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Original Article

Prevalence of hemoprotozoan parasites in small ruminants along a human-livestock-wildlife interface in western Uganda

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ABSTRACT

Small ruminants are important to community livelihood in developing countries; however information on the role of hemoprotozoan parasites is scanty. The objective of the study was to determine hemoprotozoan parasitic prevalence in western Uganda and identify major areas associated with these infections. This was a cross sectional study conducted at the edge of Budongo Conservation Forest in Masindi district of western Uganda in which 712 small ruminants were sampled. Blood from the jugular vein was collected from caprines and ovines and placed in an EDTA tube, and transported to the laboratory for examination. Thin and thick smears were prepared and examined by microscopy for hemoprotozoan parasites, and DNA was extracted and examined by PCR for *Trypanosoma* spp. A total of 13 villages in Budongo sub-county were surveyed and the study showed that caprines were the major small ruminants of importance to the community. Prevalence of hemoprotozoan parasites was as follows; anaplasmosis (3.65%) > theileriosis (0.45%) > trypanosomiasis (0.15%) and babesiosis (0%) by microscopy. Infections were found in the young with the exception of *Anaplasma* spp. while coinfections of anaplasmosis and theileriosis were high. Molecular analysis showed an overall trypanosome prevalence of 9.27% (PCR), mainly due to *Trypanosoma brucei* and *T. congolense* forest. Villages with trypanosomiasis were found in lowlands and swamps. The current trypanosomiasis prevalence in small ruminants of Uganda was 10 times greater than that previously reported showing that the disease burden has increased overtime within Uganda. A prevalence of 0.14% (95% CI: 0.00, 0.78) for the SRA gene showed that small ruminants would be important reservoirs of infection to humans. Hemoprotozoan parasites are a threat to community livelihood in developing countries and the role of molecular diagnostic techniques in disease monitoring was re-emphasized by this study. Information on primary hosts involved in the propagation of hemoprotozoan parasites in Uganda would help streamline prospective disease surveillance and control efforts.

1. Introduction

Hemoprotozoan parasites are a major limitation to the rearing of livestock, due to their ability to affect livestock productivity (Elsify et al., 2015; Maharana et al., 2016); and developing countries are affected the most due to a lack of appropriate diagnostic and management techniques and presence of specific vectors as compared to farming communities in developed nations (Altay et al., 2012; Aouadi et al., 2017; Maharana et al., 2016). This is because hemoprotozoan parasitic infection has been associated with disruption of the physiological status in different animal species (Cornelius et al., 2014). In developing countries of East Asia and Africa, there is a close relationship between humans and livestock due to the community reliance on livestock for protein, social-cultural status and decision making (Herrero et al., 2013; Randolph et al., 2007). For example, communities use animals as dowry in weddings, land settlements, and sources of food, thus showing their close importance to man in these parts of the world.

The epidemiology of hemoprotozoan parasites has shown that vectors play a key role in the propagation of infection within herds (Maharana et al., 2016). For example, ticks have been implicated in the

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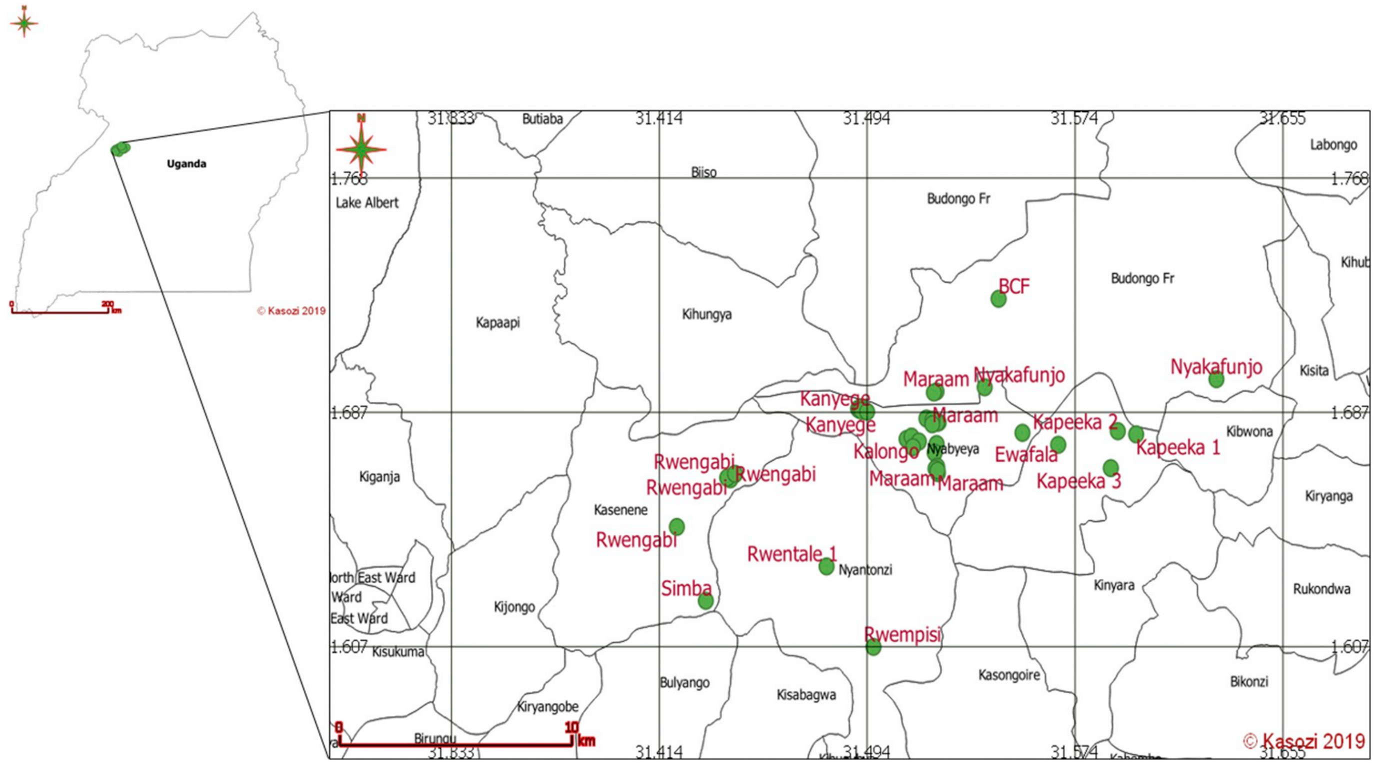

