

Laboratory Based Study of Bovine Trypanosomiases in Bokkos LGA of Plateau State, Nigeria

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Abstract: Trypanosomes are flagellated protozoa that belong to the genus *Trypanosoma*, they cause diseases that are generally referred to as trypanosomiasis. Trypanosomiasis is identified by World Health Organization (WHO) as a neglected tropical disease; of which the clinical manifestation depends on the host and the infecting species. In this study, we investigated trypanosome infection in Bokkos LGA of Plateau State, Nigeria. The aim of the study was to determine the prevalence of bovine trypanosomiasis in Bokkos LGA of Plateau State. Specifically, to determine the prevalence of trypanosomiasis using conventional parasite detection and nucleic acid detection methods, and also to characterize the Trypanosome species using Polymerase Chain Reaction (PCR) method. A total of 110 heads of cattle were sampled from the study area. Blood was collected using a systematic random sampling method from the cattle. Sample DNA was extracted from the blood Buffy coat layer (BCT). The electrophoresis protein bands were read with the aid of a trans-illuminator equipped with software meant for that purpose. From the one hundred and ten blood samples analysed, 3 (2.7%) were positive for Trypanosome parasite. The prevalence of Trypanosome species by the BCT was 2.72% and 4.54% by PCR. The specie *Trypanosoma congolense* forest, *Trypanosoma congolense* Kenya, and *Trypanosoma brucei* were characterised. Our study has shown that PCR can be used to effectively study and validate carrier status of trypanosome infection in the field. It also suggests that *T. congolense* is the most prevalent species in this study area.

Key words: Trypanosome, Bokkos, Nigeria, Bovine

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I. Introduction

Trypanosomes are flagellated protozoa that belong to the genus *Trypanosoma*. They cause diseases that are generally referred to as trypanosomiases, clinical manifestations of the diseases depends on the host and the infecting species. *Trypanosoma cruzi* cause chagas disease, *T. brucei rhodesiense* and *T. brucei gambiense* cause sleeping sickness in humans, *T. congolense*, *T. vivax* and *T. brucei brucei* cause Nagana in livestock while, *T. evansi* causes surra in camels and horses.

Trypanosomiases is a disease identified by World Health Organization (WHO) as a neglected tropical disease.¹ Bovine trypanosomiases is an endemic animal disease that cause a drain on the financial resources of livestock farmers and the productivity of their livestock.² Nigeria is one of the approximately 30 African countries most affected by this disease, that impacts negatively on the income and nutritional status of rural women and children. Generally milk yield by local breeds of cow is low and but maybe further aggravated by trypanosome infections.

Investigations have revealed a popular complain by many Fulani women who hawk milk and milk products indicating a decrease in milk yield over the years in Bokkos Local Government Area (LGA) of Plateau State in Nigeria. While poor animal nutrition and environmental factors may contribute to such a decrease, we proposed trypanosome infection as a contributory factor in this otherwise low trypanosomiases prevalent ecological zone.

In this study, we investigated trypanosome infection in Bokkos Local Government Area of Plateau State, Nigeria to establish a prevalence and species of trypanosomes to provide crucial evidence for prioritising and targeting cost-effective intervention as well use as a tool for advocacy. A prevalence rate of 66.7%, due to *T. vivax*, 25% due to *T. congolense* and 8.3% due to *T. brucei* was reported by Dede *et al*, 2005 in this same area.³ That study however, was based only on conventional parasite detection method.

Gathering of accurate data regarding the diagnosis of trypanosomiasis is crucial for the development of good treatment and control strategies thus, Polymerase Chain Reaction (PCR) is used in this study to overcome the limitation of the conventional parasite detection technique.^{4,5} The diagnosis of bovine trypanosomiasis

including determination of carrier status has been improved by the application of DNA based techniques such as PCR, which is very sensitive and effective method for the detection of chronic stage or prepatent period of disease.⁶ Killed Trypanosome DNA does not remain in the blood for more than 24-48 hours, thus PCR based assay helps in the detection of only active infections after the drug therapy.⁷ The aim of this study was to determine the prevalence of bovine trypanosomiases in Bokkos LGA of Plateau State, Nigeria. Specifically, the Objectives were

1. To determine the prevalence of trypanosomiases in the study area using both conventional nucleic acid detection methods.
2. To characterize the Trypanosome species using Polymerase Chain Reaction (PCR) method.

The study was conducted in Bokkos LGA of Plateau State, Nigeria. Bokkos LGA is located 77 kilometres southwest of Jos City, the capital of Plateau State, Nigeria. This LGA is located between 9°18'00"N and 9° 00'00"E. It encompasses a total land area of 1, 682 Km² and has a population of 178, 454 people as at the 2016 Nigeria national census.

