Original Research Article

The Prevalence and Detection of *Trypanosoma Congolense* by Microscopy and Polymerase Chain Reaction in Cattle from Kawo and Tudun -Wada Abattoirs in Kaduna State, Nigeria

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ABSTRACT

Detection of *Trypanosoma congolense* was carried out by microscopy and PCR in cattle from selected abattoirs in Kaduna metropolis, Nigeria. Two hundred blood samples were randomly collected from cattle in Kawo and Tudun-wada abattoirs, 100 samples each. A total of 38 blood samples representing 19.0% from the study were positive from the selected abattoirs using microscopy. Tudun-wada had a higher prevalence of 12% and not statistically significant at $p < 0.05$ and Kawo abattoir had a lower prevalence of 07%. 12 samples from both abattoirs were subjected to PCR amplification for confirmation of the presence of the parasite gene in blood samples obtained from the abattoir. After gel electrophoresis, *T. congolense* were seen at lanes 2 and 12 at 316bp. Therefore, it was concluded from the study that PCR should be used alongside Microscopy for effective surveillance and monitoring of the parasite in the case of prevalence studies.

Keywords: Abattoir, Cattle, Microscopy, PCR, Prevalence, *T. congolense*.

INTRODUCTION

Trypanosomes are unicellular protozoan parasites causing diseases in Africa and South America. [1-3] The *Trypanosoma* genus contains many different species which cause disease in humans and livestock. [4] Trypanosomes are transmitted through the saliva of the tsetse fly (Glossina genus) by biting its hosts. [5] This disease in animals is known as African Animal Trypanosomosis (AAT).

AAT is a parasitic disease, which causes serious economic losses in livestock ranging from anaemia, loss of appetite and emaciation and if untreated could lead to death. It is found mainly in sub-Saharan regions of Africa where its biological vector, the tsetse fly, exists and can also be transmitted by other biting flies acting as mechanical vectors (*Tabanids* and *Stomoxys*). [6-8] Protecting animals from trypanosomosis is difficult in endemic areas, as bites from tsetse flies and a variety of other insects are not prevented in other to limit the spread of the disease. [9] It infects 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 main clinical signs of high intermittent fever, anaemia, and loss of weight, oedema, nervous symptoms and abortion. [10] The parasite is also responsible for major production loss which is considered as a permanent constraint in livestock productivity. [11,8]

Microscopy is the only available tool for the detection of parasites through inspection of blood smears and also the easiest technique for detection of

trypanosomes in peripheral blood is by direct microscopic examination, although, detection of the disease is based on the clinical evidences with parasitological tests observed from the animals. [12]

Molecular techniques are suitable for detecting parasites in animals and insect vectors. Currently, Polymerase chain reaction (PCR) technique is the main sensitive tool for detection of diseases; it is based on the use of enzyme DNA polymerase that will amplify the sequences of DNA bases, until sufficient DNA material is produced to detectable levels. [13-15] Therefore, the aim of this study is to detect *Trypanosoma congolense* by microscopy and PCR and its prevalence in cattle slaughtered in Kawo and Tudun-wada abattoirs in Kaduna State.

MATERIALS AND METHODS
Collection of samples
A total of 200 blood samples were collected from different cattle at point of slaughter between Kawo and tudun- wada abattoirs in Kaduna State, Nigeria. During the study, 20 blood samples were randomly collected every two days interval for a period of four months from 6:00 -9:00 am. 5 ml (Five) of blood sample was collected by a veterinary doctor from jugular vein of each cattle at point of slaughter from each abattoir. The blood was collected using a sterile bottle containing EDTA and placed in cooler with ice packs. It was transported to Nigerian Institute for Trypanosomiasis

Research (NITR) (No 1 surami road Ungwan rimi, Kaduna State, Nigeria) and the blood samples were immediately prepared for analysis upon arrival for detecting the presence of Trypanosomes.

Wet mount
This was carried out using the method described by Cheesebrough. [16] A drop of blood was placed on a clean glass slide, covered with a cover slip and then 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.

