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Species Diversity of Trypanosomes in Cattle from Selected Abattoirs in Kaduna State, Nigeria Using Parasitological and Molecular Methods

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Abstract

A total of 500 blood samples were collected and analyzed from two abattoirs using Standard Trypanosome Detection Methods (STDM) and molecular methods (Polymerase Chain Reaction- PCR and Gel Electrophoresis). A prevalence of 2.6% was obtained using Standard Trypanosome Detection Methods (STDM) with the concentration methods having the highest detection in; Buffy Coat Method (BCM) and Haematocrit Centrifugation Technique (HCT), while 25% prevalence was obtained using polymerase chain reaction (PCR). The species of trypanosomes identified included: *Trypanosoma brucei* (76.9%), *Trypanosoma vivax* (7.69%), *Trypanosoma evansi* (7.69%) & *Trypanosoma congolense* (7.69%) with the parasitological analyses while the molecular technique identified Trypanozoon group (52%), *Trypanosoma vivax* (8%), *Trypanosoma theileri* (20%) and a mixed infection of Trypanozoon group with either of *T. vivax* (4%) or *T. theileri* (16%).

Keywords: Trypanosoma species, STDM, PCR

INTRODUCTION

Trypanosomiasis, an important protozoan disease caused by the genus *Trypanosoma is* transmitted through bites by different species of *Glossina* and mechanically by a number of biting flies such as *Tabanus* and *Stomoxys spp*. It is a serious disease in domestic livestock that causes a significant negative impact in food production and economic growth of many parts of the world, particularly in Sub-Saharan Africa (Griffith, 2010). African bovine trypanosomosis is a collective term for a group of diseases brought about by one or more of the pathogenic trypanosome species namely: *T. vivax*, *T. congolense* and *T. brucei*. The disease is characterized by a slow and progressive loss of condition accompanied by increasing anemia and weakness to the point of extreme emaciation, collapse and death (Harley *et al.,* 2011). Diagnosis is an essential element in the management of disease, both at the level of individual patient care and at the level of disease-control in populations. Diagnostic tests should be simple, rapid, specific and highly sensitive, also should ideally differentiate between closely related parasites, especially if the disease syndromes they cause require different management approaches as well should be suitable for field application (Shaker and Elsharkawy, 2015). Parasitological detection techniques are highly specific, but their sensitivity is relatively low. Polymerase chain reaction (PCR) has been used to amplify trypanosome DNA from genetic material isolated from both blood and buffy coat preparations. Rapid, efficient and reproducible procedures for isolating DNA before PCR amplification are essential for confirmation of infection (Zhou *et al.,* 2014). The Parasite concentration techniques such as the haematocrit centrifugation technique (HCT) described by Woo (Fagnani *et al.,* 2011) and its improved version, the buffy coat technique (BCT) described by Murray (Melgar *et al.,* 2010) were developed to improve the detection capacity of parasitological technique. The study aimed to identify the different species of trypanosomes affecting cattle in the region, providing insights into their prevalence and distribution. The integration of traditional parasitological methods with modern molecular techniques, such as Polymerase Chain Reaction (PCR), represents an advancement in the accurate detection and identification of trypanosome species. Comparing these methods can highlight their strengths and weaknesses, ultimately leading to improved diagnostic capabilities.

MATERIALS AND METHODS

The study was cross sectional and abattoir based. Abattoir is a converging area for animals coming from different local government areas of Kaduna and other neighboring states, as well as farm houses and ranches. The abattoir was visited for seven days to estimate slaughters per day. Cattle recruited in the study were selected by systematic sampling technique by every n^{th} unit in the population sampled according to Putt *et al.* (1988).

Collection of Blood Samples: A total number of 500 cattle were sampled and examined during the period of study. Five (5) ml of blood were collected at slaughter point into sterile Ethylene Diamine Tetraacetic Acid (EDTA) bottles and identified appropriately.

Parasitological Analyses

Wet preparation: A drop of blood was placed on a clean glass slide and covered with a coverslip to spread the blood at a mono layer of cells. This was examined using microscope of x40 magnification to detect motile trypanosomes seen either directly,

moving between the blood cells, or indirectly, as they cause the blood cells to move. The lower the magnification, the larger the field observed, and the faster the examination (Cheesbrough, 2006).

