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Food-borne pathogens at the livestock-wildlife-human interface in rural western Uganda

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Running head: Zoonotic food-borne pathogens in rural Uganda

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Conflict of Interest

The authors declare that they have no conflict of interest.

Title

Food-borne pathogens at the livestock-wildlife-human interface in rural western Uganda

Abstract

Foodborne pathogens are an important cause of morbidity and mortality worldwide. To assess the presence of *Salmonella*, *Campylobacter* and *Arcobacter* spp. in livestock, wildlife, and humans from different regions across western Uganda, 479 faecal samples were tested by PCR. *Salmonella* and *Campylobacter* spp. were more frequently detected in livestock (5.1% and 23.5%, respectively) compared to wildlife (1.9% and 16.8%, respectively). Wildlife from remote areas showed lower *Salmonella* and *Campylobacter* spp. occurrence than in areas where interactions with livestock are common, suggesting that spill-over may exist from livestock or humans. Further studies are needed to better understand the transmission dynamics of these pathogens at the wildlife-livestock-human interface in western Uganda.

Foodborne pathogens are an important cause of morbidity and mortality worldwide, threatening both human health and economic growth. *Salmonella* and *Campylobacter* spp. are important foodborne pathogens in developing countries, which are transmitted to humans through food contamination, but also by direct contact with animals, including wildlife, and by contaminated environment (Havelaar et al. 2015). In the recent years, *Arcobacter* species (Family *Campylobacteriaceae*) have been identified as emerging foodborne pathogens throughout the world in animals and humans (Ramees et al. 2017).

Despite important advances in the understanding of enteric pathogens' transmission, the presence of these infectious agents and their transmission dynamics in the African continent is still poorly understood (Penakalapati et al. 2017). In East-Africa, livestock is considered a common source of *Salmonella* and *Campylobacter* spp. to people (Thomas et al., 2020). However, the environmental reservoirs (soil and water sources) and transmission pathways of these pathogens among livestock, wildlife, and humans are poorly understood. Moreover, there are no studies in East Africa that assessed the presence and transmission of *Arcobacter* spp..

The aims of this study are to compare the presence of *Salmonella* spp., *Campylobacter* spp. and *Arcobacter* spp. among domestic animals, wildlife, and humans, in six rural areas from western Uganda with different levels of interaction.

Faecal samples were collected between 2015 and 2018 from six areas of western Uganda (Figure 1), namely: Mgahinga Gorilla National Park (MGNP; 1°22′10″S, 29°38′25″E), Murchison Falls National Park (MFNP; 2°11′15″N, 31°46′53″E), Budongo Central Forest Reserve (BCFR; 1°43′27″N, 31°32′45″E), Queen Elizabeth National Park-Northern sector (QENP-N; 0°8′14″S, 30°02′28″E), Queen Elizabeth National Park-Southern sector (QENP-S; 0°33'00"S, 29°53'00"E), and Hoima District (HD; 1°25′55″N, 31°21′09″E). The areas sampled in this study are part of the Albertine Rift and represent different levels of interaction between livestock and wildlife, classified as high, medium, low and no interaction (Supplementary

material). We defined high interaction when there is high density of human population in the area with settlements surrounding the park and there are no physical barriers separating protected areas (MFNP southern sector and QENP-N); medium interaction when density of human population is high but there are permeable barriers or when density of human population is medium to low but interactions occur regularly (MGNP, BCFR); low interaction when human population density is low but there are no physical barriers separating the protected area (QENP-S); no interaction when there is only presence of livestock (HD) or wildlife (MFNP northern sector) within or around the sampling area.

