

Molecular detection of *Trypanosoma species* and haematological alterations in four trypanosome-infected Nigerian horses

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Abstract

Trypanosomes cause anaemia and are responsible for widespread morbidity and mortality particularly in imported breeds of animals found in sub-tropical and tropical parts of the world. Light microscopy and polymerase chain reaction (PCR) were used to detect trypanosomes in naturally infected Nigerian crossbred horses at Obollo-Afor abattoir, Udenu Local Government Area, Enugu State Nigeria. Blood was collected via the jugular outflow from a total of 200 horses of varying ages and either sex. Conventional procedures were followed during the PCR assay, parasite identification in wet mount, Leishman-stained thin blood and buffy coat smears on glass slides. Light microscopy revealed *Trypanosoma* species with an elongated, streamlined and tapered body, highly suggestive of *T. brucei brucei* or its subspecies *T. evansi* or *T. equiperdum*. PCR assay produced the expected fragment size of 700 bp specific for ITS-1 region of the 18SrRNA gene of *Trypanosoma species* in 4 (2%) of 200 blood samples against the routine blood and buffy coat smear examination, which revealed trypanosomes in 3 (1.5%) out of 200 blood samples. Sex and age were not significantly ($p > 0.05$) associated with the trypanosome infection. One of the *Trypanosoma* infected anaemic horses had microcytic normochromic anaemia, high erythrocyte sedimentation rate and normal leukocyte count, while one of the *Trypanosoma species* infected non-anaemic horses had erythrocytic parameters and ESR values that are within the reference range, with leukocytosis. It was concluded that the prevalence of equine trypanosomosis was very low, and it's characterized by mild to moderate anaemia in clinical cases.

Keywords: anaemia; Nigerian horses; PCR assay; prevalence; *Trypanosoma* species

Introduction

Trypanosomosis is a disease caused mainly by trypanosomes such as *Trypanosoma vivax*, *T. congolense*, *T. brucei*, *T. equiperdum* and *T. evansi*, and which is responsible for huge production losses in the livestock industry (Seidl *et al.*, 1998). Trypanosomes are extracellular, microscopic and elongated protozoan parasites

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belonging to the genus *Trypanosoma* that thrive in the peripheral blood circulation and in tissues (Radostits *et al.*, 2007). This haemoprotozoan is virulent, inoculable but not contagious, except for *Trypanosoma equiperdium* that is transmitted by coitus (Radostits *et al.*, 2007). These parasites are commonly found in the tropics and affect a wide variety of domestic and wild animals (Ugochukwu, 2009). It causes high morbidity and high mortality in infected herds, thereby making the disease very difficult to manage in endemic areas (Ugochukwu, 2009).

These trypanosomes of veterinary and economic importance are cyclically transmitted by tse-tse (*Glossina* spp.) and mechanically transmitted by other biting flies such as *Tabanus* spp. and *Stomoxys calcitrans* (Mijares *et al.*, 2010). The severity of the infection is dependent upon the species, co-infection, age and nutritional status of the horse. A disease condition known as Nagana (African trypanosomosis) is caused by *T. congolense* and *T. brucei brucei* in horses while Surra is caused by *T. evansi* and *T. vivax*. In general, trypanosomosis is characterized by macrocytic hypochromic anaemia, leukopenia, thrombocytopenia, increased erythrocyte sedimentation rate, monocytosis, increased serum activities of liver enzymes, elevated blood urea nitrogen, hypoalbuminemia and hypergammaglobulinemia, fever, anorexia, dullness, weight loss, tissue damage, immunosuppression and in some cases, death (Anosa, 1988a, 1988b; Kihurani *et al.*, 1994; Auty *et al.*, 2008; Mijares *et al.*, 2010; Hussain *et al.*, 2014; Agina and Ihedioha, 2016; Agina, 2017). Trypanosomosis caused by *T. brucei brucei* infection characterized by presence of subcutaneous oedema of the dependent areas such as the ventral abdominal wall, thorax and limbs, keratoconjunctivitis, ataxia and paralysis (Radostits *et al.*, 2007). *Trypanosoma vivax* or *T. congolense* usually produce the chronic form and may cure spontaneously in local breeds (Taylor and L-Authie, 2004). Surra (Mal de Caderas) caused by *Trypanosoma evansi*, is an endemic disease of mainly horses and camels in the tropics and subtropics. The disease is associated with fever, subcutaneous oedema, nervous signs and death. *Trypanosoma evansi* is transmitted mechanically by blood sucking horse flies, from one susceptible host to another and does not possess the insect stage (Lai *et al.*, 2008; Luckins, 1998). At the molecular level, *T. evansi* is said to possess an akinetoplastic DNA (complete loss of kinetoplastid DNA) and this locks the trypanosome in the bloodstream form (Lai *et al.*, 2008). Dourine (mal de coit) is a venereal disease of donkeys, horses, mules and water buffaloes caused by *T. equiperdum*. The disease is characterized by low morbidity and high mortality. *Trypanosoma equiperdum* is one of the pathogenic trypanosomes that does not require an arthropod vector for its transmission, and at the molecular level, they possess partial loss of kDNA (dyskinetoplastid) which also locks it in the bloodstream stage (Lai *et al.*, 2008). Natural transmission occurs only by coitus as the organism inhabits the mucosa of genital organs. Donkeys have some degree of resistance to the infection but may be compromised by any form of stress (Agina, 2017).

