

Molecular Identification and Prevalence of Animal African Trypanosomes Among Cattle Distributed within Jebba Axis of River Niger, Kwara State.

ISSA FUNSHO HABEEB (✉ issahabeeb1284@gmail.com)

Ahmadu Bello University Faculty of Science

GLORIA DADA CHECHET

Ahmadu Bello University Faculty of Science

JACOB K. P. KWAGA

Ahmadu Bello University Faculty of Veterinary Medicine

Research Article

Keywords: Trypanosomiasis, Cattle, ITS-1, Prevalence, Jebba, Geospatial Distribution, River Niger.

Posted Date: June 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-586022/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Trypanosomiasis is a fatal disease that threatens the economy of at least 37 countries in Sub-Saharan Africa most especially livestock farming. In this study, we sought to investigate the prevalence of trypanosome infection in cattle, the potentials of these livestock as reservoirs of human-infective trypanosomes and the spatial distribution of trypanosome infected herds.

Methods: The survey was conducted at the midland between the Northern and Southern part of Nigeria, an area perceived to have harboured migrating animals over the years due to insecurity in the north. A randomized cross-sectional study was conducted along the Jebba axis of river Niger, Kwara state by screening cattle from 36 herd clusters by nested PCR using ITS-1 generic primers. Data generated were analyzed using the Chi square test at 95% confidence interval.

Results: Microscopic screening identified 3/398 samples representing 0.75% prevalence while twelve animals, representing 3.02% of the 398 sampled were detected as positive by PCR. Our result showed a decline in the PCV of infected animals (24.7%). The infection rate were categorized as single infection 11/12 (91.67%) and mixed infection 1/12 (8.33%). Animals were more susceptible to *Trypanosoma congolense* infection (50%) with *T. congolense* Savannah being the most prevalent Sub-specie (71.4%). Consequently, Trypanosome infections were more prevalent among female animals (4.30%), young animals (10.0%), White Fulani breeds (3.7%), animals with residency period of three years or less (3.18%), Transhumance animals (3.6%), animals with the diseases history (4.05%), animals with no history of drug administration (3.1%), animals close to river Niger (56.2%), larger herds (33.3%) and animals that have travelled to trypanosome endemic areas (50.0%). Aside age and distance of animals from river Niger, statistical difference in every other parameter tested were based on mere probabilistic chance. Spatial data showed that the disease is prevalent among herd located in less than 3Km distance from the river Niger which may represent a key risk factor.

Conclusion: It is concluded that our study area may not be classified endemic but the epidemiological significance of this finding is that at least cattle populations may play a vital role in the maintenance and possible resurgence of the disease in the study area.

Introduction

African Trypanosomosis (AT) are parasitic diseases of public health concern, and limits agricultural productivity in almost all developing countries in Sub-Saharan Africa. The disease is caused by blood parasites, belonging to the genus *Trypanosoma* and widely transmitted in Africa by tsetse flies (1, 2). In excess of 30 tsetse fly species and subspecies pervade a territory of 33% of Africa's landmass and influences animals and humans in at least 36 sub-Saharan Africa countries (2, 3). It is generally classified as Neglected Tropical Disease (NTD), either as African Animal Trypanosomiasis (AAT) or Human African Trypanosomiasis (HAT) (4, 5). In Africa, the disease affects around 100 million heads of cattle and in Nigeria, 6 million are estimated to be at risk out of a cattle population that is presently estimated to be

20 million (6). The World Health Organization (WHO) in the year 2000 gave an estimate of between 50 to 70 million individuals were at the risk of tsetse bites in Africa, which causes sleeping sickness. The disease advances rapidly from less virulent to a chronic animal disease. The actual number of cases on yearly estimate ranges from between 300,000 and 500,000 people and with a direct economic loss of between \$4–5 billion in term of Gross Domestic Product (4, 5, 7).

In natural conditions, parasite transmission is majorly through nibbles of infected tsetse flies including *Glossina* species. In ruminants, three major species are responsible for these parasitic infections namely *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax* all of which are cyclically transmitted by tsetse flies and mechanically by other blood-feeding insects (8). In humans, *Trypanosoma brucei gambiense* causes chronic infection while *Trypanosoma brucei rhodisiense* causes acute cases (9), with recent reports showing cross infection of human-type trypanosomes in animals (10, 11).

Identification of this parasite is very fundamental to evaluate the overall threat presented by tsetse specie (12). In recent years, epidemiology has been advanced tremendously with tools from molecular biology in the study of disease transmission and distribution. It has also permitted the zoonotic capability of unidentified agents to be determined (13). The utilization of conventional microscopic method is not adequate to recognize the trypanosomes in the host (14). A very sensitive diagnostic tool for speciation and identification of low cases of parasitaemia will help address the limitations associated with conventional strategies as regards parasite detection specificity and sensitivity (15). Though ELISA discovery was an incredible improvement in regards to sensitivity of pathogen determination, antigen detection using MAb-based ELISA is unreliable due to the presence of immune active agents in the blood even after animals are treated and cannot distinguish between active and cured infection (14).

Advent of PCR as a novel technique for distinguishing parasite DNA are more sensitive and reliable than those utilized previously. However, PCR with primer pairs does not differentiate band patterns in gels for specific DNA segments and thus cannot differentiate isolates of the same species of trypanosomes and those that utilizes similar developmental sites such as the subspecies of *T. brucei* (16), and parasite species in the case of mixed and/or immature infections (17). Consequently, generic primers (12) has proven suitable for the identification of all known trypanosomes transmitted by tsetse in the amplification of ribosomal RNA gene loci within the internal transcribed spacer (ITS-1) region of the trypanosome genome. This is as a result of its inter-species length variation and high copy number. In this regards, species of trypanosome can be recognized by different band sizes produced due to DNA fragment amplification within the ITS-1 region (17, 18). Various researches concentrating on trypanosomiasis transmission have been conducted in some parts of Nigeria. To date, there is no research interest that seeks to characterize and estimate the overall prevalence of AAT in Kwara State. Hence the need to study the occurrence and distribution of this parasitic infection in this agro-ecological zones of the middle belt.

Methods

Study area

This study was conducted along the Jebba axis of River Niger, Kwara State. Jebba is located on a geographical coordinate of 9°9'14"N 4°48'43"E with views of the River Niger. In light of its area, it is alluded as "Midland" and the "Door" between Southern and Northern parts of Nigeria (19). According to the latest census, the city's population in 2006 was 22,411 and it is approximately 500 Km away from Abuja, 306 Km and 600 Km from Lagos and Kaduna respectively.

Study population

Our study population consisted of mostly transhumance cattle, having the possibility of mixing with sentinel animals. For parasite identification, animals with recent administration of trypanocidal drugs were excluded from the study

Study design

A cross-sectional study was embarked upon that captures cattle distribution across the Jebba axis of river Niger, Kwara State and its tributaries to assess trypanosome distribution/infection across the geographical area, June 2019.

Sampling methods

Sample frame and sample size determination

Systematic random sampling techniques was employed in this study. Herds of cattle in each coordinate were pooled and considered as a cluster from where animals were sampled by systematic randomisation. Sampling frame was identified by listing herds (Myetti-Allah) locations across Jebba and samples were obtained in each cluster based on proportion to size of herd i.e. 6% per herd size is equalled to sample size per herd. Systematic random sampling technique was used to select animals in each of the randomized cluster whereby the sampling interval was generated by dividing the herd size by the sample size required for that herd. The first study subject was randomly selected from among cattle, while others to be included in the sample were selected after every j th interval (20). Due to the complete absence of previous record on the prevalence of trypanosomiasis in our study area, 50% prevalence was assumed and the sample size was estimated in accordance with the method of (21). A minimum total sample size of 384 was drawn across all the identified clusters. The Sample size calculation was based on the assumption of a 95% confidence level, 50% assumed prevalence, 0.05 tolerable error. In each cluster, all animals regardless of the health status was considered in randomization so as to give an overall current infection rate status of the herd. Animals aged one year and younger were considered as young calves, while those over one year were regarded as adults. Dentition was used to determine the ages of animals while body conditions score (BCS) were assessed and adequately scored. Other parameters like breed, sex, source and location of the cattle were recorded. Herd data was collected to include the residency, travel history, herd size, history of trypanocidal treatment and history of disease.

