Heterogeneity in Prevalence of Bovine Trypanosomosis and its Associated Risk Factors in Pastoral and Agro Pastoral Communities Surrounding Murchison Falls National Park, Uganda.

Daniel Kizza (danielkizza8@gmail.com)
Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity
https://orcid.org/0000-0001-6446-2894
Michael Ocaido
Makerere University College of Veterinary Medicine Animal Resources and Biosecurity
Anthony Mugisha
Makerere University College of Veterinary Medicine Amimal Resources and Biosecurity
Rose Azuba
Makerere University College of Veterinary Medicine Animal Resources and Biosecurity
Sarah Nalule
Makerere University College of Veterinary Medicine Animal resources and Biosecurity
Howard Onyuth
Makerere University College of Veterinary Medicine Animal Resources and Biosecurity
Simon Peter Musinguzi
Kabale University Faculty of Agriculture Department of Animal Science
Rodney Okwasiimire
Makerere University College of Veterinary Medicine Animal Resources and Biosecurity
Charles Waiswa
Makerere University College of Veterinary Medicine Animal resources and Biosecurity

Research

Keywords: ITS-PCR T.vivax, T.congolense, Risk factors, Prevalence, tsetse

Posted Date: March 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-256519/v1

Abstract

Background: Bovine trypanosomosis transmitted by tsetse flies is a major constraint to cattle health and productivity in sub- Saharan countries including Uganda. The objectives of this study were to determine the prevalence of bovine trypanosomosis and its associated risk factors and the species of trypanosomes associated with the disease.

Methodology: A cross sectional study was conducted around Murchision Falls National Park, Uganda from January 2020 to April 2020. Blood samples were collected from 460 cattle and were screened for trypanosomes using ITS-PCR.

Results: From 460 samples collected, 136 (29.6%) of the samples were positive for trypanosomosis infections while 324 (70.4%) samples were negative. The overall trypanosome prevalence was 29.6% (95% CI 25.4-33.8). *T. vivax* (n = 130, 28.3%) was the most prevalent trypanosome specie detected and two mixed infection types *(T.vivax + T. congolense* (n = 2, 0.4%) and *T.vivax + T. brucei* (n = 1.0, 0.2%*)* were detected during the analysis. There was a significant difference when Trypanosome prevalence was compared with sex, age, breed of the sampled animals ($P < 0.05$ for sex), ($P = 0.0043$ for age) and ($P = 0.001$ for breed).

Conclusion; Trypanosomosis is still a major limitation to cattle production around Murchision Falls National Park and interventions are urgently needed .The prevalence of trypanosomosis was high and *T.vivax* was identified as the major trypanosome species in the cattle herd.

Background

Trypanosomosis is a disease of economic importance affecting both humans and animals caused by several trypanosome species. The disease is majorly transmitted by tsetse flies of genus *Glossina* while other trypanosome species especially Trypanosoma vivax and *T. evansi* are also transmitted through non-cyclical mechanical means by biting flies: Tabanus and Stomoxys (Auty et al., 2015).

Trypanosomosis is one of the disease that constrains productive livestock farming, impedes economic development and can cause a huge toll on human health in Sub Saharan Africa (Angwech et al., 2015) and (Madalcho, 2019).

The most important trypanosomes affecting livestock are *T. congolense* and *T.vivax* in cattle, *T. evansi* affecting horses, camels, water buffalos and cattle and *T. equiperdium* in horses and donkeys (Giordani et al., 2016). In Uganda, *Trypanosoma brucei brucei, T. Congolese* and *T. vivax* are species of economic importance that cause bovine trypanosomosis and are transmitted by several species of tsetse (*Glossina)* (Biryomumaisho et al., 2013). The human African trypanosomiasis commonly found in Uganda are caused by two trypanosoma sub species: *Trypanosoma brucei gambiense* in North West and *Trypanosoma brucei rhodensiense* in South East and North East of the country (Ford, 2007). Trypanosoma brucei population in cattle and wild animals are also a reservoir for human infective sub

species *T.b. rhodensiense*.(Waisswa, C., Olaho-Mukani, W., Katunguka-Rwakishaya, 2003), (Hamill et al., 2017),(Wamwiri & Changasi, 2016) and (Charles Waiswa & Wangoola, 2018).

Identification of heterogeneities characteristic with vector borne diseases is necessary for the development of local and adaptive control measures that efficiently uses limited resources (Lambrechts et al., 2009).

There is need to understand the link between the trypanosome species and the way the disease manifests in livestock so that to have the ability to diagnose and identify correctly the trypanosome species found in animals. The multiple parasite species causing bovine trypanosomosis have an effect for the epidemiology, severity and management of the disease (Adams et al., 2010). The correct detection and identification of the different parasite species using PCR based tools is crucial in the management and control of bovine trypanosomosis ((Desquesnes & Dávila, 2002). Analysis using PCR technique involves gene encoding a little ribosomal subunit in order to identify and differentiate most clinically important African trypanosome species and subspecies. PCR is more economic simple and sensitive and provides a detailed information (Cox et al., 2005).

