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MOLECULAR DETECTION AND CHARACTERIZATION OF TRYPANOSOMES INFECTING TRADITIONALLY MANAGED CATTLE IN THE TROPIC WARM SUB-HUMID ZONE OF NIGERIA

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ABSTRACT

Traditionally managed cattle constitutes the main source of animal protein to humans in Nigeria. However, seasonal migration in search of pasture exposes them to several vector-borne infections such as the African Animal Trypanosomosis (AAT), which limits their productivity. In this study, blood samples from 130 cattle in Plateau and Nasarawa states collected from May to June, 2021 were examined by the Polymerase Chain Reaction (PCR) and sequencing methods to determine the prevalence of pathogenic trypanosomes. Overall, the DNA of *T. vivax* was detected in 19 out of the 130 (14.6 %) samples examined by the PCR. However, using the micro-hematocrit centrifugation technique, motile haemoparasites were detected in only six (4.6 %, confidence interval [CI] 0.5—6.9 %) of the samples. The higher prevalence of *T. vivax* was recorded in samples sourced from the abattoir than in samples submitted from the field in Plateau state (16.7 % versus 11.5 %). However, the reverse was the case in Nasarawa state (2.9 % versus 37.5 %). The difference in prevalence of *T. vivax* between the abattoir and field samples was significant (P=0.009) in Nasarawa state, but not in Plateau state (P=0.55). The mean PCV (Packed Cell Volume) of the trypanosome infected animals was lower than that of the non-infected animals, but the difference was not significant (P=0.29). The internal transcribe spacer region (ITS) nucleotide sequences of *T. vivax* generated in this study were 100 % identical to each other and formed a monophyletic cluster with the sequences of *T. vivax* from different countries in the GenBank. AAT remains a major constraint to profitable cattle production and food security in Nigeria and deserves more attention.

Key words: cattle; ITS; mHCT; Nigeria; PCR; Trypanosomes

INTRODUCTION

Pastoral cattle production in the form of semi-sedentary, agro-pastoralists and transhumance are the dominant cattle management systems in most parts of Nigeria [3]. These practices do not only exert stress on the animals, but also expose them to disease vectors during the course of migration across different ecological zones [21]. One of such diseases is the African Animal Trypanosomosis (AAT). There are 11 different pathogenic trypanosomes

known to exist in Africa [15, 22]. AAT has a significant impact that can lead to reduced animal productivity as much as 38% in high risk areas [28]. The condition is characterized by: fever, hair loss, stupor, oedema, anaemia, bilateral lacrimation, keratitis, weight loss, abortion and eventually death. The name Nagana is derived from the Zulu word "N'gana", meaning "useless", because of the progressive, wasting nature of the disease [25]. In addition, AAT can also cause immunosuppression, making the host more susceptible to coinfections [27]. The common cause of AAT in cattle in sub-Saharan Africa are: *Trypanosoma congolense*, *Trypanosoma brucei*, and *Trypanosoma vivax* leading to a chronic wasting disease [22, 28]. Trypanosomes are mainly transmitted biologically between mammalian hosts by the bite of an infected *Glossina* species [8]. However, some *Trypanosoma* spp. such as *T. vivax* and *T. evansi* can be mechanically transmitted by hematophagous Diptera belonging to the Tabanidae, Stomoxyinae, and Hippoboscidae [13]. Therefore, *T. vivax* and *T. evansi* are found in areas beyond the range of the tsetse flies [6]. Taken together, the epidemiology of trypanosomosis is not dependent on the availability of the biological vectors only, but also on the cattle management system, trade in livestock, and wildlife, socio-cultural, and political factors [21]. The Standard Trypanosomes Detection Methods (STDMs); wet film, thick and thin smear, microhaematocrit centrifugation technique and animal inoculation are the commonly used methods for the diagnosis of AAT in Nigeria. However, these techniques have low sensitivity and specificity and lack the ability to accurately differentiate between the various trypanosomes [15]. The polymerase chain reaction (PCR) has been employed for the diagnosis of trypanosomes with high efficiency, although, technical requirement and cost have precluded its widespread use [5, 22].

