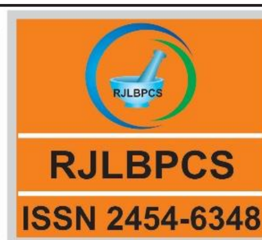

Original Research Article **DOI - 10.26479/2017.0205.16**

DIAGNOSIS OF TRYPANOSOMA BRUCEI IN CATTLE FROM THREE SELECTED ABATTOIRS AND THREE FARMS IN KADUNA STATE

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ABSTRACT: A total of 150 blood samples were collected from catlles in selected three abattoirs and three farms for diagnosis of T.b.brucei in Kaduna State. The prevalence of 21% from abattoirs was recorded as compared to the 1% recorded in farms which is statistically different at $p<0.05$ but between each abattoir, there was no significant difference at $p<0.05$ indicating no relationship between each study site using microscopy. Further analysis was carried out using PCR with specie-specific primer for T.b.brucei at 230bp, bands were seen after gel electrophoresis confirming the presence of the parasite. In conclusion, this study shows that blood samples from the abattoir had more infection than those collected from the farms indicating that the animals brought to the abattoirs are mostly in critical health conditions. Therefore, there is a need for proper diagnosis and treatment of animals so as to promote more meat and milk production for human consumption.

KEYWORDS: Abattoirs, Blood, Cattle, Farms, T.b.brucei, Trypanosomiasis

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1. INTRODUCTION

Trypanosomes are unicellular protozoan parasites that belong to the genus Trypanosoma causing animal Trypanosomiasis, a disease complex affecting both domestic and wild animals. The parasite is transmitted to mammalian hosts through the bite of an infected tsetse fly which causes “nagana” in cattle. Species of major threat to cattle, sheep and goats include *Trypanosoma vivax (T. vivax),*

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Trypanosoma congolense (T. congolense), and *Trypanosoma brucei brucei (T.b. brucei)* [1,2]. Although, small ruminants may not often show clinical signs of the disease, it is assumed that the ruminants are rarely affected under natural conditions [3]. *T. b. brucei* can cross to the blood brain barrier of cattle in its chronic stage, thus, economically important as it causes reduced milk, meat and draught power for agriculture production.In general, the disease is characterized by severe anaemia, weight loss, reduced productivity, infertility and abortion, with death occurring in some animals during the acute phase of the disease. Animals which survive often remain infected for several months or years, exhibiting a low level of fluctuating parasitaemia which serves as a reservoir for the disease, although, the infected animals may undergo spontaneous recovery [4,5]. Owing to these varied clinical manifestations, diagnosis of trypanosomiasis cannot be based on clinical signs alone therefore,laboratory confirmation of the diagnosis is an absolute necessity. Introduction of DNA based methods such as species specific DNA probes in diagnostic tests for the detection of infection with African trypanosomes in animals (hosts) and tsetse fly (vector) has improved the specificity and sensitivity of parasite detection and identification [6,13]. The use of PCR for detecting trypanosome DNA is a reliable and accurate technique available for the specific identification of natural animal infections for most trypanosome species and sub-species [14,15]. Species-specific DNA targets have been identified for the most important pathogenic Trypanosoma species that occur in cattle (*Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax*) and PCR based methods for their amplification developed. Therefore, this study is aimed at diagnosing *T.b.brucei* from three selected abattoirs and farms each using microscopy and PCR.

2. MATERIALS AND METHODS

Study Area

Kaduna state is located at the center of northern Nigeria with the coordinates 10°31′N 7°26′E 10.517°N 7.433°E. The study was conducted within three selected Abattoirs and three confined Feedlots in parts of Kaduna State, Nigeria.

Collection of blood samples

A total of 150 blood samples were collected from animals in study areas. 34 blood samples were collected from Tudun-wada abattoir, 33 from Kawo and Zango abattoirs each. 17 blood samples were collected from Simbafid and Hairi farms each and 16 samples for Malonagge farm. Five milliliters (5ml) of blood was collected into Ethylene tetra-acetic acid (EDTA) bottle. Each sample was kept cool by placing in a box containing ice pack and transported to the laboratory for examinations.

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Thin film

Smear of the blood sample was made by placing a drop of blood from a microhaematocrit capillary tube on a clean microscope slide. Using the edge of another clean slide at 20 milimeters from one end, the smear was spread at 30 degrees to the first slide and drawn back to make contact with the blood droplet. The blood was allowed to run along the edge of the spreader and pushed to the other end of the slide in a fairly rapid but smooth motion. The slide was air dried, fixed with methanol for 2min, stained with Giemsa reagent and viewed using the x100 oil immersion objective lens.

