NIGERIAN VETERINARY JOURNAL

Molecular Survey of *Trypanosoma congolense* "Forest-Type" in Nigerian Cattle

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SUMMARY

African animal trypanosomosis is an important livestock disease in Nigeria which is considered as a threat to the on-going effort on poverty alleviation in the continent. The disease is caused by several *Trypanosoma* species which are protozoan parasites transmitted by tsetse. Trypanosomosis is characterized by tissue injury including overwhelming activation of Classical Myeloid cells that results in destruction of the liver and uncontrolled parasite growth. Reduction in production and sometimes death are disease sequella. Hence, proper surveillance of the disease using a sensitive tool is very necessary for monitoring and control of trypanosomosis. Therefore this study concentrated on providing knowledge on the true infection rate of *Trypanosoma congolense* "forest type" and its effect on body weight and haematological parameters. Blood sample was collected from 180 cattle, DNA was extracted and PCR technique was adopted for prevalence study. Haematological analysis was carried out using Auto-haemo-analyser. Data generated were analysed using SAS statistical package. A prevalence rate of 91.67% was recorded. There were significant (p<0.05) differences between Body-weight, Red Blood Cell count, Mean Corpuscular Volume and Monocyte number of infected and un-infected cattle. The haematology reveals that hosts were able to respond to anaemia compensatorily as perceived in the macro-cytic, normo-chromic status of the peripheral blood erythrocytic generation. The study concluded that the true prevalence of trypanosome infection is high and has both epidemiological and economic importance. This study `provides information that could facilitate future monitoring and control of the disease in the study area.

Key words: Blood picture; disease surveillance; Nigerian indigenous cattle, PCR; trypanosome

INTRODUCTION

African bovine trypanosomosis (nagana) is a disease caused by extracellular haemoprotozoan parasites; *Trypanosoma (T) congolense, T. vivax and T. brucei brucei* transmitted by tsetse *(Glossina* species) (Oluwafemi *et al*., 2007). The disease is characterized by pyrexia, emaciation, loss of appetite, weakness, corneal opacity, parasitaemia, generalized lymphadenopathy and anaemia as the most prominent features of the disease (Fasanmi *et al*., 2014). Other pathologic effects are circulatory disturbances, leukopaenia, low serum complement levels,

lymphoid tissue hyperplasia and later hypoplasia, immunosuppression and sometimes death (Taylor and Authié, 2004). Trypanosomosis is consequently a severe constraint to animal agriculture in many parts of sub-Saharan Africa (McDermott and Coleman, 1982). There are three control strategies in cattle that are often used: trypanocidal drugs, vector control and the use of trypanotolerant cattle, none of which is fully effective in the long term (McDermott and Coleman, 1982). The control strategy with the greatest impact would have been vaccination. However, due to the antigenic variations of the parasite surface antigens, attributed to variable expression of antigenic forms of the variant surface glycoprotein (VSG), development of a vaccine is proving to be a difficult task. A vaccine based on VSG would have to cover the entire repertoire of antigenic types, which is not feasible (Taylor, 1998). Diagnosis or detection of trypanosome infection has involved the use of parasitological methods, immunodiagnostic and molecular diagnostic techniques (PCR). However, PCR is the most sensitive method as it detects specific parasite DNA with the added advantage of differentiating trypanosomes which have a similar morphology but very different economic impacts (Picozzi *et al*., 2002).

Desoxyribonucleic acids (DNA) probes have allowed the identification of four different sub-species of *T. congolense* in different ecological zones: *T. congolense* "forest type", *T. congolense* "savannah type", *T. congolense* "Kilifi type" and *T. congolense* "Tsavo" (Majiwa *et a*l., 1985; Masiga *et al.,* 1992; Majiwa *et al.,* 1993; Clausen *et al.,* 1998). These variants of *T. congolense* are pathogenic and develop high parasitaemia accompanied by anemia and leucopenia (Sidibe *et al.,* 2002; Bengaly *et al.,* 2002a, b). However, there are clear differences in the pathogenicity among the various types of *T. congolense.* For example, experimental studies comparing the virulence of one strain of each subgroup in mice and cattle have shown differences between the subgroups (Bengaly *et al.,* 2002a, b).

