IJSAR, 10(5), 2023; 42-52

International Journal of Sciences & Applied Research

www.ijsar.in

CONVENTIONAL AND MOLECULAR DETERMINATION OF *TRYPANOSOMA* INFECTION IN APPARENTLY HEALTHY SMALL RUMINANTS IN NORTH CENTRAL- NIGERIA

***Peter, J. G[1]., Adeiyongo, C. M[2].,Yohanna, J. A[2]., Arin, R.Y.,[1]Tyem, D. A.,[3] and Peter, Y.J.,[4]**

[1]Parasitology Division, National Veterinary Research Institute Vom, Plateau State, Nigeria [2]Department of Zoology, University of Jos, Jos-Nigeria
[3]Biotechnology Division, National Veterinary Research Institute Vom, Plateau State-Nigeria, [4]Department of Medical Microbiology and Parasitology, University of Abuja, Nigeria.
Corresponding Author email: jaypee1410@gmail.com
Celine Movihinze Adeiyongo, Email: adeiyongoceline@gmail.com
Jael Asabe Yohanna, Email: jaelyohanna@yahoo.com
Rebecca Yakubu Arin, Email: rebyaku@yahoo.com
Dinchi Andrew Tyem, Email: jasminetyem@yahoo.com
Peter Yoa'seem Jonah, Email: jonah.peter@uniabuja.edu.ng

ABSTRACT

Small ruminants such as sheep and goats are a common economically low-cost livestock investment for smallholder farmers in Nigeria with several disease limitations affecting livestock productivity, among which is African Animal Trypanosomosis (AAT). In apparently healthy animals, trypanosomosis can best be diagnosed by molecular method compared to the conventional clinical diagnosis and standard trypanosome detection method (STDM). A positive test shows active infection and nucleotide sequences specific for a particular trypanosome species or subspecies. To assess the prevalence of trypanosomes in local breeds of sheep and goats in Bassa and Jos East areas, a total of 400 blood samples collected at random from June to August 2018 were subjected to Polymerase Chain Reaction (PCR) molecular method and Standard Trypanosome Detection Method (STDM) using the microscope for examination [Thin, Thick, Buffy Coat smears and Packed Cell Volume (PCV). The PCV of each animal sampled was assessed. There was no significant difference in the mean PCV between infected Caprine and non-infected Caprine in Bassa and Jos East areas ($P>0.05$). A significant difference in the mean PCV between infected Ovine and non-infected Ovine in Bassa and Jos East areas ($P<0.05$) was observed. Results showed 109 (27%) infected with *Trypanosoma brucei* and 01(0.25%) *Trypanosoma vivax* by PCR and 0.0% STDM (Thin, Thick, Buffy Coat smears and PCV). The results further showed that small ruminant trypanosomosis even at the sub-clinical level is prevalent in the areas studied.

Towards improved diagnosis and enrichment of the trypanosomosis database, the study recommends the inclusion of molecular diagnostics for more effective planning and implementation of control strategies towards African trypanosomosis elimination in Nigeria.

Keywords; Sheep, Goats, Trypanosomosis, PCR, Plateau State, Nigeria.

INTRODUCTION

Trypanosomosis is a widespread disease of livestock in Nigeria. *Trypanosoma* species are transmitted by *Glossina* flies also known as tsetse flies. *Glossina tachinoides* and *Glossina palpalis* are the species identified in Plateau State (Majekodunmi et al. 2013). An early report showed a widespread bovine trypanosomosis in Nigeria with the infection rate in Plateau State ranging from 1.9 % to 4.5% (Pollock, 2001). A survey spanning Jos North, Jos South, Bassa, and Jos East Local Government Areas (LGAs) of Plateau State reported an overall infection rate of 7.9% in cattle and sheep (Shamaki et al. 2002). A projection from these earlier reports is likely to provide a high infection rate which can be blamed on the absence or non-implementation of specific control programs. High rates of trypanosome parasite infection culminate in a decreased yield in meat and milk production and hence increased poverty and food insecurity for smallholder farmers in such areas. African Animal Trypanosomosis [AAT] in small ruminants is known to be a significant productivity-limiting disease that contributes to poverty and food insecurity in rural households (Swallow, 2000). An empirical study in Ethiopia showed that trypanosomosis is a major livestock production constraint (Abro et al. 2021). Some authors have projected that the presence of AAT in an area could reduce the total number of livestock by 25–50% and reduce agricultural gross domestic product (GDP) by 5 -10% (Isaac et al.2007; Odeniran et al. 2018). The population of sheep and goats in Nigeria are estimated to be 89.84 million providing nutrition, income, food security, and cultural value to smallholder farmers (Fadiga et al. 2013). The Food and Agricultural Organization (FAO) advocates that to achieve Sustainable Development Goals (SDGs). AAT is a constraint that must be addressed to provide an improved livelihood for rural smallholder farmers (FAO,2017). The economic importance of small ruminants is primarily associated with their small size, as it favours low-cost investment, smaller risk of loss, and high fecundity (Peter et al. 2015). However, the benefits derived could be below expectation due to low productivity which could be caused by diseases, poor management, and lack of proper breeding policies [Peter et al. 2015; Anyaegbunam and Okafor, 2013). Sheep and goats could naturally be infected with *Trypanosoma* species and may produce acute, sub-acute, chronic or sub-clinical forms of the disease in these animals (Anyaegbunam and Okafor, 2013; Samdi et al, 2008). Sub-clinical or asymptomatic infections where local breeds of sheep and goats appear healthy in the face of underlying trypanosomosis in men and animals (Anyaegbunam and Okafor, 2013; Samdi et al, 2008; Yanan et al. 2007; Ayana et al. 2015; Dede et al. 2005). It is generally believed that inadequate information on the true prevalence status using advanced techniques has prevented proper planning for control in several endemic countries including Nigeria. (Anyaegbunam and Okafor, 2013; Samdi et al, 2008). This study describes the diagnosis of sub-clinical *Trypanosoma brucei* and *Trypanosoma vivax* infections in apparently healthy local breeds of sheep and goats by Polymerase chain reaction molecular method which may have been missed by Standard Trypanosome Detection Method (STDM) -conventional methods.