Determining the prevalence and identifying the trypanosome species in a geographical region is essential for the understanding of the epidemiology of the disease especially zoonotic sleeping sickness. Sleeping sickness is still a challenge in Uganda and the drivers for the persistence of zoonotic sleeping sickness in certain areas, the role of wild animals and cattle reservoirs or other drivers of persistence including silent disease carrier need urgent attention (Welburn et al., 2016). Furthermore, rapid changes in human behavior, resource utilization continue to threaten to the distribution of several endemic and traditionally neglected zoonoses in many developing regions worldwide (Okello et al., 2014). Trypanosome species that cause Trypanosoma brucei rhodensiense in humans also exists in non-human hosts like tsetse, wildlife and domestic animals with several other non-human infective pathogenic trypanosomes (C. Waiswa et al., 2020). Information on the species of trypanosomes circulating among livestock in the pastoral and agro pastoral communities surrounding Murchison Falls National Park is important to guide the trypanosomiasis control intervention by several stakeholders. Therefore, the objectives of this study were (a) to determine the prevalence of bovine trypanosomosis and its associated risk factors (b) to determine the species of trypanosomes associated with the disease.

Material And Methods

MAP OF UGANDA SHOWING THE STUDY DISTRICT.

Study design and sampling-

Study area -Buliisa district located at (02 11 N 31 24 E) neighbouring Murchison Falls National Park was purposively selected due to its location in the cattle corridor and its proximity to Murchision Falls National Park. The socio- economic activities in the district include pastoralism, agro pastoralism, fishing and subsistence agriculture. The district experiences a bimodal type of climate with 2 rainy seasons (March to

May and August to November) and the vegetation is classified into forest, savannah, grassland and swamp. The forest vegetation include Budongo forest while savannah vegetation comprises of perennial grasses, scattered trees and shrubs. Murchison Falls National Park and Bugungu Game reserve contributes to grassland and woodland cover. The national population and housing census in 2014 by Uganda Bureau of Statistics (UBOS) reported the total population of Buliisa district as 113,161 people while the total cattle population is about 34,800 heads of cattle. Bullisa Sub County was targeted for the study as it had the highest cattle population in Buliisa district. In addition Bullisa Sub County is the only sub-county in the district where livestock, people and wildlife share the same environment.

The animals sampled in the study were selected from Kataleba village in Bugaana parish, Kabolwa and Kijanji villages in Kigoya Parish.

Study animals- The animals sampled in this study belonged to the following breed categories: local Zebu, Boran, crosses of Zebu and Boran and crosses of Zebu and Friesian. The farmers' village, cattle breed (local or crossbred), sex (male or female) and age (calf, heifer, steer or adult) of the animals were recorded. Animals were grouped based on their body condition score (thin, borderline or moderate) using the rib appearance and dorsal spines.

Sample size determination- The sample size was determined by the following formula:

$$n = \frac{(Z)^2 pq}{e^2}$$

Where n = sample size, Z = Z value 1.97 at 95% confidence level, e = desired level of precision (5%), p = estimated proportion of an attribute that is present in the population (50%), q = 100- p (Israel, 2012). Based on the formula the minimum number of sample size was 388 animals. A total of 460 animals of both sexes, local and crossbred and of different ages were randomly sampled in the study.

Study methodology- A cross sectional study design was conducted from January 2020 to April 2020. Preliminary reconnaissance visit was done to the study area through the Coordinating Office for the Control of Trypanosomosis in Uganda.

Collection of information on host related risk factors. Information on sex of the animals, age, breed and body condition score of all the sampled animals was recorded at the time of collecting blood samples from the animals.

The target population was all cattle keeping farming house hold in Bugaana and Kigoya parishes in Buliisa Sub County. The sampling frame consisted a list of all cattle keeping households in the Bugaana and Kigoya parishes provided by the sub county veterinary staff. The respective sample size of respondents were drawn from the list cattle keeping households in parish by simple random sample using Microsoft Excel. The selection criteria of study participants was voluntarily consenting to allow their animals participate in the study.

Taking blood samples

Animal bleeding was performed from community crushes by trained field veterinarians. Blood samples were collected by puncturing the marginal ear vein with lancet and collected into two micro haematocrit tubes. Blood from the micro haematocrit tubes was applied onto a designated sample area of Whiteman TM flinders technology associates (FTA) cards taking precaution to avoid cross contamination (Angwech et al., 2015) and (Muhanguzi et al., 2014b). The spotted FTA cards were air dried and correctly labelled. Later, the spotted FTA cards were packed in foil pouches containing silica gel desiccants and transported to College of Veterinary Medicine, Animals Resources and Biosecurity, Makerere University for PCR analysis. Following reception at the Central Diagnostic laboratory (CDL), the samples were critically examined to ensure that they met the recommended acceptance criteria. Accepted samples were accessioned and allocated laboratory numbers.

Dna Extraction

DNA was extracted from discs punched out of the sample cards following procedures modified from the GE Healthcare Application note 28-9822-22 AA.(Healthcare, 2010). Harris micro punches, 70% ethyl alcohol, FTA purification reagent, T.E buffer, chelex resin, Nuclease free water and a heat block were used in the DNA extraction process.

