Microscopic and molecular detection and characterization of *Trypanosomes* in cattle fattening cohorts in Plateau State, Nigeria

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Abstract

Cattle fattening is a thriving agribusiness in many parts of Nigeria. However, diseases like the Trypanosomiasis caused by various species of trypanosomes significantly affect the health, productivity, and market value of susceptible farm animals, thereby constituting a major constraint to food safety, security, and the economy. This study was designed to examine blood samples from 500 cattle from fattening units in Qua'an-Pan and Mikang Local Government Areas of Plateau State, Nigeria, for the prevalence of trypanosomes species using microscopy and molecular methods. Trypanosomes were detected in 22 out of 500 (4.4%) samples by microscopy and from 14 out of 200 (7.0%) samples by Polymerase Chain Reaction (PCR). More samples from Mikang than Qua'an-Pan (8% vs 6%) were positive but the difference was not significant (p=0.81). Similarly, there was no significant association between sex of animals and trypanosomes infection (p=0.052). However, adult cattle (p=0.0001), animals in fair body condition (p=0.009) and samples collected during the dry season (p=0.01) were significantly at risk of Trypanosomiasis than young (Juveniles and the Calves) cattle, those in poor or good body conditions or samples collected in the wet season, respectively. BLASTn search and Phylogenetic analysis of the nucleotide sequences generated in this study confirmed the presence of Trypanosoma vivax and Trypanosoma evansi in the cattle fattening units. The trypanosomes detected in this study have high identity (>99%) and clustered with high bootstrap value (>70%) with corresponding sequences in the GenBank. Findings from this study call for the implementation of adequate control measures to mitigate the impact of the disease on cattle production in Nigeria.

Keywords: Trypanosomiasis, Cattle fattening, PCR, Mikang, Qua'an-Pan, Nigeria

Détection et caractérisation microscopiques et moléculaires des *trypanosomes* chez des cohortes de bovins à l'engraissement dans l'État du Plateau, au Nigéria

Résumé

L'engraissement des bovins est une entreprise agricole prospère dans de nombreuses régions du Nigéria. Cependant, des maladies comme la trypanosomose, causées par diverses espèces de trypanosomes, affectent considérablement la santé, la productivité et la valeur marchande des animaux d'élevage sensibles, constituant ainsi une contrainte majeure pour la sécurité sanitaire des aliments, la sécurité alimentaire et l'économie. Cette étude visait à examiner des échantillons de sang de 500 bovins provenant d'unités d'engraissement dans les zones de gouvernement local de Qua'an-Pan et Mikang de l'État du Plateau, au Nigéria, pour déterminer la prévalence des espèces de trypanosomes en utilisant des méthodes microscopiques et moléculaires. Les trypanosomes ont été détectés dans 22 échantillons sur 500 (4,4 %) par microscopie et dans 14 échantillons sur 200 (7,0 %) par réaction en chaîne par polymérase (RCP). Plus d'échantillons de Mikang que de Qua'an-Pan (8 % contre 6 %) étaient positifs, mais la différence n'était pas significative (p=0,81). De même, il n'y avait pas d'association significative entre le sexe des animaux et l'infection par les trypanosomes (p=0,052). Cependant, les bovins adultes (p=0,0001), les animaux avec un état corporel moyen (p=0,009) et les échantillons collectés pendant la saison sèche (p=0,01) étaient significativement plus à risque de

trypanosomose que les jeunes bovins (juvéniles et veaux), ceux ayant un état corporel médiocre ou bon, ou les échantillons collectés en saison des pluies, respectivement. La recherche BLASTn et l'analyse phylogénétique des séquences nucléotidiques générées dans cette étude ont confirmé la présence de Trypanosoma vivax et Trypanosoma evansi dans les unités d'engraissement de bovins. Les trypanosomes détectés dans cette étude ont une identité élevée (>99 %) et se regroupent avec une valeur bootstrap élevée (>70 %) avec les séquences correspondantes dans la GenBank. Les résultats de cette étude appellent à la mise en œuvre de mesures de contrôle adéquates pour atténuer l'impact de la maladie sur la production bovine au Nigéria.

