

Original Research Article

## Evaluation of Microscopy and Polymerase Chain Reaction Methods for Identification of *Trypanosoma Vivax* in Cattle from Three Selected Abattoirs in Kaduna State, Nigeria

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### ABSTRACT

Microscopy and Polymerase Chain Reaction technique was used to evaluate 150 blood samples from cattle in three abattoirs in Kaduna State to detect the presence of *Trypanosoma vivax*. Out of the 150 blood samples collected and tested for the presence of *T. vivax* using Microscopy, 13 samples from Tudun-wada, Kawo and Makera abattoirs were found to be positive giving a total prevalence of 26.0%. Tudun-wada abattoir had the highest prevalence with 16.0% while 3.0% and 2.0% for Kawo and Makera abattoirs respectively. Tudun-wada abattoir at 0.016 was seen to be significantly different at  $p < 0.05$  from the remaining two abattoirs with 0.950 and 0.934. Thereafter, these samples were further subjected to PCR to detect the presence of this parasite, only 3 bands were confirmed to contain DNA for *T. vivax* as seen in Plate II after agarose gel electrophoresis at 150 bp and Plate I showing no band at 150bp. Thus, indicating a false positive result from 13 samples obtained from Microscopy in this study. It was then concluded that Microscopy which is the easiest technique for parasitic detection is not a gold standard for detection of *T. vivax* but PCR which is two to three fold more sensitive and specific.

**Keywords:** Abattoir, DNA, Microscopy, PCR, Primer, *T. vivax*.

### INTRODUCTION

Trypanosomiasis is one of the neglected tropical diseases in Africa caused by the genus *Trypanosoma*, affecting both humans and animals (domestic and wild).<sup>[1,2]</sup> The course of Trypanosomal infection varies considerably which depends on both the species of trypanosome and host species involved. This disease is generally characterized by the presence of parasite in

the blood, and continuous intermittent fever. Anemia develops mostly in affected animals, followed by loss of body condition (i.e. body weight, appetite, hair loss and dullness) reduced productivity, which if not treated consequently leads to death.<sup>[3]</sup>

*Trypanosoma vivax* is of veterinary importance in Africa and South American countries, it affects a large number of domestic ungulate species. Transmission is

either biological with tsetse fly as its vector or mechanical via other blood sucking dipterans. [1] Mechanical transmission across cows has been experimentally demonstrated for tabanids, [4] however in Africa, mechanical and cyclic transmission co-exist in the field but only mechanical transmission can explain the permanent presence of *T. vivax* outside tsetse belt region.

Routine diagnosis of Trypanosomes using microscopy shows poor sensitivity and specificity under field conditions, [5,6] however this limitation of microscopy for detecting the parasite led to the development of a range of serological tests such as Complement Fixation Test (CFT), Indirect Fluorescent Antibody Test (IFAT) and Enzyme-Linked Immunosorbent Assay (ELISA). These methods were unable to differentiate between existing infection and previous exposure which lack specificity. [5,6] However, in recent years, polymerase chain reaction (PCR), has been widely used for detection of *T. vivax* and it has shown to be highly specific and sensitive. [7] The use of this technique in detecting the parasite from its DNA is the most reliable technique for specific identification in infected animal. [8,9] Species-specific primer targets DNA and identifies the presence of pathogenic species which is highly sensitive and specific. [6] Therefore, this study is aimed at identifying *T. vivax* using specie-specific primer for PCR in blood from cattle in Kaduna metropolis, Kaduna state.

## MATERIALS AND METHODS

### Study Area

This study was conducted in Kaduna metropolis, Kaduna State, Nigeria. Kaduna State lies between latitude 9°30' 0 N and 11°0'0 N, longitude 6°0'0"E and 11°0'0"E with population of 6,066,562 and growth rate of 2.27% according to C-GIDD report of 2008. [10] and National bureau of statistics

(NBS). [11] Farming is the major practice in the state with about 80% of the people actively engaged in animal and crop farming. [12] Kaduna State is a major transportation route for animals from northern to southern part of Nigeria. [12]

### Collection of samples

Blood samples were collected from 150 cattle at point of slaughter daily for a period of 5 months. Fifty blood samples were randomly collected from cattle of each abattoir from 6:00 -9:00 am making a total of 150 blood samples. Five ml of blood sample was collected by a veterinary doctor from jugular vein of each cattle at point of slaughter from each abattoir using a sterile bottle containing EDTA and it was placed in cooler with ice packs which was immediately transported to the Nigerian Institute for Trypanosomiasis Research (NITR) Kaduna State, Nigeria and prepared for analysis upon arrival for the presence the parasite in blood samples collected.