Trypanosome Its-pcr

The various trypanosome species were screened following the internal transcribed spacer based PCR primers (ITS1 CF, 5'-CCGGAAGTTCACCGATATTG-3' and ITS1 BR, 5'-TTGCTGCGTTCTTCAACGAA-3') as previously described (Njiru et al., 2005) and (Thumbi et al., 2008). In addition to 10µM of each of the above primers, 2X Go Taq Green Master mix, Nuclease free water and the extracted DNA were added to the PCR mix and the PCR amplification was done as follows: initial step at 94˚C for 5 min, followed by 35 cycles of 94˚C for 40 s, 58˚C for 40 s, 72˚C for 90 s, and final extension at 72˚C for 5 min.

Agarose Electrophoresis

Agarose electrophoresis
Following PCR amplification as described above, the PCR amplicons together with a standard molecular ladder were electrophoresed in TBE buffer on 1.5% agarose gels stained with ethidium bromide and visualized/photographed on a UV trans-illuminator.

Data analysis-Individual cattle sampled in the study was assigned a serial number. The animals' sex, body condition score, age, breed was entered into Microsoft Excel. Other information captured in excel included cattle name, village and parish where the animals originated. Information in excel was exported to SPSS software (IBM version 20). Descriptive statistics were generated and Chi- square test was used to

determine the association between trypanosomosis infection rates and the risk factors. In all analyses, the confidence interval level was 95% and P value less than 0.05 was considered as significant.

Results

Following reception, accessioning and quality checking, all the 460 samples received qualified to be analyzed using internal transcribed spacer PCR. 136 (29.6%) of the samples provided positive results while 324 (70.4%) samples were negative. The results showed an overall prevalence of 29.6% (95% CI 25.4–33.8).

T. vivax (n = 130, 28.3%) was the most prevalent trypanosome specie detected and two mixed infection types *(T.Vivax + T. Congolense* (n = 2, 0.4%) and *T.Vivax + T. Brucei* (n = 1.0, 0.2%*)* were detected during the analysis. The representative gel image of the results obtained during the sample analysis was shown in Fig. 1.

The description of the animal population was shown in Table 1

Table 1
Description of animal population

Study population attribute	Attribute level (N = 460)	
	Sampled (n)	(%)
Age		
Adult > 36 months	212	46.1
Heifer/steers 12–30 months	137	29.8
Calves < 12 months	111	24.1
Sex		
Male	73	15.9
Female	387	84.1
Breed		
Local	157	34.1
Crossbred	303	65.9

As Table 1 shows, the majority of animals sampled were adult female crossbred cattle.

The specific species prevalence of different trypanosomes was presented in Table 2.

Table 2
Prevalence of trypanosomosis based on species level

Trypanosome species	Number examined	Number of positives	Prevalence (%)	χ^2 (P-value)
T.brucei	460	1	0.2	485 (P < 0.05)
T.congolense	460	2	0.4	
T.vivax	460	130	28.3	
T.vivax, T.brucei	460	1	0.2	
T.vivax, T.congolense	460	2	0.4	

***** Denotes statistical significance at 5% level

From the data in Table 2 it is apparent that *T.vivax* was the most common trypanosome species identified in the sampled animals followed by *T. Conglense* and mixed *T.congolense T.vivax.*

The prevalence of bovine trypanosomosis according to sex, breed, and age and body condition score in Bugaana and Kigoya Parishes, Buliisa Sub County was presented in Table 3.

Table 3
Prevalence of bovine trypanosomosis according to sex, breed, age and body condition score in Bugaana and Kigoya parishes

Host related risk factors	Number of examined cattle	Number of infected cattle	Prevalence (%)	χ^2 (P-value)
Sex				
Female	387	114	84	62 (P < 0.05*)
Male	73	22	16	
Total	460	136		
Age				
Adult	212	59	43.38	6.28 (P = 0.043*)
Heifers/steers	137	37	27.20	
Calves	111	40	29.41	
Total	460	136		
Body Condition Score (BCS)				
Thin	142	48	35.3	2.4 (P = 0.301)
Borderline	143	37	27.2	
Moderate	175	51	37.5	
Total	460	136		
Breed				
Local breed	157	49	36	10.61 (P = 0.001*)
Cross breed	303	87	64	
Total	460	136		
* Denotes statistical significance at 5% level				

In Table 3, there was significant difference between trypanosomosis prevalence and sex, age and breed of the cattle sampled. From this data, there were more female animals sampled compared to males. According to age, more adult animals were sampled compared to steers/heifers and calves.

Discussion

In this study, a total of 460 cattle blood samples were tested using ITS PCR to determine the presence of bovine trypanosomosis. The overall prevalence of bovine trypanosomosis in the study location was

29.4% (95% CI 25.4–33.8) Table 2. This result is higher compared with the national mean prevalence of 14.28% (95%10.39–18.67) (Ebhodaghe et al., 2018).The study finding was slightly lower from a similar study conducted by (Angwech et al., 2015) that used ITS PCR found the overall prevalence of 41% of bovine trypanosomosis in Nwoya and Amuru districts. Another study by Muhanguzi et al., (2014a) in South East Uganda reported a lower prevalence rate of 15.3% compared to the findings form our study. The difference in prevalence rate can be explained in part by the difference in vegetation types and the difference in periods when the studies were conducted. The difference in vegetation type and period of study affects the tsetse fly population and ultimately influences the prevalence rates of the disease.

