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REGULAR ARTICLES

Prevalence and control implications of bovine trypanosomes in endemic areas of northern Uganda

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Abstract

African animal trypanosomiasis (AAT), a disease complex caused by tsetse fly–transmitted *Trypanosoma brucei brucei*, *T. congolense* savannah ITS, *and T. vivax*, continues to inflict heavy losses to the animal industry in terms of decreased livestock production and productivity. Live bait technology and chemotherapy have been used as a control strategy in northern Uganda since 2006 with minimal success. Here, we report the results of a cross-sectional study carried out in Lango subregion, Uganda, to assess the species prevalence of bovine trypanosome in cattle using the internal transcribed spacer (ITS) of trypanosome ribosomal DNA (rDNA). Blood samples were collected from 1090 cattle by ear vein puncture and screened using a single pair of primers designed to amplify ITS ribosomal DNA (rDNA). Our results indicate an overall prevalence of 40.18% (438/1090, 95% CI 30.82–54.51). *T. vivax* constituted 32.66% (356/1090), *T. congolense* 2.39% (26/1090), *T. brucei* 1.28% (14/1090), *T. godfreyi* 0.09%(1/1090), *T. brucei* and *T. congolense* 0.36% (4/1090), *T. brucei* and *T. vivax* 1.47% (16/1090), *T. vivax* and *T. congolense* 1.65% (18/1090), *T. vivax* and *T. simiae* 0.18% (2/1090), *and T. vivax* and *T. godfreyi* 0.09% (1/1090) of infections. Over 91.7% of infections involved single species, while 9.5% were mixed infections. Over 90.2% (37/41) of the mixed infections involved *T. vivax* as one of the species, while 53.7% (22/41) involved *T. congolense.* The high prevalence of AAT and the continued presence of *T. brucei* raise public health concerns because of the zoonotic implications. An integrated approach that involves mass treatment of cattle, vector, and animal movement control should be adopted to reduce the risk of both AAT and HAT.

Keywords African animal trypanosomiasis · Deoxyribonucleic acid · Polymerase chain reaction · Prevalence

Introduction

African animal trypanosomiasis (AAT) affects the health and productivity of livestock, and it is estimated that 45 to 60 million cattle and tens of millions of small ruminants are at risk from trypanosomiasis (Chadenga 1994). In cattle, the disease is caused by *Trypanosoma vivax*, *Trypanosoma congolense*, and *Trypanosoma brucei brucei* (Clarkson 1976; Magona et al. 2005). Trypanosomes are transmitted cyclically by tsetse flies (Glossinidae), but these and other biting flies such as tabanids can also transmit *T. vivax* mechanically. Consequently, AAT caused by *T. vivax* has been reported outside the tsetse belts of Africa (Desquesnes and Dia 2003). Four species of tsetse flies, namely, *Glossina fuscipes fuscipes*, *G. morsitans submorsitans*, *G. pallidipes*, and *G. brevipalpis* are important in the epidemiological cycle of AAT in Uganda. Clinically animals affected by AAT are weak, are lethargic, and have anemia and fever, which leads

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to weight loss as well as infertility and reduction of milk production.

From the human health perspective, human African trypanosomiasis (HAT) or sleeping sickness caused by *Trypanosoma brucei rhodesiense* is a major public health concern in over 36 sub-Saharan African countries (Lundkvist et al. 2004; Simarro et al. 2011). The presence of a wide range of wild and domestic animals that act as reservoirs throughout southern and Eastern Africa makes it possible for *T. brucei rhodesiense* to circulate, thereby making transmission to humans possible in the presence of the tsetse fly vectors (Hide et al. 1996; Waiswa et al. 2003, 2006; Welburn et al. 2009). For example, a prevalence of *Trypanosoma brucei (subspecies T. brucei brucei and T. brucei rhodesiense)* of up to 40% has been reported among some Ugandan cattle populations (Welburn et al. 2009). In addition to the animal reservoirs, the epidemiology of HAT is influenced by several other factors, including the nature of the parasite, tsetse flies, human populations, and biophysical phenomena whose complex interactions drive the emergence of epidemics (Anderson et al. 2011). Civil conflicts have also been blamed for epidemiological shifts of the disease. For example, the Lord's Resistance Army (LRA) insurgency in Northern Uganda was partly blamed for the outbreaks of HAT in northern Uganda (Berrang-Ford et al. 2011; Selby et al. 2013; Tong et al. 2011).