So far, there are a few studies on AAT in Nigeria using the PCR. The prevalence of 77%, 46% and 37% of AAT have been reported in cattle in Ogun, Plateau and Kaduna states, respectively, using the PCR [17, 29], suggesting widespread AAT across the ecological zones of Nigeria. Thus, the aim of this study was to apply PCR and sequencing approach to determine the prevalence and characterize the *Trypanosoma* spp. infecting cattle in two contiguous states; Plateau and Nasarawa located in tropic sub-humid zone of Nigeria.

MATERIALS AND METHODS

Ethical approval

Approval for this study was granted by the Institutional Animal Care and Use Committee (IACUC), National Veterinary Research Institute (NVRI), Vom, Nigeria, approval number: AEC/03/108/21. Oral consent was obtained from the management of the abattoirs and cattle owners before the animals were sampled.

Study Area

The study was conducted in Plateau and Nasarawa states located in the tropic sub-humid zone of Nigeria (Fig. 1) [11]. The climate over this region is the tropical savanna climate and exhibits a well-marked rainy season and a dry season. The single dry season experienced in the study areas begins from October to May and the rainy season lasts from June to September [3, 21]. The temperatures are above 18 °C, and the average annual precipitations ranges from 750 mm to 1,100 mm. The vegetation cover is predominantly made up of plains of tall grass which are interrupted by trees, that provide a suitable land for crop and animal production. Furthermore, the near temperate climate experienced in this region, coupled with the availability of good vegetation cover and crop residues as a source of animal feed makes this zone an ideal choice for livestock production [16].

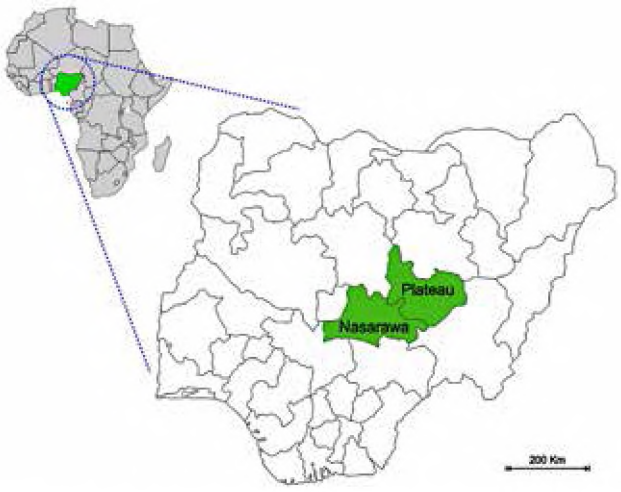

Fig. 1. Map of Nigeria showing the study areas (shaded)

Study design and blood sample collection

Samples analysed in this study were obtained from two sources. The first group consisted of anticoagulated blood samples from cattle in Plateau and Nasarawa states, submitted to the Parasitology Laboratory, NVRI Vom, for parasitological analyses. The second group consisted of blood samples collected from cattle slaughtered in the main abattoirs in Jos and Lafia; the capital cities of Plateau and Nasarawa states, respectively. In the abattoirs, the samples were collected at random from the jugular vein before slaughter over a period of two months (May – June, 2021). Approximately 3 ml of blood samples were transferred into ethylene diamine tetraacetic acid (EDTA) tubes, labelled and kept on ice before they were transported to the Parasitology laboratory NVRI Vom and processed accordingly.

The sex, age and breed of the animals were recorded before the collection of blood samples.