MATERIALS AND METHODS

The study was carried out from June to August 2018 in two Local Governments Areas (LGAs) of Plateau State, Nigeria across eight (8) communities with the following coordinates; Dusen Kura (110 26” 57’ N; 70 41” 10’E), Kishika (100 00” 58’ N; 80 40” 57’E), Kwall (90 48” 39’N; 80 39” 18’E), Rafin Bauna (90 52” 6’N; 80 47” 10’E), in Bassa LGA; and Febas (90 51’ 14” N; 90 4’ 16” E) Federe (90 51’ 03” N; 90 4’ 57” E), Fobur (90 52’ 02” N; 90 1’ 59” E) and Rafin Sanyi (90 49’ 02” N; 90 1’ 59” E) in Jos East LGA. The study areas are majorly Guinea Savannah Forest, and the climate is cold with a mean annual temperature of 22℃ (Adzandeh et al. 2015).

Simple balloting (personal communication) was carried out on livestock owners in areas known to have animals in high and medium numbers. Oral interview (about the nature of the research and to get their consent) of smallholder farmers was initially conducted and thereafter blood samples were collected from their small ruminants. The animals to be sampled were selected through a systematic random sampling technique as described by (Peter and Peter 2017; Takeet et al. 2013). The sex and age of sampled animals were recorded against unique identification codes by the accompanying research assistants who are animal health officers. A total of 400 small ruminants from Bassa and Jos East LGAs, comprising sheep and goats aged between 9 months to 5 years were sampled. Using a suitable sterile syringe, 5mls of blood sample was collected from the jugular vein of each animal into Ethylene Diamine Tetra Acetic (EDTA) bottles and immediately rocked gently, labelled, and stored in a portable cold storage chamber. The blood samples collected were immediately transported to the National Veterinary Research Institute for analysis.

Procedure for Microscopic Examination (STDM)

The Standard Trypanosome Detection Method - Thin, Thick, buffy coat smears and Packed Cell volume) as described by Soulsby (Soulsby, 1982), was carried out as described briefly. Thin smears were made from whole blood samples. Each drop of the blood sample was placed at the edge of a clean grease-free microscope slide and with the aid of a spreader, it was moved in a quick and forward direction to obtain a thin smear. It was allowed to air dry, and smears were labelled accordingly. For a thick smear, a drop from each sample was placed at the centre of a clean grease-free microscope slide, a circular smear was made at the centre. It was air-dried and labelled accordingly. Only thin smears were fixed in absolute alcohol (Methanol) and were air-dried. Freshly prepared and filtered Giemsa solution (10%) was flooded onto each pre-dried slide and was allowed to act for 45 minutes. It was differentiated in phosphate-buffered saline at p^H 7.2. It was air-dried and stained blood smears were examined under the microscope using the oil immersion objective (x100) for the detection of *Trypanosoma* species. A minimum of 50 microscopic fields were examined before the result was determined. STDM - microscopic examinations were processed accordingly.

Procedure for Packed Cell Volume (PCV)

Blood was drawn into a heparinized tube by capillary attraction. It was filled to 3/4 capacity and the blood stain was wiped with a clean dry cotton wool. One end of the tube was carefully sealed with a crystal seal and loaded symmetrically in a microhematocrit centrifuge and spun for five minutes at 1200 rpm. It was read with the aid of a haematocrit reader and results were expressed as percentages.

Procedure for Buffy Coat Smear

The capillary tube was broken 1mm above the buffy-coat layer. The contents were partially expelled and smeared unto a clean grease-free slide. It was air-dried, labelled, and fixed in methanol, was air-dried and flooded with fresh and filtered dilution of 10% Giemsa solution. It was timed for 60 minutes and was differentiated with phosphate-buffered saline at P^H 7.2. It was air-dried and viewed using the oil immersion objective. (x100).(Murray et al. 1977).