Differential Morphology

Any organism with a free flagellum, very well developed undulating membrane and a small sub-terminal kinetoplast was classified as *T.b.brucei* [16]

DNA extraction

Two hundred and fifty microlitres (250µl) of blood was put into 1.5µl of effendorff tubes, 5µl of lysis buffer was added and mixed thoroughly by vortexing. The sample was then incubated at room temperature for 30mins, and then mixed by invasion and put into spin column and centrifuged at 12,000g for 5min. The flow through was discarded and 500µl of wash buffer. It was added and centrifuged at 12, 000g for 2min, and the flow through was discarded. 500µl of wash buffer II was added and centrifuged at 12,000g for 2min, and the flow through was discarded. The spin column was centrifuged empty at 12,000g for 2min. The column was then placed in microcentrifuge tube and 80µl of elusion buffer was added, and was incubated for 5mins at room temperature, then centrifuged at 12,000g for 2min. Seventy microlitres (70µl) more of the elusion was added and centrifuged at 12,000g for 2min. The extracted DNA was then stored in the freezer for downstream application.

Cocktail preparation of isolates for Polymerase chain reaction

The cocktail was prepared using 12.5µl of Master mix. This was mixed with 1µl of Reverse Primer, 1µl of Forward Primer, 3.5µl of Nuclease-free water, and 7µl of DNA Template. The tubes were incubated at 94°C for 5min in an initial denaturation step, followed by 30 cycles of 30sec at 94°C, followed by annealing step at 56°C for 30sec, 72°C for 30sec for initial extension step, and final extension was processed at 72°C for 7min. The PCR reaction was started by adding the Taq polymerase before the first annealing step.

Detection of PCR products on agarose gel

A mixture of 9µl PCR products and 1µl of loading buffer, was applied onto 1.5% agarose gel stained with ethidium bromide. Electrophoresis was run at 120V for 1hr in an electrophoresis set containing 1X Tris Borate EDTA and photographed under UV illumination. DNA ladder was used to determine the size of the PCR products.

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Primer sequences for PCR amplification of trypanosome DNA for *T.b.brucei*

Primer	Primer sequence (5′-3′)	Size	Reference
TB1	5'-GAA TAT TAA ACA ATG CGC AG-3'	**230 bp**	Masiga et al. (1992)
TB2	5'-CCA TTT ATT AGC TTT GTT GC-3'	**230 bp**	Masiga *et al.* (1992)

TB – *Trypanosoma brucei brucei*

DATA ANALYSIS

The data obtained were analyzed using Statistical Package for Social Sciences version 20.00 using Fisher's exact and paired T-Test. Results were reduced to percentages and presented in tables and figures. Values of $p<0.05$ were considered significant at 95% confidence interval.

3. RESULTS AND DISCUSSION

A total of 150 blood samples collected from animals from both abattoir and farms for diagnosis of T.b.brucei showed a mean prevalence of 21% and 1% respectively which showed a significant value at 0.000 at $p<0.05$. Table 2 shows the mean PCV in bulls and cows for each study site, there was no significant difference observed at 0.756, 0.456 and 0.221 with mean of 21% each in Tudun-wada, Kawo amd Zango abattoirs while mixed infection was recorded in 4 samples for both Tudun-wada and Kawo abattoirs while Zango abattoir had 3 samples with mixed infection. Results obtained from the farm had no significant value at 1.001, 1.000 and 1.002 with only 1 sample recorded positive in Malonagge farm. Samples were further subjected to PCR analysis and only lanes 2 and 8 had visible bands while lanes3-7 were faintly visible after gel electrophoresis at 230bp using specie-specific primer for T.b.brucei.

Table 1: The mean Packed Cell Volume (PCV) (%) of T.b.brucei between abattoirs and farms.

Sample size = 150

Study area	Total	Mean PCV (%)	Sig. (2-tailed)
Abattoirs	100	21 (33.87)	0.000
Feedlots (Farms)	50	1 (33.33)	

P≤0.05 (Paired Samples T-Test)

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Table 2: The rate of infection associated with T.b.brucei and mean PCV between each study area.

Sample size = 150

Study area	Sample size	Mean PCV(%)	*T.b.brucei* (+)	Mixed infection	Exact Sig.
Tudun-Wada Abattoir	34	21	7	4	0.756
Kawo Abattoir	33	21	7	4	0.456
Zango Abattoir	33	21	7	3	0.221
Simbafid Farm	17	0	0	0	1.001
Hairi Farm	17	0	0	0	1.000
Malonagge Farm	16	6	1	0	1.002
Total	150		22	11	

