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Original Article

Gastrointestinal Parasites of Dogs (*Canis Familiaris*, Linnaeus, 1758) in the Conservation Areas of Bwindi, Mgahinga, and Queen Elizabeth in Uganda

James Robert Ochieng^{1*}, Marta Planellas Bachs², Anthony Mutebi Nsubuga¹, Innocent B. Rwego¹, Benard Matovu¹, Simon Peter Lowopek³, Jaume Gardela², Jesus Muro Figueres¹ & John Joseph M. Kisakye¹

¹ Makerere University, P. O. Box 7062, Kampala, Uganda.

² Universitat Autònoma de Barcelona, P. O. Box 08193, Bellaterra, Spain.

³ Tororo District Local Government, Uganda, P. O. Box 1, Tororo, Uganda.

*Author for Correspondence ORCID ID: <https://orcid.org/0000-0003-0765-4958>; Email: jjamesro@gmail.com

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Western Uganda.*

If not treated, gastrointestinal parasites (GIPs) can negatively affect dog fitness, performance, and growth and cause severe sickness and/or death. Dogs harbour parasites responsible for zoonosis, and this is likely to be worst in contiguity to conservation areas having many parasite reservoirs. To understand the dog GIPs status in contiguity to Bwindi, Mgahinga, and Queen Elizabeth conservation areas in western Uganda, faecal samples (n = 284) collected from domestic dogs in July 2022 were subjected to parasitological analysis using sodium nitrate floatation and formol-ether sedimentation before microscopic examination. Hookworm-positive samples were further subjected to molecular analysis to aid worm differentiation. A total of 18 parasites were detected, and 258 (90.8%) of the samples tested positive with at least two parasites: protozoa (n=6), trematode (n=1), cestodes (n=3), and nematodes (n=8). All the detected parasites have zoonotic potential and are of public health concern. The most prevalent parasite was *Entamoeba coli* (56%), followed by *Entamoeba histolytica* (25%) and *Trichuris vulpis* (21%), and the least prevalent was *Diphylllobothrium* sp. (4%). There was no significant statistical difference ($P > 0.05$) in the overall parasite prevalence rate across the study sites and between sexes, but a significant statistical difference ($P < 0.05$) in the parasite prevalence across age, mode of life, and breed. Some of the recognised parasites, like *E. histolytica*, *Cryptosporidium*, *Giardia* species, and *A. caninum* are responsible for serious zoonosis and raise the need for awareness on parasite prevention and establishing dog veterinary care services in the study area.

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INTRODUCTION

Parasites affect hosts' ecology and evolution of inter-specific interactions, population growth, performance, and fitness, increasing vulnerability to diseases and/or fatality if not treated (Esch & Fernandez, 1993; Hudson et al., 2002). GIP infections in dogs have been reported as an important public health problem worldwide, including in Australia (Palmer et al., 2008), Argentina (Fontanarrosa et al., 2006), Thailand (Rojekittikhun et al., 2014), Greece (Haralabidis et al., 1988), Sri Lanka (Perera et al., 2013), Iran (Mirzayans et al., 1972; Sarvi et al., 2018), and Spain (Martínez-Moreno et al., 2007). Canine gastrointestinal (GI) parasitosis is described as more severe and abundant in developing countries in sub-Saharan Africa, namely, Ethiopia (Kebede, 2019), Nigeria (Ayinmode et al., 2016; Sowemimo, 2009), Gabon (Davoust et al., 2008), South Africa (Verster, 1979), Egypt (Abbas et al., 2023), Rwanda (Ntampaka et al., 2021), Kenya (Wyckliff et al., 2017) and Uganda (Inangolet et al., 2010) where preventive medicine seems limited and/or not established and dog health monitoring seems minimal. These studies reported cases of several dog GIPs, including *E. coli*, *E. histolytica*, *Cryptosporidium* sp., *Eimeria* sp., *Dipylidium caninum*, *Sarcocystis* sp., *Schistosoma* sp.,

Echinococcus granulosus, *Taenia taeniaeformis*, *Toxocara canis*, *Toxocara cati*, *Ancylostoma* spp., *Ascaris* sp., *Trichuris vulpis*, *Strongyloides* sp., among others. Although today dogs are the most successful canids adapted to human habitation worldwide (Paul et al., 1900; Tannen, 2004), their close human-dog relationship plays a major role in public health, including the transmission of parasites of zoonotic potential (Chidumayo, 2018; Perera et al., 2013), yet fewer studies exist in relation with canine GIPs in low developed countries including Uganda.

Related previous dog parasitosis studies in the conservation areas of Bwindi, Mgahinga, and Queen Elizabeth by Ochieng et al. (2022) discovered that communities living in the current study areas prioritize livestock farming and keeping dogs for various purposes, including security, herding, hunting, companionship, and scaring off vermin. The majority of the dogs, except the kenneled ones, roam freely in the conservation, rural, and urban areas, increasing their chances of consuming decomposed food, viscera, and unsanitary water, but also contaminating the environment with their faeces. This increases dogs' potential as a link for parasite exchange among humans, livestock, and wildlife, which needs to be intervened. Previous related studies in conservation

areas have noted that dogs are also a source of zoonosis to wildlife, such as canine distemper, cryptosporidiosis, and toxoplasmosis, among other parasitosis (Alexander & Appel, 1994; Bowser & Anderson, 2018).