Procedure for DNA Extraction

The five millilitres (5ml) of whole blood were spun at 10,000g for 5 minutes. Two hundred microliters (200µl) buffy coat was harvested into labelled cryovials and stored at -20°C until

needed for DNA extraction. Total DNA was extracted from the buffy coat using the zymo Quick-gDNA™ Miniprep Extraction Kit (ZYMO Research, USA catalogue number; D3025) according to the manufacturer's instructions. The frozen labelled samples were allowed to thaw at room temperature. 400µl of genomic lysis buffer was pipetted into labelled Eppendorf tubes. 100µl of each buffy coat (sample) was pipetted and carefully added into each labelled tube and was mixed completely by vortexed for six (6) seconds and was allowed to stand at room temperature for ten (10) minutes. The mixture was transferred to the Zymo-spinTM IIC column in a collection tube and was individually centrifuged with the aid of the Eppendorf Centrifuge at 10,000 x g for one (1) minute. The collection tubes were discarded with the flow through. The Zymo-spinTM IIC column was transferred to a new collection tube. DNA pre-wash buffer was pipetted in 200µl aliquot and was added to the spin columns, centrifuged with aid of Eppendorf Centrifuge at 10,000.00 x g for one (1) minute. DNA wash buffer was pipetted in 500µl aliquot and was added to the individual spin column. It was centrifuged with aid of Eppendorf Centrifuge at 10,000.00 x g for one (1) minute. The spin columns were transferred to clean pre-labelled microcentrifuge tubes. DNA elution buffer was pipetted in a 60µl aliquot and was added to the spin column. It was incubated at room temperature for five (5) minutes. It was centrifuged with aid of Eppendorf Centrifuge at 14,000.00 x g for 30 seconds. (This is to elute the DNA). The eluted DNA was carefully packaged in cellophane and stored at -20°c for molecular-based application.

PCR

The touchdown PCR primers (Kin1 and Kin 2) gave an amplification product of 540bp which was equivalent to the amplification product of *Trypanosoma brucei* reported by McLaughlin et al. (1996). Representative touchdown PCR is depicted in Table 1. DNA amplification was viewed under Ultraviolet and documentation was with Gel Documentation System (Synegene, Indonesia).

Table 1: Set of Primers used for PCR

Primer	Primer Sequence (5’- 3’)	Size(bp)	Reference
Kin 1 Forward	GCGTTCAAAGATTGGGCAAT	540	McLaughlin et al.1996
Kin 2 Reverse	CGCCCCGAAAGTTCACC	540	McLaughlin et al.1996
Tv.80.24F	CAGTGCTCCCGCTCGTACACGGAC	266	Gall et al. 2004
Tv.322.24R	GCACGCCACATAGCCGGGGAACAG	266	Gall et al. 2004

To ensure that all animal *Trypanosoma* species present in the samples collected were detected, a *Trypanosoma vivax* PCR detection protocol developed by Gall et al., (2004) was also used to analyse all the samples. A separate *T. vivax* PCR protocol was used because the Touchdown PCR protocol has a low limit of detection when *T. vivax* parasitaemia is very low.

Positive and Negative Controls

For positive control, Purified Trypanosome DNA of *Trypanosoma brucei* ILTat 1.4 (originally isolated from a steer in Uhembo, Kenya (Gall et al. 2004). Positive control used for *Trypanosoma vivax, IL* 1392 Zaria Y 486 originating from a steer in Zaria, Nigeria (Gall et al. 2004). Nuclease free sterile water was used as the negative control.

PCR Amplification of *T. brucei* DNA Extract

The PCR amplification of the samples was performed in a 25µl reaction that contained 3µl of genomic DNA extract, 0.25µl of 20µM primer (Kin1 and Kin2), 12.5µl of one Taq® Quick-Load® 2x Master Mix with Standard Buffer (New England Bio Labs) and the volume was made up with 9.0µl Nuclease Free Water (Promega®). This was taken to a thermocycler that was pre-set and pre-heated - Applied Biosystem®9700 PCR Machine for amplification. The reaction conditions were: initial denaturation at 94°C for 5 minutes, followed by 4 cycles of denaturation at 93°C for 30 seconds, annealing at 58°C for 30seconds, extended at 2°C for 1 minute, followed by 8 cycles of denaturation at 94°C for 30seconds, annealing at 56°C for 30 seconds, extended at 72°C for 1minute, followed by 30cycles of denaturation at 94°C for 30seconds, annealed at 54°C for 30seconds, extended at 72°C for 1minute. It held at 4°∞ until ready for electrophoresis. PCR products (Amplicons) were visualized in 1.5% Agarose gel stained with ethidium bromide. The gel was observed for the appropriate size DNA band under a UV trans-illuminator.