The horse is a domesticated animal used for transport, draft, sports, recreation, research purposes and as food (source of meat) worldwide) (Edwards, 1994). The African breeds of horses include the Dongola breed, West African Barb, Nigerian local (Arewa) breed of horses and their crosses with Arabian, Dongola, Barb and Sudanese breeds. Their coat colours are a deep reddish bay, sometimes chestnut or black (Hendricks and Dent, 2007). They are commonly found in the northern part of Nigeria (Ihedioha and Agina, 2013, 2014). The largest category of horses in this region is the mixed 'Arewa' breed and is kept for rides during festivals (Garba *et al.*, 2011).

There is little information on the molecular prevalence of *Trypanosoma* species in Nigerian horses. Therefore, this study was conducted to ascertain the prevalence of naturally occurring trypanosome infections in Nigerian horses at Obollo Afor horse lairage, Udenu Local Government, Enugu State, Nigeria and to determine the haemato-pathological features of the disease. To the best of our knowledge, this is the first molecular detection of trypanosomes in Nigerian horses.

Materials and Methods

Ethical approval

This research was done in accordance with the Ethics and Regulations guiding the use of animals as approved by the University of Nigeria Senate committee on Medical and Research ethics, ECUN/77932.

Study design

The study was a cross-sectional survey of Nigerian horses at the Obollo Afor horse lairage, Udenu Local Government Area, Enugu State, Nigeria. Obollo Afor is located between latitude 6° 54' 56" and longitude 7° 30' 55". The Obollo Afor horse lairage is the transit and sales point for horses mainly of the Arabian, Barb-Arab and Dongola breeds and cross-bred horses shipped from Northern to Eastern Nigeria for agricultural and teaching purposes (Ihedioha and Agina, 2013, 2014) The horses are kept in the lairage for 1-4 weeks after arrival before slaughter for meat.

Sample size determination

To study the status of the prevalence of trypanosome infection, the expected prevalence was considered 2.4%.¹⁰ with confidence limits of 95% and a desired absolute precision of 5% to collect a minutes number of samples, according to (Thrusfield, 2005) The number of samples thus calculated was adjusted for finite population and was correlated with 200 samples (200 horses). Blood samples were collected every two weeks throughout the 5-month study period from July – November 2017. These horses were subjected to comprehensive physical examination and aged before blood samples were collected for parasitological evaluations.

Aging of the horses

The ages of the horses were estimated based on tooth eruption and wear (Ensminger, 1969).

Blood sample collection

Blood for parasitological and haematological determinations was collected from the jugular outflow at slaughter into ethylenediaminetetraacetic acid (K₂EDTA) blood tubes. Blood sample (125 µL) for PCR assay were placed in a circular motion onto designated spots on Whatman® FTA® classic cards (Sigma-Aldrich, USA), the blood was air-dried for 60 minutes. The blood sample-FTA cards were stored at room temperature of approximately 25 °C until further use.

