RESEARCH ARTICLE

Identification of human African Trypanosomiasis foci using school-going children in post-conflict era in Nwoya District, Northern Uganda: A cross-sectional study [version 1; peer review: 1 approved, 2 approved with reservations, 1 not approved]

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Abstract

Background: Human African Trypanosomiasis (HAT) is fatal if untreated; the drugs to treat it are toxic making its management difficult and diagnosis complex. Nwoya district has a long history of sleeping-sickness dating back to pre-colonial times. The civil war of 1986-2008 displaced many who upon return complained of cattle and dogs dying of unknown causes alongside increased tsetse flies infestation hence, the needs for the study.
Methods: We enrolled local 3,040 pupils and recorded their social-demographic characteristics and access to different domesticated animals/fowls in their homes. Screening for HAT using the card agglutination test for trypanosomiasis (CATT) was performed; positive individuals had their titres determined, followed by microscopy and loop mediated isothermal amplification analysis (LAMP). R was used for analysis where associations were sought between dependent and independent variables. Any factor with P-value <0.05 was taken as statistically significant.
Results: HAT serological prevalence of 1.2% (95% CI 0.8-1.6) was obtained, 58.3% being boys while 41.7% were girls with titres ranging from 1:2 - 1:16. Two schools alone, constituted 47% of the CATT positive cases.
Pupils who came from homes with dogs were more likely to be CATT/ *Trypanosoma brucei gambiense* positive; (adjusted odds ratio = 3.12, 95% CI 1.41-6.99 & p=0.005).
Conclusions: Though no parasites were detected, with prevalence of CATT positive at 1.2%, active surveillance in the district is still recommended. CATT positive cases needs follow-ups were immune trypanolysis test done to ascertain their exposure.

Keywords

HAT, CATT/T. b. gambiense, Northern Uganda, Nwoya District, LAMP

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Introduction

Human African Trypanosomiasis (HAT) commonly known as sleeping sickness is still active in over 30 sub-Saharan African countries[1], and found within the regions between latitude 14^0 North and 20^0 South in more than 250 active recognized different foci[2]. If left untreated, it may lead to death[3–7]. The etiologic agents are; *Trypanosoma brucei rhodesiense,* the East African type that runs an acute course, and *T. b. gambiense,* the West African type that runs a chronic course[4,8]. The two species, unfortunately are morphologically undistinguishable, with characteristically different epidemiological features and drugs of treatment[9,10]. Up to 70 million people are at risk of this debilitating and fatal disease with 30,000 individuals estimated to be infected[11]. Since 2010, *T. b. gambiense* was still endemic in at least 24 countries in Africa, and accounted for over 95% of HAT reported cases[2,7,12].

Uganda is the only country that harbours both species[1,13], with *T. b. rhodesiense* having afflicted the South, through Eastern into mid-northern Uganda, while *T. b. gambiense* is endemic in North-West Nile districts and part of the Amuru district[12].

In Northern Uganda, HAT was the main cause of relocations, a policy of compulsory evacuation of the natives from the affected areas[4,14], an action that remained in force until the early 1950's[15]. Large swaths of land, especially in Anaka and Purongo sub-counties, had to be bush cleared in an attempt to rid the vectors of suitable habitation (The Monitor Newspaper of 22nd Nov. 1999). Tsetse Control Camps were permanently maintained in several places that bordered the Murchison National Game Park and East Madi game reserve up to the period just before the northern insurgency in 1986.

Over two decades of civil war (1986–2008) led to mass displacement of people leading to total breakdown of social and physical infrastructures[16]. This same region is reported to be a potential site for a possible overlap of the two pathogenic *brucei* subspecies estimated to be within 160 km apart, and yet the so called frontier that divides the two seems to be only imaginary[1,2,17].

Our study was therefore designed to use primary school-going children to ascertain the prevalence of HAT, identify and map the current possible HAT foci, and to determine if there is any association among card agglutination test for trypanosomiasis (CATT) positive individuals and domestic animals/fowls kept at homes.

Methods

Study area

Nwoya district is located in mid-northern Uganda and lies within 02^038N, 32^0 00E covering 4,736.2 km^2 (1,736.2 sq miles) with a population of 54,000 people. Subsistence agriculture and livestock husbandry is the main economic activities besides tourism however; currently, there is steady increase in commercial agriculture according to Uganda Bureaus of Statistics, 2011.

The district is made up of 4 sub-counties (Koch Goma, Alero, Anaka and Purongo) in addition to Nwoya town council. Koch Goma and Purungo sub-counties form part of Murchison National Game Park; and thus are heavily infested with tsetse flies which act as vectors for sleeping sickness. In 2013, there were 44 Government-aided primary schools in the district with 39,632 pupils; 15,428 being boys while 14,436 were girls according to records obtained from district education office.

Study design

Our study was a cross-sectional survey; questionnaire was used to collect socio-demographic characteristics and risk factors while laboratory form was used for registering blood sample. The study was conducted in schools in sub-counties that form parts of the Murchison National Game Park and those closer to River Nile, were eligible for sampling. In total we purposively selected 19 Government-aided and 2 community schools since they are closer to the game park or river Nile which are foci for tsetse flies. In each school, we recruited pupils using consecutive sampling methods in each class. Pupils were drawn from primary three to primary seven, except for Gony-Cogo community school that had the whole school enrolled due to their small numbers and the children generally being more mature with one only who was 4 years old.

Data collection

Socio-demographic characteristics. Data on socio-demographic characteristics of each pupil; past history (e.g. places where they could have lived other than their homes), and the different animals/fowls kept at home were recorded. A Global Positioning System (GPS) was used to record locations of salient features; schools, sub-county headquarters, health units, district headquarter and homes of pupils who were found to be CATT/*T.b. gambiense* positive for easy follow-ups in the future.