PCR Amplification of *T. vivax DNA Extract*

The PCR amplification of the samples was performed in a 25µl reaction that contained 2.5µl of genomic DNA extract, 0.5µl of 20µM primer (Tv.80.24F and Tv.322.80R), 12.5µl of one Taq® Quick-Load® 2x Master Mix with Standard Buffer (New England Bio Labs) and the volume was made up with 9.0µl Nuclease Free Water (Promega®). This was taken to a thermocycler that was pre-set and pre-heated - Applied Biosystem®9700 PCR Machine for amplification. An initial denaturation at 95°C for 10 minutes, was followed by 34cycles of denaturation at 94°C for 60 seconds, annealed at 60°C for 30 seconds, extended at 72°C for 30 seconds, followed by another 34 cycles of denaturation at 94°C for 60seconds, annealed at 60°C for 30 seconds, extended at 72°C for 60seconds and was repeated until the 34th cycle in an Applied Biosystems®9700 PCR Machine It held at 4°∞ until ready for electrophoresis. The PCR products (Amplicons) were visualized in 2% Agarose gel stained with ethidium bromide. The gel was observed for the appropriate size DNA band under a UV trans-illuminator.

Preparation of Agarose Gel

1.5g of agarose powder was weighed on a weighing balance and was made to dissolve in 100 ml of Tris Boric Ethylene Diamine Tetra Acetic acid (TBE) buffer. The mixture was heated in an oven for 3 minutes for it to properly dissolve. It was allowed to cool in a ventilated hood. 0.5 µl ethidium bromide (Fermentas®) was added to the molten gel and stirred. It was poured on a clean gel plate containing a well borer. It was allowed to solidify in the ventilated hood. The borer was carefully removed thereby, leaving the gel with holes in the gel holder.

Preparation of 2% Agarose Gel for *Trypanosoma vivax*

2g of agarose powder was weighed on a weighing balance and was made to dissolve in 100 ml of TrisBoric Ethylene Diamine Tetra Acetic acid (TBE) buffer. The mixture was heated in an oven for 3 minutes for it to properly dissolve. It was allowed to cool in a ventilated hood. 0.5 µl ethidium bromide was added to the molten gel and stirred. It was poured on a clean gel plate containing a well borer. It was allowed to solidify in the ventilated hood. The borer was carefully removed thereby, leaving the gel with holes in the gel holder. 100 base pair (100bp) molecular ladder was used for *T. brucei* and *T. vivax*.

Data Analysis

Data generated during the study were analysed using the statistical software program SPSS version 26.0. The chi-square test was used to determine the association between infection and epidemiological variables while the student's t-test was used to determine the significant difference between the mean Packed Cell Volume (MPCV) of infected and non-infected. The p-value was set at $p \leq 0.05$.

RESULTS

Of all the 400 small ruminants examined, 204 (51.00%) were Goats, and 196 (49.00%) were Sheep. Among the Goats, 47 (23.03%) were males, while 157 (76.96%) were females. Among the Sheep, 51 (26.02%) were males, while 145 (73.97%) were females. Of the 400 blood samples that were subjected to Giemsa stained thin, thick, and buffy coat smear microscopy examination, none was found to be positive for *Trypanosoma* species. The results of the mean Packed Cell Volume are presented in Table 2.

Table 2: Mean Packed Cell Volume (MPCV) for infected and Non-Infected Caprine and Ovine in Bassa and Jos East Local Government Areas of Plateau State, Nigeria.

LGA/Animal Type		N	MEAN (%)	SD	DF	T-VALUE	P-VALUE	DECISION
Bassa Caprine	Infected	10	31.20	6.55	58	1.53	0.129	Not Sig
	Non-Infected	50	34.00	4.97				
Ovine Bassa	Infected	38	25.37	4.36	138	2.79	0.005	Sig
	Non-Infected	102	27.60	4.15				
Caprine Jos East	Infected	52	35.57	4.15	144	1.86	0.065	Not Sig
	Non-Infected	94	34.02	5.11				
Ovine Jos East	Infected	9	35.33	5.32	52	2.14	0.036	Sig
	Non-Infected	45	31.27	5.18				

There was a significant difference in the mean PCV between those infected and those not infected ($P<0.05$). Of the 400 blood samples examined by PCR, among the 47 (11.75%) male Goats, 13 (3.25%) were infected and among the 157 (39.25%) female Goats, 49 (12.25%) were infected. Among the 51 (12.75%) male Sheep, 20 (5.00%) were infected and among the 145 (36.25%) female Sheep, 27 (6.75%) were infected. In all, 62 (30.39%) of the Goats examined were infected and 47 (23.97%) of the Sheep examined were infected in the study areas. The representative of the gel electrophoresis is presented in figures 1, 2 and 3.