II. Materials and Methods

The sample size was determined using the formula described by Thrusfield.⁸ A total of 110 heads of cattle were sampled from in the study area. Five millilitres of blood was collected at random from each cattle, from the jugular vein using 10mls syringe and immediately transferred into bijoux bottles containing EDTA. This was stored in a flask containing icepacks and transported to Parasitology laboratory of National Veterinary Research Institute (NVRI) Vom. Conventional laboratory examination techniques (Wet preparation, Haematocrit Centrifugation Technique (HCT), Buffy Coat Technique (BCT), Packed Cell Volume (PCV) screening tests, thin and thick blood stained films were used to process the blood.

Using the QIAmp[®] DNA mini kit according to manufacturer's specification DNA was extracted from Buffy-coat layer of each of the samples. Electrophoresis was done with 8µl of PCR Amplicons in a 1.5% Agarose gel stained with Ethidium bromide in the presence of 2µl of gel loading buffer (Fermentas[®]), positive and negative controls were included. The electrophoresis was carried out at 100 volts for one hour. One hundred base pair DNA Marker (Roche, Mannheim, Germany) was used as DNA molecular size marker. DNA amplifications were viewed under UV light and documented with Gel Documentation system (Synegene, Indonesia). The electrophoresis protein bands were read with the aid of a trans-illuminator equipped with software meant for that purpose.

III. Results

Four (Wet preparation, Buffy coat technique, Thin smear, and Thick smear) of the standard trypanosomiasis detection methods were employed to determine the presence or other wise of bovine *trypanosoma* species in the 110 bovine blood samples collected. The Prevalence of trypanosomiases in the Fulani white breed of cattle according to sex is presented in Table 1 below.

Table 1: Prevalence of Bovine Trypanosomes in Bokkos LGA

Cattle	No. Examined	No. Positive (%)
Male	30	1 (3.3)
Female	80	2 (2.5)
Total	110	3 (2.7)

There is no significant difference in prevalence between the sex of the animals. $P < 0.05$

Table 2: Sensitivity, Specificity, Positive and Negative Predictive values of the conventional methods used in relation to the PCR method.

Method used	Number positive	Sensitivity (%)	Specificity (%)	Positive Pred. Value (%)	Negative Pred. Value (%)
Wet preparation	2	40.0	100.0	100.0	97.2
Thin Smear	2	40.0	100.0	100.0	97.2
Thick Smear	2	40.0	100.0	100.0	97.2
Buffy Coat	3	60.0	100.0	100.0	98.1

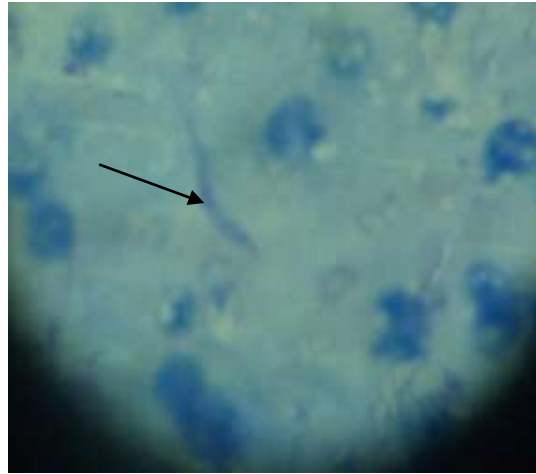


Figure1: *Trypanosoma* species in a Buffy coat layer smear surrounded by white blood cells,

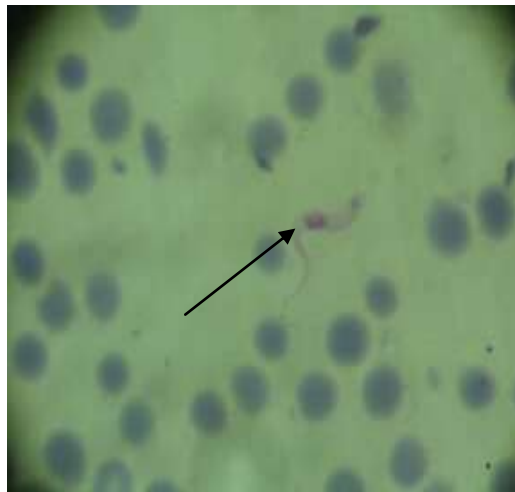


Figure 2: *Trypanosoma* specie (arrowed) on a thin blood film smear surrounded by red blood cells. The morphology is best studied in this thin smear presentation.

