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THE PREVALENCE OF NATURAL MIXED INFECTION OF *T. congolense* and *T. vivax* IN CATTLE FROM SELECTED ABATTOIRS IN KADUNA METROPOLIS, KADUNA STATE, NIGERIA.

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ABSTRACT

Animal trypanosomiasis is a complex disease of animals found in Africa causing serious economic loss in animal production. This study was carried out to investigate the prevalence of natural mixed infection in cattle from selected abattoirs in Kaduna metropolis. Wet mount and polymerase chain reaction (PCR) was used to detect the presence of trypanosomes in blood of cows which showed a prevalence of 17% for *T. congolense* and 4.3% for *T. vivax* while mixed infection of 1.3% was observed from two abattoirs. Tudun-wada abattoir was statistically significant at $p<0.005$. Further analysis conducted using PCR technique showed the presence of genomic DNA of trypanosomes from positive samples obtained from wet mount method as seen in plate 1. Primers TCS 344-345 for *T. congolense* at 437bp and TVW1-2 for *T. vivax* at 399bp confirmed the presence of both parasites as seen in plate 2. In conclusion, this study showed the presence of both parasites in natural mixed infection in cattle from Tudun Wada and Kawo abattoirs in 4 cows each. This also indicates that natural mixed infection can occur in the field invading the immune system of affected hosts. Therefore, we suggest that microscopy and PCR should be used side by side for effective detection of these parasites so as to proffer appropriate control measure in combating the menace of African animal trypanosomiasis.

Keywords: African trypanosomiasis, Wet mount, *T. vivax*, *T. congolense*, PCR.

INTRODUCTION

African trypanosomiasis is a complex and debilitating disease of both humans and animals found in sub-Saharan Africa (Nimpaye *et al.*, 2011). Human African trypanosomiasis (HAT) or the human form commonly known as sleeping sickness is found in the western and eastern parts of Africa; while African Animal trypanosomosis (AAT) commonly known as "*Nagana*" (which is derived from a Zulu term meaning to be in low or depressed spirits) or *sammorre* (in Fulfulde language), found in Africa is a leading cause of serious economic loss in animal production (Nimpaye *et al.*, 2011; Omeje *et al.*, 2018). The causative agents of this disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts which are transmitted by the bite of infected tsetse flies (*Glossina* spp.) (Prowse, 2005). The distribution of this disease in Africa corresponds to the range of tsetse flies and comprises currently an area of 8 million km^2 between 14 degrees North and 20 degrees South latitude (Prowse, 2005; Dede *et al.*, 2007). Throughout history, African trypanosomiasis has severely repressed the economic and cultural development of the continent (Urquhart *et al.*, 1996).

African animal trypanosomiasis is recognised as both a serious health problem and a severe constraint to Africa's socio-economic development, which claims the lives of over 3 million livestock causing huge economic loss and untold human misery (Dede *et al.*, 2007). It has been documented that Nigeria has an

estimated population of over 19.5 million cattle, 72.5 million goats, 41.3 million sheep, 7.1 million pigs, 28,000 camels and 974, 499 donkeys (NASS, 2012)**.** Infection with trypanosome in animals may result in a chronic, debilitating, emaciating and often fatal disease but the outcome of the infection differs substantially between trypanosome species or sub-species, between livestock species and within livestock species among breeds depending on the challenge and virulence of the strains (Connor and Van Den Bossche, 2004). Due to their frequencies, pathogenicity and consequence on productivity, *T. congolense* and *T. vivax* are amongst the principal trypanosomes which infects domestic animals (Wellde *et al.,* 1983; Trail *et al.,* 1991). Infection with both trypanosomes results in sub-acute, acute or chronic disease characterized by intermittent fever, anaemia, occasional diarrhoea and rapid loss of condition and often terminates in death (Omeje *et al.,* 2018). This hematic trypanosomes (*T. congolense* and *T. vivax*) in mixed infection cause injury to the host mainly by the production of severe anaemia, which is accompanied in the early stages of the disease by leucopoenia and thrombocytopenia (Dhollander *et al.,* 2006; Desquesnes *et al.,* 2011). In the terminal stages of the disease caused by the hematic trypanosomes, focal polio-encephalomalacia probably results from ischemia due to massive accumulation of the parasites in the terminal capillaries of the brain (Sidibe *et al.,* 2002; Bengaly *et al.,* 2002a,b). Although, animals affected by *T. vivax* have been reported to be less pathogenic for cattle than *T. congolense*, nevertheless it is one of the most important cause of AAT in West African cattle. However, due to their genetic diversity, both species have been shown to cause serious infections in cattle, horses and asses which lead to abortion and death (Authié *et al.,* 1999; Faye *et al.,* 2001; Dhollander *et al.,* 2006).