In addition to the regular rabies vaccination widely carried out in Uganda aimed at having rabies free dogs, investigation on the level of dog GI parasitosis is also pertinent to having healthy dogs in community settings. The goal of the present survey was to obtain an overall picture of the dog GIPs and to investigate the hookworm species in dogs in the conservation areas of Bwindi, Mgahinga, and Queen Elizabeth in western Uganda. We believe having such knowledge documented can allow better preparedness and response in case of an outbreak of dog parasitic infections in Uganda and beyond. The study findings can be used directly by communities, the Ministry of Health, Uganda, and stakeholders in decision-making towards mitigating the risk of GIP zoonotic spillover from dogs to humans. This study will help generate new information on canine GIPs in the conservation areas of Western Uganda, increase awareness of zoonotic risks, and define the appropriate safety measures required. This can contribute to the prevention, control, and policy intervention in disease management to save lives.

MATERIALS AND METHODS

Study Site

The study was carried out in the conservation areas of Bwindi Impenetrable National Park (NP), Mgahinga Gorilla NP, and Queen Elizabeth NP in western Uganda (Figure 1). The Bwindi-Mgahinga Conservation Area (BMCA) comprises Bwindi Impenetrable NP and Mgahinga Gorilla NP. The BMCA and Queen Elizabeth NP possess a diversity of fauna and flora, hence serve purposely for biodiversity conservation, but can be hotspot zones for pathogens, including those responsible for zoonosis (Karesh et al., 2012; Keesing & Ostfeld, 2021). The reasons for choosing these conservation

sites included, but were not limited to designing management strategies for zoonosis, particularly GI parasitosis spread to communities through dogs. We trust that having healthy communities and livestock free from pathogens of wildlife origin in the study areas will help reduce wildlife-community conflicts, improve biodiversity, and promote conservation.

The three NPs are briefly described as follows; Bwindi Impenetrable NP (BINP) (331km²: 0.53-1.08° N; 29.35° -29.50° E) in Kanungu district, southwestern Uganda, bordered west by the Democratic Republic of Congo which is on the eastern edge of the Albertine Rift Valley, bordered south and southeastern by Kisoro and Kabale districts, respectively. BINP is an afro-montane moist evergreen rainforest with an altitude range of 1,160 to 2,607 m, characterized by steep-sided hills, peaks, narrow valleys, and an average daily rainfall of 1.25 to 3.88 ml and a temperature range of 19.8 to 27.7 °C (Mcneilage et al., 2001). This “impenetrable rain forest” is one of the most diverse forest ecosystems in East Africa, with at least 223 known tree species of approximately 53% of Uganda’s tree flora, and provides shelter to several birds including 23 Albertine Rift endemics, non-human primates and other wild faunas (McLennan & Plumptre, 2012; Rothman et al., 2007). Currently, BINP protects at least 400 mountain gorillas (*Gorilla gorilla beringei*), which is roughly half the world’s population, including several habituated groups that can be tracked.

Mgahinga Gorilla NP (MGNP), created in 1991, is located in Kisoro district, southwestern Uganda, and is small, occupying 3,390 ha (33.9 km²), bordering Rwanda to the south and the Democratic Republic of Congo (DRC) to the west. MGNP is contiguous to Virunga NP of Rwanda. MGNP was established mainly to protect mountain gorillas, vulnerable populations of indigenous fauna and flora endemic to the area, and other ecological resources (Butynski & Kalina, 1993).

Queen Elizabeth NP (QENP), gazetted in 1950, located within the Albertine Rift in western Uganda, covers a total area of 1,978 km², comprised of hills, plains, forests, and swamps, and touches the border of the Democratic Republic of Congo. QENP is the second largest NP in Uganda, spreading over the districts of Bushenyi, Kasese, Rukungiri, Kanungu, and Ibanda. QENP includes more than half the Uganda shoreline of the great lakes Edward and George, as well as the 20-mile-long Kazinga Channel, which connects the two and is listed as a world biosphere reserve (Keesing & Ostfeld, 2021; Mcneilage et al., 2001).

During the whole data collection process, we categorised the study sites as rural, suburban, and urban. 1) Rural sites included areas less than 1.5 km from the protected NP boundary. This consisted of the less densely human-populated and underdeveloped communities, with homes scattered far away from each other, 2) suburban sites: located about 3 km from the NP boundary, consisted of fairly developed areas, with a moderately dense human population than in the rural sites, and 3) urban sites: located over 4 km from the NP boundary, characterised with a dense human population, well-built houses, with some families living in secured fenced household premises.

Figure 1. Left to Right: Map of Western Uganda Showing Queen Elizabeth National Park, Bwindi Impenetrable NP, Mgahinga NP, and the Study Areas Marked in Red Dots where We Sampled the Dogs.