T.vivax was found as the most common trypanosome species in the study area contributing to about 95.6% of all the trypanosome infections (Table 2). The finding is consistent with other previous studies by (Biryomumaisho et al., 2013) (Waisswa, C., Olaho-Mukani, W., Katunguka-Rwakishaya, 2003) and (C. Waiswa et al., 2006) in Uganda that reported similar findings. The cause for T. vivax being the most prevalent is due to the fact that unlike other trypanosomes, it can also be transmitted non cyclically (mechanically) by other biting flies (Musinguzi et al., 2016). Another possible explanation for the predominance of *T. vivax* compared to other trypanosome species might be that *T.vivax* has a shorter lifecycle in the tsetse fly proboscis Gardiner & Wilson, (1987), Jones & Dávila, (2001) Jefferies et al., (1987) and a quick multiplication of parastaemia in their host which could led to high detection in cattle (Osório et al., 2008). The observed predominance of *T.vivax* prevalence compared to other trypanosome species may be due to the high presence of *Glossina fuscipes fuscipes* species that is known to transmit *T.vivax* compared to other species of *Glossina (G.Pallidipes)* (Moloo et al., 1980) and (Albert et al., 2015). The prevalence of other trypanosome species *(T. congolense, T.brucei mixed infection of T.vivax + T.bruce and T.congolense + T.vivax)* were extremely low compared to *T.vivax*. These findings could suggest that the vegetation type in the study area might have influenced ambient temperature which determined the unfavorable micro climate for other *Glossina* species. The type of micro climate in the study area could have been more favorable to *G.f.fuscipes* which may have contributed to predominance of *T.vivax*. It seemed possible that there could be few mammalian hosts for other *Glossina* species that transmit other trypanosome species other than *T.vivax* as explained by findings in studies done by (Sow, 2013) and (Jordan, 1974).

From Table 3, the animals sampled were predominantly female and a significant difference between sex of the animals and prevalence of bovine trypanosomosis ($P < 0.05$) was observed. There were more female animals infected by trypanosomosis compared to males. The difference in prevalence of trypanosomosis according to sex may be explained by the fact that farmers keep fewer male animals in their herds compared to female animals which constitute the largest proportion of animals in their herds. In this study there were few male animals sampled in the study compared to female animals. A study by (Magona et al., 2008) showed significant difference between trypanosomosis infection and sex of the animals. In contrast a study in Tanzania by Nonga H E and Kambarage DM, (2009) revealed that sex and breed were not significant risk factors affecting trypanosome infection. A significant difference was found between age and prevalence of trypanosomosis ($P = 0.0043$). Cattle of different ages in the study area probably were subjected to the same risk of exposure to the vector. The difference can be attributed

to tsetse flies attraction to mature bigger animals than calves (Torr et al., 2006). In addition adult cows produce more odour plumes that attract tsetse flies due to their large sizes compared to those produced by calves (Simukoko et al., 2007). Calves on the other hand could have been grazed in areas close to households with less risk of tsetse infestation compared to high risk distant grazing lands where heifers/steers graze as explained in the focus group discussions.

The results did not reveal significant difference between body condition and disease prevalence (P = 0.301). The body condition score of animals was associated with their health and nutritional status. Cattle suspected to be infected with trypanosomosis in advanced stage (chronic) were classified as thin-3 while cattle suspected to be free from trypanosomosis and looked healthy were graded as moderate-5. Although from our study, there was no significant difference between bovine trypanosomosis prevalence and body condition (Table 3), animals in good body condition score are more resilient to trypanosomosis infection compared to those in poor body condition in wet season as reported in Cameroon by (Gimonneau et al., 2016). The most likely possible explanation for lack of significant difference was that at the time of conducting this study, animals nutritional body reserves were not yet depleted and therefore no considerable variation in the body condition score was evident. The results (Table 3) showed significant difference between breed and trypanosomosis prevalence (P = 0.001) with a higher prevalence observed in crossbred animals compared to local breed animals. The local breed included the Ankole, Zebu and Boran while the crossbred animals were mainly crosses of Ankole - Friesians, Zebu - Friesian and Boran - Friesian. Farmers were crossbreeding their local cattle with exotic breeds as an overall strategy to migrate from a low input low output system to high input high output system as stated by (Leroy et al., 2015). The strategy was targeting to reduce poverty through increased animal performance in purely extensive production system which was common in the study area. However, providing the necessary health inputs was a challenge. Crossbred animals demanded regular spraying with insecticide to control the disease vector, prompt curative treatment of suspected sick animals and periodic preventive treatment of susceptible animals which costs were likely to drain away the anticipated income accruing from improved animal performance.

Conclusion

Trypanosomosis was still a major limitation to cattle production in Buliisa Sub County. The results from this present study revealed the prevalence of trypanosomosis was high compared to the national mean prevalence and *T.vivax* was identified as the main trypanosome species in the cattle herd. Age, breed and sex of the animals were significant risk factors for bovine trypanosomosis prevalence., The vector control strategies targeting both tsetse and biting flies (*tabanids)* are still very important for the improvement of cattle production in Uganda.

