Tsetse fly distribution and occurrence of *Trypanosoma* species among cattle and goats around Queen Elizabeth National park, Uganda

Mallion Kangume
Africa Centers for Disease Control and Prevention, AU Commission
Denis Muhangi
College of Veterinary Medicine Makerere University
Joseph Byaruhanga
College of Veterinary medicine Animal Resources and Biosecurity
Aggrey Agaba
Africa One health University Network
Joachim Sserunkuma
Makerere University College of Veterinary Medicine Animal Resources and Biosecurity ences
Stallon Justus Kisembo
Africa One Health University Network
Paul Bogere
Busitema University
Patrick Vudriko
Makerere University COllege of Veterinary Medicine Animal Resources and Biosecurity
Innocent Bidason Rwego (irwego@covab.mak.ac.ug)
Makerere University College of Biosecurity Animal Resources and Veterinary medicine
https://orcid.org/0000-0003-1274-7788

Research article

Keywords: Glossina species, Trypanosoma species, co-infections, neglected tropical diseases

Posted Date: October 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32266/v2

Abstract

Background: African Animal Trypanosomiasis (AAT) is an infectious disease of economic and veterinary importance in Sub-Saharan Africa. The current study aimed at providing baseline information on tsetse fly distribution and occurrence of *Trypanosoma* species in cattle and goats within and around Queen Elizabeth National Park (QENP), in western Uganda. A minimal entomological survey was conducted in April 2017 while blood samples collected from cattle (n = 576) and goats (n = 319) in June 2015 and May 2017 were subjected to Polymerase Chain Reaction (PCR) to determine the occurrence of *Trypanosoma* species.

Results: *Glossina pallidipes* and *G. fuscipes* were the only tsetse fly species trapped in the study area with apparent density of 20.6. The overall prevalence of *Trypanosoma* spp. was 27% for goats and approximately 38% for cattle. The most prevalent *Trypanosoma* spp. in goats was *T. brucei* (n = 60, 18.8%) while the most prevalent in cattle was *T. congolense* (n = 102, 27.1%). In both cattle and goats, a dual infection of *T. brucei* + *T. congolense* was most encountered. In goats a triple infection of *T. brucei* + *T. congolense* + *T. vivax* was higher than that in cattle.

Conclusions: Current findings show that there are two species of tsetse flies, and three species of *Trypanosoma*, important in transmission of AAT in both cattle and goats. Control efforts of AAT have mainly focused on cattle and this study proves that prevention and control efforts should also involve goat farmers.

Background

Animal African trypanosomiasis (AAT), or Nagana, is an infectious disease of economic and veterinary importance that affects livestock negatively affecting food production and economic growth in most parts of the world, especially sub-Saharan Africa (1-3). Tsetse flies play a big role in the transmission and spread of AAT among the various domestic and wild animals. In Uganda, over 70% of land is estimated to be infested with tsetse flies with approximately 60% of livestock at risk of AAT (4, 5). The main sub-species of tsetse flies documented in Uganda are *Glossina fuscipes* and *G. pallidipes* (4). *Glossina fuscipes* is the most dominant species around the Lake Victoria basin, Eastern Uganda and Northern part of the country (4, 6). Queen Elizabeth National park (QENP) is historically known to be infested with tsetse flies. However, there is no recent study that has been conducted within and around QENP to ascertain the distribution of different species of tsetse fly especially at the livestock-wildlife interface.

A range of wildlife serve as reservoirs of infection in domestic animals (5). Trypanosomes, the causative agents of the disease are vectored by tsetse flies of the genus *Glossina* and their distribution coincides with that of their vector (3, 7, 8). Trypanosomes are parasites that cause trypanosomiasis in livestock posing a serious threat to agriculture and hindering use of millions of square kilometres of productive lands in sub-saharan Africa including Uganda (1, 3, 8, 10-12). Areas infested with tsetse flies are faced with food insecurity because of the millions of heads of cattle and other livestock species that are at risk.

For instance, it is estimated that not less than three million livestock die each year due to presence of tsetse flies and trypanosomiasis. The infection also causes reduced calving, reduction in milk yield, decreased numbers of livestock and reduced work efficiency in draught animals (13). Factors such as interaction between tsetse flies, source of vertebrate blood (either from livestock or wildlife) and season determine the epidemiology of trypanosomiasis and its impact on livestock production (2).

Studies conducted on *Trypanosoma* species in Uganda have mainly been in cattle (8, 10, 12) and less in goats even though the latter have been shown to play an important role in the distribution and dissemination of the disease (2, 13, 14). A study conducted by Biryomumaisho et al. (14) in the districts of Kasese, Jinja and Rakai showed the prevalence of *Trypanosoma* species to be 7.6% in cattle and 0.7% in goats. In QENP ecosystem, there is direct and indirect interaction between livestock and wildlife through sharing of common grazing land. Wildlife have been thought to be an important maintenance host for AAT (1, 6). The current study looked at the distribution of tsetse fly species and occurrence of *Trypanosoma* species in communities within and around QENP in cattle and goats. Data collected in 2017 followed a request from the farms to the International Development Research Centre (IDRC)-Canada funded Ecohealth project to determine whether the farmers cattle had *Trypanosoma* species or not. Therefore, the authors compared data collected in 2015 with that in 2017. This information was used to inform management of tsetse flies and trypanosomiasis especially in cattle farmers at the wildlife-livestock interface.