Parasitological diagnosis

Trypanosome identification by direct examination techniques

The wet blood film (wet mount) was done by placing a drop of blood on a clean grease-free microscope slide and covering it with a clean cover slip. The wet mount was examined with a light microscope at 10 × 10 magnification for the movement of the trypanosomes which is used as a means of identification of the specific *Trypanosoma* species. A thin blood smear was made with the aid of a clean cover slip after placing about a drop of blood on another clean grease-free microscope slides. The slides were air dried and stained following the Leishman technique and examined under oil immersion using a light microscope at 10 × 100 magnification. Identification of parasites was done using morphological description by Hoare (1972).

Parasite concentration technique (microhaematocrit centrifugation/buffy coat technique)

This involved the centrifugation of a microhaematocrit capillary tube containing the blood sample and microscopic examination of the buffy coat/plasma junction (Murray *et al.*, 1977). Two-thirds of the micro-hematocrit capillary tubes were filled with blood by capillary action and one end was sealed with plasticine. The tubes were placed in a micro-hematocrit centrifuge (Techmel and Techmel, USA) and spun at 10,000

revolutions per minutes (1120 x g) for 5 minutes. The tubes were cut at the buffy coat/plasma junction and extruded onto a clean microscope slide and covered with a coverslip and examined using a light microscope.

Conventional PCR assay

Polymerase chain reaction (PCR) primers were selected based on previously published work (Adams *et al.*, 2006). Details of the primers are presented in Table 1.

Table 1. Genus primer for trypanosome species gene amplification

Parasite	Primer sequence (5'-3' Forward) and Reverse	Gene	Amplicon size (bp)	Reference
<i>Trypanosoma spp.</i>	TRYP3: TGC AAT TAT TGG TCG CGC TRYP4: CTT TGC TGC GTT CTT	ITS-1	150-750	Adams <i>et al.</i> , 2006

Genomic DNA extraction

Genomic DNA was extracted from 150 randomly selected designated spots on blood sample-FTA cards using Chelex 100 resin. Briefly, a 5% w/v suspension of Chelex 100 resin in sterile water was prepared prior to use and stirred to obtain a suspension. Harria Uni-Core disposable punch (2.0 mm) was used to remove sample discs from the centre of a dried blood sample spot. The discs were placed in a clean RNase/DNase free 1.5 mL micro centrifuge tube. Discs were washed by adding 1 mL of sterile water to each sample tube and incubated at room temperature for 10 minutes with occasional vortexing. The water was removed, washing was repeated by adding 1 mL of sterile water to each sample tube and incubated at room temperature for 10 minutes with occasional vortexing. The samples were centrifuged for 3 minutes at 14,500 rpm. The supernatant was removed and discarded. 200 µL of Chelex 100 resin suspension was added using a large-bore pipette tip to the sediment. The samples were incubated at 56 °C for 20 minutes with vortexing every 10 minutes. The samples were vortexed for approximately 15 seconds and incubated at 95 °C for 10 minutes. The samples were vortexed for approximately 15 seconds and centrifuged at 14,500 rpm for 5 minutes. The supernatant was transferred (about 150 µL) to a sterile pre-labeled tube being careful to avoid taking the Chelex 100 resin pellet. The eluted DNA was stored at -20 °C.