All the field and laboratorial procedures were authorized by the Uganda Wildlife Authority (UWA) (EDO/35/01 and COD/96/02), the Higher Degrees Research Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity (SBLS/HDRC/20/011), and the School of Biomedical Sciences Research and Ethics Committee (SBS-REC-824). Overall, 479 fresh faecal samples were collected from domestic, wild animals, and humans: 139 cattle, 59 goat, 30 sheep, 27 chicken, 118 African buffalo (*Syncerus caffer*), 53 Uganda kob (*Kobus kob thomasi*), 19 African elephant (*Loxodonta africana*), 19 Anubis baboon (*Papio anubis*), and 15 humans (Table 1). Faecal samples were obtained by purposive sampling in both wildlife and livestock, selecting sampling points representative of each study area and rural communities surrounding natural protected areas. Finally, human stools were collected in QENP-N from herd owners who voluntarily provided the samples. Fresh faecal samples were collected immediately after defecation, using sterile sample bags. All samples were maintained at 4-8ºC for 24-48h during transportation and were stored at -20ºC at the laboratory until analyses.

DNA extraction of stool specimens was performed using QIAamp DNA Stool Mini Kit® (QIAGEN, Germany), according to the manufacturer's instructions. The presence of

Salmonella, *Campylobacter* and *Arcobacter* spp. was determined by PCR, using previously reported primers and PCR conditions (Table 2). *Campylobacter*-positive samples were screened to identify *Campylobacter* species using species-specific primers (Table 2). *Salmonella*-positive samples were further screened to identify serovars Enteritidis and Typhimurium (Table 2).

To assess differences of *Salmonella*, *Campylobacter* and *Arcobacter* spp. detection between study areas and animal species, and pathogen co-ocurrence, Pearson's Chi-squared test χ^2 (with Yates' correction when appropriate) was performed using the R software (R Core Team 2021). Bonferroni post-hoc tests were also conducted to assess the relationship between study areas and *Campylobacter* and *Salmonella* spp. detection. *p*-Values lower than 0.05 were considered statistically significant. The map was done with QGIS (QGIS Development Team 2022).

The sample prevalence was higher in livestock (*Salmonella* spp. 5.1%, $CI_{95\%}$ 2.7-8.6; and *Campylobacter* spp. 23.5%, $CI_{95\%}$ 18.5-29.2) compared with wildlife (*Salmonella* spp. 1.9%, $CI_{95\%}$ 0.5-4.8; and *Campylobacter* spp. 16.8%, $CI_{95\%}$ 12.0-22.5), but differences were not statistically significant. However, *Campylobacter*'s detection in wildlife samples was significantly lower in MFNP northern sector than in areas categorized with high wildlife-livestock interaction ($\chi^2 = 33.1$, df = 3, $p = 0.03$).

Salmonella spp. was detected in 18 samples (3.8%; $CI_{95\%}$: 2.2-5.9) and was not associated with any specific host species (Table 1). However, HD had a significant highest percentage of samples positive to *Salmonella* spp. (22.7%; $CI_{95\%}$: 7.8-45.4) compared to areas with medium and low interaction ($\chi^2 = 27.9$, df = 5, $p < 0.001$). Among the *Salmonella*-positive samples, serovars could only be identified in two samples as *Salmonella enterica* serovar Typhimurium, belonging to one cow and a herder from HD and QENP-N, respectively.

Campylobacter spp. was detected in 99 samples (20.7%; $CI_{95\%}$ 17.1-24.6) from all the domestic and wild species sampled, except elephants. Significant higher sample frequency was found in sheep (53.3%), goats (37.3%), kobs (30.2%) and humans (26.7%), compared to Anubis baboon (15.7%), cattle (15.1%), buffalo (13.6%) and chicken (3.7%) (χ^2 = 48.9, df = 8, p < 0.001). *Campylobacter* spp. detection was significantly different between study areas (χ^2 = 29.4, df = 5, p < 0.001), being higher in MGNP (35.1%; $CI_{95\%}$: 26.4-44.6) (Table 1). Five *Campylobacter* species were identified from 17 *Campylobacter*-positive samples: *C. jejuni* (6 cattle and 1 sheep), *C. lari* (1 cattle), *C. lanienae* (1 cattle and 6 goat), *C. fetus* (1 goat) and *C. hyointestinalis* (1 cattle).

