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Apparent density, trypanosome infection rates and host preference of tsetse flies in the sleeping sickness endemic focus of Northwestern Uganda

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Abstract

Background: African trypanosomiasis, caused by protozoa of the genus Trypanosoma and transmitted by the tsetse fly, is a serious parasitic disease of humans and animals. Reliable data on the vector distribution, feeding preference and the trypanosome species they carry is pertinent to planning sustainable control strategies.

Methodology: We deployed 109 biconical traps in 10 villages in two districts of northwestern Uganda to obtain information on the apparent density, infection rates and blood meal sources of tsetse flies. A subset (272) of the collected samples was analyzed for detection of trypanosomes species and sub-species using a nested PCR protocol based on primers amplifying the Internal Transcribed Spacer (ITS) region of ribosomal DNA. 34 blood-engorged adult tsetse midguts were analyzed for blood meal sources by sequencing of the mitochondrial cytochrome c oxidase 1 (COI) and cytochrome b (cytb) genes.

Results: Out of the 109 traps deployed, we captured 622 Glossina fuscipes fuscipes tsetse flies (269 males and 353 females). Apparent density (AD) ranged from 0.6 to 3.7 flies/trap/day in the two districts. 29 (10.7%) of the flies were infected with one or more trypanosome species. Infection rate was not significantly associated with neither age group ($\chi2 = 5.001$, df=2, 0.082), sex of the fly ($\chi2 = 0.099$, df = 1, p = 0.753), district of origin ($\chi2= 0.629$, df = 1, p = 0.428) nor village ($\chi2= 9.252$, df = 9, p = 0.414). Nested PCR revealed several species of trypanosomes: T. vivax (6.62%), T. congolense (2.57%), and T. brucei and T. simiae each at 0.73%. Blood meal analyses revealed five principal vertebrate hosts, namely, cattle (Bos taurus), humans (Homo sapiens), Nile monitor lizard (Varanus niloticus), African mud turtle (Pelusio schapini) and the African Savanna elephant (Loxodonta africana).

Conclusion: We found a moderately high infection rate of 10.78%, with all infections attributed to trypanosome species that are causative agents for the animal disease only. However, more validation using large-scale passive and active screening of human and tsetse samples should be done. Cattle and humans appear to be the most important tsetse hosts in the region and should be considered in the design of interventions.

Background

African trypanosomes, members of the genus *Trypanosoma*, are flagellated parasitic protozoa that cause zoonotic diseases generally known as African trypanosomiasis. The diseases are known as sleeping sickness (or human African trypanosomiasis, HAT) and Nagana (or animal African trypanosomiasis, AAT) and are inextricably linked in humans and livestock, respectively. HAT still remains one of the most devastating neglected tropical diseases of poverty that causes severe morbidity and mortality, with over 70 million people at risk of being infected in Sub-Saharan Africa [1]. AAT on the other hand is a major constraint to livestock production causing massive economic burden in sub-Saharan Africa. The Food and Agricultural Organization of the United Nations (FAO) estimates that Africa loses up to US$1.5 billion annually as a result of the disease [2]. Thus, these diseases together, have both negative health and economic impacts.

In Uganda, vector-borne diseases, notably AAT presents a major constraint to livestock productivity [3,4]. Poor livestock health as a result of AAT denies farmers draught power and manure thereby contributing to poverty and hunger in the tsetse-infested areas [5,6]. Regarding HAT, despite the current small number of cases, Uganda is the only country where both the chronic (caused by *T. b. gambiense*) and acute (caused by *T. b. rhodesiense*) forms of the disease occur. The *T. b. gambiense* form occurs in the northwestern corner of the country (where this study was conducted) while *T. b. rhodesiense* is in the Eastern and Southern part of Uganda. Evidence already point to a danger of merger of the two HAT belts, fueled by animal movements [7–9], and vector migration northwards [10–12]. This underscores the need for research geared towards providing information to support sustainable control in the country.

Despite the existence of various drugs effective against AAT, chemoprophylaxis and treatment are expensive and usually ineffective, besides it is impossible to treat all wild and domestic hosts [13]. Furthermore, improper use of therapeutic drugs has led to the emergence of drug resistance in animal trypanosomes [14,15]. Vector control therefore offers a more viable strategy to disease management [16,17] and it remains the only available strategy capable of protecting human individuals from acquiring infection [13]. However, effective vector control requires reliable and accurate data on distribution, trypanosome infection status and preferred hosts of the vectors. Therefore, in this study, we assessed the preferred hosts of tsetse (blood meals sources), distribution of trypanosomes and trypanosome infection rates of tsetse flies in the West Nile districts of Adjumani and Moyo. We used the mitochondrial cytochrome c oxidase 1 (COI) and cytochrome b (cyt b) gene sequences for identification of tsetse fly blood meal sources collected from tsetse midguts. We also detected trypanosomes species in tsetse using a nested PCR protocol based on primers amplifying the ITS region of ribosomal DNA.