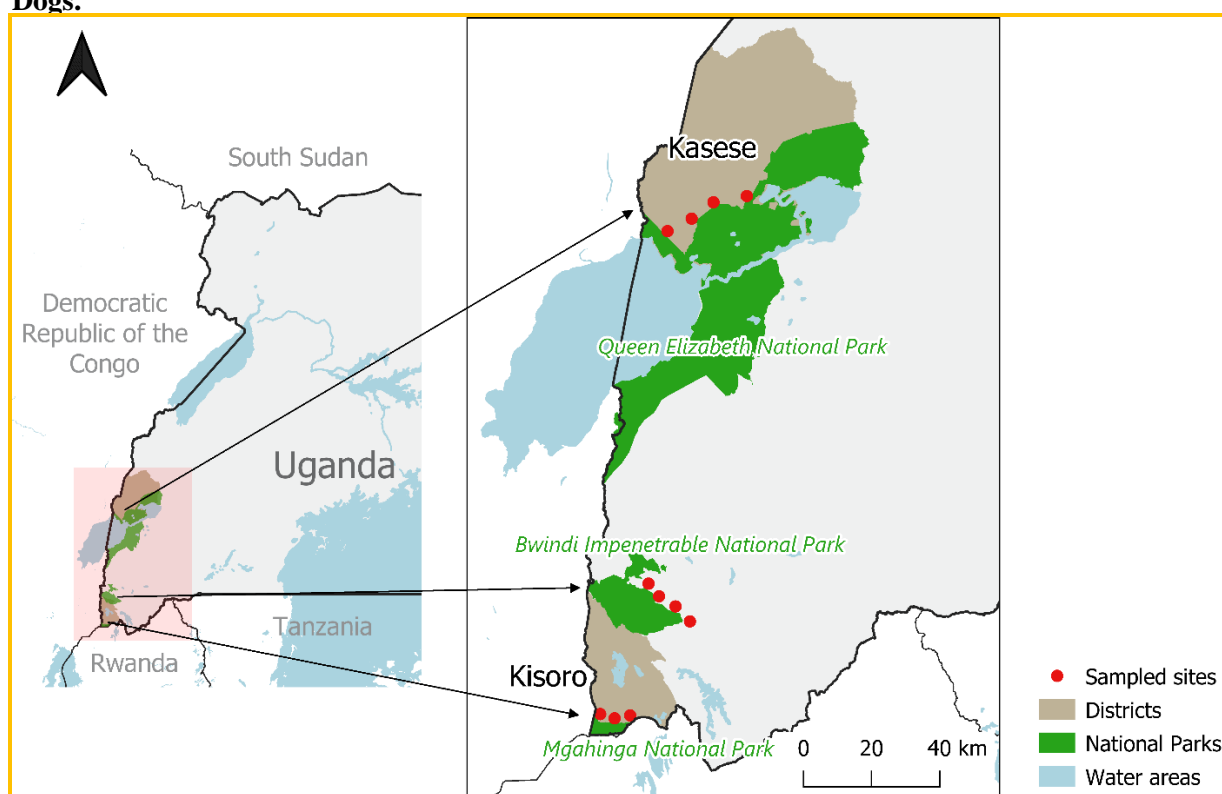


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Sample Collection and Microscopic Examination

Faecal samples were collected from identified 284 domestic dogs in the conservation areas of Queen Elizabeth NP (25 females, 59 males), Bwindi Impenetrable NP (45 females, 61 males), and

Mgahinga NP (31 females, 63 males) during Rabies vaccination by Daktari East Africa NGO (<http://www.daktariandorra.org>) and Mission Rabies UK team in July 2022. Daktari East Africa NGO is a veterinary NGO that, among other

activities, provides dog rabies vaccination, deworming, treatment, neutering (castration and spaying), and surgeries in East Africa. Fresh faecal samples were removed from the rectum, and one gram of each faecal sample was mixed with 8 ml of 10% formalin in a 20 ml vial and stored at room temperature. The other fresh faecal portions were stored in ice packs during the sampling process and later shifted to -20°C until laboratory analysis. All sampled dogs were $6 \geq$ months, and were ranked following the international veterinary age categories (Harvey, 2021): juvenile (6-12 months), young adult (aged 1 year or 12–24 months), mature adult (2-6 years), senior adults (7-11 years), and geriatric (above 11 years). Demographic data, signalment (age, sex, breed, mode of life, season, and occupation of the dog's owner), and clinical signs like diarrhoeic or non-diarrhoeic were recorded for each dog.

According to the owners' information, the sampled 241 free-ranging dogs had never received any anthelmintic and/or antiprotozoal treatment, unlike some of the 43 kenneled dogs included in the study. The 10% formalin stored samples were divided into two portions: one subjected to sedimentation with formol-ether, and the other to floatation using the sodium nitrate standard method as described by Ahmadi and Damraj (2009) and Broussard (2003). These were microscopically examined for the presence of parasite eggs, oocytes, cysts, and tapeworm segments as prescribed by Monica (2006) and Soulsby and Lawrence (1968). The parasite egg and cyst length and width were measured using a calibrated ocular micrometre, and representative micrographs were taken.

DNA Extraction on Hookworm Positive Samples

Fifty-one samples ($n=51$) that tested positive for hookworm microscopically were subjected to a conventional polymerase chain reaction (cPCR) to confirm the hookworm species. Portions of a fresh faecal sample previously stored at -20°C were used. 300 μg of each faecal sample was thawed, and DNA was extracted using ASL (QIAamp DNA Mini

Stool Kit) following the manufacturer's instructions.