Mots-clés : Trypanosomose, Engraissement des bovins, RCP, Mikang, Qua'an-Pan, Nigéria

Introduction

Livestock production occupies a crucial place in most rural farming communities in Nigeria and cattle are considered one of the most economically important livestock animals in Nigeria (Habeeb *et al.*, 2021). The predominant livestock production system in Nigeria is the pastoral system and modifications thereof (FAO, 2009). Furthermore, these farmers engage in subsistence production of grains and cereals to provided food and cash crops that serve as a source of income for the family upkeep. To further boost the family income, farmers select some of their animals or purchase some cattle from the livestock market for fattening under intensive or semi- intensive system. This practice is gaining popularity among livestock farmers in most parts of Nigeria (Shettima *et al.*, 2019; Jibrin *et al*., 2023). However, threats of diseases and high cost of feeds and veterinary care pose significant challenge to the profitability of this venture (Jibrin *et al.*, 2023). Among the diseases of concern is the Animal African Trypanosomiasis (AAT) caused by *Trypanosoma* species; *T. congolense*, *T. vivax*, and *T. brucei brucei* transmitted cyclically by tsetse flies (Gaithuma *et al.*, 2019). AAT affects domestic animals, including cattle, goats, sheep, and pigs, and its pathogenicity differs according to the host species (Peter *et al*., 2023). The disease manifests varying clinical signs including but not limited to fever, anemia, loss of weight and productivity, abortion, decreased fertility, edema, paralysis, and even death (Marsela *et al.*, 2020). The disease remains a major threat to animal health and stock farming within the tsetse belt (Gondwe *et al.*, 2009). Of concern is the fact that unlike *T. congolense* and *T. brucei*, which are transmitted biologically by tsetse flies, *T. vivax* and *T. evansi* can also be transmitted mechanically by other hematophagous flies; as a result, it has a broader geographical distribution (Mamman *et al.*, 2021).Therefore, AAT is a major concern in sub-Saharan Africa, with detrimental effects on both human and animal health and causing significant losses to affected countries (Alibu *et al.*, 2015).The control of AAT is challenging due to the complexity of the disease epidemiology associated with variations in pathogen, host and vectors as well as the involvement of wildlife reservoirs (Isaac *et al.*, 2017). This has resulted to atypical manifestation of AAT in cattle in Nigeria characterized by the development of chronic hemolymphatic stage without the formation of a chancre making the diagnosis by the traditional methods difficult to achieve accurately (Mamman *et al*., 2021). To overcome this problem, the superiority of molecular methods over the Standard *Trypanosoma* Detection Methods (STDM) and serology has been reported (Ahmed *et al.*, 2013). Therefore, the use of molecular techniques such as the Polymerase Chain Reaction (PCR) and sequencing is advocated for the accurate identification and characterization of *Trypanosoma* species infecting cattle in a particular area. There are reports of the successful use of PCR targeting different genes or fragments to accurately identify and characterize *Trypanosoma* species in Nigeria and other countries (Majekodunmi *et al.*, 2013a; Greninger *et al.*, 2015; Cuypers *et al*., 2017). It is therefore desirable to integrate

PCR and sequencing methods in the detection and characterization of *Trypanosoma* species in monitoring the health of animals under the fattening programs. This will constitute a holistic approach needed to elucidate the roles of the various component of the disease epidemiology in order to formulate a cost effective control measures. This study aimed to detect and characterize *Trypanosoma* species infecting cattle under fattening operations in Mikang and Qua'an-Pan Local Government Areas (LGA), Plateau State, Nigeria and to recommend appropriate control measures.

Materials and Methods

Study area

The study was conducted at Ba'ap in Qua'an-Pan (7º05′00″N 6º45′00″E) and Tunkus in Mikang (9º00″N 9º 35′′E) Local Government Areas of Plateau state located in the North central region of Nigeria (Fig. 1). The climate of the two study areas is composed of a wet season; from April – October and dry season; from November− March. The mean annual rainfall varies from 131.75cm to 146cm. The annual mean temperature of the study areas is 32°C (monthly mean temperature ranges between 25.8°C to 35.7°C). The vegetation is Guinea Savanna type, which is characterized by the presence tall grasses, scattered trees and shrubs (Hudu and Ibrahim, 2021).