PCR analysis

Polymerase chain reaction was performed in a 25 µL final reaction volume comprising 12.5 µL of Dream Taq 2x mix (ThermoFisher Scientific, USA), 0.1 µL of each primer (100µM), 5 µL of DNA template, 7.3 µL of nuclease free water (Promega, USA). Negative control (template DNA substituted with nuclease free water) and positive test controls (*Trypanosoma brucei brucei* J10 and *Trypanosoma congolense savannah* WG81) were incorporated into each run. The PCR tubes were placed in a thermocycler (Bio-Rad, USA). The thermocyclic conditions were as follows: pre-denaturation at 95 °C for 3 minutes followed by 35 cycles of 95 °C for 1 minute, 54 °C for 1 minute, 72 °C for 30s, and followed by final extension of 72°C for 5 minutes. Fifteen microliters of the PCR products were electrophoresed through 1.7% agarose gel in 1x TAE (Tris-acetic acid-EDTA) buffer at 100V for 60 minutes, along with 15 µL Hyperladder™ Marker 100 bp DNA (Bioline, UK). Gels were stained with ethidium bromide at 5µL/100 mL of the agarose gel suspension. Gels were viewed under UV transilluminator (Blook™, Genedirex). Images were captured using an AlphaImager Hp System (Protein Simple, USA). To prevent cross-contamination, work areas were designated solely for DNA extraction and PCR amplification. All reagent preparation was done in a dedicated biosafety cabinet and was UV illuminated before and at the end of each session to avoid DNA contamination.

Haematological analysis

The packed cell volume (PCV) was determined by the micro-haematocrit method (Thrall and Weiser, 2002) while haemoglobin concentration (Hbc) was determined by the cyanomethaemoglobin method (Higgins *et al.*, 2008). Red blood cell (RBC) counts and total leukocyte counts (TLC) were carried out by

haemocytometer method (Thrall and Weiser, 2002). Differential leukocyte count was done by making a blood smear made on clean glass slide and stained following the Leishman technique. The different cells of the leukocytic series were enumerated by the battlement counting method (Thrall and Weiser, 2002). The mean corpuscular values – mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) was calculated using the standard formulae (Coles, 1986). Erythrocyte sedimentation rate was determined by Westergren method (Coles, 1986).

Ethical issues

This research was done in accordance with the Ethics and Regulations guiding the use of animals as approved by the University of Nigeria Senate committee on Medical and Research ethics, ECUN/77932.

Statistical analysis

Data obtained from this study were subjected to descriptive statistics with the aid of the Statistical Package for Social Sciences (SPSS) version 16 (Chicago, USA). Chi-square or Fisher exact test was used to analyse for associations between sex (male and female), horse age (young, adult, and old), and infection with trypanosomes. Values of $p < 0.05$ were considered statistically significant. The prevalence of trypanosome infections in the horses is expressed as the percentage of the sampled horses.

$$\text{Prevalence} = \frac{\text{Number of Trypanosome positive horses}}{\text{Number of horses sampled}} \times 100$$

Results

Demographic data on the horses sampled.

Out of the 200 horses sampled, 7 (3.5 %) were young (1-4 years old), 175 (87.5%) were adults (5-11 years old), and 18 (9 %) were old (>12 years old). A total of 138 (69.0%) were females (mares) while 62 (31.0%) were males (stallions) (Table 2).

Table 2. Distribution of Trade horses sampled by their demographic characteristics

Demographic characteristics	Total number sampled	Percentage (%)
Age		
Young (1 – 4 years)	7	3.5
Adult (5 – 11 years)	175	87.5
Old (> 12 years)	18	9
Sex		
Female (Mare)	138	69
Male (Stallion)	62	31
Health Status		
Trypanosoma positive horses	4	2.0
Trypanosoma negative horses	196	98

Trypanosome identification

None of the wet mounts examined under the light microscope showed evidence of movement/motility (No motile parasites were seen). The trypanosome species identified in a Leishman-stained thin blood smear under x1000 magnification of the light microscope was highly suggestive of *T. brucei* (Figure 1). It has an elongated body, a streamlined and tapered shape, and its kinetoplast lies near the basal body. A single flagellum runs towards the anterior end, and along the body surface, the flagellum is attached to the cell membrane forming an undulating membrane (Figure 1). The buffy coat concentration technique yielded one positive result (Table 5).