Methods

Study sites and sample collection

The study was carried out in Adjumani (3.3784° N, 31.7822° E) and Moyo districts (3.6527° N, 31.7281° E) in northwestern Uganda (Fig. 1). Northwestern Uganda has traditionally been known as an endemic sleeping sickness focus but recent surveys show that there has been a decrease in new cases of HAT [18]. Regarding AAT, there are numerous confirmed and anecdotal reports of the presence of the disease among cattle keeping households. North-western Uganda has two rainfall periods that run from March-May and from July-November, with a short dry spell in June and a fairly long period of dryness from December to February. The vegetation is a mixture of forests and savannah, with open woodland, grassland, and shrubbery. Besides, there are several fast running streams passing through subsistence farms with low plains and rolling hills and valleys that slope towards the river Nile. The typical riverine habitats are suitable for *Glossina fuscipes fuscipes*, the principal tsetse vector in this area. The population is largely rural, practicing mixed crop and livestock farming, consisting of food and cash crops such as tobacco, and livestock, mainly cattle, goats, sheep and pigs.

[Figure 1]

Tsetse trapping was conducted using biconical traps [19] baited with acetone and cow urine, deployed along suitable habitats, targeting majorly areas of human and animal activities (Fig. 2). At each site, an average of 10 traps were mounted approximately 100 m apart in different vegetation types for three consecutive days and in well shaded areas to minimize fly mortality due to excessive heat. The traps were deployed the villages of Olwi, Olobo, Oringya, Osugo East and Pagirinya in Adjumani district, and in Orubakulem, Ori, Lea, Cefo and Moyipi villages in Moyo district. The geographical coordinate of each trap was recorded using a handheld Global Positioning Systems (GPS) unit (GPS 12 XL, Garmin Ltd. 2003, Olathe, Kansas, USA). We also captured information on trap code, sex of the captured flies and dates of collection. To prevent the attack of ants on the flies in the traps, each supporting pole was smeared with grease.

Trapped tsetse flies were collected every 24 hours for at least three consecutive days [20]. After each collection, tsetse flies were identified morphologically, counted and sorted into teneral and non-teneral as described by Laveissière et al. [21]. The tsetse flies were assigned to one of the six age categories, according to the degree of wear or fraying observed on the hind margin of the wing as described by Jackson [22]. After categorizing the wing fray (WF), the actual age of the fly was estimated using directions for estimating the mean age of a sample of tsetse flies as outlined in the FAO Training manual for tsetse control personnel [23]. The ages of tsetse flies based on wing fray categories were later pooled as “young tsetse” (WF1-2), “old tsetse” (WF 3-4) and “very old tsetse” (WF 5-6) for statistical analysis. Only fresh and non-teneral flies were then selected for dissection of midguts, salivary glands and proboscis. The teneral flies are newly emerged individuals that have not yet fed, so were excluded from the analyses. The dissections were carried out as described in the FAO Training Manual for Tsetse Control Personnel [23]. Trapped flies were dissected in phosphate buffered saline in 2% glucose under a dissecting microscope. Samples of midguts, salivary glands and proboscis from dissected flies were preserved in 70% ethanol in sealed eppendorf tubes until required for subsequent DNA extraction and PCR assays.

[Figure 2]

DNA extraction

The ethanol preserved tissues (miguts, proboscis and salivary glands) of each tsetse sample was pooled together in a single tube and genomic DNA extracted using Purelink^{TM} extraction kit from Invitrogen following the manufacturer’s instructions. The supernatant was used either directly for PCR or stored at -20°C. Prior to their use or storage, DNA samples were electrophoresed in a 1% agarose gel in 0.5× TBE buffer at 100 V for 45 mins. The quality of DNA in the sample was then estimated by comparing florescent yield of

the sample with standard cut Lambda DNA run alongside the DNA samples. Spectrophotometry (Nano-Drop Technologies, Wilmington, USA) was used for checking the purity of DNA extracted, with the optical density (OD) taken at 260nm and 280nm, and a ratio 260: 280 (260nm: 280nm) was calculated to provide an estimate of the purity of nucleic acids.