### Wet mount

The method described by Cheesebrough [13] was used. A drop of blood was placed on a clean glass slide and covered with a cover slip to spread, it was examined at x40 magnification to detect motile trypanosomes which was seen either directly, moving between the red blood cells, or indirectly, as they cause the blood cells to move.

### Polymerase Chain Reaction (PCR)

This is a molecular based assay for detection of infectious diseases which amplify single copy to few copies of parasitic DNA from samples obtained. This technique includes extraction of the DNA, PCR amplification and agarose gel electrophoresis of PCR products (amplicons). In this study, the method of Morlais *et al.* [14] was employed with slight modifications. Table 1 shows the specific primer for *T.vivax* with its amplicon size.

Table 1: primer sequence for *T. vivax* and its amplicon size

Primer name	Primer sequence	Amplicon size	Reference
TW1	5'CTG AGT GCT CCA TGT GCC AC-3'	150bp	Morlais <i>et al</i> 1998
TW2	3'CCA CCA GAA CAC CAA CCT GA-5'		

TW1 and 2 (*T. vivax* forward and reverse)

### Extraction of parasitic DNA from blood samples

Blood samples collected from abattoirs and reagent kits for PCR stored in refrigerator were brought to room temperature. 700µl of genomic buffer (PBS) lysis solution and 500µl of blood sample were mixed into a tube, placed in water bath at 52°C for 10 minutes. Stock solution was poured into collection tube and centrifuged for 12,000 rpm and the supernant was discarded. 500µl of wash buffer 1(WB1) was added and centrifuged, it was discarded. 500µl of wash buffer 2 (WB 2) was added, centrifuged and discarded thoroughly by shaking, it was centrifuged, emptied and collection tube was changed, 100µl of DNA pre-wash buffer was added, centrifuged and not discarded, 50µl of DNA pre-wash buffer was added and centrifuged before it was discarded. 50µl of nuclease free water was added onto DNA binding solution and centrifuged at 12,000 rpm for 1 minute. [15]

### PCR Amplification

Amplification was carried out using specific primer for *T. vivax* as shown in Table 1 for preliminarily screening of DNA samples. [14] Initially, 2µl of template DNA was transferred into a PCR tube and 13µl of PCR mix (10x PCR buffer, 2 mM dNTP mixture, triple distilled water, and 0.5 Taq polymerase (Inqaba biotec, Zymo Research) and primers were added into the sample. PCR was performed in a thermal cycler programmed to a temperature-step cycle of 94°C at 3 min, 94°C at 30 min, 60°C at 30 sec, followed by 30 min extension at 72°C for a total of 30 cycles. The final extension was carried out at 72°C for 5 min. The products were analyzed by electrophoresis in 2% TAE (Tris-acetate-EDTA) agarose gel together with 100 bp DNA ladder as a standard molecular weight marker.

### Gel Electrophoresis of Amplicons

The PCR product was visualized in 2% agarose gel. Two grams of agarose (Oxoid, UK) powder was dissolved in

100ml of 1 × Tris –acetate buffer (TAC) and heated to melt the agarose. 5µl of *Ethidium bromide* was added to the heated mixture and poured into a gel casting tray with comb inserted. The gel was allowed to cool and solidify at room temperature. The comb was removed and 300ml of 1 × Tris-acetate was dispensed on the gel tank. The wells were loaded with 5µl of PCR product mixed with 1µl of loading dye; the gene ladder was loaded in wells. At about 20-30 minutes, the products migrated based on their molecular weight at 60 volts. PCR products were placed under ultra- violet light to detect the amplicons using gel documentation system. [15]

### Data Analysis

The data obtained were analyzed using Statistical Package for Social Sciences version 20.00. Results were reduced to percentages and presented in tables and figures. Inferential statistics (Analysis of variance) was used to conclude the result. Values of  $p < 0.05$  were considered significant at 95% confidence interval.