Blood collection and preparation. At each selected schools, ethical procedures were observed e.g. explaining the purpose of the study and why they needed to participate although they would experience some slight pain during sample collection. After obtaining their assent, they were then enrolled by giving them identification numbers before going through the questionnaires to capture demographic information as well as animals/fowls kept at home. Finally, 2–3 ml of whole blood was collected aseptically following vein puncture in the *cubital fossa* into sterile plasma tubes 4.0 ml (BD, Franklin Lakes, NJ, USA) spray-coated with 60 USP (Units of Sodium Heparin) as an anticoagulant[18]. The blood was gently homogenized with the anticoagulant, 3% Phosphate Saline Glucose (PSG) was added to keep the trypanosomes active for a prolonged period. Samples were kept in cold boxes at temperature not exceeding 20^0C to avoid exposure to heat and direct sunlight.

CATT test and microscopy. Screening was performed using CATT/*T. b. gambiense* in accordance to the manufacturer's (ITM, Antwerp, Belgium) manual by diluting blood 1:2 in CATT buffer. Briefly, a drop of whole blood was mixed with a

corresponding amount of the reagent and rotated onto a flat orbital rotator for 5 minutes at 60 rpm. Both positive and negative controls were set along test samples and results read as positive, if there were visible agglutinations with the naked eye[19,20]. Titres were obtained by making twofold dilutions of 1:4, 1:8, 1:16 and 1:32 plasma in CATT buffer. 25 µl freshly reconstituted CATT/*T. b. gambiense* reagent (Institute of Tropical Medicine, Antwerp, Belgium) was added to each dilution, mixed and rocked for 5 minutes at 60 rpm. Titre was read as the highest dilution where visible agglutination was observed.

Wet preparations were made, mounted with cover slips 24 × 32 mm and examined using Olympus CX21 microscope under x20 and x40 magnifications and the results recorded. Thick blood smears of CATT positive samples were made, air dried, labeled and packaged into slide folders for eventual staining with *Giemsa* stain (SIGMA-ALDRICH®, Catalog No. GS) for laboratory examinations under oil immersion (x100).

Sample preparations & detection of repetitive insertion mobile element (RIME) using loop mediated isothermal amplification (LAMP). Homogenized whole blood from plasma tubes were sucked in heparinized capillary tubes sealed at one end with plasticine and spun at 800 x g (M24 Hematocrit centrifuge, LW Scientific) for 10 minutes to separate the different blood constituents. Buffy coats are located at the interface of packed cells on the lower end and serum at the top end. Using diamond pencil the tube is cut just above the packed cells and the Buffy coat was applied carefully onto labeled FTA® Classic Cards (Lot No. 5114552C, Whatman International Ltd, Maidstone, UK) beginning from the centre moving outwards within the circle, air dried, packaged in self-sealing plastic bags containing desiccants, and stored in a lockable cupboard/drawer for LAMP analysis.

Loopamp™ *Trypanosoma brucei* detection kit *version3.11* was used to carry out the analysis by following the most recently revised standard protocol[21,22]. Test sample was positive if florescence was present indicating the availability of trypanosome DNA and negative if there was no fluorescence[21–23]. Both positive and negative controls were checked to confirm the validity of the test before results were read and recorded.

Data management and analysis

Data were entered in *Microsoft Office Excel 2007*, exported to R *version 3.2.3* converted to comma delimitated (.csv) file, cleaned, edited and exported to STATA version 11 for analysis. Continuous variables were summarized using mean and categorical data were summarized in terms of frequencies and percentages. Univariate analysis was performed for both dependent and independent variables. Prevalence of HAT was obtained by dividing those who were CATT positive with the total number of pupils screened. Bivariate logistic regression was performed to determined association between the independent variables and presence of HAT. We reported Odds Ratio, 95% CI and P-value. Multivariate logistic regression analysis was used to assess for association between presence of HAT and the independent predictors. We run the stepwise logistic regression while adjusting for other independent variables in the model. We also adjusted for clustering of data around schools since the study was a survey. We calculated the adjusted Odds ratio, 95% confidence interval and P-value. Any variable with P-value ≤0.05 was taken as a significant predictor of HAT presence.

Ethical clearance

Ethical clearance (Ref. No. GU/IRC/01/11/11) was sought from Institutional Review Board of Gulu University. Study approval was granted by the Uganda National Council for Science and Technology (UNCST). The school management (Head teacher) was briefed about the study and the pupils were briefed about the study by the investigators. After thorough explanation in Acholi, the local language, the purpose of the study and the procedure involved, the pupils were given an informed consent form to take to their parent and return with it the following day when reporting to school before the study was conducted. The pupils who accepted to participate in the study signed an assent form. Pupils whose parents did not provide consent were excluded from the study and pupils who expressed fear of being pricked with needle even if their parent had consented and had first assented to participate in the study were excluded from the study, because that was a sign of withdrawal of assent. Children who were found to be CATT positive were followed home for a brief of their parents about the results and offer guidance where to seek treatment.

Results

A total of 3,040 pupils were enrolled for participation; 49.4% (n=1,501) were boys and 50.6% (n=1,539) were girls. The prevalence of CATT/*T. b. gambiense* positive was 1.2% (n=36), (95% CI 0.8 – 1.6%); 58.3% (n=21) were boys while 41.7% (n=15) were girls. All the 36 pupils had neither parasites detected in their blood by microscopy, nor did they show swollen lymph nodes on palpation. Table 1, below summarizes the results for schools surveyed.

Paraa P/S in Purongo and Lutuk Community School in Koch Goma sub-counties had the highest number of pupils who tested CATT positive with 9 and 8 respectively. Those with titre ¼ (moderate reactions) were 15 while those with strong reactions 1/8 and 1/16 were 12.

School enrolment is at 50–50 for girls and boys although those involved in the study where mainly in the age group 13–18 years (63%). Fowls/animals that are mostly kept at home in the district are chicken, goats, and dogs with pigs and cattle are steadily on the increase over the years (Table 2).

With the use of GPS; villages, schools, sub-counties, district headquarter, hospital/health centres and salient features such as district and sub-county boundaries, road networks and streams/rivers are located on the district map for ease of follow ups especially the CATT positive cases even by someone who has not been involved in the study (Table 3; Figure 1).

Of the 36 CATT/*T. b. gambiense* positive samples, 34 were subjected to LAMP and all of them were found to be negative. The results were as shown in Figure 2a and 2b.

Table 1. Primary schools surveyed in Nwoya district along with their CATT results and titres.