Animal Trypanosomosis is an important livestock disease in Nigeria which is considered as a threat to the on-going effort on poverty alleviation in the continent (Kalu *et al*., 2001) as it is still extensively distributed in all agro-ecological regions. Although, studies have been conducted in some of these areas (Murray *et al*., 1982; Paris *et al*., 1992; Ekejindu *et al*., 1992; Fakae and Chiejina, 1993; Samdi *et al*., 2010; Samdi *et al*., 2011), adequate information on the true prevalence of the disease in these areas is still scanty and not current to enable proper surveillance of the disease. Also, the diagnostic techniques used in some of these previous studies are less sensitive than PCR. These along with other factors hinder proper control of the disease (Maikaje, 1998; Enwezor and Ukah, 2000). This study investigated the true prevalence of *Trypanosoma congolense* "forest type" in herds of cattle in south western Nigeria using molecular tools and its effect on bodyweight and haematological parameters.

MATERIALS AND METHODS
Study 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). Blood samples were collected from Muturu cattle 149 in number from Ipokia Local Government Area of Ogun State. The location was considered to be natural habitat for Muturu herds in Nigeria. A total of 180 cattle were sampled for this study. The other breeds used in this study were White Fulani cattle (11), N'Dama cattle (3), crosses of Muturu and White Fulani (10), crosses of White Fulani and N'Dama (3), crosses of Muturu and N'Dama which were sampled in Alabata, Odeda LGA of Ogun State. The animals sampled were apparently healthy and were all sampled within the Tse-tse infested

zone hot-humid forest zone. The Muturu cattle which forms the greater percentage of cattle breed sampled were managed under the sedentary farming system where the animals were tethered in harvested farm lands and the animals feed on crop residues within a radius. It was obvious that these animals lacked veterinary care and was the only cattle breed kept by these traditional people in Ipokia LGA.

Phenotypic data collection and analysis of haematological parameters

The ages of the sampled cattle were determined using dentition while weights were determined

using a weigh-band. Five millilitres (5ml) of blood was collected via jugular venipuncture using EDTA vacutainer kits for analysis. Blood samples were analysed using an auto-haemo-analyzer (model BC2800VET). The haematological parameters analysed were; red blood cell, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemaglobin, mean corpuscular haemoglobin concentration, white blood cell, lymphocyte number, monocyte number, granulocyte number, lymphocyte percentage, monocyte percentage and granulocyte percentage.

TABLE I: The *Trypanosoma congolense* "forest-type" reference primer sequences used for the PCR analysis

Specificity of Primers	Forward primer	Reverse primer	Expected product size (bp)	Reference
T.congolense "forest type" (VSG gene)	5'- GGACACGCCAGAAGGTACTT-3' (TCF-F)	5'- GTTCTCGCACCAAATCCAAC-3' (TCF-F)	350	Masiga *et al.*, 1992

VSG gene- Variant surface glycoprotein gene

Analysis for prevalence of trypanosome infection using PCR technique

Genomic DNA was extracted from the whole 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. PCR diagnostic technique was used for the detection of *Trypanosoma congolense* infection using the Reference genotype-specific primer (Forward and Reverse) for the Variant Surface Glycoprotein (VSG) gene of the parasite. The oligonucleotide (Reference primers; Masiga *et al.*, 1992) used for this analysis is shown in Table I while the PCR conditions for the amplification of the gene is shown in Table II. Genomic DNA was used as a template in the PCR protocol. Every sample with amplification

size of 350bp on the agarose gel was considered positive or infected with trypanosome infection while the sample without amplification was considered negative or un-infected.

PCR protocol, Agarose gel preparation, electrophoresis and visualisation of PCR products

The PCR was carried out using a thermal-cycler (MultiGene OptiMax Thermal Cycler; TC9610/TC9610-230 Ver. 1.0 manufactured by Labnet International, Inc. 31 Mayfield Ave. Edison, NJ 08837, USA) following the Laboratory protocol and PCR conditions for the gene after the primer optimization. A 1% (w/v) agarose was prepared. Then the PCR products were loaded after the size marker GENEMate Quanti-Marker 100 bp DNA ladder was loaded.

The detection of the amplified fragments was done under UV light using a transilluminator, while the presence of the fragments of interest (350bp) was verified.