Results

Entomological findings

A total of 5,003 tsetse flies, *G. pallidipes* (99.5%) and *G. fuscipes* (0.5%), were captured in communities within and around QENP with other biting flies (n = 20,237) (Table 1). BT traps caught both *G. pallidipes* (99.3%) and *G. fuscipes* (0.67%) while F3 traps caught only *G. pallidipes.* The other biting flies were mainly in the genus of *Stomoxys* and Tabanids. *Stomoxys* had the highest number among other flies captured (Table 1).

Table 1. Frequency of tsetse fly species and other flies captured in and around QENP

Type of Fly	Sex		Total
	Male No. (%)	Female No. (%)	
Tsetse fly			
G. pallidipes	368 (7%)	4,611 (93%)	4,979
G. fuscipes	7 (29%)	17 (71%)	24
Other flies*			
Stomoxys	-	-	18,310
Tabanids	-	-	428
Muscids	-	-	278
Hymenopteran	-	-	117
Lepidopterans	-	-	14

*No sex determination was done for other flies.

The computed apparent fly density (FTD), the average number of flies caught per trap per day for the entire surveyed area was 20.6. BT traps caught a total of 3,566 tsetse flies with an average of 63.67 and the FTD of 21.22 tsetse flies per day while F3 caught a total of 1,437 tsetse flies with an average of 62 and FTD of 21 tsetse flies per day (Table 2).

Table 2. Tsetse fly and flies caught per trap type in different communities around QENP

Community Site	Type of Trap	No. of Traps	Tsetse flies/trap	Other flies	FTD	Mean ± SE*
Hamukungu	BT	10	2278	282	74.3	222.87 ± 50.6
	F3	5	1065	275		
Nyakatonzi	BT	9	1187	10068	38.1	114.23 ± 40.3
	F3	4	298	1832		
Karusandara	BT	9	31	2601	0.9	2.79 ± 1.68
	F3	5	8	2601		
Muhokya	BT	9	61	447	1.7	5.08 ± 2.55
	F3	4	5	65		
Ibuga	BT	10	1	1389	0	0.07 ± 0.07
	F3	5	0	393		
Busunga	BT	9	8	245	2.1	6.27 ± 3.756
	F3	2	61	39		
Totals		**81**	**5003**	**20237**	**20.6**	

FTD - Apparent fly density; BT and F3 - Types of tsetse fly traps; *Mean number of Tsetse flies caught in each community (SE - Standard Error)

Hamukungu community had significantly higher numbers (p = 0.001) of tsetse flies caught than in the rest of the communities (Table 2). The mean numbers of *G. pallidipes* (61.5 ± 14.7) captured were significantly higher than that of *G.fuscipes* (0.3 ± 0.2) in the whole study area (p = 0.001). There was significantly higher apparent density (FTD) of *G. pallidipes* caught by BT than the *G. fuscipes* captured by the same trap type (F-Statistic = 48.4, p = 0.001). There was no *G. fuscipes* trapped by F3 trap but still there was a statistically significant difference for FTD between *G. pallidipes* and *G. fuscipes* (F- Statistic = 16.4, p = 0.031).

Furthermore, the results showed a significant difference in the number of tsetse flies caught in different vegetation types in the study area (F – Statistic = 8.56, p = 0.001). Thickets and shrubs had significantly higher numbers of tsetse flies trapped than those trapped in the open savannah grasslands (D = 138.465, p = 0.001) and marshy riverine woodland (D = -138.0, p = 0.002). However, the difference was not statistically significant with grassland and thickets (D = 98.444, p = 0.198). There was a significant difference in the number of tsetse flies caught in different vegetation types in the study area (Post hoc Analysis F = 8.56, p = 0.001). Marshy riverine woodland had significantly higher number of tsetse flies than open savannah grasslands (D = 271.3, p = 0.001) and grassland and thickets (D = 231.3, p = 0.001).

Trypanosoma species in cattle and goat blood samples collected in June 2015

In 2015, a total of 445 blood samples were collected from both cattle and goats with 71.7% (n = 319) of the samples being goats. Three types of *Trypanosoma* species, namely *T. brucei* (480 bp), *T.* congolense (700 bp) and *T. vivax* (250 bp) were identified using PCR (Fig 1).

The overall prevalence of *Trypanosoma* spp in cattle and goat blood samples collected in 2015 was 38.9% and 37% respectively. However, the prevalence in cattle was not significantly different from that of goats (F = 1.654, p = 0.4870). *Trypanosoma brucei* was the most prevalent *Trypanosoma* species in both cattle (23%, 29/126) and goats (18.8%, 60/319). This was followed by *T. congolense* in cattle (17.5%, 22/126) and goats (16.9%, 54/319). *Trypanosoma vivax* was the least prevalent in cattle (11.1%, 14/126) and goats (12.9%, 41/319).

The proportion of animals with single infection was higher than for mixed infection in both cattle and goats. In both cattle and goats, mono-infections were more prevalent than mixed infections. The commonest dual infections in both cattle (4.8%, 6/126) and goats (4.1%, 13/319) were due to *T. brucei* and *T. congolense*. Other dual infections were due to *T. brucei* and *T. vivax* in both cattle (3.2%, 4/126) and goats (0.6%, 2/319). In addition, there was a dual infection of T. congolense and T. vivax in cattle (0.8, 1/126) and goats (1.3, 4/319). Meanwhile, triple infections involving all the three species were higher in goats (2.8%, 9/319) than in cattle (2.4%, 3/126).