Identification of different trypanosome species

To detect trypanosome DNA, we employed the nested PCR protocol described by Cox et al., [24], using the same primer sequences but with slight modifications in amplification conditions. The outer primer sequences were ITS1 (5-GAT TAC GTC CCT GCCATT TG-3), and ITS2 (5-TTG TTC GCT ATC GGTCTT CC-3) and inner primer sequences ITS3 (5-GGA AGC AAA AGT CGT AACAAG G-3), and ITS4 (5-TGT TTT CTT TTC CTCCGC TG-3). All primers were obtained from the Bioneer Corporation. PCR amplifications were performed in two PCR rounds. The first round was performed in a final reaction volume of 20 µL containing 10pmol of each primer, the Bioneer*AccuPower®* PCR premix (Bioneer Corporation), and 2µL of each DNA template. The amplification conditions began with 1 cycle of denaturation at 95 °C for 5min followed by 40 amplification cycles at 94 °C for 1min, 55 °C for 1min, and 72 °C for 2minutes. In the second round, 2µL of the amplified product from the first round was placed in a fresh tube and 20µL of the reaction mixture was added as described above for the outer primers, except that the outer primers (ITS1 and 2) were substituted with the inner primers (ITS3 and 4). The amplifications conditions were identical to the one described for the first PCR round. To minimize bias due to false positives during repeated PCRs, negative controls in which DNA templates were replaced with sterile distilled water as well as positive control DNAs (of each trypanosome species) were included in all PCR reactions. All reactions were carried out in a highly sterilized and dedicated PCR diagnostics lab environment of the BecA-ILRI Hub laboratories with a GeneAmp 9700 thermal cycler PCR system (Applied Biosystems). After the nested PCR, 5µL of the amplified products were loaded on a casted 1.8% agarose gel, which was subsequently stained with a Gel Red nucleic acid stain, with a 75bp gene marker. These were run in a Mupid®-exu Sub-marine electrophoresis gel tank (Helix Technologies Inc. MEXO 0800137) for 45 mins at 100V in 0.5X TBE buffer. The gels were then visualized under ultra-violet illumination and photographed. Trypanosomes species and subspecies were identified by comparing the molecular sizes of their DNA fragments with the documented band sizes of trypanosome species [24]. For *T. brucei*, further investigation was done by running a second PCR for diagnosis of *T. b. gambiense* employing a nested-PCR with a first reaction using TgsGP1/2 primers [25] and a second one with TgsGP sense2/antisense2 primers described by Morrison et al., [26].

Identification of tsetse blood meal sources

To test for the presence of DNA from the blood of vertebrate hosts, samples of DNA from residual blood meal in tsetse midguts were amplified using PCR with universal primers complementary to the conserved region of mitochondrial DNA (mtDNA) CO1 and cytb gene as detailed in Muturi et al. [27]. The primer sequences for the CO1 gene was VF1d_t1 (5’ TGTAAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG- 3’) and VR1d_t1 (5’-

CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCRAARAAYCA- 3') and for the Cyt b was (Cb5'-CCATCCAACATCTCAGCATGATGAAA-3') and (Cb2 5'-CCCCTCAGAATGATATTTGTCCTCA-3') as described in Ivanova et al.,[28] and Kocher et al., [29], respectively. Both PCR reactions were performed in a total volume of 20μl containing 10pmol of each primer, 10 mMTris-Cl, pH 8.3 and 50 mMKCl, 1.5 mM $MgCl_2$, 2.5 mMdNTPs, 2μL of the DNA template and 1unit DreamTaq™ DNA polymerase (Fermentas Life Sciences). The PCR was then carried out in a GeneAmpPCR System 9700 (Applied Biosystems) thermocycler. The conditions for CO1 PCR were as follows: initial denaturation at 94°C for 5 minutes, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 minute, and primer extension at 72°C for 30 seconds. For Cyt b PCR, conditions were: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 sec, and primer extension at 72°C for 30 s. Positive and negative controls were included in each PCR assay. The positive controls were cattle genomic DNA obtained from the Animal Health Unit at ILRI Research Laboratories. Negative controls consisted of the Master Mix for each PCR and fresh Milli Q water obtained from the Central Core of the BeCA hub laboratories. Five microlitres of the amplified products were loaded into a casted 1.8% agarose gel stained with gel red stain, with a 75bp gene marker, positive controls and negative controls. These were run in either a Mupid®-exu Sub-marine electrophoresis (Helix Technologies Inc. MEXO 0800137) or BIO-RAD Sub-cell®GT 9204355RevA gel tanks for 45 mins and 1 hour respectively at 100V in 0.5X TBE buffer. The amplified products were visualized under ultra-violet illumination and a picture of the gel taken using the gel documentation and analysis systems mounted with a high-performance CCD Camera-COHU. The bands were then used to confirm the amplicon size of the CO1 and cyt b genes.

The PCR products were gel-purified using the GeneJet™ kit (catalog no K0702 EU) following the manufacturer's instructions and submitted to the BeCA-ILRI Hub Sequencing Unit (Segolip) for sequencing using Bigdye™ Terminator. Sequencing was done bi-directionally using the inner amplification primers for the CO1 and cyt b genes. Consensus sequences were then generated by contiguation functions of the CLC Main Workbench Version 6.6.2 and manually edited, where necessary, by reference to the chromatograms. Vertebrate species were confirmed by sequence alignments with those already deposited in GenBank database using the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) [30]. Sequences of a given pair-wise alignment from positive PCR products with high percentage similarity (identity matches of 90-98%) and lowest E- values were selected as the most likely species of host.

Data analyses

The average Apparent density (AD), expressed as the average number of flies caught per trap per day (flies/ trap/ day or FTD), was calculated to obtain the data on tsetse distribution in the area for each trapping site using the formula: $FTD=\Sigma F/T\times D$, where, ΣF is the total number of tsetse flies caught, T is the number of traps deployed and D is the number of days of trapping in the field. Tsetse infection rates were calculated by dividing the number of flies infected with trypanosomes by the total number of flies analysed, and expressed as percentages. Pearson chi-square goodness of-fit-tests (χ^2) were employed to determine the association of tsetse infection rates with fly's sex, district of origin, and age based on wing fray categories. Values of p-

value < 0.05 were considered significant at 95% confidence interval (CI). Independent samples t-test was conducted to compare whether the average number of flies caught per trap per day differed between the two districts under study. All statistical tests were performed using SPSS software (version 21.0.1, SPSS Inc., Chicago, IL, USA).