Packed cell volume
Three quarters level of blood was filled into a sterile heparinized capillary tube, it was sealed using modeling clay sealant and centrifuged in a microhematocrit rotor for 5 minutes at 12 000 rpm. It was thereafter read using a microhematocrit reader for the percentage level of the blood collected from the abattoir for anaemia as described by Cheesbrough. [16]

Polymerase Chain Reaction Method
This is a sensitive method for the detection of DNA from blood samples of cattle for trypanosomes. It involves extraction of the DNA, PCR amplification and detection of PCR products by electrophoresis. The method of Baticados *et al*. was employed with slight modifications. [17] Table 1 shows the specific primer for the parasite with its amplicon size.

Table 1: Specific primer for detection *T. congolense* with its amplicon size

Primer name	Primer sequence	Amplicon size	Reference
TCS(F)ILO344	5'-CGA GAA CGG CAC TTT GCG A-3'	316bp	Baticados *et al*., (2005).
TCS(R)ILO345	3'GGA CAA ACA AAT CCC GCA CA-5'		

TCS-*Trypanosoma congolense*

Extraction of Trypanosomes for Polymerase Chain Reaction
All samples and reagents kits were stored at room temperature. Seven hundred microlitres of genomic buffer (PBS) lysis solution were pipette into tube with 500µl of blood sample and placed in water bath at 52^0C 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) were added to it and centrifuged at the same rate, and then discarded. However another five hundred microlitres of wash buffer 2 (WB 2) were added, centrifuged and discarded thoroughly by shaking. After that, the collection tube was centrifuged, empty for the preparation

of another sample. 100µl of DNA pre-wash buffer was added, centrifuged and was not discarded, and again 50µl of DNA pre-wash buffer was added and centrifuged before it was discarded. Thereafter, fifty microlitres (50µl) of nuclease free water were added onto DNA binding solution and centrifuged at 12,000 rpm for 1 minute. [17]

PCR Amplification

The PCR was performed using specific primer for *T. congolense* (Table 1) for preliminarily screening of DNA samples. [17] 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 PCR 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. [17]

Detection of PCR products by Electrophoresis

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. Five microlitres of *Ethidium bromide* was added to the heated mixture and poured into a gel casting tray and comb was inserted. The gel was allowed to cool and solidify at room temperature. After solidifying, the comb was removed and wells were created. Three hundred ml (300 ml) of 1 × Tris-acetate was dispensed into the gel tank. The wells were loaded with 5µl of PCR product mixed with 1µl of loading dye and the gene ladder was also loaded in the wells. This preparation for electrophoresis was carried out for 20-30 minutes at 60 volts; this allowed the products to migrate based on their molecular weight. PCR products were placed under ultra- violet light to detect the amplicons using gel documentation system. [17]

Data analysis

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

RESULTS

The results on the presence and detection of *T. congolense* in cattle from two selected abattoirs in Kaduna metropolis by Microscopy and PCR techniques are given below. Out of the 200 blood samples tested for *T. congolense*, 38 were positive, giving a prevalence of 19.0 % by microscopy which was seen to be not-statistically significant at $p < 0.005$ as shown in Table 2. The highest prevalence was recorded in samples collected for microscopy from Tudun-wada abattoir at 12% and Kawo abattoir with 07% for 24 and 14 samples respectively.

Table 2: The prevalence of *T. congolense* by microscopy in the selected abattoirs in Kaduna metropolis, Nigeria

Abattoirs	Sample size	*T. congolense* (%)	p-value
Tudun-wada	100	24 (12%)	0.018 (p>0.05)
Kawo	100	14 (07%)	
Total	200	38(19.0%)	

Identification of *Trypanosoma congolense* by microscopy

On wet mount when viewed under x40 magnification, it was seen moving between the blood cells directly or indirectly as it causes the blood cells to move. It has medium-sized marginal sub terminal kinetoplasts, no free flagella, and poorly-developed undulating or inconspicous membrane. Further analysis for confirmation of the parasite using PCR, shows the agarose gel electrophoresis obtained after amplification of *T. congolense* with specific sequences of 316 bp. Out of the thirty eight blood samples, 12 samples were randomly tested from the two