Fig. 1. Map showing study location in Uganda. The villages in the parishes visted are shown in green, all located within Budongo subcounty. Several village points are plotted with name repeats representing consecutive samples collected from households in the same village. Br = Budongo forest, BCF = location of Budongo Conservation Field station relative to the study villages.
In brief, samples were only collected from Kasenene, Nyantonzi, Nyabyeya and Kabango parishes. These parishes lie adjacent to the forest and some homesteads in Nyakafunjo (Nyabyeya parish) had encroached on the forest.

spread of *Babesia spp., Anaplasma spp., Theileria spp.,* and *Ehrlichia spp.* (Ghai et al., 2016). Also, tsetse flies and biting flies (such as stomoxys and tabanids) have been shown to play a crucial role in the propagation of extracellular *Trypanosoma* spp. in livestock (Cnops et al., 2015; Svobodová et al., 2017). In addition, cross species infection especially of livestock close to the wildlife interface makes control of infections difficult (Kabuusu et al., 2013). The role of community farming practices, such as the irrational usage of pesticides, lack of veterinary consultations has subsequently favored the propagation of hemoprotozoan parasitic infections (Kasozi et al., 2014). In Africa, the prevalence of hemoprotozoan parasites has been shown to range from 25.7% in West Africa (Kamani et al., 2010) to 47.4% in East Africa (Kasozi et al., 2014). In Uganda, control of intracellular piroplasms infections in farming communities has been shown to be complicated by the development of acaricide resistance (Vudriko et al., 2016, 2017), while trypanosome resistance to drugs has also been reported (Matovu et al., 2001). In East Africa, the trypanosomiasis prevalence has subsequently been shown to be variable i.e. 0.7% in Uganda (Biryomumaisho et al., 2013), 21.4% in Kenya (Ng'ayo et al., 2005) and 5.1% in Ethiopia (Dinka and Abebe, 2005).

In northeastern Uganda, prevalence of *Anaplasma* (19.5%), *Babesia* (16%) and coinfections were high in small ruminants (Lolli et al., 2016). In southern Uganda, no trypanosomes were detected in small ruminants (caprine), while cattle had a prevalence of 7.6% (Biryomumaisho et al., 2013). In another study carried out at the edge of Kibaale National Forest, *Trypanosoma* prevalence in small ruminants was 0% (microscopy) as compared to 4.3% in bovines (Weny et al., 2017), showing the importance of cattle in these regions. These observations may imply that small ruminants are not important reservoirs of *Trypanosoma*; however, these need to be interpreted with caution, since most of the studies in Uganda were carried out in cattle predominant communities. This is because in northwestern Uganda, trypanosome prevalence in caprines (0.2%) and ovines (5.0%) has already been reported (Balyeidhusa et al., 2012), showing the role of small ruminants in the epidemiology of hemoprotozoan parasites.

Bearing in mind that animal trypanosomiasis has been recognized as a zoonotic disease (Musaya et al., 2017), the role of *T. brucei rhodesiense*, which is common to East Africa and *T. brucei gambesiense* (common to West and Central Africa) (Welburn et al., 2001) has been explored extensively, however information on its presence in small ruminants in East Africa is scarce. Currently, the presence of the serum resistant associated (SRA) gene has been used as a diagnostic tool to differentiate between these two sub species of *T. brucei* (Radwanska et al., 2002), however, there have been limited studies investigating the prevalence of *T. b. rhodesiense* in small ruminants within Uganda.

In Uganda, encroachment by humans and livestock onto national forest reserves and game park is a common threat being faced by the national government (Laudati, 2010) including Masindi district where Budongo Forest is located. Budongo Forest reserve is one of the many wildlife habitats that have been affected by heavy human encroachment activities, and efforts to conserve the fauna in the community are facing serious resistance (Webber et al., 2007). Important fauna that had been common in the forest in the last century were elephants, monkeys, chimpanzee and antelopes, however, these animal species currently exist in the forest in lowered numbers, and some are no longer present (Fairgrieve and Muhumuza, 2003; Hill, 1998; Plumptre and Reynolds, 1996; Tweheyo et al., 2005; Tweheyo and Lye, 2003). In view of the challenges faced by the fauna in the forest, the Budongo Conservation Field Station (BCFS), previously known as the Budongo Forest Project was founded with a primary interest on the chimpanzee community (Mitani, 2006; Newton-Fisher et al., 2002; Tweheyo and Lye, 2005; Waller and Reynolds, 2001). Increased human settlement at the edge of national parks has been shown to be a risk factor for hemoprotozoan parasitic infection in small ruminants (Weny et al., 2017);

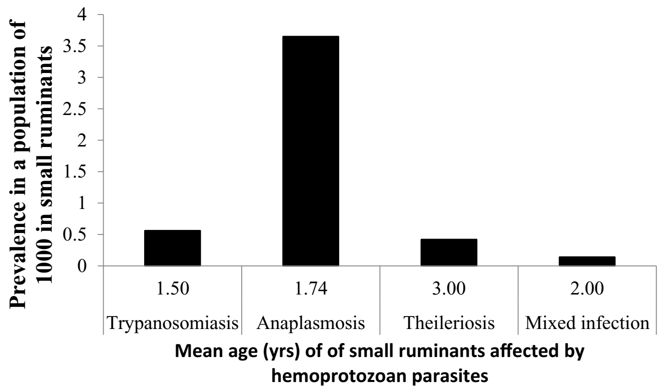

Fig. 2. Relationship of hemoprotozoan parasite prevalence and age of small ruminants.

however information from the farming communities at the edge of the Budongo forest, remained to be established. In addition, Masindi district had been shown to contribute to the ever increasing high hemoprotozoan parasitic burden in livestock of Uganda (Kasozi et al., 2014), however, studies in the small ruminant population in western Uganda are scanty. The objective of the current study was to establish the hemoprotozoan prevalence in small ruminants of Masindi district.

2. Methods

2.1. Study area

The study was conducted in Budongo sub-county of Masindi district in Western Uganda during the month of November 2017, towards the end of the wet season and since we faced funding restrictions, only one period of sampling could be undertaken. Masindi district is located in western Uganda at coordinates 1.4920° N, 31.7195° E and the village locations as well as altitude were taken using a GPS Garmin device at an accuracy of 3 m. The study covered 13 villages, 120 homesteads and 712 livestock. BCFS is located in the heart of Budongo Forest, in Budongo sub-county of Masindi district in Uganda as shown in Fig. 1.