Sample Size

Sample size was estimated as given below (21):

$$N = \frac{z^2 pq}{d^2}$$

Z = Confidence level/Z-score, p = Assumed Prevalence, q = Complementary probability, d = percentage error. Ninety five percent confidence level was used which statistically equate to 1.96, and which also amount to a percentage error of 5%. Estimate for the sample size of the cattle is then given as: The assumed prevalence of trypanosome infection for the said region stood at P=50%

$$\text{Sample size } N = \frac{1.96 * 1.96 * (0.5) * (1 - 0.5)}{0.05 * 0.05} = 384.16$$

Sample size, N is approximately 384

Proportion to size of herd

$$\text{Proportion to size of herd (\%)} = \frac{\text{Total sample size required}}{\text{Total population size}} * 100$$

$$\text{Proportion to size of herd (\%)} = \frac{384}{6603} * 100$$

Proportion to size of herd (%) is approximately 6%

Number of sample per herd = 6% of herd size

Sample collection and parasitological analyses

Five milliliter (5 mL) of blood samples were collected from the jugular vein of each randomized animal using a sterile vacutainer needle into tubes containing anti-coagulant (Ethylenediaminetetraacetic acid) (7). Each sample were identified by a unique barcode system that correspond to the name of the village, herd cluster and sample number. The samples were transported in ice box to the laboratory and stored at 4°C prior to laboratory analysis. Parasitological examination was done in the laboratory using the standard trypanosome detection Method i.e. Hematocrit Centrifugation Technique HCT (22), Buffy Coat Method, BCM (20), parasite load estimation (23) and Giemsa stained thick and thin films (24). The Packed Cell Volume (PCV) of each animal was also determined while the parasites were identified (25, 26)

DNA template extraction and PCR cycling

As prescribed by the manufacturer, Quick-gDNA™ Mini-Prep kit from Zymo Research Corporation, Irvine, CA, USA was used for gDNA extraction from the blood as prescribed by the manufacturer. DNA yield and purity assessment was done using Nanodrop ND-100 UV Spectrophotometer (Nanodrop Tech., Inc. DE, USA) while the DNA eluted was stored at -20°C until further use (20). PCR amplification was carried out

with slight modification (12). In the first round of the reaction, three microliter of DNA was added into the reaction mix. PCR was performed in a total reaction volume of 25 μ L containing 2.5 μ L standard Taq buffer, 1.0 μ L dNTPs, 1.0 μ L of each primer (25 μ M), 0.25U μ L Taq DNA polymerase, 3.0 μ L template and nuclease-free water was added to a final volume of 25 μ L. Cycling conditions was set as follows:

$$\begin{array}{l} \text{First run } 95 \int 120, \quad 94 \int 30, \quad 54 \int 30, \quad 72 \int 60, \quad 72 \int 300, \quad 4 \int \alpha \\ \text{Second run } 95 \int 120, \quad 94 \int 30, \quad 55 \int 30, \quad 72 \int 60, \quad 72 \int 300, \quad 4 \int \alpha \\ \qquad \qquad \qquad x \int y \\ \qquad \qquad \qquad x \text{ is Temperature in } (^{\circ}\text{C}) \\ \qquad \qquad \qquad y \text{ is Time in (sec.)} \end{array}$$

For nested PCR, two sequential runs were done using two primer sets. In the first reaction run, TRYP 3 and 4 were used as outer primer followed by TRYP 1 and 2 which served as inner primers in the second reaction run. From the first run, 2.0 μ L of the PCR product from the first run was added to 23 μ L of the mix in the second round of the reaction and in a fresh PCR tube. Cycling conditions were the same as the standard PCR cycling (Except for 1⁰C rise in annealing). Positive and a negative control was included in each set of reaction run (27). Reference DNA used in testing the sensitivity and specificity of primers was *T. congolense* specie identified by microscopy from the field. The amplified DNA was resolved on 2% agarose gel, visualized under a UV trans-illuminator and photographed with a Gel documentation apparatus (Molecular Image Gel-doc with Image Lab Bio-Rad Lab. Inc. Framework V 3.0) for clear visualization and reference purposes. The nucleotide sequence of the primer used for the PCR is as shown below (12).

Inner primers

TRYP 1 F' 5'AAGCCAAGTCATCCATCG3' TRYP 2 R' 5'TAGAGGAAGCAAAG3'

Outer primers

TRYP 3 F' 5'TGCAATTATTGGTCGCGC3' TRYP 4 R' 5'CTTTGCTGCGTTCTT3'

DNA Sequencing and phylogenetic analysis

The PCR products were purified and 20 μ l of the PCR products was sequenced using Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). As described by Applied Biosystems, Product of PCR were sequenced using Applied Biosystems Cycle Sequencing Kits (BigDye Terminator v1.1 and v3.1 kits). The sequence data obtained were viewed on Finch Trace Viewer v.1.4.0 (Applied Biosystems, Foster City, CA, USA), while flanking regions of high Noise-to-Signal ratio were trimmed off the sequence to improve the accuracy and precision of sequence data obtained. Ambiguous nucleotides were edited and replaced with conventional ones based on the highest peak recorded on the electropherogram. Each edited sequence were BLAST searched against the DNA sequence database

(NCBI) and/or the published databases for various trypanosome species (Tri-Tryp) explicitly for trypanosomes. Sequences of the ITS-1 region were aligned using ClustalW against known sequences in order to confirm species identity. Molecular Evolution Genetic Analysis Version 7.0.2.6 (MEGA7) was used to construct phylogenetic tree to observe their evolutionary trend and variation over time.

Statistical Analysis

The results obtained from this study were subjected to descriptive statistics to determine the frequency and distribution of trypanosome infection across the study area. The prevalence rates among localities, breeds of cattle, age and sex of the animals was expressed as percentages of the total number of animals sampled. This was done by dividing the number of infected animals by the total number of animals examined and expressed as percentages. Categorical values were evaluated using the Chi Square to measure the strength of between variables at 95% confidence interval. All data obtained were analyzed using SPSS statistical software version 20.0. Values of $P < 0.05$ were considered significant

Results

Across the study area, seventy two (72) herd clusters were identified as sample frame from where 50% of the herds representing 36 clusters were balloted by simple random sampling. In all, 398 blood samples were obtained from across the study area, three of which were screened positive by microscopy, representing 0.75% prevalence, while twelve samples representing 3.02% were tested positive by nested PCR with distinct band sizes characteristic of the specie involved. Sequencing of the PCR products and bioinformatic analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) further validated the species and sub-species of the Trypanosomes.

Table 1: Prevalence of trypanosome infection among cattle in Jebba by microscopic examination (June 2019)

Cluster	Total Cluster	Sampling Point	Herd Size	Total Examined	+Ve	-Ve	Prevalence (%)	Specie
Positive	3	JH	183	11	1	10	9.1	T. c spp.
		JAA	172	10	1	9	10	T. b spp.
		JAG	92	6	1	5	16.7	T. b spp.
Negative	33	Others	6156	371	0	371	0	Nil
Total	36		6603	398	3	395	0.75	

+Ve=Positive sample, -Ve= Negative sample, T. c=*Trypanosoma congolense*, T. b=*Trypanosoma brucei*

Table 2: Comparison of mean PCV among cattle breeds infected with *Trypanosoma* specie in Jebba, Kwara State (June 2019).

Cattle Breed	No of Animal Infected	Haematocrit Values (%)			Average Haematocrit (%)	
		Infected	Not Infected	Total	Infected (Mean±SEM)	Not Infected (Mean±SEM)
Muturu	Nil	Nil	591	591	Nil	32.8±1.23
Red Bororo	1	18	1230	1248	18.3±0.84	34.2±1.15
Sokoto Gudali	3	78	4443	4521	26.0±1.82	35.0±1.84
White Fulani	8	199	7344	7543	24.9±1.70	35.7±2.62

PCV values are means of three replicates and are expressed as Mean±SEM

Table 3: Comparison of mean PCV of cattle infected with *Trypanosoma* species in Jebba, Kwara State (June 2019).

S/NO	Specie Infection	Number of Animal Infected	PCV (%)
1	Non-infected	386	36.2±3.71
2	<i>T. congolense</i>	7	23.8±1.15
3	<i>T. brucei</i>	2	30.3±0.92
4	<i>T. evansi</i>	1	20.0±0.32
5	<i>T. theileri</i>	1	19.2±1.12
6	<i>T. simiae</i>	1	22.0±0.63

PCV values are means of three replicates and are expressed as Mean±SEM

Table 4: The prevalence of trypanosomosis detected by PCR according to sex, age, cattle breed, residency/stay period, animal origin, disease and treatment history (June 2019).

Category	Sub-Category	No. of Cattle Screened	No. of Trypanosome Infection	Prevalence (%)	χ^2	P-value
Sex	Male	165	2	1.20	3.133	0.077
	Female	233	10	4.30		
	Total	398	12	3.02		
Age (years)	≤ 1	70	7	10.0	14.172	0.000
	> 1	328	5	1.50		
	Total	398	12	3.02		
Breed	W.F	214	8	3.7	1.172	0.760
	S.G	130	3	2.3		
	R.B	36	1	2.8		
	MUT	18	0	0.0		
	Total	398	12	3.02		
Animal residency (years)	≤ 3	126	4	3.18	0.016	0.899
	>3	272	8	2.94		
	Total	398	12	3.02		
Animal origin	Sentinel	174	4	2.3	0.542	0.461
	Transhumance	224	8	3.6		
	Total	398	12	3.02		
Disease history	History	148	6	4.05	0.870	0.351
	No history	250	6	2.40		
	Total	398	12	3.02		
Treatment history	History	366	11	3.0	0.001	0.970
	No history	32	1	3.1		
	Total	398	12	3.02		

W.F: White fulani, R.B: Red bororo, S.K.: Sokoto gudali, MUT.: Muturu χ^2 =chi-square, Test of association were carried out at 95% Confidence Interval

Table 5: Prevalence of trypanosomosis detected by PCR according herd location, herd size and travel history to endemic area (June 2019).

