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DETECTION OF *T. VIVAX* AND *T. CONGOLENSE* IN CATTLE FROM SELECTED ABATTOIRS IN KADUNA METROPOLIS, KADUNA STATE, NIGERIA.

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ABSTRACT

African trypanosomiasis is disease caused by protozoan parasite in sub-Saharan Africa that affects human and animals. In animals it causes serious economic loss in livestock as a result of anaemia, loss of appetite and emaciation and when not treated death occurs. This study therefore, was aimed at detecting *T. vivax and T. congolense* in cattle from selected abattoirs in Kaduna State using the conventional wet mount technique and PCR. A total of 300 blood samples were obtained from the study area with 100 samples from each of three abattoirs. Out of the 300 samples, 64 were positive at a prevalence of 21.3% using wet mount, while for individual abattoir, the lowest prevalence was observed in Makera at 5.0% for 15 cows, Kawo at 5.7% for 17 cows; while Tudun-wada recorded the highest prevalence of 10.7% for 32 cows, respectively. *T. congolense* recorded a higher prevalence when compared to *T. vivax* with 17% for the former and 4.3% for the latter. Seventeen positive blood samples were randomly selected and subjected to PCR to confirm the presence of the parasites using specific primers. In addition to confirming the presence of both parasites with the PCR, false positive result were detected in the gel picture after electrophoresis compared to the earlier result of the microscopy. In conclusion, microscopy and PCR should be used complimentarily for the confirmatory diagnosis of trypanosomal infection in cattle in order to carry out effective prophylactic and therapeutic control measures and curb the menace of this neglected tropical disease.

Keywords: Abattoir, Trypanosomes, Cattle, *T. congolense, T. vivax,* Wet mount, Polymerase Chain Reaction

INTRODUCTION

Trypanosomes are unicellular protozoan parasites causing diseases in sub-Saharan Africa and South America (OIE, 2008; Mejia-Jaramillo *et al.*, 2011; Kneeland *et al.*, 2012; Odeniran and Ademola, 2018). The *Trypanosoma* genus contains many different species several among which cause disease in humans and livestock (Grebaut *et al.*, 2009). Trypanosomes belong to the order Kinetoplastida because of the large DNA-containing structure, the kinetoplast found at the base of the flagellum manage to escape the host's immune response due to the consequences of the immune reactions by switching the variable surface glycoprotein, a phenomenon known as antigenic variation (Damian, 1997; Brown, 2008; Amit *et al.*, 2015).

African Animal Trypanosomiasis (AAT) causes serious economic losses in livestock as a result of anaemia, pruritus, ocular discharge, loss of appetite and emaciation and if untreated could lead to death. It is found mainly in those regions of the sub-Saharan Africa where its biological vector, the tsetse fly, exists (Acha *et al.*, 2003; Bhatia *et al.*, 2006; Amit *et al.*, 2015), and can also be transmitted by other biting flies acting as mechanical vectors (*Tabanids*, *Hippoboscide* and *Stomoxys* spp.). The most important species include *Trypanosoma congolense, T. vivax* and *T. brucei brucei*. Other species such as *T. simiae* and *T. godfreyi* can also infect animals, concurrent infections can occur with more than one species of trypanosome (Njiokou *et al.*, 2004).

The three species earlier mentioned are of economic importance in cattle (Fajinmi *et al.*, 2007). They infect various species of domestic and wild animals such as cattle, deer, elephants, sheep, goats, donkeys, horses, camels, buffaloes, mule, dogs, pigs, foxes, tiger and jackals, with clinical signs such as intermittent fever, anaemia, loss of weight, oedema, nervous symptoms and abortion in female animals (Samdi *et al.*, 2011). It is also responsible for major production loss in the agricultural and livestock industry (Desquesnes *et al.*, 2013; Amit *et al.*, 2015). The fastest technique for detection of trypanosomes in peripheral blood is by direct microscopic examination, but generally,