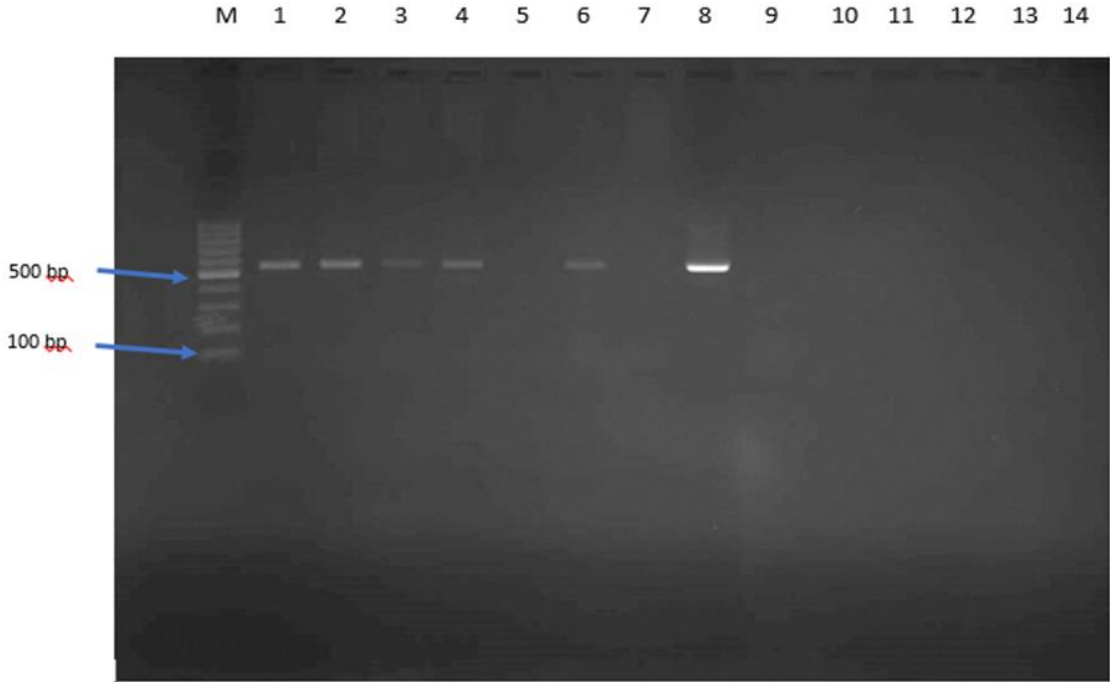

Figure 1: Representative of Gel Electrophoresis from Amplicons of *T. brucei* DNA from Caprine samples. Lane M. Maker (100bp) Lanes 1,2,3,4 & 6 are positive for *T. brucei* (540bp). Lanes 5, 7, 9, 10, 11, 12 &13 are Negative. Lanes 8 & 14 are Positive & Negative Controls, respectively.

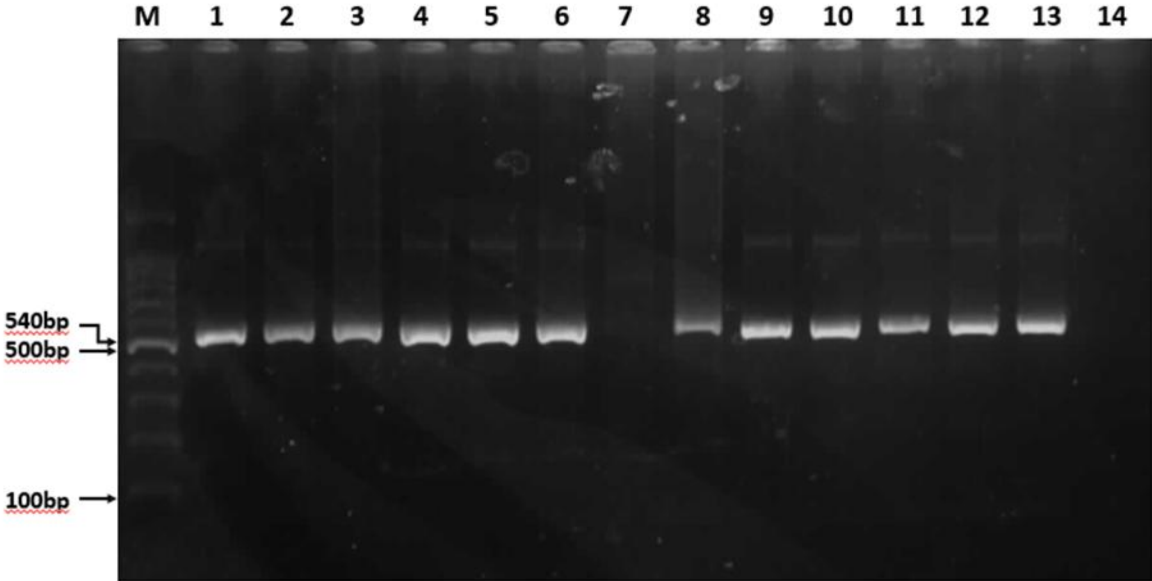

Figure 2: Representative of Gel Electrophoresis from Amplicons of *T. brucei* DNA from Ovine samples. Lane M. Maker (100bp) Lanes 1-6 and lanes 8-12 are positive for *T. brucei* (540bp) Lane 7 is Negative. Lanes 13 & 14 are Positive & Negative Controls, respectively.