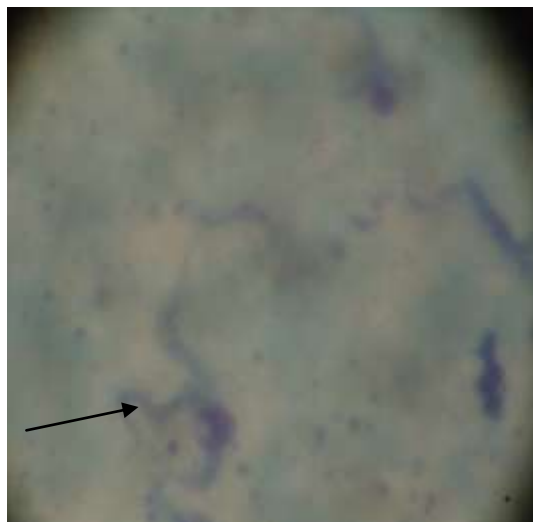


Figure 3: *Trypanosoma* specie (arrowed) in a thick blood film smear surrounded by lysed red blood cells.

The PCR detected bovine trypanosome parasite DNA from the field samples at different bands as shown in figure 4, 5 and 6 below.

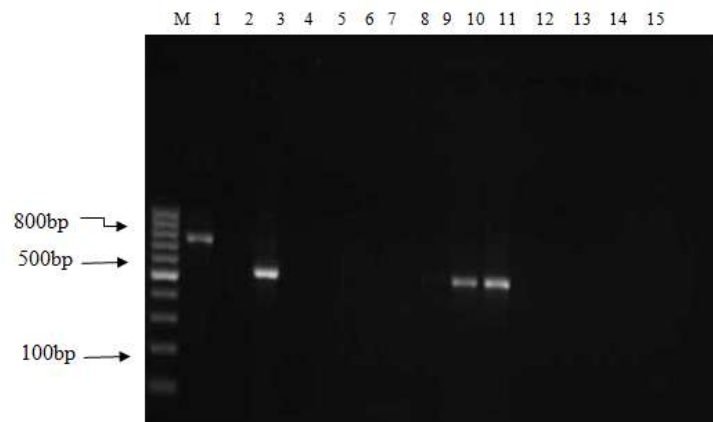


Figure 4: The 1.5% Agarose gel electrophoresis of PCR products using kin 1 and kin 2 specific primers. Lane M: 100bp DNA molecular marker (Fermentas®). Lane 1- *T. congolense* (780bp). Lanes 3 and 9 - *T. brucei* (540bp). Lanes - 10 and 15 are the positive and negative controls respectively.

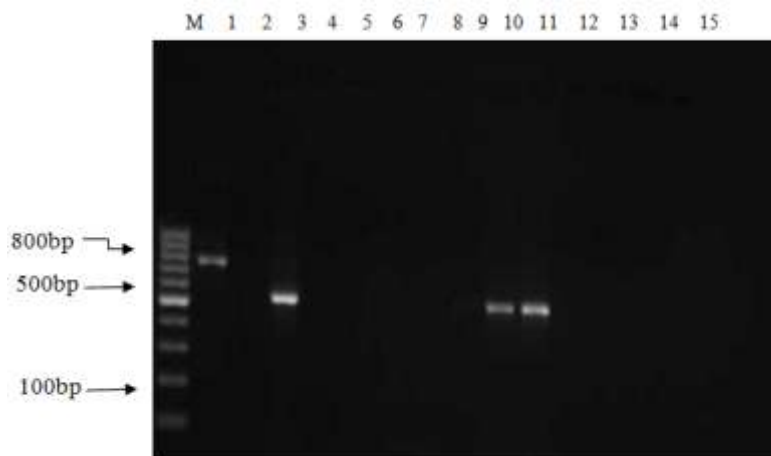


Figure 5: 1.5% Agarose gel electrophoresis of PCR products using kin 1 and kin 2 specific primers. Lane M: 50bp DNA molecular marker (Fermentas®). Lanes 1and 2 – *T. congolense* (680bp). Lanes 4 and 17: Positive and Negative controls respectively.