Determination of the packed cell volume (PCV) and microhaematocrit centrifugation technique (mHCT)

The presence of motile parasites in the blood was determined using the mHCT according to standard procedures [32]. Briefly, for every blood sample, a microhaematocrit capillary tube was filled to 75% and stoppered with a sealant. After centrifugation at 12,000 rpm for 5 min, the PCV was recorded. The tubes were placed on a grease free microscope slide and examined under the microscope at 100× magnification with low light intensity for the presence of motile parasites at the level of the buffy coat.

DNA extraction

DNA extraction was achieved using a commercially available kit; Quick-DNATM MiniPrep (ZymoResearch). Approximately 150 µl blood was subjected to genomic DNA extraction according to the manufacturer's instructions. DNA was eluted in 50 µl and stored at –20 °C until use.

Amplification of *Trypanosoma* species DNA using conventional PCR

All the DNA samples were initially screened for the presence of the DNA of pathogenic trypanosomes. The initial PCR was conducted using the internal transcribed spacer one (ITS1) ribosomal DNA (rDNA) based primers reported to efficiently detect the DNA of all pathogenic trypanosomes at different base pairs in a single reaction. Amplification was performed in 25 µl reaction volumes, containing 12.5 µl of 2X PCR Mastermix with standard buffer (New England Biolabs Inc), 0.5 µl of each primer (10 mM), 5 µl of template DNA and 6.5 µl of nuclease-free water (BioConcept, Switzerland). Amplification involved 30 s at 94 °C initial denaturation followed by 40 cycles of 1 min at 94 °C, 1 min at 60 °C, and 45 s at 68 °C followed by a final elongation at 68 °C for 7 min. The conventional PCR was conducted on a GenAMP 7400 (Applied Biosystems, Foster City, CA). Positive samples were further amplified using species-specific primers to differentiate the presence of different trypanosomes. The various primers and reaction conditions used in the study are listed in Table 1.

Table 1. Primers and reaction conditions for the detection of *Trypanosoma* spp. in cattle in north-central Nigeria

Code	Primer sequence (5'-3')	Annealing Temperature [°C]	Amplicon length [bp]	Reference
TITS1F	CCGGAAGTTCACCGATATTG	60	250—710	[24]
TITS1R	TTGCTGCGTTCTTCAACGAA			
TCFF	GGACACGCCAGAAGGTACTT	55	350	[18]
TCFR	GTTCTCGCACCAAATCCAAC			
TCSF	CGAGCGAGAACGGGCAC	55	316	[18]
TCSR	GGGACAAACAAATCCCGC			
TCKF	GTGCCCAAATTTGAAGTGAT	55	294	[19]
TCKR	ACTCAAAATCGTGCACCTCG			

Bp—base pairs.

The PCR products were electrophoresed in a 1.5% agarose gel stained with SafeViewTM Classic (Applied Biological Materials, Richmond, BC, Canada) and were visualized under a Blue light Transilluminator (Cleaver Scientific, UK).

Positive amplicons were sequenced at a commercial facility (Inqaba Biotech West Africa Ltd, Ibadan, Nigeria) using the PCR primers. Sequences were edited manually using the Bioedit Software [10]. The nucleotide sequences were checked using a BLASTn search hosted by the National Centre for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for the comparison with other known nucleotide sequences in the GenBank. Sequences generated in this study were deposited in the GenBank under the accession numbers ON139612–ON139618.

Phylogenetic Analysis

The phylogenetic tree was inferred using the Neighbor-Joining [26], and the p-distance method [23]. Percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [7]. The evolutionary analyses were conducted in MEGA11 [30].

Data analysis

The data generated in this study were recorded in Microsoft excel and analyzed using descriptive statistics. Furthermore, the Odd ratio was computed to determine the association between the prevalence of trypanosomes and samples collected from abattoirs and those submitted from farms using the MedCalc® Statistical Software version 20.015 [20]. The effects of trypanosomes on the PCV was determined using the student t-test. P-values <0.05 were considered significant.