Results

Entomological survey

We caught 622 tsetse flies (269 males and 353 females) (Table 1) from 109 traps deployed in 10 villages (five in each district). Of these, 320 flies were caught from Adjumani and 302 from Moyo districts, respectively. All the tsetse flies caught belonged to the *Glossina fuscipes fuscipes* species. Apparent density (AD) ranged from 0.58 to 3.67 tsetse flies per trap per day for all villages. However, the average FTD in the two districts did not differ significantly (Independent samples t-test, t = 0.35, df = 8, p=0.458). The average age of flies caught in Adjumani district was 30 days (Mean Wing Fray Value, MWFV = 3.8) while for Moyo district was 25 days old (MWFV = 3.3) (Table 1).

[Table 1]

Trypanosome infection rates

We analysed 43% (272/622) of the collected tsetse samples for detection of trypanosomes. The overall infection rate was 10.67% (29/272). Trypanosome infection was not significantly associated with sex (χ^2 = 0.099, df = 1, p = 0.753) and age based on wing fray category (χ^2 = 5.001, df=2, 0.082). Furthermore, the trypanosome infection rate also showed no association with district (χ^2= 0.629, df = 1, p = 0.428) and village (χ^2= 9.252, df = 9, p = 0.414). In Adjumani district, the village with the highest infection rates was Olobo (18.3%), followed by Oringya (14.2%), Osugo East (11.4%) and Pagirinya (3.45%). In Moyo District, the highest infection rates were recorded in Orubakulem (11.67%), followed by Ori (6.25%) and Lea (10%). There was no infected fly in Cefo and Moyipi villages (Table 2).

[Table 2]

Molecular identification of trypanosomes species and sub-species

Out of the 29 infected tsetse flies found in both districts, *Trypanosoma vivax* was the most prevalent species accounting for 6.62.1% of the infections, followed by *T. congolense* (all three sub types Kilifi, Forest and Savanna; 2.57%), and *T. brucei* and *T. simiae* each at 0.73% (Table 2). The results of investigation for *T.*

brucei gambiense turned out negative, and since we did not expect the other Trypanozoons (*T. evansi* and *T. b rhodesiense*) to be present in this geographical area, we concluded that the subspecies was *T. brucei brucei*. There were two cases of mixed infections with *T. brucei brucei/T. vivax*. The overall prevalence of the different species and sub-species in the two districts is shown in Figure 3.

Blood meal identification

A total of 34 blood-engorged flies' midgut samples was analyzed to determine the sources of the blood meal. Amplification of the COI and *Cyt b* genes all gave a 650 bp and 359bp PCR products, respectively, on 1.8% agarose gels (Fig. 4).

[Figure 4]

Hosts were identified in 88.2% (30/34) analyzed samples, with sequence identities ranging from 96-100% in homology with reference sequences in Genbank. The four samples consistently amplified tsetse DNA, implying probably degraded blood meal DNA. Tsetse hosts identified included cattle (14/30), Humans (8/30), Nile monitor lizard (5/30), African mud turtle (2/30) and the African savanna elephant (1/30) (Table 3).

[Table 3]

Discussion

This study assessed tsetse fly populations in Adjumani and Moyo Districts in northwestern Uganda, a known African trypanosomiasis endemic focus. The only species of tsetse caught was *Glossina fuscipes fuscipes.* This is probably because of the nature of our sampling scheme that mainly targeted the edges of major rivers and streams as well as peri-domestic environments. *Glossina fuscipes fuscipes* is known to generally disperse along waterways, following riverbeds or the edges of gallery forests, where they are able to survive low humidity conditions during dry seasons [31–33]. Female flies were consistently higher than males in most traps in both Adjumani and Moyo Districts. This finding is similar to results of a survey in Mukono district, southeastern Uganda where the percentage of females was higher than that of males in all study months [34]. The high percentage of females than males caught by our traps could be due to the fact that there may have simply been more females than males in the study area, and thus our catches was representative of the sex ratio in the population [35]. Females are also known to be more prolific feeders; male flies feed every 3-4 days [36]whereas females take blood meals regularly due to their role in reproduction [37], which increases their chances of getting trapped as they seek for blood meals.

In the present work, we found no significant difference in apparent density between the two districts implying that the similarity in biotopes present favorable habitats for tsetse in both districts. The average AD obtained in this study is similar to the findings by Azabo et al., [38] in mid northern Uganda. However, it's lower than those reported in southeastern Uganda by Waiswa et al., [39]. This observed difference could be attributed to variations in the environmental and agro-ecological conditions and the season in the study areas. Northern and northwestern Uganda differ significantly in climate, especially in annual precipitation, from the southern part of the country [40]. Southern Uganda has a somewhat cooler climate and is less humid, with mean annual rainfall near Lake Victoria often exceeding 2100– 3000 mm; the high temperature varies by 2–3 °C over the year, with a mean daily high being around 26 °C. In the north and northwest, the rainfall is between 1000 and 2000 mm, and temperature varies by 5 °C over the year, with the mean daily high being 29 °C [41]. The presence of unique climatic background create different tsetse population dynamics, and may be responsible for the observed difference in AD.