Kashaka landing site (50%, n = 25/50) had the highest *Trypanosoma* infection rates followed by Kisenyi landing site (43.9%), Rwenshama (33.1%) and lowest in Kirugu sub-county (32.6%). While the prevalence was high in Kashaka fish landing site (Table 3), the difference compared to other areas was not statistically significant (p = 0.106). Cattle in Nyakatonzi community had a *Trypanosoma* spp. prevalence of 38.9%.

Table 3. Prevalence of *Trypanosoma* spp. in cattle and goats in Year 2015

Category		Prevalence (%) in Cattle					Prevalence (%) in Goats				
		No.*	*T. congolense*	*T. brucei*	*T. vivax*	P#	No.*	*T. congolense*	*T. brucei*	*T. vivax*	P#
Location											
	Kashaka	-	-	-	-	-	50	12.0	16.0	34.0	50.0
	Kirugu	-	-	-	-	-	89	10.1	19.1	6.7	32.6
	Kisenyi	-	-	-	-	-	41	31.7	22.0	19.5	43.9
	Rwenshama	-	-	-	-	-	139	18.7	18.7	7.2	33.1
	Nyakatonzi	-	12	17.5	23.0	11.1	38.9	-	-	-	-
Sex	Male	23	17.4	39.1	0.0	47.8	57	10.5	26.3	3.5	33.3
	Female	103	17.5	19.4	13.6	36.9	262	18.3	17.2	14.9	37.8
Age	Adult	70	21.4	22.9	11.4	44.3	186	17.2	17.2	12.4	34.4
	Sub-adult	56	12.5	23.2	10.7	32.1	131	16.8	20.6	13.7	40.5
	Young	-	-	-	-	-	2	0.0	50.0	0.0	50.0

*Data represents percentages of number of positives for *Trypanosoma* spp.; # Represents overall prevalence per category. (-) means no cattle samples were collected. There no cattle in Kashaka, Kirugu, Kisenyi and Rwenshama fishing villages.

Trypanosoma spp. in cattle blood samples collected in May 2017

A total of 250 blood samples were collected from cattle in May 2017. No blood samples were collected from goats during this time. The overall prevalence of *Trypanosoma* spp. was 38% (n = 95) in cattle. The prevalence for *T. congolense* (n = 80, 32.4%), *T. vivax* (n = 17, 6.8%) and *T. brucei* (n = 16, 6.4%) were statistically different ($F = 12.76$; $p < 0.002$). Post hoc analysis showed that *T. congolense* was significantly more prevalent than *T. brucei* and *T. vivax*. A co-infection of *T. brucei* and *T. congolense* was most encountered in the study area (n = 19; 7.4%). Only 3.2% (n = 8) of the cattle were co-infected with all the three *Trypanosoma* spp.. There was no mixed infection of *T. brucei* and *T. vivax* recorded in the study area for this period.

By individual location, the prevalence of *Trypanosoma* spp. was high in Muhokya (56.3%; n = 27) followed by Lake Katwe (42.9 %, n = 15) and less prevalent in Kahendero (24.1%, n = 13). The prevalence of *T. congolense* was highest in all the sub-counties of study followed by *T. vivax* (except in Nyakatonzi) while *T. brucei* was least prevalent (Table 4). There was a significant difference in prevalence of *Trypanosoma* spp. by location ($p = 0.039$). Using the Post-hoc analysis, Muhokya was found to have a

significantly higher prevalence than Kahendero (p = 0.001). The overall prevalence of *Trypanosoma* spp. was higher in adult cattle (37.1%, n = 75) than in young ones (31.3%, n = 15). The prevalence of *Trypanosoma* spp. was high in local breeds (38.1%, n = 69) followed by cross breeds (37.1%, n = 23) and least prevalent in Friesian breeds (14.3%, n = 1). There was no case of *T. brucei* recorded in the Friesian breeds.

Table 4. Prevalence of Trypanosoma spp. in cattle in May 2017

Category		Prevalence (%)				
		No.#	*T. congolense*	*T. brucei*	*T. vivax*	Overall*
Location	Kahendero	54	18.5	3.7	5.6	24.1
	Karusandara	35	37.1	8.6	11.4	37.1
	L. Katwe	35	37.1	5.7	8.6	42.9
	Muhokya	48	47.9	4.2	6.3	56.3
	Nyakatonzi	78	28.2	10.3	5.2	34.6
Sex	Male	22	27.3	4.5	4.5	27.3
	Female	228	32.9	7	7	36.8
Age	Young	48	29.2	4.1	8.2	31.3
	Adult	202	33.2	7.4	6.4	37.1
	Local	181	32.6	7.7	6.6	38.1
Breed	Cross	62	33.9	4.8	6.5	37.1
	Friesian	7	14.3	0	14.3	14.3

- Number of cattle sampled per category; *Overall prevalence. Only young and adult animals were sampled in Year 2017.

Discussion

Species of tsetse flies in and around QENP

Glossina fuscipes, G. pallidipes and *G. brevipalpis* are sub-species of tsetse flies previously reported to exist in Uganda (4, 8) (15, 16). The current study showed the presence of only two species of tsetse flies, *G. fuscipes* and *G. pallidipes,* in rangelands within and around QENP ecosystem and surroundings. Similar results have been reported in Uganda by Albert (4) and Waiswa et al. (17) in the Lake Victoria basin and eastern Uganda, respectively. *Glossina pallidipes* was the most predominantly caught species in communities around Queen Elizabeth national park, south western Uganda. The results are different

from those of previous studies in Uganda (4, 17) and Ngonyoka et al (18) in Tanzania that all showed *G. fuscipes* as the dominant species.