Sub-County	Primary Schools accessed	No. Screened	CATT +ve	TITRES			
				1/2	1/4	1/8	1/16
Koch Goma	Wlilacic	224	3	1	0	1	1
	Goro	96	0	0	0	0	0
	Koch Goma	211	1	0	1	0	0
	Koch Lii Pakiya	125	0	0	0	0	0
	Koch Lii	197	0	0	0	0	0
	Koch Lila	169	4	2	0	0	2
	Lutuk Community	128	8	2	3	2	1
	Gony Cogo Comm	62	1	0	1	0	0
Purongo	Got Apwoyo P/S	193	0	0	0	0	0
	Wii Anaka P/S	146	5	3	1	1	0
	Purongo P/S	186	1	0	0	0	1
	Paraa P/S	212	9	1	7	1	0
	Olwiyo P/S	128	1	0	1	0	0
	Purongo Hills P/S	219	1	0	0	0	1
	Aparanga P/S	149	0	0	0	0	0
Anaka	Agung P/S	142	1	0	0	1	0
	St Kizito Bidati P/S	159	1	0	1	0	0
Alero	Lulyango P/S	153	0	0	0	0	0
	Lungulu P/S	144	0	0	0	0	0
4	**19**	**3043**	**36**	**9**	**15**	**6**	**6**

Table 2. Socio-demographic characteristics of the pupils that participated in the study and the animals/fowls kept at home.

Variables	Frequency	Percentage
Sex		
Male	1,501	49
Female	1,539	51
Age groups		
4 – 12 Years	1,120	37
13 – 18 Years	1,921	63
Presence of dogs at home		
Yes	1,742	57
No	1,299	43
Presence of chicken at home		
Yes	2,811	92
No	230	8
Presence of ducks at home		
Yes	465	15
No	2,576	85
Presence of turkeys at home		
Yes	92	3
No	2,949	97
Presence of cattle at home		
Yes	925	30
No	2,116	70
Presence of goats at home		
Yes	2,482	82
No	559	18
Presence of sheep at home		
Yes	602	20
No	2,439	80
Presence of pigs at home		
Yes	771	25
No	2,270	75

Table 3. Crude Odds Ratio and Adjusted Odds Ratio for the predictor of being CATT positive.

Variables	c OR	95% CI	P-value	a OR	95% CI	P-value
Chicken	0.40	0.17 - 0.98	0.044	0.47	0.18 - 1.24	0.128
Ducks	0.69	0.24 - 1.96	0.486	0.64	0.22 - 1.90	0.423
Turkeys	1.91	0.45 - 8.05	0.381	2.59	0.56 - 11.87	0.221
Cattle	0.88	0.42 - 1.83	0.729	0.95	0.43 - 2.07	0.902
Goats	0.51	0.25 - 1.04	0.063	0.49	0.22 - 1.12	0.089
Sheep	0.98	0.43 - 2.24	0.958	1.09	0.46 - 2.62	0.843
Pigs	0.59	0.24 - 1.41	0.234	0.57	0.22 - 1.43	0.229
Dogs	**2.26**	**1.06 - 4.81**	**0.035**	**3.12**	**1.41 - 6.94**	**0.005**