Our results showed that 10.7% of tsetse flies in both Adjumani and Moyo Districts were infected by at least one trypanosome species. This figure is relatively high given that infection rates are known to be generally low in tsetse flies [42]. Other studies in Uganda have reported lower rates; Azabo et al., [38] got an infection rate of 5.6% in tsetse flies from mid northern Uganda districts of Lira, Apac and Kole. Waiswa et al., [39] got an even lower rate (1.55%) in southeastern Uganda, although their study was based on microscopic examination only. Our comparatively high infection rates can be explained by the fact that livestock owners in Adjumani and Moyo Districts are not keen on use of trypanocidal drugs due to high costs associated with purchase (Personal Communication, Mathias Dramwi, District Veterinary Officer Adjumani, 2019).

Our results also indicated that sex of fly had no significant association with infection rates. This contrast with the suggestion that fly sex appears to influence susceptibility to trypanosome infection [43–46]. Some authors suggest that female flies should have higher infection rates than males as they live longer than males and thus they have higher chances of getting infection[47]. Moreover, the presence of high number of females caught in this study might result in high population and probably high infection rate [48]. However, there have been instances where higher number of infections was associated with male flies [43,44,46]. Thus, the precise associations of trypanosome infection with sex remain unresolved. Trypanosome infection rates were also not correlated with age, contrary to the suggestion that older flies have higher risk of infection than their younger counterparts. This could be due our selection criteria wherein we left out all teneral (non-fed) and examined only relatively older flies. Our results of ageing using wing fray technique indicated a relatively old population of approximately 30 days in Adjumani district and 25 days in Moyo district, which could also explain the relatively high overall infection rate in the two districts.

The most common trypanosome species in tsetse flies in the two districts was *T. vivax*, accounting for 67% of the infected flies. Magona et al. [49] found similar results in studies of *Glossina fuscipes fuscipes* tsetse flies in Southeast Uganda. In this study, our traps caught only *Glossina fuscipes fuscipes*, for which better competence in the transmission of *T. vivax* has been reported compared to *G. pallidipes* [50]. *Trypanosoma vivax* is also known to have a shorter developmental life cycle and can be mechanically transmitted by haematopahgous insects, which may explain its predominance in the surveyed samples [51]. An earlier study by Angwech et al.[52] in Amuru and Nwoya districts, which border the study area in the present study, found that *T. vivax* was also the most predominant trypanosome species in cattle. Our results are also consistent with several other previous studies on tsetse and trypanosomes ecology in Uganda [39,53,54].

We did not find any evidence of human infective trypanosomes in the tsetse flies sampled, in agreement with a recent epidemiological data reporting no cases in the neighbouring Arua and Koboko districts [55], all of which are within the same gambiense focus. It is possible that the transmission of *T. b. gambiense* has been decisively suppressed and prevalence reduced to near elimination. The driver behind this unprecedented decline has probably been the large active and passive screening programme by Ugandan National Sleeping Sickness Programme and Medecins Sans Frontières (MSF) France between 1987 and 2002 [56]. In West Nile, the last large-scale active screening was undertaken by MSF Spain and the Ministry of Health (MoH) supported by WHO in 2010 and 2011[18]. However, more extensive screening in the vectors is paramount given our relatively small sample size, since it's known that the prevalence of gambiense HAT disease amongst wild tsetse populations is often extremely low [57–59].

In the present study, we used two genetic markers (COI and cytb) for identification of tsetse blood meal sources in Adjumani and Moyo. The COI gene has a higher taxonomic coverage in reference databases than the cyt b gene. However, *Cyt b* has been shown to be better able to detect the origin of blood meals in some insect vector e.g., female mosquitoes with digested blood in their abdomens [60], and therefore increases the accuracy of blood meal identification. Muturi et al., [27] consistently failed to amplify tsetse vertebrate host DNA from *Glossina swynnertoni* using the COI gene for flies caught in Serengeti. However, by complementing the analyses of the COI gene with the analyses of *Cyt b*, the authors were able to identify blood meal sources of the caught tsetse. Conversely, in the study of Pettersson et al.,[61], the authors used COI for blood meal analyses for samples that did not produce results with *Cyt b* primers in order to increase the opportunity for host identification, Therefore, where possible, the use of both markers for detection of blood meal sources in vertebrates is a logical application since the COI and *Cyt b* DNA barcoding regions from more species are being sequenced and submitted to public databases.