The detection of trypanosomes relies mainly on conventional methods such as (wet mount, dry thick and thin film, buffy coat method) in addition to clinical symptoms, anamnesis, travel history and geographic location of animals (Omeje *et al.,* 2018). *T. vivax* and *T. congolense* are characteristically present in the bloodstream of infected hosts when viewed under the microscope in fresh blood films (Prowse, 2005). *T. vivax* can be differentiated from the other specie by its movement rapidly across the field in fresh unfixed blood films with broad and round posterior end and a short free flagellum, while *T. congolense* has no free flagellum with a blunt shaped posterior end (Urquhart *et al.,* 1996; Prowse, 2005; El-Metanawey *et al.,* 2009; Baticados *et al.,* 2011). Polymerase chain reaction (PCR) assays is specific for the diagnosis of trypanosomiasis via detection of trypanosomal DNA in the blood samples of infected hosts (Desquesnes, 1997; Masake *et al.,* 1997; Ventura *et al.,* 2002; Sudan *et al.,* 2014; Parashar *et al.,* 2015). Studies have reported that PCR technique is more reliable than microscopy, as result obtained from it can be more specific and sensitive especially from very low-parasitized samples including those from asymptomatic animals (Thumbi *et al.,* 2008; Tamarit *et al.,* 2010; Baticados *et al.,* 2011; Shahid *et al.,* 2013; Bal *et al.,* 2014; Gadahi *et al.,* 2014). Development of DNA amplification technique on the detection of trypanosomes has broadened and improved method of detection from samples of suspected hosts (Gadahi *et al.,* 2014). However, natural mixed infections have been reported (Baticados *et al.,* 2005; Baticados *et al.,* 2011), there is paucity of information on their prevalence reported in abattoirs. Therefore, this study investigated the prevalence of mixed infection of *T. congolense* and *T. vivax* in cattle in selected abattoirs of Kaduna metropolis.

MATERIALS AND METHODS

Study Area

This study was conducted in Kaduna State which lies between latitude 9°30′ 0 N and 11°0′0 N, longitude 6°0′0"E and 11°0′0"E with population of 6,066,562 according to Lakner and Milanovic (2013). This study was carried out in cattle (male and female) from three slaughtering abattoirs during the early hours of the morning (6:00 am) in Kawo, Tudun Wada and Makera abattoirs.

Sample Collection

Five mL of blood from each cow was collected at point of slaughter at every two days interval for a period of four (4) months via jugular venipuncture. One hundred blood samples were randomly collected from cattle from each of the abattoirs at about 6:00-9:00 am making a total of 300 blood samples. The blood was collected using a sterile bottle containing EDTA and placed in a cooler with ice packs which was immediately transported to the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna State and subsequently prepared for analyses upon arrival for detection presence of trypanosomes.

Wet mount

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 (Cheesbrough, 2005).

Packed cell volume

Three quarters level of blood was filled into a sterile heparinized capillary tube, it was sealed using modelling clay sealant and centrifuged in a micro-haematocrit rotor for 5 minutes at 12 000 rpm. It was thereafter read using a micro-haematocrit reader for the percentage level of the haematocrit (Cheesbrough, 2005).

DNA extraction, PCR and Gel electrophoresis

This is the most sensitive method for the detection of DNA trypanosomes from blood. All samples and reagents kits were brought to room temperature. The methods of Baticados *et al.* (2005) and Morlais *et al.* (1998), were employed with slight modifications for extraction of DNA, PCR amplification and detection of PCR products by gel electrophoresis.

Data Analysis

Results were reduced to percentages and presented in Table and Figures. Inferential statistics was used to conclude the result and values of $p<0.05$ were considered significant at 95% confidence interval (CI).

RESULT

This study showed 1.3% prevalence of mixed infection in cattle from Kawo and Tudun Wada abattoirs. However, for individual abattoir, four animals had mixed infection for both parasites each, *T. congolense* and *T. vivax* showed a prevalence of 17% and 4.3%, respectively as shown in Figure 1.