Category	Sub-category	No of herds screened	No of herd(s) infected	Prevalence (%)	χ^2	P-Value
Location (Km) (Distance From River Niger)	Far (> 3 Km)	20	1	5.00	11.638	0.001
	Close (\leq 3 Km)	16	9	56.2		
	Total	36	10	27.8		
Herd size	Large \geq 200	15	5	33.3	0.396	0.529
	Small < 200	21	5	23.8		
	Total	36	10	27.8		
Travel history to endemic areas	Endemic	8	4	50.0	2.532	0.112
	Not endemic	28	6	21.4		
	Total	36	10	27.8		

Travel history: Kaduna, Ogun, Oyo, Jos, Benue and Delta χ^2 =chi-square, Test of association were carried out at 95% Confidence Interval

Discussion

Low prevalence reported in this study by microscopic screening (Table 1) is not surprising considering the low sensitivity imminent of parasitological diagnostic method (28, 29, 30). This is especially so for field animals characterized by low parasitaemia. The superiority of PCR over MHCT have been widely demonstrated in the epidemiological study of animal trypanosomosis (3, 20, 32, 33). These differences are due to sensitivity thresholds of the techniques. As against the prevalence by microscopy, nested Polymerase Chain Reaction (PCR) method gave an overall prevalence of 3.02%. Each species tested

produced amplicons of between 200–700 bp in length (Figs. 1 and 2). The ITS-1 PCR product size of *T. evansi* was similar to that of *T. brucei* and sequencing analysis was key to differentiating between the two PCR products. Sample JT11 was further confirmed to be *T. evansi* (supplementary data) suggesting the possible role of cattle as reservoirs of *T. evansi*. Generally, bands obtained from the amplification result were in agreement with previous studies (12, 31, 34, 35, 36, 38, 39, 40, 41). Samples JH4 and JM8 resulted in band sizes of approximately 700bp and which were confirmed by Sequence analysis to be *Trypanosoma congolense* Savannah sub-specie. Molecular characterization of *Trypanosoma* species using ITS-1 generic primers and/or its slight modification gave an estimated range of ITS-1 band sizes with a maximum amplicon length of 640bp (12) but went further in noting that all species amplification using generic primers could lead to a bands size of between 150–750bp in length as evidenced in this study and those previously reported (17, 31, 39, 42). An amplicon length of 210bp for *T. vivax* was not reported previously which may be an indication that *T. vivax*'s 18 rRNA is fast evolving at 7 to 10 times the rate of non-salivarian trypanosomes and also significantly evolving faster than all other trypanosomes. Although, no living specimen of this trypanosome was isolated, our conclusion could only be based on DNA sequence analysis while its taxonomic relationships were deduced from phylogenetic analysis of the amplified ssuRNA (70). Further biological characterization will depend on isolation of a living specimen into culture. The ability to identify this trypanosome by the distinct size of the ITS-1 region has provided preliminary information on its distribution and prevalence that should help track it down in the field. To make an evolutionary inference, all isolates aligned with the salivarian group except JO6 (*Trypanosoma theileri*) which fell in the stercoraria group. ssrRNA from JT11 (*T. evansi*), JAD7 (*T. brucei*), JAG2 (*T. brucei*) fell in the same branch but different clades which depicts a logical evolutionary event emanating from within these species (Fig. 3). *Trypanosoma evansi* is widely known to have evolved from *Trypanosoma brucei* and all of which were rooted on *Trypanosoma vivax* (16).

A drastic fall in PCV is traditionally considered a warning sign of the trypanosomiasis (43, 44). Classically, infection with trypanosome species that are pathogenic in local breeds of cattle result in retarded growth and anemia while nutritional status is a determining factor of infection (45, 46). From our findings, there is a PCV declined in trypanosome-positive cattle (Table 2) possibly due to the effect of parasites on blood cells. Similarly, the average hematocrit values varied between cattle breeds. However, the very low PCV presented by the red bororo breed may not be a true reflection of the PCV trend as only one animal was screened positive. The type of trypanosome specie infection impacted differently on the PCV of animals with average falling below the standard obtainable for cattle (24%-46%) except for the animals infected with *Trypanosoma brucei* with an average Packed Cell Volume of 30.3 ± 0.92 . The animal infected with *Trypanosoma theileri*, a non-pathogenic trypanosome of cattle had the lowest PCV value (19.2 ± 1.12) (Table 3). From our findings, it may be illogical to conclude that this comparative decrease in the PCV is due to *T. theileri* infection as only one animal was infected. However, it is possible that the parasite may have transited from a non-pathogenic to pathogenic form, hence the need to have a controlled experiment aimed at monitoring the PCV in the face of *Trypanosoma theileri* infection and other trypanosome species.

This study showed 3.02% overall prevalence which compares well with 4.3% national prevalence as reported by European Economic Commission project of 1989 and 1996 (47), 3.9% in Ogbomosho (48), 4.69% in Oyo (49) and 9.4% in Kaduna (50) as against high prevalence of 53.4% in Kaura, Kaduna (51) and 46.8% in Jos (52). These contradictory findings might reflect seasonal or local differences in tsetse populations, sample size and site, improved sensitization among nomads on grazing course, better implementation on the use of trypanocidal drugs and urbanization which may have perturbed the ecology of the transmitting vector, leading to ecological migration to a more favourable ecosystem, hence low prevalence recorded in our study.

Although there is no any significant difference in the infection rate between male and female animals (Table 4), our results showed that females were more infected. This observed differences may be attributed to livestock management adopted in the farming community where larger numbers of males are frequently sold off the herd at any early age while the rest are kept for breeding or animal traction. Also female animals persist longer in herds for the purpose of breeding, thus allowing the chronic infection to be maintained for very long period. As a result, the remaining males are more closely monitored while the females are readily exposed to hazard in the population vis a vis multiple copulation with limited male animals in the herd. Also the larger population of females (59%) obtained in this study by simple random sampling may account for this difference. Previously, 199 male cattle and 121 female were examined with no statistical difference in the infection prevalence (49). However, occurrence of any disease is dependent on many factors of which sex is just one of them. Factors other than sex relating to the host or its environment could therefore have played a role in influencing the susceptibility of animals to infection which has been documented in several studies (8, 10, 53).

From our study, there is a decreases in disease prevalence as animals get older probably due to age-acquired immunity which could represent a key positive factor and bearing in mind that trypanocidal treatments are more frequently used on adults by local farmers. In addition, young animals are more vulnerable to tsetse bites due to their skin fragility. Moreover, they are not agile enough to ward-off insects away along the grazing route as the adults. The tsetse flies also frequently target weak animals as a source of food in order to avoid being crushed by moving animals (54). In this study, despite the very low number of young animals randomly sampled, prevalence of 10.0% (7/70) for younger animals and 1.5% (5/328) were recorded for adult which is statistically significant ($P < 0.05$) (Table 4). This indicated that the incidence rate was not similar in young and adult animals (8). Although not significant, the infection rate differ among cattle breed. The prevalence of trypanosome infection was lowest in Sokoto gudali (2.3%), a breed not known for trypanotolerance (56, 57), and may have resulted from adaptability of this breed to its environment. The higher prevalence observed among the White Fulani breed may be attributed to their trypano-susceptibility and perhaps due to their higher representation in the sampling (52.8%). Of the four cattle breeds studied, the White Fulani are usually raised under the nomadic system of management. This may be another possible explanation for the higher prevalence recorded by this cattle group (10).

Although our test of statistic showed no significant difference in the infection rate among cattle in regards to period of residency, the infection was more prevalent among animals that were recently domiciled (3.18%). This observation may well be attributed to recent influx of herders down south due to insecurity and ban on open grazing in some parts of Nigeria and which has forced nomadism away from the north. Similarly, our findings revealed that infection was found to be more prevalent among transhumance animals (3.6%) probably due to exposure to tsetse bites while pervading territories of different endemic locals in a bid to having greener pastures. Sentinel animals appear to be more protected from tsetse bites due to livestock management style and guided path to grazing by cattle keepers. Despite the difference in infection rate, the statistical test showed that the observed difference was due to mere probabilistic chance (Table 4).

Having excluded animals with recent administration of trypanocidal drugs, a high prevalence of the disease was noted among animals having disease history (4.05%). An explanation to this could be that animals having the disease history may have not been well treated to clear the parasite in their blood in the first place or it may be that the treatment administered during the last infection may not be effective/efficacious or the parasite itself may have developed resistance to the administered drugs. During our survey, 92% of sampled animal had history of trypanocidal drugs treatment (Table 4) which may explain the low infection prevalence generally recorded with possible indications of drug resistance as seen in animals known to have had history of the disease and treatment but still reported in the study as infected animal.

The prevalence of trypanosome infection was significantly higher in locations closer to river Niger as compared to those further away (Table 5 and Fig. 6). This may be attributed to differences in herd management practices, grazing route which predisposes the herd to tsetse bites, herd composition and frequent exposure to trypanocidal drugs which may differ in each herd. The river could be a positive factor for the vector transmitting the disease as well as a source of water for grazing animals which could expose them to risks of bite by riverine species of the flies (58).

Although not significant, the disease rate was high among larger herds (33.3%) as compared to smaller ones (23.8%) (Table 5). It may be that smaller herds are more closely monitored and easily managed and treated before the transmission sets in as compared to larger herds where animals are seen as a single entity. Furthermore, animals that had travel history to trypanosomiasis endemic zone of the country (Benue, Jos, Kaduna, Delta, Oyo and Ogun as published in literatures) were more infected (50.0%) probably due to contact of travelling animals with infected sentinels in endemic zones and exposure to tsetse bite during trans-boundary movement.