Sequence analyses showed various blood meal sources, with cattle and humans being the predominant hosts. *Glossina fuscipes fuscipes* is known to be a non-preferential feeder, with blood meals taken from the most available hosts [62]. However, in Adjumani and Moyo districts, tsetse mostly feed on humans and cattle

due to the fact that the environment is highly anthropized and wild animals are rare, except in Zoka forest, which has abundant wildlife [63], an area we did not sample. Another probable reason for the high cases of blood meal from cattle, and to some extent humans, is that this survey was done in the dry season when it is common that animals, especially cattle, are taken to the banks of rivers and streams to graze and drink water. As such, *Glossina fuscipes fuscipes*, a riverine species, gain access to the cattle and humans who attend to the animals and utilize the water for domestic and other uses. There was, however, one curious case of a blood meal taken from an elephant. This particular fly was trapped from Arinyapi sub-county in Adjumani district, which borders southern Sudan. Trans-boundary elephants especially from Nimule National Park in South Sudan that have on several occasions destroyed numerous acres of food crops belonging to hundreds of small-scale farmers in the two sub-counties of Arinyapi and Dzaipi [64] are seemingly potential hosts of *Glossina* tsetse. Results of blood meal analyses furthermore highlight the importance of reptiles, especially monitor lizards and African mud turtles, as important hosts for *Glossina fuscipes fuscipes* tsetse. Previous studies in southeastern Uganda [39,62,65] and on the shores of Lake Victoria in Kenya [66] identified monitor lizards as an important food source for tsetse. It has been proposed that the preference for reptiles may simply reflect an ecological 'concordance' between flies and lizards; both are cold-blooded and probably with similar diurnal pattern in the environment where they co-exist [66]. Reptiles are even suspected to be natural cryptic reservoirs of human infective trypanosomes [67].

Conclusion

The present study has shown a moderately high infection rate at 10.78%, with all infections attributed to trypanosome species that are causative agents of the animal disease only. The absence of detection of human infective trypanosomes in the tsetse flies indicates that HAT may not be so highly prevalent in this endemic focus anymore. However, this result has to be validated using large-scale passive and active seasonal entomological and human screening. Cattle and humans appear to be the most important tsetse blood meals sources in the region and should be considered in the design of tsetse and trypanosomiasis control interventions.

Abbreviations

AD: Apparent Density; COI: Cytochrome Oxidase I; FAO: Food and Agricultural Organization; HAT: Human African Trypanosomiasis; AAT: Animal African Trypanosomiasis; OD: Optical Density; PCR: Polymerase Chain Reaction; TgsGP: *Trypanosoma gambiense* specific Glycoprotein; WHO: World Health Organization

Declarations

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Authors’ contributions

RO1, RE and EAO conceived and designed the study. RO1, MN, and MM collected data and performed the lab analyses. RO1, RO2 performed initial analyses and wrote initial draft of manuscript. GMM, EN, RE, EAO, FAO, AH and GO critically revised the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The data supporting the conclusion of this study is available from the link: https://osf.io/jhtu3/quickfiles

Ethics approval and consent to participate

This research was approved by the Gulu University Institutional Review Board and by the Uganda National Council for Science and Technology.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1: Results of entomological surveys

District	Village	X	Y	No of traps	Male	Female	Total	FTD	MWFV
Adjumani	Olwi	3.149863	31.681045	10	10	18	28	0.93	3.8
	Osugo East	3.200765	31.722688	10	32	49	81	2.70	2.8
	Olobo	3.384375	32.011374	12	55	60	115	3.19	4.3
	Oringya	3.486135	32.010335	11	14	21	35	1.06	4.5
	Pagirinya	3.377809	31.994145	10	29	32	61	2.03	3.6
Moyo	Ori	3.647330	31.789970	10	10	29	39	1.30	4.2
	Orubakulem	3.691983	31.780385	13	64	79	143	3.67	3
	Lea	3.592517	31.606833	11	30	49	79	2.39	2.8
	Cefo	3.605500	31.651733	10	12	8	20	0.67	3.5
	Moyipi	3.637940	31.718250	12	13	8	21	0.58	3
Total				109	269	353	622		

*MWFV refers to Mean Wing Fray Value; X and Y refer to the values of X and Y coordinates of the villages

Table 2: Rate of trypanosome infection in the villages of Moyo and Adjumani districts, northwestern Uganda

District	Village	Total examined	Total infected	Infection rate (%)	*T. vivax*	*T. brucei*	*T. simiae*	*T. Congolense*
Adjumani	Olwi	12	0	0	0	0	0	0
	Osugo East	35	4	11.42	3	0	0	1
	Olobo	60	11	18.33	7	1	1	2
	Oringya	14	2	14.2	1	0	0	1
	Pagirinya	29	1	3.45	1	0	0	0
Moyo	Ori	16	1	6.25	1	0	0	0
	Orubakulem	60	7	11.67	3	1	1	2
	Lea	30	3	10	2	0	0	1
	Cefo	8	0	0	0	0	0	0
	Moyipi	8	0	0	0	0	0	0
Total		272	29	10.67	18 (6.62%)	2(0.73%)	2(0.73%)	7(2.57%)

Table 3: Details of identification and accession numbers for all reference sequences for COI and cytb gene sequences generated

Host species identified	N (n=30)	Closest match on BLAST - Genbank ID (COI)	Closest match on BLAST - Genbank ID (Cyt b)
Humans (Homo sapiens)	08	JF739540.1 JQ705025.1 JQ705660.1 JQ704657.1	AY509658 GU123040.1 HM036565 GU123040.1
Varanusniloticus	05	HQ219067.1 AB185327.1 HQ219069.1	ND
Cattle (Bos taurus)	14	JN817351 JN817314 JN817330 JN817351	EU365345 AY682374 EU365345
Pelusios chapini	02	HQ329729.1	U81347.1
African Savanah Elephant (Loxodonta africana)	01	AB443879.1	AY768855

*Data combine both cytb and COI, and GenBank accession numbers for representative sequences given, and where not determined indicated as ND.