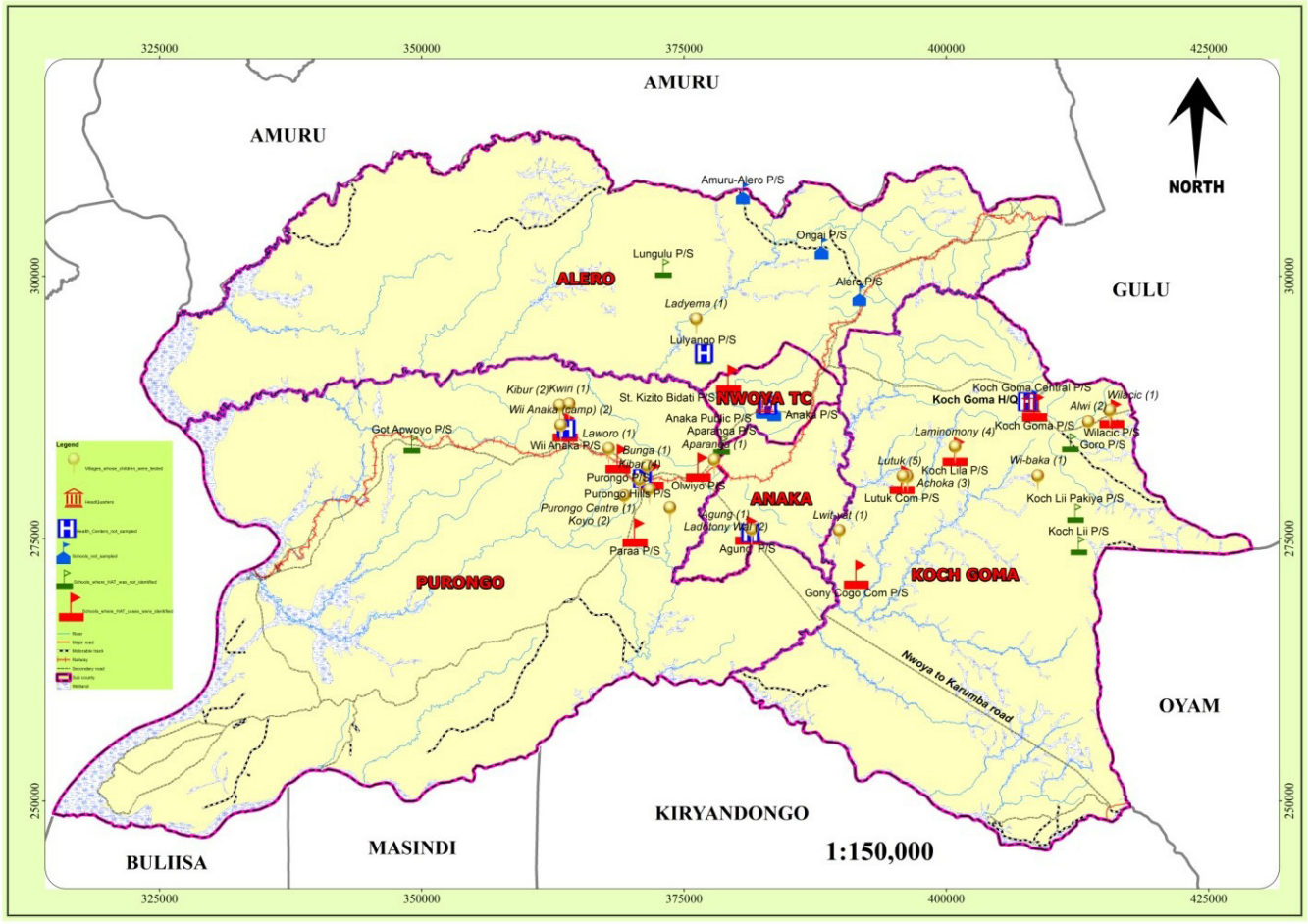

Figure 1. Map of Nwoya district showing sub-counties boundaries, schools accessed, and physical features such as rivers, roads, health units, and GPS locations of homes of CATT positive cases.

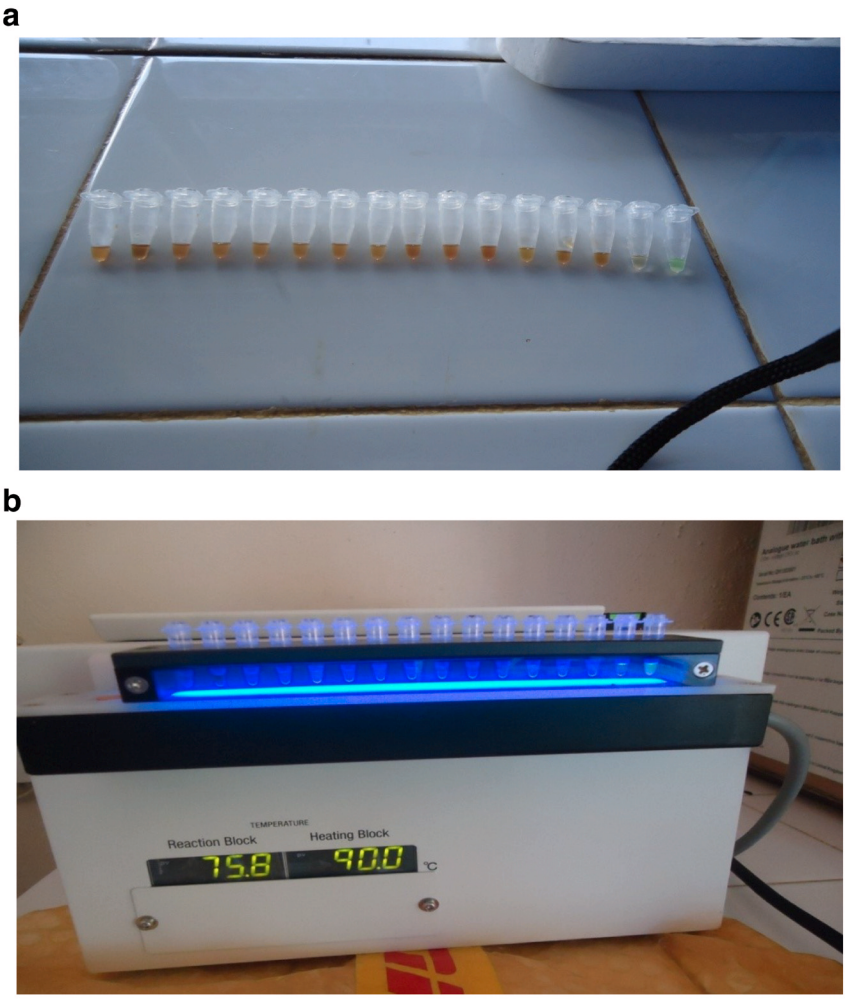

Figure 2. (**a**) Prepared tests samples set along with both negative and positive controls in reaction tubes ready for reading before being inserted in visualization chamber. Tubes labeled 1 to 14 (from left to right) are test samples; tube 15 is a negative control (colorless) and tube 16 is a positive control (Light green). (**b**) Picture showing test samples in a fluorescence unit of the LAMP incubator. Tubes 1 to 15 showing no fluorescence in the test and negative control fluids, whereas there was intense fluorescence in tube 16 (positive control) as seen to the naked eye.

Discussion

A study conducted in Taraba state, Nigeria screened n=400 using CATT/*T. b. gambiense* obtained a prevalence of 1.8% (7/400). Males tested more positive than their female counterparts[23] and the difference in the number of males and females who were CATT positive was statistically significant, p=0.05. Although this agrees with our study; males (21/36) being more CATT positive than females (15/36), the difference in the sexes was not statistically significance. Due to a small sample size of Taraba study, this could have influenced the prevalence causing a significant difference between the sexes in terms of infectivity. HAT is a disease that is related to behavioural risk factors through complex interactions; environmental and behavioural risk factors, vector and the human host[24].

Considering the fact that our subjects were school-going children, they were engaged in similar activities in disregard to their sexes and this could have been a key factor of having no statistical difference in the way the two sexes were exposed. In villages where girls go out in search of firewood in the park or forests, as seen in Laworo and Wii Anaka in Purongo sub-county, they got more exposed than the boys, whereas in areas where boys herd animals, go digging or playing in the fields, they were more exposed than girls, as observed in Alwi and Laminomony villages in Koch Goma sub-county. In some cases, pupils who travelled long distances leave schools nearby; Paraa Primary School (P/S)coming from Purongo trading centre, St Kizito Bidati P/S from Ladyema in Alero sub-county, Olwiyo P/S from Aparanga and Koch Goma P/S from

Wi-Baka. These pupils were likely to have been exposed along the way as they travelled to school; indicated by the fact pupils who attended schools nearby were CATT negative.