Molecular Identification of Hookworm Species by Polymerase Chain Reaction

The extracted DNA samples were subjected to a cPCR in 40 μl reaction volumes containing 2 μl of the extracted faecal DNA sample, 25 pmol/l of forward and reverse primer, 250 μM of each dNTP, 3 mM MgCl_2 , and 2 U Taq polymerase (Promega). Control samples with genomic DNA of adult *Ancylostoma duodenale* and double distilled water were used as positive and negative controls, respectively. Amplification was performed in two rounds, with the first one involving primers NC1 (forward: 5'ACGTCTGGTTCAGGGTTGTT-3') and NC2 (reverse: 5'-TTAGTTTCTTTTCCTCCGCT-3'). The PCR cycling conditions were programmed as prescribed in Joshi and Deshpande (2011); initial denaturation at 94°C for 5 min, followed by 35 amplification cycles each consisting of 1 min denaturation at 94°C , 1 min primer annealing at 60°C , and 1 min polymerization at 72°C , with a final extension at 72°C for 5 min. Thereafter, 2 μl of the first-round amplicon were subjected to a second amplification round using primers AD1 (forward: 5' - CGACTTTAGAACGTTTCGGC-3') and NC2. The cycling conditions were as follows: 94°C for 5 min, 35 cycles at 94°C , 1 min; 60°C , 1 min; 72°C , 1 min, then 72°C for 5 min. The amplified products were subjected to electrophoresis through 1.5% w/v agarose gels containing 0.5 $\mu\text{g}/\text{ml}$ ethidium bromide, pH 9.0, and flooded with $1\times$ TBE buffer. The electrophoresis conditions were 55 V and 250 mA, and a run for 40 min. Hookworm species determination involved comparing individual sample PCR amplicons with the corresponding band size of the positive control bands (Verweij et al., 2001). The presence of two or more bands indicates mixed hookworm infections, while single bands signify monolithic infections.

Statistical Analysis

Microsoft Excel spreadsheets were used for raw data entry and management. Diversity denoted the number of GIP species and the relative abundance of each species in the dogs per conservation area. The prevalence rate was defined as the proportion of GIPs infected dogs among all the dogs examined during the study. Species richness denoted the number of different GIP species in dogs per conservation area, and evenness denoted the relative abundance of parasite species present in dogs per conservation area. The Shannon–Wiener diversity index was used to calculate the parasite species diversity (H') and evenness (J') (Shannon, 1948). All analyses were carried out using the IBM SPSS Statistics 23 package. Pearson Chi-square test was used to assess any statistical difference in the GIPs diversity and prevalence rate about age, gender, mode of life, breed, and habitat across the three conservation areas. The p -value of 0.05 was considered significant.

RESULTS

The GIPs cysts, oocysts, eggs, and larvae detected are shown in Table 1, Figure 2, and Figure 3. A total of 18 parasites, all responsible for zoonosis were detected: protozoa ($n=6$), trematodes ($n=1$), cestodes ($n=3$), and nematodes ($n=8$) were detected across the three conservation areas. The most prevalent were protozoan parasites (Table 1). Of the 284 dogs examined, 258 (90.8%) were infected with one or more parasite species. Of the fifty-one dogs ($n=51$) that were microscopically positive for hookworms, 40 were confirmed positive for *A. caninum* using PCR, so we concluded that 40 dogs were *A. caninum* positive (Table 1).

While 17(94%) of the parasite species were shared across the three conservation areas, for no clear reasons, only *Diphyllbothrium* sp. was not detected in the conservation area of Mgahinga NP

(Table 1). The GIP species richness ranged from 84.4% to 100%, and the parasite diversity ranged between $2.55 \leq H' \leq 2.73$ (Table 2). This lies in the normal standard range of $1.5 \leq H' \leq 3.5$ (Clarke et al., 2014; Clarke & Warwick, 2001). The parasite species evenness was $0.882 \leq J' \leq 0.94$ (Table 2), which also lies within the normal range ($0 \leq J' \leq 1$), indicating that there is uniformity in parasite species diversity among the dogs in the three conservation areas (Barirega et al., 2010).

There was a significant statistical difference ($P < 0.05$) in the prevalence of seven individual parasites across the three conservation areas (Table 1), but no significant statistical difference ($X^2 = 6.32$, $df = 2$, $P = 0.09$) in the overall parasites prevalence rate. The most prevalent parasite was *Entamoeba coli* (56%), followed by *E. histolytica* (25%), *Trichuris vulpis* (21%), *Eimeria* sp. (17%), and *Toxocara canis* (17%). Moderately prevalent (5-15%) parasites included *Eimeria* sp., *Cryptosporidium* sp., *Giardia* sp., *Isospora* sp., *Taenia* sp., *A. caninum*, *Toxascaris* sp., and *Ascaris* sp., among others, and the least prevalent parasites were *Diphyllbothrium* sp. (4%) and *Strongyloides* sp. (5%) (Table 1).

In the aspect of dog's age, gender, mode of life, breed, and site location, GIP prevalence was higher in juvenile (94.1%), female (95%), free-ranging (95.9%), African shepherd (94%) and rural sites (51.8%), respectively (Table 4). In general, there was a significant statistical difference ($P < 0.05$) in the overall parasite prevalence across age, mode of life, and breed (Table 4). Of the 18 detected parasite species, only 14 showed a significant statistical difference ($P < 0.05$) between gender (Table 3), but there was no significant statistical difference ($P > 0.05$) in the overall parasite prevalence between gender and site location (Table 4). These findings exhibit substantial interest and have the potential to significantly contribute to the formulation of intervention measures.

Table 1: Prevalence (%) of Canine Gastrointestinal Parasites Recovered from Dog Faecal Samples in the Conservation Areas of Bwindi, Mgahinga, and Queen Elizabeth, Uganda.

