

Molecular Diagnosis of Natural Infection of *Trypanosoma Congolense* in a Dog in Abeokuta, Nigeria: A Case Report

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Abstract: A case of trypanosomosis was reported in a 3-year old male Rottweiler breed of dog in Abeokuta Metropolis, Nigeria. The dog was presented with clinical signs of epistaxis, bilateral corneal opacity, anorexia, vomiting brownish materials and recumbency. Haematological examination revealed high lymphocytes count, decreased red blood cells, Pack Cell Volume, haemoglobin concentration, MCH, MCHC and platelet count. Diagnosis was made using wet mount and Polymerase chain reaction (PCR) amplification of the DNA using a primer set targeting the internal transcribe spacer 1 (ITS1) of rDNA, revealed single infection with *Trypanosoma congolense*. The dog was treated with a single dose of diminazene aceturate at the dose rate of 3.5 mg/kg body weight, intramuscularly along with the supportive therapy. The dog recovered 9 days post therapy.

Keywords: Abeokuta, canine trypanosomosis, dog.

I. Introduction

African animal trypanosomosis constitutes a major impediment to livestock production and economic development in several parts of sub-Saharan Africa, including Nigeria [1,2] despite decades of attempts to control the disease and its vectors [3]. All species of *Trypanosoma*, with the exception of some strains of *T. vivax* which produce a hyper acute and acute infection, are characterized by high parasitemia, fever, severe anaemia and haemorrhages on the mucosal and serosal surfaces [4]. Dogs are particularly susceptible to *Trypanosoma congolense*, which is transmitted cyclically by Glossina species. Dogs pose a minimal risk for human infection; however, they seem to be important as a sentinel for infection [5]. Anaemia is a cardinal feature of the disease in which red blood cells are removed from the circulation by the expelled mononuclear phagocytic system. Later, in infection of several months duration, when the parasitaemia become low and intermittent, anaemia may resolve to a variable degree [4]. There are a number of effective trypanosomacidal agents for dogs including suramin, quinapyramine and diminazene but single dose of diminazene diacetate is effective in eliminating the natural trypanosomiasis infection in canine [6]. The parasitological detection of trypanosomosis, based on detection of trypanosomes in blood of animals, has low sensitivity and is been hampered by fluctuating parasitemia [7]. In the continued efforts to explore an alternative means of diagnosing trypanosomosis, polymerase chain reaction (PCR) was found suitable and adapted for detection of trypanosome DNA using relevant primers [8].

However, there is a dearth of information on the incidence or molecular diagnosis of natural infection or disease in dogs in endemic areas of trypanosomosis such as Nigeria. This paper is to report a case of molecular diagnosis of natural infection of trypanosome in a Rottweiler in Abeokuta, Nigeria.

II. Case History

A 3-year old male Rottweiler dog was present to the small Animal Clinic of the Veterinary Teaching Hospital of the Federal University of Agriculture Abeokuta, with the complaint of vomiting, loss of appetite, sudden recumbency, nose bleeding and weakness, a day prior to presentation. History revealed that the dog was acquired as a puppy within Abeokuta metropolis and that it had neither travelled out of the town nor strayed. It was the only dog in the compound.

III. Clinical Examination And Management

Physical examination revealed high rectal temperature (40.8^oC), tachycardia, pale mucous membrane, generalized superficial lymphadenopathy, serous ocular discharges, bilateral corneal opacity (Fig 1), generalized debility, vomiting of brownish materials and epistaxis (Fig 2). Blood sample (4ml) was collected from the dog into EDTA bottle using the cephalic vein, for wet mount, complete blood count and polymerase chain reaction. The dog was given a single dose of diminazene aceturate at dose rate of 3.5 mg/kg body weight intramuscularly

along with supportive therapy. On day 4 post treatment, parasitological examination was carried to evaluate the level of parasitemia using Wet mount and thin blood smear methods. The complete blood count was performed using haematological auto-analyzer machine (Mindray).



Figure 1: Dog showing corneal opacity.



Figure 2: Dog showing epistaxis and corneal opacity

IV. DNA Extraction

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, 400µl 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. The eluted DNA was stored at -20° until use.

V. Primer Sets And Optimization

Samples were initially tested by species-specific primers directed against multi-copy satellite repeats of each species and strains. Two sets of primers were selected for optimization based on the published work. The primer sets include TBR 1 & 2 [9] and ITS1 CF & BR [10].

VI. Parasitological Detection Of Trypanosomes

From the anti-coagulated blood, two capillary tubes were filled and sealed at one end with plasticin. The capillary tubes were spun in microhaematocrit centrifuge at 10,000 rpm for 3 minutes. After centrifugation, the buffy coat and upper most layer of red blood cells was examined as described by [11] for the presence motile trypanosomes. Not less than 50 fields were examined before positive or negative was declared for each sample. While the haematocrit centrifugation technique (HCT) positive samples were further processed as thin smear stained with Giemsa for trypanosome species identification at (x 1000 magnification).