Thick film: A drop of blood was placed on a clean slide and allow to dry,it was then fixed with absolute methanol and stained with 5% Giemsa stain and was examined microscopically using x100 magnification.

Thin film: A drop of blood was thinly smeared on a clean glass slide, slide was allowed to dry before dripping in Giemsa stain for 10minutes, washing stain away and drying. Stained slide was viewed under x100 magnification.

Concentration method: Three quarter level of blood was filled into a heparinized capillary tube sealed using modeling clay sealant and centrifuged in a microhaematocrit rotor for 3 minutes at 3,000rpm. It was seen that most of the white cells settled at a slower rate than the red cells as their specific gravity is a little less than that of the red cells, the capillary tube was placed on a glass slide and viewed at x40 magnification for the presence of parasites while the buffy coat that formed between the much thicker red cell layer and the column of plasma was viewed (Cheesbrough, 2004).

PCR amplification of the ITS1 gene

Extraction of trypanosome DNA was carried out using the extraction kit method according to the manufacturer's procedure (Bioneer Cooperation). Extracted DNA from the recovered trypanosomes were subjected to polymerase chain reaction amplification involving three amplification cycles viz: Denaturation (melting), annealing and polymerization (extension). Amplification was carried out using PCR premix in 20µl reaction mixtures containing 10x reactions Buffer (10Mm Tris-HCl), pH 8.3, 50Mm KCl; 1.5mM, 200mM each of the four deoxynucleoside triphosphates (dNTPs). Primers, DNA template and distilled water were added.

The targeted DNAs containing sequence to be amplified were subjected to 40 cycles at 94°C denaturation for 5mins, 48°C annealing for 30 sec and 72°C elongation step for 1min after pre-denaturation at 95°C for 5 mins (Desquesnes *et al.*, 2001). The amplified target gene bands were further viewed using the Agarose gel electrophoresis procedure.

Table 1: Primers Used For Amplification of ITS1 of the Trypanosomes

Primer Name	Primer Sequence	As	References
KIN1	5′-GCG TTC AAA GAT TGG GCA AT -3′ (reverse)	300-800bp	Desquesnes *et al.,* 2001
KIN2	5′-CGC CCG AAA GTT CAC C -3′ (forward)		

As - Amplicon size
KIN - *Kinetoplast*

RESULTS

Prevalence of *Trypanosoma* Infection in Cattle: Out of the five hundred (500) samples of blood collected and analyzed, thirteen were positive for *Trypanosoma* infection giving a prevalence of 2.6% (13/500) in the two study areas.

The highest numbers of trypanosome cases were detected using HCT and BCM with infection prevalence of 2.6% each and one percent prevalence was detected using the wet preparation

method. No positive cases were identified with either thick or thin film (Table 2).

Species diversity of *Trypanosoma* species among cattle at the study area: Based on the morphological identification, *Trypanosoma brucei* was detected (76.9%) which exist as a complex of subspecies and variant that is widely spread across sub-saharan Africa (Table 3).

Table 2: Occurrence of Trypanosomes using Standard Trypanosome Detection Methods (STDM)

Method of screening	No. examined	No. positive (%)	χ^2-value	*p*-value
Wet mount	500	5 (1.0)	27.894	< 0.001
HCT	500	13(2.6)		
Buffy coat	500	13(2.6)		
Thick film	500	0(0.0)		
Thin film	500	0(0.0)		

Table 3: Species diversity of Trypanosomes among cattle slaughtered in selected abattoirs in Kaduna State using STDM

Trypanosoma species	No. of positive (%)
T. congolense	1 (7.69)
T. brucei	10 (76.9)
T. vivax	1 (7.69)
T. evansi	1 (7.69)
Total	13