The validity of CATT results has been a subject of controversies in the wake of low endemicity since it's known to work well in endemic areas with prevalence above 5%[25]. In our study however, the distribution of pupils who were CATT positive had a very striking pattern; in most cases, where there were several positive cases arising from a particular school, the pupils tended to come from the same villages as was observed in the villages of Laminomony, Wii Anaka area, Lutuk, Kibar, Koyo and Alwi. In one scenario, 2 cases from Lutuk community school were siblings from the same homestead in Bargunya village. Lutuk had a tsetse control camp in the 1980's due to HAT outbreak then, these results could indicate that it is on the verge of HAT re-emergence once again. While Wii Anaka and Paraa are settlements close to Murchison National Game Park; a place known to be infested with tsetse flies. Like Lutuk, Wii Anaka also had a nearby tsetse control camp at Got Apwoyo that now lies in ruins. Our findings therefore, reconfirms that most of the CATT positive results were certainly not due to errors or cross-reactions as other previous authors have suggested[13], it's likely that these children could have been exposed, and either the parasites failed to be maintained or got neutralized by their strong immunity due to their long period of stay in this HAT endemic region[26]. It's also likely that over time *T. b. gambiense* could have under gone a lot of changes while in the human host; a study conducted in northern Uganda had found that 75% of those who were found to be CATT positive where parasitologically positive with careful wet preparations and thick blood smear microscopy[27]. Currently, numerous studies are reporting lots of aparasitaemic cases with gross variations in parasitaemia across foci[24,28]. Humans, like animals, are believed to possess trypanotolerance that protect them from the disease[29].

Cut-off titres at some points were used to define disease cases; 1:4 required parasitological confirmation whereas 1:16 was regarded as indication of infection that required treatment even without parasites being seen[13]. In our study the following CATT positive cases were detected; 1:2 (n=9), 1:4 (n=15), 1:8 (n=6), and 1:16 (n=6) (see Table 1). Our findings cast doubts on reliability using CATT titres, as high as 1:16 did not reveal presence of parasites or their DNA. In a related study that was done in 2012, a case from Onigo village, Miniki sub-county in Adjumani district that was CATT positive with a titre of 1:4 who was aparasitaemic at the time, turned out to be a stage 2 case after trypanosomes were recovered from his CSF within 2 weeks from the time CATT screening was performed [unpublished study, Luryama Moi K, Anywar D and Madra P].

Though it has now been proven that animals play a yet unclear role in the maintenance of *T. b. gambiense* even when they have been eliminated from the human population, explaining in parts the reason behind re-emergence of HAT[30]. In a study conducted in West Africa, out of 397 domestic animals sampled, 64% were CATT positive for *T. b. gambiense* and when PCR analysis was done, 15.4% of sheep, 11.6% of pigs, 3.5% of goats and a low number of dogs where confirmed as infected with HAT[31]. Meanwhile a study carried out in Cameroon did not find any *T. b. gambiense* infection in dogs; this could have been due to a small number of dogs sampled[32]. In our study however, we did not sample the animals but instead tried looking at possible associations of those pupils who were CATT positive and the domesticated animals. After analysis for possible associations we found that those who had dogs were more than 3 times at increased risk of being CATT positive; Adjusted OR 3.12, (95% CI 1.41-6.99 and p = 0.005). This relationship could be explained in two ways; dogs could be acting as a reservoir of *T. b. gambiense*, a threat to re-introduction into human population. The other explanation could be due to the fact that dogs are social animals that are routinely used for hunting and herding animals in this region, and it could simply mean that those with dogs spend more time outside and therefore at higher risk of exposure than those without dogs.

All our CATT positives turned out to be negative by LAMP even those with high titres of 1:16. Studies in one of the sleeping sickness treatment centre at Omugo Health Centre (level IV) located in Arua district, north-western Uganda where SD BIOLINE HAT™ (Alere Inc., Waltham, MA, USA) is currently used to test for *T. b. gambiense* VSG *LiTat* 1.3 and 1.5 antibodies found that; out of 72 SD BIOLINE positive, only 2 were positive RIME LAMP [unpublished study, Luryama Moi K and Louga A]. As suggested by Mitashi *et al.* (2013), we also recommend that LAMP may still require more evaluation studies before it's adopted as a gold standard in the diagnosis of *T. b. gambiense*.

Study limitations

This was a one off survey and therefore we cannot account for information on pupils who refuse to participate or were absent from school nor children who have dropout of schools. However, our sample was large enough to be representative of the general pupils' population in the schools in the sub-counties which form foci for tsetse fly.

The screening test used only targeted *T. b. gambiense* and as such making only 36 samples to qualify for testing using LAMP that detects both *T. b. gambiense* and *T. b. rhodesiense*. However, the information is good enough for baseline data upon which future research can build on.

Recommendations/conclusion

Pupils who were CATT/*T. b. gambiense* positive need a follow-up study for repeat CATT test, as well as performance of an Immune trypanolysis test to establish their infectivity status as the latter test seems to be more accurate. There is need to for active screening of populations using superior screening tests that combines both, *T. b. gambiense* and T. *b. rhodesiense* for regions that faces the threats of merger. Health workers at lower health facilities need sensitization and skill development in identifying cases with HAT. In conclusion, we confirm that the use of school-going children offers the most efficient means in the identification of HAT foci in places that accessibility is difficult or participation from the community is low.

Data availability

Data underlying the study are available on OSF: http://doi.org/10.17605/OSF.IO/DM7FT[33]

Competing interests

No competing interest were disclosed.

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Data Source

Open Peer Review

Current Peer Review Status: ✔ ? ✘ ?

Version 1

Reviewer Report 08 May 2018

https://doi.org/10.21956/aasopenres.13917.r26327

?

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The manuscript describes a school-based approach to investigate HAT. This approach (unlike in the case of diseases such as schistosomiasis) is not normally taken in HAT, perhaps because most cases affected are among the middle age-group as a consequence of longer-term exposure via occupational activities that bring them into contact with the Tsetse fly. Nevertheless, the authors have shown that taking a school-based approach, one is able to zero in on microfoci where transmission might be taking place.

In the background part of the abstract, it is indicated that cattle and dogs were dying of undiagnosed disease, in presence of heavy tsetse infestation. It would have been interesting to do a pilot sampling of these to show any evidence for trypanosomes as a basis to suspect possible presence of HAT. But dogs are known to be susceptible to *T. brucei* group and typically die of symptoms similar to those of late stage HAT, corneal opacity is also a common manifestation of Trypanosomiasis in dogs.

