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A survey of intestinal helminths in domestic dogs in a human–animal–environmental interface: the Oloisukut Conservancy, Narok County, Kenya

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Research Paper

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Abstract

Dogs living in a domestic–wildlife interface can serve as reservoirs and sentinels of parasites shared among humans, domestic animals and wildlife. In Kenya, the epidemiology of intestinal parasites of dogs and their role as reservoirs of zoonoses is poorly understood, especially in domestic–wildlife interfaces. This study aimed to determine the occurrence of intestinal helminths in domestic dogs in the Oloisukut Conservancy. One hundred dog faecal samples were collected per rectum and examined microscopically following zinc chloride flotation and formal-ether concentration techniques. Genotyping of helminths was achieved by nested polymerase chain reaction of NADH dehydrogenase subunit 1, cytochrome oxidase 1 and partial sequencing. Nine genera were detected by microscopy in 65 (65%) dog faecal samples from 54/76 (71.05%) households. The most frequent helminths were hookworm (39%), *Spirometra* spp. (17%), taeniids (13%), *Toxocara* spp. (10%), *Trichuris* spp. (10%), *Spirocerca lupi* (5%), *Physaloptera* spp. (2%), *Dipylidium caninum* (1%) and *Strongyloides* spp. (1%). *Ancylostoma caninum* was the only hookworm species detected in dogs, while *Taenia serialis* and *Taenia madoquae* were detected in four and one faecal samples, respectively. This study reports for the first time the molecular detection of the cestodes *Spirometra theileri*, *D. caninum* and *Mesocestoides* sp. in dogs in Kenya. The presence of zoonotic helminths in dogs indicates that the residents of this conservancy are exposed to public health risks. The helminths reported here confirm the interaction of domestic dogs with wildlife. An integrated control programme involving the medical, veterinary and wildlife conservation professionals is needed to avert transmission of infectious diseases to humans, domestic animals and wildlife.

Introduction

Intestinal parasites constitute some of the most common infections in humans and animals worldwide (Dado *et al*., 2012; Pullan *et al*., 2014). Dogs are reservoirs of several zoonotic parasites of public health significance worldwide (Deplazes *et al*., 2011) and are also linked with mechanical transmission of intestinal parasites such *Ascaris lumbricoides* (Shalaby *et al*., 2010), *Toxoplasma gondii* (Lindsay *et al*., 1997) and *Hymenolepis* (Meloni *et al*., 1993). In addition to being reservoirs of infectious agents to humans, animals and wildlife, intestinal parasitic infections in dogs are either asymptomatic or lead to severe clinical symptoms including anaemia, diarrhoea, vomiting, anorexia, dermatitis or even death, especially in puppies (Bowman, 2014).

Domestic dogs are potential reservoirs of infectious agents which are transmissible to wildlife, resulting in severe disease outbreaks such as that of canine distemper virus in the Serengeti–Mara ecosystems (Alexander & Appel, 1994; Roelke-Parker *et al*., 1996; Cleaveland *et al*., 2000) and Chile (Acosta-Jamett *et al*., 2011). The survival of wildlife is also threatened by emerging diseases in humans and domestic animals such as toxoplasmosis and cryptosporidiosis (Bowser & Anderson, 2018). Wild carnivores may also act as reservoirs of human and domestic animal infectious agents as a result of spill-over events (Otranto & Deplazes, 2019). In the domestic–wildlife interface areas, pastoralists keep dogs to help them in herding and guarding their livestock from wild carnivores. This practice leads to leaving these shepherd dogs without food, shelter and veterinary care and, therefore, encourages free roaming and scavenging in wildlife habitats, thus posing a health risk to humans, domestic animals and wildlife (Sparkes *et al*., 2015). Mass vaccination of dogs against rabies and canine distemper coupled with anti-parasitic treatment against helminths can help reduce the risk of

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dogs transmitting these infectious diseases (Cleaveland *et al.*, 2018; Dantas-Torres *et al.*, 2020). In Kenya, the human–domestic animal–wildlife interactions are common because of encroachment into national parks/game reserves by pastoral communities during grazing of livestock (Løvschal *et al.*, 2019).

Considering the frequent interactions between humans, domestic and wild animals, and the possibility of disease transmission, this is an ideal environment for the application of the One Health approach in disease control. The One Health approach has been defined by the American Veterinary Medical Association (AVMA) as 'the collaborative effort of multiple disciplines working locally, nationally and globally to attain the optimal health for people, animals and our environment'. The use of One Health has been encouraged by the Ministries of Health and Agriculture, Livestock and Fisheries in Kenya, and especially by the Zoonotic Disease Unit (ZDU) (Munyua *et al.*, 2019); thus, this study presented yet another opportunity for its application.

