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Research Article

Detection and Identification of Pathogenic Trypanosomes across Niger Republic–Nigeria Border by Polymerase Chain Reaction: A Case Study at Maigatari International Livestock Market, Northern Nigeria

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ABSTRACT

Trans-border trade among African countries has been a major route for transfer of goods and animals for centuries influencing socio-economic dynamics of regions and countries. Animal trading across borders has been implicated in the spread of several diseases and constitute a major risk to public health.The present study is aimed to investigate the role of livestock trade across Niger Republic-Nigeria border on the spread of animal African trypanosomiasis (AAT) using Maigatari International Livestock market in Northern Nigeria as a case study.To achieve this, blood samples were collected from 200 animals comprising 40 each of horses, donkeys, goats, camels and cattle brought for trading. Each sample was analysed for the presence of trypanosome *spp* by polymerase chain reaction using ITS1 CF and BR generic primers.The results showed an overall prevalence of 60% trypanosomes infection, with 16/40 (40%) for horse, 20/40 (50%) for donkey, 36/40 (90%) for goat, 36/40 (90%) for camel and 12/40 (30%) for cattle, respectively.The ITS1 CF and BR primers gave band sizes of 250 bp, 480 bp and above 580 bp for *T. vivax*, subgenus trypanozoon and *T. congolense*, respectively. The most prevalent trypanosome species detected were *T. vivax* single infection (86.7%), *T. vivax*/subgenus *trypanozoon* mixed infection (10%) and *T. congolense*/subgenus *trypanozoon* mixed infection (3.3%). These results confirmed the threat to public health pose by animal trade across Niger Republic-Nigeria border and call for strategic measures for the control and management of the spread of animal African trypanosomiasis especially as the disease is zoonotic in nature.

Keywords: *Animal African Trypanosomiasis, Niger Republic-Nigeria, Trade, Public Health*

INTRODUCTION

African Trypanosomiasis (AT) is a protozoan parasitic disease of serious public health concern. The disease is caused by blood parasites, belonging to the genus *Trypanosoma* and widely transmitted in Africa by tsetse flies (Adams and Hamilton, 2008; Habeeb *et al.,* 2021).Most trypanosomes are heterogeneous (requiring more than one obligatory host to complete life cycle). In animals, the disease is referred to as animal African trypanosomiasis (AAT) while in humans it is referred to as human African trypanosomiasis (HAT), also known as "sleeping sickness".

The trypanosomes pathogenic to animals are *Trypanosoma brucei* spp, *Trypanosoma evansi, Trypanosoma equiperdum* (*Trypanozoon* subgenus), *Trypanosoma vivax, Trypanosoma simiae*, and the three main *Trypanosoma congolense* sub-species (savannah, forest, and kilifi) (Masiga *et al*., 1992; Clausen *et al*., 1998). These *species* are the causative agents of animal African trypanosomiasis in wildlife and domestic animals. In cattle, the disease is called "*Nagana*" meaning depressed spirit in

the Zulu language. The disease in domestic animals, particularly in cattle, is a major hindrance to socio-economic development of affected rural areas (WHO, 2022).

The impact of AAT on a community is the result of complex interactions between environmental, political, socio-cultural, entomological and livestock management factors (Bouyer *et al.,* 2013). It was estimated that Nigeria has 19.5 million cattle, 72.5 million goats, 41.3 million sheep, 7.1 million pigs, 28,000 camels and 974, 499 donkeys (Isaac *et al.,* 2017). Many of these animals especially cattle, camel, and goats are brought into Nigeria from neighbouring countries especially through Niger Republic-Nigeria border for trading because of existing large market for livestock in the country.

These animals may be carrying different kind of infections that poses a serious threat to public health especially as some of these infections could be zoonotic in nature. The unavailability of reliable data on the matter further compounded the problem.

It is for this reason, the study was undertaken to investigate the presence of trypanosome infections in animals brought across Niger Republic-Nigeria border from different countries for trading at Maigatari International Livestock Market, Northwest Nigeria, which is few kilometres from Niger Republic-Nigeria border.