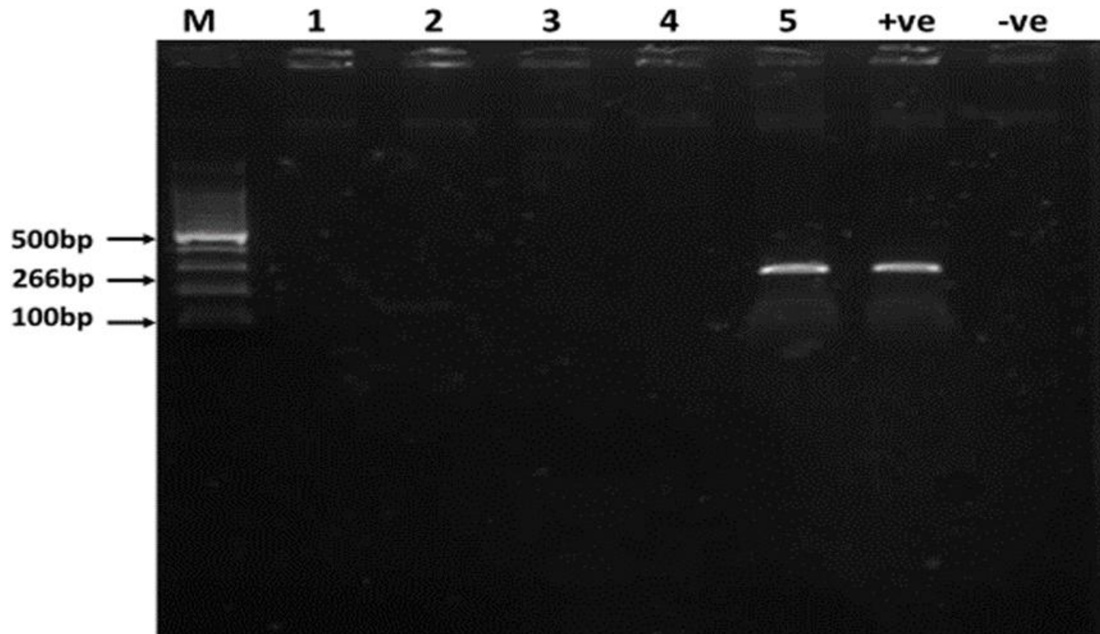

Figure 3: 2% Agarose gel electrophoresis of PCR products using Tv.80.24F and Tv.322.80R specific primers. Lane M: 100bp DNA molecular marker (Fermentas®). Lanes 1, 2, 3 & 4 are negative. Lane 5 is positive for *Trypanosoma vivax* from an ovine in Jos East LGA. Lanes 6 and 7 are positive and negative controls, respectively.

Touchdown PCR detected the presence of *Trypanosoma* species. Out of which 109 (27.27%) samples were positive for *Trypanosoma brucei* DNA amplification product of 540bp and 1(0.25%) sample was positive for *Trypanosoma vivax* DNA amplification product of 266bp (Table 1).

DISCUSSION

Sheep and Goats will continue to constitute an important part of the lives and livelihood of subsistence farmers in Nigeria and especially in Plateau State, both as sources of low-cost economic investment and a source of animal protein. However, diseases such as trypanosomosis cause a setback to small ruminant productivity and may pose a threat to livestock owners. Accurate diagnosis and characterization of trypanosomosis are essential in determining the identity and prevalence of *Trypanosoma* species in livestock, which will aid the development of appropriate control programs. In our study, all the 400 blood samples examined by the Standard Trypanosome Detection Method did not yield any positive results. This may be due to the scanty presence of the parasite in peripheral blood combined with the insensitive nature of microscopy as recorded by earlier authors. (Peter and Peter 2017; Takeet et al. 2013; Ohaeri,2010; Tilki et al. 2016; Enwezor et al. 2012; and Kalu et al. 2001). According to Ohaeri, (2010), a 1.2% prevalence rate from 956 small ruminants was obtained by microscopy. This could be due to the differences in study areas and our lower sample size. In this study, the West African Dwarf breed of Goats recorded a high trypanosome infection rate of 62 (30.39%), while the Yankasa breed of

sheep recorded 47 (23.97%). Carrier status sustenance in the environment may be due to the ability of the West African Dwarf to maintain chronic trypanosome infection without obvious clinical signs, while the Sokoto red breed is more susceptible (Peter et al. 2015). We concluded that the infection rate based on the PCR results was 27.25% and 0.25% for *Trypanosoma brucei* and *Trypanosoma vivax* respectively among small ruminants.