Both the acute (Rhodesian) and chronic (Gambian) type of HAT have been reported in Uganda. Rhodesian HAT is endemic in the Southeast of the country, while Gambian HAT is endemic in the Northwest of the country and extending to most parts of South Sudan, although the distribution of the different trypanosome species is shifting (Picozzi et al. 2005; Selby et al. 2013; Wardrop et al. 2013). The movement of cattle from endemic areas of southeast Uganda to eastern and more northerly districts has introduced the disease into areas previously free of these zoonotic species, resulting in sleeping sickness epidemics in humans (Fèvre et al. 2001; Wendo 2002).

Materials and methods

Study location

The study was conducted in Uganda, a country situated in East Africa and located between 4.220 to − 1.480 N latitude and 29.570–35.040 E longitude. It covers a land area of 197,100 km^2 with a human population of 41 million. Uganda largely depends on agricultural production and it is also a home for millions of heads of cattle. The country has a diverse topography, which forms the basis for several agro-climatic zones. Specifically, the study was done in Lango subregion located 32.8333–33.0030 E longitude and 2.2143–2.4028 N latitude, approximately 350 km north of Kampala. The 20 villages are scattered in 13 subcounties in three districts of Kole, Alebtong, and Lira (see Electronic Supplementary Material). The human population in the region is about 2.1 million according to the 2014 population census. The Northern region sometimes experiences relatively high temperatures exceeding 30 °C. The region experiences one major rain season from April to October, while the period from November to March receives minimal rain. The vegetation is mainly savannah grassland.

The 2015 Uganda Bureau of Statistics (UBOS) statistical abstract estimates the national cattle population to be about 14 million. Northern Uganda has a cattle population of about 1.6 million (14.4%) with Lango subregion having about 643,395 cattle (40.2%). The three districts were selected purposively because of the persistence of the earlier government-supported intervention to stamp out sleeping sickness in high-risk areas using diminazene di-acetate. It was therefore important to assess whether the cattle had been cleansed of the trypanosome infections.

Study design, sample size determination, and sampling strategy

The sample size was estimated using the formula suggested by Wiegand (1968) for cross-sectional studies $N = (Z\alpha^2\,P\,(1\text{-}P))/d^2$, where N = sample size estimate; P = assumed true population prevalence from a previous study (35%) (Muhanguzi et al. 2014), 1-P = the probability of not having the disease (65%), $Z\alpha$ = standard normal deviation at 95% confidence interval corresponding to 1.96, d = absolute error between the estimated and the true prevalence of the disease which is 5% (0.050.

$$\mathrm{N} = (1.96)^2 * 0.35 * 0.65\Big) / (0.05)^2$$

$$N = 0.873964/0.0025$$

$$N = 349.5856$$

The minimum number of cattle in the sample (n) was determined to be 350. Sampling sites were identified and cattle owners encouraged bringing their cattle to the site. However, a total 1090 cattle from 387 households were presented at the different sites and all of them were sampled. The cattle in this study were mainly shorthorn zebu raised under traditional communal grazing system.

Blood sample collection and processing

About 125 μL of blood were collected from the middle ear vein using capillary tubes and applied onto the sample area of the classic Whatman FTA® cards (Whatman Bioscience Ltd., Cambridge, UK) (Ahmed et al. 2011; Muhanguzi et al. 2014;

Picozzi et al. 2002). Each sample was applied in the designated sample area, avoiding sample cross-contamination. Four blood samples were collected on each FTA® card and allowed to air-dry thoroughly at ambient temperatures and then labeled serially using district, village, name of farmers, and animal number codes. Samples were packed in foil pouches with a silica gel desiccant (Sigma-Aldrich, St Louis, USA) and shipped to Makerere University Central Diagnostic Laboratory for analysis.

