# PREVALENCE AND RISK FACTORS ASSOCIATED WITH HEMOPARASITES IN CATTLE AND GOATS AT THE EDGE OF KIBALE NATIONAL PARK, WESTERN UGANDA

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ABSTRACT: Livestock production is a major sector of the Ugandan economy. Ugandan ruminant livestock (principally cattle and goats) are susceptible to hemoparasites that can cause serious clinical disease and production losses. Kibale National Park, in western Uganda, is a protected forest ecosystem surrounded by small-scale farms where cattle and goats are raised. We conducted a cross-sectional study of cattle and goats in this area and diagnosed hemoparasite infections by microscopy. We collected data on animal characteristics and management practices to assess risk factors associated with infection. We studied 186 cattle and 317 goats from 20 villages, including 16 villages directly adjacent to Kibale and 4 villages  $\geq$ 3 km from the park boundary. Hemoparasites detected in cattle and goats were of the genera Theileria, Anaplasma, and Trypanosoma with a prevalence of 15.1%, 1.6%, and 4.3% respectively in cattle, and 10%, 6.0%, and 0.0%, respectively in goats. Trypanosomes infected approximately 8% of cattle in villages bordering Kibale but were never detected in cattle in "control" villages  $\geq 3$  km from the park. Trypanosomes were approximately 7 times more likely to infect animals in households that did not provide veterinary care to their animals than in households that provided routine veterinary care. Within cattle, Theileria infections were approximately 7 times more likely to occur in cross-bred cattle than in indigenous pure breeds. Anaplasma infections were approximately 3.5 times more likely to occur in cattle than in goats (no goats were diagnosed with Trypanosoma infection). These data suggest that proximity to the park, provision of veterinary care, and breed are significant risk factors for hemoparasites in this population of ruminants, and that, in general, cattle are more susceptible than goats.

Hemoprotozoan diseases, especially babesiosis, anaplasmosis, theileriosis, and trypanosomosis, are considered major impediments to the health and production of livestock in Sub-Saharan Africa (Rajput et al., 2005), including Uganda (Kasozi et al., 2014). Generally, hemoparasite infection is thought to result from complex interactions among pathogens, vectors, vertebrate hosts, and the environment (Opiyo, 2011). These interactions are modified by community farming activities (Kasozi et al., 2014). In Uganda, Trypanosoma prevalence is 4 times greater in the western region than in the central region (Kasozi et al., 2014), and prevalence of hemoparasites in cattle varies with agroecological zones and management practices in eastern Uganda (Rubaire-Akiiki et al., 2004). The western region of Uganda contains several national parks, which are foci of biodiversity but also contain sylvatic hosts that serve as sources of infection for livestock (Kabuusu et al., 2013). However, epidemiological studies of how demographic, locational, and environmental factors affect the risk of infection have been rare in Uganda and similar settings.

In East and Central Africa the disease with the highest health impact is East Coast fever (ECF), which is caused by *Theileria parva*, transmitted by *Rhipicephalus appendiculatus* ticks (Muhanguzi et al., 2014). *Anaplasma marginale* and *Anaplasma centrale* have been detected in livestock in East Africa in both small and large ruminant populations; these agents are transmitted by *Boophilus decoloratus* ticks, with mechanical transmission during herd vaccinations also reported (Wesonga et al., 2010). Anaplasdata on its occurrence are limited (Angwech et al., 2011), in part because clinical outcomes range from severe disease to subclinical infection (Uilenberg, 1995). Among trypanosomes, the most important species in cattle are *Trypanosoma vivax*, *T. congolense*, *T. brucei*, *T. evansi*, and *T. equiperdum* (Leak, 1998). Trypanosomiasis also affects sheep, goats, pigs, camels, and buffalo (Tussy Musiime, 1979), although it has been far less well studied in these species. In cattle, the effects of trypanosomiasis vary with breed, strain, and infectious dose of the hemoparasite, with African livestock breeds being relatively resistant (Martins et al., 1995; Morton, 2010).

mosis is endemic in parts of Uganda; however, epidemiological

Recent studies in Uganda have shown that certain wild ungulates in Queen Elizabeth National Park (which abuts the southern end of Kibale), such as the Cape buffalo (*Syncerus caffer*), may act as reservoirs for certain hemoparasites (Kabuusu et al., 2013). Such findings suggest that a "wildlife-livestock interface" may modify the epidemiology of hemoparasite transmission (Kabuusu et al., 2013). Unfortunately, limited access to veterinary diagnostics and molecular data limit the ability to assess the impact of sylvatic infections on domestic livestock (Kasozi et al., 2014). For these reasons, and because of economic constraints, integrated, ecological control programs are generally not in place in Uganda (Kasozi et al., 2014).