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2.2. Study design

The choice in sampling of ovines and caprines was decided after consulting the local government offices (veterinary officer), since bovines are sparse in the area. A list of villages in the sub-county was acquired from the Budongo sub-county local government veterinary officer, and villages samples were written in Microsoft Excel and villages were picked randomly by typing out their names in Microsoft Excel. These were then assigned a random number, auto-arranged and the first 13 villages were picked; while all animals were selected due to the low household livestock ratio in the community. Strategic mobilization of the communities was conducted in support with the BCFS staff with a goal of promoting synergistic surveillance action with BCFS through on-going disease control efforts in the community. Community local elders and chairpersons where consulted prior to sample collection, and after acquiring informal consent, animal blood samples were collected.

2.3. Ethical approval

This was acquired from the Ethical Review Board at the University

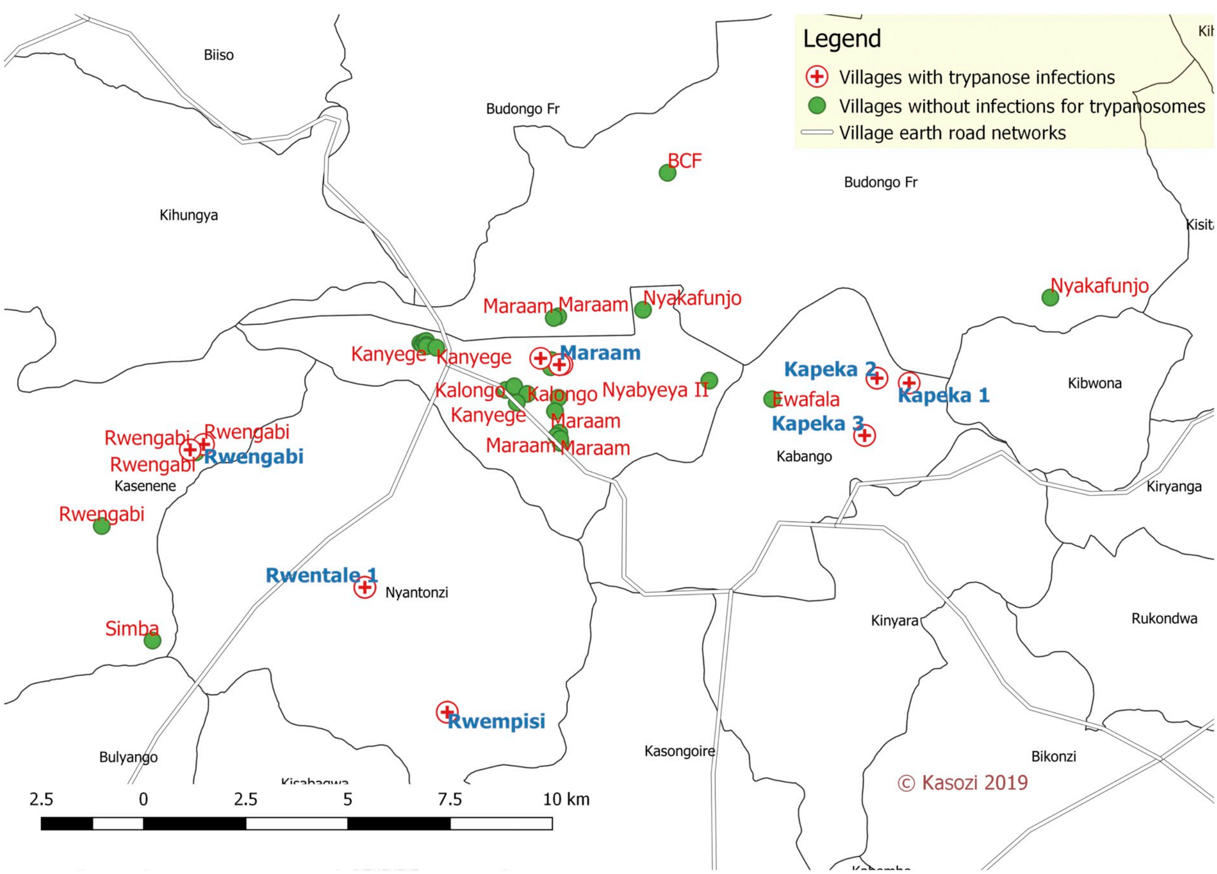

Fig. 3. Villages in which trypanosomes are prevalent within Budongo sub-county. In the community, some households had infections while others didnt and yet these lived in the same villages. This showed that trypanosome occurence was related to household specific practices and not generally diffuse.