Glossina pallidipes was predominantly found in the thickets and shrubs vegetation. The current study area was generally a savannah land dominated with invasive species of thickets and shrubs. Species, for example, *Acacia* spp, *Eurphobia sp* and *Lantana camara* among others, with small trees less than 3 metres above the ground, were the dominant species. This probably explains the presence of high numbers of *G. pallidipes* in the study area as such land cover provides unique ecological conditions ideal for survival and infestation of *G. pallidipes* (3, 4, 16, 19). The thickets and shrubs had the highest number of tsetse fly catches. The results agree with literature which suggests that *G. pallidipes* inhabits woodland savannas and thickets (16, 20, 21). Tsetse flies are very sensitive to changes in the environment and the ecology of an area. Factors such as temperature, humidity and vegetation cover are important in provision of shade and maintenance of suitable micro-climate and a habitat for hosts of tsetse flies (4, 19, 20, 22).

The sex ratio of caught tsetse flies indicated that higher numbers of females were recorded during the study. Similar results have been reported elsewhere (1, 4, 19). The high number of females present in the study area could result in future high population density of tsetse flies and ultimately infection rate (1). The high proportion of females in the current study can probably be attributed to the fact that females live longer than males (mean female fly life span is 8 weeks, but only 4 weeks for the males).

There was an existence of more biting flies each trap than tsetse flies. This is in agreement with previous literature which suggest that parts of south west and mid central Uganda have lower populations of tsetse flies than other biting flies (4). Presence of biting flies could pose a threat to livestock production since biting flies such as *Stomoxys* and Tabanids among others have been associated with mechanical transmission of some livestock diseases such as AAT (23, 24). Severe biting by these flies may be a nuisance to animals resulting in physical discomfort, wounds, reduced weight and milk production in livestock (23-25).

Different types of traps have been designed to catch different species of tsetse flies and these traps have been reported to have varying effectiveness in trapping different tsetse fly species (26-28). In this study, two different traps (Biconical and F3) were used in the capture of tsetse flies within and around QENP and their efficiency was determined. Generally, Biconical traps (BT) were more effective in trapping tsetse flies in the study area than F3 traps regardless of the tsetse fly species. Higher number of tsetse flies captured by biconical traps in this study could probably be explained by nearly twice the numbers of biconical traps deployed as compared to the F3 traps. Overall, 5003 tsetse flies were captured in the study with the calculated FTD of 20.6 which is comparable to that reported by Apaatah (1) and Salekwa et al. (21). However, it is higher than that previously reported by Albert (4).

Prevalence of *Trypanosoma* spp. in goats and cattle

The prevalence of *Trypanosoma* spp. in both cattle (37%) and goats (38.9%) for samples collected in 2015 based on PCR was higher than that reported by other studies in the same animal species in Uganda, Nigeria and Zambia (2, 5, 13, 14, 23). In addition, the overall prevalence of *Trypanosoma* spp. among cattle (38%) samples collected in 2017, based on PCR results was higher than what has been reported in previous studies in the country. For instance, the prevalence was 7.6% in Kasese, Rakai and Jinja districts of Uganda (14), 2.4% in Mbarara district, western Uganda (5) and 15.3% in Tororo district, eastern Uganda (11).This prevalence is also higher than in studies carried out in other tsetse fly endemic countries in Africa (1, 2, 25, 29, 30). However, some studies have reported almost similar prevalence of *Trypanosoma* spp. in cattle. For example, reports in Zambia and Ivory Coast showed prevalence of 33.5% and 22.31% in cattle in Zambia (2) and Ivory Coast (30) respectively. The higher prevalence of *Trypanosoma* spp. in this study could probably be explained by the close proximity of the study sites to the QENP savannah grasslands that hosts various wildlife species that are possible reservoirs of *Trypanosoma* spp. Wildlife has previously been reported to be important reservoir and maintenance hosts of *Trypanosoma* spp. (5, 8, 31). In addition, the difference in prevalence observed in Uganda could be due to variations in different geo-ecological zones and time of year for sampling (5, 14, 24, 30). The type of husbandry practices such as communal grazing, free range grazing and climatic variations across different geographical regions can influence the survival of both the parasites and vectors. Fishing communities within QENP keep goats that directly and indirectly interact with wildlife species. In addition, it is not uncommon to find wildlife interacting with cattle around QENP especially in communities that neighbour the national park.

The prevalence of *Trypanosoma* spp. was higher in cattle than in goats, for samples collected in 2015. The chances of an animal being infected with *Trypanosoma* spp. vary with the geographical location, the rate at which the host is being fed on by the infected tsetse fly (2, 14), the detection of carbon dioxide emitted by potential vertebrate host, the short-range visual and olfactory stimuli by a tsetse, and the behavior of a particular host (2). Cattle have larger body sizes compared to goats and therefore are most likely to emit more odor (carbon dioxide) and therefore likely to attract more tsetse flies than goats (2, 14).