Figures

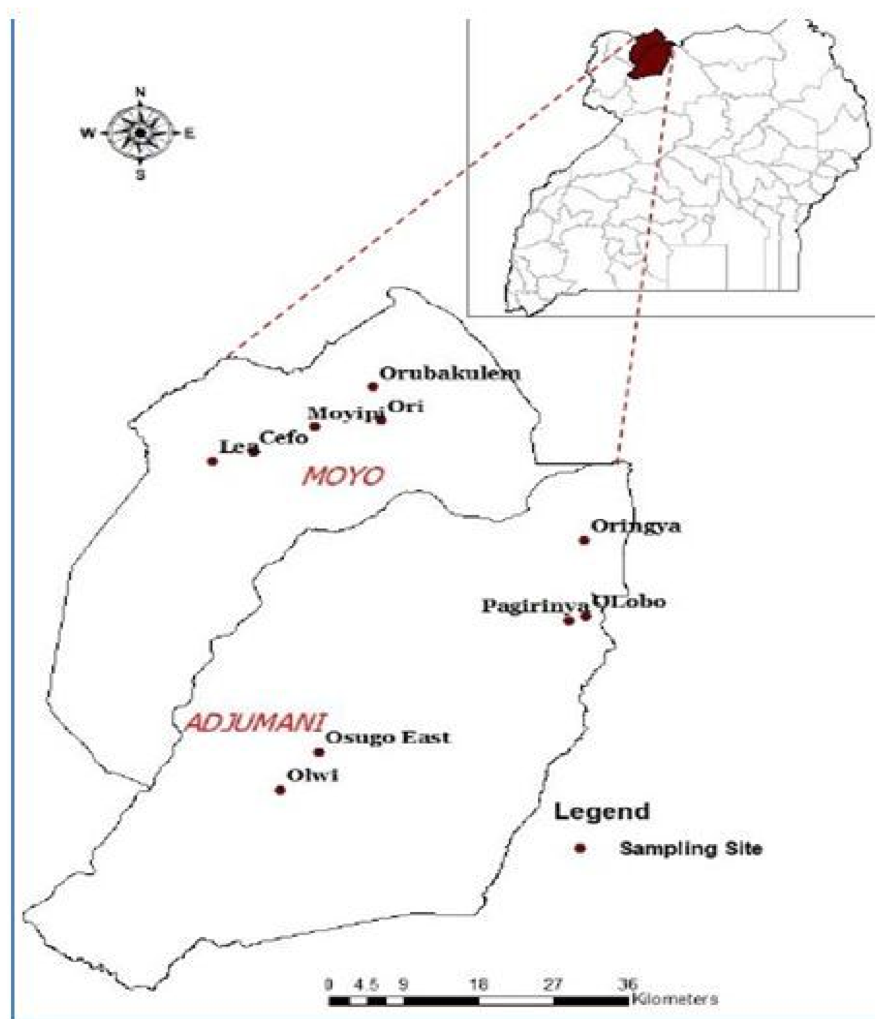

Figure 1

Map of study area showing sampled villages. Map was created by the authors using ArcGIS version 10.3.1.

Figure 2

A biconical trap baited with cow urine and acetone to enhance trapping efficiency deployed along a river in the study area.