Majority of the trypanosomes in cattle were *T. congolense* and *T. brucei* which accounted for 50.0% (6/12) and 16.67% (2/12) (Fig. 5) respectively with nearly half of the overall infection due to *Trypanosoma congolense* Savannah sub-specie (Fig. 6), possibly as a result of large host range or probably due to the fact that riverine species of tsetse are generally considered susceptible to *T. congolense* infections (69). High prevalence of *T. congolense* infection is an indication of the dominance

of *G. morsitans* species of the fly (7, 8, 18, 20, 52, 59, 60, 52) and could be that its transmission is highly favoured by the obligate cyclical vector or the *T. vivax* and *T. brucei* respond better to the trypanocidal drugs, diminazene aceturate and homidium chloride administered by farmers. A high prevalence of the Savannah subgroup in cattle may also indicate that the parasites were introduced recently into the tested herds coupled with its reported virulence as compared to other sub-specie (55). The low prevalence of *T. brucei* infection may relate to reported resistance of indigenous West African cattle to the parasite (61). However, the detection of *T. brucei* and *T. evansi* in Nigerian cattle might portend serious danger not only to cattle and other livestock but also to livestock owners and the host communities at large as *T. evansi* infection has been reported in cattle and humans in India (62, 63). Low prevalence of *T. simiae* infection is an indication of low transmission of the parasite as animals infected with this species will probably not survive the acute and severe nature of this parasite (64, 65). Double infections in animals are a normal occurrence in the field (66). This study identified *T. congolense* Kilifi and *T. vivax* mixed infection in only one of the herd clusters. In Nigeria, previous surveys identified mainly *T. congolense* and *T. vivax* as animal pathogenic trypanosomes (67) and co-circulation has been reported in studies conducted in northern Nigeria (20, 41, 68). Co-infections with multiple *Trypanosoma* species have also been documented previously due to bites from tsetse flies carrying more than one *Trypanosoma* infections or successive bites from flies with different *Trypanosoma* species (8, 16, 37).

Conclusion

Within the Jebba axis of River Niger, an overall prevalence of cattle trypanosomosis by PCR was 3.02% as against 0.75% recorded by microscopy. Despite the low prevalence reported in this study, the present findings should be of interest to veterinarians and health workers. Sex, breed, animal stay period, animal origin, disease and treatment history did not significantly influence the rate of trypanosome infections (Tables 4 and 5). However, the test of statistics showed that age and relative distance of the herds to River Niger may be a contributory risk factor in the disease prevalence. This study has evidenced the circulation of six trypanosome species with all isolates having appreciable homology (> 80%) with what was already established in the NCBI database. Comparatively with set threshold of EEC (4.3%), the study area may not be classified endemic but the epidemiological significance of this study is that at least cattle population may play important role in the possible resurgence of the disease in this region. Factors such as geographical distribution of all trypanosome species can be used as a guide to improve control measures. The knowledge and awareness of trypanosome infection will also enhance concrete human-based control measures in this local. This situation has determined the potential zone to be placed under surveillance in the case of disease outbreak in the country.

Abbreviations

AAT: African Animal Trypanosomiasis, HAT: Human Africa Trypanosomiasis, PCR: Polymerase Chain Reaction, ITS-1: Internally Transcribed Spacers-1, HCT: Hematocrit Centrifugation Technique, BCM: Buffy Coat Method, PCV: Packed Cell Volume (PCV)

Declarations

Acknowledgements

Thanks are extended to staff and students of the Department of Biochemistry and Center for Biotechnology Research and Training, ABU Zaria Nigeria in particular Prof. Junaid Kabir, Prof. Muhammed Mamman, Prof. Y.K.E. Ibrahim, Dr. Emmanuel O. Balogun, Dr. Y. Y. Pala, Lamin B. S. Dibba and Musa M. Shuaibu for their technical support.

Authors' contributions

IFH: collected samples, analyzed samples by PCR and sequencing, analyzed data, drafted and reviewed the manuscript. GDC: designed specific primers for trypanosomes, reviewed and edited the manuscript, facilitated the support of traditional and administrative authorities, supervised the field and laboratory experiment. JKPK: Designed the project, facilitated the support of traditional and administrative authorities, supervised the fieldwork and laboratory experiments.

Funding

This work was funded by the Africa Centre of Excellence for Neglected Tropical Disease and Forensic Biotechnology (ACENTDFB), Ahmadu Bello University, Zaria, Nigeria. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary data files.

Ethics approval and consent to participate

Approval to collect blood from cattle was obtained from the community head, local cattle breeders (miyetti Allah) and the Kwara State Ministry of Agriculture and Rural Development.

Consent for publication

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication

Competing interests

There are no competing interests in this research.

Author details

¹Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria.

²Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. ³Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria, Nigeria.

References

1. Adams ER. and Hamilton PB. New molecular tools for the identification of trypanosome species. *Future Microbiology*. 2008;3(2):167-176.
2. Food and Agriculture Organisation of the United Nations, (FAO). Food, agriculture and food security: The global dimension, 2002. WFS02/Tech/Advanced Unedited Version. Rome p. 19–28.
3. Food and Agriculture Organisation of the United Nations, (FAO). Food, agriculture and food security: Trypanosomiasis 2003. Available from: <http://www.spc.int/rahs/> (Accessed on August 27, 2019).
4. World Health Organization Report on Elimination of African Trypanosomiasis (*Trypanosoma brucei gambiense*) Geneva, Switzerland 2012.
5. World Health Organization. Human African Trypanosomiasis, (sleeping sickness) 2016. Accessed May 26, 2019. https://www.who.int/trypanosomiasis_african/en
6. Shiferaw S, Muktar Y, Belina D. A review on trypanocidal drug resistance in Ethiopia. *Journal of Parasitology and Vector Biology*. 2015;7(4):58-66.
7. Ravel S, Mediannikov O, Bossard G, Desquesnes M, Cuny G, Davoust B. A study on African Animal trypanosomiasis in four areas of Senegal. *Folia Parasitologica*. 2015;62:44. <https://doi.org/10.14411/fp.2015.044>
8. Farougou S, Allou SD, Sankamaho I, Codjia V. Prevalence of Trypanosome Infections in Cattle and Sheep in the Benin's West Atacora Agro-ecological zone. 2012;30(3):141–146.
9. Kyambadde JW, Enyaru JCK, Matovu E, Odiit M, Carasco JF. Detection of trypanosomes in suspected sleeping sickness patients in Uganda using the polymerase chain reaction. *Bulletin of the World Health Organization*. 2000;78(1):119–124.
10. Karshima SN, Lawal IA, Bata SI, Barde IJ, Adamu PV, Salihu A, et al. Animal reservoirs of *Trypanosoma brucei gambiense* around the old Gboko sleeping sickness focus in Nigeria. *Journal of Parasitology and Vector Biology*. 2016;8(5)47–54. <https://doi.org/10.5897/JPVB2015.0228>.
11. Umeakuana PU, Gibson W, Ezeokonkwo RC Anene BM. Identification of *Trypanosoma brucei gambiense* in naturally infected dogs in Nigeria. *Parasites and Vectors*. 2019;12:420.
12. Adams ER, Malele II, Msangi AR, Gibson WC. Trypanosome identification in wild tsetse populations in Tanzania using generic primers to amplify the ribosomal RNA ITS-1 region. *Acta Tropica*. 2006;100:103–109. <https://doi.org/10.1016/j.actatropica.2006.10.002>.
13. Traub RJ, Monis PT, Robertson ID, Molecular epidemiology: A multidisciplinary approach to understanding parasitic zoonoses. *International Journal of Parasitology*. 2005;35:1295–1307. <https://doi.org/10.1016/j.ijpara.2005.06.008>.