Limitation Of The Study

The cross-sectional approach of the study did not allow to collect blood samples from animals during both wet and dry seasons to evaluate the seasonal effect on disease prevalence. The study could not

differentiate between the different species of subgenus trypanozoon (*T.evansi and T.b.brucei).*

Due to limited resources the study was only conducted in one Sub County and there was no entomological survey conducted to identify the major species of tsetse flies prevalent in the area.

Declarations

Ethics approval and consent to participate; the study protocol was approved by Makerere University School of Veterinary Medicine Animal Resources (SVAR) higher degrees, SVAR research and ethics committees (SVAREC /19/2018). Study participants signed a voluntary consent to participate in the study.

Availability of data and materials: The dataset(s) supporting the conclusions of this article is (are) available from the corresponding author on reasonable request

Consent for publication: All the authors have approved the manuscript for submission.

Competing interests: The authors report no conflict of interest.
Funding Acknowledgement to the Government of Uganda support, Uganda Trypanosomiasis Control Council (UTCC) through the Coordinating Office for the Control of Trypanosomosis in Uganda (COCTU) and World Bank supported African Centre of Excellence in Agro ecology and livelihood Systems (ACALISE) for funding this study.

References

Adams, E. R., Hamilton, P. B., & Gibson, W. C. (2010). African trypanosomes: Celebrating diversity. *Trends in Parasitology*, *26*(7), 324–328. https://doi.org/10.1016/j.pt.2010.03.003

Albert, M., Wardrop, N. A., Atkinson, P. M., Torr, S. J., & Welburn, S. C. (2015). Tsetse Fly (G.f. fuscipes) Distribution in the Lake Victoria Basin of Uganda. *PLoS Neglected Tropical Diseases*, *9*(4). https://doi.org/10.1371/journal.pntd.0003705

Angwech, H., Nyeko, J. H. P., Opiyo, E. A., Okello-Onen, J., Opiro, R., Echodu, R., Malinga, G. M., Njahira, M. N., & Skilton, R. A. (2015). Heterogeneity in the prevalence and intensity of bovine trypanosomiasis in the districts of Amuru and Nwoya, Northern Uganda. *BMC Veterinary Research*, *11*(1), 1–8. https://doi.org/10.1186/s12917-015-0567-6

Auty, H. K., Torr, S. J., Michoel, T., Jayaraman, S., & Morrison, L. J. (2015). Cattle trypanosomosis: the diversity of trypanosomes and implications for disease epidemiology and control. *Rev. Sci. Tech. Off. Int. Epiz*, *34*(2). https://doi.org/10.1017/S0031182016001268

Biryomumaisho, S., Rwakishaya, E. K., Melville, S. E., Cailleau, A., & Lubega, G. W. (2013). Livestock trypanosomosis in Uganda: Parasite heterogeneity and anaemia status of naturally infected cattle, goats

and pigs. *Parasitology Research*, *112*(4), 1443–1450. https://doi.org/10.1007/s00436-013-3275-9

Cox, A., Tilley, A., Mcodimba, F., Fyfe, J., Eisler, M., Hide, G. V, & Welburn, S. (2005). *A PCR based assay for detection and differentiation of African trypanosome species in blood*. *111*, 24–29. https://doi.org/10.1016/j.exppara.2005.03.014

Desquesnes, M., & Dávila, A. M. R. (2002). Applications of PCR-based tools for detection and identification of animal trypanosomes: A review and perspectives. *Veterinary Parasitology*, *109*(3–4), 213–231. https://doi.org/10.1016/S0304-4017(02)00270-4

Ebhodaghe, F., Isaac, C., & Ohiolei, J. A. (2018). A meta-analysis of the prevalence of bovine trypanosomiasis in some African countries from 2000 to 2018. *Preventive Veterinary Medicine*, *160*(August), 35–46. https://doi.org/10.1016/j.prevetmed.2018.09.018

Ford, L. B. (2007). *Civil conflict and sleeping sickness in Africa in general and Uganda in particular*. *10*, 1–10. https://doi.org/10.1186/1752-1505-1-6

Gardiner, P. R., & Wilson, A. J. (1987). Trypanosoma (Duttonella) vivax. *Parasitology Today*, *2*, 49–52.

Gimonneau, G., Alioum, Y., Abdoulmoumini, M., & Zoli, A. (2016). *Insecticide and Repellent Mixture Pour-On Protects Cattle against Animal Trypanosomosis*. 1–16. https://doi.org/10.1371/journal.pntd.0005248

GIORDANI, F., MORRISON, L. J., ROWAN, T. G., DE KONING, H. P., & BARRETT, M. P. (2016). The animal trypanosomiases and their chemotherapy: a review. *Parasitology*, *143*(14), 1862–1889. https://doi.org/10.1017/S0031182016001268

Hamill, L., Picozzi, K., Fyfe, J., von Wissmann, B., Wastling, S., Wardrop, N., Selby, R., Acup, C. A., Bardosh, K. L., Muhanguzi, D., Kabasa, J. D., Waiswa, C., & Welburn, S. C. (2017). Evaluating the impact of targeting livestock for the prevention of human and animal trypanosomiasis, at village level, in districts newly affected with T. b. rhodesiense in Uganda. *Infectious Diseases of Poverty*, *6*(1), 16. https://doi.org/10.1186/s40249-016-0224-8

Healthcare, G. (2010). *Reliable extraction of DNA from Whiteman FTA cards*.