Parasite	QENP (n= 84) n (%)	BINP (n= 106) n (%)	MGNP (n= 94) n (%)	Total prevalence n (%)	Pearson Chi-square test X ²	p-value
Protozoa						
<i>Entamoeba coli</i>	39 (46.4)	68(64.2)	51 (54.3)	158 (56)	6.07	*
<i>Entamoeba histolytica</i>	17 (20.2)	19 (17.9)	36 (38.3)	72 (25)	12.58	**
<i>Eimeria</i> sp.	22 (26.2)	7 (6.6)	19 (20.2)	48 (17)	13.9	**
<i>Cryptosporidium</i> sp.	14 (16.7)	7 (6.6)	23 (24.5)	44 (15)	12.27	**
<i>Giardia</i> sp.	11 (13.1)	8 (7.5)	16 (17.0)	35 (12)	4.2	0.12
<i>Isospora</i> sp.	10 (11.9)	6 (6.7)	8 (8.5)	31 (11)	2.36	0.31
Trematoda						
<i>Fasciola</i> sp.	4 (4.8)	6 (5.7)	13 (13.8)	23 (8)	6.25	*
Cestoda						
<i>Taenia</i> sp.	15 (17.9)	19 (17.9)	5 (5.3)	39 (14)	8.4	*
<i>Diphyllbothrium</i> sp.	9 (10.7)	2 (1.9)	0 (0.0)	11 (4)	15.47	***
<i>Dipylidium caninum</i>	8 (9.5)	4 (3.8)	7 (7.4)	19 (6.7)	2.61	0.27
Nematoda						
<i>Trichuris vulpis</i>	20 (23.8)	20 (18.9)	19 (20.2)	59 (21)	0.72	0.7
<i>Toxocara canis</i>	13 (15.5)	23 (21.7)	12 (12.8)	48 (17)	3.32	0.19
<i>Toxascaris</i> sp.	9(10.71)	7(6.60)	16(17.02)	32(11.3)	5.44	0.07
<i>Spirocerca lupi</i>	6 (7.14)	12 (11.3)	8 (8.5)	26 (9)	1.05	0.59
<i>A. caninum</i>	18 (21.4)	13 (12.3)	9 (9.6)	40 (14.1)	5.6	0.06
<i>Ascaris</i> sp.	10 (12.0)	8 (7.5)	13 (13.8)	31 (11)	2.14	0.34
<i>Chitwoodspirura</i> sp.	7 (8.3)	15 (14.2)	9 (9.6)	31 (11)	1.89	0.39
<i>Strongyloides</i> sp.	4 (4.8)	9 (8.5)	2 (2.1)	15 (5)	4.1	0.13

Key: QENP= Queen Elizabeth NP, BINP= Bwindi Impenetrable NP, MGNP= Mgahinga NP, n = number of dog faecal samples collected from each conservation area, X² = Pearson Chi-square value, P-value = Chi-square P- value, *, ** and *** = P-value less than 0.05, 0.01 and 0.001 respectively.

Table 2: Canine Gastrointestinal Parasite Species Diversity, Richness and Evenness in the Conservation Areas of Bwindi, Mgahinga and Queen Elizabeth, Uganda.

Conservation areas	Number of GIP species detected	Parasite richness	Diversity index (H')	Evenness (J')
Queen Elizabeth	18	18/18 (100%)	2.73	0.944
Bwindi	18	18/18 (100%)	2.55	0.882
Mgahinga	17	17/18 (94.4%)	2.59	0.914

Abbreviation: GIP= Gastrointestinal parasite, J'= Pielou's evenness.

Figure 2: Overview of Micrographs of Protozoan Cysts Recovered from Dog Faecal Samples During the Study: (a) Nucleated *Entamoeba coli* Cyst 10×10 µm, 7 nuclei (b) Nucleated *E. histolytica* cyst 7×7 µm, 4 nuclei, (c) Sporulated *Eimeria* sp. oocyst 15×15 µm, (d) Unsporulated *Cryptosporidium* sp. cyst 9×9 µm, (e) *Giardia* sp. Young Cyst with Two Haploid Nuclei and 1 ring, (f) Unsporulated *Isospora* sp. cyst.

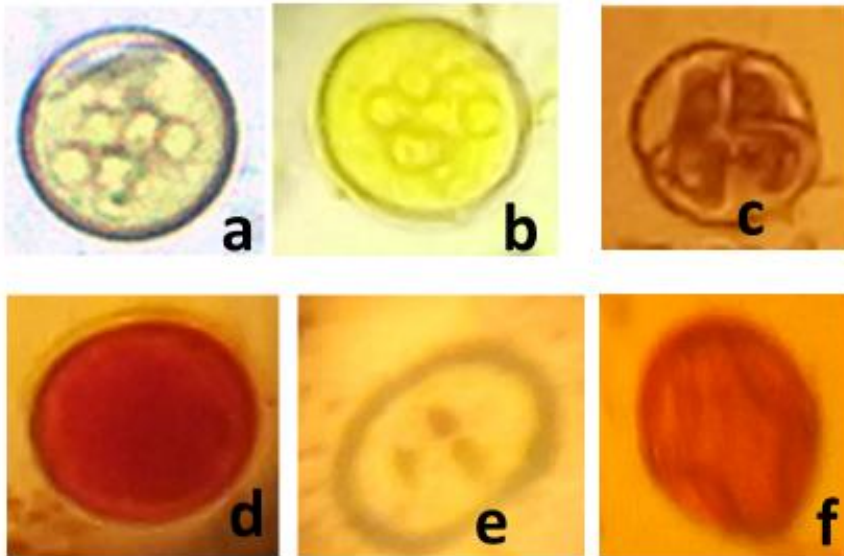


Photo © James Robert Ochieng.

