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Sequence Analysis of Human Trypanosome Detected in a 1-Year Old Boer-boel with a Case of Trypanosomosis in Abeokuta, Nigeria

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Abstract
African trypanosomosis is a devastating protozoan disease affecting man and animals. The species *Trypanosoma brucei gambiemse* and *T.brucei rhodiense* are specifically responsible for causing sleeping sickness in human in Africa. In this study, *T. brucei gambiemse* was molecularly detected in a dog presented to the Veterinary Teaching Hospital, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria with signs of corneal opacity, mucopurulent ocular discharges, pale mucous membrane and recumbency. Haematological examination revealed PCV of 12%, decreased lymphocyte and neutrophil counts. Gel electrophoresis of polymerase chain reaction (PCR) product revealed a band of 580bp corresponding to *T. brucei*. Sequencing of the PCR product revealed *T. brucei gambiemse*. We concluded that *T. brucei gambiemse* (a human infective trypanosome) is capable of causing clinical trypanosomosis in dogs. This report presents sequence analysis of human infective species of trypanosome molecularly detected in a 1-year old Boer boel breed of dog in Abeokuta, Nigeria.
Keywords: Sequences, *Trypanosoma brucei gambiemse*, Boerboel, Nigeria.

1. Introduction

African trypanosomosis is a hemoprotozoan disease caused by parasites of the genus *Trypanosoma*. The disease is widely distributed in tropical and subtropical regions and its epidemiology is determined by the ecology of its vector, the tsetse. According to World Health

Organization (W.H.O., 1998), the disease is found in many regions of the world, but mainly in sub-Saharan Africa between latitude 14ºN and 29ºS. In tsetse-infested sub-Saharan African countries, pathogenic trypanosome species are transmitted to a wide range of susceptible mammalian hosts, including dogs, through infective tsetse (*Glossina spp*) bites when taking blood meals (Brun *et al*., 2010). Dogs are specifically infected by *Trypanosoma congolense*, *T. brucei* -subspecies and *T. evansi* (Matete, 2003; Gow *et al*., 2007; Elov and Lucheis, 2009), causing canine African trypanosomosis (CAT). In almost all societies, dogs are widely used for various purposes, especially for companionship and security (Wells, 2007). Although, dogs pose a minimal risk for human infection, they seem to be an important sentinel for human infection (Greene, 2006). Dogs infected with human-infective *T. brucei rhodesiense* and *T. brucei gambiense* may serve as sources of infection for humans and other domesticated animals (Matete *et al*., 2003; Ezeokonkwo *et al*., 2010; Namangala *et al*., 2013).

1.1 History

A 1-year old female Boer-boel breed of dog weighing 26 kg, was presented to Ogun State Veterinary Clinic, Abeokuta, Nigeria with complaint of bilateral corneal opacity, anorexia and emaciation. History revealed that the pet owner recently relocated from a cattle farm settlement within the State (Plate 1).

1.2 Physical examination

On physical examination, rectal temperature, pulse rate, heart rate and respiratory rate were 39.3 ºC, 120 beats/minute, 116 beats/minute and 25 cycles/minute, respectively. There was lethargy, Pale ocular mucous membrane, bilateral mucopurulent ocular discharge, bilateral corneal opacity, emaciation and enlargement of superficial lymph nodes.

1.3 Tentative diagnosis:
A tentative diagnosis of canine trypanosomosis was made.

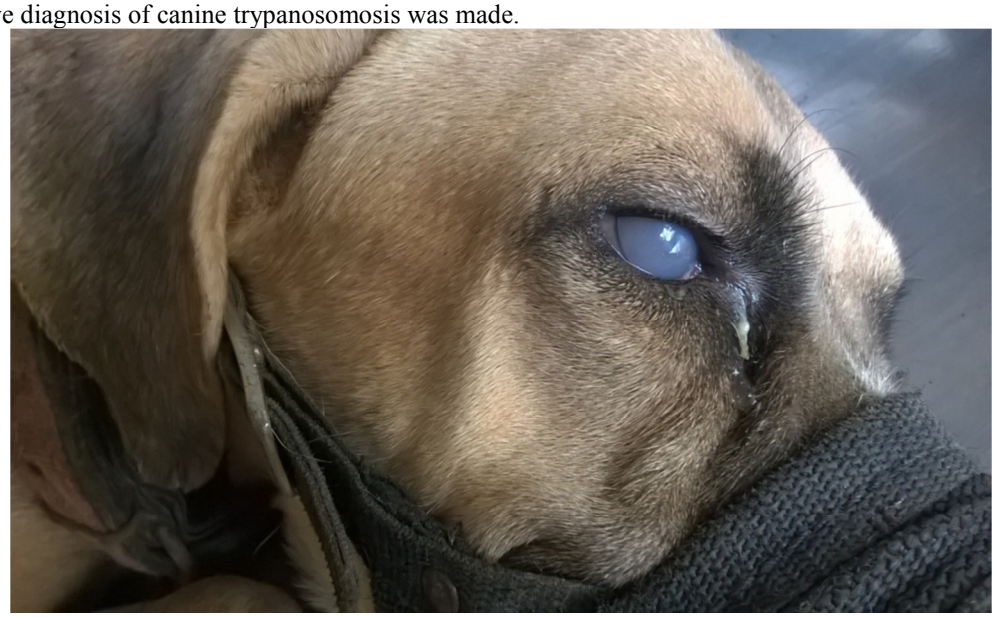

Plate 1: Mucopurulent ocular discharges and bilateral corneal opacity in dog infected with *T. brucei gambiense*