Israel, G. D. (2012). *Determining Sample Size*.

Jefferies, D., Helfrich, M. P., & Molyneux, D. H. (1987). Parasitology. *Parasitology Research*, 289–292.

Jones, T. W., & Dávila, A. M. R. (2001). Trypanosoma vivax - Out of Africa. *Trends in Parasitology*, *17*(2), 99–101. https://doi.org/10.1016/S1471-4922(00)01777-3

Jordan, A. M. (1974). Recent developments in the ecology and methods of control of tsetse flies (Glossina spp.) (Dipt., Glossinidae)—a review. *Bulletin of Entomological Research*, *63*(3), 361–399. https://doi.org/10.1017/S0007485300040876

Lambrechts, L., Knox, T. B., Wong, J., Liebman, K. A., Albright, R. G., & Stoddard, S. T. (2009). Shifting priorities in vector biology to improve control of vector-borne disease. *Tropical Medicine and International Health*, *14*(12), 1505–1514. https://doi.org/10.1111/j.1365-3156.2009.02401.x

Leroy, G., Baumung, R., Boettcher, P., Scherf, B., & Hoffmann, I. (2015). Review: Sustainability of crossbreeding in developing countries; definitely not like crossing a meadow... *Animal*, *10*(2), 262–273. https://doi.org/10.1017/S175173111500213X

Madalcho, E. B. (2019). *A Study on the Prevalence of Bovine Trypanosomiasis and its Associated Risk Factors in Tarcha Zuria District , Dawuro Zone*. *7*(8), 4–10.

Magona, J. W., Walubengo, J., & Odimin, J. T. (2008). Acute haemorrhagic syndrome of bovine trypanosomosis in Uganda. *Acta Tropica*, *107*(2), 186–191. https://doi.org/10.1016/j.actatropica.2008.05.019

Moloo, S. K., Kutuza, S. B., & Boreham, P. F. L. (1980). Studies on Glossina pallidipes, G. fuscipes fuscipes and G. brevipalpis in terms of the epidemiology and epizootiology of trypanosomiases in south-eastern Uganda. *Annals of Tropical Medicine and Parasitology*, *74*(2), 219–237. https://doi.org/10.1080/00034983.1980.11687333

Muhanguzi, D., Picozzi, K., Hattendorf, J., Thrusfield, M., Kabasa, J. D., Waiswa, C., & Welburn, S. C. (2014a). The burden and spatial distribution of bovine African trypanosomes in small holder crop-livestock production systems in Tororo District, south-eastern Uganda. *Parasites & Vectors*, *7*(1), 603. https://doi.org/10.1186/s13071-014-0603-6

Muhanguzi, D., Picozzi, K., Hattendorf, J., Thrusfield, M., Kabasa, J. D., Waiswa, C., & Welburn, S. C. (2014b). The burden and spatial distribution of bovine African trypanosomes in small holder croplivestock production systems in Tororo District, south-eastern Uganda. *Parasites and Vectors*, *7*(1), 1–10. https://doi.org/10.1186/s13071-014-0603-6

Musinguzi, S. P., Suganuma, K., Asada, M., Laohasinnarong, D., Sivakumar, T., Yokoyama, N., Namangala, B., Sugimoto, C., Suzuki, Y., Xuan, X., & Inoue, N. (2016). A PCR-based survey of animal African trypanosomosis and selected piroplasm parasites of cattle and goats in Zambia. *Journal of Veterinary Medical Science*, *78*(12), 1819–1824. https://doi.org/10.1292/jvms.16-0240

Njiru, Z. K., Constantine, C. C., Guya, S., Crowther, J., Kiragu, J. M., Thompson, R. C. A., & Dávila, A. M. R. (2005). The use of ITS1 rDNA PCR in detecting pathogenic African trypanosomes. *Parasitology Research*, *95*(3), 186–192. https://doi.org/10.1007/s00436-004-1267-5

Nonga H E and Kambarage DM. (2009). *Prevalence of Bovine Trypanosomosis in Morogoro Tanzania* (Vol. 8, Issue January, pp. 208–213). Pakistan Journal of Nutrition.

Okello, A. L., Bardosh, K., Smith, J., & Welburn, S. C. (2014). One Health: Past Successes and Future Challenges in Three African Contexts. *PLoS Neglected Tropical Diseases*, *8*(5), 1–7. https://doi.org/10.1371/journal.pntd.0002884

Osório, A. L. A. R., Madruga, C. R., Desquesnes, M., Soares, C. O., Ribeiro, L. R. R., & Da Costa, S. C. G. (2008). Trypanosoma (Duttonella) vivax: Its biology, epidemiology, pathogenesis, and introduction in the New World - A review. *Memorias Do Instituto Oswaldo Cruz*, *103*(1), 1–13. https://doi.org/10.1590/S0074-02762008000100001

Simukoko, H., Marcotty, T., Phiri, I., Vercruysse, J., & Van den Bossche, P. (2007). Heterogeneity in the trypanosomosis incidence in Zebu cattle of different ages and sex on the plateau of eastern Zambia. *Acta Tropica*, *103*(2), 98–101. https://doi.org/10.1016/j.actatropica.2007.05.011

Sow, A. (2013). *Trypanosomosis risk factors and impact assessment of a tsetse and trypanosomosis eradication campaign in Burkina Faso*. 165.