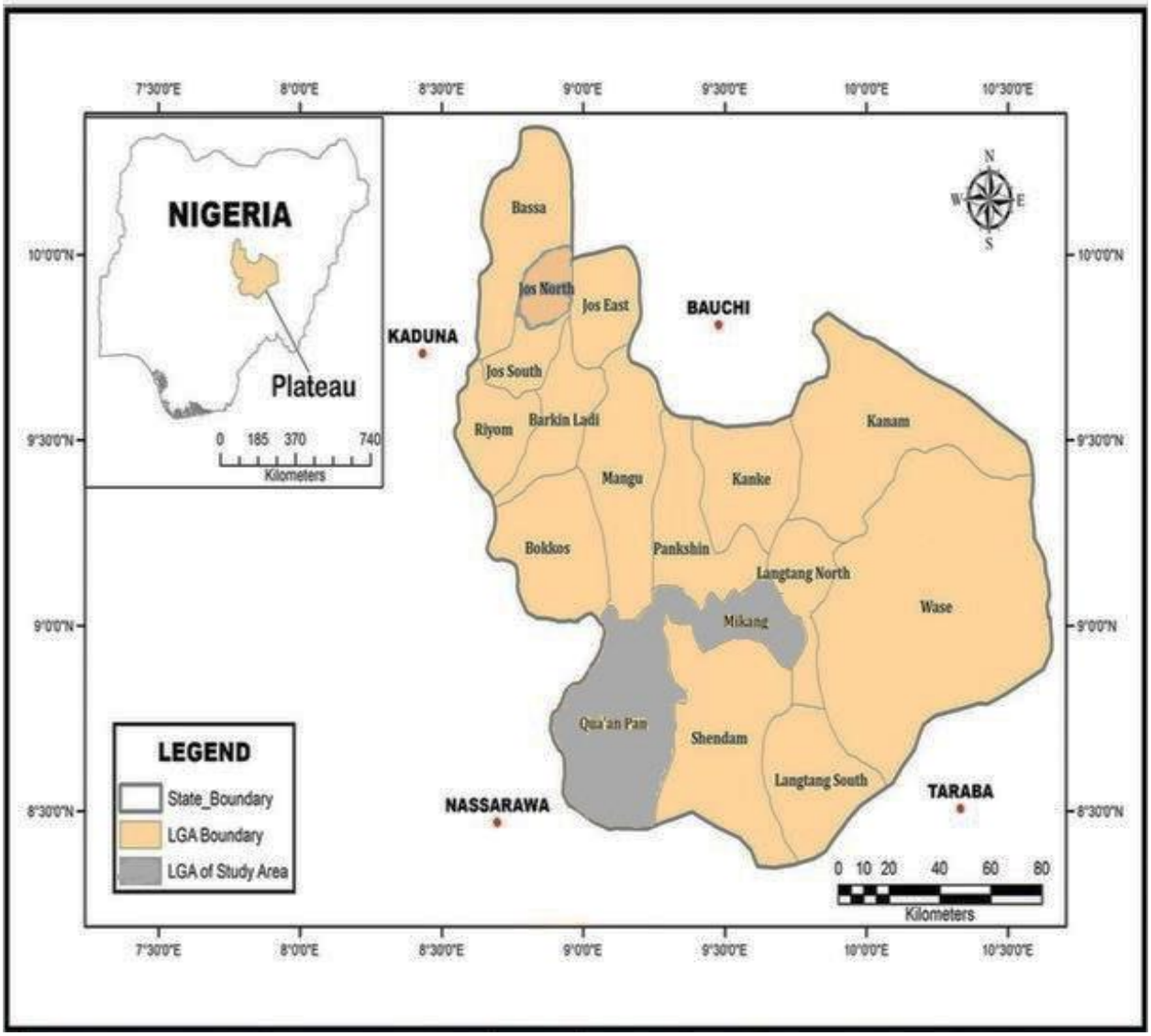

Figure 1: Map of Plateau State showing Qua'an-Pan and Mikang LGA
Source: Plateau State Ministry of Lands and Survey, 2023

Ethical approval

All experimental protocols and animal work were approved by the Animal Use and Care Committee of the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria with the certification ID: NVRI AEC REF No. ACE/02/88/20.