14. Geysen D, Delespaux V, Geerts S. PCR–RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. *Journal of Veterinary Parasitology*. 2003;110:171–180.
15. Koffi M, Kouadio KI, Sokouri DP, Wognin MT, N’Guetta A. Molecular characterization of trypanosomes isolated from naturally infected cattle in the "Pays Lobi" of Côte d’ivoire. *Journal of Applied Biosciences*. 2014;83:7570–7578.
16. Malele I, Craske L, Knight C, Ferris V, Njiru Z. The use of specific and generic primers to identify trypanosome infections of wild tsetse flies in Tanzania by PCR. *Infection, Genetics and Evolution*. 2003;3:271–279. [https://doi.org/10.1016/S1567-1348\(03\)00090-X](https://doi.org/10.1016/S1567-1348(03)00090-X)
17. Desquesnes M, McLaughlin G, Zoungrana A, Davila AMR. Detection and identification of Trypanosomes of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. *International Journal of Parasitology*. 2001;31:610–614.
18. Njiru ZK, Makumi JN, Okoth S, Ndungu JM, Gibson WC. Identification of trypanosomes in *Glossina pallidipes* and *longipennis* in Kenya. *Infection, Genetics and Evolution*. 2004;4:29–35. <https://doi.org/10.1016/j.meegid.2003.11.004>.
19. Oyebanji JO. Quality of life in kwara state, Nigeria. An exploratory geographical study: *JSTOR* 1982;13(2):34-40.
20. Takeet IM, Fagbemi BO, De Donato M, Yakubu A, Rodulfo H E, Peters SO, et al. Molecular survey of trypanosomes in naturally infected Nigerian cattle. *Research in Veterinary Science*. 2013;94:555–561. <https://doi.org/10.1016/j.rvsc.2012.10.018>.
21. Thrusfield M, *Veterinary Epidemiology*. 3rd edition oxford: Blackwell Science; 2007.
22. Ledoka MV. Molecular Characterization of Trypanosomes Commonly Found In Cattle, Wild Animals and Tsetse Flies In Kwazulu. Being a thesis submitted for an award of the master’s degree in the Department of Veterinary Tropical Disease, Faculty of Veterinary Science, University of Pretoria; 2008.
23. Herbert WJ, Lumsden WH. *Trypanosoma brucei*: A rapid “matching” method for estimating the host’s parasitemia. *Experimental Parasitology*. 1976;40(3):27–31.
24. Gagman HA, Ajayi OO, Yusuf AS. A Survey for Haemo-Parasite of Pigs Slaughtered in Jos Abattoir Plateau State Nigeria, *Bayero Journal of Pure and Applied Sciences*. 2014;7(2):59–63. <http://dx.doi.org/10.4314/bajopas.v7i2.12> ISSN 2006 – 6996
25. Cheesbrough M. *District laboratory practice in tropical countries, part 2*. Press syndicate of the University of Cambridge U.K; 2000. p. 320–321.
26. Chandhri SS, Goupte SK. *Manual of General Veterinary Parasitology*. First edition, International book distributing co; 2003. p. 19–48.
27. Ng’ayo MO, Njiru ZK, Muluvi GM, Osir EO, Masiga DK. Kinetoplastid Biology and Disease Detection of trypanosomes in small ruminants and pigs in western Kenya: Important reservoirs in the epidemiology of sleeping sickness. *Kinetoplastid Biology and Disease*. 2005;4(5):1–7. <https://doi.org/10.1186/1475-9292-4-5>.

28. Picozzi K, Tilley A, Fèvre EM, Coleman PG, Magona JW, Odiit M, et al. The diagnosis of trypanosome infections: applications of novel technology for reducing disease risk, African Journal of Biotechnology. 2002;1(2)39–45.
29. Abenga JN, Enwezor FNC, Lawani FAG, Osue HO, Ikemereh ECD. Trypanosome prevalence in cattle in Lere area in Kaduna State. Revue Elev. Med. Vet. Pay. Trops. 2004;57(1–2):10–13.
30. Enwezor FNC, Samdi SM, Ijabor O, Abenga JN. The prevalence of bovine trypanosomes in parts of Benue State, north-central Nigeria. Journal of Vector Borne Disease. 2012;49:188–190.
31. Da´vila AM, Herrera HM, Schlebinger T, Souza SS, Traub-Cseko YM. Using PCR for unraveling the cryptic epizootiology of livestock trypanosomosis in the Pantanal, Brazil. Veterinary Parasitology. 2003;117:1–13.
32. Herrera HM, Da´vila AMR, Norek A, Abreu UGP, Souza SS, Dandrea OS et al. Enzootiology of *Trypanosoma evansi* in Pantanal, Brazil. Veterinary Parasitology. 2004;125:263–275.
33. Ferná´ndez D, Gonza´lez-Baradat B, Eleizalde MC, Gonza´lez-Marcano E, Perrone T, Mendoza M. *Trypanosoma evansi*: a comparison of PCR and parasitological diagnostic tests in experimentally infected mice. Experimental Parasitology. 2009;121:1–7.
34. Gonzales JL, Jones TW, Picozzi K, Cuellar, RH. Evaluation of a polymerase chain reaction assay for the diagnosis of bovine Trypanosomiasis and epidemiological surveillance in Bolivia. Kinetoplastid Biology of Disease. 2003;2:8–11.
35. Gonzales JL, Chacon E, Miranda M, Loza A. and Siles L.M. Bovine trypanosomosis in the Bolivian Pantanal. Veterinary Parasitology. 2007;146:9–16.
36. Mekata H, Konnai S, Witola WH, Inoue N, Onuma M. Ohashi K. Molecular detection of trypanosomes in cattle in South America and genetic diversity of *Trypanosoma evansi* based on expression site-associated gene 6. Infectious and Genetic Evolution. 2009;9:1301–1305.
37. Mekata H, Konnai S, Simuunza M, Chembensofu M, Kano R, Witola WH, et al. Prevalence and Source of Trypanosome Infections in Field-Captured Vector Flies (*Glossina pallidipes*) in Southeastern Zambia. Journal of Veterinary Medicine and Science. 2008;70:923–928.
38. Rama´rez-Iglesias JR, Eleizalde MC, Reyna-Bello A, Mendoza M. Molecular diagnosis of cattle trypanosomes in Venezuela: evidences of *Trypanosoma evansi* and *Trypanosoma vivax* Journal of Parasitology and Disease. 2017;41(2):450–458 DOI 10.1007/s12639-016-0826.
39. Nakayima J, Nakao R, Alhassan A, Mahama C, Afakye K, Sugimoto C. Molecular epidemiological studies on animal trypanosomiases in Ghana. Parasites and Vectors. 2012;5:217.
40. Garcí´a HA, Garcí´a ME, Pe´rez G, Bethencourt A, Zerpa E, Pe´rez, H. et al. A. Trypanosomiasis in Venezuelan water buffaloes: association of Packed-Cell Volumes with seroprevalence and current trypanosome infection. Annals of Tropical Medicine and Parasitology. 2006;100:297–305.
41. Weber JS, Ngomtcho HSC, Shaida SS, Chechet GD, Gbem TT, Nok JA, et al. Genetic diversity of trypanosome species in tsetse flies (*Glossina*) in Nigeria. Parasites and Vectors. 2019;12:481.
42. Njiru ZK, Constantine CC, Guya S, Crowther J, Kiragu JM, Thompson, RC et al. The use of ITS-1 rDNA PCR in detecting pathogenic African trypanosomes. Parasitology Research. 2005;95:186–192.

43. Esievo KAN, Saror DI, Ilemobade AA Hallaway MH. Variation in erythrocyte surface and free serum sialic acid concentration during experimental *Trypanosoma vivax* infection in cattle. *Research in Veterinary Science*. 1985;32:1–5.
44. Doko-Allou S, Farougou S, Salifou S, Ehile E. Geerts S. Dynamique des infections trypanosomiennes chez les bovins Borgou sur la ferme de l'Okpara au Bénin. *Tropicultura*. 2010;28(1):37-43.
45. Holmes PH, Katunguka-Rwakishaya E, Bennison JJ, Wassink GJ, Parkins, JJ. Impact of nutrition of pathophysiology of bovine trypanosomiasis. *Parasitology*. 2000;120:573–585.
46. Murray M, D'leteren GDM. Teale AJ. From Trypanotolerance. In *The Trypanosomiasis*. Edited by Maudlin I, Holmes PH, Miles MA. Wallingford: CABI Publishing; 2004. p. 48.
47. Onyiah JA. African Animal Trypanosomosis: An overview of the current status in Nigeria. *Tropical Veterinary*. 1997;15:111-116.
48. Ameen SA, Joshua RA, Adedeji OS, Raheem AK, Akingbade AA, Leigh OO. Preliminary studies on prevalence of Ruminant trypanosomosis in Ogbomoso area of Oyo State, Nigeria. *Middle East Journal of Scientific Research*. 2008;3(4):214–218.
49. Fasanmi OG, Okoroafor UP, Nwufoh OC, Bukola-Oladele, OM, Ajibola ES. Survey for *Trypanosoma* species in cattle from three farms in Iddo Local Government Area, Oyo State. *Sokoto Journal of Veterinary Sciences*. 2014;12(1):57-61.
50. Agu WE, Kalejaiye JO, Olatunde AO. Prevalence of bovine trypanosomosis in some parts of Kaduna and Plateau State, Nigeria. *Bulletin of Animal Health and Production in Africa*. 1990;37(2):161-166.
51. Maikaje DB. Some aspects of the epidemiology and drug sensitivity of bovine trypanosomiasis in Kaura LGA of Kaduna State. PhD Thesis, Ahmadu Bello University, Zaria, Nigeria; 1998.
52. Majekodunmi AO, Fajinmi A, Dongkum C, Picozzi K, Thrusfield M V, Welburn SC. A longitudinal survey of African animal trypanosomiasis in domestic cattle on the Jos Plateau, Nigeria : Prevalence, distribution and risk factors. *Parasites and Vectors*. 2013;6(1):1. <https://doi.org/10.1186/1756-3305-6-239>.
53. Shah SR, Phulan MS, Memon MA, Rind R, Bhatti A. Trypanosome infections in camels. *Pakistan Veterinary Journal*. 2004;24(4):209-210.
54. Bengaly Z, Ganaba R, Sidibe I, Duvallet G. Infections trypanosomiennes dans la zone Sud-soudanienne du Burkina Faso. *Rev. Elev. Méd. Vétérinaire Pays tropical*. 1998;51:225-229.
55. Bengaly Z, Sidibe I, Ganaba R, Desquesnes M, Boly H, Sawadogo L. Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. *Veterinary Parasitology*. 2002;108:1–19.
56. Ogunsanmi AO, Ikede BO, Akpavie SO. Effect of management, season, vegetation zones and breed on the prevalence of bovine trypanosomosis in Southwestern Nigeria. *Israel Journal of Veterinary Medicine*. 2000;55(2):25-43.
57. Talabi AO, Otesile EB, Joshua RA, Oladosu LA. Clinical observations on three Nigerian Zebu cattle breeds following experimental *Trypanosoma congolense* *Bulletin of Animal Health Production in Africa*. 2012;60(2):187– 192.