Sixteen of the 18 *Salmonella*-positive samples were also positive to *Campylobacter* spp., but co-occurrence of both pathogens was not statistically significant. All samples analysed were negative to *Arcobacter* spp.

The present study assesses a wide geographic distribution of both *Salmonella* and *Campylobacter* spp. in wildlife and livestock from remote areas in western Uganda at the Albertine Rift Valley. Agricultural changes and the expansion of livestock production, especially in proximity to wildlife habitats, creates attraction points for both domestic animals and wildlife, facilitating pathogens' spill-over and zoonotic disease emergence at the livestock-wildlife-human interface (Caron et al. 2013). In this sense, the study areas that had a more likely interaction between livestock and wildlife (MGNP, MFNP and QENP-N) also had a higher frequency of *Salmonella* and *Campylobacter* spp. in wildlife. This result suggests that livestock might be the primary reservoir hosts for these food-borne pathogens, but also highlights that spill-over into wildlife probably occurs around natural protected areas in western Uganda.

The detection of *Salmonella* spp. in human wastewater treatment plants, slaughterhouses and farms has been widely reported in Uganda (Ikwap et al. 2014; Afema et al. 2016). The frequency detection of *Salmonella* in livestock samples from was lower

compared with previous data on different African countries that ranged from 3.4 to 13.9% (Thomas et al. 2020). This may be caused due to different factors such as differences in herd size (<50 individuals in our study), different contacts between herds or local differences in transmission due to presence and distribution of aggregation points such as water sources or grazing areas (Caron et al. 2013). The finding of the serovar Typhimurium in the present study, in one cattle and one human sample, together with the reports in pigs (Ikwap et al. 2014), and amongst severely malnourished children with bacteraemia in Uganda (Bachou et al. 2006), indicates a widespread occurrence of this zoonotic pathogen in Uganda and a potential public health threat in this country.

The frequency of *Campylobacter* spp.-positive samples we have found has also been reported in other areas within East-Africa (Hlashwayo et al. 2021), and its detection in multiple species supports the multi-host nature of this bacterial organism. The high proportion of *Campylobacter* spp.-positive samples in Uganda kob from QENP-N (n = 16/53) has not previously been reported in any other wild antelope in Africa. Despite sample size was low for stool samples from people (n = 15), *Campylobacter* spp. was more frequently detected (26.7%) than the pooled prevalence of this pathogen in West (10.7%) and South Africa (8.5%) where similar sample sizes were used (Hlashwayo et al. 2021). These results suggest that in rural areas, not only livestock but also wildlife, may harbour these zoonotic enteric bacteria, and control measures should be focused on both niches to mitigate the risk of transmission.

Although the most prevalent *Campylobacter* species detected in livestock from sub-Saharan Africa are *C. jejuni* and *C. coli*, especially in cattle and poultry (Hlashwayo et al. 2020), in this study *C. jejuni* has only been identified in cattle and sheep at a low frequency. Similar to other African regions, *C. lari* and *C. hyointestinalis* were detected in cattle (Hlashwayo et al. 2020). Despite the primary reservoirs of *C. fetus* are cattle and sheep, it has been described in

domestic goats from Sudan (Elbrissi et al. 2017). In addition, the present study extends the known distribution range of *C. lanienae* within the African continent.

All samples were negative to *Arcobacter* spp. despite this pathogen has been detected in animal and human faecal samples from other sub-Saharan African countries (Adesiji et al. 2011). Additional studies are needed to clarify its distribution and pathogenic role in East-Africa.

In conclusion, *Salmonella* and *Campylobacter* spp. detection was higher in livestock than wildlife from western Uganda. Moreover, the frequency of detection of these bacteria in wildlife from remote areas was low compared to areas with high livestock-wildlife-human interaction. Molecular epidemiology and metagenomic studies are needed to better understand the transmission dynamics of these food-borne pathogens in wild hosts and their interface with livestock and humans in western Uganda.