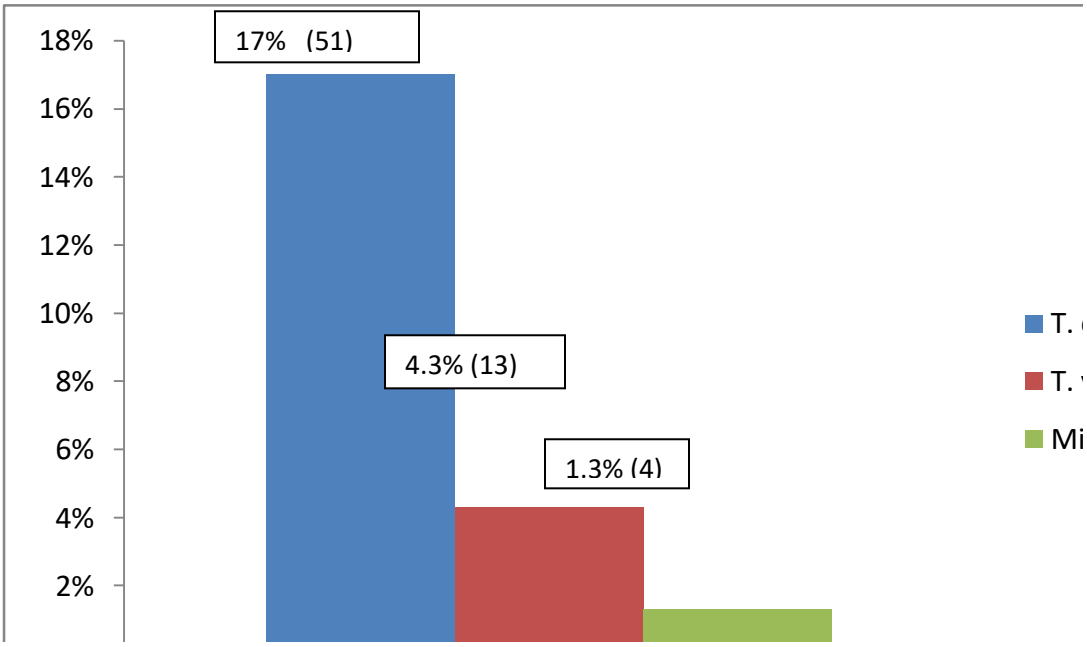

Fig.1. Prevalence of *T. congolense*, *T. vivax* and mixed infection from selected abattoirs in Kaduna metropolis.

Further analysis carried out on positive samples from microscopy using PCR to indicate presence of trypanosomes in blood sample from selected abattoirs. Lane 1 shows the 100bp DNA marker while lanes 2-10 indicates the presence of both parasites showing the genomic DNA as seen in Plate 1.

Plate 1: Showing the extracted Genomic DNA for both species of Trypanosomes

Primers TCS 344-345 for *T. congolense* and TVW1-2 for *T. vivax* were used for DNA amplification. Lane 1 shows the DNA marker at 100bp and Lanes 2-5 indicates the presence of mixed infection from the samples processed at 399 bp and 437 bp, respectively.