The study confirmed presence of *T. congolense*, *T. vivax* and *T. brucei* in both goats and cattle for years 2015 and 2017. However, *T. vivax* was the least prevalent of all the species. This is contrary to other studies that have reported *T. vivax* as the most predominant species in the endemic parts of Uganda (5, 11, 14, 24). However, the results are consistent with studies in Zambia (2, 32, 33), Nigeria (25, 34), Ghana (1) and some parts of Uganda (35) where *T. congolense* was reported to be the most prevalent species. The high prevalence of *T. congolense* could be attributed to the complementary roles played by major cyclic vectors like *G. pallidipes*. In addition, this could be due to high sensitivity and specificity of PCR methods used in the current study that can detect even extremely low parasitaemia. *Trypanosoma congolense* and *T. vivax* species may also be mechanically transmitted by biting flies (36). The low prevalence of *T. vivax* could be attributed to the low densities of *G. fuscipes* in the current study. *Glossina fuscipes fuscipes* is well known to be a good vector for *T. vivax* (37, 38).

Mixed infections for all the species for T*. brucei* and *T. vivax* were only in cattle and goats sampled in 2015. In both years, there was mixed infections for all species of *Trypanosoma* in both cattle and goats

though it was less prevalent in cattle samples of 2017. *Trypanosoma brucei* and *T. congolense* mixed infections was most prevalent for both years in all animals. Presence of mixed infection was consistent with the findings by Apaatah (1), Kouadio et al. (30) and Samdi (25). Presence of biting flies such as Stomoxys, Tabanids, *G. fuscipes* and *G. pallidipes* may explain the mixed infection in cattle and goats. Mixed infections may also be due to close interaction between wildlife and domestic animals. Tsetse flies may be getting blood meals from cattle, goats and wildlife leading to mixed infections.

For samples collected in 2015, there was no major variations in the prevalence of *Trypanosoma* spp. infections in all the study sites. The results were consistent with those reported in a previous report (1). This could suggest that climatic and ecological conditions which influence tsetse fly distribution in these communities sampled may be homogeneous. However, for samples collected in 2017, there was a statistically significant difference in the prevalence of *Trypanosoma* spp. by location with Muhokya and Lake Katwe, that are closely bordering the national park, having higher prevalence compared to other sites. The results are consistent with studies elsewhere which showed that prevalence of *Trypanosoma* species are higher in sites that are in close proximity with wildlife areas (14, 30). The pastoralists around QENP practice similar traditional husbandry management practices such as communal grazing and sometimes graze near or inside the national park. They expose their animals to tsetse flies that are more common inside than outside the national park. The occurrence of *Trypanosoma* species causing AAT directly corresponds to the availability of the vector (especially tsetse fly) (1, 26).

The survey for tsetse fly distribution was not conducted widely nor done in both the years to allow for comparison. The infection rates of tsetse flies captured was not investigated as well for seasonal variations in the tsetse fly abundance. The prevalence of *T. brucei* and *T. congolense* was high for samples collected in 2015 and 2017 respectively. This may be a true picture of prevalence since we tested the samples using same method of PCR. In this study, there was a time lag between sample collection and direct examination in the laboratory which could easily affect the results of this study. In addition, we neither sampled the same farms nor households for cattle in Years 2015 and 2017. Goats were sampled only in 2015 since farmers were more interested in sampling cattle in 2017. However, these were the same village in most instances.

Conclusions

This study showed that QENP ecosystem and the surroundings areas are highly infested by tsetse flies of mainly *G. pallidipes* and *G. fuscipes* sub-species. *Glossina fuscipes* sub-species were also recorded in Nyakatonzi community. Both goats and cattle were highly infected with *Trypanosoma* species. Therefore, there is need to intensify tsetse fly and trypanosomiasis control effort and measures in the communities around Queen Elizabeth National Park. The high proportion of goats infected with *Trypanosoma* species points to their role as domestic livestock reservoirs in the epidemiology of the disease in livestock. This implies that goats should also be given attention as much as cattle in the control and prevention of AAT. The presence of *T. brucei* sub-species in livestock that share rangelands with wildlife and humans pose a significant public health threat since T. brucei may cause Nagana that

disrupts livestock production. There is need for urgent attention from all relevant stakeholders to intensify wide vector and disease management of tsetse fly infestation and Nagana in communities bordering the national park in order to safeguard livelihoods.

Methods

Study area

The study was conducted in selected communities within and around QENP, southwestern Uganda. It is adjacent to the Democratic Republic of Congo (DRC) and is situated at the equator (00^0 12'S 30^0 00'E) within the western Albertine Rift Valley. The park occupies an estimated area of 1,978 km^2 and covers part of the Greater Virunga landscape, which comprises of multiple wildlife reserves that include Kyambura Game Reserve, Kibale Forest National Park, Kigezi Game Reserve all in Uganda and Virunga National Park in DRC among others (40). QENP further extends from Lake George in the north-east to L. Edward to the south-west and includes Kazinga channel connecting the two lakes, a range of wetlands and crater lakes (40). The study area is situated in a semi-arid area with mainly two dry and two wet seasons annually. Dry seasons are estimated to be from December to February and June to August while wet seasons are March to May and September to November. The mean annual rainfall varies within the park with a mean of 1,200 mm near the Rift Valley wall and 500 mm around Lakes George and Edward (40).