TABLE II: PCR condition for *Trypanosoma congolense* "forest-type" primer sequences used for amplification

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

TCF R/F-Trypanosoma congolense forward and reverse; VSG gene-Variant Surface Glycoprotein gene

TABLE III: Prevalence of *Trypanosoma congolense* "forest-type" based on sex, age and breed of Nigerian cattle

Factor	Sub-class	Number Tested	Number –ve	Number +ve	Percentage	Prevalence Rate (%)
Sex	Male	42	2	40	24.24	22.22
	Female	138	13	125	75.76	69.44
	Total	**180**	**15**	**165**	**100.00**	**91.67**
Age(years)	0 – 1	43	3	40	24.24	22.22
	>1 ≤ 2	46	2	44	26.67	24.44
	>2 ≤ 3	64	8	56	33.94	31.11
	>3	27	2	25	15.15	13.89
	Total	**180**	**15**	**165**	**100.00**	**91.67**
Breed	Muturu	149	9	140	84.34	77.78
	White Fulani	11	2	9	5.42	5.00
	N'Dama	3	–	3	1.81	1.67
	*WF x Muturu	10	2	8	4.82	4.44
	*WF x N'Dama	3	1	2	1.20	1.11
	*Muturu x N'Dama	4	–	4	2.41	2.22
	Total	**180**	**14**	**166**	**100.00**	**92.22**

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

Statistical analysis

Data was analysed using SAS 9.1 Statistical Package. Also means were separated for haematological parameters of infected and un-infected cattle using student t-test.

RESULTS

The prevalence rate of *Trypanosoma congolense* "forest-type" based on sex, age and breed of Nigerian cattle

The prevalence rate of *Trypanosoma congolense* based on sex, age and breed of Nigerian Cattle in the survey is represented in Table III. The overall prevalence rate was 91.7%. Twenty-two point twenty-two per cent (22.2%) was due to infection in the males while 69.4% of the infection was recorded in the female cattle. Prevalence based on Age(years): 0-1 was 22-22%; >1- ≤ 2 was22-44%; >2 - ≤ 3 was 31.11%; >3 was 13.89%. Prevalence based on Breed: Muturu was 77.78%; White Fulani was 5%; N'Dama was 1.67%; White Fulani and Muturu cross was 4.44%; White Fulani and N'Dama cross was1.11%; Muturu and N'Dama cross was 2-22%.

TABLE IV: Effect of *Trypanosoma congolense* "forest-type" infection on body weight and haematological parameters of Nigerian cattle

Parameters	Unit	Un-infected	Infected
Body weight (BWT)	Kg	179.47 ± 25.34^a	141.08 ± 5.77^b
Red blood cell (RBC)	$\times 10^{12}$/l	8.96 ± 1.26^a	4.98 ± 0.35^b
Haemoglobin (HB)	g/l	106.27 ± 7.05	106.14 ± 1.96
Packed cell volume (PCV)	%	31.37 ± 1.83	29.15 ± 0.51
Mean corpuscular volume (MCV)	Fl	27.78 ± 1.18^b	31.75 ± 0.44^a
Mean corpuscular haemoglobin (MCH)	Pg	61.67 ± 8.42	66.29 ± 2.65
Mean corpuscular haemog. Conc. (MCHC)	g/l	2375.67 ± 387.59	2166.23 ± 91.50
White blood cell (WBC)	$\times 10^9$/l	10.31 ± 1.38	12.64 ± 0.41
Lymphocyte (LYM)	$\times 10^3$/µl	6.05 ± 0.82	7.72 ± 0.30
Monocyte (MON)	$\times 10^3$/µl	0.63 ± 0.17^b	1.00 ± 0.05^a
Granulocyte (GRAN)	$\times 10^3$/µl	3.79 ± 0.47	4.13 ± 0.16
Lymphocyte percentage (LYMPC)	%	57.71 ± 2.72	58.64 ± 0.92
Monocyte percentage (MONPC)	%	9.55 ± 1.10^b	6.89 ± 0.29^a
Granulocyte percentage (GRANPC)	%	38.41 ± 2.36	34.13 ± 0.88

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

The effect of *Trypanosoma congolense* "forest-type" infection on body weight and haematological parameters of Nigerian cattle