In this study, we sought to estimate the prevalence of hemoparasite infection in cattle and goats along the western edge of Kibale, as well as to assess risk factors for infection. We designed the study to look explicitly at proximity to the park (i.e., the wildlife–livestock interface) as a potential risk factor, and simultaneously to examine household- and individual-level factors. The translational goal of the study was to provide information to subsistence farmers in the region through the Ugandan Ministry of Agriculture, Animal Industry and Fisheries,

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and through the District Veterinary Office, so that an ecologically informed management plan might be designed and implemented.

# MATERIALS AND METHODS

#### Study area

Kibale is a 795-km<sup>2</sup> protected area near the foothills of the Rwenzori Mountains (the "Mountains of the Moon") in western Uganda (Chapman et al., 2007). Kibale contains diverse landscapes, from grassland to dense evergreen forest. Kibale ranges in altitude from approximately 1,700 meters above sea level (masl) in the north to approximately 900 masl in the south (Chapman et al., 2007). Kibale is home to mammals, including wild ruminants such as red duiker (*Cephalophus natalensis*), blue duiker (*Philantomba monticola*), waterbuck (*Kobus ellipsiprymnus*), bushbuck (*Tragelaphus scriptus*), sitatunga (*Tragelaphus spekii*), kob (*Kobus kob*), buffalo (*Syncerus caffer*), and elephant (*Loxodonta africana*), as well as wild suids such as warthog (*Phacochoerus africanus*), bush pig (*Potamochoerus larvatus*), and giant forest hog (*Hylochoerus meinertzhageni*) (Kingdon, 1971).

Two predominant tribes, the Batooro and Bakiga, as well as several minor ethnic groups, inhabit the area around the park, with subsistance agriculture and animal husbandry as their principal economic activities. Both indigenous (Ankole/Zebu) and cross-bred cattle (indigenous crossed with Holstein Friesians) are kept in small herds of approximately 1–20 animals, grazed on pasture. Goats are also common. Other less common domestic livestock are sheep, pigs, rabbits, chicken, ducks, and turkeys (Odrek and Karibwije, 2005).

#### Selection of study households

We studied goats and cattle, the 2 most common ruminant livestock species in the area, in 16 villages directly bordering Kibale. We randomly selected households within 500 m of the park boundary from each of the 16 villages. When a randomly selected household had no cattle or goats, another was randomly selected until one with cattle or goats was enrolled. For every 4 villages along the park boundary, we selected a "control" village. Control villages were at least 3 km from the park boundary. This distance was chosen because previous research shows that such villages are socioeconomically and culturally similar to villages bordering the park, but that a 3-km distance is sufficient to "protect" the village from the direct influence of the park, such as from crop raiding by the park's resident wildlife (Naughton-Treves et al., 2011; Goldberg et al., 2012).

We visited each household once between June and December 2013, to collect data and clinical samples from cattle and goats. Variables recorded included sex, age, and breed of the animals, farm characteristics, grazing system, and whether or not the farmer engaged the services of a veterinarian routinely.

# Animal selection and sample collection

Because of uncertainty in determining age, cattle estimated to be younger than 2 yr and goats younger than approximately 6 mo (criteria based on Kugonza et al., 2011) were classified as "juveniles," whereas older animals were considered "adults." Breeds were classified as indigenous (Ankole cattle and Mubende goats) and crosses (Ankole cattle  $\times$  Holstein Friesians and Mubende goats  $\times$  Boer goats from South Africa). Only animals that had been in the study area for more than 3 mo were enrolled. For herds or flocks that had more than 10 animals, we selected and included only 10 animals for physical examination and blood sampling. In this case, animals were sampled to capture the full range of sex, age, and breed present in the herd.

Jugular venipuncture was used to collect 2 ml of blood from each animal using a sterile, disposable needle and evacuated blood collection tube, after disinfecting the collection site using 70% isopropanol. Each sample was then labeled and transported in a cooler with ice packs to a field laboratory for processing. The sample size of animals and households was planned in advance using a power analysis based on an expected prevalence of 10% for mixed hemoparasite infections in Ugandan cattle (Magona and Mayende, 2002) and 28% in Ugandan goats (Ssenyonga et al., 1992).