2. Laboratory examinations
2.1 Extraction of De-oxyribosomal nucleic acid (DNA)
Blood was collected into sample bottle containing Ethylene di-ammine tetracetic acid (EDTA) for analysis by Wet mount and polymerase chain reaction (PCR) techniques. DNA was extracted from the blood in EDTA bottle using Quick-gDNA™ MiniPrep (Zymo Research Corporation, Irvine, CA 92614, U.S.A) as described by the manufacturer. Briefly, 400 *µl* of genomic lysis buffer was added to 100 *µl* of blood, thoroughly mixed by vortexing for 4-6 seconds and incubated at room temperature for 5 – 10 minutes. The mixture, blood and the lyses buffer, was transferred to a spin column in a collection tube and centrifuge at 10,000 x g for 60 seconds after which the collection tube with the flow through was discarded and the spin column transferred to a new collection tube. 200 µl of prewash buffer was added to the spin column and centrifuged at 10,000 x g for 60 seconds after which 500 µl of genomic DNA wash buffer was added to the spin column and centrifuged at 10,000 x g for 60 seconds. To elute the DNA, the spin column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of nuclease free water was added to the spin column and incubated at room temperature for 2-5 minutes, centrifuge at 16,000 x g for 30 seconds.

2.2 Primer sets and optimization
Extracted DNA was subjected to a PCR test, which amplifies the ITS1 region of the rDNA gene of all African trypanosomes by using ITS1 CF/BR, CF:5'-CCGGAAGTTCACCGATATTG-3', BR: 5'-TGCTGCGTTCTTCAACGAA-3' as described by (Njiru *et al*., 2005). The 700bp, 480bp, 400bp, and 250bp of PCR products corresponding *to T. congolense*, *T. brucei* subspecies and *T. evansi, T. simiae* and *T. vivax* respectively, were amplified using MJ MiniTM Gradient Thermal Cycler (BIORAD, USA). This involved the initial denaturation of the of the DNA at 95^{o}C for 5 min followed by 35 cycles of 95^{o}C for 1 min, 58^{o}C for 1 min and 72^{o}C for 1 min with final extension at 72^{o}C for 10 min.

2.3 Gel Electrophoresis
One percent agarose gel containing 5-µl ethidium bromide in 1 x electrophoresis buffer was prepared. The electrophoresis chamber was filled with buffer solution (1 x electrophoresis buffer) until the top surface of the gel submerged by approximately 1 mm (about 400 ml). Three microliters (3 µl) of the sample buffer were mixed with 10 µl PCR product. The products (10 µl) and DNA ladder of 100 bp (10 µl) were placed into the agarose gel wells. The chamber was connected to a 90 Volt power supply and allowed to run for 40 minutes. The separated DNA products were viewed using UV trans-illumination and the bands were photographed using camera (Samsung, UAE).

2.4 Sequencing of PCR products
The PCR product of ITS-1 was sent to Cornell University Core Laboratory, Ithaca NY for sequencing, using Big dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) with the forward

amplification PCR primers and Ampli Taq-FS DNA polymerase. The sequences obtained were viewed and compared on Finch television (TV) and Sequence Scanner (Applied Biosystems) before they were aligned with each other and with published sequences of various *Trypanosoma spp* using the Molecular Evolutionary Genetic Analysis software (MEGA 5.05).

3. Laboratory result

3.1 Wet mount and haematological result

Wet mount film revealed flagellated organism moving fast across the microscopic field. Hematological results were PCV of 12%, total whit blood counts of 6.3 $\times 10^6$, neutrophil Counts of 77%, lymphocyte counts of 11% and monocyte counts of 6%.

3.2 Polymerase chain reaction (PCR) Result

The gel electrophoresis of the ITS-1 PCR product revealed band size of 480 bp which corresponded with the expected band sizes of *T. brucei*. Sequencing and sequences homology search revealed that *T. brucei* sequence had 96% homology with *T. brucei gambiense* (Accession number; FN554966).

3.3 Alignment of *T. brucei* sequence

The aligned *T. brucei* sequence with that obtained from GenBank showed that the sequence is less polymorphic. The sequence is characterized by insertion at point 179 (A), alteration at points 216 (A⇢G), 218 (T⇢A), 257 (A⇢C), 261 (A⇢C) and deletion at point 336 (T) (Figure I).