Study population and sampling

Our study population consisted of cattle selected from transhumance cattle for fattening. Animals that were treated with trypanocidal drugs two week preceding the sampling time were excluded from the study. Apparently healthy cattle kept for fattening were purposively selected for the study. A total of 500 animals were sampled in the two LGAs. The age of the cattle was determine based on dentition (Johnson, 2003) and were categorized into Juvenile (0 –11months), Calf (1-2years), and Adults (2+years). Furthermore, the cattle were categorized based on the body conditions score as M (Fair), M- (Poor) and M+ (Good) according to Nicholson and Bitterworth, (1986). Animals were properly restrained and about 4 mL of blood was collected by jugular venipuncture and aliquot into sterile ethylene diamine tetra acetic acid (EDTA) tubes for parasitological and molecular analysis. In the field, the blood samples were kept in a cold box packed with ice and transported to the

Parasitology Division Laboratory, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria where they were stored at − 20 °C until further analysis.

Microscopic and molecular detection and characterization of trypanosomes

Five hundred blood samples from the two study areas were initially screened for trypanosomes by the Hematocrit Centrifugation Technique (HCT) and microscopic examination of Giemsa stained smears according to standard protocols (Soulsby, 1982; Luckins, 1992).

Two hundred anticoagulated whole blood samples including samples that were positive for trypanosomes by microscopy and additional randomly selected were submitted to Genomic DNA extraction using quick-DNA miniprep kit (Qiagen QIAamp^{TM}) according to the manufacturer's instructions. The eluted DNA was then stored at − 20 °C until PCR analysis. Published primers, ITS1-FOR-(5′-CCGGAAGTTCACCGATATTG -3′) and ITS1-REV-(5′-TTGCTGCGTTCTTCAACGAA -3′) for the amplification of variable regions of *Trypanosoma* species: 250-bp of *T. vivax*, 475-bp of *T. evansi*, 387-bp of *T. simiae*, 470-bp of *T. brucei* and 750-bp of *T. congolense* were used (Njiru *et al*., 2005).The PCR amplification was performed in a total volume of 30 µL consisting of 5 µL template DNA, 0.5 µL each primer, 15 µL 2x PCR Master mix (Thermo-Scientific) and 9 µL PCR grade water. A tube containing all the reaction mix except template DNA was included in the reaction set-up non-template controls (NTC). Amplification was conducted on an GenAMP 7400 (Applied Biosystems, Foster City, CA, USA) programmable PCR thermocycler in the Molecular Biology Unit, Parasitology Division, NVRI Vom under the following conditions: initial denaturation at98^{o}C for 10 secs, followed by 40 cycles of 98^{o}C for 1 sec, 60^{o}C for 45 secs (annealing), 72^{o}C for 45 secs (extension) and final elongation at 72^{o}C for 3 minutes. Amplicons were electrophoresed on a 1.2% agarose gel stained with Safe View and run at 95V for 30 minutes. The result was evaluated in comparison with a 100 bp molecular ladder and was viewed under a blue-light Trans-illuminator and documented using a digital camera.

Positive amplicons were sent to a commercial sequencing company (Inqaba, Ibadan-Nigeria) for sequencing in the forward direction. Sequences obtained were manually edited and compared with the sequences available in the GenBank database using the Basic Local Alignment Sequence Techniques (BLASTn) algorithm hosted by the National Centre for Biotechnology Information, Bethesda, MD, USA (www. blast. ncbi. nlm. nih.gov/ blast. cgi). Phylogenetic analysis was performed to compare the relationship between nucleotide sequences detected in this study with those in GenBank database. Sequences were retrieved from the GenBank and aligned with sequences obtained in the study using the Muscle alignment and they were used to infer the evolutionary history using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model. The bootstrap consensus tree was inferred from 1000 replications. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 14 nucleotide sequences. *Leishmania major* and *L. brazilensis* were used as outgroup. Evolutionary analysis was conducted in the Molecular Evolutionary Genetics Analysis package (MEGA 11).