## RESULTS

This study was conducted to determine the presence of trypanosomal infection in cattle from three selected abattoirs in Kaduna metropolis by Microscopy and PCR methods. Out of the 150 blood samples collected and tested for the presence of *T. vivax*, a total prevalence of 26.0% was seen to be positive for samples subjected to microscopy. For individual study area, 8 samples were found to be positive in Tudun-wada, 3 samples for Kawo and 2 for Makera abattoirs respectively. The highest prevalence was recorded in Tudun-wada abattoir with 16.0%, Kawo abattoir with 6.0% and Makera had the least prevalence of 4.0 % as shown in Table 2. At  $p < 0.05$ , Tudun-wada abattoir with the highest prevalence is significantly different from the remaining two abattoirs.

The percentage prevalence by microscopy of *T. vivax* in selected abattoirs in Kaduna metropolis, Nigeria

Study area	Samples	No of positive	% Percentage prevalence	p-Value(p<0.05)
Tudun wada	50	8	16.0	0.016
Kawo	50	3	6.0	0.950
Makera	50	2	4.0	0.934
<b>Total</b>	<b>150</b>	<b>13</b>	<b>26.0</b>	

These samples were further subjected to PCR to confirm the presence of *T. vivax*. Plate I shows the agarose gel electrophoresis obtained after amplification

of *T. vivax* with primer sequence of 150 bp with no bands present for *T.vivax* and Plate II showing three bands indicating the presence of *T.vivax*.

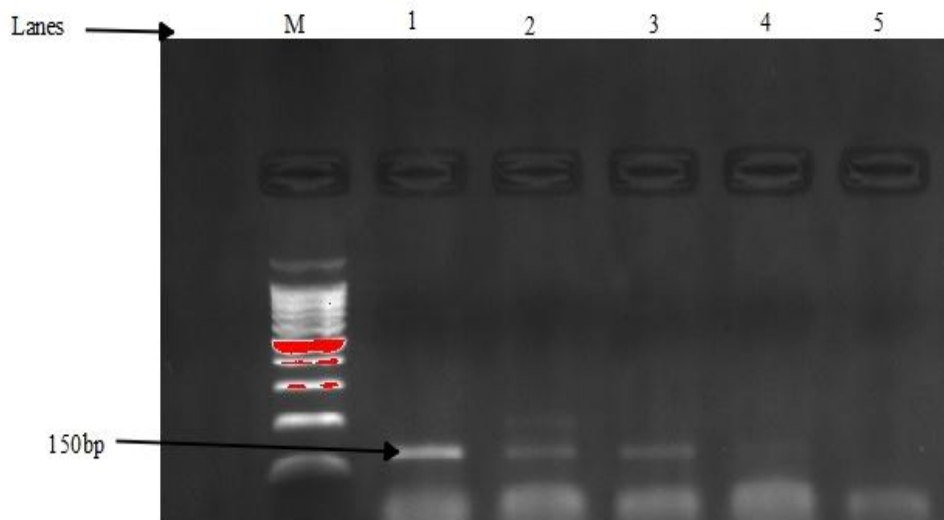
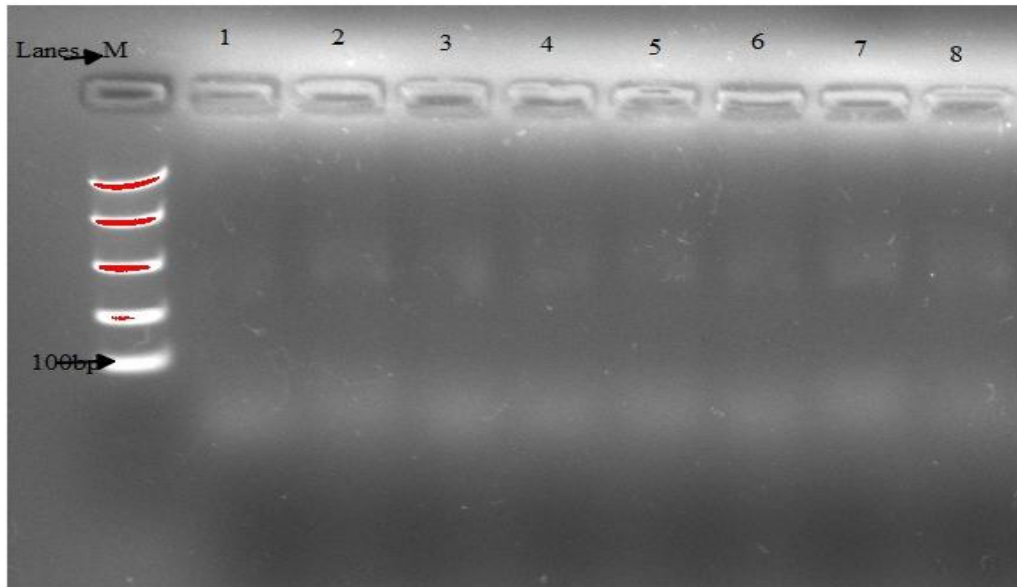


Plate II: The Agarose gel electrophoresis obtained after amplification of *T. vivax* at 150bp.