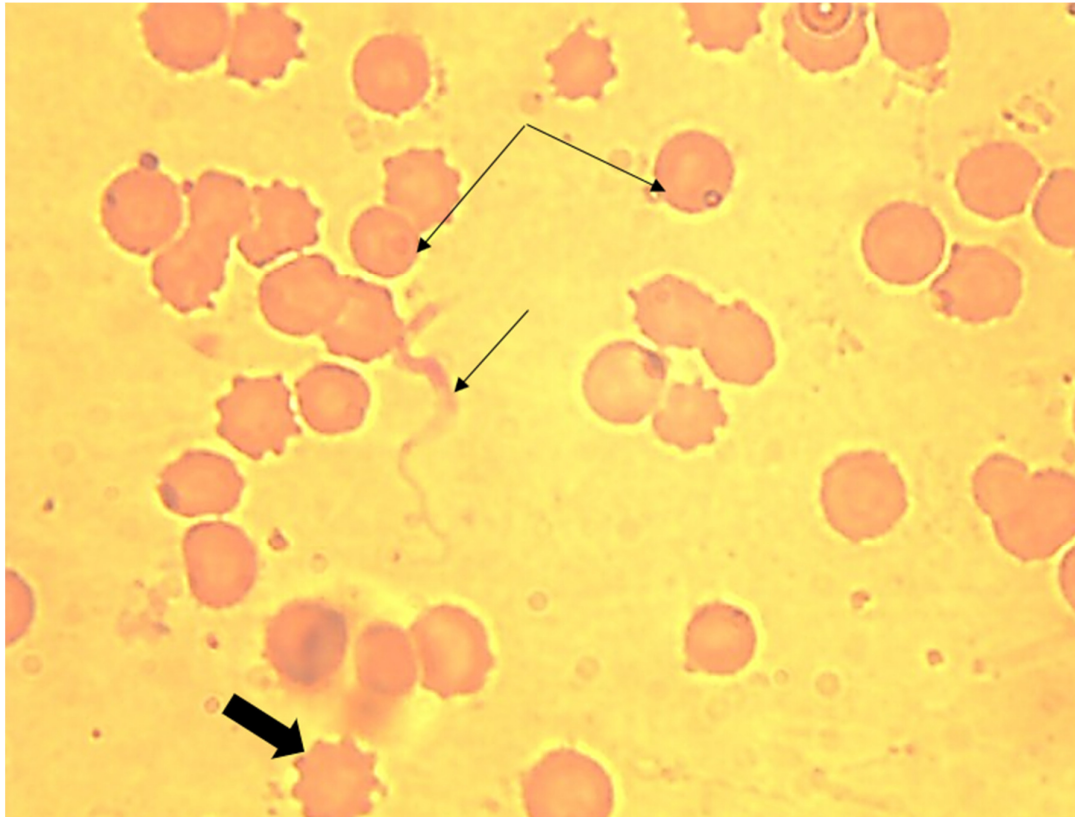


Figure 1. The blood picture of *Trypanosoma species*(arrow) infected horses showing the echinocyte (block arrow) and red blood cells of different sizes (double arrow) X1000 magnification

Prevalence of trypanosome infections in the horses

The Leishman-stained blood smears showed that 3 (1.5%) out of the 200 horses sampled were found to have trypanosomes in blood (trypanosome positive), while 197 (98.5%) were trypanosome negative (Table 2). The females (mares) had a 2.2% prevalence rate of trypanosomosis while males (stallion) had 1.6% prevalence rate (Table 3). There were no significant association ($P=0.176$) between the prevalence of trypanosomosis and sex of horses (Table 3).

Table 3. Chi-square test-based association analysis of trypanosomosis in male and female trade horses

Sex	Number sampled	Number Positive	Chi-square	Fisher's exact test	P value
Female	138	3 (2.2%)	1.834	1.356	0.176
Male	62	1 (1.6%)			

The young horses had the highest prevalence of trypanosomosis (14.3%), followed by the old horses (5.6%), and 1.1 (%) prevalence of trypanosomosis in adult horses (Table 4). There was no significant association ($P=0.077$) between the prevalence of trypanosomosis and age of horses (Table 4).