#### Laboratory analyses

Two thin blood smears were prepared from each blood sample following Emberth (1986) and allowed to air dry before fixation. Smears were fixed in methanol (HEMA Fixative, Thermo Fisher, Waltham, Massachusetts) and air dried. Each slide was then stained with Wrights-Giemsa for 30 min, washed with water, and air dried. Thin smears were observed at 100× under oil immersion with the use of a light microscope (ACCU-SCOPE 3002 Microscope series, New York Microscope, Inc., Hicksville, New York). Each slide was scanned for hemoparasites by a single trained observer (G.W.), and 50 fields from each stained slide were examined under microscope (×100) for identification of hemoparasites at genus level (Warhurst and Williams, 1996).

# Data analyses

Analyses were performed with GraphPad Prism (GraphPad Software, Inc., La Jolla, California). Prevalence was calculated as the proportion of infected animals, based on microscopic evaluation of blood, with 95% confidence intervals generated using the modified Wald method (Agresti and Coull, 1998). Odds ratios and Fisher's exact tests were used to examine associations between hemoparasite occurrence and location, age, sex, breed, grazing system, and veterinary care. Multivariate logistic regression was planned for examining the individual and interactive effects of risk factors on infection, but such analyses proved unnecessary (see below).

#### RESULTS

A total of 186 cattle and 317 goats were sampled from 40 households in 16 villages within 500 m of the park boundary, and from 15 households in 4 control villages  $\geq$ 3 km from the park boundary (Table I). The overall prevalence of hemoparasitemia was 30.0% in cattle (12.9% in communities at the park edge and 3.7% in control communities) and 16.4% in goats (12.9% in communities at the edge and 2.2% in control communities) (Table II). Prevalence of specific genera of hemoparasites in cattle was *Theileria*, 15.1%; *Trypanosoma*, 4.30%; and *Anaplasma*, 1.61%, and prevalence in goats was: *Theileria*, 10.4%; *Trypanosoma*, 0.0%; and *Anaplasma* 5.68% (Table II).

Cattle were more likely than goats to be infected with *Theileria*, whereas goats were more likely than cattle to be infected with *Anaplasma* (Table III). Cattle in households bordering the park

TABLE I. Characteristics of cattle and goats included in the study.

		Frequency (%)					
Factor	Value	Cattle $(n = 186)$	Goats (n = 317)				
Sex	Male	43 (23.1%)	61 (19.2%)				
	Female	143 (76.9%)	256 (80.8%)				
Age	Adults	111 (59.7%)	242 (76.3%)				
-	Juveniles	75 (40.3%)	75 (23.7%)				
Breed	Cross	112 (60.2%)	52 (16.4%)				
	Indigenous	74 (39.8%)	265 (83.6%)				
Location*	<0.5 km	124 (66.7%)	255 (80.4%)				
	>3 km	62 (33.3%)	62 (19.6%)				
Grazing system <sup>†</sup>	Communal	147 (79.0%)	78 (24.6%)				
	Restricted	39 (21.0%)	239 (75.4%)				
Veterinary care	None	69 (37.1%)	151 (47.6%)				
2	Routine	117 (62.9%)	166 (52.4%)				

\* Distance to the Kibale National Park boundary.

<sup>†</sup> Communal: animals were grazed on common pasture. Restricted: animals were tethered or grazed in fenced pastures.

had higher prevalence of *Trypanosoma* than cattle from control villages  $\geq$ 3 km from the park boundary, where no *Trypanosoma* infections were observed at all (Table III). *Trypanosoma* was not detected in goats in any location. Mixed infections were observed in 5 cattle; 3 cattle were infected with both *Trypanosoma* and *Theileria* and 2 cattle were infected with both *Theileria* and *Anaplasma*. In goats, only 1 case of mixed infection of *Theileria* and *Anaplasma* was detected.

The odds of *Theileria* infection in cattle were significantly higher in cross-bred cattle than in indigenous breeds (OR = 6.8, Fisher's exact P < 0.05; Table III). Sex, age, location, grazing system, and routine veterinary care were not significantly associated with *Theileria* infection in cattle (Table III). However, cattle that received no routine veterinary care had higher odds of infection with *Trypanosoma* than did cattle that received routine veterinary care (OR = 13.1, Fisher's exact P < 0.05; Table III). As with *Theileria* infection, sex, age, breed, and grazing system were not significantly associated with *Trypanosoma* infection in cattle (Table III). No factor measured was associated with *Anaplasma* infection in cattle (Table III). Similarly, no factor measured was significantly associated with *Theileria* or *Anaplasma* infection in goats (Table IV).

# DISCUSSION

Our data show overall hemoparasite infection prevalences of 30.0% in cattle and 16.4% in goats. These estimates are higher than reported by Magona and Mayende (2002), who estimated a 10% prevalence of hemoparasite infection in Ugandan cattle. However, our estimate for goats is lower than that reported by Ssenyonga et al. (1992), who documented 28% prevalence in southwestern Uganda. These differences are minor, however, and our study generally agrees with former assessments that hemoparasite infections are frequent in Ugandan livestock.

