Prevalence of *Trypanosoma vivax* Infection of Cattle in south-west, Nigeria

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Abstract

In south-west Nigeria, several *Trypanosoma* species are prevalent. These parasites cause trypanosomosis in livestock and man with cellular changes and tissue damages resulting in great loss in production and sometimes death. Hence, continuous surveillance is very necessary for monitoring and control of trypanosomosis. This study concentrated on the survey of trypanosome infection and its effect on both weight and haematological indices of Nigerian cattle. One hundred and eighty cattle were used in the study. The sex of cattle was noted while the age of cattle was determined using dentition and weight was determined using weigh-band. Blood sample was collected from 180 cattle, DNA was extracted from each cattle blood sample and PCR technique was adopted as a molecular diagnostic tool for prevalence study. Reference primer specific for *Trypanosoma vivax* (Variant Surface Glycoprotein (VSG) gene) was used as a probe for the detection of the presence of parasite while gene amplification on agarose gel was considered positive for the cattle under consideration. The haematological analysis was also carried out using Auto-haemo-analyser. The prevalence rate of *Trypanosoma vivax* based on sex, age and breed of cattle was determined. The result revealed a prevalence rate of 87.22%. About 19.44% was due to infection in the males while 67.78% of the infection was recorded in the female cattle. For the prevalence within the age group; 0 -1 year was 21.11%, >1 - 2 years was 21.11%, >2 - 3 years was 32.22%, >3 years was 12.78%. There was no significant ($p > 0.05$) difference between the weight, haematological parameters determined and the infection status. In the phase of infection, the hosts were able to maintain their red blood cell counts from a perceived compensatory response which was revealed in the erythrocytic indices and packed cell volume. Though the prevalence rate recorded was high, the hosts were able to maintain the haematimetric indices, it can then be concluded that Muturu cattle has displayed trypanotolerant property.

Keywords: Epidemiological survey, trypanosome, haematology, Nigerian cattle, PCR.

Introduction

In south-west Nigeria, just as in the other tsetse fly infested areas of Africa, several trypanosomes species, including *Trypanosoma vivax*, *Trypanosoma brucei* and *Trypanosoma congolense* are prevalent which affect the health of cattle and other livestock. Great loss in production indices have been recorded in affected herd. This disease caused by trypanosomes is

trypanosomosis and it is characterized by pyrexia, emaciation, generalized lymphadenopathy, anaemia, abortion, immune-depression, splenomegaly and hepatomegaly. This disease has zoonotic importance as the causative agents of human trypanosomosis (sleeping sickness) are *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* but are non-pathogenic in livestock and wild

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bovids (Picozzi *et al.*, 2002). Of particular importance to public health is the new possibility of rapidly identifying animals that serve as reservoirs for human infective trypanosomes. The risk of spreading sleeping sickness is closely related to the movement of cattle populations, and the screening and treatment of livestock to prevent disease spread has been advocated (Fevre, *et al.*, 2001). **The Muturu cattle in Nigeria has been well adapted to the tsetse infested zone of the middle belt and southern parts of the country.**

Diagnosis or detection of trypanosome infection has involved the use of parasitological methods, immunodiagnostic and molecular diagnostic techniques. However, the molecular technique using Polymerase Chain Reaction (PCR) is the most sensitive method as it detects specific parasite DNA and as such, establishes current infection status in a population. Also, the PCR technique is known to combine the advantages of a greater sensitivity than the parasitological or immunological techniques with the added advantage of differentiating trypanosomes which have a similar morphology but very different economic impacts (Picozzi *et al.*, 2002).

It is estimated that agricultural produce worth US\$ 4.75 million is lost each year as a result of trypanosomosis (Public Health Agency of Canada (PHAC) Newsletter, 2001). Hence, continuous surveillance is very necessary to monitor the effectiveness of control measures and also provide epidemiological data for future monitoring and control of the disease. Reports in some other parts of Nigeria had revealed the epidemiological importance of such studies (Agu *et al.*, 1989; Ekejindu *et al.*, 1992; Ahmed and Agbede, 1993; Fakae and Chiejina, 1993; Daniel *et al.*, 1994;

Kalejaiye *et al.*, 1995; Nawathe *et al.*, 1995; Kalu *et al.*, 1996). Therefore, this study concentrated on the survey of *Trypanosoma vivax* infection and its effect on both weight and haematological indices of Nigerian cattle.