Statistical analysis

Data generated during the study were entered into Microsoft Excel and analyzed using the R statistical software (R Core Team. 2013). The results obtained from this study were subjected to statistics to determine the prevalence by localities, body condition score, age and sex of the animals and were expressed as percentages of the total number of animals sampled. The association between prevalence and risk factors

was assessed using the chi-square test. The level of significance was set at $p \leq 0.05$.

Results

Overall, trypanosomes were detected in 22 out of the 500 (4.4%) and 14 out of the 200 (7.0%) samples screened by micros
copy and PCR, respectively (Table 1). Although, 22 samples were scored as positive by microscopy only 14 (63.6%) of them turned out to be positive by PCR amplification. Therefore, the risk factor analysis was based on the PCR results. More samples from Mikang than Qua'an-Pan (8% vs 6%) were positive but the difference was not significant (p=0.81). Similarly, there was no significant association between sex of animals and trypanosomes infection. However, samples collected during the dry season, from adult cattle and those in fair body condition were significantly infected (p<0.05) (Table 1). The PCR amplification produced bands of appropriate sizes corresponding to *T. vivax* (250 bp) and *T. evansi* (475 bp). Sequencing was successful in four amplicons. The sequences obtained from this study were submitted to the GenBank and were assigned Accession numbers: PQ758634−PQ758636.

BLASTn search showed that the nucleotide sequences obtained in this study were 99−100% identical to sequences of *T. vivax* and *T. evansi* from different countries in the NCBI GenBank database.

Furthermore, a Maximum Likelihood (ML) Phylogenetic tree placed the sequences generated from fattening cattle in this study in distinct clusters with the sequences of their corresponding species in the GenBank (Fig.2).

Table 1. Prevalence of trypanosomes in fattening cattle in Mikang and Qua'an-Pan LGA, Plateau Sate by microscopy and PCR

Variables	Diagnostic method						Risk factors	
	Microscopy			PCR				
	No. positive /number tested (n=500) (%)			No. positive /number tested (n=200) (%)			Chi square	p-value
	Study area		Total	Study area		Total		
	Mikang	Qua'an-Pan		Mikang	Qua'an-Pan			
Body condition score								
M	7/99 (7.1)	5/108 (4.6)	12/207 (5.8)	7/34 (20.6)	3/34 (8.8)	10/68 (14.7)	9.4	0.009*
M-	3/71 (4.2)	1/69 (1.4)	4/140 (2.9)	1/34 (2.9)	1/34 (2.9)	2/68 (2.9)		
M+	1/80 (1.3)	3/73 (4.1)	4/153 (2.6)	0/32 (0.0)	2/32 (6.3)	2/64 (3.1)		
Sex								
Female	3/102 (2.9)	2/110 (1.8)	5/212 (2.4)	1/50 (2.0)	2/50 (4.0)	3/100 (3.0)	3.8	0.052
Male	10/148 (6.8)	7/140 (5.0)	17/288 (5.9)	7/50 (14.0)	4/50 (8.0)	11/100 (11.0)		
Age								
Adults	9/87 (10.3)	7/105 (6.7)	16/192 (8.3)	7/34 (20.6)	5/34 (14.7)	12/68 (17.6)	18.4	0.0001*
Juveniles	2/114 (1.8)	2/111 (1.8)	4/225 (1.8)	1/34 (2.9)	1/34 (2.9)	2/68 (2.9)		

Calves	0/49 (0)	0/34 (0)	0/83 (0)	0/32(0.0)	0/32(0.0)	0/64 (0.0)		
Season								
Wet	3/83 (3.6)	2/98 (2.0)	5/181 (2.8)	1/50 (2.0)	1/50 (2.0)	2/100 (2.0)	6.2	0.01*
Dry	10/167 (6.0)	7/152 (4.6)	17/319 (5.3)	7/50 (14.0)	5/50 (10.0)	12/100 (12.0)		
Overall	13/250 (5.2)	9/250 (3.6)	22/500 (4.4)	8/100 (8.0)	6/100 (6.0)	14/200 (7.0)	0.06	0.81

Values with asterisk (*) are statistically significant.