The area comprises of a diverse range of vegetation types which includes open grasslands, grassland with thickets, thickets and shrubs, forests, wetlands, riverine vegetation species and about 250 km of lakeshore (40). A cross-sectional study was conducted in 12 selected communities which extended in three districts of Kasese, Rubirizi and Rukungiri all bordering the QENP. Some of the communities lie within the national park in about 11 fishing enclaves carrying out mainly fishing activities but keep livestock that they graze illegal in the national park. Other communities that border the national park practice crop growing and livestock keeping (pastoralism).

Collection of tsetse fly samples (Entomological survey)

This minimal entomological survey was conducted in May 2017 which coincides with the rainy season in the study area (Fig 2), to provide baseline data for decision makers after an outcry from the communities that border the national park. During the survey, biconical (n = 56) and F3 traps (n = 23) were set up at an interval of about 200 metres to 250 metres apart in vegetations close to rivers, streams and in savanna rangelands. The sampled points were geo-referenced. Vegetation types in the study area were observed, categorized and recorded around the trap sites and they included; thickets and shrubs, open savannah grass land, marshy riverine woodland, and grassland and thickets. Traps were set in selected communities within and around QENP and left at each site for about 72 hours. The trapped tsetse flies were then retrieved, preserved in 70% alcohol and then transferred to the College of Natural Sciences at Makerere University for identification and storage. Tsetse fly species were identified based on the

morphological characteristics as previously described by Leak et al. (26). Other flie, including biting flies such as *Stomoxys* and Tabanids, were also identified and separated according to their morphological characteristics such as colour, size, proboscis and wing structure (26).

Collection and processing of blood samples

The study utilized blood samples from cattle (n = 126) and goats (n = 319) collected and archived in June 2015. In addition, the study utilized blood samples collected from only cattle (n = 250) between April to May 2017 upon request from farmers who had reported problem of tsetse flies affecting their animals. Simple random sampling was used to select the villages and farms to be sampled. Simple random sampling technique was used to determine the goats and cattle to be sampled in each pastoralist community while in communities with very small livestock numbers less than ten, all the cattle and goats present were sampled. Specifically, blood samples from cattle and goats were collected from the jugular vein after restraining the animal. The samples were collected by veterinarians using a protocol approved by the ethics committee at the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University. Approximately 5 mls of whole blood were collected and stored in a vacutainer tube containing Ethylenediaminetetraacetic acid (EDTA). The vacutainer tubes with whole blood were placed in a cool box containing ice packs and transported to the Research Center for Tropical Diseases and Vector Control laboratory at COVAB. The blood samples were aliquoted into 2 ml serum tubes for storage at -30^{0}C until time for analysis.

Deoxyribonucleic Acid (DNA) extraction and amplification

Stored whole blood was thawed and a 200 µl of whole blood was pipetted into labelled sterile 1.5 ml Eppendorf tubes. DNA was extracted from the whole blood samples using Qiagen blood mini-kit (Qiagen, Duesseldorf, Germany) following the manufacturer's instructions. The eluted DNA (50 µl) was stored at -20^{0}C for long storage or at 4^{0}C for short storage.

Amplification of *Trypanosoma* DNA was performed in a total reaction volume of 12.5 µl PCR reaction mixture containing 0.25 µl of polymerase enzyme (KOD FX Neo, Toyobo, Japan), 2.5 µl of PCR water, 1.25 µl of Deoxynucleoside triphosphates, 6.25 µl of buffer, 2 µl of template and 0.125 µl of each of the primers (ITS1 CF: 5'CCGGAAGTTCACCGATATTG-3' and ITS1 BR 5'TTGCTGCGTTCTTCAACGAA-3') designed to amplify internal transcribed spacer (ITS1) of different *Trypanosoma* ribosomal deoxyribonucleic acid (rDNA) (8). ITS1 TF/BR primers, priming the ITSI of rDNA of different *Trypanosoma* species were expected to generate bands in the regions of 700, 480 and 250 base pairs that correspond to *T. congolense, T. brucei,* and *T. vivax* respectively. Sterile double stilled water (with no template DNA added) was used as the negative control and a positive control, *T.b.b* GVR-35 strain (Molecular laboratory, MUK-COVAB) was used. PCR was carried out in a SimpliAmp® ThermoCycler (Thermofisher, USA) at the following cycling conditions; denaturation step at 94 °C for 5 minutes, 35 cycles of denaturation at 94 °C for 1 minute each cycle, annealing at 60 °C for 1 minute, extension at 72 °C for 1 minute and a final elongation step at 72 °C for 5 minutes. PCR products were electrophoresed in 1.5% agarose gel and

stained with ethidium bromide. The gels were visualized on an ultraviolet trans illuminator following the methods described previously (5).

Data Analysis

Data from laboratory analysis and entomological survey was entered into Microsoft excel spread sheets, cleaned and exported to Statistical Package for Social Sciences (SPSS) (version 20.0) to generate descriptive statistics. Descriptive results were summarized using frequencies and percentages and presented as charts and tables. Spatial analysis was done using Arc GIS version 10.2 to generate tsetse fly distribution maps. The apparent density, which is relative to the type of sampling trap used, was expressed as the average number of tsetse flies caught per trap used per day (FTD) (26). The apparent density was calculated for each trap used (T) and the number of days (D) for which a particular trap was operational (17). Therefore, FTD = F/TxD. If the trap was destroyed or not operation for some reason, then the trap-day was excluded. Post Hoc Analysis was conducted to find out differences in tsetse fly caught per vegetation type. Independent sample T-test was done to analyse the difference in prevalence of *Trypanosoma* species between the sampled cattle and goats in communities around QENP. Analysis of Variance (ANOVA) was used to test the difference in prevalence of trypanosomes among the study communities, cattle breeds, *Trypanosoma* species and vegetation type. The analysis was done at 95% confidence interval and 5% level of significance. All variables in inferential analysis with P-values less than 0.05 were considered significant.