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                                                          ***************
T.b.gambiense      63 -------------------------------------------CTGATATCCATTATA 123
31.T.b.gambiense   67 TAAAAGCGTGCAGTTACTGTAGGTGACCTGCAGCTGGATCATTTTCTGATATCCATTATA 127

                      ************************************************************
T. b.gambiense    124 CAAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCAAAAATATACA 184
31.T.b.gambiense  128 CAAAAAAGAGCATATTTATGTGCATGTATAAATTGCACAGTATGCAACCAAAAATATACA 188

                      ************************************************************ *
T. b.gambiense    185 TATATGTTTTACATGTATGTGTTTCTATATGCCGTTTGACATGGGAGATGAGGGATGC-T 245
31.T.b.gambiense  189 TATATGTTTTACATGTATGTGTTTCTATATGCCGTTTGACATGGGAGATGAGGGATGCAT 248

                      ********************************** * ***********************
T. b.gambiense    246 ATACATAGTTCTGTTATTTTCTATCATGTATGTGTATTAGAGTGTCTGTGTTAATATACT 306
31.T.b.gambiense  249 ATACATAGTTCTGTTATTTTCTATCATGTATGTGTGTAAGAGTGTCTGTGTTAATATACT 309

                      ***************** **  ***************************************
T. b.gambiense    307 TTTTAATGCATGCTCTACATAATATACAGTAGTAATAACACAGAGAATACGTATGGAATG 367
31.T.b.gambiense  310 TTTTAATGCATGCTCTCCACCATATACAGTAGTAATAACACAGAGAATACGTATGGAATG 370

                      ********************************** * * ******** ** ******  **
T. b.gambiense    368 CGTATCTCTCTATATATATTTATGTATATATGCTATGTGTATATCAACCTCGCATA--TT 428
31.T.b.gambiense  371 CGTATCTCTCTATATATATTTATGTATATATGCCA-GGGTATATCACCCCCGCATATTTT 431
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Figure 1: Aligned *T. brucei gambiense* ITS1 rDNA sequences showing minimal points of deletion, insertions and alterations.

Key: T. b.gambiense = sequence obtained from GenBank, 31.T.b.gambiense = sequence obtained from the sample

4. Treatment

Diminazene aceturate at dose of 91 mg was given as single dose intramuscularly. Surportive therapies were iron dextram, 200mg and vitamin B complex, 2ml intramuscularly. On day 3 post-treatment, blood sample was taken for re-evaluation. The PCV increased to 17% while, the parasite was not seen on examination by wet mount technique, however, the corneal opacity did not subside. The client informed the clinic on day 10 post-treatment that the dog was improving but corneal opacity persisted. The client did not report to the clinic again for further re-evaluation of the health status of the patient.

5. Discussion

In this study, we attempted to shed light on the molecular characteristics of the trypanosomes detected in naturally infected dogs using a partial region of internal transcribed spacer 1 of ribosomal DNA sequences analysis. The aligned *T. brucei gambiense* sequence from this study with sequences obtained from Gen Bank was less polymorphic. This finding could not be compared due to lack of data on molecular characterization of *T. brucei gambiense* from Nigeria. The dog was presented with bilateral corneal opacity, this has been reported in several cases of Trypanosomosis (Matete, 2003; Abakpa *et al*., 2013).

Other clinical signs observed in this case were anemia, emaciation, ocular discharges, bilateral corneal opacity and they are similar to the report of Rani and Suresh (2007). The World Health Organization (2012) reported that *T. brucei gambiense* is responsible for chronic form of sleeping sickness in West and Central Africa, and accounts for more than 95% of reported cases of sleeping sickness. The detection of *T. brucei gambiense* which is of zoonotic importance, in this report, suggests that dogs can be infected by the human species, and this poses great risk to human beings in the area. This finding is in agreement with reports of Ezeokonkwo *et al*., (2010) and Namangala *et al*., (2013) that dogs serve as source of infection for humans. The pet owner was reported to have recently relocated from a cattle farm settlement. One can speculate that the infection must have been acquired from the cattle or cattle owners. This might be a window for spread of this zoonotic disease. After treatment with Diminazene aceturatee, the parasite was cleared from the blood thereby proving the efficacy of Diminazene aceturate (Berenil®) against trypanonosomosis in dogs (Rashid *et al*., 2008). Detection of human infective species (*T. brucei gambiense*) was an indication that dogs are means of transmission of *T. brucei gambiense* to human being.

6. Conclusion

The human infective specie of trypanosome (*T. brucei gambiense)* is capable of infecting dogs which can result in clinical condition. Diaminazene aceturate is effective against against *T. brucei gambiense* infecting dogs in Abeokuta, Nigeria.

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