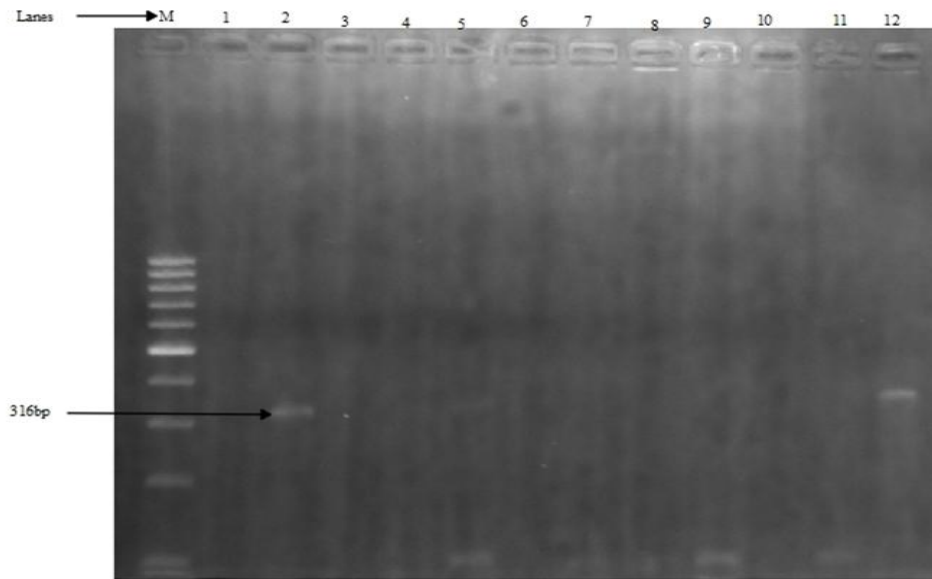abattoirs detected by microscopy, only lanes 2 and 12 were confirmed positive for *T. congolense* using PCR.

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

DISCUSSION

This study was observed to have 19.0% prevalence from the two abattoirs. The results observed in this study is similar to study carried out in Adamawa State (A state in northeastern Nigeria) which indicated a prevalence of 17.8%-50% as reported by Qadeer *et al*. [18] but higher than 3.9% and 1.2% previously reported in Ogbomoso, Ogun state and Zaria, Kaduna State respectively. [19,20] The change observed in this study is as a result of different animal husbandry practices adopted, which can influence survival of parasite and also, it has been observed that *T. congolense* is more prevalent in cattle than other species of trypanosomes that affect animals. [21] Prolong treatment with same antitrypanosomal drugs by the farmers or herd's men without consulting veterinary doctors probably led to the parasites becoming resistant to drugs; hence increase in the prevalence rate observed in Tudun-wada abattoir which showed a high prevalence of 12% as shown from the study.

The presence of the parasitic gene was examined in twelve blood samples by PCR amplification at 316bp of blood from cattle obtained from two selected abattoirs.

It was seen from the bands that *T. congolense* was present in both abattoirs. This coincides with the report of Baticados *et al*. [22] on parasitological and PCR detection of *Trypanosoma evansi* in buffalos. Therefore, PCR is an additional method for effective detection of Trypanosomes as microscopy is the only readily tool available for diagnosis of this parasite from blood of animals using wet mount examination, but microscopy is laborious and time consuming as individual slide had to be examined, however the use of specific primer will target only the parasitic gene present in the blood samples obtained. Thus, it is easier as more samples can be examined especially in the case of prevalence study where samples are many. In this study, it was observed that microscopy can be used alongside PCR for effective surveillance and monitoring of *T. congolense* for detection of true prevalence of the disease.

CONCLUSION

In conclusion, both methods (Microscopy and PCR) should be used alongside for effective detection of the parasites. These methods showed promising

results in this prevalence study carried out. This could enhance better treatment of the disease which is mostly caused by this parasite in cattle. It has been observed from this study that blood samples collected from Tudun-wada abattoir had a higher prevalence than Kawo abattoir.

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