MATERIALS AND METHODS

Study area:

This study was conducted at Maigatari International Livestock Market, Maigatari Local Government Area, Jigawa State, Nigeria, 140 kilometres from Dutse, the state's capital. Maigatari is located on the latitude 12° 23′00″ - 12° 48′26″N and longitude 9° 27′05″ - 9°30′05″E and it covers an area of 1250 km^2 (Yakubu, 2014) with a population of 179,715 according to 2006 census. The Local Government shares borders with Niger Republic to the North, Gumel Local Government Area in the South, Sule Tankarkar Local Government Area in the West and Kaugama Local Government Area to the East. The principal inhabitants are Hausa, Fulani and Kanuri that are predominantly cattle rearers, farmers and traders.

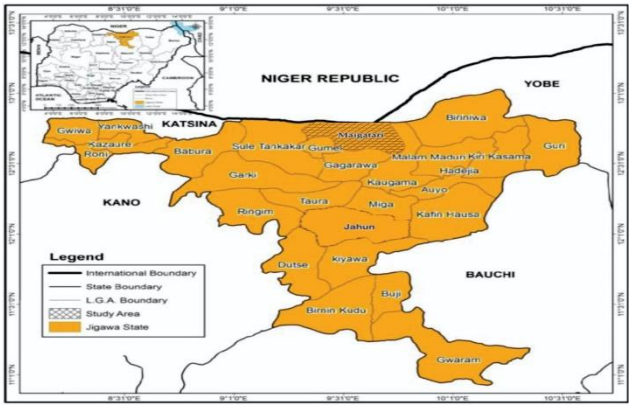

Figure 1: Map of Jigawa state showing Maigatari Local Government Area as highlighted
(Sources: Google map)

Field blood sample collection:

Whole blood sample (5 mL) was collected from the jugular vein of 200 restrained animals comprising 40 each of horses, donkeys, goats, camels and cattle respectively, and dispensed into EDTA bottles. The samples were placed in a cold box containing ice-bergs and transported to the Biotechnology Research Laboratory Unit, Nigerian Institute for Trypanosomiasis Research, Kaduna State, for analysis.

DNA Extraction:

Genomic DNA was extracted from the whole blood samples using AccuPrep® Genomic DNA Extraction Kit (Bioneer Cooperation, South Korea), according to the manufacturer's instruction. The DNA was then quantified using Nanodrop 2000c Spectrophotometer (Thermo scientific, Germany) and stored at -20ºC until required.

Polymerase Chain Reaction (PCR):

PCR was performed using Accupower®HotStart PCR premix (Bioneer Cooperation, South Korea) for detection of trypanosome species by amplifying the internal transcribed spacer of 18S rDNA by ITS1 CF (5′-CCGGAAAAGTTCACCGATATTG-3′) and BR (5′-TTGCTGCGTTCTTCAACGAAA-3′) primers as described (Njiru *et al.*, 2005) in a 20 µL reaction containing 1 µL each of 10 µM ITS1 CF and BR primers, 1 µL of gDNA template and 17 µL of double distilled water. The PCR conditions were: initial denaturation at 95°C for 5 mins, followed by 35 cycles of 94°C for 30 seconds, 49°C for 40 secs, 72°C for 1 min and final extension at 72°C for 10 mins. The PCR products were resolved on 1% agarose gel run at 100 V for 1 hr.

RESULTS AND DISCUSSION

Identification of Trypanosome species

The PCR using ITS1 CF and BR primers detected and identified different trypanosome species and gave distinct bands with amplicons at the expected sizes: *T. vivax* at 250 bp, subgenus trypanozoon (*T. brucei* , *T. evansi* and *T. equiperdum*) at 480 bp and *T. congolense* at above 580 bp. *T. vivax* was the predominant trypanosome spp detected and responsible for 80.7% single infections and 10% mixed infections (Plate I and Table 2).

The ITS1 CF and BR primers detected trypanosome DNA in the different blood samples: 16/40 (40%) in horse, 20/40 (50%) in donkey, 36/40 (90%) in goat, 36/40 (90%) in camel and 12/40 (30%) in cattle. In total, 120/200 animals screened were positive for trypanosome DNA representing a prevalence of 60%. Camels have highest trypanosome infections (80%), while cattle showed least trypanosome infections (10%) (Table 2).