Abbreviations

AAT – African Animal Trypanosomiasis; ANOVA - Analysis of Variance; BT – Biconical traps; COVAB - College of Veterinary medicine Animal Resources and Biosecurity; DNA- Deoxyribonucleic Acid; EDTA - Ethylenediaminetetraacetic acid; FTP – Flies per trap per day; PCR – Polymerase Chain Reaction; PCV – Packed Cell Volume; QENP – Queen Elizabeth National Park; rDNA - ribosomal deoxyribonucleic acid;

Declarations

Ethics approval and consent to participate

The study was submitted to Makerere University's COVAB ethics review board for approval. Ethical clearance approval for human sample collection was provided by the College of Health Sciences, School of Biomedical Sciences, Higher Degrees Research and Ethics Committee (SBS-HDREC) with award file number (SBS-191). A written informed consent was obtained from each of the respondents to participate. Goats and cattle were neither euthanized nor treated after sample collection. All insect samples were kept in sample tubes for future analysis if and when needed.

Consent to publish

Not applicable

Availability of data and materials

All the data generated or analyzed during this study are included in the published article. All the animals from which the blood samples were collected were returned back to the farmers.

Competing interests

The authors declare that they have no competing interests.

Funding

All the work was funded by the International Research Development Center (IDRC) - Canada Ecohealth Chair Grant (Grant No. 107345-001) to IBR. The funder did not have any role in the study design, collection, analysis and the interpretation of data and in drafting the manuscript.

Authors' contributions

AA, JB and IBR conceived the study; AA, SJK, IBR and JS collected tsetse fly and blood samples from the field. AAMK, JB, JS and PV conducted laboratory data analysis. JM, DM, PB and IBR were responsible for data analysis and drafting of the manuscript; DM and IBR were overall coordinate of manuscript writing. All authors have read, edited and approved the submitted version of the manuscript.

Acknowledgments

We would like to thank the Kasese and Rubirizi District Veterinary Offices for the support in collection of tsetse fly and blood samples. In addition, we thank Hellen Kiconco, Yona Kajuna, Henry Mugaya, Joseph Masinde, Ronah Asasira, and Patrick Abila for support in the field during collection of samples.

