence of the fragments of interest was verified and then a photograph was taken showing samples and marker in their lanes. The samples with amplification was considered positive (infected) while non-amplified samples were considered negative (un-infected).

RESULTS AND DISCUSSIONs

The prevalence rate of *Trypanosoma congolense* "savannah-type" (TCS) based on sex of Nigerian cattle: *Trypanosoma congolense "*savannah-type" (TCS) was detected using PCR. The prevalence rate of *Trypanosoma congolense "*savannah-type" based on sex of Nigerian cattle in this study is presented in Table 2. The prevalence rate for TCS infection was 93.89%. Twenty-three point thirty-three per cent (23.33%) was due to infection in the males while 70.55% of the infection was recorded in the female cattle. These prevalence rates observed in this study is also high compared to report of by Okwelum *et al*. (2014) who reported a prevalence rate of 4%, the parasitological method adopted was blood smear techniques which is far less sensitive than the PCR technique.

The effect of *Trypanosoma congolense* "savannah-type" on body weight and haematological parameters of Nigerian cattle: The effect of *Trypanosoma congolense "*savannah-type" (TCS) on body weight and haematological parameters of Nigerian cattle is shown in Table 3. There were significant differences ($p<0.05$) in body weight and all the measured haematological parameters except for haemoglobin (HB), PCV, Mean Corpuscular Haemoglobin (MCH) and lymphocyte percentage (LYMPC). The body weight (BWT) of infected animals was significantly lower than that of un-infected animals. The lower RBC, haemoglobin concentration and haematometric indices of infected animals as compared to the un-infected cattle is clear evidence of shift towards anaemia which is associated with the TCS infection. Also the lower values of WBC fractions in the un-infected cattle as compared to the values of the infected cattle was a sign of leukocytosis is associated with the infection of TCS.

Table 1. PCR Conditions for *Trypanosoma congolense* "savannah-type" oligonucleotide primers

Species specific	Identification	Amplification Conditions
Trypanosoma congolense "savannah-type" (VSG gene)	TCS F/R	30 cycles (denaturation) 94°C for 60 s, (annealing) 55°C for 120 s, (extension) 72°C for 120 s.

TCSF/R-Trypanosoma congolense "savannah-type" forward and reverse; VSG-Variant surface glycoprotein

Table 2. The prevalence rate of *Trypanosoma congolense* "savannah-type" based on sex of Nigerian cattle

Factor	Sub-class	Number Tested	Number –ve	Number +ve	Percentage Sampled	Prevalence Rate (%)
Sex	Male	42	–	42	24.85	23.33
	Female	138	11	127	75.15	70.55
	Total	**180**	**11**	**169**	**100.00**	**93.89**

Table 3. Effect of *Trypanosoma congolense* "savannah-type" on body weight and haematological parameters of Nigerian cattle

Parameters	Unit	Un-infected	Infected
Body weight (BWT)	Kg	255.55 ± 21.92^{a}	137.03 ± 5.50^{b}
Red blood cell (RBC)	$X10^{12}/L$	11.19 ± 0.87^{a}	4.93 ± 0.34^{b}
Haemoglobin (HB)	g/L	94.18 ± 3.12	16.93 ± 1.98
Packed cell volume (PCV)	%	28.82 ± 1.17	29.39 ± 0.52
Mean corpuscular volume (MCV)	Fl	24.03 ± 0.08^{b}	31.90 ± 0.42^{a}
Mean corpuscular haemoglobin (MCH)	Pg	80.95 ± 0.84	64.93 ± 12.67
Mean corpuscular haemog. Conc. (MCHC)	Pg/L	3327.09 ± 168.22^{a}	2070.21 ± 91.85^{b}
White blood cell (WBC)	$X10^{9}$	5.3 ± 0.70^{b}	12.91 ± 0.40^{a}
Lymphocyte (LYM)	$X10^{3}/uL$	3.09 ± 0.55^{b}	7.87 ± 0.29^{a}
Monocyte (MON)	$X10^{3}/uL$	0.05 ± 0.02^{b}	1.03 ± 0.05^{a}
Granulocyte (GRAN)	$X10^{3}/nL$	2.31 ± 0.22^{b}	4.21 ± 0.16^{a}
Lymphocyte percentage (LYMPC)	%	55.36 ± 0.81	58.77 ± 0.89
Monocyte percentage (MONPC)	%	0.55 ± 0.16^{b}	7.09 ± 0.27^{a}
Granulocyte percentage (GRANPC)	%	44.09 ± 3.81^{a}	33.86 ± 0.83^{b}

$^{a, b}$ means with different superscripts across the same row are significantly ($P < 0.05$) different.

CONCLUSION

The study showed that 93.89% prevalence rate of *Trypanosoma congolense* "savannah-type" infection was high and considered to be of economic and epidemiological importance in the study area. The quantity of beef derivable from infected animals may drastically reduce due to poor body condition arising from muscle wastage.

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