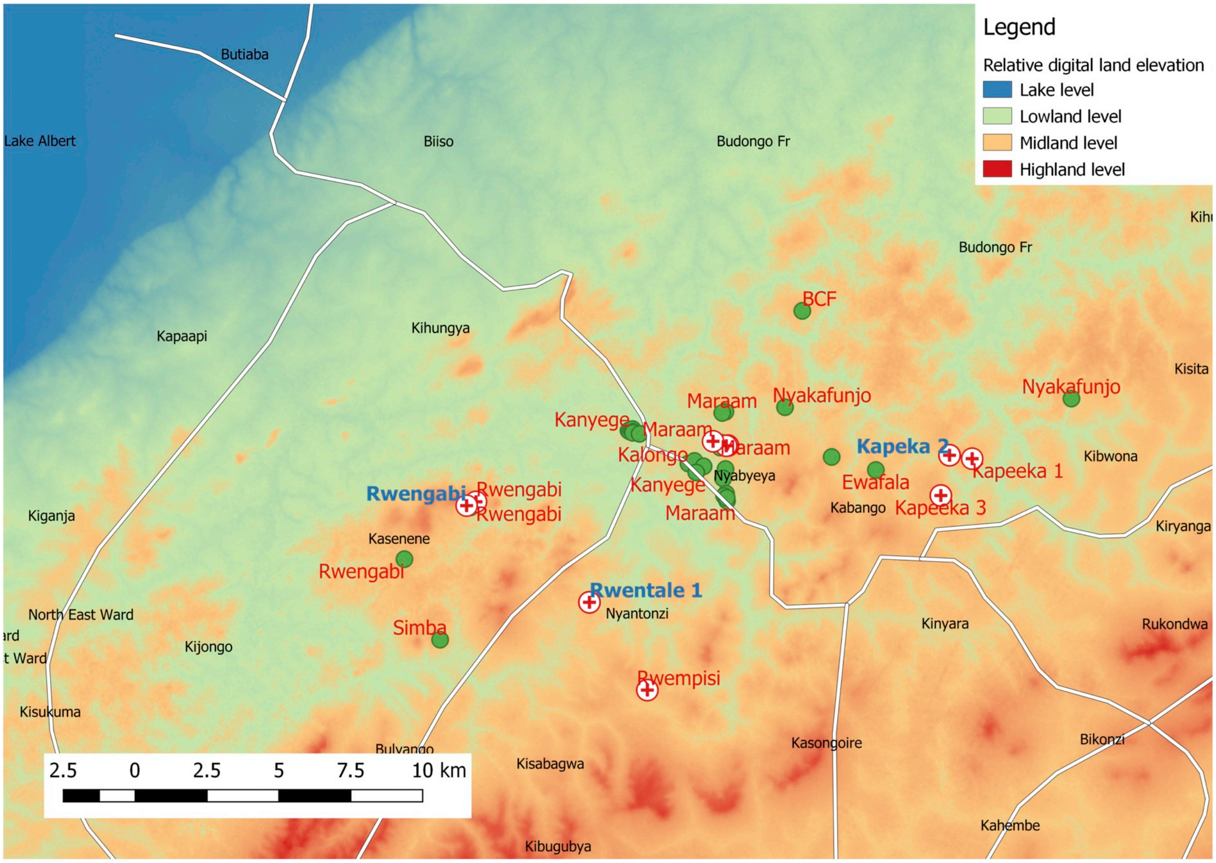

Fig. 4. Trypanosome occurrence in low lands of Budongo sub-county of Uganda.
KEY: Deep brown is high elevation, and light brown is low land. Majority of cases associated with low lands within community. Information acquired from the GPS device showed that in the trypanosome infested villages, the altitude was found to be in the order of Maraam (1084) < Rwentale 1 and Kapeeka 2 (1097) < Kapeeka 3 (1108) < Rwengabi (1110) < Kapeeka 1 (1112) < Ewafula (1121) < Rwempisi (1128). The highest village trypanosome prevalence was reported in Rwentale 1 (23.60%) and Kapeeka 3 (38.71%) villages which are relatively within the same altitude. In addition, both the Rwentale1 and Kapeeka (1, 2, and 3) communities are located within the sugarcane plantations, thus offering a conducive environment for disease transmission due to increased water accessibility in low and midlands.

of Edinburgh and clearance from the Uganda National Council of Science and Technology (UNCST) with reference numbers OS5–17 and A570 respectively. Since the sample size per homestead was found to be low per homestead ($N < 5$), all small ruminants located at each homestead were included in the study.

2.4. Animal blood samples

Blood was collected from the jugular vein of both caprines and ovines that were found in the community. This was then placed in a vacutainer with anticoagulant (EDTA), placed in an ice box, and transported to the laboratory for smear preparation and storage.

2.5. Microscopic examination of blood samples

Using guidelines from the International Organisation for Animal Health (OIE), blood samples were analyzed by microscopy for the major hemoprotozoan parasites (OIE, 2014, 2015). Thick blood smears were prepared by spreading a drop of blood in a circular pattern so that it was transparent enough for one to read across it and this was examined at x400 using a microscope. In addition, thin blood smears were prepared from each blood sample, fixed in absolute methanol for 2 min and stained with 5% Giemsa; this was subsequently rinsed with water. Examination for hemoprotozoan parasites was done at a magnification of ×1000 under oil on a Nikon microscope.

2.6. DNA extraction

DNA from blood samples was extracted using Himedia® HiPura™ blood genomic DNA Miniprep purification spin kit (Jaswal et al., 2014) as described by the manufacturer.

2.7. PCR for trypanosome screening

After preliminary trials, minor modifications were made to optimize the PCR conditions i.e. ddH_20 at 1.35 µl, 2× Ampdirect plus® 5 µl, 10 µM each of forward and reverse Internal transcribed spacer 1 (ITS) of rRNA primers (Gaithuma et al., 2019) 0.3 µl each, 0.05 µl of 5 U/µl Taq polymerase (KAPA®), and DNA template at 3 µl to make a 10 µl reaction volume in each PCR tube for a 1× reaction. In particular, ITS forward and reverse primer sequences used are as shown in Table 3. PCR conditions were as follows; an initial denaturation step of 95 °C for 10 min, followed by 35 cycles of at 94 °C for 30 s, 60 °C for 60 s, 72 °C for 1 min, finally an extension step at 72 °C for 7 min for A-tailing. A 1% gel was used to run the samples with a 100 kb gene ladder, supplied by Wako Nippon Gene®. Band visualization was subsequently done under UV light. PCR conditions as described above were used during the SRA – PCR with an annealing temperature of 68 °C after optimization. Furthermore, a nested PCR for the SRA gene was carried out as described by Matovu and colleagues (Matovu et al., 2010) using the same primers shown in Table 3.