diagnosis of the disease is based on the clinical evidences and parasitological tests carried out on observed animals. However, clinical signs like emaciation, fever, anaemia, lacrimation, corneal opacity and diarrhoea (Chaudhary and Iqbal, 2000; Amit *et al*., 2015) are not exclusive of trypanosomal infection and are insufficient for confirmatory diagnosis. Therefore, molecular diagnostic technique such as polymerase chain reaction (PCR) has proven to be a reliable technique for the confirmatory diagnosis of trypanosomiasis based on the detection of trypanosomal DNA in the blood samples. It is a highly sensitive and specific method for detection with specie specific primers targeting different subgroups of trypanosomes (Desquesnes, 1997; Masake *et al.,* 1997; Ventura *et al.,* 2002; Tavares *et al.,* 2011; Sudan *et al*., 2014; Parashar *et al*., 2015). The aim of the study therefore was to detect *T. vivax* and *T. congolense* by microscopy and PCR, and compare the sensitivity and specificity of both technique in the screened blood of cattle from selected abattoirs in Kaduna North and South LGAs of Kaduna State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in 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 a population of 6,066,562 according to C-GIDD (2008). One abattoir in Kaduna North (Kawo) and two abattoirs in Kaduna South LGAs (Tudun-wada and Makera) as shown in fig.1 below were selected

Fig 1: The map Kaduna showing the selected study area

Sample Collection

The sample size was determined using a reported mean prevalence of 24% by Majekodunmi *et al.* (2013) and the formula described by Naing *et al*., (2006) was adopted. The size calculated was 280, but a total of 300 was collected with 100 sample from each abattoir. One hundred blood samples were randomly collected from cattle of each abattoir from 6:00 -9:00 AM for a period of four months. Five mLs of blood sample was collected by a Veterinary Doctor from jugular vein of each cattle at point of slaughter into a sterile EDTA bottle and placed in cold box containing ice packs, it was immediately transported to Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna State and prepared for subsequent analyses for detection of trypanosomes.

Wet Mount

The method described by Cheesebrough (2005) was adopted for microscopic examination of the parasites. 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 blood cells, or indirectly, as they cause the blood cells to move. The notable distinguishing features between both trypanosomes was that; *Trypanosoma congolense* is smaller with no free flagella while *T. vivax* is large with a distinct free flagella.

Polymerase Chain Reaction Method (PCR)

This is a sensitive method for the detection of DNA from blood of cattle for trypanosomes. It involves extraction of the DNA, PCR amplification and detection of PCR products by electrophoresis. The methods of Baticados *et al*. (2005) and Morlais *et al*. (2008) were employed with slight modifications.

Table 1: Result showing the specific primers for both parasites with their amplicon size during the experimental period

Primer name	Primer sequence	Amplicon size	References
TCS(F)ILO344	5'-CGA GAA CGG CAC TTT GCG A-3'	316bp	Baticados *et al*.,
TCS(R)ILO345	3'GGA CAA ACA AAT CCC GCA CA-5'		(2005).
TVW1	5'CTG AGT GCT CCA TGT GCC AC-3'	150bp	Morlais *et al*., (2008).
TVW2	3'CCA CCA GAA CAC CAA CCT GA-5,		

Key: TCS-*Trypanosoma congolense* ; TVW-*Trypanosoma vivax*

DNA extraction of parasite from whole blood

All refrigerated samples and reagents kits were brought to room temperature. Seven hundred microlitres of genomic buffer (PBS) lysis solution was pipetted into tube with 500µl of blood sample and placed in water bath at 52 °C for 10 minutes. Sample mixture was poured into collection tube and centrifuged for 12,000 rpm and the supernatant was discarded. Five hundred microlitres of wash buffer 1(WB1) was added, centrifuged, and supernatant was discarded. Five hundred microlitres of wash buffer 2 (WB 2) was also added, centrifuged and supernatant discarded after shaking it thoroughly, it was centrifuged again, 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. Fifty microlitres of nuclease free water was added onto DNA binding solution and centrifuged at 12,000 rpm for 1 minute (Baticados *et al*., 2005).

