

# 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 packed cell volume 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 confirms the presence of both as seen in plate 2. In conclusion, this study shows the presence of both parasites in natural mixed infection in cattle from tudun-wada and kawo abattoirs in 4 cows each. Therefore, it shows that *T. congolense* and *T. vivax* as single and mixed infection can impact negatively on the health of cattle showing severe and mixed infection occurs in the field. This also indicates that natural mixed infection can occur in the field invading the immune system of affected hosts. This suggests that microscopy and PCR should be used alongside for effective detection of these parasites so as to combat the menace of African Animal Trypanosomiasis.

**Keywords:** Wet mount, Packed cell volume, *T. vivax*, *T. congolense*, Mixed infection, PCR.

## INTRODUCTION

African trypanosomiasis is a complex and debilitating disease of both

humans and animals found in sub-Saharan Africa. [2] 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) is widely known as “Nagana” (which is derived from a zulu term meaning to be in low or depressed spirits) or sammore, found in Africa causing serious economic loss in animal production. [1-2] 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 sp.*) [35]. The distribution of this disease in Africa corresponds to the range of tsetse flies and comprises currently an area of 8 million km<sup>2</sup> between 14 degrees North and 20 degrees South latitude [35, 37]. Throughout history, African trypanosomiasis has severely repressed the economic and cultural development. [36]

African Animal Trypanosomosis is recognized 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 [37]. 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 [33]. 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 subspecies, between livestock species and within livestock species among breeds depending on the challenge and virulence of the strains [27]. Due to their frequencies, pathogenicity and consequence on productivity, *T. congolense* and *T. vivax* are the principal trypanosomes which infect domestic animals. [32, 30] Infection with both trypanosomes results in subacute, acute or chronic disease characterized by intermittent fever, anemia, occasional diarrhea and rapid loss of condition and often terminates in death. [1] This hematic trypanosomes (*T. congolense*

and *T. vivax*) in mixed infection cause injury to the host mainly by the production of severe anemia, which is accompanied in the early stages of the disease by leucopenia and thrombocytopenia [10, 29]. In the terminal stages of the disease caused by the hematic trypanosomes, focal polioencephalomalacia probably results from ischemia due to massive accumulation of the parasites in the terminal capillaries of the brain. [31, 25-26] 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 causes of AAT in West African cattle. However, due to their genetic diversity, both [34] species have been shown to cause serious infections in cattle, horses and asses which leads to abortion and death. [28-29]

The detection of trypanosomes rely mainly on conventional methods in addition to clinical symptoms, clinical history, travel history and geographic location of animals. [1] *T. vivax* and *T. congolense* are characteristically present in the bloodstream of infected hosts when viewed under the microscope in fresh blood films [35] *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. [35-36, 11, 4] Polymerase chain reaction (PCR) assays is specific for the diagnosis of Trypanosomosis via detection of Trypanosomal DNA in the blood samples of infected hosts [9, 14, 24, 19, 16] Studies have reported that PCR technique after Microscopy can be more specific and sensitive especially from very low-parasitized samples including those from asymptomatic animals [23, 22, 5, 21, 4, 13]. Development of DNA amplification technique on the detection of trypanosomes has broaden and improve method of detection from samples of suspected hosts. [13] However, natural mixed infections have been reported, [5, 6] little or no information has been reported in abattoir.

Therefore, this study investigates the prevalence of mixed infection between *T. congolense* and *T. vivax* in cattle and the possible significance this could have on livestock farming in 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 C-GIDD report [7]. This study was carried out among cattle from three abattoirs including male and female cattle slaughtered 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 between any other two days interval for a period of four months by a veterinary doctor from the jugular vein. One hundred blood samples were randomly collected from cattle of each abattoir 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 cooler with ice packs which was immediately transported to Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna State and prepared for analysis upon arrival for 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. [8]

### 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 microhaematocrit 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. [8]

### Polymerase Chain Reaction Technique

This is the most sensitive method for the detection of DNA trypanosomes from blood. The methods of Baticados [6] and Morlais [15] were employed with slight modifications for extraction of DNA, PCR amplification and detection of PCR products by electrophoresis.