Word count = 1654

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Table 1. Samples and results from the molecular detection of *Salmonella* and *Campylobacter* spp., in faeces from animals and humans from western Uganda, 2015-2018.

	N	***Salmonella* spp.**	***Campylobacter* spp.**
Mgahinga NP (MGNP)	**114**	**3 (2.6%)**	**40 (35.1%)**
Cattle	35	2 (5.7%)	9 (25.7%)
Chicken	9	0	0
Goat	23	0	8 (34.8%)
Sheep	25	1 (4%)	15 (60%)
Buffalo	22	0	8 (36.4%)
Murchison Falls NP (MFNP)	**102**	**1 (0.9%)**	**12 (11.8%)**
Cattle	30	0	1 (3.3%)
Chicken	12	0	0
Goat	20	1 (5%)	9 (45%)
Sheep	5	0	1 (20%)
Buffalo	20	0	1 (5%)
Kob	15	0	0
Queen Elizabeth NP N) (QENP-N)	**176**	**9 (5.1%)**	**38 (21.6%)**
Cattle	52	0	6 (11.5%)
Chicken	6	0	1 (16.7%)
Goat	16	4 (25%)	5 (31.2%)
Buffalo	41	1 (2.4%)	7 (17.1%)
Elephant	12	0	0
Kob	34	3 (8.8%)	15 (44.1%)
Human	15	1 (6.7)	4 (26.7%)
Queen Elizabeth NP (S) (QENP-S)	**46**	**0**	**1 (2.2%)**
Buffalo	35	0	0
Elephant	7	0	0
Kob	4	0	1 (25%)
Hoima District (HD)	**22**	**5 (22.7%)**	**5 (22.7%)**
Cattle	22	5 (22.7%)	5 (22.7%)
Budongo Central Forest Reserve (BCFR)	**19**	**0**	**3 (15.8%)**
Anubis baboon	19	0	3 (15.8%)
TOTAL	**479**	**18 (3.8%)**	**99 (20.7%)**