DNA extraction

Deoxyribonucleic acid (DNA) was extracted and eluted from FTA® sample discs according to a protocol previously described by (Becker et al. 2004). Briefly, each FTA® card was placed on a supporting base (Whatman Bioscience Ltd), and from each of the individual samples, five discs were punched out using a Harris 3.0-mm Micro Punch (Whatman Bioscience Ltd) and discharged into 1.5-mL Eppendorf tubes. The Micro Punch was cleaned after punching each sample by punching at least the same number of discs from a clean filter paper (Muhanguzi et al. 2014). A negative control was prepared by punching the same number of discs from a sterile chromatography paper (Whatman Bioscience Ltd) and was processed together with field samples. Samples were incubated twice for 15 min with 1.0 ml FTA® Purification Reagent (Whatman Bioscience Ltd) at room temperature, followed by two rinses of 15 min with 1 ml TE-1 buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) at room temperature. After drying for 45 min at 37 °C, the test sample or control discs were heated at 90 °C for 30 min in a DNA Engine Dyad® Cycler PTC-0221 (Bio-Rad Laboratories Inc., Hercules, USA). 100 μL of 5% (*w*/*v*) aqueous suspension of Chelex 100 resin was added and vortexed. The mixture was centrifuged to remove the Chelex, and the discs were later air-dried and DNA eluted in 20 μL of water at 90 °C (Ahmed et al. 2011; Becker et al. 2004; Muhanguzi et al. 2014; Picozzi et al. 2002, 2008). The eluted DNA was kept at −20 °C for future use in PCR analyses as described by Njiru et al. (2005).

Internal transcribed spacer polymerase chain reaction analysis for trypanosome detection

Eluted DNA samples were screened for different trypanosome species using a single pair of primers (ITS1 CF,5'CCGGAAGTTCACCGATATTG-3′ and ITS1 BR, 5'TTGCTGCGTTCTTCAACGAA-3′) previously designed to amplify internal transcribed spacer (ITS1) of different trypanosomes ribosomal deoxyribonucleic acid (rDNA)(Njiru et al. 2005). The 250, 480, and approximately 700 base pair fragments corresponding to *T. vivax, T. brucei s.l.*, and *T. congolense* (*savannah*) were amplified generated by ITS1 CF/BR primers. The PCR was carried out in 25 μL reaction volume, 2.5 μL of which was the PCR master mix containing 10x-reaction buffer (Fisher Biotech), 2 μM each of the CF and BR primers, 0.5 U of *Taq* DNA polymerase (Fisher Biotech), 17 μL RNase-free water, and 1 μL of extracted test sample DNA or positive control DNA or negative control elute. PCR was carried out in a thermal Cycler (PTC-0221, Bio-Rad Laboratories Inc.) at cycling conditions including a denaturation step at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 40 s, annealing at 58 °C for 40 s, extension at 72 °C for 1 min, and a final elongation step at 72 °C for 5 min (Njiru et al. 2005) and at 4 °C infinity. PCR products were electrophoresed in 1.5% Agarose (Bio Tolls Inc., Japan), stained in GelRed™ (Biotium Inc., Hayward, USA) and visualized on a UVIDOC HD6-gel documentation system (Cleaver Scientific, UK) for fragment size determination.

Data analysis

Data was entered in Microsoft Excel version 14.1.0 (2010 Microsoft corporation) and exported to RStudio version1.0.153 (© 2009–2016 RStudio, Inc.) for analysis. The data was summarized into proportions, and the prevalence of each trypanosome species according to ITS1-PCR was analyzed in relation to the age of the animal and the district of origin.

Results

Distribution samples per village

A total of 1090 blood samples were collected from the 20 villages in Lira, Alebtong, and Kole. The villages include Bung cell, Abunguwube, Akaidebe, Otyang, Amonomito, Akwele, Oluru, Elupe, Ogora, Acanakwo a, Acungkena a, Bar dyang, Bar dyel, Abur ward, Amiabil, Bar nyang, Alilo, Obele, and Barr opok. The distribution of sample per village is shown in Fig. 1 below;

Prevalence of trypanosomes per species

The descriptive statistics indicate an overall prevalence of 40.18% (438/1090, 95% CI 30.82–54.51). *T. vivax* constituted 32.66% (356/1090), *T. congolense* 2.39% (26/1090), *T. brucei s.l.* 1.28% (14/1090), *T. godfreyi* 0.09% (1/1090), *T. brucei* and *T. congolense* 0.36% (4/1090), *T. brucei* and *T. vivax* 1.47% (16/1090), *T. vivax* and *T. congolense* 1.65% (18/1090), *T. vivax* and *T. simiae* 0.18% (2/1090), *and T. vivax* and *T. godfreyi* 0.09% (1/1090) of infections. Over 91.7% of infections involved single species, while 9.5% were mixed infections. Over 90.2% (37/41) of the mixed infections involved *T. vivax* as one of the species, while 53.7%(22/41) involved *T. congolense* (Fig. 2).