Materials and methods

Sampling sites and sampling

The study was carried out in the Federal University of Agriculture Abeokuta. Abeokuta is in the south-western zone of Nigeria (Google Earth, 2006).

Animals and management

Blood samples were collected from Muturu cattle in Ipokia Local Government Area (LGA) of Ogun State. The location was considered to be natural habitat for Muturu herds in Nigeria. A total of 180 cattle were sampled. The other breeds used in this study were sampled in Alabata, Odeda LGA of Ogun State.

Collection of phenotypic data

The ages were determined using dentition while weights were determined using a weigh-band. About 5mL of blood was collected via jugular venipuncture using EDTA vacutainer kits for haematological analysis and PCR.

Analysis for prevalence of trypanosome infection using (PCR) technique

Polymerase chain reaction (PCR) diagnostic technique was used for the detection of *Trypanosoma vivax* infection using the species specific primer (Forward and Reverse) for the Variant Surface Glycoprotein (VSG) gene for the parasite. The primers used for this analysis is shown in Table 1 while the PCR conditions for the amplification of the gene is shown in Table 2. Genomic DNA was extracted from the whole blood in EDTA, this DNA was used as a template in the PCR protocol. Every sample with amplification on the agarose gel was considered positive or infected with trypanosome infection while the sample

without amplification was considered negative or un-infected.

Analysis of haematological parameters

Blood samples were analysed using auto-haemo – analyzer for haematology. The Machine model is BC2800VET. The haematological parameters analysed were; red blood cell (RBC), haemoglobin (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), lymphocyte number (LYM), monocyte number (MON), granulocyte number (GRAN), lymphocyte percentage (LYMPC), monocyte percentage (MONPC) and granulocyte percentage (GRANPC). Samples were then stored in the Freezer under -4°C and were used later for genomic studies (PCR).

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).

Purified DNA samples were stored at -4°C.

PCR protocol

The PCR Master-Mix (Tag buffer, MgCl₂, Tag polymerase) (5 µl) was added into a PCR tube. Then 3 µl of Reference Primers (forward and reverse) was added. Nuclease free water (1 µl) was added. And 1 µl of DNA template was added. This gave a total of 10 µl reaction volume for one sample. Then the tubes were loaded into the PCR machine (thermocycler) and the machine was set according to the PCR conditions for each gene. The Reference Primers are listed in Table 1 and the PCR conditions are shown in Table 2.

Table 1: The oligo-nucleotide primers sequences used in the PCR analysis

Specificity of primers	Forward primer	Reverse primer	Expected base size	Literature reference	Gene bank reference
<i>T. vivax</i> (VSG gene)	5'-TCG CTA CCA CAG TCG CAA TCG TCG TCT CAA GG- 3' (TVX-F)	5'CAG CTC GGC GAA GGC CAC TTG GCT GGG GTG- 3' (TVX-R)	400	Masake <i>et al.</i> , 1997	U43183

VSG gene -Variant surface glycoprotein; ITS gene - Internally transcribed spacer gene; IL-10 -Interleukin – 10 gene

Table 2: PCR conditions for the various primers

Species specificity	Identification	Amplification conditions
<i>Trypanosoma vivax</i> (VSG gene)	TVXF/R	30 cycles (denaturation) 94 °C for 45 s, (annealing) 60°C for 60 s, (extension) 72°C for 30 s.

TVXF/R-*Trypanosoma vivax* forward and reverse; VSG gene-Variant surface glycoprotein gene

Agarose gel preparation and electrophoresis

A 1% (w/v) agarose, 200 ml of 1 × TAE buffer was prepared. The solution was allowed to cool down, 0.5 µg/mL of ethidium bromide was then added. The gel solution was then carefully poured into the casting tray and left for about 35 minutes to set. This was then placed into electrophoresis chamber submerged in 1 × TAE buffer. After the PCR, the amplified fragments were run on gel. at 80 volts,

118mA for 30minutes. Before loading, the PCR products were mixed with the gel-loading dye (bromophenol blue). For each well 10µl of sample mixed (PCR product mixed with the loading dye) was loaded. In the first well of each row, a size marker GENEMate Quanti-Marker 100 bp DNA ladder (BioExpress, Kaysville, UT, USA) was loaded.