Our data support our main hypothesis that the wildlifelivestock interface is a high-risk area for hemoparasite infection. Cattle in villages bordering park had higher odds of infection with Trypanosoma than cattle in villages  $\geq 3$  km from the park boundary, where Trypanosoma infection was not detected at all. Although the mechanisms for this difference remain unclear, these results concord with findings from Morrison et al. (1981), who documented high rates of Trypanosoma infection in wildlifelivestock interface habitats, with the ultimate effect of the virtual exclusion of cattle from forested areas. Similarly, Gachohi et al. (2009) found that geographical variation in prevalence of Trypanosoma in cattle may reflect differences in management, such as regular use of trypanosome prophylaxis, breed resistance to parasites, and the distribution of tsetse fly vectors. In our setting, the park edge is associated with the presence of wild ungulates (e.g., buffalo) that can serve as reservoir hosts for Trypanosoma (van den Bossche et al., 1999; Kabuusu et al., 2013), suggesting wildlife-to-livestock transmission. However, we cannot rule out indirect environmental effects, such as locational differences in nutritional quality of forage or vector habitat suitability.

TABLE II. Prevalence (%) of hemoparasites in cattle and goats, stratified by individual and herd-level factors.

Factor			Cattle $(n = 186)$	Goats $(n = 317)$		
	Value	Theileria	Anaplasma	Trypanosoma	Theileria	Anaplasma
Age	Juvenile	17.3	2.7	0.9	9.3	8.0
-	Adult	13.5	0.9	6.3	10.7	5.4
Sex	Male	20.9	0.0	2.3	6.6	11.5
	Female	13.3	2.1	4.9	11.8	4.9
Breed	Indigenous	4.1	0.0	4.1	9.4	5.7
	Cross	22.3*	2.7	4.5	15.4	7.7
Location <sup>†</sup>	<0.5 km	15.3	0.8	6.5*	11.4	5.9
	$\geq$ 3 km	14.5	3.2	0.0	6.5	6.5
Grazing system <sup>‡</sup>	Communal	15.0	2.0	4.8	14.1	7.7
	Restricted	15.4	0.0	2.6	9.3	5.5
Veterinary care	Routine	12.0	1.7	0.9	9.6	7.8
-	None	20.1	1.4	10.1*	11.3	4.0
Total		15.1	1.6	4.3	10.4	5.7

\* Statistically significant at P < 0.05.

† Distance to the Kibale National Park boundary.

‡ Communal: animals were grazed on common pasture. Restricted: animals were tethered or grazed in fenced pastures.

Factor Va			Theileria		Anaplasma			Trypanosoma					
	Value	Pos (%)	OR	95% CI	P value	Pos (%)	OR	95% CI	Р	Pos (%)	OR	95% CI	P value
Sex	Male	20.9	1.7	0.7-4.1	0.32	nil	0.5	0.02-11.0	0.61	2.3	0.4	0.05-3.86	0.82
	Female	13.3				2.1				4.9			
Age	Adult	13.5	0.7	0.3-1.7	0.30	0.9	0.3	0.02-3.7	0.71	6.3	4.9	0.6-41.3	0.20
-	Juvenile	17.5				2.7				0.9			
Breed	Cross	22.3	6.8	1.9-23.5	0.0003	2.7	4.1	0.2-82.5	0.70	4.5	1.1	0.2-5.8	0.60
	Indigenous	4.1				0.0				4.1			
Location <sup>†</sup>	<0.5 km	15.3	1.1	0.5-2.5	0.54	0.8	0.2	0.02-2.7	0.52	6.5	*	1.1-infinite	< 0.05
	>3 km	14.5				3.2				0.0			
Grazing system <sup>‡</sup>	Communal	15.0	1.0	0.4-2.6	0.60	2.0	1.6	0.1-33.1	0.69	4.8	2.2	0.3-18.1	0.82
	Restricted	15.4				0.0				2.6			
Veterinary care N	None	20.1	1.9	0.8-4.2	0.09	1.4	0.8	0.1-9.5	0.69	10.1	13.1	1.6-108.9	0.004
-	Routine	12.0				1.7				0.9			

TABLE III. Risk factors associated with hemoparasite infection in cattle near Kibale National Park, Uganda.

\* Undefined.

† Distance to the Kibale National Park boundary.

‡ Communal: animals were grazed on common pasture. Restricted: animals were tethered or grazed in fenced pastures.