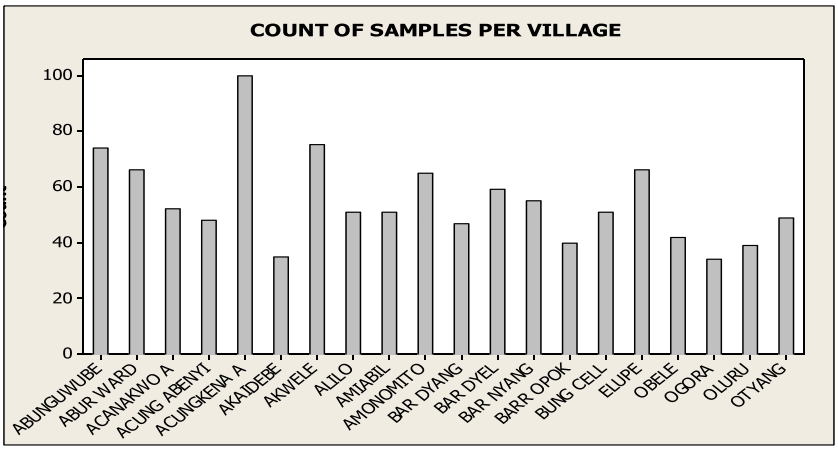

Fig. 1 Distribution of samples per village

Prevalence of trypanosomes by age of cattle

Calves (< 6 months) had a prevalence of 51.8% (86/166), older cattle (> 2 years) had a prevalence of 39.06% (266/681), and the heifers > 6 months and < 1.5 years had 35.39%(86/243) as shown in Table 1.

Prevalence of trypanosome infections at district

At district level, Kole had the highest prevalence of 46.44% (254/547) while Lira had the lowest prevalence of 20.8(40/192). The differences in the above prevalence rates were significant at $P = 0.015$ (Table 2).

Discussion

Trypanosome prevalence by species

Our findings indicated a high prevalence of trypanosomes in calves less than 6 months 51.8% (86/166) than adult cattle (> 2 years and heifers > 6 months and < 1.5 years standing at 39.06% (266/681) and 35.39% (86/2430, respectively. These findings are in contrast with the study carried out by Waiswa and Katunguka-Rwakishaya (2004) in Kashari and Angwech et al. (2015) in Amuru and Nwoya. In the Kashari study, the prevalence rates in calves and adults were 1.6% and 3.4%, respectively. In the Amuru study, the risk of trypanosome infections was nine times higher in males above 48 months of age