Polymerase Chain Reaction

A conventional PCR was performed using primers specific for *T. vivax* and *T. congolense* as shown in Table 1 for preliminarily screening of DNA samples using the methods of Baticados *et al*. (2005) and Morlais *et al*. (2008). Initially, 2 µl of template DNA was transferred into a PCR tube and 13 µl 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 PCR products were analysed by electrophoresis in 2% TAE (Tris-acetate-EDTA) agarose gel together with 100 bp DNA ladder as a standard molecular weight marker (Baticados *et al*., 2005).

Agarose gel Electrophoresis

The PCR product was visualized in 2% agarose gel. Two grams of agarose (Oxoid, UK) powder was dissolved in 100 mLs of 1 × Tris –acetate buffer (TAC) and heated to melt the agarose. Five microlitres of ethidium bromide was added to the heated mixture and poured into a gel casting tray and combs inserted. The gel was allowed to cool and solidify at room temperature. The comb was removed and 300 mLs of 1 × Tris-acetate was dispensed on the gel tank. The wells were loaded with 5 µl of PCR product mixed with 1 µl of loading dye; the gene ladder was loaded in wells. The preparation of electrophoresis for 20-30 minutes at 60 volts, the products migrated based on their molecular weight. PCR products were placed under ultra- violet light to detect the amplicons

using gel documentation system (Baticados *et al.*, 2005).

Data Analysis

Results were presented as percentages. Chi square test was used to test for association between categorical variables using Statgraphics 5.0 (Statpoint Technologies, Inc., Warrenton, VA, USA) package. Values of $p<0.05$ were considered significant at 95% confidence interval (CI).

RESULT

Out of the 300 blood samples collected from cattle in the study area, 64 were positive for both *T. congolense* and *T. vivax* at a prevalence of 21.3% using microscopy as shown in Table 1 below.

Table 1: Total prevalence of Trypanosomes isolated from selected abattoirs in the study area

Abattoirs	Total examined	*T. vivax*	*T. congolense*	Total	p-Value
Kawo	100	3	14	17(5.7%)	0.018
Tudun-wada	100	8	24	32(10.7%)	
Makera	100	2	13	15(5.0%)	
Total	300	13(4.3%)	51(17.0%)	64 (21.3%)	

Trypanosoma congolense showed a higher prevalence at 17.0% when compared to *T. vivax* with a prevalence of 4.3%. However, for individual abattoirs, samples from Kawo had a prevalence of 5.7% for both parasites, while Makera recorded 5.0%, the highest prevalence was recorded for Tudun Wada abattoir at 10.7%. A total prevalence of 21.3% is reported for all the selected abattoirs which shows a no significance at p=0.018.

A total of 17 blood samples were randomly selected from 64 positive using microscopy and subjected to PCR for both parasites. Twelve positive samples for *T. congolense* using wet mount were subjected to PCR, but only lanes 2 and 12 were confirmed positive for *T. congolense* at 316bp as shown in Plate I.

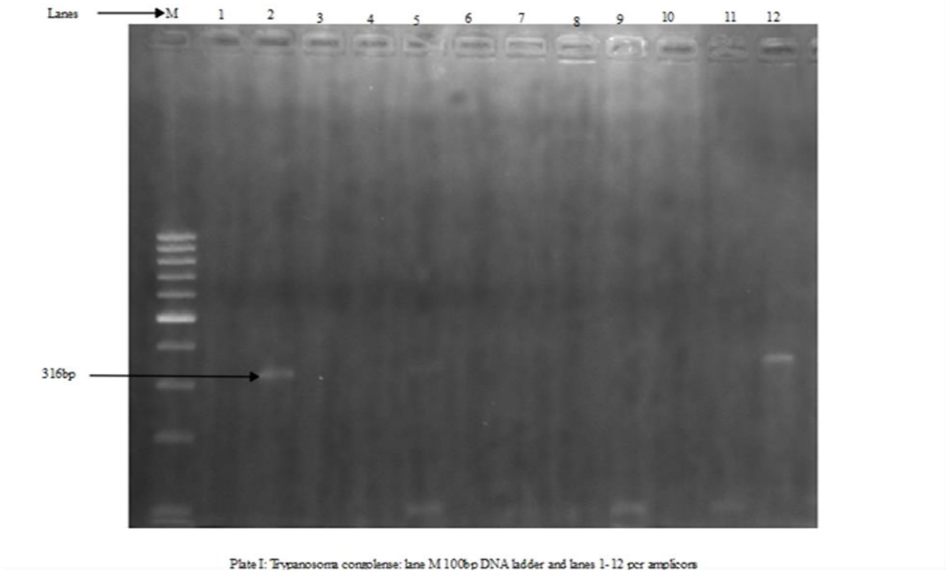

Plate I: Trypanosoma congolense: lane M 100bp DNA ladder and lanes 1-12 pcr amplicons