CONCLUSION

Sub-clinical trypanosomosis as found in this study could be a potential salient contributory factor to decreased livestock productivity for smallholder farmers in the study areas. Therefore, for a more accurate and confirmatory diagnosis, the study recommends the inclusion of molecular diagnostics for enriched data generation required for more effective planning and implementation of control strategies towards African trypanosomosis elimination in Nigeria.

ACKNOWLEDGEMENT

We acknowledge the contributions of various Staff of the National Veterinary Research Institute (NVRI), and the Nigeria Institute for Trypanosomosis Research (NITR) domiciled in Vom, Plateau State, Nigeria, for the Laboratory analysis and Mrs. Pauline D. Chomo, Mr. James Budaye, and Mr. Aliyu Gankyu is especially appreciated.

DECLARATIONS

Ethical Approval: The study protocol was approved by the Institutional Animal Use and Care Committee (AUCC), National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria with the certification ID: NVRI/AEC/Ref No. ACE/02/45/18.
Consent to participate: The livestock owner's consent was obtained for the study before using their animals.

AUTHORS CONTRIBUTION

Joana Garba Peter, Celine Movihinze Adeiyongo, and Jael Asabe Yohanna conceptualized and designed the study. Jonah Yoa'seem Peter, Dinchi Andrew Tyem, and Joana Garba Peter participated in fieldwork and data collection. Joana Garba Peter, Rebecca Arin Yakubu and Dinchi Andrew Tyem performed laboratory work. Rebecca Arin Yakubu and Jonah Yoa'seem Peter interpreted the data. Rebecca Arin Yakubu and Jonah Yoa'seem Peter contributed new reagents. Joana Garba Peter prepared the first draft of the manuscript, reviewed by Celine Movihinze Adeiyongo, Jael Asabe Yohanna and Jonah Yoa'seem Peter. All authors contributed to the development of the final manuscript and approved its submission.

CONFLICT OF INTEREST: There are no conflicts of interest regarding the publication of this paper.

FUNDING: None

REFERENCES

Abro, Z., Kassie, M., Muriithi, B., Okal, M., Masiga, D., Wanda, G., Gisèle, O., Samuel, A., Nguertoum, E., Aime, R., Phile´mon, M., Adam, Y., Camara, M., Olet, P., Boucader, D., Jamal, S., Garba, A.I., Ajakaiye, J.J., Kinani, J.F., Hassan, M.A., Nonga, H., Daffa, J., Gidudu, A., Chilongo, K.,2021.The potential economic benefits of controlling trypanosomosis using a

waterbuck repellent blend in sub-Saharan Africa. *PLoS ONE* 16(70),DOI:10.1371/journal.pone.0254558

Adzandeh, E.A., Akintunde, J., Akintunde, E.A., 2015. Analysis of urban growth agents in Jos metropolis, Nigeria. *International Journal of Remote Sensing*, 4, 41–50. https://www.researchgate.net/publication/324942965.

Anyaegbunam L. C., Okafor O. J., 2013. Trypanosomosis in Red Sokoto and West African Dwarf Goats at Ikpa Abattoir, Nsukka, Enugu State, Nigeria. *Journal of Entomology and Zoology Studies*, 1(5), 35-37.

Ayana, Z., Lemma, D., Abera, B., Eticha, E.,2015. Prevalence of small ruminant trypanosomosis in Assosa and Homosha districts, Benishangul Gumuz Regional State, Northwest of Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 7(5), 186-192.

Dede, P., Halid, H., Omoogun, G., Uzoigwe, N., Njoku, C., Daniel, A., Dadah, A.,2005. Current tsetse and trypanosomosis situation on Jos Plateau, Nigeria. Epizootiological factors that may enhance disease transmission and spread. *Revue d'elevage et de médecine vétérinaire des pays tropicaux*, 58,1-2, 31-35.

Enwezor, F.N.C., Samdi, S.M., Ijabor, O., and Abenga, J.N.,2012. The prevalence of bovine trypanosomes in parts of Benue state, north-central Nigeria. *Journal of Vector Borne Diseases*, 49, (3), 188–190.

Fadiga, M., Jost, C., Ihedioha, J.,2013. Financial costs of disease burden, morbidity, and mortality from priority livestock diseases in Nigeria. Disease burden and cost-benefit analysis of targeted interventions. ILRI Research Report 33. Nairobi, Kenya: ILRI.