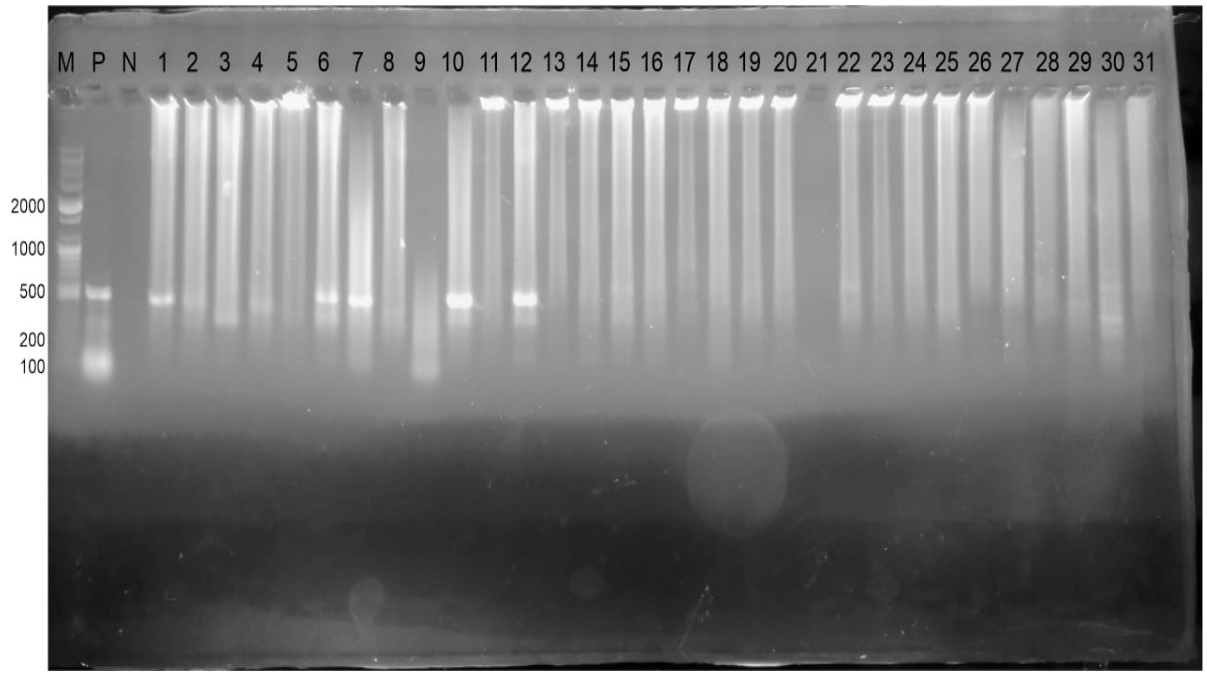

Plate 1: Agarose gel image of PCR products for identification of *Trypanosoma* spp

Table 4 shows a prevalence of 25% (25 out of a total of 100 samples analysed) using molecular techniques. The most predominant species isolated were subgenus *Trypanozoon* with 52% prevalence, the trypanozoon consist of 5 different species including *Trypanosoma brucei g ambiense* and *Trypanosoma brucei rhodesience* (the human type) with *Trypanosoma brucei brucei, Trypanosoma evansi* and *Trypanosoma equiperdum* (which are of the animal type).Other species included *Trypanosoma vivax* and *Trypanosoma theileri.*

Table 4: Species diversity of Trypanosomes in cattle from the study area using molecular technique

Species	No of positives	Prevalence (%)
Trypanosoma vivax	2	8
Trypanosoma theileri	5	20
Trypanozoon group	13	52
Trypanozoon/T.theileri	4	16
Trypanozoon/T.vivax	1	4
TOTAL	**25**	**100**

DISCUSSION

The 2.6% prevalence reported in this study is higher than 0.8% reported in Kwara State, Nigeria (Habeeb *et al.,* 2021), 1.2% in Zaria reported in 2013 (WHO) according to a fact sheet by HAT, 1.5% reported in Obudu cattle ranch in Cross river (Anene *et al.,* 1991) and 1.8% reported in Sokoto (Fajinmi *et al.,* 2010).

The lower prevalence reported in those areas is not surprising because they were earlier designated as tsetse and trypanosomiasis free zones (Anene *et al.,* 1991; Fajinmi *et al*., 2010; Habeeb *et al*., 2021) and it could also be because of improper report of survey or lack of thorough surveillance carried out.