58. Munang'andu MH, Siamudaala V, Munyeme M, Nalubamba KSA. Review of Ecological Factors Associated with the Epidemiology of Wildlife Trypanosomiasis in the Luangwa and Zambezi Valley Ecosystems of Zambia, Interdisciplinary Perspectives on Infectious Diseases. 2012;20:13 doi:10.1155/2012/372523.
59. Peacock L, Ferris V, Bailey M, Gibson W. The influence of sex and fly species on the development of trypanosomes in tsetse flies. Public Library of Science Neglected Tropical Disease. 2012;6(2):115-19.
60. Simukoko H, Marcotty T, Phiri I, Geysen D, Vercruyssen J, Van den Bossche P. The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. Veterinary Parasitology. 2007;147(3-4):231-8.
61. Kalu AU. Current status of tsetse fly and animal trypanosomiasis on the Jos Plateau, Nigeria. Preventive Veterinary Medicine. 1996;27:107-113.
62. Laha RE, Sasmal NK. Detection of *evansi* infection in clinically ill cattle, buffaloes and horses using various diagnostic tests. Epidemiology and Infection. 2009;137:1583-1585.
63. Joshi PP, Shegokar VR, Powar RM, Herder S, Katti R., Salkar HR, et al. Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. American Journal of Tropical Medicine and Hygiene. 2005;73:491-495.
64. Sidibe I, Bengaly Z, Boly H, Ganaba R, Desquesnes M, Sawadogo L. Differential Pathogenicity of *Trypanosoma congolense* subgroup: Implication for the strategic control of trypanosomiasis. Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV). 2002;6:33-35.
65. Simo G, Asonganyi T, Nkinin SW, Njiokou F, Herde S. High prevalence of group 1 in pigs from the Fontem sleeping sickness focus in Cameroon. Veterinary Parasitology. 2006;139:57-66.
66. Mugittu KN, Silayo RS, Majiwa PAO, Kimbita EK, Mutayoba BM, Maselle R. Application of PCR and DNA probes in the characterization of trypanosomes in the blood of cattle in farms in Morogoro Tanzania. Veterinary Parasitology. 2000;94:177-189.
67. Odeniran PO, Ademola IO. A meta-analysis of the prevalence of African animal trypanosomiasis in Nigeria from 1960 to 2017. Parasitology and Vectors. 2018;11:280.
68. Isaac C, Ciosi M, Hamilton A, Scullion KM, Dede P, Igbinosa IB. Molecular identification of different trypanosome species and subspecies in tsetse flies of northern Nigeria. Parasites and Vectors. 2016;9:301.
69. Reifenberg JM, Cuisance D, Frezil JL, Cuny G, Duvallet G. Comparison of the susceptibility of different Glossina species to simple and mixed infections with *Trypanosoma* (Nannomonas) *congolense* savannah and riverine forest types. Medical and Veterinary Entomology. 1997;11:246-52.
70. Stevens J, Rambaut A. Evolutionary rate differences in trypanosomes. Infectious and Genetics Evolution. 2001;1:143-150.

Figures

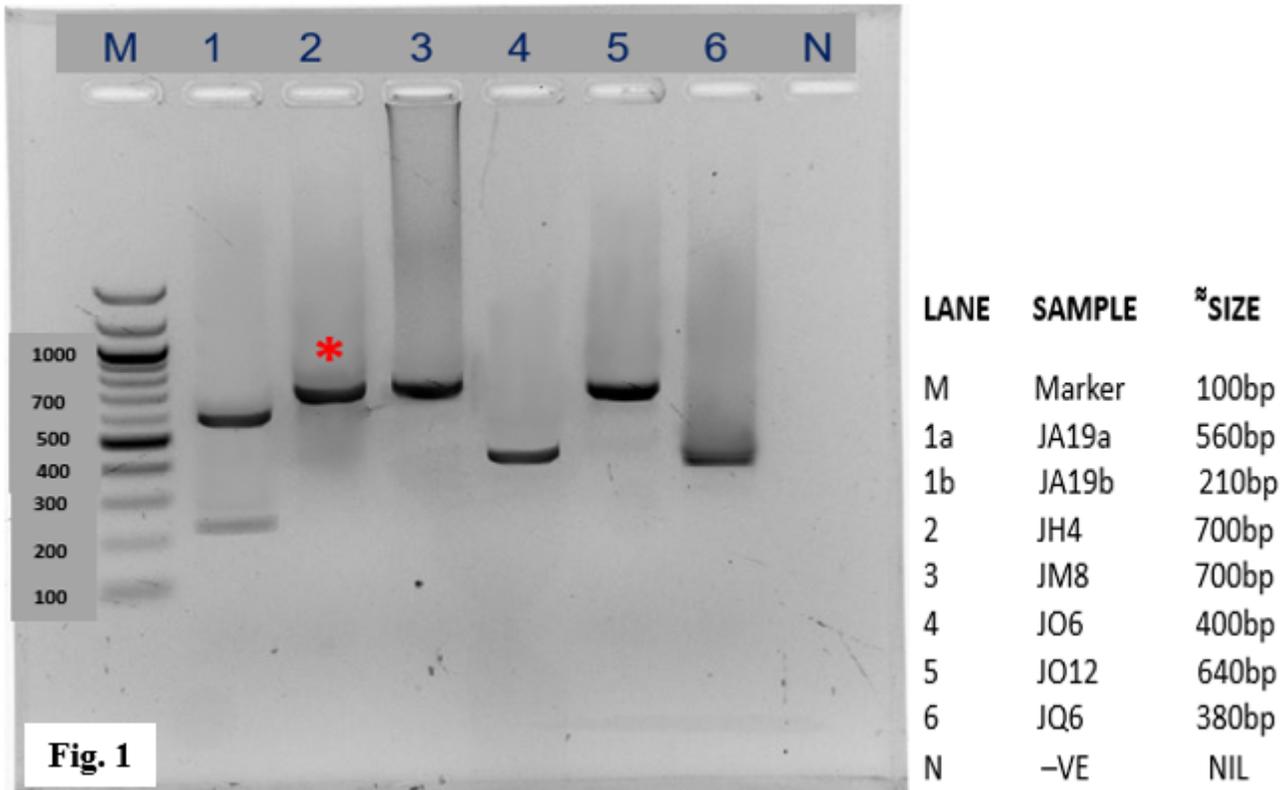


Figure 1

PCR amplification of trypanosome template DNAs. *: Positive for microscopy, Lanes M: 100bp supperladder-mid, (ABgene), Lane 1 (JA19a and JA19b): A mixed infection with *Trypanosoma congolense* Kilifi and *Trypanosoma vivax*, Lane 2 (JH4): *Trypanosoma congolense* Savannah, Lane 3 (JM8): *Trypanosoma congolense* Savannah, Lane 4 (JO6): *Trypanosoma theileri*, Lane 5 (JO12): *Trypanosoma congolense* Savannah, Lane 6 (JQ6): *Trypanosoma simiae*, Lane 7 (JT4): *Trypanosoma congolense* Savannah, Lane 8 (JT11): *Trypanosoma evansi*, Lane 9 (JY5): *Trypanosoma congolense* Savannah, Lane 10 (JAA7): *Trypanosoma brucei brucei*, Lane 11 (JAD7): *Trypanosoma congolense* Forest, Lane 12 (JAG2): *Trypanosoma brucei brucei*, Lanes N: Negative control.