Five positive samples for *T.vivax* using wet mount were subjected to PCR but confirmed positive were seen in lanes 1-4 while lane 5 showed a negative band as shown in plate II.

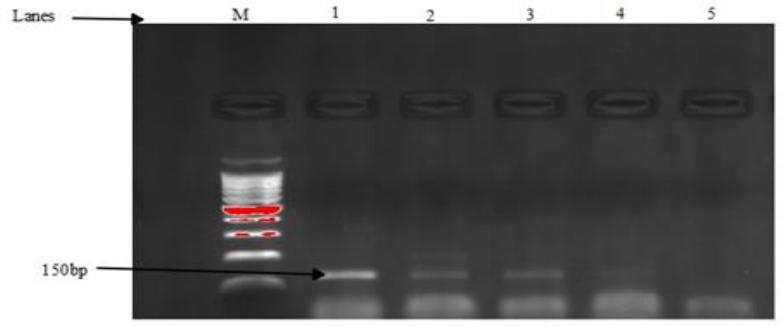

Plate II: TheAgarose gel electrophoresis obtained after amplification of T. vivax at 150bp.

DISCUSSION

This study was aimed at detecting *T. congolense* and *T. vivax* by microscopy and PCR in cattle from selected abattoirs in Kaduna metropolis. The 21.3% prevalence observed from the three abattoirs is similar to the 17.8% - 50% reported by Qadeer *et al*. (2009) but relatively higher than the 3.9% and 1.2% previously reported in Ogbomoso, Ogun State and Zaria, Kaduna State, respectively (Ahmed and Agbede, 1993; Ameen *et al*., 2008). The prevalence recorded in this study disagrees with the report of Nabulime *et al*. (2014) and Samdi *et al*. (2011). The prevalence of trypanosomal infection observed in this study could be as a result of extensive system of animal husbandry practice adopted, which influenced survival of both vector and parasite (Fasanmi *et al*., 2014). It could also be as a result of the animals being exposed to trypanosome vectors for a long time during grazing or treatment of the disease repeatedly using the same drugs resulting in the parasite becoming resistant, thus availing the prevalence reported in the study.

This study was able to determine the presence of trypanosomes using microscopy and molecular technique. In addition to the determination of the presence of trypanosomes through the use of Polymerase Chain Reaction, it was observed that samples positive using wet mount where found to be negative using PCR as observed in Plates I and II for both parasites in the three abattoirs. In this regard, the PCR detection test could be viewed as an additional method for the effective monitoring and diagnosis of trypanosomes. Also specific primer for each trypanosome specie was used to target only the parasitic DNA which offers an advantage that only blood samples with TCS ILO 344-345 for *T. congolense* and TVW1-2 for *T. vivax* hybridized with the DNA probed. The washing steps remove non-specific reactions which may interfere with the specific ones, thus, making PCR highly specific and sensitive for detection of trypanosomes. Several works have documented similar findings as recorded in this study, where it was noted that PCR technique is more specific and sensitive than microscopy particularly in the area of confirmatory diagnosis of many diseases including animal African trypanosomiasis (Valkiunas *et al.*, 2008; Ndao, 2009; Momcilovic *et al.*, 2019)

CONCLUSION

In conclusion, microscopy though remains a gold standard in parasite diagnosis as demonstrated in this study with the presence of trypanosome in the blood sample of cattle, therefore, we conclude that microscopy and PCR can be used complementarily for the effective diagnosis of *T. congolense* and *T. vivax* in domestic animals.

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