### DNA Extraction

All samples and reagents kits were brought to room temperature. Seven hundred microlitres of genomic buffer (PBS) lysis solution were pipetted into tube with 500µl of blood sample and placed in water bath at 52°C for 10 minutes. Sample mixture was poured into collection tube and centrifuged for 12000 rpm and the supernatant was discarded. Five hundred microlitres of wash buffer 1 was added and centrifuged and discarded and five hundred microlitres of wash buffer 2 was immediately added, centrifuged and discarded thoroughly by shaking, it was centrifuged, emptied and collection tube was changed. 100µl of DNA pre-wash buffer was added, centrifuged and not discarded; 50µl of DNA pre-wash buffer was added and centrifuged before it was finally discarded. Finally, fifty microlitres of nuclease free water was added onto DNA binding solution and centrifuged at 12,000 rpm for 1 minute. [6]

### Polymerase Chain Reaction

This was performed using primers for both trypanosomes. Initially, 2µl of DNA template 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. [6]

### Gel 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 combs inserted. The gel was allowed to cool and solidify at room temperature. The comb was removed and 300ml of 1 × Tris-acetate was dispensed on the gel tank. The wells were loaded with 5µl of PCR product mixed with 1µl of loading dye; the gene ladder was loaded in wells. The preparation of electrophoresis for 20-30 minutes at 60 volts, the products migrated

based on their molecular weight. PCR products were placed under ultra- violet light to detect the amplicons using gel documentation system. [6]

### Data Analysis

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

## RESULT

This study showed 1.3% prevalence of mixed infection in cattle from Kawo and Tudun wada abattoir. However, for individual abattoirs, 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.

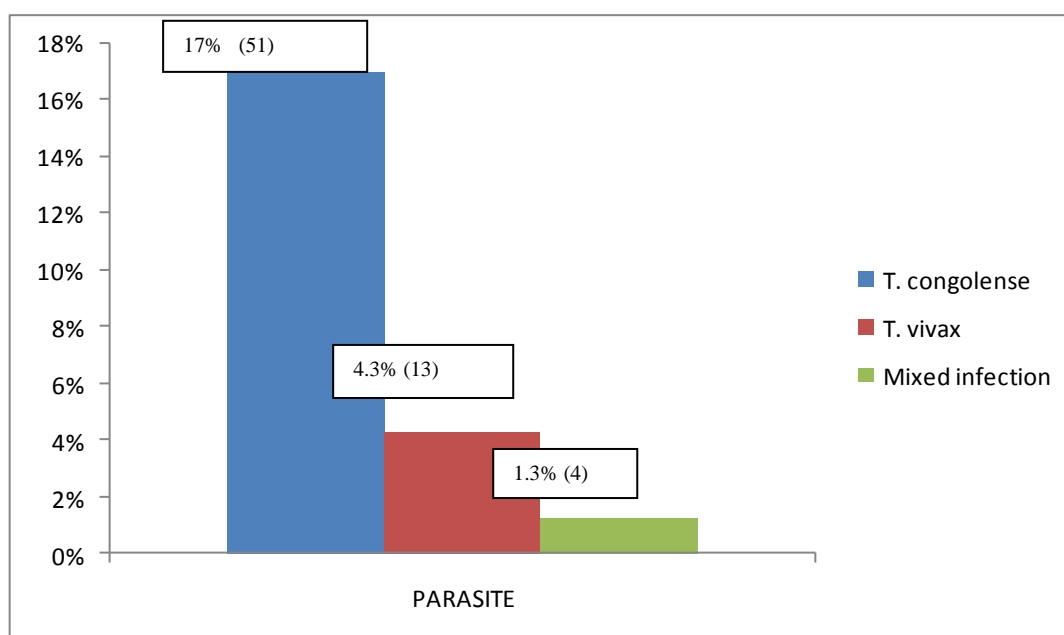


Fig:1 Prevalence of *T. congolense*, *T. vivax* and mixed infection from selected abattoirs in Kaduna metropolis.

The mean PCV obtained for samples collected from the animals showed a statistically significant difference at  $p < 0.05$  (0.016) for Tudun- wada abattoir with results from Kawo (0.950) and Makera (0.934) abattoirs, which were found to be non- significant as showed in Table 1.

The mean Packed Cell Volume (PCV) of samples obtained from the selected abattoirs

Abattoir	Sample	Mean PCV (%)	<i>T. congolense</i>	<i>T. vivax</i>	Mixed Infection	Std error	p-Value
Kawo	100	25	14	3	4	0.748	0.950
T/wada	100	25	24	8	4	0.748	0.016
Makera	100	25	13	2	0	0.748	0.934

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.

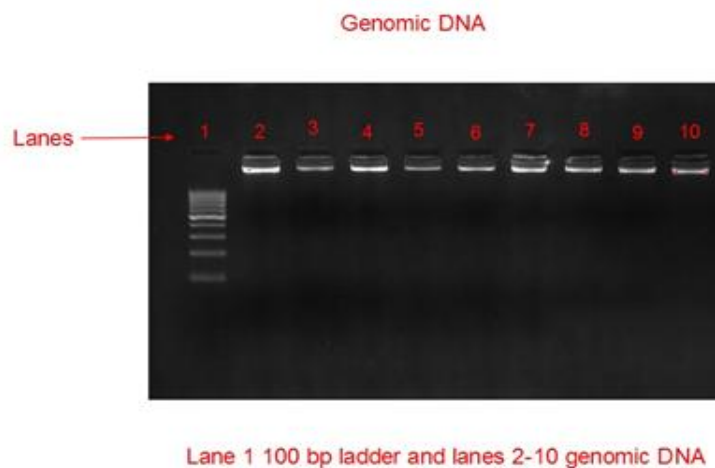


Plate 1: Showing the extracted Genomic DNA for both species of Trypanosomes

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 399bp and 437bp respectively.