Visualization of PCR products

The detection of the amplified fragments was done under UV light using a

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transilluminator, while the presence of the fragments of interest was verified and then a photograph was taken showing samples and marker in their lanes. Amplification of *Trypanosoma vivax* gene on gel was recorded positive for that particular sample whereas absence of amplification or band meant negative to the infection and as such the cattle is considered un-infected.

Statistical analysis

Data was analysed using ANOVA in General Linear Model (GLM) using SAS 9.1 Statistical Package. Means were separated using Duncan's multiple range test of the same package. Also means were separated for haematology of infected and un-infected cattle using t-test.

Results

The prevalence rate of *Trypanosoma vivax* (TVX) based on sex, age and breed of Nigerian cattle in the survey is represented in Table 3. The prevalence rate for TVX infection was 87.22%. Nineteen point forty-four per cent (19.44%) was due to infection in the males while 67.78% of the infection was recorded in the female cattle. For the prevalence within the age group; 0 -1 year was 21.11%, >1 2 years was 21.11%, >2 3 years was 32.22%, >3 years was 12.78%. The Muturu breed accounted for 72.77% of the total prevalence, White Fulani was 5.56%, N'Dama was 1.67%, cross of White Fulani and Muturu was 5.00%, Cross of White Fulani and N'Dama was 1.11% while cross of Muturu and N'Dama was 1.11%.

Table 3: Prevalence of *Trypanosoma vivax* based on sex, age, and breed of Nigerian cattle

Factor	Sub-class	Number Tested	Number -ve	Number +ve	Percentage Sampled	Percentage Infected
Sex	Male	42	7	35	22.29	19.44
	Female	138	16	122	77.70	67.78
	Total	180	23	157	100	87.22
Age(years)	0 – 1	43	5	38	24.20	21.11
	>1 2	46	8	38	24.20	21.11
	>2 3	64	6	58	36.94	32.22
	>3	27	4	23	14.65	12.78
	Total	180	23	157	100	87.22
	Breed					
	Muturu	149	18	131	83.44	72.77
	WF	11	1	10	6.37	5.56
	N'Dama	3	–	3	1.91	1.67
	*WF x Muturu	10	1	9	5.73	5.00
	*WF x N'Dama	3	1	2	1.27	1.11
	*Muturux N'Dama	4	2	2	1.27	1.11
	Total	180	23	157	100	87.22

* Breed classification was based on physical observation of predominant phenotypic traits of pure breeds in the mixed-bred cattle population; WF-White Fulani

The effect of *Trypanosoma vivax* infection on body weight and the measured haematological parameters of the Nigerian cattle is shown in Table 4. There was no

significant difference ($p>0.05$) between the infected and un-infected cattle in the body weight and in all the haematological parameters measured.

Table 4: Effect of *Trypanosoma vivax* on body weight and haematological parameters of Nigerian cattle

Parameters	Unit	Un-infected	Infected
Body weight (BWT)	Kg	141.83 ± 12.51	144.63 ± 6.32
Red blood cell (RBC)	x10 ¹² /L	5.21 ± 0.94	5.33 ± 0.37
Haemoglobin (HB)	g/L	110.57 ± 5.93	105.50 ± 1.98
Packed cell volume (PCV)	%	29.78 ± 1.46	29.27 ± 0.52
Mean corpuscular volume (MCV)	fL	31.71 ± 1.11	31.738 ± 0.46
Mean corpuscular haemoglobin (MCH)	Pg	61.17 ± 6.41	66.60 ± 2.74
Mean corpuscular haemog. Conc. (MCHC)	Pg/L	1972.17 ± 237.18	2172.73 ± 96.84
White blood cell (WBC)	x10 ⁹	13.72 ± 1.20	12.26 ± 0.42
Lymphocyte (LYM)	x10 ³ /uL	836 ± 0.85	7.47 ± 0.30
Monocyte (MON)	x10 ³ /uL	1.08 ± 0.13	0.75 ± 0.05
Granulocyte (GRAN)	x10 ³ /nL	4.24 ± 0.34	4.08 ± 0.17
Lymphocyte percentage (LYMPC)	%	59.90 ± 2.40	58.37 ± 0.93
Monocyte percentage (MONPC)	%	7.04 ± 0.74	6.63 ± 0.31
Granulocyte percentage (GRANPC)	%	32.92 ± 2.25	34.72 ± 0.89