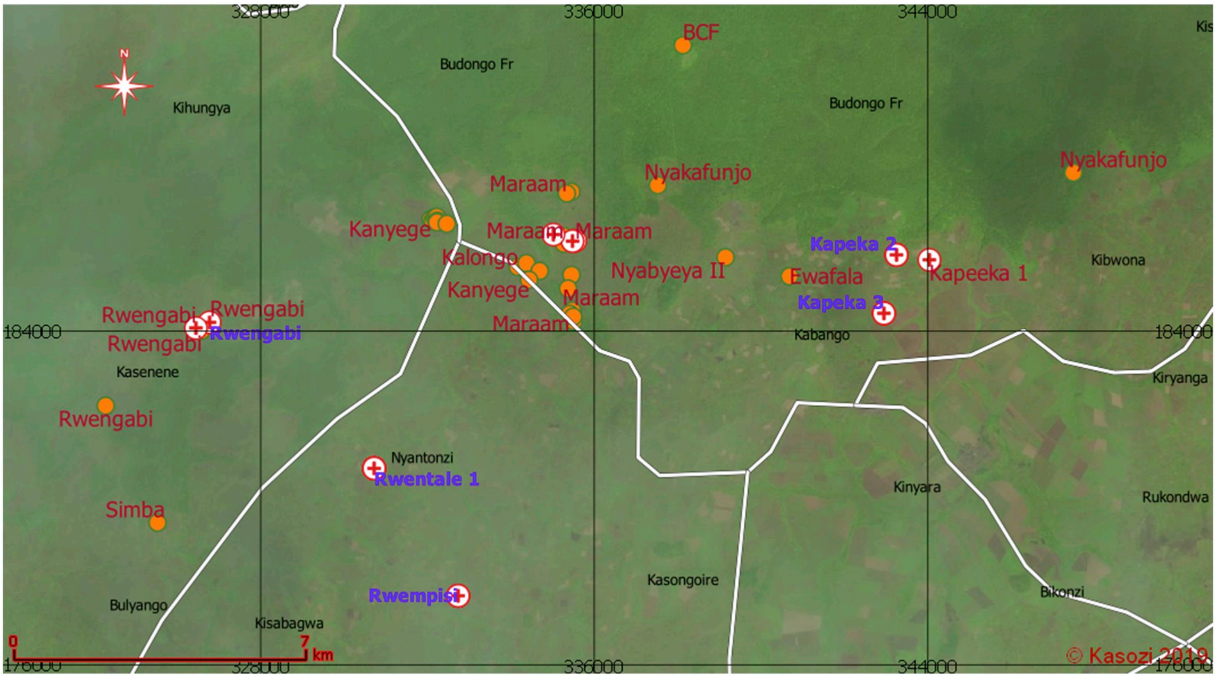

Fig. 5. Trypanosome occurrence and vegetation cover in Budongo sub-county.
Most of the cases were in swampy areas at the forest edge. Furthermore, most of the infected villages are located close to each other probably due to the communal grazing practices, and all animals sampled in Nyantonzi had trypanosomiasis, although these sites were over 3 km from the forest edge. These villages in Nyantonzi are in areas which have been reclaimed from the forest due to the increasing human population at the forest edge. Brown rectangular and circular-conical shades represent households while deep green represents the forest and light green shades are human agricultural activities.

2.8. Identification of trypanosome species

This was done in line with methods on ITS (Njiiri et al., 2015) specific to Trypanozoon (480 bp), *T. congolense* Forest (710 bp), *T. congolense* Savannah (700 bp) *T. congolense* Kilifi (620 bp), *T. vivax* (250 bp), *T. godfreyi* (300 bp), *T. simiae tsavo* (370 bp) and *T. simiae* (400 bp) while SRA primers (Table 3) were used for further identification of Trypanozoons (Gustave et al., 2012).

2.9. Statistical analysis

Data was recorded in Microsoft Excel and descriptive statistics was conducted using WinPepi® to generate the 95% confidence interval and odds ratios for infections. Digital land elevation and land cover satellite images were used to determine risk areas for trypanosomiasis infection in the study area and presented on maps using qGIS® open source software. Using an image acquired from USGS Aster global Dem Satellite image number ASTGM2_N01E031_dem land elevation analysis was done and vegetation cover analysis was conducted by using an image acquired from USGS Sentinel-2 satellite, image number 20171224T091914.

3. Results

3.1. Descriptive of the animal distribution in the study area

A total of 39 village points in 4 parishes of Budongo sub-county of Masindi district were visited during the study period. In particular, 4 village points were visited in Kabango parish and from these, a total of 255 (35.8%) of the animals sampled were caprines (238) and ovines (17). In Kasenene parish, 6 village points were visited and 108 (15.2%) of the animals sampled were mainly caprines (107) and one ovine. In Nyabyeya parish 27 village points were visited and 229 (32.2%) of the animals sampled were caprines (205) and ovines (24). Finally, 2 village points in Nyantonzi parish were visited from which 120 (16.9%) of the animals sampled, 116 and 4 were caprines and ovines respectively as shown in Table 4.

The study also showed that a majority of small ruminants were female (89.9%), and most of these were collected from Kabango parish (35.8%) and significant differences exist between caprines and ovines in sex and parish ($P < .05$). In addition, a majority of the animals were adults (61.0%) as shown in Table 5.

3.2. Hemoprotozoan and trypanosome prevalence in study area

Anaplasma species were found to be prevalent in small ruminants (3.65%) overall and 3.6% in caprines and 4.5% in ovines only, showing that < 1 caprine per 10 small ruminants was at risk of acquiring Anaplasmosis as compared to ovines (aR = −0.9). In addition, *Theileria* and trypanosome prevalence was 0.45% and 0.15% only in caprines by microscopy. Molecular identification showed that 42 small ruminants had single infections i.e. 41 small ruminants with only *T. brucei* (i.e. ovines were over 2 times at risk of infection than caprines, aR = 2.84). Also, one caprine which had only *T. congolense* Kilifi (0.15%), while the rest of the positive isolates were mixed infections of *Trypanosoma* parasites. In addition mixed infections contributed 0.98% to the burden i.e. at 0.3% and 0.7% involving two infections and four infections respectively. Furthermore, *T. congolense* Forest detected in 5/712 and *T. congolense* Kilifi (6/712) were the major *T. congolense* spp. identified as shown in Table 6.

Trypanosoma and *Theileria* infections were found majorly in young animals while *Anaplasma* was common amongst the adults and older small ruminants as shown in Fig. 2.

3.3. Trypanosoma distribution in the study area

The village communities of Kapeeka 1, 2 and 3 were endemically infected with *Trypanosoma* ranging from *T.congolense* forest, *T. congolense* Kilifi, *T. brucei* and *T. godfreyi*. These infections were complicated by the presence of multiple infections as shown in Table 7.

Table 1
Prevalence of hemoprotozoan parasites in small ruminants of East Africa.

Country	Prevalence in small ruminants (references)			
	Theileriosis	Babesiosis	Anaplasmosis	Trypanosomiasis
Uganda	10% in caprines (Weny et al., 2017)	16.00% in caprines and ovines (Lolli et al., 2016)	19.50% in caprines (Weny et al., 2017)	0.7% in caprines (Biryomumaisho et al., 2013)
Kenya	58–71.4% ovines (Ghai et al., 2016)	3.3% in ovines (Khansa, 2017)	80% ovines and 85% in caprines (Ngeranwa et al., 2008)	21.4% in caprines and ovines (Ng'ayo et al., 2005)
Ethiopia	92% ovines and 0.4% caprines (Gebrekidan et al., 2014)	0% in ovines and caprines (Tomassone et al., 2012)	47.4% in caprines and 50% in ovines (Lee et al., 2018)	5.1% in caprines and ovines (Dinka and Abebe, 2005)

In East Africa anaplasmosis is the major tick borne disease while the burden of trypanosomiasis seems to be low amongst small ruminants.