RESULTS

A total of 130 cattle were examined in this study. This included 80 (61.5%, CI 50.8—68.7%) from Plateau state and 50 (38.5%, CI 31.3—49.1%) from Nasarawa state. Seventy-eight (67.7%, CI 61.6—78.2%) of these samples were sourced from abattoirs while 42 (32.3%, CI 21.8—38.4%) were samples submitted to our laboratory from cattle herds for parasitological analyses (Table 2). In Plateau state, a higher prevalence of *T. vivax* was recorded in samples sourced from the abattoir than in samples submitted from the field (16.7% versus 11.5%). However, the reverse was the case in Nasarawa state (2.9% vs. 37.5%). The difference in prevalence of *T. vivax* between the abattoir and field samples was significant (P=0.009) in Nasarawa state, but not in Plateau state (P=0.55). Near equal prevalence (15.0 % vs. 14.0%) of *T. vivax* DNA was recorded in samples from Plateau and Nasarawa states (Table 2). Overall, the DNA of *T. vivax* DNA was detected in 19 out of the 130 (14.6%) of samples examined by PCR. However, using the mHCT, motile haemoparasites were detected in only six (4.6%, CI 0.5—6.9%) of the samples.

Table 2. Prevalence of *Trypanosoma vivax* in cattle in north-central Nigeria

Sample location	Source of sample	Number positive/ No. tested [%]	OR	95 % CI	Z	P
Plateau	Abattoir	9/54 (16.7)	1.53	0.38—6.22	0.60	0.55
	Farms	3/26 (11.5)				
Total		**12/80 (15.0)**				
Nasarawa	Abattoir	1/34 (2.9)	0.05	0.005—0.47	2.62	0.009
	Farms	6/16 (37.5)				
Total		**7/50 (14.0)**				
Overall	Abattoir	10/88 (11.4)	0.47	0.18—1.26	1.50	0.13
	Farms	9/42 (21.4)				

OR—Odds ratio; Z—Z- statistics; CI—confidence interval; P—Significance level

Table 3. Detection of trypanosomes by mHCT and PCR in cattle in Nigeria

		PCR		
		Positive	Negative	Total
mHCT	Positive	5	1	6
	Negative	14	110	124
	Total	19	111	130

Table 4. Effects of *Trypanosoma* spp. infection on PCV of cattle in Nigeria

Sample location	Infection status	Mean PCV (%) ± SD (Normal value = 25—46)	95 % CI	T	P
Plateau	Non-infected	33.1 ± 6.6	0.07—0.23	0.2	0.42
	Infected	32.6 ± 7.5	0.77—0.93		
Nasarawa	Non-infected	30.2 ± 10.9	0.73—0.94	0.35	0.36
	Infected	29.0 ± 5	0.06—0.27		
Overall	Non-infected	31.65 ± 9.0	0.79—0.91	0.56	0.29
	Infected	31.41 ± 6.2	0.08—0.21		