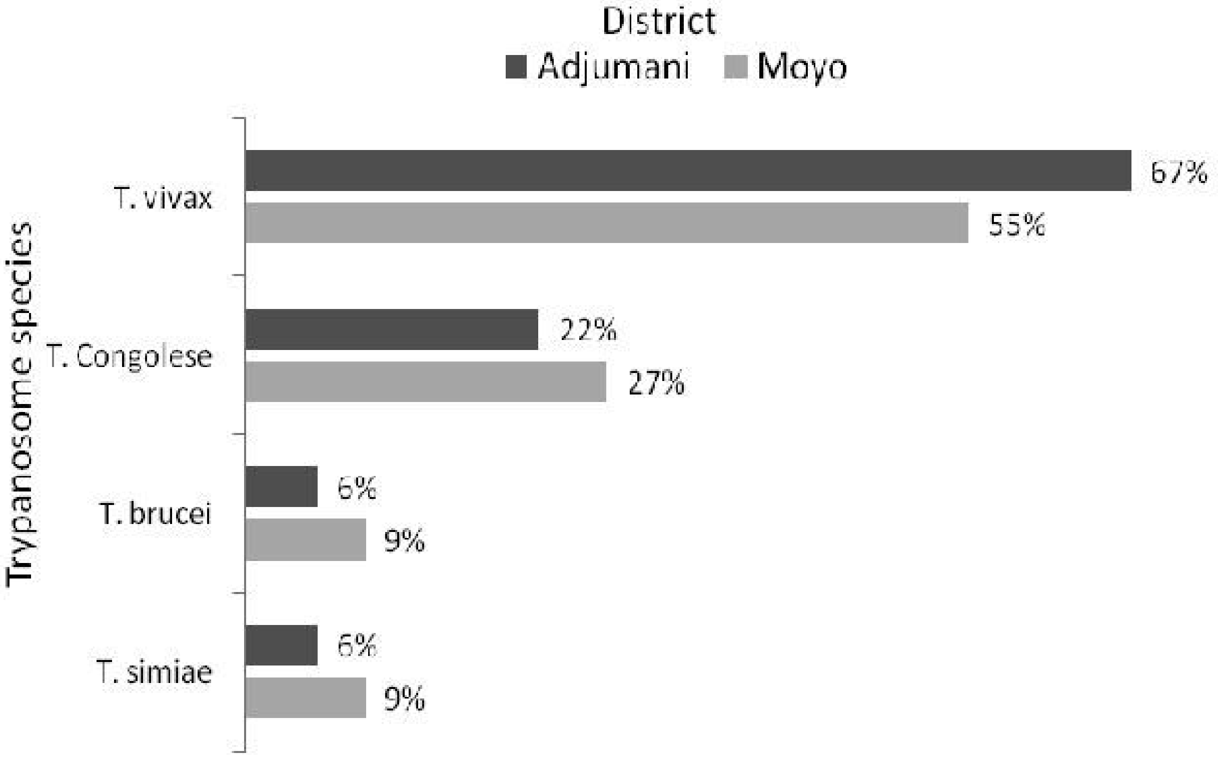

Figure 3

Overall prevalence of the different trypanosome species and subspecies in Moyo and Adjumani Districts, Northwestern Uganda.

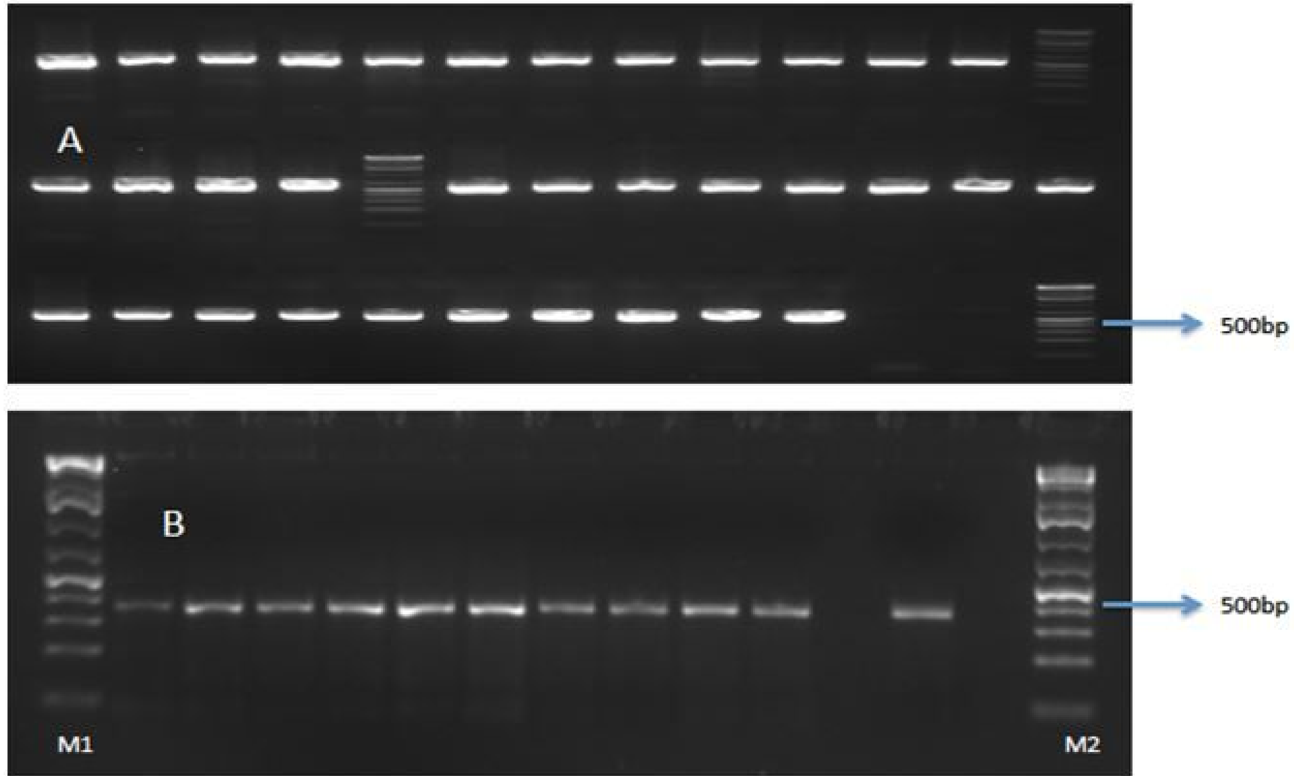

Figure 4

Representative gel photograph showing 650bp (A) and 359 bp (B) fragments size of COI and cyt b gene amplification products. Lanes M1, M2: 75 bp marker.