Our data also indicate that cattle receiving no routine veterinary care were more prone to Trypanosoma infection than those receiving routine veterinary care. This finding is consistent with current Ugandan livestock husbandry practices and agricultural policy, in which decisions about whether or not to prevent or treat infections are made at the level of the household (mostly based on economic considerations) and larger, coordinated preventive veterinary medical efforts are rare. Common veterinary interventions in this region consist of antibiotics, antiprotozoan agents, and acaricides (Kasozi et al., 2014). Ugandan livestock herds also likely contain chronic carriers, and inconsistent chemotherapeutic interventions may facilitate drug resistance (Angwech et al., 2011). This situation is worsened by limited resources available to local farmers (Naughton-Treves et al., 2011). These observations underscore the need for uniform regulations for livestock hemoparasite control in Uganda, as well as for improved diagnostics (Kasozi et al., 2014) and better regulation of drugs (Okello-Onen et al., 1998).

Our data also show a significant effect of breed on infection with *Theileria*, with cross-bred cattle nearly 7 times more likely to be infected than indigenous breeds. A similar study done by Norval et al. (1992) revealed that African Short horn Zebu (Nkedi) and other African local breeds were relatively resistant to ticks and tick-borne hemoparasite infections (as compared to exotic and cross-bred animals) and, when infected, were less likely to develop clinical disease. Similarly, Siddiki et al. (2010) showed that a lower prevalence of hemoparasite infections in indigenous cattle compared to cross-bred cattle reflected higher exposure rates and impaired acquired immunity in crosses.

The higher prevalence of infection of goats with *Anaplasma* than cattle in this study differs from findings by Angwech et al. (2011), who showed that species was not a significant predictor of infection with *Anaplasma*. Nevertheless, *Anaplasma* infections in goats in Sub-Saharan Africa are generally high and in line with our estimates (Bell-Sakyi et al., 2004). We were unable to determine the species of *Anaplasma* infecting cattle and goats in our study; *Anaplasma ovis* in goats and *Anaplasma marginale* 

TABLE IV. Risk factors associated	with hemoparasite infection in go	bats near Kibale National Park, Uganda.
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Factor	Value	Theileria				Anaplasma			
		Pos. (%)	OR	95% CI	P value	Pos. (%)	OR	95% CI	P value
Sex	Male Female	6.6 11.8	0.5	0.2–1.6	0.39	11.5 4.9	2.5	0.9–6.6	0.13
Age	Adult Juvenile	10.7 9.3	1.2	0.5–2.8	0.72	5.4 8.0	0.7	0.2–1.8	0.56
Breed	Cross Indigenous	15.4 9.4	1.7	0.7–4.1	0.15	7.7 5.7	1.4	0.4–4.4	0.40
Location*	<0.5 km >3 km	11.4 6.5	1.9	0.6–5.5	0.18	5.9 6.5	0.9	0.3–2.8	0.99
Grazing system <sup>†</sup>	Communal Restricted	14.1 9.3	1.6	0.7–3.5	0.31	7.7 5.5	1.4	0.5–3.9	0.63
Veterinary care	None Routine	11.3 9.6	1.2	0.6–2.4	0.77	4.0 7.8	0.5	0.2–1.3	0.23

\* Distance to the Kibale National Park boundary.

† Communal: animals were grazed on common pasture. Restricted: animals were tethered or grazed in fenced pastures.

would be expected, however (De Waal, 2000), and may have different ecologies of transmission.

Because the light microscopic methods used in this study were conducted in the field, where only microscopy was available, it proved difficult to identify hemoparasites to the species level. Different species of hemoparasite may have different ecologies of transmission, as mentioned above, but more detailed work (e.g., molecular identification) would be required to detect such differences (Muwanika et al., 2016), although this was beyond the scope of the current study. Also, we did not identify an epidemiological mechanism to explain our finding of an effect of proximity to the National Park on the prevalence of *Trypanosoma* in cattle. Although the concept of the wildlife–livestock interface suggest transmission from wild ruminants as a possible mechanism, so too are alternative mechanisms such as locational differences in vector habitat suitability, host genetics, or husbandry practices.

Regardless of mechanism, the hemoparasite genera *Theileria*, *Anaplasma* and *Trypanosoma* infect cattle and goats in western Uganda at high rates, and cattle along the edge of park are most frequently infected, especially if they are cross breeds that do not receive routine veterinary care. We recommend that control programs begin by targeting these risk factors. We also recommend targeted educational efforts in areas where special control measures (e.g., vector control programs, wildlife management programs, alternative husbandry practices) might be most beneficial.

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