T—T-test; P—significance level; PCV—packed cell volume; SD—standard deviation

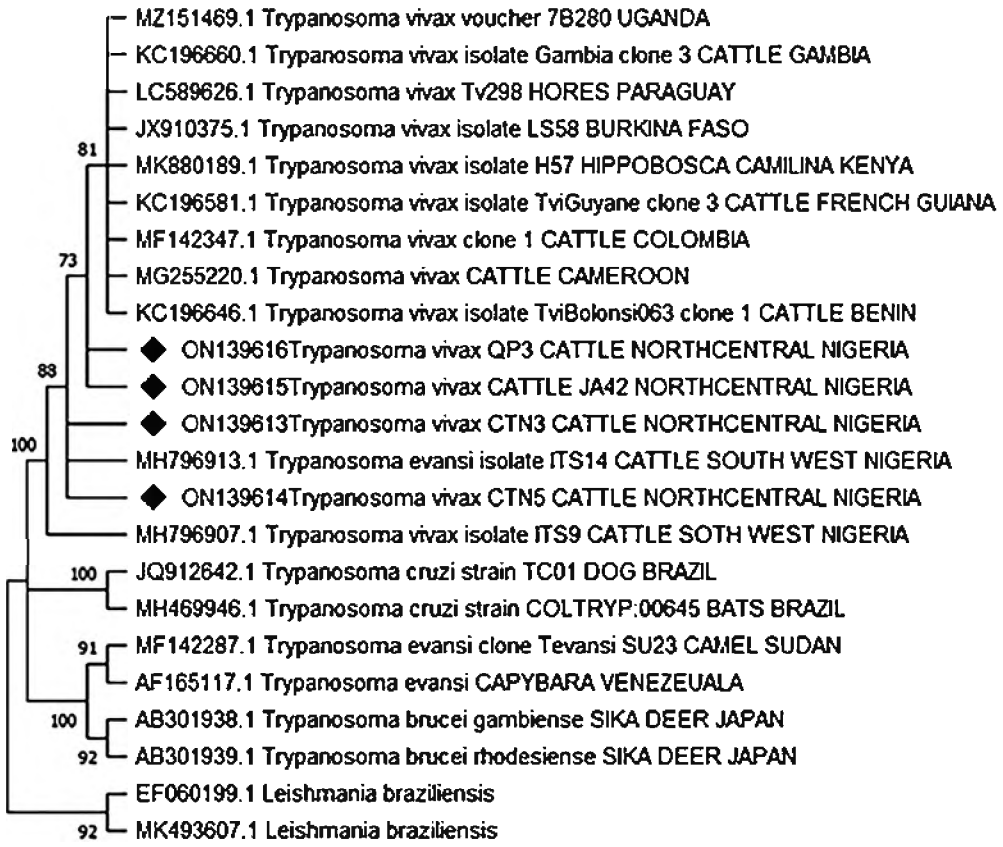

Fig. 2. Neighbor-Joining phylogenetic tree showing the relationship of sequences of *T. vivax* obtained in the study (black rhombus) and other sequences in the GenBank

Countries and host species where sequences were obtained are indicated. Percentage of 1000 replicate are shown next to the branches. *Leishmania brazilliensis* (EF060199 and MK493607) were used as outgroups

All the samples positive in the mHCT except one were positive for the DNA of *T. vivax* in the PCR test (Table 3). Similarly, all the mHCT positive samples were from those submitted from farms for the parasitological analyses. Generally, the mean PCV of trypanosome infected animals was lower than that of non-infected animals, but the difference was not significant (T=0.56; P=0.29) (Table 4). None of the positive samples were amplified using any of the *Trypanosoma congolense* species-specific primers used in this study.

The nucleotide sequences generated in this study were 100% identical to each other and 98.5—100% identical to *T. vivax* sequences in the GenBank. The *T. vivax* sequences from this study and formed a monophyletic cluster with sequences of *T. vivax* from different countries in the GenBank (Fig 2).

DISCUSSION

The primer pair ITS1 F and ITS1 R used in this study is reputed to amplify the ITS1 region of all pathogenic trypanosome species, producing PCR products with species-specific sizes and thus enabling the multiplex detection of different parasite species [24]. Amplification of an approximately 250bp was achieved in 19 out of the 130 samples (14.6%) screened in this study, suggesting that the amplified DNA belonged to *T. vivax*. The analysis of the ITS1 nucleotide sequences obtained in this study showed 99—100% identity to sequences of *T. vivax* in the GenBank. Thus, confirming *T. vivax* as the trypanosome species infecting the cattle examined in this study. This is not surprising as *T. vivax* can be transmitted both biologically and mechanically and is reputed as the most widespread species infecting cattle in Nigeria.