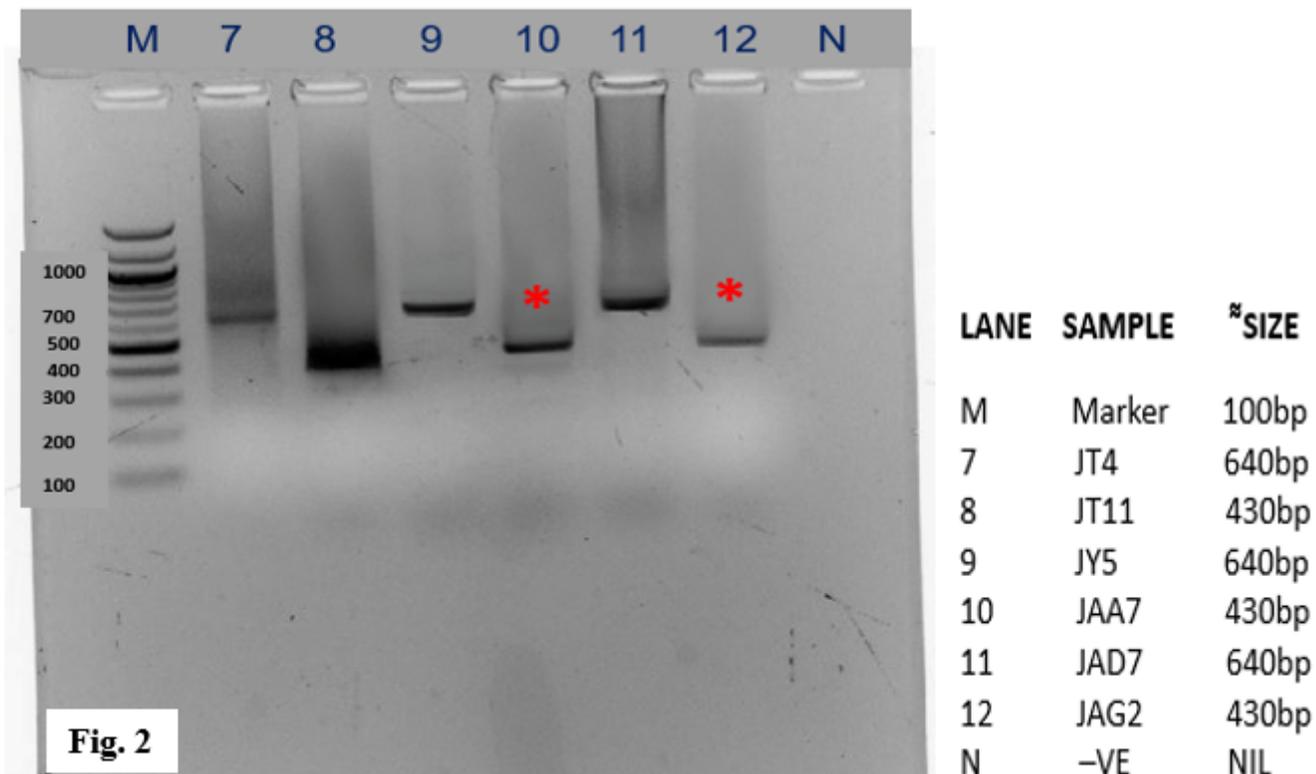


Figure 2

PCR amplification of trypanosome template DNAs. *: Positive for microscopy, Lanes M: 100bp supperladder-mid, (ABgene), Lane 1 (JA19a and JA19b): A mixed infection with *Trypanosoma congolense* Kilifi and *Trypanosoma vivax*, Lane 2 (JH4): *Trypanosoma congolense* Savannah, Lane 3 (JM8): *Trypanosoma congolense* Savannah, Lane 4 (JO6): *Trypanosoma theileri*, Lane 5 (JO12): *Trypanosoma congolense* Savannah, Lane 6 (JQ6): *Trypanosoma simiae*, Lane 7 (JT4): *Trypanosoma congolense* Savannah, Lane 8 (JT11): *Trypanosoma evansi*, Lane 9 (JY5): *Trypanosoma congolense* Savannah, Lane 10 (JAA7): *Trypanosoma brucei brucei*, Lane 11 (JAD7): *Trypanosoma congolense* Forest, Lane 12 (JAG2): *Trypanosoma brucei brucei*, Lanes N: Negative control.

substitutions per site. The analysis involved 39 nucleotide sequences and 77 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

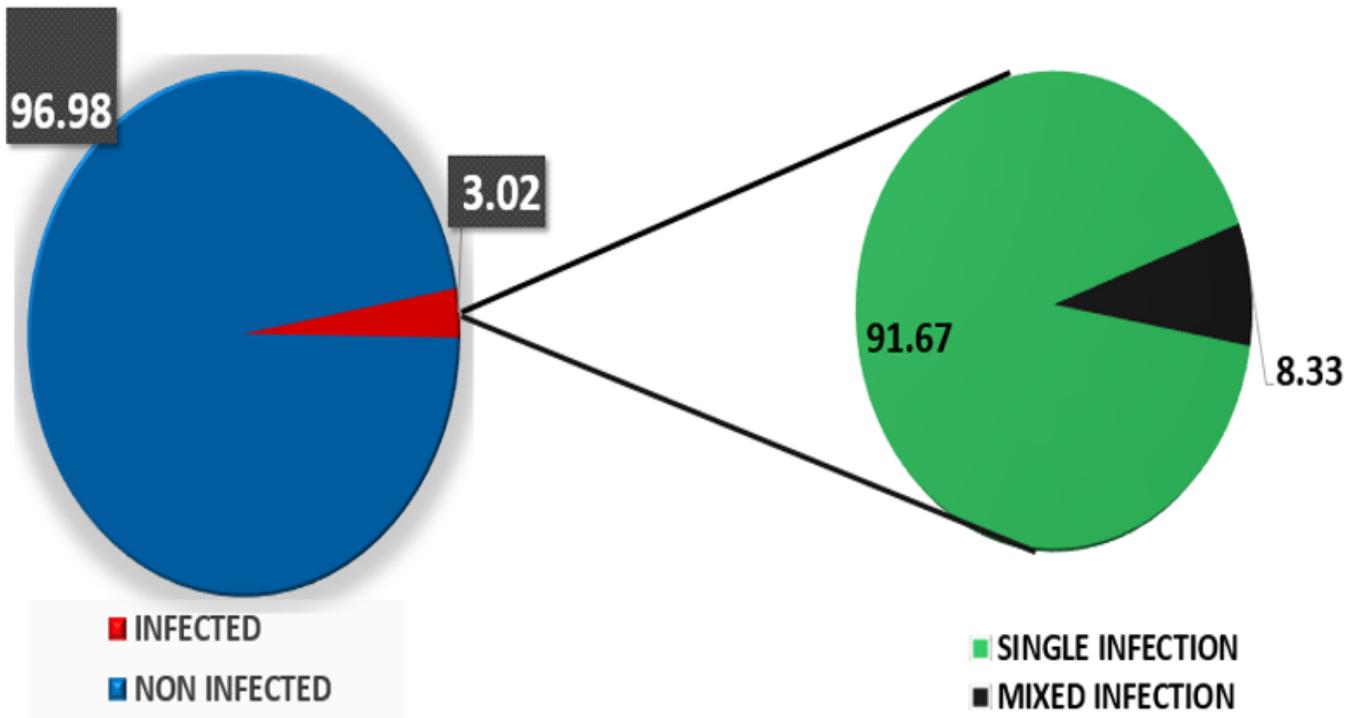


Figure 4

Prevalence of single and mixed infection with trypanosome among cattle distributed within Jebba axis of River Niger, Kwara State (June 2019).

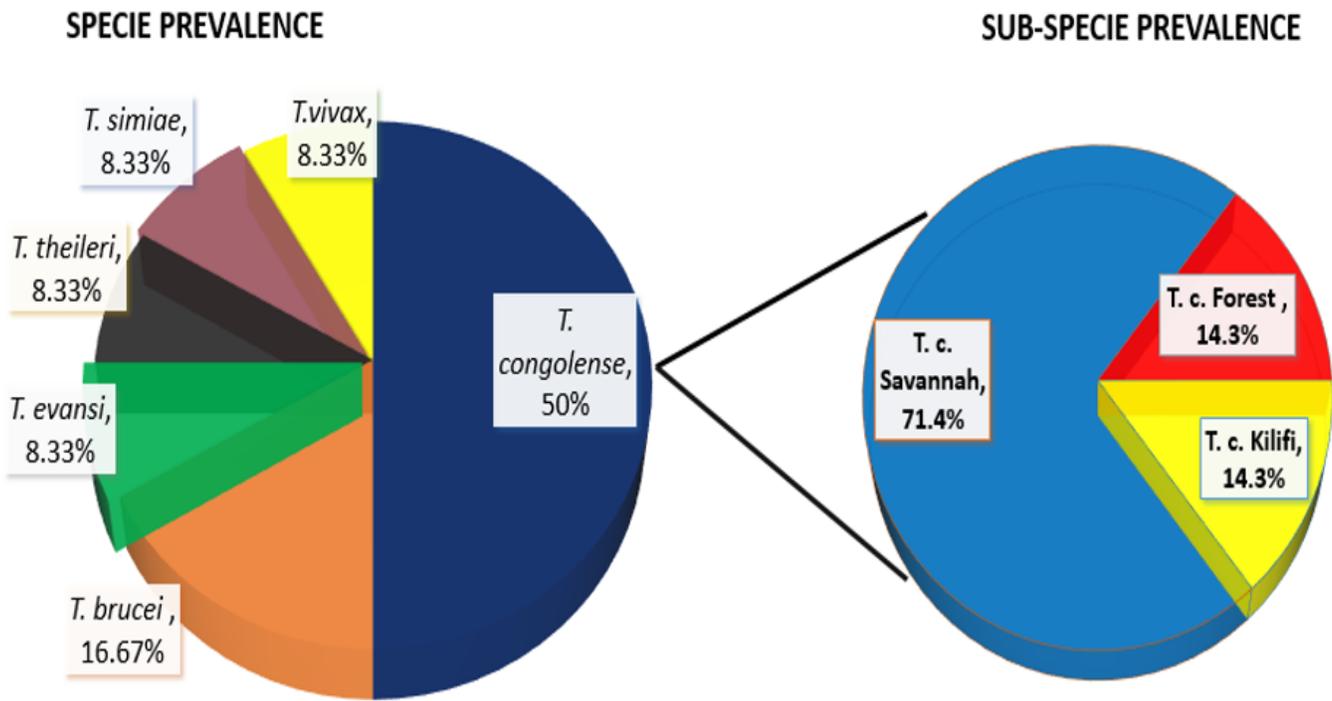


Figure 5

Specie and Sub-Specie prevalence of trypanosome infection within the Jebba axis River Niger, Kwara State (June, 2019)