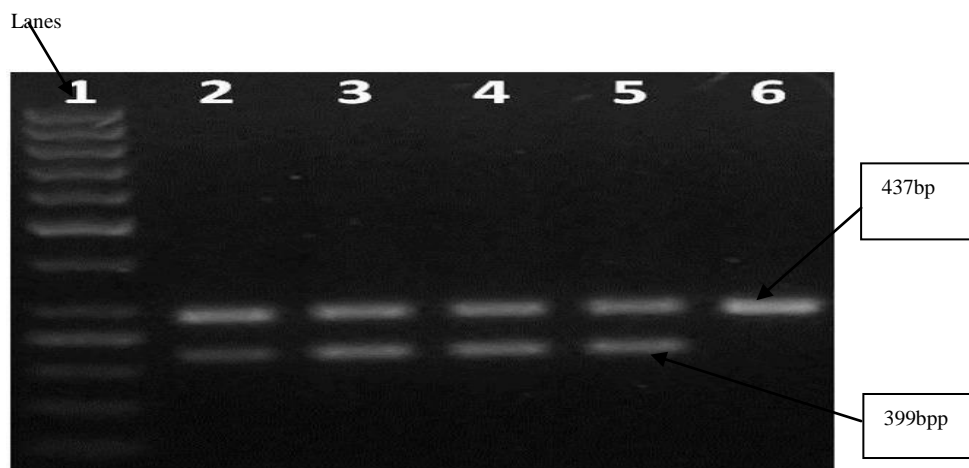


Plate 2: Showing bands of mixed infection of *T. congolense* and *T. vivax*

## DISCUSSION

*Trypanosoma vivax* and *Trypanosoma congolense* are major causes of AAT in cattle, causing 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 [20] stating that associating both parasite 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 becoming mixed infection but this findings agrees with the report of [3]. *T. congolense* showed a higher prevalence of 17% as compared to *T. vivax*

(4.3%) in the three selected abattoirs. This is because cattle have more *T. congolense* infection than *T. vivax* as *T. congolense* infection decreases with age and significantly lower in sheep than cattle but the reverse is the case for *T. vivax*. Infections at birth, and in line with a previous report, the adult and old cattle were more affected by *T. congolense* than *T. vivax* [20]. The findings 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 [12] and the animals would have been exposed to trypanosome vectors during long time grazing. In field conditions, tsetse flies can be infected by several trypanosome species or subspecies which can be transmitted to the same hosts leading to natural mixed infection in host species.

Packed cell volume gives an indication of anaemia and disease status [38]. This study revealed that anaemia was influenced by infection as in the case of Tudun Wada abattoir. Cattle in Tudun-Wada abattoir showed a higher positive value in the mean PCV for *T. congolense* 24 and *T. vivax* at 8 respectively, showing mixed infection in 4 cows with p-value of 0.016, which is statistically significant at  $p < 0.05$ . Thus showing a significant difference in samples from Tudun-wada abattoir when compared to the remaining two abattoirs and this agrees with the report of Samdi et al. [17] on the prevalence of trypanosomiasis at slaughter in Kaduna central abattoir while Kawo abattoir showed mixed infection in 4 cows. This could be as a result of repeated exposure to the same population of trypanosomes in a given area, higher animal and tsetse contact resulting from the concentration of the flies in the area and number of animals slaughtered daily. [17]

This study was able to confirm 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 and used side-by-side with microscopy, which is 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 [5] on Parasitological and PCR detection of Trypanosomal infection. PCR is a very good molecular technique for the detection of parasite DNA in blood samples and highly sensitive in terms of parasitic detection [5]. Primers for each trypanosome specie was used in the cause of this study and compared with a previous report by [14] which offers 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 detection of trypanosomes. [18]

## CONCLUSION

It can be concluded from this study that *T. vivax* and *T. congolense* as single and mixed infections impacted negatively on cattle health. It was observed that cows infected with both parasites showed more severe symptoms. This also indicates that natural mixed infection can be occurred in the field invading the immune system of affected hosts. The use of microscopy and PCR should be used alongside for effective detection of these parasites so as to combat the menace of African Animal Trypanosomiasis.

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