VII. Trypanosomes Detection By Pcr

Polymerase Chain Reaction amplification using species specific primer sets was performed in 20µl final reaction volume containing equivalent of 20ng of genomic DNA, 10mM Tris-HCl, pH 8.3, 1.5mM MgCl₂, 50µM KCl, 200µM each of dNTPs, 40ng of each of the primers and 1unit of *Thermus aquaticus* DNA polymerase (Bioneer USA). The reactions were placed in MJ Mini™ Gradient Thermal Cycler (BIORAD, USA). The reaction conditions were as follows TBR 1&2; Initial denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 30sec, 55°C for 30 sec, and 72°C for 30 sec; and final extension at 72°C for 5 min. The multiplex primer sets, ITS1 CF & BR were also used with initial denaturation of 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 58°C for 1 min and 72°C for 1 min with final extension at 72°C for 10 min. Ten microliter of the PCR products were electrophoresed through 1.0% agarose gel in 1 x TAE (40 mM TRIS-acetate and 1 mM EDTA) at 90 V for 60 min along with 10µl of biological marker, GENEMate Quanti-Marker 100 bp DNA ladder (BioExpress, UT, USA). Gels were stained with Ethidium bromide at 5µl/100ml of the agarose gel suspension. After electrophoresis, the PCR products were visualized using ultra violet transilluminator (Spectroline^R TC 312 E) before the gels was photographed

VIII. Result

8.1. Haematological values and parasitological results

Haematology revealed high lymphocytes (31.6 %), decreased red blood cells ($4.83 \times 10^{12}/L$), haemoglobin concentration 8g/L, PCV (32%), MCH (16.5pg), MCHC (246g/L) and platelet count ($27 \times 10^9/L$). Examination of the buffy coat revealed a sluggishly moving trypanosome and the Giemsa stained smear made from the second buffy coat from the second capillary tube revealed trypanosomes with marginally paced kinetoplast.

8.2 Gel electrophoresis and Polymerase Chain Reaction (PCR)

Agarose gel electrophoresis of the PCR products revealed a band size of about 700bp (Fig. 3)

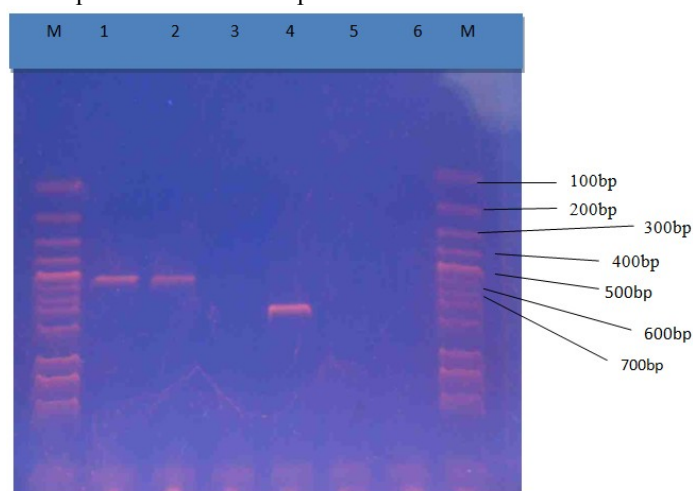


Figure 3: Electrophoresis analysis of the PCR product Trypanosomes

M: Molecular marker

1-2: Trypanosoma brucei, 3-5: Trypanosoma congolense, 6: Negative control

IX. Discussion

Canine trypanosomiasis is a disease which its prevalence in Nigeria has been neglected in Nigeria. The frequency of re-emerging unpublished field cases call for concern. The clinical signs observed in this case were, high temperature ($40.8^{\circ}C$), pale mucous membrane, bilateral corneal opacity, epistaxis, splenomegaly, lymphadenopathy and generalized debility. These observations were in agreement with the findings of [6] who reported *T. evansi* organism in peripheral blood with history of inappetence, dullness and persistent fever for five days and bilateral corneal opacity which is a characteristic finding in chronic trypanosomiasis [12]. Anaemia, though not pathognomonic, is common feature in trypanosomiasis. In this case, the Red Blood Cells, Packed Cell Volume, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet were decreased. The results of the present study were similar to the observations of [13] who studied clinical hematology of canine chagas disease in Texas and reported decreased Packed Cell Volume and also recorded hypoproteinemia, hypoalbuminemia and thrombocytopenia. The diminazene aceturate given intramuscularly once at a dosage of 3.5 mg/kg was effective and the parasitemia was cleared on day 4 post therapy. Similar results were recorded by [6] who treated trypanosomiasis in Pomeranian dog with a single dose of diminazene aceturate.

X. Conclusion

In conclusion, canine trypanosomiasis may be prevalent among dogs in Nigeria and elsewhere than reported. We recommended that veterinarians in the field should endeavour to rule out canine trypanosomiasis when diagnosing infection due to haemoparasites.

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