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Figures

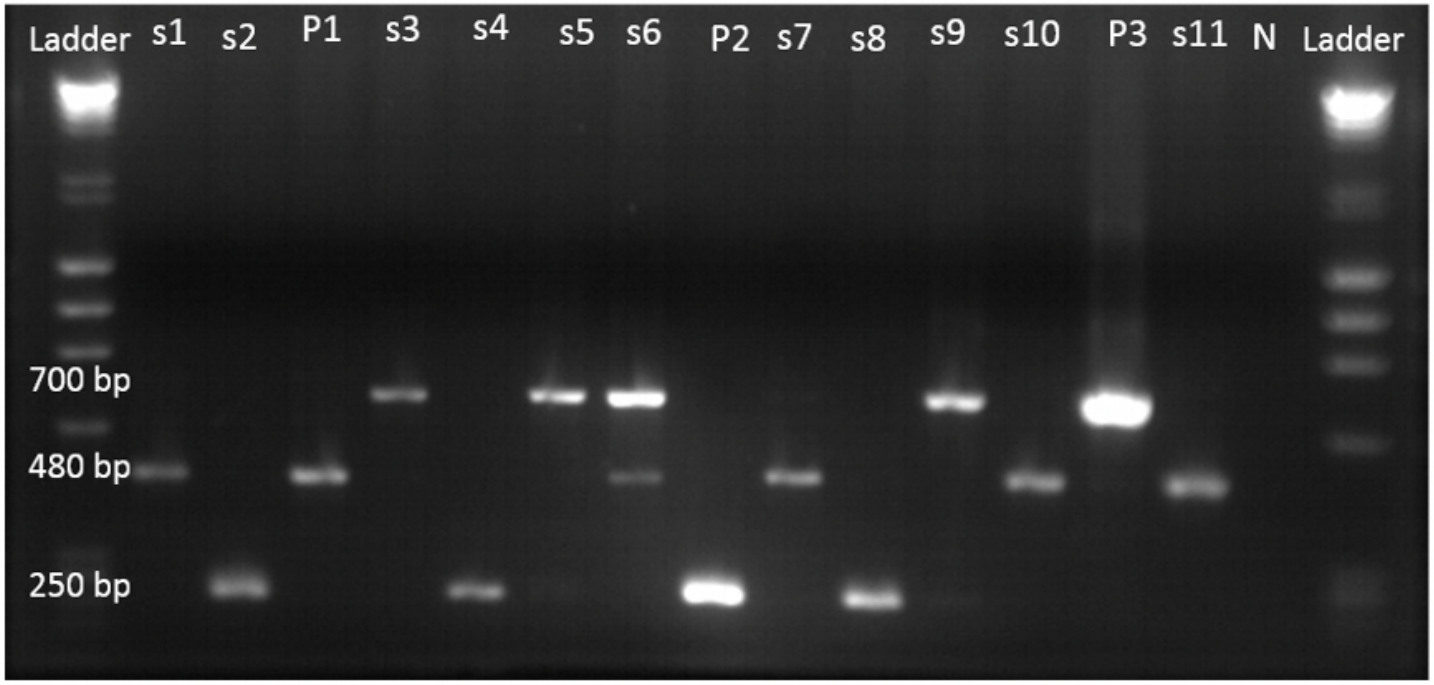

Figure 1

Uncropped Gel for the PCR products of the different Trypanosomes s1 to s11 correspond to test samples positive for trypanosomes and N indicates the Negative control while P1 is positive control for T. brucei, P2; positive control for T. vivax and P3 is positive control for T. congolense . 700bp indicates T. congolense, 480bp indicates T. brucei and 250bp represents T. vivax. Sample (s6) is positive for both T. congolense and T. brucei. DNA ladder (100bp) was used as indicated on the gel above.

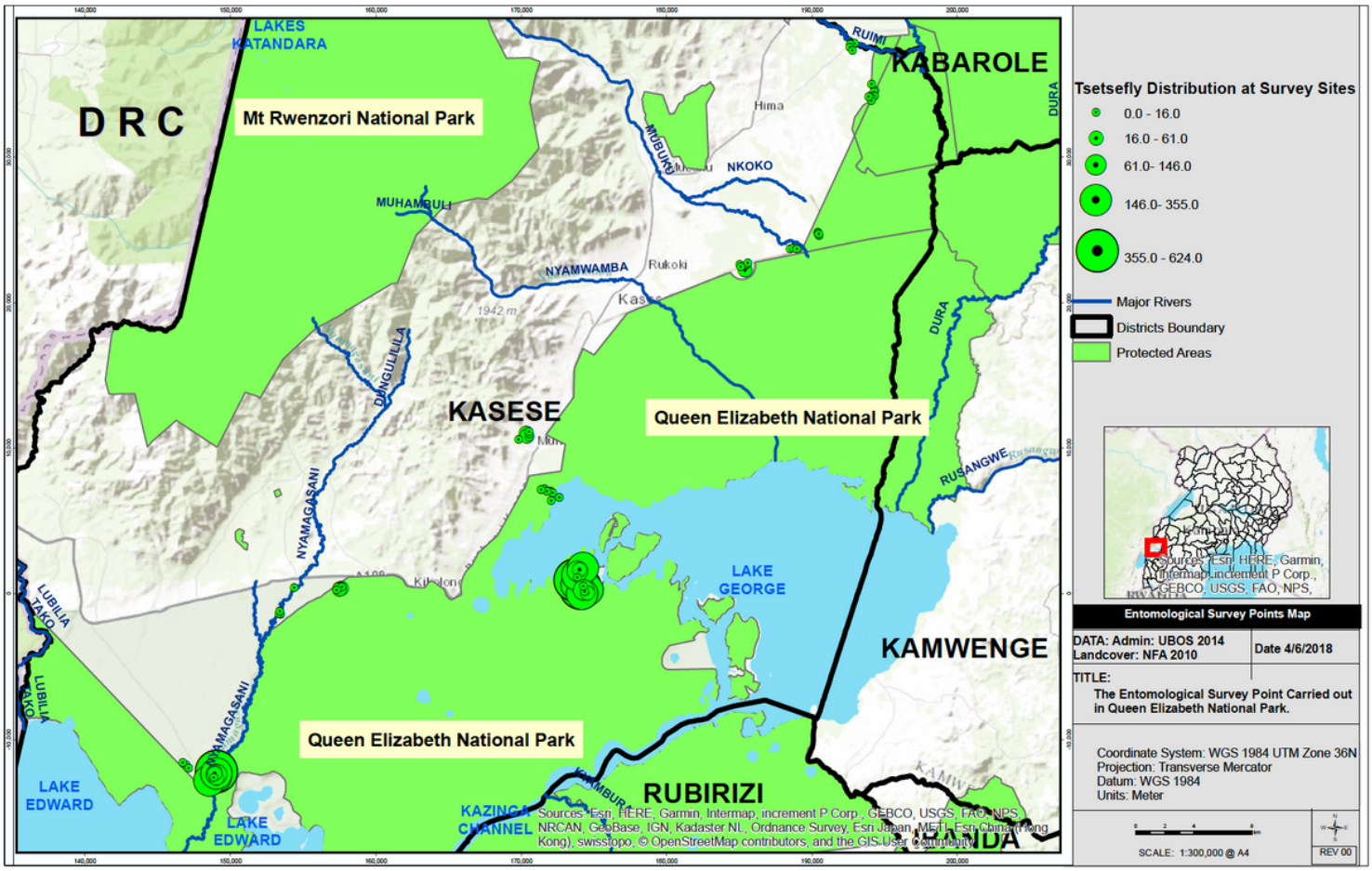

Figure 2

Distribution of Tsetse Flies in and around QENP (Source: Authors). The Datum, UTM, WGS 84, 36N, for presence of tsetse were taken in the field. The tsetse fly numbers at each of the sampling locations were categorized into different ranges using symbology functions in ArcGIS version 10.2 and later shapefiles of different features namely rivers, protected area, and district boundaries were overlaid against the sampling location coordinates to indicate the position of the sampling location in relation to these features. The data was then cleaned and displayed in ArcGIS.