CATT is an antibody test, known to cross react with other parasite antigens. Thus false positives are expected, even when executed outside HAT endemic foci. However, its specificity inside HAT endemic areas is considerable high, making it a useful test to screen for potential HAT cases. The authors should make it clear that they investigated sero-prevalence rather than HAT prevalence since a CATT positive reaction does not exclusively point to HAT presence. There is also need to use generally accepted terminologies e.g. Their CATT 1/2 might be referring to CATT whole blood. Normally in screening we talk about CATT whole blood, CATT ¼, 1/8, and 1/16; 1:4 is not the same as 1/4. Also clarify that the wet smears were read under x20 and x40 objectives; the magnifications were x200 and 400 respectively. Noteworthy is that being an area at the periphery of a T. b. gambiense endemic districts, more sensitive parasitology such as the heamatocrit centrifugation technique (HCT) would have been more appropriate, given the characteristically lower parasiteamia of that subspecies in humans.

For LAMP, the authors give no details of how they processed template before running the test, so it is not clear if it was performed with the best possible template. LAMP is a good molecular test but can not be considered a gold standard. The HAT case definition set by the WHO remains demonstration of the parasite itself in blood, lymph node aspirates, or cerebrospinal fluids; these comprise the so-called

"composite reference standard". The authors could also have considered PCR alongside, to show if any signals indicative of T. brucei would have been obtained.

In the discussion, please refer to seropositive individuals as suspects rather than cases.

The major arguments in the discussion regarding trypanotolerance would hold water, however in absence of any evidence for human infective trypanosomes circulating in humans, animal reservoirs or vectors in Nwoya, they remain very highly speculative. The only pointer to HAT is the CATT seropositivity whose results alone remain far from conclusive (it is a screening test that must be follwed by confirmatory parasitology); more techniques need to be deployed in the area in order to make more valid conclusions.

Other minor comments: use either "North west Uganda" or "West-Nile" districts. The words "were" and "where" have been interchangeably used, please rectify. Under recommendations, TL would help establish "infection", not "infectivity" status.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology, HAT surveillance and epidemiology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 08 May 2018

https://doi.org/10.21956/aasopenres.13917.r26363

José R. Franco
Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, World Health Organization, Geneva, Switzerland

There are important conceptual misunderstandings in the manuscript reviewed that can give a wrong impression about the epidemiological situation of HAT in the area studied.

1. The CATT is a serological test used for screening and therefore with a specificity limited and well described cross reactions (false positives), as for instance with transient infections by nonhuman trypanosomes[1]. This could be the case in the studied area. In areas of very low or zero HAT prevalence, the predictive positive value of the test is very low and the presence of CATT seropositives does not give any indication about the presence of HAT. No trypanosome was observed in the serological suspects after parasitological test, and therefore with the data presented it is not possible to infer that there is a presence or risk of the disease in the area. To talk than about “HAT serological prevalence” is confusing and wrong (The authors define “Prevalence of HAT” as CATT positive individuals divided by screened population). The statement indicating that these CATT results are not due to errors or cross reactions is not adequately justified.

2. Nwoya district was affected in the beginning of 20th century by gambiense sleeping sickness[2]. In different outbreaks in the mid-20th century, the distribution of cases did not reach the area of Nwoya (Jonan and Okoro districts)[3]. At the end of the last century (from 80’s), an important outbreak of gambiense HAT was described in West Nile[4], but no cases of gambiense HAT have been diagnosed in the area of Nwoya since that time[5,6,7].

3. Sporadic cases of rhodesiense have been diagnosed in tourists visiting the neighbouring National Park of Murchinson Falls but these cases are due to *T. b. rhodesiense* (e.g. http://www.nicd.ac.za/assets/files/NICD-NHLS%20Communicable%20Disease%20Communiqu%C) and linked to the presence of *T.b rhodesiense* in wild animals. To investigate local populations with gambiense CATT would not have any interest to asses’ rhodesiense HAT infections, which could happen in the studied area. At the same time primary school children are not a group at high risk for rhodesiense HAT, usually linked with activities related to wild animals and people entering in the protected areas (rangers, poachers, tourist guides, herders, honey and firewood collectors,…).

4. It is important to clearly distinguish between gambiense and rhodesiense HAT and also to underline that the presence of tsetse fly and even animal trypanosomiasis does not implies the presence of human African trypanosomiasis.

    1. The presence of animal trypanosomiasis in the area is well-known[8].

    2. The presence of *T.b gambiense* has been described in different animals but the epidemiological role that animals can play as reservoir for gambiense HAT is not clear[9].

5. There would be an ethical concern as if the authors consider the CATT seropositives as possible cases and no action has been taken to follow them up to confirm the presence or not of a disease that is considered as lethal.

The authors confirm “that the use of school children offers the most efficient means in the identification of

HAT foci in places that accessibility is difficult or participation from the community is low". According to the existing epidemiological data, the district of Nwoya cannot be currently considered as a HAT focus. The data presented in the paper (some children presenting CATT positive reactions but no presence of trypanosomes after parasitological tests) does not allow considering the area as a focus with active transmission of HAT.

The study tries to ascertain the prevalence of HAT using primary school children, and the conclusion should be that just based in some CATT positive results, it is not possible to conclude that there is transmission of HAT in the area.

It is not clear if authors want to assess the situation of gambiense HAT or rhodesiense HAT, and it would be important to clarify it.

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Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 30 April 2018

https://doi.org/10.21956/aasopenres.13917.r26362

?

Mike Brown
North Central London South Hub TB Clinic, Whittington Health, London, UK

This is a useful surveillance study covering an important region, from which there have been few data published in the last 15 years. It addresses an important public health issue which is the potential role of domestic livestock in limiting the effectiveness of HAT elimination programmes. The methodology used was good, and the analyses and conclusions fair.

I have the following comments covering sections that would need major revision.

1. The introduction, while providing some background on HAT in Uganda, is not sufficiently focused on the question of interest which is the role of domestic animals in HAT transmission. It needs to be shortened and re-focused on this topic.