Though a higher prevalence than our findings was reported in the same area (Kaduna) by other authors such as Kalu *et al*. (1991) (53.4%), Abenga *et al*. (2004) (9.1%), Samdi *et al*. (2011) (9.1%), Hargrove *et al*. (2012) (8.4%) and Kneeland *et al*. (2012) (9.4%). The lower prevalence in our finding could be as a result of serious migration along the cattle route reported in the area because of insecurity. However, this lower prevalence if not properly managed could lead to higher one due to spread.

The Standard Trypanosome Detection Method (STDM) used in the study is to avoid misdiagnosis of the infection as it's a combination of different techniques. The buffy coat and haematocrit centrifugation methods detected more of the infection (2.6%) than other methods. This could be as a result of the samples been in concentrated forms hence the higher chance of detecting the parasites. Our finding is in agreement with Omotainse *et al.* (2004) and Fajinmi *et al.* (2010).

The predominant species detected was *Trypanosoma brucei*. This agrees with Majekodunmi *et al.* (2013) that isolated *T. brucei* the most. The detection of *T. brucei* in Nigerian cattle might portend serious danger not only to cattle and other livestock but also to livestock owners and the communities at large as it had been reported in cattle and humans (Fevre *et* al., 2001; Welburn *et al.,* 2001 and Joshi *et al.,* 2005).

Studies have shown that molecular diagnostic technique is more accurate than the conventional

parasitological techniques in detection of trypanosomal infection from very low parasitized samples including those from asymptomatic animals (Baticodos *et al.,* 2011; Bal *et al.,* 2014; Gadahi *et al.,* 2014; Shaida *et al.,* 2018).

Several sequences have been used for the sensitive and specific PCR-based detection of livestock trypanosome DNA in host blood and for this study in order to identify the parasites accurately and detect the presence of other genotypes in the area if possible.

The molecular technique employed in our study showed that 25 out of the 100 samples analysed were positive for trypanosomes using the multi specie-specific primer (**KIN1:** 5- GCG TTC AAA GAT TGG GCA AT-3 (reverse) and **KIN 2:** 5- CGC CCG AAA GTT CAC C-3 (forward) the sampling was based on our result of the mean PCV value of (25%) obtained from our data that were considered to be anemic according to Mamoudou *et al*. (2016).

Using the KIN primers, 25% prevalence was obtained as positive for trypanosomiasis which is far higher than 2.6% gotten using the conventional methods, because out of the 500 cattle examined using the standard trypanosome detection method (STDM), only 2.6% were infected with trypanosomes and 97.4% as negative whereas 25% prevalence was obtained of the 100 samples analysed using molecular technique.

In an early study by Takeet *et al*. (2013), microscopy detected 15.1% positive infection of at least one of *Trypanosoma brucei, Trypanosoma congolense* or *Trypanosoma vivax,* while PCR detected 63.7% positive infection of at least one of those species which lends support to our current finding. Microscopy has been shown to be far less sensitive and accurate than DNA-based detection and identification methods (Desquesnes and Davila, 2002), while DNA-based methods have the advantage of being more sensitive and able to identify trypanosomes to the subspecies level as well as mixed infections.

Our results of the PCR showed that cattle in the two study sites harbor mature infections of *T. vivax, T. theilleri* and members of the subgenus Trypanozoon. This implies that there is an active transmission of these trypanosomes in the two sites sampled; indeed the presence of *Trypanosoma* species could affect the growth of farm animal (Ng'ayo *et al.,* 2005).

Among the trypanosomes species associated with infections in cattle, the Trypanozoon subgenus and *T. theilleri* are likely to be the main parasites responsible for African Animal Trypanosomiasis (AAT) in livestock. The demonstration that the Trypanozoon and *T. theilleri* were the predominant species in our study area is unexpected; this is at variance with previous reports on the prevalence of *T. vivax* in several cattle species in endemic areas (Takeet *et al.,* 2013; Mamoudou *et al.,* 2016; Isaac *et al.,* 2016).

The mixed infection we observed in cattle could be explained by exposure of animals to infected tsetse flies (Nakayima *et al.,* 2012) hence the presence of some *Trypanosome* species could negatively impact human and animal health. Mixed infections are becoming common occurrences globally, especially in areas with significant endemicity for bovine trypanosomiasis (Morenikeji *et al.,* 2023).