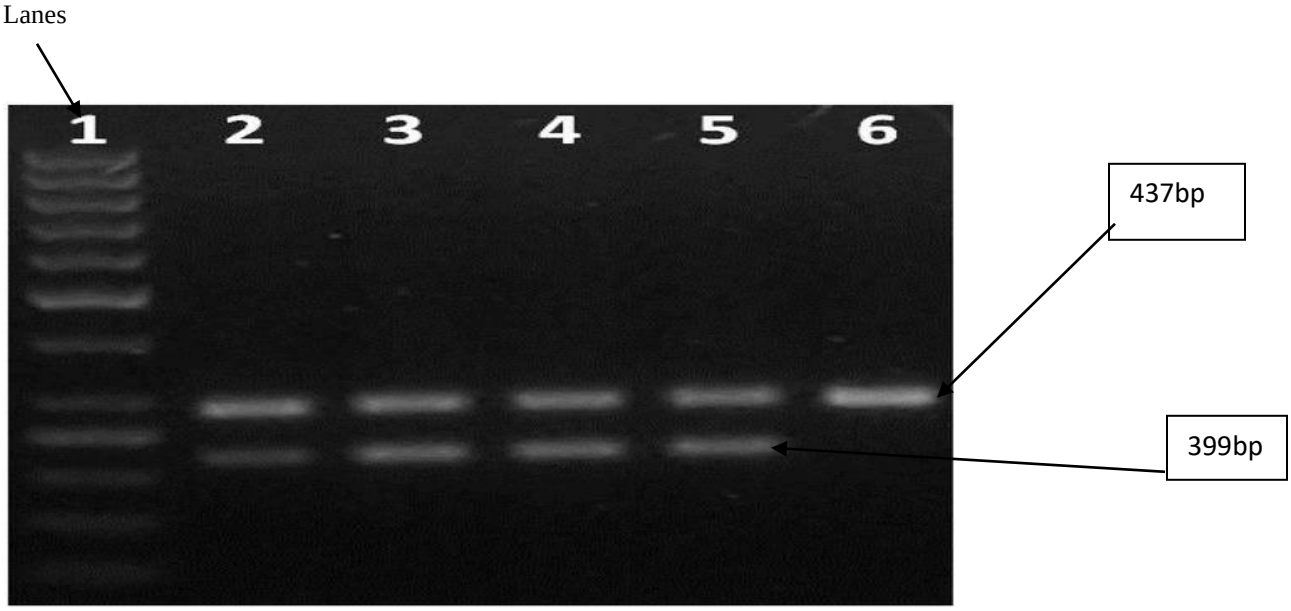

Plate 2: Showing bands of mixed infection of *T. congolense and T. vivax*

DISCUSSION

Trypanosoma vivax and *Trypanosoma congolense* are major causes of AAT in cattle, leading to serious health problem and severe constraint to Africa's socio-economic development. The prevalence of both parasites was observed in the three abattoirs selected from Kaduna metropolis during the study. Mixed infection of *T. congolense* and *T. vivax* of 1.3% reported in this study disagrees with the report of Tasew and Duguma (2012), which documented that locating both parasite in a host is very scarce but this

could have occurred as a result of the vector feeding on infected host and subsequently feeding on another infected host of different species which pre- establish the possibility of mixed infection. However, this finding agrees with the report of Gillingwater *et al.* (2010) which reported mixed infection of two different types of *T. congolense* in Kwa-Zulu Natal region of South Africa in their work. *T. congolense* showed a higher prevalence of 17% as compared to *T. vivax* (4.3%) in the three selected abattoirs, this is probably because *T. congolense* have more predilection for cattle than *T. vivax* whereas the reverse is the case for *T. vivax* in sheep. Furthermore, it has been documented in previous report that both adult and old cattle were more affected by *T. congolense* than *T. vivax* (Tasew and Duguma, 2012). In addition, the finding observed in this study could be as a result of climatic variations across different geographical regions and type of animal husbandry practices adopted as most of the animals were brought in from different locations to the abattoir for slaughter (Fasanmi *et al.*, 2014) and the animals could have been exposed to numerous trypanosome vectors during long time grazing. In field conditions, tsetse flies can be infected by several trypanosome species or sub-species which can be transmitted to the same hosts leading to natural mixed infection in the host species.

This study confirmed the presence of trypanosomes using PCR and to determine both parasites in mixed infection from positive samples obtained from wet mount. In this regard, the PCR detection test was viewed as an additional method for the effective monitoring and surveillance of the parasite which could be used side-by-side with microscopy, being the gold standard widely used for routine diagnosis of trypanosomes. The results also indicated that there were mixed infections in trypanosomal species in cattle from Kawo and Tudun-wada abattoirs, respectively which coincides with the report of Baticados *et al.* (2011), on parasitological and PCR detection of trypanosomal infection. Their report affirmed PCR to be a very good molecular technique for the detection of parasite DNA in blood samples and highly sensitive in terms of parasitic detection (Baticados *et al.*, 2011). Primers for each trypanosome specie was used in the cause of this study and compared with a previous report by Masake *et al.* (1997), which offered an advantage that only blood samples with TCS 344-345 at 437bp for *T. congolense* and TVW1-2 at 399bp for *T. vivax* hybridized with the DNA probe. The washing steps remove non-specific reactions which may interfere with the specific ones, thus, making PCR highly specific and sensitive for the detection of trypanosomes). (Sow *et al.*, 2006

CONCLUSION

It is concluded that natural mixed infection does occur *in-situ* invading the immune system of affected hosts which could expose such animals to concurrent infection and grievous disease manifestation. Finally, microscopy and PCR should be used concomitantly for effective detection of these parasites so as to combat the menace of African animal trypanosomiasis.

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