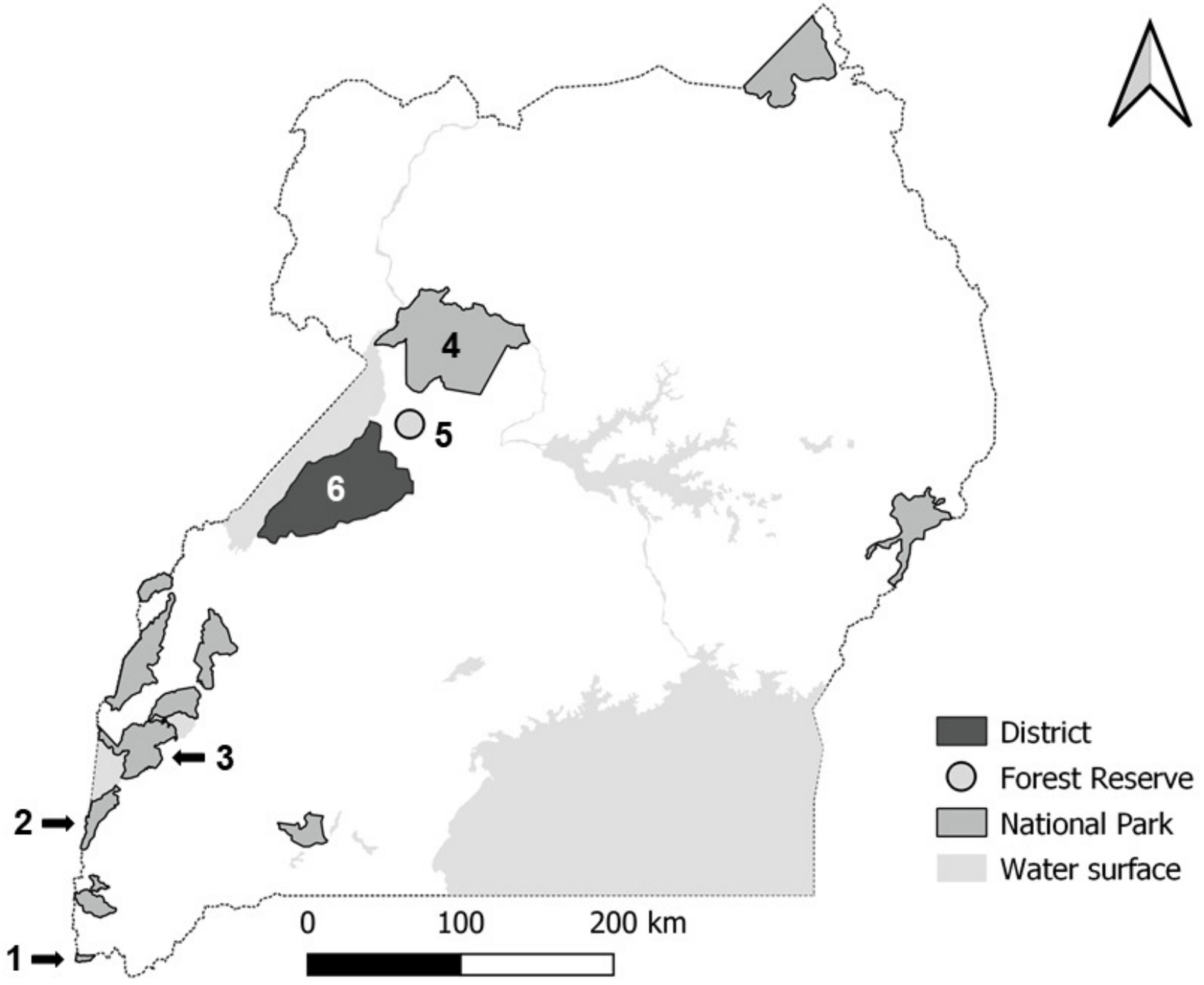
Table 2. Primer sequences used in this study.

Species	Primer	Nucleotide sequence (5' to 3')	Fragment length (bp)	Target gene
***Salmonella* spp.**	invA-F[a]	GTG AAA TTA TCG CCA CGT TCG GGC AA	284	*invA* (Rahn et al. 1992)
	invA-R[b]	TCA TCG CAC CGT CAA AGG AAC C		*invA* (Rahn et al. 1992)
***Salmonella* Enteritidis**	sefA-F[a]	TGT GCG AAT GCT AAT AGT TG	526	*sefABC* (Thomas 1994)
	sefA-R[b]	CTG CTG AAC GTA GAA GGT CG		*sefABC* (Thomas 1994)
***Salmonella* Enteritidis and Typhimurium**	fljBA-F[a]	CTG GCG ACG ATC TGT CGA TG	250 and 1000	*fljB-fljA* (Echeita et al. 2001)
	fljBA-R[b]	GCG GTA TAC AGT GAA TTC AC		*fljB-fljA* (Echeita et al. 2001)
***Campylobacter* spp.**	C412F[a]	GGA TGA CAC TTT TCG GAG C	857	16S rRNA (Linton et al. 1996)
	campR2[b]	GGC TTC ATG CTC TCG AGT T		16S rRNA (Katzav et al. 2008)
C. jejuni	lpxA-J[a]	ACA ACT TGG TGA CGA TGT TGT A	331	lpxA (Klena et al. 2004)
C. coli	lpxA-C[a]	AGA CAA ATA AGA GAG AAT CAG	391	lpxA (Klena et al. 2004)
C. lari	lpxA-L[a]	TRC CAA ATG TTA AAA TAG GCG A	233	lpxA (Klena et al. 2004)
C. upsaliensis	lpxA-C.Ups[a]	AAG TCG TAT ATT TTC YTA CGC TTG TGT G	206	lpxA (Klena et al. 2004)
C. jejuni, C. coli, C. lari, C. upsaliensis	lpxARKK2m[b]	CAA TCA TGD GCD ATA TGA SAA TAH GCC AT		lpxA (Klena et al. 2004)
C. lanienae	CLAN76F[a]	GTA AGA GCT TGC TCT TAT GAG	920	16S rDNA (Logan et al. 2000)
	CLAN1021R[b]	TCT TAT CTC TAA GAG GTT CTT A		16S rDNA (Logan et al. 2000)
C. fetus	JH0087[a]	TGA GGC TGT TAC AAG CGA GTT A	100	*cpn60* (Chaban et al. 2009)
	JH0088[b]	TGA GCT ATC GCT ATT TGC TGA A		*cpn60* (Chaban et al. 2009)
C. hyointestinalis	ChII-spBU8[a]	CCT AGT AGC GCT ACT TAG	215	*cdt* (Kamei et al. 2016)
	ChII-spBR8[b]	CAA ATA CCC TAC CTG TAG C		*cdt* (Kamei et al. 2016)
***Arcobacter* spp.**	Arco I[a]	AGA GAT TAG CCT GTA TTG TAT C	1223	16S rDNA (Harmon and Wesley 1997)
	Arco II[b]	TAG CAT CCC CGC TTC GAA TGA		16S rDNA (Harmon and Wesley 1997)