Thumbi, S. M., McOdimba, F. A., Mosi, R. O., & Jung'a, J. O. (2008). Comparative evaluation of three PCR base diagnostic assays for the detection of pathogenic trypanosomes in cattle blood. *Parasites and Vectors*, *1*(1), 1–7. https://doi.org/10.1186/1756-3305-1-46

Torr, S. J., Mangwiro, T. N. C., & Hall, D. R. (2006). The effects of host physiology on the attraction of tsetse (Diptera: Glossinidae) and Stomoxys (Diptera: Muscidae) to cattle . *Bulletin of Entomological Research*, *96*(1), 71–84. https://doi.org/10.1079/ber2005404

Waisswa, C., Olaho-Mukani, W., Katunguka-Rwakishaya, E. (2003). Domestic animals as reservoirs for sleeping sickness in three endemic foci in south–eastern Uganda. *Annals of Tropical Medicine & Parasitology*, *97*(2), 149–155. https://doi.org/10.1179/000349803235001688

Waiswa, C., Azuba, R., Makeba, J., Waiswa, I. C., & Wangoola, R. M. (2020). Experiences of the one-health approach by the Uganda Trypanosomiasis Control Council and its secretariat in the control of zoonotic sleeping sickness in Uganda. *Parasite Epidemiology and Control*, *11*. https://doi.org/10.1016/j.parepi.2020.e00185

Waiswa, C., Picozzi, K., Katunguka-Rwakishaya, E., Olaho-Mukani, W., Musoke, R. A., & Welburn, S. C. (2006). Glossina fuscipes fuscipes in the trypanosomiasis endemic areas of south eastern Uganda: Apparent density, trypanosome infection rates and host feeding preferences. *Acta Tropica*, *99*(1), 23–29. https://doi.org/10.1016/j.actatropica.2006.06.005

Waiswa, Charles, & Wangoola, M. R. (2018). *Sustaining Efforts of Controlling Zoonotic Sleeping Sickness in Uganda Using Trypanocidal Treatment and Spray of Cattle with Deltamethrin*. *XX*(Xx), 1–6. https://doi.org/10.1089/vbz.2018.2382

Wamwiri, F. N., & Changasi, R. E. (2016). Tsetse Flies (Glossina) as vectors of human African Trypanosomiasis: A review. *BioMed Research International*, *2016*. https://doi.org/10.1155/2016/6201350

Welburn, S. C., Molyneux, D. H., & Maudlin, I. (2016). Beyond Tsetse - Implications for Research and Control of Human African Trypanosomiasis Epidemics. *Trends in Parasitology*, *32*(3), 230–241. https://doi.org/10.1016/j.pt.2015.11.008