Table 2
Hemoprotozoan parasite prevalence, detection method in major small ruminants of Uganda.

Hemoprotozoan parasites	*Theileria*spp.	*Anaplasma* spp.		*Babesia* spp.	*Trypanosoma* spp.				
Prevalence	10.00%	19.50%	6.00%	16.00%	2.90%	4.40%	0.20%	5.00%	0.00%
Detection method	Microscopy				ITS-PCR		TBR-PCR		Microscopy
Small ruminants	Caprine	Caprine and ovine	Caprine	Caprine and ovine	Caprine and bovine	Caprine and bovine	Caprine	Ovine	Caprine
Region of Uganda	West (Kibaale region)	Northeast (Karamoja region)	West (Kibaale region)	Northeast (Karamoja region)	Southwest (Kasese region)	South (Rakai region)	Northwest (Moyo region)		West (Kibaale region)
Reference	Weny et al. (2017)	Lolli et al. (2016)	Weny et al. (2017)	Lolli et al. (2016)	Biryomumaisho et al. (2013)		Balyeidhusa et al. (2012)		Weny et al. (2017)

KEY: ITS = Internal transcriber spacer, PCR = Polymerase chain reaction, TBR = *Trypanosome brucei rhodesiense.*
In small ruminants of Uganda, the disease burden of hemoprotozoan parasites has been greatest in the following order; Anaplasmosis > Babesiosis > Theileriosis > Trypanosomiasis. In these studies, a majority of samples have been detected through microscopy and PCR which has offer more diagnostic information in the caprine and ovine population. These studies have mainly been conducted all over Uganda i.e. North, East, South, West and Central regions of the country, thus offering a holistic picture of the country status.

Table 3
PCR gene targets with corresponding primer sequences.

Sn.	Gene target	Forward primer sequence	Reverse primer sequence	Band size	Primer reference
1	ITS	5'CGAAAGTTCACCCGATATTGC-3'	5'AGGAAGCCAAGTCATCCATC-3'	Methods by Njiri (Njiiri et al., 2015)	Gaithuma et al. (2019)
2	SRA	5'ATAGTGACAAGATGCGTACTCAACGC-'	5'AATGTGTTCGAGTACTTCGGTCACGCT-3'	363 bp	Gustave et al. (2012)
3	Nested SRA	SRA-out-s (5'-CCTGATAAAACAAGTATCGGCAGCAA-3') and SRA-out-as (5'-CGGTGACCAATTCATCTGCTGCTGTT-3');	SRA-inner-s (5'-ATA GTG ACA TGC GTA CTC AAC-3' GC-3') and SRA-inner-as (5'-AAT GTG TTC GAG TAC TTC GGT CAC GCT-3').	284 bp	Matovu et al. (2010)

In addition, Rwentale 1 village had the highest *T. brucei* prevalence of 23.6%. *Trypanosoma brucei* was the most prevalent (5.76%) species identified in small ruminants, followed by the presence of multiple coinfections (Mixed infection B).

3.4. Disease map showing spatial distribution of trypanosomes in Budongo sub-county

The disease map showed that a majority of trypanosome cases were located in villages that are not immediately adjacent to the forest edge, with the exception of Maraam village as shown in Fig. 3.

All trypanosome cases reported in the study were found to be located mainly in low lands (Fig. 4).

In addition, the infected small ruminants were also found to be located in close proximity to wetlands Fig. 5.

3.5. Prevalence of SRA gene and sensitivity of the diagnostic tests used

Of the 49 positive isolates, only one sample was positive (caprine) by nested SRA-PCR, thus showing a prevalence of 0.14% (95% CI: 0.00, 0.78). Using results in Table 6 on trypanosomiasis diagnosis for microscopy and ITS – PCR, SRA – PCR and nested SRA – PCR, the sensitivity was shown to be low and high respectively as shown in Table 8.

4. Discussion

This study showed that in Masindi district (Fig. 1), the major livestock species in the district are small ruminants and this was in agreement with ruminant distributions in eastern Africa (Table 1 and Table 2). This was because in this particular community, ovines are scarce in comparison to caprines (Table 4 and Table 5), while cattle are close to non-existent due to the low financial status of the community (refugees). In addition, limitation of farming space and pastures implied that homesteads are forced to graze their animals much closer to Budongo conservation forest and practice tethering to avoid these from straying into neighboring gardens (field observation), thus leading to increased human – domestic – wildlife interactions. In addition, tethering animals, has also been associated with high incidence of hemoprotozoan burden due to limited freedom for the animals to escape from the vectors (Siefert and Opuda-Asibo, 1992). In this study, emphasis was placed on hemoprotozoan burden due to its heavy economic importance in Uganda (Kasozi et al., 2014).

Microscopy examination showed that *Anaplasma* was a threat to small ruminant health (Table 6). These observations are in agreement with a previous study although the *Anaplasma* prevalence in the current study (prevalence of 3.6%) was slightly lower lower than the study by Weny et al. (2017) (prevalence of 6.0% in caprines alone). In Uganda microscopic diagnosis of hemoprotozoan parasites is a major practice in several rural veterinary office offices such as one at Budongo Conservation Field Station and this has been instrumental in disease surveillance, however, for effective control of hemoprasites at the human – domestic – wildlife interface, a lot of effort has to be placed on strengthening molecular techniques within the region (Altay et al., 2012; Aouadi et al., 2017; Maharana et al., 2016; Njiiri et al., 2015). A majority of old small ruminants were endemically infected with anaplasmosis while the young had either trypanosomiasis or theileriosis (Fig. 2). These findings are in agreement with the epidemiology of hemoparasites due to their ability to effectively evade the immune system in the weak and immune compromised (Cnops et al., 2015; Ghai et al., 2016; Kabuusu et al., 2013; Maharana et al., 2016; Svobodová et al., 2017). These observations would subsequently make the community control of hemoprotozoan parasites challenging without professional assistance, since farmers in Uganda have been shown to have a low reliance on veterinarians following the liberalization of the animal industry (Kasozi et al., 2014; Read and Parton, 2009). In addition, the presence of coinfections of hemoprotozoan parasites demonstrated in

Table 4
Number of animals sampled in each parish, showing number of village centers covered, mean animal, households ratio in Budongo sub-county of Masindi district in Uganda.