The prevalence of 14.6% *Trypanosoma* spp. DNA in samples from the Plateau state in this study by PCR is higher than the earlier reports of 7.8% and 5.1% by classical methods [4, 14), but lower than the 46.8% by the PCR [17]. However, the prevalence of 14.0% recorded for samples from Nasarawa state in this study was lower than the 53.3% previously reported for abattoirs samples from the same location [12]. These differences may be attributed to the variation in the study design, sample size, and importantly, the diagnostic methods employed in each of the studies. It is evident even from our report that the PCR was three times better at detecting trypanosomes than the mHCT. Another factor, that may account for differences in the results is that the method of preservation and the duration before testing. The integrity of samples may be compromised with poor preservation over prolonged periods. We had higher detection rates in samples submitted from the field than samples from abattoirs, being that samples from the field were collected and transported to the laboratory and analysed within a short time period (within 24 hours). This was not the case with most of the samples collected in the abattoirs, especially those from Nasarawa state, where they were preserved for 48—72 hours before they were transported to the laboratory for analysis. Our finding of the DNA of *T. vivax* only in cattle in this study is at variance with previous reports where in addition to *T. vivax*, *T. congolense*, and *T. brucei* were also reported in the study area [4, 12, 14, 17]. Although, most of the results were based on morphological identification of the trypanosomes, which is highly subjective, one of the studies was based on PCR and sequencing [17]. Although, the presence of *T. congolense* and *T. brucei* infection in cattle in Plateau state had been earlier reported, the relatively small sample size and the short sampling period (3 months) in this study might have limited our scope of trypanosome species detection. Seasonal variation in trypanosomes prevalence, with the lowest prevalence in the early wet season which coincided with our sampling period have been reported in Plateau state [17]. Drivers of livestock migration on the Jos Plateau have been identified to include: lack of water, pasture, lack of access to grazing land or water and avoidance of tsetse flies [16]. It should be noted, however, that seasonal variation in trypanosome prevalence does not always follow a clear cut pattern. It would appear that multiple factors, such as: management practice, veterinary care, nutrition and inter-current diseases superimpose and alters known epidemiological factors [21]. Another worrisome dimension is the prevailing unsupervised veterinary practice in Nigeria. It has been observed that herdsmen administer drugs to their animals without adequate supervision, therefore, the total curative effects may not be achieved. Hence, a chronic carrier state may be the norm rather than the exception. This may explain the deviation from the predictive seasonal prevalence of trypanosomes [1, 31]. Therefore, it will be interesting to also obtain information on the use of trypanocide among herdsmen during survey studies to evaluate perception, handling, administration and efficacy of the treatments.

We observed that there was no significant association between trypanosome infection and PCV of infected cattle compared to those that were not infected. This is in accord with the report of B i r h a n u et al. [2] in Ethiopia but at variance with the reports from Nigeria [17, 29]. Pastoralist cattle in Nigeria are usually infected with several haemoparasites in a state of endemic stability and therefore may not manifest severe haemolytic signs as in naïve or exotic breeds [9]. The majority of the samples examined in this study were obtained from animals in the abattoir. Under the traditional cattle production system in Nigeria, cattle ownership is a family prestige and status symbol, hence, sales of animals are highly restricted. Whenever it becomes necessary, aged cows or those in poor health condition are the prime candidates for sale. This may explain the low PCV value in most of the animals examined in this study regardless of their trypanosome infection status. The *T. vivax* nucleotide sequences generated in this study were 100% identical to each other and 98.5—100% identical to sequences in the GenBank suggesting as evolution of this species.

CONCLUSIONS

In conclusion, only *T. vivax* DNA was found in blood samples of cattle from Plateau and Nasarawa states in Nigeria. The persistence of this parasite in areas at the fringe or outside the known tsetse fly distribution zone is attributed to its ability to be transmitted by mechanical and biological routes. Recent events in Nigeria such as insurgency and conflicts between farmers and herders over grazing land have led to displacement of many livestock farmers consequently altering the epidemiology of AAT. More studies are needed to determine the nationwide prevalence, species distribution and vector and reservoirs of AAT in Nigeria. This will form an essential first step in the design of cost effective control measures for the disease.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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