Figures

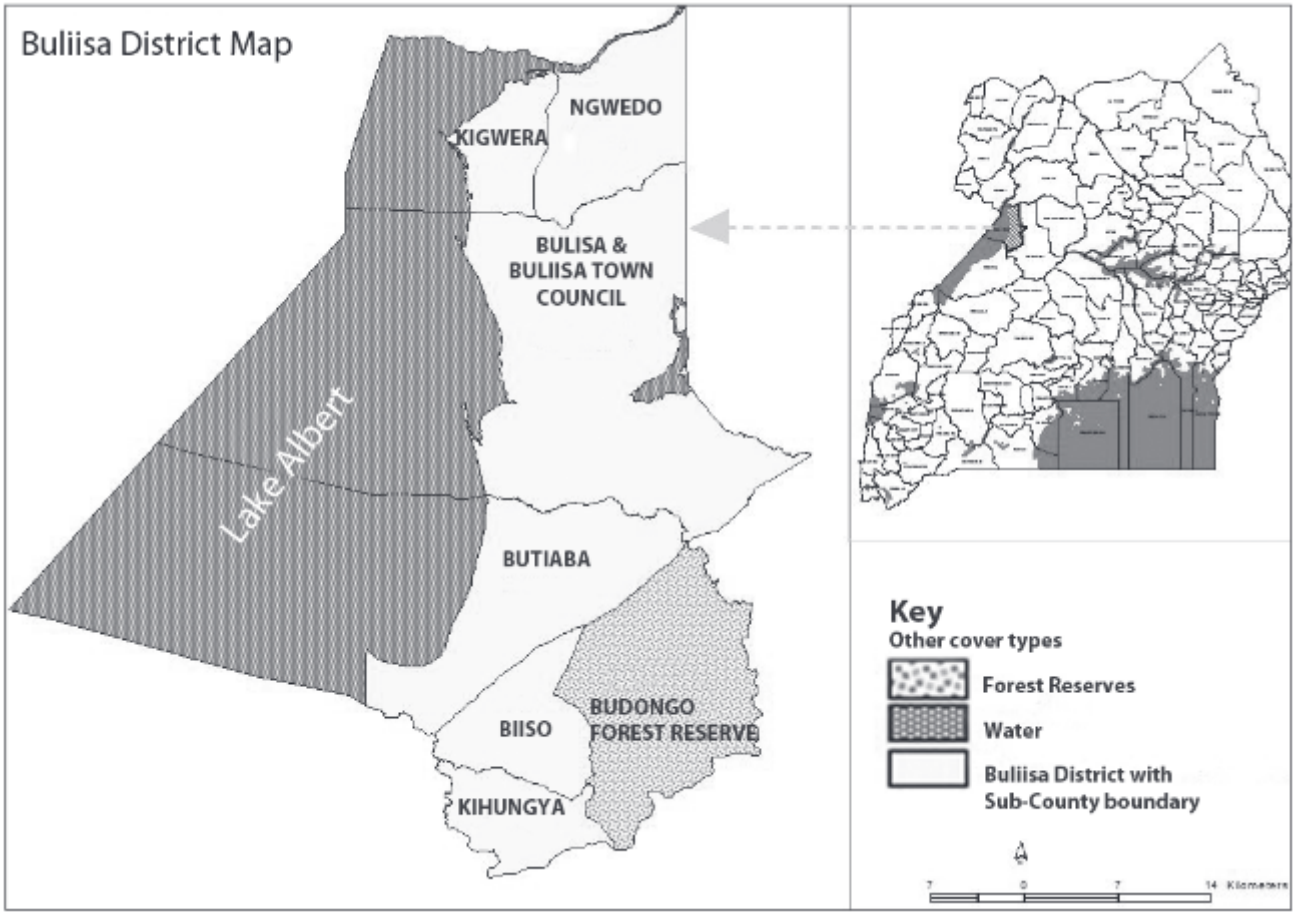

Figure 1

Map of Uganda showing the study district (Source: Arch GIS) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

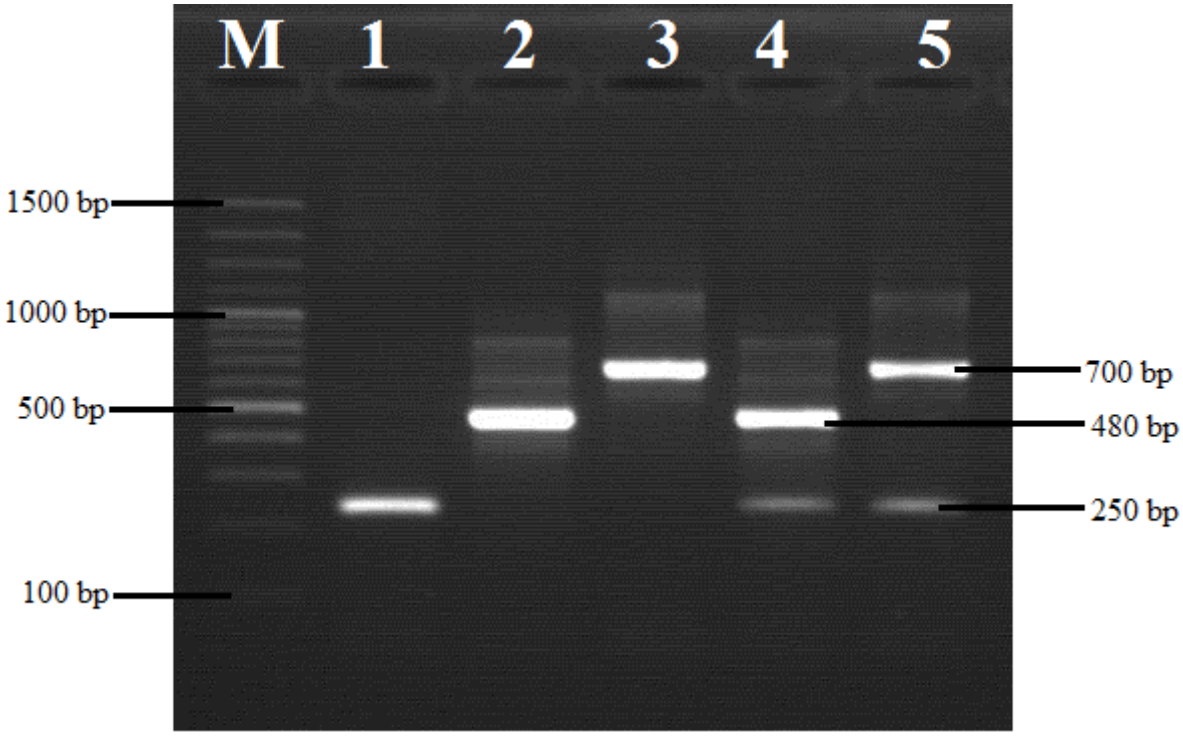

Figure 2

Representative gel image of the results obtained during the sample analysis. Lane M: 100 bp DNA Ladder, Lane 1: T. Vivax (250 bp), Lane 2: T. Brucei (480 bp), Lane 3: T. Congolense (700 bp), Lane 4: T. Vivax + T. Brucei (Mixed infection) and Lane 5: T. Vivax + T. Congolense (Mixed infection)

Supplementary Files

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

- GraphicalAbstract1.pdf