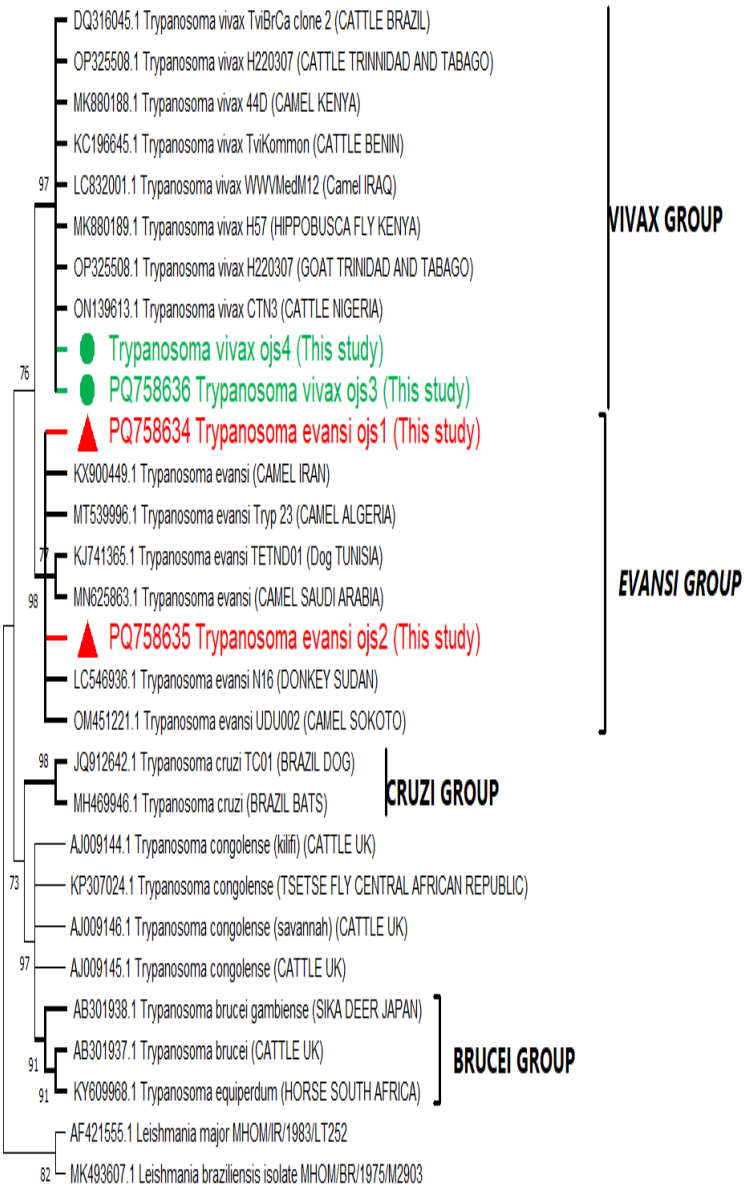

Figure 2: Maximum Likelihood Phylogenetic tree based on the Kimura 2-parameter model. The bootstrap consensus tree was inferred from 1000 replications. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Sequences in this study are color coded; green (*T. vivax*) or red (*T. evansi*).