Figure 3: Overview of Micrographs of Helminths Recovered From Dog Faecal Samples During the Study: (a) *Fasciola* sp. Egg, Blue Arrow Shows the Operculum 120×80 µm, (b) *Taenia* sp. Head, Neck and Proglottids, (c) *Taenia* sp. egg, (d) *Dipylidium caninum*, Egg Packet Containing at Least 11 eggs, (e) *Trichuris vulpis* egg 65×28 µm, (f) Unembryonated *Diphyllobothrium* sp. Egg 65×38 µm, (g) Embryonated *Spirocerca lupi* with Its Transparent Thick-shell in Lugol Iodin Stain, (h) Thin Shelled *A. caninum* egg 65×38 µm, this was Further Confirmed by PCR, (i) Fertilized *Ascaris* sp. egg, (j) *Chitwoodspirura* sp. egg 56×30 µm, (k) Thick Smooth Shelled *Toxascaris* sp. egg, (l) Zygoted *Toxocara canis* egg with a Sub-spherical Yellow-brown Thick Rough Outer Shell, 78 µm Wide, (m) Large *Strongyloides* sp. Egg (n) *Strongyloides* sp. Egg with Emerging Larvae, (o) Rhabditiform larva of *Strongyloides* sp.

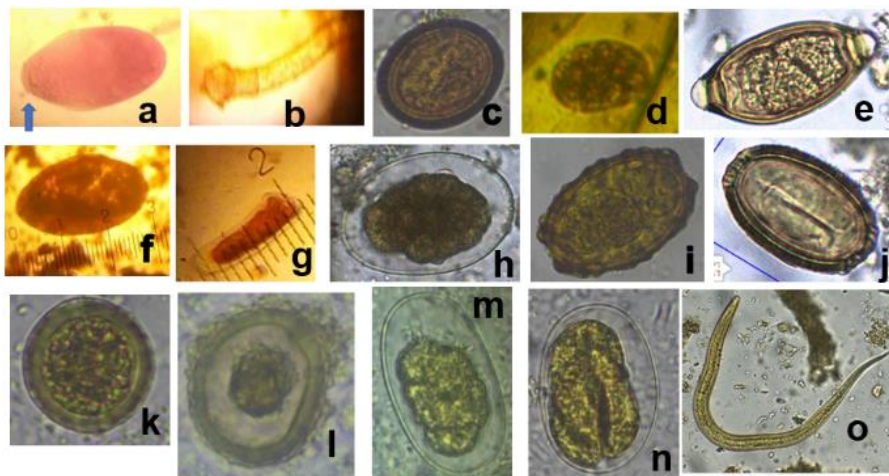


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Table 3: Canine Gastrointestinal Parasites Prevalence (%) in Relation to Sex in the Conservation Areas of Bwindi, Mgahinga and Queen Elizabeth, Uganda.

Parasite	QENP (n=84)		BINP (n=106)		MGNP (n=94)		Pearson Chi-square test	
	Male (n=59) n (%)	Female (n=25) n (%)	Male (n=61) n (%)	Female (n=45) n (%)	Male (n=63) n (%)	Female (n=31) n (%)	X ²	p-value
Protozoa								
<i>Entamoeba coli</i>	17(28.8)	22(88)	24(39.3)	44(97.8)	23(36.5)	28(90.3)	91.19	***
<i>Entamoeba histolytica</i>	7(11.9)	10(40)	9(14.8)	10(22.2)	17(27.0)	19(61.3)	33.6	***
<i>Eimeria</i> sp.	12(20.3)	10(40)	0(0.0)	7(15.6)	10(15.9)	9(29.0)	25.75	***
<i>Cryptosporidium</i> sp.	8(13.6)	6(24)	4(6.6)	3(6.7)	8(12.7)	15(48.4)	33.94	***
<i>Giardia</i> sp.	1(1.7)	10(40)	0(0.0)	8(17.8)	10(15.9)	6(19.4)	35.86	***
<i>Isospora</i> sp.	2(3.4)	8(32)	0(0.0)	6(13.3)	8(12.7)	0(0.0)	31.22	***
Trematoda								
<i>Fasciola</i> sp.	0(0)	4(32)	0(0.0)	6(13.3)	0(0.0)	13(41.9)	67.57	***
Cestoda								
<i>Taenia</i> sp.	6(10.2)	9(36)	9(14.8)	10(22.2)	0(0.0)	5(16.1)	24.07	***
<i>Diphyllobothrium</i> sp.	7(11.9)	2(8)	0(0.0)	2(4.4)	0(0.0)	0(0.0)	17.55	**
<i>Dipylidium caninum</i>	4(4.7)	4(16)	3(4.9)	1(2.2)	4(6.3)	3(9.7)	6.16	0.29
Nematoda								
<i>Trichuris vulpis</i>	12(20.3)	8(32)	12(19.7)	8(17.8)	9(14.3)	10(32.3)	6.31	0.28
<i>Toxocara canis</i>	3(5.1)	10(40)	6(9.8)	17(37.8)	6(9.5)	6(19.4)	34.07	***
<i>Toxascaris</i> sp.	9 (15.2)	0(0.0)	5(8.2)	2(4.4)	10(15.9)	6(19.4)	10.15	0.07
<i>Spirocerca lupi</i>	0(0.0)	6(24)	4(6.6)	8(17.8)	0(0.0)	8(25.8)	33.77	***
<i>A. caninum</i>	10(17)	8(32)	0(0.0)	13(28.9)	3(4.8)	6(19.4)	30.59	***
<i>Ascaris</i> sp.	2(3.4)	8(32)	1(1.6)	7(15.6)	3(4.8)	10(32.3)	38.24	***
<i>Chitwoodspira</i> sp.	3(5.1)	4(16)	12(19.7)	3(6.7)	3(4.8)	6(19.4)	13.1	*
<i>Strongyloides</i> sp.	2(3.4)	2(8)	3(4.9)	6(13)	1(1.6)	1(3.23)	8.59	0.13