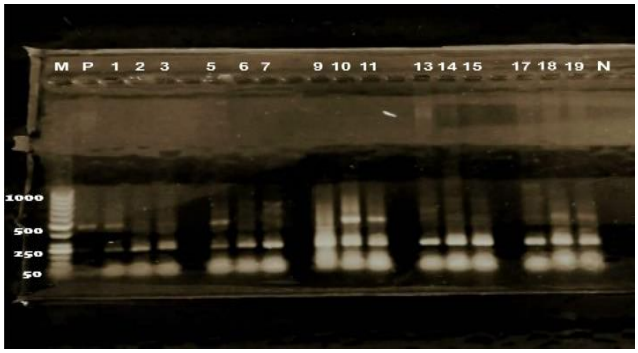

Plate I. PCR Amplification of Internal Transcribed Spacer Region of 18S rDNA by ITS1 CF and BR Primers for Identification of Trypanosome *spp*.

M: molecular DNA marker (50 bp, Thermo Scientific, Germany); P: positive control (subgenus Trypanozoon), lanes 1-3 horses (*T. vivax*/subgenus Trypanozoon, *T. vivax*, *T. vivax*); lanes 5-7 donkey (*T. vivax* /subgenus Trypanozoon, *T. vivax*, *T. vivax*); lanes 9-11 goats ,(*T. vivax*, *T. vivax*/*Trypanozoon*, *T. vivax*/subgenus Trypanozoon), lanes 13-15 cattle (*T.vivax*); lanes 17-19 camels (*T. vivax*, *T. vivax*/subgenus Trypanozoon, *T. vivax* / subgenus Trypanozoon) N: negative control (uninfected blood).

Distribution of Trypanosome infection in the animals from different countries

The distribution and pattern of trypanosome infections in different animals brought to Maigatari International Livestock Market is shown in Table 1. Out of the 200 animals screened, 144 were from Niger Republic and 56 from Nigeria, with 120 infected with trypanosome spp indicating a prevalence of 60%. More trypanosomes infections were detected in animals from Niger Republic (63.9%) compared to Nigeria (50.0%).

Table 1. Geographical Distribution of Screened Animals and Pattern of Trypanosome Infections.

Animals/Country of Origin	Nigeria (n=56)	Niger Rep. (n=144)	Total (n=200)
HORSE	12	28	40
Infected	4	12	16 (40%)
Not infected	8	16	24 (60%)
DONKEY	10	30	40
Infected	6	14	20 (50%)
Not infected	4	16	20 (50%)
GOAT	18	22	40
Infected	16	20	36 (90%)
Not infected	2	2	4 (10%)
CAMEL	0	40	40
Infected	0	36	36 (90%)
Not infected	0	4	4 (10%)
CATTLE	16	24	40
Infected	2	10	12 (30%)
Not infected	14	14	28 (70%)
Total			
Positive	28	92	120 (60%)
Negative	28	52	80 (40%)

Table 2. Prevalence of Trypanosome Species in Animals at Maigatari International Livestock Market, Northwest Nigeria.

Trypanosome Species	**PCR product band size obtained (Njiru *et al.*, 2005)**	**Horse (n=40) No. of positive n (%)**	**Donkey (n=40) No. of positive n (%)**	**Goat (n=40) No. of positive n (%)**	**Camel (n=40) No. of positive n (%)**	**Cattle (n=40) No. of positive n (%)**	**Total (n =200)**
T. vivax	250 bp	*14* (35%)	18 (45%)	30 (75%)	32 (80%)	10 (25%)	104 (86.7%)
T. vivax* +*T. congolense	250/580 bp	-	-	2 (5%)	-	2 (5%)	4 (10%)
***T. vivax* + subgenus Trypanozoon (*T. brucei* group, *T. evansi*, *T. equiperdum*)**	250/480 bp	2 (5%)	2 (5%)	4 (10%)	4 (10%)	-	12 (3.3%) Total = 120/200(60%)

Discussion

Successful and sustainable strategies for the control of AAT require early diagnosis and correct treatments especially for animals that are domesticated in one area such as farms or Fulani "ruga". However, thousands of other animals are traded across international borders carrying different diseases, and thus could constitute a high risk to public health.