2. There is little data here on current knowledge (or lack thereof) of active case finding studies in this area i.e. is the current study embedded within an existing surveillance programme or is there no current active case finding there, and what did prev data show? What does passive case finding data demonstrate to justify looking in this school-age population to answer the question around domestic livestock. Why schoolage children?

3. It does not follow, to the reader, why "near Murchison.....thus are heavily infected....". Please clarify

4. What was delay from sample collection to wet microscopy?

5. What was access to treatment? It would be unethical to embark on this screening strategy without positive participants being able to access treatment. A short sentence describing treatment provision in the area would be appropriate.

6. Paragraph 4 of results. It is unclear what this has to do with results. I do not understand the paragraph.

7. It is unclear what potential explanatory factors were analysed in univariate analyses, that were then included in multivariate model. Were there measures of socioeconomic status? Were all the different animals included in the model? if so why? i.e. what is the logic of adjusting for e.g. chickens in exploring relationship between dogs and CATT positivity? It looks like this has been done but am not sure it is appropriate. If you collected GPS data shouldn't you have adjust for e.g. distance from Murchison or from Nile?

8. In the discussion I think the authors need to convince more fully that CATT positivity meant current HAT infection. None of the positive subjects had any other tests to support the diagnosis, and a serological test is poor evidence in itself of infection, esp in a setting where people may be more exposed to other infections that might drive hypergammaglobulinaema e.g. malaria, HIV, etc. I think that the real story in this paper is (A) domestic livestock have been shown to harbour HAT and this may have a role in HAT transmission. (B) in an area where there has historically been a strong HAT public health problem and despite control programmes and treatment access this has not completely disappeared, domestic livestock could be a reason for ongoing infection (C) we looked for associations between potential HAT infection/exposure as measured by CATT and domestic animals (D) we found an association esp with dogs BUT (E) no patients were parasitaemic and so there are limitations on whether these were true cases and (F) most cases were found in wards closest to large tsetse populations - dogs/etc may not have been on the causal pathway but may reflect confounding by activities such as hunting, socioeconomic status, etc.

9. There is a publication in Vet Parasitol 2012 (Balyeidhusa et al) showing no PCR evidence of human tryps in animals in NW Uganda. This has not been referenced or discussed.

10. PCR is a tool increasingly used (see studies by Jammoneau et al for example) to detect true infection in aparasitaemic CATT positive patients. Authors might recommend this technique in their concluding remarks.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 30 April 2018

https://doi.org/10.21956/aasopenres.13917.r26364

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Abstract

Conclusions (under Abstract): Pg 1
"CATT positive cases needs follow-ups were immune trypanolysis test done to ascertain their exposure"
This sentence is unclear, thus re-write to correct grammatical mistakes.

Keywords: Pg 1
CATT/T. b. gambiense = Follow rules when writing a scientific name. The genus and species names are always italicized; *T. b. gambiense*

Introduction: Pg 3
1st paragraph, 3rd sentence- the word 'runs' is repeated two times in the same sentence. In the 4th sentence, substitute *'undistinguishable'* with the more commonly used *'indistinguishable'*
3rd paragraph, 1st sentence; In Northern Uganda… = In northern Uganda…

Methods: Pg 3
Study area
Re-write the second sentence in the 1st paragraph;
Subsistence agriculture and livestock husbandry is the main economic activities besides tourism however; currently, there is steady increase in commercial agriculture according to Uganda Bureaus of Statistics, 2011.

Study design: Pg 3
1st sentence on 1st paragraph;
Rephrase the following sentence

Our study was a cross-sectional survey; [revise usage of semi-colon] questionnaire was used to collect socio-demographic characteristics [data] and risk factors[,] while laboratory form was used for registering blood sample [laboratory forms were used to record the details of collected blood samples].

In the 2nd & 3rd sentences, include punctuation marks (e.g. commas) where appropriate ['In total [insert a comma] we…'], in another case, a comma was wrongly inserted into the sentence (see '… and those closer to River Nile, [delete comma] were eligible…').

Last sentence under study design;
Pupils were drawn from primary three to primary seven, except for Gony-Cogo community school that had the whole school enrolled due to their small numbers and the children generally being more mature with one only who was 4 years old.

Question;
How did you define maturity? What age bracket is mature? This statement on maturity is subjective.

Data collection: Pg 3
Socio-demographic characteristics.[:] Data on socio-demographic characteristics of [delete to avoid repetition]; past history (e.g. places where they could have lived other than their homes [*insert*: within the study sites]), and the different [domestic] animals/fowls kept at home were recorded [*insert:* for each pupil].

Blood collection and preparation
Rephrase the following statement;
"After obtaining their assent, they were then enrolled by giving [= assigning] them [= unique] identification numbers before going through the questionnaires to capture demographic information as well as animals/fowls kept at home" [This is repetition, see the above sub-title on socio-demographic characteristics]

2nd sentence
"…whole blood was collected aseptically following vein puncture in the cubital fossa into sterile [insert=4.0 ml] plasma tubes (BD, Franklin Lakes, NJ, USA)…"

CATT test and microscopy
Screening was performed using CATT/*T. b. gambiense* in accordance to [= with] the manufacturer's (ITM, Antwerp, Belgium) manual by diluting blood 1:2 in CATT buffer (ITM, Antwerp, Belgium).
Comment: By using CATT/*T. b. gambiense* test alone, the authors seem to have overlooked the possibility of *T. b. rhodesiense* infections in the study area, even though past history of participants living in other regions was recorded. Yet they clearly state that "the so called frontier that divides the two pathogenic *brucei* subspecies separated by a distance of about 160km in northern Uganda seems to be only imaginary" This is supported by recent findings by Opiro et al., 2017.
- Does CATT/*T. b. gambiense* kit detect *T. b. gambiense* only?
Based on the findings of LAMP assay used in this study, the authors have raised doubts on its sensitivity to detect *T. b. gambiense* and *T. b. gambiense.*

2nd sentence
"…corresponding amount of the reagent and rotated onto [= on] a flat orbital rotator for 5 minutes at 60 rpm"

3rd sentence, on page 4
"Both positive and negative controls were set along [=alongside] test samples and results read as positive, if there were visible agglutinations with the naked eye" *rephrase the statement to make it succinct, i.e if agglutinations were observed.*

Last sentence of 1st paragraph;
"Titre was read as the highest dilution where visible agglutination [Delete the word 'visible'] was observed"

Next paragraph, 1st sentence; page 4
Wet preparations [of what?] were made, mounted with cover slips 24 × 32 mm and examined using Olympus CX21 microscope under x20 [= 20×] and x40 [= 40×] magnifications and the results recorded.