Food and Agriculture Organization (2017). The State of Food Security and Nutrition in the World 2017. Building resilience for peace and food security. Rome,http://www.fao.org/3/a-I7695e.pdf

Gall, Y., Woitag, T., Bauer, B., Sidibe, I., McDermott, J., Mehlitz, D., Clausen, P.H., 2004. Trypanocidal failure suggested by PCR results in cattle field samples. *Acta Tropica*, 92,1, 7- 16. https://doi.org/10.1016/j.actatropica.2004.04.003

Isaac, C., Ohiolei, J.A., Ebhodaghe, F., Igbinosa, I.B., Eze, A.A., 2017. Animal African trypanosomosis in Nigeria: a long way from elimination/eradication. *Acta Trop*ica, 176, 323–31. doi:10.1016/j.actatropica.2017.08.032

Kalu, A.U., Oboegbulem, S.I., Uzoukwu, M., 2001. Trypanosomosis in small ruminants maintained by low riverine tsetse population in central Nigeria. *Small Ruminant Research,*40, 109–115.

Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., Thrusfield, M.V., Welburn, S.C.,2013. A longitudinal survey of African animal trypanosomiasis in domestic cattle on the Jos Plateau, Nigeria: prevalence, distribution, and risk factors. *Parasites and Vectors* (6), 239-248

McLaughlin, G.L., Ssenyonga, S.S., Nanteza, E., Rubaire-Akiki, Wafula, O., Hansen, R.D., Vodkin, M.H, Novak, R.J, Gordon, V.R., Montenegro-James, S., James, M., Aviles, H., Armijos, R., Santrich, C., Weigle, K., Saravia, N., Wozniak, E., Gaye, O., Mdachi, R., Shapiro, S.Z., Chang, K.P., Kakoma, I., 1996. PCR- based detection and typing of parasites. In: Parasitology for the 21st Century. Aczel, M.A., Alkan, M.Z. (Eds.), CAB International, Wallingford Oxon. 261-287.

Murray, M., Murray, P.K., McIntyre, W.I.M.,1977. An improved Parasitological Technique for the Diagnosis of African Trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 71, 325-326. http://dx.doi.org/10.1016/0035-9203.

Odeniran, P. O., Ademola, I.O., Macleod, E.T., Welburn, S.C., 2018. Bovine and small ruminant African animal trypanosomiasis in Nigeria, Reginal Studies, and Reports – A review. *Veterinary Parasitology*, 13, 5–13.

Ohaeri, C. C.,2010. Prevalence of Trypanosomosis in Ruminants in Parts of Abia State Nigeria. *Journal of Animal and Veterinary Advances*, 99 (18), 2422-2426. DOI: 10.3923/java.2010.2422.2426

Peter, I.D., Dauda, Y., Thalma, P.B., Ndahi, J.J., Madziga, H.A., Stephen, J., Mustapha, A., 2015. A Retrospective Study of Small Ruminant Diseases Identified at the State Veterinary Hospital Maiduguri, Nigeria. *Journal of Animal Health and Production*, 3 (4), 88-93. https://doi.org/10.14737/journal.jahp/2015/3.4.88.93

Peter, J. G., Peter, Y. J.,2017. Laboratory-Based Study of Sheep Trypanosomiases in Bokkos LGA of Plateau State, Nigeria. *Journal of Agriculture and Veterinary Science,* 10,8, 9-14.

Pollock, J., 2001. Trypanosomosis: Country Report—Nigeria. *Tsetse and Trypanosomosis Information Quarterly* 24 (3), 80–83FAO, Rome, Italy.

Samdi, S., Abenga, J.N., Fajinmi, A., Kalgo, A., Idowu, T., Lawani, F., 2008. Seasonal Variation in Trypanosomosis Rates in Small Ruminants at the Kaduna Abattoir, Nigeria. *African Journal of Biomedical Research*, 11, 229 – 232.

Shamaki, B.U., Yanan, E.G., Omotainse, S.O., Balak, C.G., Halid, I.,2002. Ruminant Trypanosomosis in Plateau State of Nigeria: Current status. Book of Abstract of the 39th NVMA Annual Congress, Sokoto, 27-31. October 2002.

Soulsby, E.J.L., 1982. Helminthes, Arthropods and Protozoa parasites of domesticated Animals, Bailere Tindal, London, pp 232–233.

Swallow, B.M.,2000. Impact of trypanosomosis on African agriculture. PAAT Technical and Scientific Series. Food and Agriculture Organization.

Takeet, M. I., Fagbemi, B.O., Donato, M.D., Yakubu, A., Rodulfo, H.E., Peters, S.O., Wheto, M., Imumorin, I.G., 2013. Molecular Survey of Pathogenic Trypanosome in Naturally infected Nigerian Cattle. *Research in Veterinary Science*, 94, 553-561.

Tilki, T., Dagnachew, H. M., Merdasa, Y., Mulugeta, B., Kalayou, S., 2016. Epidemiology of trypanosomal infections of caprine in Nono district, southwest Showa zone Ethiopia. *Livestock Research for Rural Development*, 28,182.

Yanan, E.G., Evutu, M.A., Shamaki, B.U., Sugun, M.Y., Dongkum, C., Ogunsan, E.A.,2007. Epidemiology of small ruminant trypanosomosis in some communities in Jos East, Plateau State: A guinea Savanna Zone. North–Central Nigeria. *Journal of Protozoology*, 17, 44-47.