Table 4. Fisher’s exact test-based association analysis of trypanosomosis in trade horses of different ages

Age	Number sampled	Number Positive	Chi-square (Fisher’s exact test)	P value
Young	7	1 (14.3)	6.522	0.077
Adult	175	2 (1.1)		
Old	18	1 (5.6)		

Clinical and haematological findings

Twenty-six (13%) of sampled horses were found anaemic. Only 2 (7.7%) out of the 26 anaemic horses were trypanosome positive while 2 (1.1%) out of the 174 non-anaemic horses had trypanosome parasite. *Trypanosome* was present in 2 anaemic horses and 2 non-anaemic horses (Table 5). The anaemic horses had lower PCV (22% and 15%), Hbc (6.05 g/dl and 6.70g/dl) and RBC counts ($4.52 \times 10^6/\mu\text{L}$ and $4.50 \times 10^6/\mu\text{L}$) than those of non-anaemic horses (41.5 % and 36%). One of the anaemic horses had mild normocytic normochromic while another had moderate microcytic normochromic anaemia. The white cell count of the non-anaemic horses was high ($15.70 \times 10^3/\mu\text{L}$ and $17.45 \times 10^3/\mu\text{L}$) when compared to the anaemic horses ($10.95 \times 10^3/\mu\text{L}$ and $8.75 \times 10^3/\mu\text{L}$). The erythrocyte sedimentation rate of horse 33 (anaemic horse) was higher (75 mm^3) than that of horse 170, the non-anaemic horse (28.26 mm^3). Clinical findings observed in the anaemic horses were paleness of the mucous membrane, weakness and mild tick infestation. One horse had opaque cloudiness of the eye (Table 5). Few ticks were found attached especially in the inguinal regions of all four horses.

Table 5. Clinical and haematological findings of four horses with *trypanosomosis*

Clinical Signs and haematological parameters	Horse 4	Horse 18	Horse 33	Horse 170
Age (years)	1	4	5	12
Sex	M	F	F	F
Breed	Crossbred	Crossbred	Crossbred	Crossbred
Clinical and haematological findings	Anaemia, pale mucous membrane, mild tick infestation	Cachexia, opaque cloudiness of the eye, leukocytosis, mild tick infestation	Anaemia, weakness, pale mucous membrane, mild tick infestation	Mild tick infestation, lame, leukocytosis
Wet mount	-	-	-	-
Buffy coat	-	-	+	-
Leishman stain thin-blood smear	+	+	-	-
PCR assay	+	+	+	+
PCV (%) [31.5 – 53.50]*	22	41.5	15	36
Hbc (g/dL) [11.53-19.86]*	6.05	12.11	6.70	13.46
RBC count ($\times 10^6/\mu\text{L}$) [6.46 – 11.16]*	4.52	9.06	4.50	7.35
MCV (fl) [38.37-60.96]*	48.67	45.81	33.33	48.98
MCHC (g/dL) [27.67-55.03]*	27.50	29.18	44.67	37.39
ESR (20 mins/ mm^3) [21.0-37.10]*	ND	ND	75	28.26
WBC count ($\times 10^3/\mu\text{L}$) [5.40-13.90]*	10.95	15.70	8.75	17.45

Note: M – Male; F – Female; ND: Not done; PCV: Packed cell Volume; Hb: Haemoglobin concentration; RBC: Red blood cell; MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; ESR: Erythrocyte sedimentation rate; WBC: White blood cell.

*Reference range of values for haematological parameters are in parentheses.

Conventional PCR

The generic PCR based assay produced an amplicon size of 700 bp of internal transcribed spacer-1 region of 18srRNA gene of trypanosome species (Figure 2), in 4 (2%) out of 200 sampled horses. The amplicon size of our samples was same with the positive control, *Trypanosoma brucei brucei* J10. The infection confirmed in Horses 18 and 170 (Table 5) by PCR were subclinical based on the absence of anaemia.