[a] Forward primers; [b] Reverse primers

Figure 1. Natural protected areas in western Uganda and study sites: 1. Mgahinga National Park; 2. Queen Elizabeth National Park-Southern sector; 3. Queen Elizabeth National Park-Northern sector; 4. Murchison Falls National Park; 5. Budongo Central Forest Reserve; 6. Hoima District.

"[insert Figure 1 here]"


4
5
6
3
2
1
0
100
200 km
District
Forest Reserve
National Park
Water surface

Supplementary Material - Food-borne pathogens at the livestock-wildlife-human interface in rural western Uganda

Study areas' description according to the levels of interaction between livestock and wildlife, based on local human population, the presence of wildlife and livestock, physical barriers, and knowledge on local wildlife-livestock interactions.

Study area	Level of interaction	Population density*	Wildlife/livestock presence	Physical barriers presence	Wildlife incursions	References
MGNP	Medium interaction	402	Yes/Yes	A stone wall separates MGNP from inhabited areas.	The wall is permeable for wildlife and indirect contact with livestock still happens.	(Babaasa et al. 2013)
MFNP northern sector	No interaction	29	Yes/No	The Nile River separates MFNP northern sector from MFNP southern sector.	There are no incursions as only wildlife is present.	(Tweheyo et al. 2005; Dowhaniuk et al. 2018; Scoon 2021)
MFNP southern sector	High interaction	250	Yes/Yes		Livestock and wildlife may interact indirectly as there are no geographical barriers separating the distribution areas of these animals and human settlements. This area also covers a large part of BFCR, which harbours chimpanzees and baboons.	
QENP-N	High interaction	909	Yes/Yes	QENP northern and southern sectors are separated by more than 100 km by road, the Kazinga channel and	Sharing of grazing areas and water sources between livestock and wildlife is common in the border of QENP-N.	(Meunier et al. 2017; Aguilar et al. 2020)

				Maramagambo forest. Non-physical barriers are present.		
QENP-S	Low interaction	198	Yes/Yes	Non-physical barriers.	Interactions between humans and elephants are common due to human pressure and uncontrolled resource use outside the protected areas.	(Keigwin 2001)
BCFR	Medium interaction	74	Yes/Yes	Non-physical barriers.	There are no livestock grazing areas within BCFR, but baboon and chimpanzee incursions are common in human settlements, with a high rate of crop-raiding incidents.	(Tweheyo et al. 2005)
HD	No interaction	156	No/Yes	Non-physical barriers.	There are no incursions as wildlife is not present in this study area.	

* Population density (number of people/km^2) per district was estimated in 2014 (UBOS 2016).

Supplementary material references

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