Discussion

Trypanosomiasis in cattle is considered an endemic disease on the Plateau (Majekodunmi *et al.*, 2013a). The overall prevalence of 4.4% and 7.0% by microscopy and PCR, respectively, detected in fattening cattle in this study is similar to the earlier reports of 5.1 and 7.8% in some parts of Plateau State (Dede *et al.*, 2005; Idehen *et al.*, 2019). However, higher prevalence of 14.6 – 46.8% has been reported in some studies in Plateau state using PCR and sequencing (Majekodunmi *et al.*, 2013a; Kamani *et al.*, 2022). The superiority of PCR detection of trypanosomes over microscopic method was demonstrated in this study. This is similar to a report where the use of microscopy detected trypanosomes in 15.1% as against 63.7% detection rate by PCR (Takeet et al., 2013). Furthermore, microscopic detection methods may not be able to convincingly determine the infecting *Trypanosoma* species. In this study, *T. vivax* and *T. evansi* were detected in fattening cattle in Mikang and Qua'an-Pan LGAs of Plateau state using PCR and sequencing approach. None of the earlier studies of trypanosomes infecting cattle in the state using microscopy and/or PCR was able to detect *T. evansi* in the analyzed samples (Dede *et al.*, 2005; Majekodunmi *et al.*, 2013a; Idehen *et al.*, 2019; Kamani *et al.*, 2022). However, an earlier study on samples from Southern and northern Nigeria reported *T. evansi* in the blood of cattle (Takeet *et al.*, 2013). The differences could be due to study design, sample population and the analytical methods employed in the various studies. Significant risk of trypanosome infection in the fattening animals was found in samples collected during the dry season, from adult cattle and those in fair body condition. Seasonal variation in trypanosome infection has been reported in Plateau state where, early wet season samples had higher chance of being positive, but mix-trypanosome species infection was higher in dry season samples (Majekodunmi *et al.*, 2013a). The higher prevalence of trypanosomes in older cattle recorded in this study is in concert with report from an earlier study in Bokkos, Plateau state (Idehen *et al.*, 2019), but inconsistent with reports of some studies conducted in Nigeria (Takeet *et al*., 2013; Kamani, 2022). In this study, there was no significant association between sex of the cattle with trypanosomes infection, similar to several reports from different parts of Nigeria (Takeet *et al.*, 2013; Idehen *et al.*, 2019; Kamani, 2022; Kamani *et al*., 2022). Trypanosomes were detected in more samples from Mikang than Qua'an-Pan LGA. Village level variation in trypanosomes infection has been reported in some parts of plateau state, and it was attributed to different socio-cultural practices (Majekodunmi *et al*., 2013b). The trypanosome species detected were characterized based on PCR amplification and nucleotide sequence analysis. Both the BLASTn search and Phylogenetic analysis confirmed the presence of *T. vivax* and *T.evansi* in the study area. Both parasites are pathogenic to cattle and the infections may result to low productivity and death in severe cases. Furthermore, both parasites can be mechanically transmitted; hence the diseases cycle can be maintained even outside tsetse infested areas. Although, the pastoral system is the predominant livestock production practice in the Sahara and Sahel agro-ecological zone (AEZ), economic challenges and the increasing need for youth empowerment has led to the establishment of cattle fattening enterprises. As such, over the years, a lot of pastorals have taken to cattle fattening as a source of livelihood (Shettima *et al.*, 2019; Jibrin *et al*., 2023). Unlike cattle raised under the pastoral system where the sociocultural attributes out-weigh the economic aspects, the reverse is the case in cattle fattening ventures. Animals earmarked for fattening are usually restricted from open grazing, fed with concentrates and given adequate veterinary care. Therefore, the finding of trypanosomes in cattle fattening cohorts attest to the endemicity of Trypanosomiasis and the threat it constitutes to profitable cattle fattening ventures in the state. In order to curtail the threat of trypanosomes to

the fattening cattle, extra attention in terms of vector control is necessary considering the fact that both *T. vivax* and *T.evansi* detected in this study can be transmitted mechanically by hematophagous flies.

Conclusion

Pathogenic trypanosomes, *T. vivax* and *T.evansi* were detected in fattening cattle in Mikang and Qua'an-Pan LGA, Plateau state, despite the extra attention given to the animals. These infections can affect the performance of the animals, consequently resulting to low productivity and profitability. More vigilance in terms of early diagnosis, prompt treatment and effective vector control are needed to curb the consequences of the disease.

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