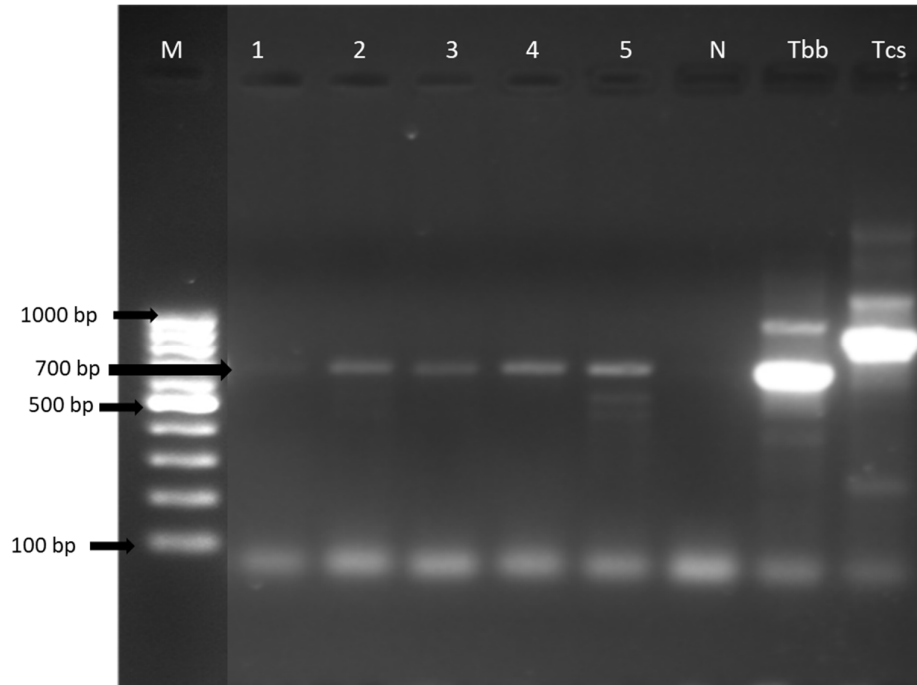


Figure 2. 1.7% agarose gel electrophoresis image showing a 700 bp band for the PCR amplification of Internal transcribed spacer- 1 (ITS-1) region of the 18S ribosomal RNA gene of *Trypanosoma species*. Lane M: 100 bp DNA Marker (Bioline); Lane 1: Negative horse sample; Lanes 2 - 5: positive horse samples; Lane N: Negative control (no DNA template); Lane Tbb (*Trypanosoma b. brucei*) J10: positive test control; Lane Tcs (*Trypanosoma congolense savannah*) WG81 positive test control. Bands were visualized by staining with ethidium bromide.

Discussion

Our PCR amplicon size compared favourably with that of the positive control, *Trypanosoma brucei brucei* J10 and this suggests that the amplified species maybe *T. brucei brucei*, *T. evansi* or *T. equiperdum*. *Trypanosoma evansi* or *T. equiperdum* were suggested because the parasite was detected in horses and are indistinguishable from each other (Li *et al.*, 2007). There are several speculations or controversy that they might all be the same species and certainly closely related to *T. brucei* (Lun *et al.*, 1992; Lai *et al.*, 2008; Carnes *et al.*, 2015; Wen *et al.*, 2016).

In this study, the prevalence of trypanosome infection was low (2.0%), and this agreed with a previous study in Eastern Nigeria where 0% prevalence was reported after 100 horses were sampled (Unpublished), followed by prevalence rate of 2.4% reported in 2016 (Agina and Ihedioha, 2016). Trypanosome identification in both studies were performed by light microscopy. The low prevalence rate recorded in this study was not in agreement with a prevalence study in Gambia where 45.5 % was documented (Faye *et al.*, 2001) and 91% documented in 2008 (Pinchbeck *et al.*, 2008), with *T. vivax* being the most prevalent *Trypanosoma* species using PCR (Pinchbeck *et al.*, 2008). The finding of a trypanosome in one buffy coat smear could be due to low