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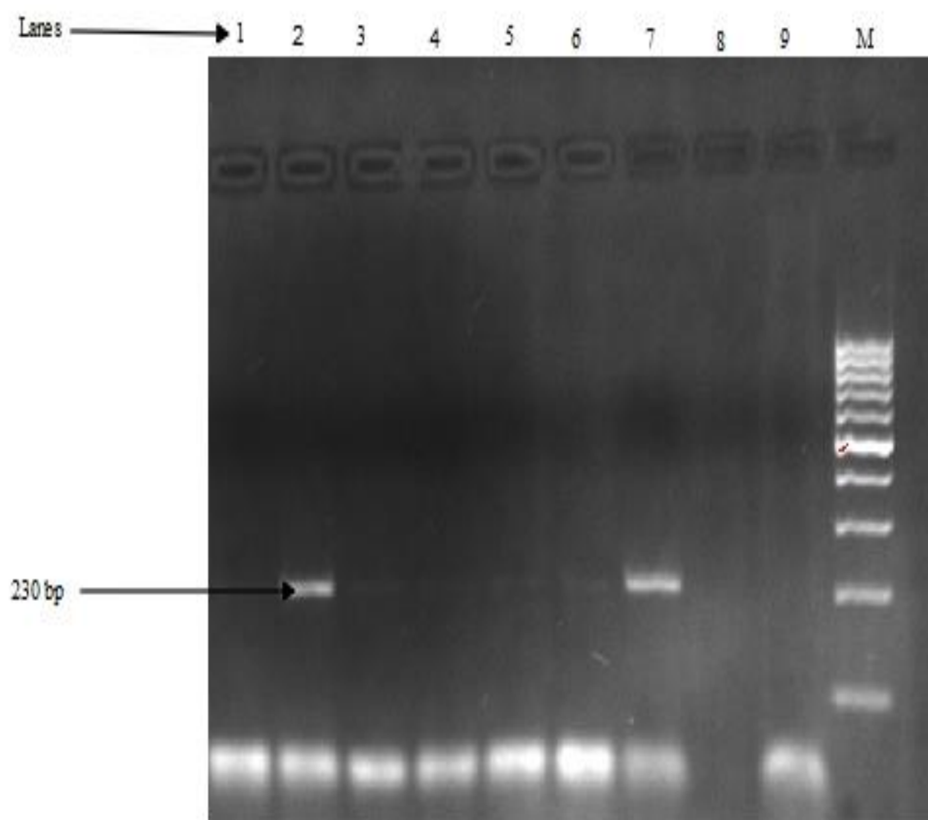

Lane M 100bp ladder, lanes 1-9 PCR amplicons of Trypanosoma brucei

Plate III: PCR photo of Trypanosoma brucei genes at 230bp

DISCUSSION

The result obtained from this study proofs that there is a difference in infection rate associated with *T.b.brucei* from samples collected from each study site. Abattoir had a low mean as observed to the mean observed in farms as seen in Table 1. This could be due to improper management of the herds and the presence of large number of infective vectors in the area where they were sourced for as compared to reduced animal and tsetsefly contact, abundance of pasture and water, coupled with reduced stress factors [17] , thus, indicating that intensive care is giving to the farm animals when compared to the animals brought to the abattoir which are mostly in critical health condition. This result attained from this study is in agreement with the work of [18] who reported a prevalence within the range of 17.8-50%. The rate of infection associated with *T. b. brucei* and mean PCV between each study area was not statistically significant showing all abattoirs had uniform infection rate with a single infection in one farm. Therefore, it showed that no relationship between each study area. The high trypanosome infection rate in the abattoirs could be linked to improper management of the herds

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and presence of larger number of infective vectors in the area during grazing. The cumulative effects of exposure to tsetse fly and new strains of trypanosomes in the abattoir probably account for the observed infection rate [19] while the lower infection observed in the farms is as as a result of the reduced animal and tsetse fly contact, abundance of pasture and water, coupled with reduced stress. The more traditional methods involving microscopy have prevailed in most field studies, despite principal problem with microscopy is the lack of effectiveness and sensitivity, furthermore, the application of DNA techniques provides much improved levels of sensitivity, such as the possibility of detection of individual organisms in samples of whole blood. Microscopy remains useful particularly as it can be carried out directly at the field level and gives immediate results. The findings from this study with earlier studies of Clausen et al.,[20] who demonstrated that the detection rate by PCR was two times higher than the detection rate with parasitological techniques and also detection of small numbers of parasites which were not seen by microscopy is possible by PCR[9], therefore, this study shows that animals from the farm is associated with a reduced trypanosome infection rate compared to those from Fulani herdsmen taken to the abattoir. The animals in abattoir are continuously exposed to new strains of trypanosomes while other adverse husbandry stress factors predispose them to increased susceptibility and high infection rate.

4. CONCLUSION

It can be concluded that the prevalence observed from study indicated abattoirs had higher values as compared to the values from the farms. It also showed that microscopy is the easiest technique for detection of T.b.brucei in abattoirs and farms but it is not specific as PCR technique for detection of this parasite. A uniform rate observed in all the abattoirs showed that the animals were infected and in unstable or critical health conditions before they were brought to slaughter in the abattoirs. Therefore, there is a need for proper diagnosis and treatment of animals so as to promote more meat and milk production for human consumption.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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