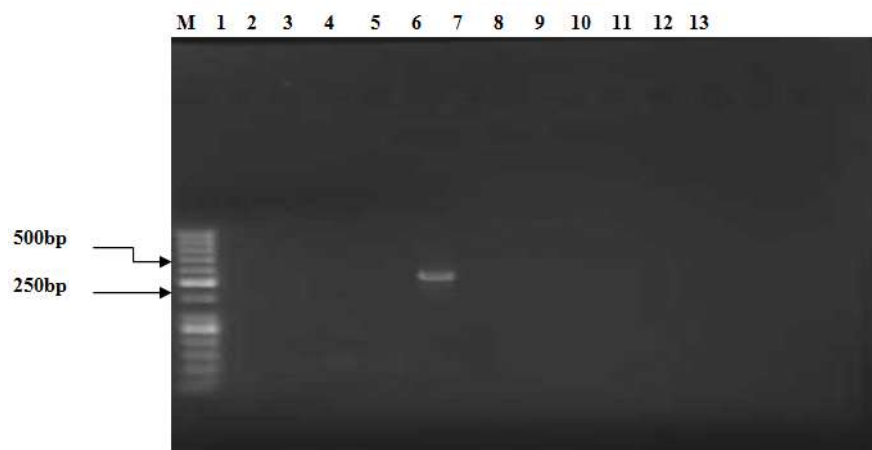


Figure 6: Absence of bands using specific primer for *Trypanosoma vivax*. Lane 5 is control.

IV. Discussion

In our study of bovine trypanosomiasis in Bokkos LGA of Plateau State, prevalence of 2.72% was detected by BCT. The BCT prevalence rate obtained in this study was quite low compared to 8.4% by Enwezor *et al* in 2009 in a grazing reserve in Kaduna State, Nigeria.⁹ Our prevalence is in agreement with the works done by Kalejaiye *et al* in 1995 and Omotainse *et al* in 2004.^{10,11} While Fajinmi *et al* got a lower infection rate of 1.8% in some ruminants by the use of BCT in some parts of Nigeria.¹²

The PCR prevalence rate of 4.54% was obtained in this study, by contrast Michael *et al* in 2013 obtained a prevalence of 63.7% by PCR.¹³ The result obtained is also at variance with the results obtained elsewhere in Africa; Vanden Bosshe *et al* in 2006 obtained a prevalence rate of 60.5% infection in South Africa, while Jesca *et al* in 2012, obtained a 57.5% infection rate in Ghana.^{14,15} The lower PCR prevalence obtained in this study could be as a result of the use of single DNA primer in our study compared to as many as six different sets of DNA primers used in other studies.

The pattern of transmitted Trypanosome distribution follows seasonal variation of the tsetse fly in the sub-humid zone, tsetse fly populations and distribution are usually lower in the dry season. Another reason for the low prevalence rate recorded in this study could be the harsh climatic conditions for Tsetse fly; temperatures are lower, humidity is higher with no available shade which limits tsetse fly dispersal.¹⁶

In our study, using conventional detection methods, BCT detected the higher number than other conventional methods, this is in agreement with the published work of Mbaya *et al*.¹⁷ Routine identification of *Trypanosome* species in our environment, is still based on morphology which is by microscopic analysis of samples from infected animal blood and/or from experimentally inoculated mice. The principal problem with microscopy is one of sensitivity. Nevertheless, the success of these methods also relies on the skills of the microscopist. The application of DNA techniques on the other hand, provides much improved levels of sensitivity, such as the possibility of detection of individual organisms in samples of whole blood. All the positive blood samples as initially revealed by the conventional detection method were also detected to be positive by the molecular method in this study. In this study Trypanosome species that could not have been characterised by the conventional parasite detection methods, were detected and characterised; *Trypanosoma congolense forest*, *Trypanosoma congolense Kenya*, and *Trypanosoma brucei*. The result obtained in this study is in agreement with the work done by Majekundun *et al* in 2013 who detected a prevalence rate of 27.7% infection due to *T. congolense* on the Jos Plateau.¹⁶ This however, is in contrast to the work of Fajinmi *et al* in 2011 who observed that *T. vivax* accounts for most of the infection in cattle trade at Sokoto Abattoir, Nigeria.¹² Michael *et al* in 2013 reported a prevalence rate of 48.7% due to *T. congolense* infection in his study covering both the northern and southern parts of the country.¹³ This study shows that PCR is a practical and specific test for the detection and characterisation of Trypanosome species.

V. Conclusion

In this study, the prevalence of Trypanosome species by the BCT is 2.72% and 4.54% prevalence by PCR. The result is lower than the results obtained from elsewhere in Africa using similar study methods. The lower prevalence indicates a low level transmission of the parasites in the study area. Our study has shown that PCR can be used to effectively study and validate carrier status of trypanosome infection in field studies. It also suggests that *T. congolense* is the most prevalent species in the study area.

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