2nd sentence (page 4)
"…for laboratory examinations under oil immersion (x100)" rephrase as "…for laboratory examinations under oil immersion using 100x objective"

Sample preparations & detection of repetitive insertion mobile element (RIME) using loop mediated isothermal amplification (LAMP)
1st sentence, page 4
"Homogenized whole blood from plasma tubes were sucked in heparinized capillary tubes sealed at one end…"

- How was homogenization of blood conducted?
- This blood collected in heparinized plasma tubes does not clot, why was homogenization necessary prior to preparation of Buffy coat?

Data management and analysis (Page 4)
1st sentence: "…converted to comma delimitated (.csv) file…" replace the word 'delimitated' with the correct one as 'delimited'

5th sentence: "Bivariate logistic regression was performed to determined association between…" Replace 'determined' with 'determine'

Last sentence: "Any variable with P-value ≤0.05 was taken as a significant predictor of HAT presence" Replace 'taken' with 'considered'

Ethical clearance (Page 4)
3rd sentence: "The school management (Head teacher) was briefed about the study and the pupils were briefed about the study by the investigators" The word 'briefed' is repeated two times, replace one with another appropriate word, e.g. 'informed'

6th sentence: "…had first assented to participate in the study were excluded from the study…" delete "from the study" to avoid repetition of "the study"

Results (Page 4)
3rd paragraph, 1st sentence: "School enrolment is at 50–50 for girls and boys although those involved in the study where mainly in the age group 13–18 years" Replace 'where' with 'were'.

3rd paragraph, 2nd sentence: "Fowls/animals that are mostly kept at home in the district are chicken,

goats, and dogs with pigs and cattle are steadily on the increase over the years" Replace "with — pigs and cattle…" with "while — pigs and cattle..."

4th paragraph: "With the use of GPS; villages, schools, sub-counties, district headquarter, hospital/health centres and salient features such as district and sub-county boundaries, road networks and streams/rivers are located on the district map for ease of follow ups especially the CATT positive cases even by someone who has not been involved in the study (Table 3; Figure 1)" This long sentence does not provide relevant information because the exact GPS coordinates are not given at all — even under **Figure 1**. Again **Table 3** is not about GPS coordinates, but predictors of being CATT positive — thus remove citation of **Table 3** in this sentence.

Table 1 (Page 5):
State the significance of titres at ½, ¼,⅛, 1/16, etc. Briefly, highlight the key message presented in the Table. The GPS coordinates could be provided in this Table that summarizes all surveyed schools.

Table 2 (Page 5):
The words kept "at home" are repeated nine (9) times. Remove these words from the Table and retain under the description.

Organize this Table by adding; specific sampling sites, GPS coordinates, number of participants from specific sites, the livestock species kept at home, etc.

Table 3 (Page 6):
The basis for selection of some domestic livestock for inclusion in this study is not highlighted. What's the key message in this Table 3? Why is the row containing 'dogs' highlighted in bold, unlike all others in the Table?

Figure 2 (Page 7): "(a) Prepared tests samples…" replace "tests" with "test"

Discussion (Page 7)
3rd sentence: "…the difference in the sexes was not statistically significance..." Replace 'significance' with 'significant'

4th sentence: "…sexes in terms of infectivity…" Replace 'infectivity' with 'infection'

3rd paragraph, page 8: "…75% of those who were found to be CATT positive where parasitologically positive with careful wet preparations and thick blood smear microscopy" Replace 'where — parasitologically positive' with 'were — parasitologically positive'

3rd paragraph, last sentence page 8: "Humans, like animals, are believed to possess trypanotolerance that protect them from the disease" This statement is misleading because it generally implies that animals [not clear if domestic or wild] are trypanotolerant. It is better to name specific animals that are known to be trypanotolerant; only some breeds of cattle are trypanotolerant e.g. some West African Taurine cattle, whereas many others are susceptible.

4th paragraph, page 8: "Cut-off titres at some points were used to define disease cases; 1:4 required parasitological confirmation whereas 1:16 was regarded as indication of infection that required treatment even without parasites being seen" This information provides the answer to my earlier question in Table 1

(page 5) on the significance of different titres. Move this to come under Materials and Methods, subsection on "*CATT test and microscopy"* (page 3).

5th paragraph, 1st sentence, pg 8: "Though it has now been proven that animals play a yet unclear role in the maintenance of *T. b. gambiense* even when they have been eliminated from the human population, explaining in parts the reason behind re-emergence of HAT" Check again the flow of information in this sentence, re-write to make it clear.

5th paragraph, 1st sentence, pg 8: "In our study however, we did not sample the animals but instead tried looking at possible associations of those pupils who were CATT positive and the domesticated animals. After analysis for possible associations we found that those who had dogs were more than 3 times at increased risk of being CATT positive" This statement is too speculative and oversimplified since the animals were not screened for trypanosome infection by PCR and gene sequencing. This is very important experiment for determining livestock reservoirs of the HAT. It will be impossible to pinpoint or even propose, without bias, the contributions of dogs in the disease epidemiology if the complex interactions between humans with other domestic, and wild animals are not well understood. In my opinion, the authors should avoid generalizations on the potential animal reservoirs of HAT due to lack of conclusive data.

Study limitations (Page 8)
"This was a one off survey and therefore we cannot account for information on pupils who refuse to participate or were absent from school nor children who have dropout of schools" Rephrase the sentence, replace 'refuse' with 'did not assent', and also replace 'dropout' with 'dropped out'

Recommendations/conclusion (Page 8)
2nd sentence: "There is need to for active screening of populations…" Delete the word "to"

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: African trypanosomiasis, molecular parasitology and entomology (the biology of tsetse fly and trypanosomes)

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.