parasitemia or that the horse might be presymptomatic or a chronic carrier, as only one trypanosome was found on an entire thin blood smear (Figure 1). In addition, there is also a possibility that the low prevalence rate might have been underestimated as small volume of blood was taken from a horse which is a large animal, and randomly selected punches from FTA cards were utilised for the PCR analysis (Cox *et al.*, 2010). Also, the low parasitemia could be because the horses might be under chemotherapy or the infection was not a recent one. The finding of a low prevalence rate of trypanosome infection in Nigerian horses as observed in this study may be attributed to the use of light microscopic identification of the parasites and conventional PCR. Nested, hemi-nested PCR and PCR-based single strand conformation polymorphism (PCR-SCPP) are most sensitive in detection of trypanosomes (Desquesnes and Tresse, 1996; Wen *et al.*, 2016). Microscopy is labour intensive, less sensitive and cannot be used to correctly differentiate between species, between current and previous infections and may not detect the parasites in blood in case of low parasitemia or during chemotherapy, thereby leading to false negative results (Desquesnes and Dávila, 2002; Li *et al.*, 2007). For example, microscopic technique cannot differentiate between *Trypanosoma brucei*, *T. evansi* and *T. equiperdum* because of the morphological similarity that exists between them, except through the molecular composition of their kinetoplast DNA (kDNA) (Sánchez *et al.*, 2015). Analysis of their total ITS sequences showed a high degree of homology by phylogenetic analysis (Wen *et al.*, 2016). PCR is very sensitive that parasitemia as low as 10 parasites per milliliter of blood can be detected (Desquesnes and Dávila, 2002; Li *et al.*, 2007). Molecular techniques such as PCR-SCPP are more sensitive and precise in the differentiation of these three members of the subgenus *Trypanozoon* and provided evidence to further support the notion that *T. evansi* and *T. equiperdum* could be subspecies or strains of *T. brucei* (Wen *et al.*, 2016).

Furthermore, the low prevalence rate of equine trypanosomosis could be that the environmental conditions in Northern Nigeria where these horses are reared are not favourable for the growth and survivability of the tse-tse or biting fly, and these horses only come down with the infection when they are brought to the South-Eastern Nigeria for sale and later slaughtered for meat.

The finding of mild normocytic normochromic anaemia in one of the *Trypanosoma* infected horses and moderate microcytic hypochromic in other horse is not in agreement with Agina and Ihedioha (2016) who reported macrocytic hypochromic anaemia in the horse. Therefore, we presumed that the infection is subclinical and the major aberrant alterations in the haematological parameters such as leukopenia are yet to manifest. Leukopenia is a major manifestation of trypanosomosis as this parasite causes myeloid hypoplasia (Stockham and Scott, 2008). The trypanosome positive horses with normal erythrocytic parameters might be chronic or asymptomatic carriers. The high erythrocyte sedimentation rate in one of the anaemic horses agrees with Agina and Ihedioha (2016) and was attributed to a smaller number of erythrocytes suspended in the plasma.

Conclusions

The prevalence of trypanosome infections in the horses sampled is 2.0% and *T. brucei* or its subspecies *T. evansi* or *T. equiperdum* might be the prevalent trypanosome species. The low prevalence of trypanosomosis could be due to absence of tsetse and biting flies in the Northern Nigeria where the horses were sourced, natural resistance of indigenous horses to trypanosome infection, and/or chronically low parasitaemias. In addition, there might be a possibility of underestimation of prevalence as small volume of blood was collected from a large animal (horse) for analysis. In our future study, species-specific primers for *T. brucei*, *T. evansi* and *T. equiperdum*, and sequencing of PCR amplicons shall be done to further confirm the parasite. Also, repeated testing shall be employed to ascertain if the estimated prevalence rate will increase.

Authors' Contributions

OAA: Data curation; formal analysis; Investigation; Methodology; Resources; Software; Validation; Writing-original draft; Writing-review & editing. JII: Conceptualization; Formal analysis; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing. TEA: Data curation; Formal analysis; Investigation; Methodology; Validation. PUU: Data curation; Investigation; Methodology; Resources; Writing-review & editing. SI: Data curation; Formal analysis; Investigation; Methodology; Software; Writing-review & editing.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

This research was done in accordance with the Ethics and Regulations guiding the use of animals as approved by the University of Nigeria Senate committee on Medical and Research ethics, ECUN/77932.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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