Parishes in Budongo sub-county	Number of village centers covered	Number (percentage) of animal species sampled			Number of households covered	Small ruminants sampled	Households covered	Household livestock ratio
		Caprines	Ovines	Total		Mean ± SEM		
Kabango	4	238 (33.4)	17 (2.4)	255 (35.8)	30	48.50 ± 11.29	7.00 ± 3.19	12.20 ± 5.16
Kasenene	6	107 (15.0)	1 (0.1)	108 (15.2)	16	18.00 ± 6.72	3.50 ± 1.73	6.94 ± 2.05
Nyabyeya	27	205 (28.8)	24 (3.4)	229 (32.2)	45	8.52 ± 1.68	1.59 ± 0.36	5.97 ± 0.60
Nyantonzi	2	116 (16.3)	4 (0.6)	120 (16.9)	29	60.00 ± 29.00	14.00 ± 8.00	6.69 ± 0.76
Total	39	666	46	712	120			

A majority of small ruminants were sampled from Kabango parish although more households were accessible in Nyabyeya parish due to convenience and proximity to BCFS. This showed that small ruminant populations were highest in the order of Nyantonzi > Kabango > Kasenene > Nyabyeya, showing that distribution was highest in hard to reach areas.

Table 5
Description of small ruminants in study Budongo sub-county.

Parameter	Variables	Number (%) of small ruminants			95% CI LL; UL	*P*-value
		Caprine	Ovine	Total		
Sex	Female	603 (84.7)	37 (5.2)	640 (89.9)	87.43; 91.94	.028
	Male	63 (8.8)	9 (1.3)	72 (10.1)	8.00; 12.57	
Parish	Nyabyeya	205 (28.8)	24 (3.4)	229 (32.2)	28.74; 35.73	.004
	Nyantonzi	116 (16.3)	4 (0.6)	120 (16.9)	14.18; 19.81	
	Kasenene	107 (15.0)	1 (0.1)	108 (15.2)	12.61; 18.02	
	Kabango	238 (33.4)	17 (2.4)	255 (35.8)	32.29; 39.46	
Age	Juvenile	260 (36.5)	18 (2.5)	278 (39.0)	35.44; 42.74	.990
	Adult	406 (57.0)	28 (3.9)	434 (61.0)	57.26; 64.56	

Key: CI = confidence intervals, LL = lower limit and UL = upper limit. Chi-square test conducted and significance measured between each parameter and small ruminants at 95% confidence.

Table 6
Hemoprotozoan prevalence by microscopy, major Trypanosomes identified by PCR, risk estimates and odds ratios in small ruminants of Uganda.

Parasites	Number (prevalence) of small ruminants			95% CI of total prevalence LL; UL	RR	aR	Odds ratios	95% CI (LL; UL)
	Caprines	Ovines	Disease		Value			
Microscopy identification								
Anaplasma	24 (3.60)	2 (4.5)	26 (3.65)	2.40; 5.30	0.8	−0.9	0.82	0.19; 7.41
Theileria	3 (0.45)	0 (0.00)	3 (0.42)	0.09; 1.23	∞	0.45	∞	0.03; ∞
Anaplasma&*Theileria* spp.	1 (0.15)	0 (0.00)	1 (0.14)	0.00; 0.78	∞	0.15	∞	0.002; ∞
Babesia	0 (0.00)	0 (0.00)	0 (0.00)	0.00; 0.52	–	–	–	–
Trypanosomes	4 (0.60)	0 (0.00)	4 (0.56)	0.15; 1.43	∞	0.60	∞	0.045; ∞
Negative	634 (95.2)	44 (95.65)	678 (95.22)	93.39; 96.67				
Total	666	46	712	–	–	–	–	–
Small ruminant HPP	32 (4.81)	2 (4.35)	34 (4.78)	3.33; 6.67	1.11	0.46		–
Trypanosome PCR identification								
Mixed infections A	0.30 (2)	0.00 (0)	0.28 (2)	0.03; 1.01	∞	0.30	0.35	0.02; 7.16
Mixed infections B	0.75 (5)	0.00 (0)	0.70 (5)	0.23; 1.63	∞	0.75	0.76	0.043; 13.67
T. brucei	5.86 (39)	8.70 (2)	5.76 (41)	4.16; 7.73	0.67	−2.84	1.35	0.34; 5.40
T. congolense Kilifi	0.15 (1)	0.00 (0)	0.14 (1)	0.00; 0.78	∞	0.15	0.21	0.009; 5.07
Total	666	46	712	–	–	–		–

KEY: The small ruminant HPP (=hemoprotozoan parasitic) prevalence was determined by adding up the sum of the infected animals with hemoprotozoan parasites. Animals defined as infected (positive cases) are those which showed presence of parasites by microscopic determination. Mixed infection A = Two infections of *T. brucei* and *T. godfreyi*; Mixed infection B = Four infections of *T. congolense* forest, *T. congolense* Kilifi, *T. brucei* and *T. godfreyi.* Total number of positive samples N (i.e. 2*A + 5*B + 41 + 1) = 66 positive isolates. Risk estimates made in reference to caprines againstovines. RR = Relative risk, aR = attributable risk. LL = lower limit, UL = upper limit.

the study, shows that disease control efforts have to be broad in target and synergistic to address all pathogens, and calls for increased reliance by the farmers on veterinarians. In northeastern Uganda, a high prevalence of coinfections in small ruminants has been reported (Lolli et al., 2016), showing that the challenge posed by coinfections in the small ruminant population could be of national concern. Efficient and timely management of hemoprotozoan parasitic infections would subsequently lead to decreased pathogenesis in small ruminants (Cornelius et al., 2014), thus supporting community livelihood.

A trypanosome prevalence by molecular detection of 9.27% was established and this was mainly due to *T. brucei* (Table 7). In addition, presence of mixed infections i.e. trypanosomes and tick borne infectious would make control of the disease more expensive and challenging. The prevalence in this study was higher than a previous study carried out by Biryomumaisho et al. (2013) in three Ugandan districts (Kasese, Jinja and Rakai) where 0.7% of goats were found to be infected through the use of the Buffy coat technique. Although Biryomumaisho et al. (2013) conducted molecular analysis i.e. 0.20% and 5.00% in caprines and

Table 7
Trypanosome prevalence in sampled villages of Budongo sub-county in Masindi district of Uganda.