In the present study, animals brought for trading from different countries across Niger Republic-Nigeria border were examined for presence of veterinary pathogenic trypanosomes at Maigatari International Livestock Market in Northern Nigeria. An overall prevalence of 60 % trypanosome infection was detected in the animals which is significantly ($P < 0.05$) higher when compared to other reports in Nigeria. For

example; a prevalence of 2.2 % in Lere Local government Area (LGA) Kaduna State (Abenga *et al.,* 2004), 3.7 % in Abia State (Ohaeri 2010), 3.8 % in Benue State (Enwezor *et al.,* 2012), 3.93 % at Kano Abattoir in Kano state (Dabo and Maigari, 2018) and 1.8 % at Sokoto Abattoir in Sokoto State (Fajinmi *et al.,* 2011) were reported. However, the prevalence was lower than in Sudan where prevalence of 99% (69/70) in cattle, 98% (61/62) in sheep, and 84% (98/116) in goats was reported in blue Nile and west Kordofan states (Mossaad *et al.,* 2020). The high prevalence reported here could be due to convergence of the animals in one place from different countries all known to have AAT as an important disease affecting their animals. As the animals are sold off and dispersed into different parts of Nigeria, the prevalence may decrease but the animals could serve as reservoirs of pathogenic trypanosomes. Travelling through tsetse belt, these animals could provide infected-blood meal for tsetse flies, thus transmitting and spreading the disease all over the country. The trypanosome parasites are transmitted by the bite of an infected tsetse fly (genus *Glossina*), and cases of AAT are only found in areas of tsetse fly infestation, which are limited to sub-Saharan Africa. Some data suggests that vector control operations covered approximately 128,000 km^2 of Africa in 2001, a mere 1.3% of the tsetse infested area (Allsopp, 2001). In areas without effective vector control, trypanocides are widely used to control AAT in cattle. However, no new veterinary drugs for the treatment of AAT have been released since 1985 (Anene *et al.,* 2001) and there is increasing resistance to the existing trypanocides.

It is difficult to explain why camels and goats had higher infection rates than donkey, horse and cattle; however, variation in the infection rates among the different animal species exists. *T. evansi* and *T. equiperdum*, the trypanosome species responsible for the disease in camels and donkeys, respectively, belong to the sub genus trypanozoon and are transmitted mechanically. The high prevalence of *T. vivax* infection agrees with similar findings where 100 % prevalence of *T. vivax* infection in cattle was reported in farms in Khartoum State, Sudan. (Ahmed *et al.* , 2016). *T. vivax* accounts for the highest number of tsetse transmitted infections (Croft *et al.*, 1984; Moloo *et al.*, 1992) due to its relatively simple cycle of development in the vector's mouthparts (Radwanska *et al.*, 2018). The ITS1 CF and BR primers used proved their ability to detect all pathogenic *Trypanosomes* in a single PCR and their specificity (no amplification of vector and host DNA) signified the high chance of detecting more pathogenic trypanosomes (*T. vivax*). The primers were designed to depress the homology with the non-pathogenic *Trypanosomes* and to improve the homology with the pathogenic *Trypanosomes* (Njiru *et al.,* 2005). The ITS1 CF and BR primers show 100% homology with the available *T. vivax* sequence (accession no. U22316) while others primers like KIN1 and KIN2 show 75%-90% homology. This signifies the high rate of ITS1 CF and ITS1 BR primers to detect more *T.vivax* than the KIN 1 and KIN 2 primers (Desquesnes *et al.,* 2001)

CONCLUSION

An overall prevalence of 60% pathogenic trypanosomes infection was detected with *T. vivax* identified to be responsible for 86.6% single infection. This may imply that highly pathogenic trypanosomes infected animals are brought into Nigeria for trading. In addition to this constituting a potential serious threat to public health, it may explain why AAT remains endemic in Nigeria. Therefore, effective and sustainable control strategies should introduce screening and treatments at border points like Maigatari International Livestock Market in order to reduce the burden and risk to public health and to facilitate sustainable agricultural practices that enhances socio-economic value chain.

AUTHORS' CONTRIBUTIONS

ABY conceptualized, designed, provided reagents, supervised the study, analysed data, edited and submitted manuscript. NK supervised the study. NIS and IBM conducted experiments and drafted the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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