Key: QENP= Queen Elizabeth NP, BINP= Bwindi Impenetrable NP, MGNP= Mgahinga NP, n = number of dog faecal samples collected for each sex from each conservation area, X² = Pearson Chi-square value, P-value = Chi-square P- value, *, ** and *** = P-value less than 0.05, 0.01 and 0.001 respectively.

Table 4: Overall Canine Gastrointestinal Parasites Prevalence (%) in Relation to Dog's Age, Gender, Mode of Life and Breed Across the Three Conservation Areas of Bwindi, Mgahinga and Queen Elizabeth, Uganda.

Category	Number examined (n)	Prevalence (%)
Age (months or years)		
Juvenile (6-12 months)	34	32(94.1)
Young adult (aged 1 year or 12–24 months)	98	92(93.9)
Mature adult (2-6 years)	112	105(93.8)
Senior adult (7-11 years)	40	29(72.5)
<i>Total</i>	284	258(90.8)
P<0.05		
Gender		
Female	101	96(95.0)
Male	183	162(88.5)
P>0.05		
Mode of life		
Free-ranging	241	231(95.9)
Kenneled	43	27(62.8)
P<0.05		
Breed		
African shepherd	268	252(94.0)
German shepherd	13	4(30.8)
Unidentified breed	3	2(66.7)
P<0.05		
Habitat/ site location		
Urban/suburban	121	111(39.1)
Rural	163	147(51.8)
P>0.05		

DISCUSSION

Results from the survey show that dogs in the conservation areas possess high parasite species diversity responsible for zoonosis. The recognised parasite cysts, oocysts, and eggs in dogs' faeces are quite tolerant to harsh environmental conditions, increasing their chances of spread. To our knowledge, the current study reports what we believe are the first findings of *Giardia* sp., *Fasciola* sp., *Diphyllbothrium* sp., *D. caninum*, *Trichuris vulpis*, *Toxocara canis*, *Toxascaris* sp., *Spirocerca lupi*, *A. caninum* sp., *Ascaris* sp., *Chitwoodspirura*

sp., and *Strongyloides* sp. in Ugandan dogs in addition to other previously detected parasites.

The pathogenic parasites reported in this study can confer a combination of pathogenic effects in dogs, such as diarrhoea, nausea, vomiting, loss of appetite, weight loss, reduced movement/lethargy, fitness, and breeding success (Smyth & Wakelin, 1994; Taylor et al., 2016). The dogs may also face physical stress, intestinal ulceration, anaemia, tissue damage, delay in puberty onset, spontaneous abortion, congenital malformation, retarded growth, and mortality if not treated (Taylor et al., 2016).

Related previous studies within Africa (Abbas et al., 2023; Ayinmode et al., 2016; Chidumayo, 2018; Davoust et al., 2008; Kebede, 2019; Ntampaka et al., 2021; Sowemimo, 2009; Verster, 1979; Wyckliff et al., 2017) and beyond Africa (Fontanarrosa et al., 2006; Haralabidis et al., 1988; Palmer et al., 2008; Perera et al., 2013; Rojekittikhun et al., 2014) shows that all the detected parasites in the current survey are responsible for zoonosis. For example, the detected *Toxocara canis* causes both visceral and ocular larval migrans in humans, and severe infections can lead to blindness, especially in children (Perera et al., 2013; Schantz & Glickman, 2010). Heukelbach & Walton (2005) also stated that dog hookworm *Ancylostoma caninum* infection is common in several resource-poor communities and causes cutaneous larva migrans. Humans acquire GIPs through accidental ingestion of infective eggs, oocysts, and/or cysts in water or food. Transmission may also occur through skin penetration of infective larvae (Habluetzel et al., 2003), or consumption of raw and/ or undercooked dog meat (Taylor, 2017).

In this study, the most prevalent parasites, like *E. coli*, *E. histolytica*, *Eimeria* sp., *Trichuris vulpis*, and *Toxocara canis*, and other moderately prevalent parasites have direct life cycles and exhibit a horizontal direct mode of transmission (Smyth & Wakelin, 1994). These highly prevalent parasites also have several reservoir hosts: insects (dung beetles, cockroaches), amphibians, reptiles, rodents, birds, bats, and livestock (Taylor et al., 2016), that are either found within or are contiguous to domestic settings, facilitating their quick transmission. These reservoir hosts may contaminate food, water, and soil with their parasite-infected faeces (Wallace & Gilles, 1995). However, other parasite species, including *Diphyllbothrium* sp., have indirect life cycles and fewer intermediate hosts (Smyth & Wakelin, 1994). So, they incur a range of environmental resistance like unfavourable moisture/hot temperatures during their free-living stages, hindering their survival (Smyth & Wakelin, 1994). The detected

Diphyllbothrium sp. could be associated with dogs eating raw or undercooked infected fish in community settings, but more intervention is still needed.