Village	Frequency (%) of trypanosome species isolated						Village trypanosome prevalence	
	Mixed A	Mixed B	Negative	*T. brucei*	*T. congolense* kilifi	Animals in village sampled	Overall N (prevalence)	95% CI: LL; UL
Ewafula	0 (0)	0 (0)	68 (9.6)	0 (0)	0 (0)	68 (9.6)	0 (0.00)	0.00; 5.28
Kanyege	0 (0)	0 (0)	42 (5.9)	0 (0)	0 (0)	42 (5.9)	0 (0.00)	0.00; 8.41
Kapeeka 1	1 (0.1)	0 (0)	26 (3.7)	0 (0)	0 (0)	27 (3.8)	2 (7.41)	0.91; 24.29
Kapeeka 2	0 (0)	2 (0.3)	120 (16.9)	7 (1.0)	0 (0)	129 (18.1)	15 (11.63)	6.66; 18.45
Kapeeka 3	0 (0)	2 (0.2)	25 (3.5)	4 (0.6)	0 (0)	31 (4.4)	12 (38.71)	21.85; 57.81
Karongo	0 (0)	0 (0)	67 (9.4)	0 (0)	0 (0)	67 (9.4)	0 (0.00)	0.00; 5.36
Maraam	1 (0.1)	1 (0.1)	84 (11.8)	1 (0.1)	1 (0.1)	88 (12.4)	8 (9.09)	4.01; 17.13
Nyabyeya 2	0 (0)	0 (0)	14 (2.0)	0 (0)	0 (0)	14 (2.0)	0 (0.00)	0.00; 23.16
Nyakafunjo	0 (0)	0 (0)	18 (2.5)	0 (0)	0 (0)	18 (2.5)	0 (0.00)	0.00; 18.53
Rwempisi	0 (0)	0 (0)	25 (3.5)	6 (0.8)	0 (0)	31 (4.4)	6 (19.36)	7.45; 37.47
Rwengabi	0 (0)	0 (0)	56 (0)	2 (0.3)	0 (0)	58 (8.1)	2 (3.45)	0.42; 11.91
Rwentale 1	0 (0)	0 (0)	68 (9.6)	21 (2.9)	0 (0)	89 (12.5)	21 (23.60)	15.24; 33.78
Simba	0 (0)	0 (0)	50 (7.0)	0 (0)	0 (0)	50 (7.0)	0 (0.00)	0.00; 7.11
Parasitic prevalence	2 (0.28)	5 (0.70)		41 (5.76)	1 (0.01)		66 (9.27)	7.24; 11.64

Key: Mixed infection A = Two infections of *T. brucei* and *T. godfreyi*; Mixed infection B = Four infections of *T.congolense* Forest, *T. congolense* Kilifi, *T. brucei* and *T. godfreyi;* N = number of positives in the village and CI = Confidence intervals.

Table 8
Sensitivity of diagnostic tests used in trypanosomiasis diagnosis in the study.

Diagnostic methods compared	Value (95% CI)	Level of agreement	Difference in sensitivities (*P* value)
Microscopy and ITS-PCR	0.08 (0.46–0.51)	Low	Significant (< .0001)
SRA-PCR and nested SRA-PCR	0.00 (0.0–0.98)	High	Insignificant (1.000)

ovines, this was only done on samples postive through microscopy rather than all goats sampled. The overall trypanosome prevalence through PCR in this study is higher than that reported in Uganda and Ethiopia. In northwestern Uganda, a low trypanosome prevalence had been reported in caprines through microscopy (0.7%) (Balyeidhusa et al., 2012) and molecular analysis was placed on *T. brucei* which showed a prevalence of 3.2% demonstrating the reliability of molecular over microscopic diagnostic techniques. Sensitivity by molecular techniques was found to be high in comparison to microscopy (Table 8), showing that a majority of these cases would be under-diagnosed or under-reported (by the local government) in the absence of robust diagnostic techniques especially amongst small ruminants in Uganda (Kasozi et al., 2014; Ndao, 2009; Weny et al., 2017).

Satellite image analysis showed that the community of Kapeeka and Maraam are in close proximity to a large water mass due to their lowland and midland elevation (Fig. 4) and at the forest edge (Fig. 5), thus presenting ideal conditions for vector disease epidemiology (Morrison, 2011; Ponte-Sucre, 2016). In Kenya, increased rainfall has been associated with high trypanosome prevalence (Griffin and Allonby, 1979), thus supporting observations in the current study on trypanosome epidemiology in these villages. Field observations showed that these communities had the highest number of animals that were thin (BCS = 2/5) in comparison to other surveyed villages (data not presented), and this is characteristic of trypanosomiasis since infection is heavily associated with severe weight loss and anemia (Morrison, 2011).

The SRA gene has been shown to be a diagnostic indicator of *Trypanosoma brucei rhodesiense* which is of public health benefit since it is a major causative agent of human African trypanosomiasis especially within the East African region (Welburn et al., 2001). A prevalence of 0.14% for the SRA gene in small ruminants was approximately 10 times lower in comparison to that reported in Ugandan cattle (Selby et al. 2013). This however provides evidence on the role of small ruminants in the propagation of infectious diseases along the human – domestic – wildlife interface since small ruminants identified in this study may act as reservoirs for *T. b. rhodesiense* in the absence of effective disease control strategies.

5. Conclusions

The high prevalence of hemoprotozoan parasites in this study could potentially limit livestock productivity. In the control of trypanosomiasis, small ruminants should be included in disease control efforts as these play a crucial role in hemoprotozoan epidemiology. In the control of disease spread, a clear balance has to be established while guiding communities on appropriate disease control efforts since water and pasture scarcity often force communities to graze in endemic lowlands. Inability to conduct molecular screening on tick borne parasites due to budget limitations as well as a lack of capacity in Uganda to carry out gene sequencing which would help identify primary hosts associated with infections are major limitations for this study, and these would have to be explored in prospective studies.

Author contributions

All authors contributed equally to this work. K.I·K, J.Y, C·S and E.M designed the study, K.I·K, M.N, A.G.K analyzed the samples, K.I·K, A.G.K, E.M, J.Y, C·S conducted data analysis while K.I·K prepared initial manuscript draft. Finally, K.I·K, M.N, A.K.G, M.M, E.M, J.Y, C·S and E.M reviewed the manuscript and approved it for submission.

Competing interests

None.

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Data availability statement

Information used in the study can be accessed at https://figshare.com/s/a576101152cab18b9ee9

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