## DISUSSION

*Trypanosoma vivax* is one of the major debilitating disease affecting cattle in

Nigeria. The result obtained from this study showed an infection rate of 26.0% which is lower than 94.9% previously reported by

Omotaise et al. [16] in central Nigeria and higher than 14.3% reported in northern Nigeria by Samdi et al. [17] This study is in concordance with the report of Agu et al. [18] Kalu et al. [19] Ohaeri. [20] This result could be due to low abundance of tsetse flies from source points where the animals were brought from and also treatment with anti Trypanosomal drugs (i.e. *Trypamidium*) by the farmers as the parasite was found to be endemic in some parts of northern Nigeria. Agriculture is one of the main activities of some indigenes in Kaduna State, therefore, the low prevalence reported (26.0%) in this study, is an evidence of endemicity as the spread of this disease is from the bite of an infected vector.

*T. vivax* was seen to have the highest prevalence in Tudun-wada abattoir with 16.0% while Kawo and Makera abattoirs had 3.0% and 2.0% respectively. This could be attributed to the fact that the cattle brought to this abattoir (Tudun-wada) have been exposed to tsetse flies carrying *T. vivax* for a long time during grazing in highly poor state. The difference in rate of infection observed from the selected abattoirs is as a result of more livestock activities carried out in Tudun-wada abattoir as compared to the remaining two abattoirs leading to the diversity in rate of infection. This 16.0% observed agrees with findings in some parts of Nigeria as reported by Agu et al. [18] Kalu et al. [21] Kalu et al. [19] Ikede, [22] Omotainse et al. [16] showing the parasite to be more prevalent in some parts of Kaduna state.

Out of the 150 samples obtained from three selected abattoirs in Kaduna State, 13 samples were found to be positive for microscopy. These samples were subjected to PCR, whereby, only three samples were seen to be carrying the DNA for *T. vivax* after gel electrophoresis using specie-specific primers. The specificity of this primer eliminates the presence of false positive result which could be as a result of contamination from host or manipulator DNA. It was observed that when *T. congolense* is in its active phase it moves

fast exhibiting the movement of *T. vivax* and  $10^4$  is the limit for detection of trypanosome per ml of blood using this conventional technique, thus the increased result obtained from this study. This correlates with the work of Fikru et al. [23] and Pillay et al. [24] on identification of *T. vivax*. Positive results obtained from PCR indicates active infection with the parasite for which the parasitic DNA will not persist for long in the host, therefore, this technique (PCR) is not only suitable for detecting parasites in the mammalian host, but also in the insect vector as reported by Dagnachew et al. [25]

Trypanosomes in cattle can be found to be acute or chronic depending on its source if it's from endemic or epidemic areas, with respect to microscopy, it exhibit very low sensitivity in the chronic phase characterized by low parasitaemia, in which PCR is two to three times more sensitive. [26] This therefore, indicates that PCR is two to three fold more sensitive in picking up DNA from blood with low or no parasitaemia, and also indicating false positive results as no bands was seen in Plate I above. From this study, it showed that proper diagnosis of *T. vivax* should be encouraged for field and epidemiological studies.

## CONCLUSION

In this study, the differences in infection rates obtained from three selected abattoirs showed that microscopy is the fastest technique for diagnosis of *T. vivax* in cattle, though it lacks sensitivity to be considered as a gold standard whereas, PCR provide an alternative gold standard for detection of this parasite. High infection rate observed in Tudun-wada abattoir showed that more activities is carried out as related to animal husbandry. The specie-specific primer makes diagnosis of *T. vivax* more accurate and also for large numbers of samples. This promises the possibility of carrying out large-epidemiological studies on *T. vivax* in a fast and accurate or effective way.

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