REFERENCES:

Abenga, J.N. and Lawal, I.A. (2004). Implicating role of animal reservoir hosts in the resurgence of Gambian trypanosomiasis (sleeping sickness). *African journal of Biotechnology,* vol. **4**(2) pp. 134-137.

Anene, B.M., Chime, A.B., Jibike, G.I. and Anika, S.N. (1991). Prevalence of trypanosomiasis in Zebu cattle at Obudu ranch, a tsetse freezone in Nigeria. *Preventive Veterinary Medicine*, **10**:257–260

Baticodos, W.N., Castro, D.I., and Baticodes, A.M. (2011). Parasitological and PCR detection of *Trypanosoma evansi* in buffaloes from Luzon, Philippines. *Ceylon journal of sciences,* **40** (2):141-146.

Desquesnes, M. and Davila, A. (2002). Applications of PCR-based tools for detection and identification of animal trypanosomes: A review and perspectives. *Veterinary parasitology.* **109**(3-4): 213-231.

Desquesnes, M., Gerald M., Adrien, Z., and Alberto, M.R.D. (2001). Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of r DNA. *Elsevier. International journal for parasitology,* **31**: 610-614.

Fagnani, R., Beloti, V., Battaglini, A., Dunga, K., Tamanini, R. (2011). Organophosphorus and carbamates residues in milk and food stuff supplied to dairy cattle. *Pesq Vet Bras* **31(7)**: 598-602.

Fajinmi, A.O., Faleke, O.O., Magaji, A.A., Daneji, A.I., and Gweba, M. (2010). Presence of trypanosome species and determination of anaemia in trade cattle at Sokoto Abattoir, Nigeria. *Research Journal of Parasitology*, ISSN 1816 – 4943/DOI: 10.3923/jp.2010

Fevre, E.M., Coleman, P.G ,Odiit, M., Magona, J.W.,Welburn S.C and Woolhouse M.E. (2001)- The origins of a new *Traypanosoma brucei rhodesiene* sleeping sickness outbreak in eastern Uganda. *Lance*t **358 (**9282) 625-628.

Gadahi, J.A., Bhutto, B., Javaid, S.B., Kashif, J., and Ducttoan, T. (2014). Diagnostic approach towards camel trypanosomiasis. *International Journal of livestock Research,* 4(1): 41-45.

Griffiths, M. (2010) Improving the safety and quality of milk: Improving quality in milk products. (Edition 1), Woodhead Publishing, Sawston, Cambridge, UK, pp. 520.

Habeeb, I. F., Gloria, D.C. and Jacob, K.P.K. (2021). Molecular identification and prevalence of trypanosomes in cattle distributed within the jebba axis of the River Niger, KwaraState , Nigeria. *Parasites and vectors.* **14**:560.

Hargrove, J.W., Ouifki, R. Kajunguri, D., Vale, G.A. and Torr, S.J. (2012). Modeling the control of trypanosomiasis using trypanocides or insecticide-treated livestock.*Tropical Disease PLoS neglected (Spanish).* **6**(5).

Harley, K.G., Huen, K., Aguilar- Schall, R., Holland, N.T., Bradman, A. (2011). Association of organophosphate pesticide exposure and paraoxonase with birth outcome in Mexican-American women. *Plosone* **6(8)**: e23923.

Isaac Clement, Marc Ciosi, Alana Hamilton, Kathleen Maria Scullion, Peter Dede, Igho Benjamin Igbinosal, Oyebiguwa P, Goddey M, Masiga D and CMR Turner (2016). Molecular identification of different trypanosome species and subspecies in tsetse flies of Northern Nigeria. *Parasites and Vectors.* **9:**301.

Joshi, P.P., Shegokar, V., Powar, S., Herder, S., Katti, R. (2005) Human Trypanosomiasis caused by Trypanosoma lewisi in India: the first case report. *Annals Journal of Tropical Medicine Hygiene* **73**:491-495.