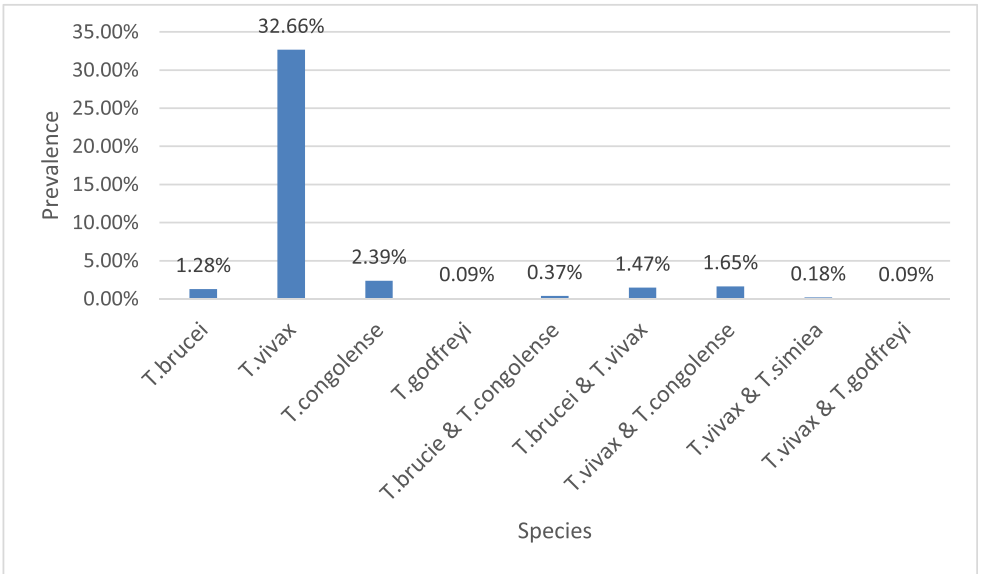

Fig. 2 Prevalence of Trypanosomes by species

Table 1 Prevalence of trypanosomes by age of cattle

Age group	No. sampled	No. positive (x)	% prevalence (x/y)
Calves (< 6 months)	166	86	51.8
Heifers (6 < x < 1.5 years)	243	86	35.39
Adult (> 2 years)	681	266	39.06
Overall	1090 (y)	438	40.18

(OR 10.0; 95% CI 5.6–17.9; $P < 0.001$) and almost six times higher in cows above 48 months of age (OR 6.1; 95% CI 3.8–9.1; $P < 0.001$) than in calves.

The high prevalence of trypanosomes in older cattle in the previous studies was attributed to differences in the period of exposure to vectors between the cattle age groups (Alemayehu et al. 2012). The older cattle also move to tsetse habitats in search for pasture and water increasing the chances of getting bitten by tsetse flies. The association between the prevalence of trypanosome infections and age of cattle might also be explained by the differences in the attractiveness of animals of different ages to tsetse flies (Stoddard et al. 2009). Further studies on host preference have also shown that savanna type of tsetse flies is attracted mostly to big and dark animals. This could be the reason why adult cattle have the highest infections. In addition, the zebu breed that is predominant in Lango subregion has mostly dark skin colors. The flies are also attracted to odors of larger or older animals than to calves (Torr et al. 2006).

This study, however, finds a higher prevalence in calves, and this could be attributed to cattle management system in this region where calves move with dams as early as 1-week post calving. The calves' immunity is still low, yet they move with the dams to risky areas as the dam looks for water and pasture. Secondly farmers spray adult cattle against tsetse flies and ticks leaving calves exposed to tsetse and other biting insects' bites exposing them to future infections. Similarly, even treatment with trypanocidals is given to older cattle.

Our results further indicate that the rate of trypanosome infection was higher in Kole (46.44%) than in Lira and Alebtong districts. This could be attributed to the many cattle markets in the district that attract several cattle from other areas. These animals are neither screened nor treated before they join the herds. Additionally, the vegetation in Kole favors tsetse fly multiplication as 70% of the district is covered by swamps. In an earlier study by Wangoola et al. (2019), it was discovered that farmers in Kole district were using Amitraz-based acaricide for tick and tsetse control yet this molecule has no effect on flies. The study also revealed that farmers were using pond water to mix trypanocidals which affects the efficacy of the drug because the water is full of impurities. This could be the reason of the higher prevalence in cattle in this area.

Table 2 Prevalence of trypanosome infections at district

District	# sampled (Y_1)	# positive (X)	District level Prev X/Y_1 (%)
Alebtong	351	144	41.03
Lira	192	40	20.83
Kole	547	254	46.44
Overall	1090	438	–

The descriptive statistics indicated an overall prevalence of 40.18% 438/1090, 95% CI 30.82–54.51) with T. *vivax* constituting 32.66% (356/1090), *T. congolense* 2.39% (26/1090), *T. brucei s.l.* 1.28% (14/1090), *T. godfreyi* 0.09%(1/1090), *T. brucei* and *T. congolense* 0.36% (4/1090), *T. brucei* and *T. vivax* 1.47% (16/1090), *T. vivax* and *T. congolense* 1.65% (18/1090), *T. vivax* and *T. simiae* 0.18% (2/1090), *and T. vivax* and *T. godfreyi* 0.09%(1/1090) of infections. The high prevalence of *T. vivax* indicates that the mechanical transmission of trypanosomiasis is taking place in this region and hence the control interventions need not only concentrate on reducing tsetse density but also other biting insects that may be playing a role in transmission.

Conclusion

The presence of *T. brucei* in the cattle reservoir in Lango subregion of northern Uganda implies that the region is a high-risk zone for zoonotic trypanosomiasis. Future studies should aim at establishing animal to human transmission of HAT. Mass treatment of cattle is appropriate for AAT control but should be done in combination with other control measures (like animal movement and vector control) in an integrated control approach.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Statement of animal rights All applicable guidelines for the care and use of animals were followed.

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