The effect of *Trypanosoma congolense* "forest-type" infection on the cattle body weight and haematological indices in this study is shown in Table IV. There were significant differences (p<0.05) in the body-weight, Red Blood Cell count, Mean Corpuscular Volume, Monocyte numbers and Monocyte Percentage between infected and un-infected cattle. The body weight of the infected cattle was significantly (p<0.05) lower than that of un-infected cattle with values as 141.08 ± 5.77kg and 179.47 ± 25.34kg respectively. The Red Blood Cell count of the infected cattle was significantly (p<0.05) lower than that of the uninfected cattle with values as 4.98 ±

0.35×10^{12}/l and $8.96 \pm 1.26 \times 10^{12}$/l respectively. The Mean Corpuscular Volume of the infected cattle was significantly (p<0.05) higher than that of the un-infected cattle with values as 31.75 ± 0.44fl and 27.78 ± 1.18fl respectively. The Monocyte number of the infected cattle was significantly (p<0.05) higher than that of the un-infected cattle with values as $1.00 \pm 0.05 \times 10^{3}$/µl and $0.63 \pm 0.17 \times 10^{3}$/µl respectively. The Monocyte Percentage of the infected cattle was significantly (p<0.05) lower than that of the un-infected with values as $6.89 \pm 0.29\%$ and $9.55 \pm 1.10\%$ respectively.

DISCUSSION

Trypanosoma congolense "forest type" was detected using a genotype-specific reference primer (Masiga *et al*., 1992) in a PCR technique. The prevalence rate was 91.67%. This rate is high compared to the rate recorded by Ahmed (2007) who reported a trypanosome infection prevalence rate of 3.98%. It is possible that the prevalence rate obtained by Ahmed (2007) during his investigation could have been higher, if a more sensitive method, such as the dot-Elisa (Bossempem *et al*., 1995; Bossempem, 1996) or PCR was used. Also, the prevalence rate recorded in this present study could have been lower than the current rate if a less sensitive parasitological method of detection was used. This prevalence rate was also 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 (Polymerase Chain Reaction) technique is currently the most sensitive diagnostic method of 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 is also

insensitive 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 high compared to report 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. 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; A 0.9% was reported in slaughtered cattle in Akwa, according to Ekejindu *et al*. (1992). 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 of the animals and thus, are hardly treated for trypanosome infection. 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.

The body-weight of the infected cattle was lower than that of the un-infected cattle. This difference may be attributed to emaciation or muscle wastage that is associated with this infectious condition (Naessens *et al*., 2002; Naessens, 2006). The value of the RBC was higher in the un-infected than in the infected cattle, this difference signifies reduction in the erythrocytes numbers which associated with the infection of *Trypanosoma* species.

The mean corpuscular volume was higher in the infected than in the un-infected cattle which may be due to the reticulocytes which is associated with the progression of the infection (Swallow, 2000; Abenga *et al*., 2002; Fajinmi

et al., 2007) as immature red blood cells are pushed into the peripheral blood. The lower red blood cell numbers of the trypanosome-infected cattle compared to parasite-free ones in this study was indicative of anaemia, which is characteristic of the infection resulting from extra vascular erythro-phagocytosis in organs such as spleen, liver, lymph nodes, haemal nodes, lungs and bone marrow (Taylor and Authié, 2004).

The monocyte number also increased in the infected cattle as compared to the un-infected cattle which have resulted from response to infection as the host immune system produces monocytes which play roles as both chemotaxic and phagocytic actions on the parasites and the affected cells. The percentage of monocytes in the infected cattle was lower than the value in the un-infected cattle which may be due to the hosts property of reducing the pathology associated with trypanosome infection by down regulating the monocyte that contribute to inflammation in the course of infection (Bosschaerts *et al*., 2008).

The prevalence rate of *Trypanosoma congolense* infection is considered to be of economic and epidemiological importance in the area of study as the quantity of beef derivable from infected animals, even at asymptomatic chronic levels, may be drastically reduced due to poor body condition scores arising from muscle wasting. The infection had influence on the haematological parameters measured. Some of the hosts were able to compensate for anaemia as response was perceived in the macrocytic status of the peripheral blood erythrocytic series and also their ability to maintain PCV value in the phase of infection.

It is recommended that animals should undergo metaphylaxis (mass treatment of animals with curative drugs) on arrival from the Northern part of the country, and also regular treatment of susceptible cattle that are reared in the south west is advised as it is evident that 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.

ACKNOWLEDGEMENT

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