Kalu, A.U., Oboegulem, S.I. and Uzoukwu, M.(1991). Trypanosomiasis in small ruminants maintained by low reverie tsetse population in central Nigeria. *Small Ruminant Research*, **40**: 109-115

Kneeland, S., Hogsette, L., Molinaochoa, L., and Foster. (2012). A century and a half of research on the stable fly, *Stomoxyscalcitrans* (L) (dipteral: muscidae), 1862-2011: *An annoted bibliography. United states department of agriculture Research services, ARS*-173

Mamoudou, Alexander Njanloga, Aliyou Hayatou, Pierre Fonghosuh and Mbunkah Daniel Achukwi (2016). Animal trypanosomes in clinically healthy cattle of north Cameroon: *Epidemiological implications*. Doi 10. 1186 13071-016-1498-1

Melgar, M., Santaeufemia, M., Garcia, M.A.

(2010). Organophosphorus pesticide residues in raw milk and infant formulas from Spanish northwest. *J Environ Sci Health B* **45(7)**: 595-600.

Murray, M., Murray, P.K., Micintyre, W.I.M. (1997). An improved parasitological technique for the diagnosis of African Trypanosomiasis. *Trans. R. Soc. Tropical Medicine Hygiene.* **71:** 325-326.

Nakayima, J., Nakao, R., Alhassan, A., Mahama, C., Sugimoto, C. (2012). Molecular epidemiological studies on animal trypanosomiases in Ghana. *Parasite vectors.* **5**: 217.

Ng'ayo, M.O, Njiru Z.K., Kenya, E.U., Muluvi, G.M., Osir, E.O. and Masiga, D.K. (2005). Detection of trypanosomes in small ruminants and pigs in Western Kenya: important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biology Disease,* **4**(1):5.

Omotainse, S.O., Edeghere, H., Omoogun, G.A., Elhassan, E.O., Thompsoon, G., Igweh, C.A., Ukah, J.A.C. Halid, M. A. (2004). The prevelance of animal trypanosomiasis in konshisha government area of benue state, Nigeria. *Israel journal of veterinary medicine* vol. **55** (4).

Rengasamy, P., Kantharimuthu, A., Balaraman, M., Sivanesan, S. (2016). Development of ELISA kit for the assay of dichlorodiphenyltrichloroethane in milk and milk products. *Afr J Food Sci* **10(3)**: 41-47.

Samdi, S.M., Fajinmi, A.O., Kalejaye, J.O., Wayo, B., Haruna, M.K., Yarnap, J.E., Mshelai, W.P., Usman, A.O., Hamra, S.M., Jijitar, A., Ogunwale, R., Ovbagbedia, R.P., and Bizi, R. (2011). Prevalence of Trypanosome in cattle slaughtered in Kaduna central Abbatoir. *Asian Journal of Animal Sciences* **5** (2): 162-165.

Shaida, S.S., Weber, J.S., Gbem, T.T., Ngomtcho, S.C.H., Musa, U.B.,Achukwi, M.D., Mamman, M., Ndams, I.S., Nok, J.A. and Kelm, S. (2018). Diversity and phylogenetic relationships of Glossina Populations in Nigeria and the Cameroonian border region. *BMC Microbiology*, **18** (suppl 1): 180 https: //doi.org/10.1186/s12866-018-1293-6

Shaker, E.M. and Elsharkawy, E.E. (2015). Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agro-industrial areas in Assiut, Egypt. *Environ Toxicol Pharmacol* **39(1)**: 433-440.

Takeet, M.I., Fagbemi, B.O., Donato, M.D., Yakubu, A., Rodulfo, H.E., Peters, S.O. (2013) Molecular Survey of pathogenic trypanosomes in naturally infected Nigerian cattle. *Research of Veterinary Science*. **94:**555-61.

Welburn, S.C., picozzi K., Fevre, E.M., Coleman, P.G., Odiit, M., carringtom, M., and Maudlin, I. (2001) –Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (SRA). *Gene lancet*, **358 (**9298), 625-628.

Zhou, S., Tang, Q., Jin, M., Liu, W., Niu, L. (2014). Residues and chiral signatures of organochlorine pesticides in mollusks from the coastal regions of the Yangtze River Delta: Source and health risk implication. *Chemosphere* **114**: 40-50.