Discussion

The prevalence rate of *Trypanosoma vivax* was 87.22%. Parasite species was detected using PCR. This prevalence rates were high compared to the reports of other researchers (Ukairokalu, 1995; Enwezor *et al.*, 2012) who observed rates as low as 25.00%. The difference in prevalence rates observed could be as a result of the less sensitive diagnostic method used in the detection of the parasites in the blood of the host by these other researchers. The PCR technique is currently the most sensitive diagnostic method or parasite detection in the hosts' blood for trypanosome infection. Abenga *et al.* (2004) reported a prevalence rate of 7% using buffy coat technique. Buffy coat technique was also less sensitive compared to the PCR technique that was used in this current study. Fajinmi *et al.* (2011) reported a prevalence rate of 15.20% in trade cattle. These prevalence rates observed in this study is also higher compared to report of 4% by Okwelum *et al.* (2014) who reported a prevalence rate of 4%, the parasitological method adopted was thin blood smear technique which is far less sensitive than the PCR technique and the buffy coat technique. The prevalence rate was dissimilar to a 5.9% rate reported for N'Dama in lower Benue river area by Kalu

(1995) and 8.5% for slaughtered cattle in Nsukka abattoir, (Agu, 2005). Many other prevalence rates have been reported in different areas of Nigeria in past years, both in abattoirs and on the field. Ferguson (1964) reported the following prevalence rates for cattle in five different abattoirs in northern Nigeria, viz. 0.08% in Maiduguri, 3.4% in Kano, 0% in Kaduna, 0% in Kebbi, and 33.9% in Ilorin; 4 out of 443 (0.9%) slaughtered cattle in Akwa, according to Ekejindu *et al.* (1992); Ahmed and Agbede (1993) reported 21% in cattle around Zaria; Kalu (1995) reported 25% in Keteku cattle in lower Benue River areas; Kalu *et al.* (1996) reported 21.3% in Katsina Ala LGA of Benue State; and Maikaje (1998) reported 63.5% in cattle during the rainy season in Kaura LGA of Kaduna State using PCR technique. The relatively higher prevalence rate observed in this study, could be due to lack of veterinary attention for most the animals and thus, are hardly treated for trypanosome infection but more importantly the resilience of the Muturu cattle breed. It is established that the southern part of Nigeria is endemic for trypanosomosis as it lies within the tsetse infested belt of the sub-Saharan Africa. There was non-significant ($p > 0.05$) difference between the body weight and all

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haematological parameter of the cattle infected with *Trypanosoma vivax*. The haematological indices did not differ statically in the infected cattle and the uninfected cattle. Generally, there was evidence of infected cattle showing ability to maintain their PCVs and other haematimetric indices which corroborates the fact that Muturu is trypanotolerant as the maintenance of PCV by hosts infected with trypanosomes is a coping mechanism in trypanotolerance.

Conclusion

The study showed that the prevalence rate of *Trypanosoma vivax* infection was 87.22% in the cattle population. The prevalence rates of trypanosome infection are considered to be of economic and epidemiological importance in the area of study. The quantity of beef derivable from infected animals, even at asymptomatic chronic levels, may be drastically reduced due to poor body condition arising from muscle wasting. The infection of *Trypanosoma species* had no influence on the body-weight and haematological parameters measured. Some of the hosts were able to prevent anaemia as compensatory response was perceived in the macrocytic status of the peripheral blood erythrocytic series and also their ability to maintain RBCs and PCVs value in the phase of infection confirm their trypanotolerance.

Recommendations

It is recommended that animals should undergo Metaphylaxis (mass treatment of animals with curative drugs) on arrival from the North, and regular treatment of susceptible cattle that are reared in the south west as it is evident that the disease (trypanosomosis) is endemic in the area of study. This will prevent the animal from

clinical infection, and also prevent the complications of muscle wasting, poor carcass quality at slaughter, abortion, and most probable further spread of the disease.

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