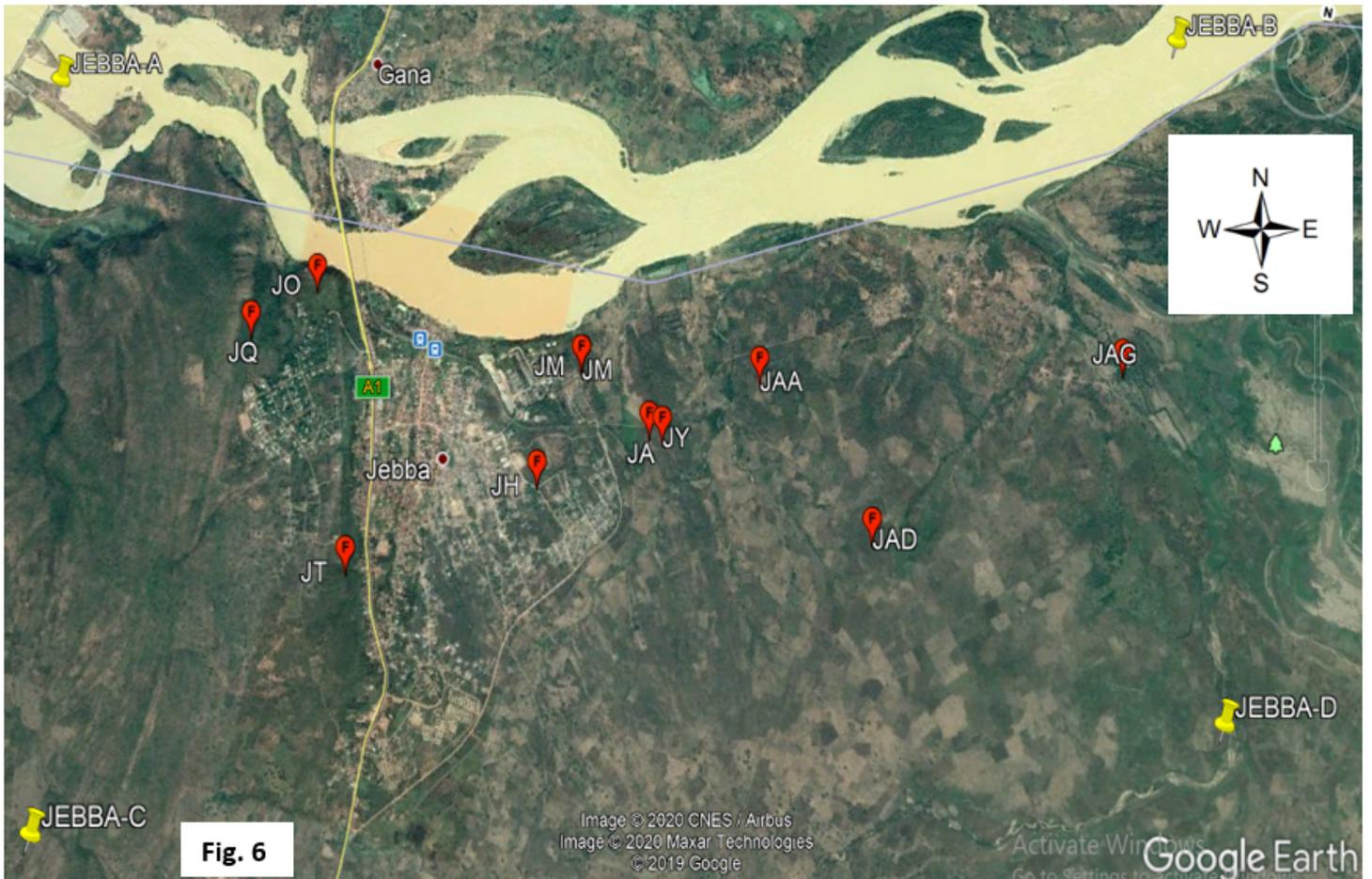


Figure 6

Spatial distribution of trypanosome-infected herds within the Jebba axis of River Niger, Kwara State Nigeria (June 2019). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

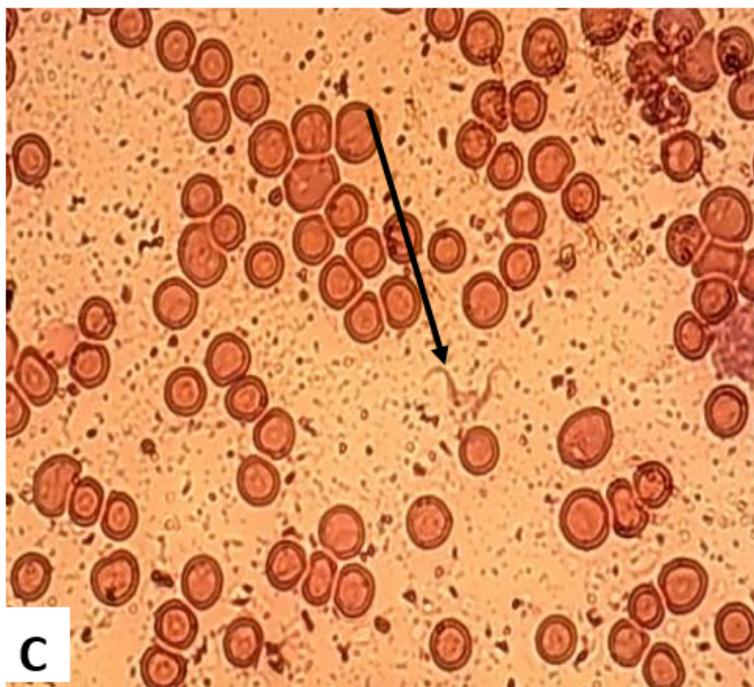
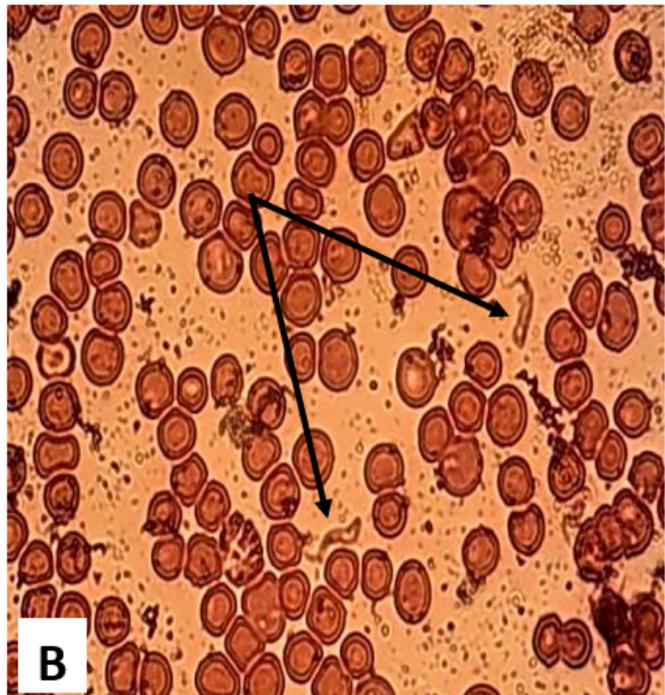
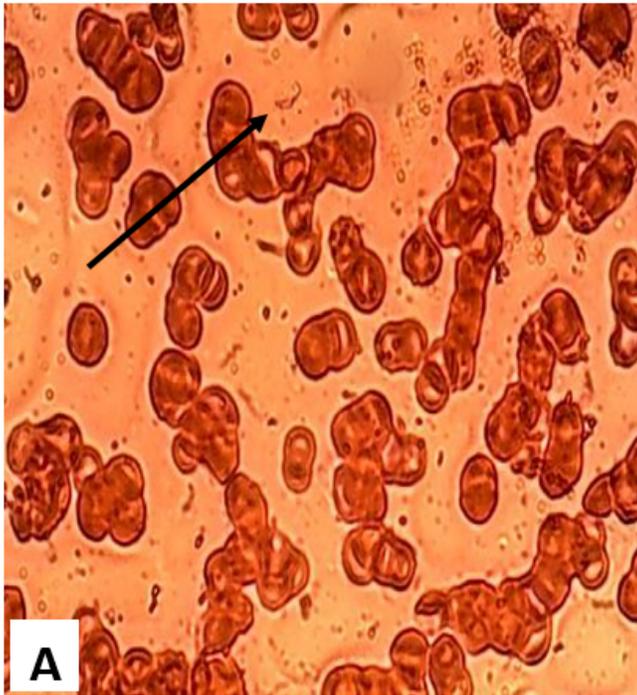


Figure 7

Plate A: Morphological identification of *Trypanosoma congolense* specie from cattle cluster JH, sample 4. Plate B: Morphological identification of *Trypanosoma brucei* specie from cattle cluster JAA, Sample 7. Plate C: Morphological identification of *Trypanosoma brucei* specie from cattle cluster JAG, Sample2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GRAPHICALABSTRACT.jpg](#)
- [Supplementarydata.docx](#)