The recognised moderately prevalent *A. caninum* (14.1%) exhibits both trans-mammary and transplacental transmission in addition to the oral route, which is the same case in *Toxocara canis* (Smyth & Wakelin, 1994). This increases the chances of their transmission compared to the least prevalent *Diphyllbothrium* sp. Also, the moderately prevalent dog tapeworm *D. caninum* (6.7%) could be associated with the high abundance of dog fleas *Ctenocephalides canis* observed during the sampling process. According to the dog owners, most of the free-roaming dogs in the study areas live in unsanitary settings, thus promoting the persistent existence of dog fleas and reinfestation. The *Ascaris* sp. detected herein could be due to coprophagy, which is common in dogs (Traub et al., 2002), and the recognized reservoirs, including pigs and non-human primates, present in the study areas.

The overall GIP prevalence (90.8%) in this study does not vary from the 94.6% and 90% recorded in Ethiopia (Kebede, 2019) and Hantana areas, Sri Lanka (Perera et al., 2013), respectively. However, the current prevalence appears to be higher compared to some previous reports: Inangolet et al (2010) reported 66.3% in Moroto district, Uganda, Wyckliff et al (2017) recorded 35.29% in Kenya, Ntampaka et al (2021) found 33.3% in Rwanda, Davoust et al (2008) detected 13.6% in northeast Gabon, Abbas et al (2023) noticed 35.9% in Egypt, Ayinmode et al (2016) stated 43.3% in Ibadan, Nigeria, Oluyomi et al (2009) confirmed 55% in Ile-Ife, Nigeria, Verster (1979) testified 74.6% in the Republic of South Africa, and Chidumayo (2018) described 71% in sub-Saharan Africa. In the current study, the highly recognized GIP prevalence in free-ranging dogs, especially in rural inhabitants, could be because they had never received any antiparasitic treatment in the previous twelve months, as proclaimed by the dog owners. This, together with

poor sanitation in rural settings, could account for the higher infection status. On the aspect of dog breeds, most of the free-roaming dogs encountered during the study were African shepherds that roam freely, unlike the kenneled German shepherds; this could also account for their high rates of infection. Furthermore, because of fewer variations in climate and environmental factors, human activities, dog breeds, and dog care practices, the GIP's status did not differ significantly among the studied areas. However, some of the studied communities, especially in rural settings, were challenged with socio-economic factors, including poverty, characterized by food shortages for both humans and dogs. Like in humans, food shortage can cause nutritional stress in dogs lowering their immunity and allowing multiple pathogenicity and reduced cure (Taylor et al., 2016). Also, some study areas had poor sanitary conditions, with no latrines, no safe drinking water, and animal cadavers thrown openly (personal observation). This, together with frequent piles of garbage, provides a fertile environment for the transmission of intestinal parasites and could account for the recognized high GIPs.

Additionally, the free-ranging dogs roam freely both in a domestic and wild environment, increasing their chances of scavenging on raw and/ or undercooked food and carcasses of infected domestic and wild animals (Craft et al., 2015; Inga et al., 2023; Jenkins et al., 2011). This, together with complex interactions (both direct and indirect contacts) with wild carnivores, increases the chances of pathogen transmission at the domestic-wild animal interface (Craft et al., 2015). This accounts for high parasite prevalence in free-roaming dogs, unlike kenneled dogs, which mostly live in good or fair sanitary settings. The high GIP prevalence in young dogs could be due to underdeveloped immunity. Also, the high GIPs in adult female dogs could be associated with reduced immunity due to the physiological peculiarities of pregnancy and nursing/lactation that may cause

stress factors and lower their immunity (Taylor et al., 2016).

CONCLUSION AND RECOMMENDATION

The overall GIP prevalence (90.8%) reveals a high level of infection, and the highly recognised parasites responsible for zoonosis call for effective control measures to mitigate parasite transmission from dogs to humans in the study area. The use of the One Health approach could widen awareness and mitigate parasite spread. We would recommend improved regular dog veterinary services: provision of prophylactic and/or persistent mass treatment of dogs with effective antiparasitic drugs. There is a need for good sanitation, fencing of abattoirs and slaughter slabs, and removing animal remains after slaughter to limit the risk of food sharing between domestic and wild carnivores in the study area. We encourage beef slaughtering in fenced boma where there are no slaughterhouses, and all animal carcasses should be buried deeply underground to reduce exposure risks. Implementing these recommendations will help secure a healthy dog population. We also recommend the wider use of molecular diagnostic methods in future studies to aid accurate parasite identification.

Conflict of Interest

The authors fully declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work and/ or findings reported herein.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon request.

Ethics Approval

The field and laboratory investigations were conducted in accordance with the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Uganda, Daktari East Africa, and Mission Rabies requirements for the purposes of

canine health management in Uganda. Therefore, this was a public health control program and did not require ethics committee approval or written consent from the dog owners.

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Authors' Contributions

JRO and JMF conceived and designed the study. JRO and JMF carried out faecal sample collection. JRO carried out laboratory sample diagnosis and statistical analysis. JG designed the study area map. JRO, JMF, and JJMK contributed to the study design and the manuscript. All authors participated in the manuscript write-up, reviewed the final version, and approved it to be submitted for publication.

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