The introduction of wildlife conservancy areas within human habitats in Kenya has increased recently. In these areas, land utilization to maximize the well-being of wildlife and livestock keeping is encouraged (Løvschal *et al.*, 2019). However, this co-existence of wildlife, domestic animals and people poses the danger of transmission of infectious diseases (Nthiwa *et al.*, 2019). In Kenya, information on the epidemiology of intestinal parasites of dogs and their role as potential reservoirs of zoonoses is limited. Further, little is known of domestic dogs living in a human–animal–environmental interface and their potential as reservoirs and sentinels for infectious diseases in Kenya. This information is important for the development of public health and veterinary strategies for treatment, control and prevention of these zoonoses and other diseases in a One Health approach. This study involved the collaboration of physicians, veterinarians and scientists, both locally and globally, to estimate the prevalence human and animal diseases of importance in the community. Hence, it used the One Health approach to collect and analyse human, domestic animals and wildlife faecal and blood samples, and aims to report the data and design appropriate control measures tailored to the study area. This study, therefore, reports the helminths detected in dogs, in a human–animal–environmental interface, Oloisukut Conservancy, Narok County, Kenya. The results from human, livestock and wildlife samples are reported elsewhere.

Materials and methods

Study area

The study was conducted in the Oloisukut Conservancy in Narok County, Kenya (fig. 1). The Oloisukut Conservancy is a community initiative that was established in 2006 and started its operations in 2010. The conservancy is part of the greater Mara–Serengeti ecosystem and has an annual temperature ranging from 14.8°C to 28°C and rainfall range of 700–2300 mm, with an average of 1500 mm. The Trans Mara sub-county lies at an altitude of 1500–2500 m above sea level. The conservancy covers an area of 23,000 acres, comprising 51 individually registered parcels of land. At the time of sampling, the conservancy had a membership of 109 heads of households, with a human population of approximately 12,500; however, some of the members reside outside the conservancy (fig. 1). The livestock population was 21,200 cattle, 35,850 sheep and goats, 250 donkeys and 881 dogs at the time of the study (Mpario, pers. comm.).

Dog faecal sampling

Dog faecal samples were collected per rectum in households that were randomly selected in April 2019 during a rabies and canine distemper vaccination and deworming campaign. Approximately 10 g of faecal samples were collected from the rectum. The faecal samples were examined macroscopically for the presence of worms or proglottids before being preserved immediately in 80% ethanol.

Detection of intestinal helminths in dog faecal samples by zinc chloride flotation-sieving and formal-ether concentration techniques

The presence of helminth eggs was determined by the zinc chloride flotation technique (Mathis *et al.*, 1996). Approximately 2–3 g of faecal samples were transferred into 15 ml falcon tubes and excess ethanol drained off. Then, 8 ml of distilled water were added into the faecal samples (1:4) to wash off excess ethanol. The pellet was washed again with 8 ml of 1 × phosphate-buffered saline containing 0.03% Tween-20 (1:4); helminth eggs were harvested by adding 8 ml (1:4) of zinc chloride (specific gravity 1.45) and then sieved through two sequential sieves of 50 and 22 microns. Helminth eggs trapped by the 22-micron sieves were washed off through a funnel into a 15 ml falcon tube. The eggs were transferred into 2 ml microcentrifuge tubes after centrifugation at 400 rpm and stored in 70% ethanol.

For the formal-ether concentration method, excess ethanol was drained off from samples. Approximately 3 g of faecal samples were rinsed by adding distilled water and thoroughly mixing by vortexing and centrifugation. To the pellet, saline (0.85%) was added up to the 7 ml marker and topped up to the 10 ml marker by adding diethyl ether. The faecal samples were thoroughly mixed by vortexing and centrifuged at 2500 rpm for 15 min (Cheesbrough, 2009). Helminth eggs were examined at X10 and identified according to Soulsby (1968) and Thienpont *et al.* (1986).

Extraction of genomic DNA from helminth eggs or proglottids

Individual taeniid eggs were picked under the microscope (X4) using a pipette and transferred into 0.2 ml thin-walled polymerase chain reaction (PCR) tubes containing 10 µl of 0.02 M sodium hydroxide (NaOH) and lysed at 99°C for 10 min. For faecal samples containing proglottids, a small proglottid tissue was cut and lysed in 50 µl of 0.02 M NaOH at 99°C for 30 min. The QIAamp® Stool Mini Kit (Qiagen, Hilden, Germany) was used to extract genomic DNA of *Dipylidium caninum*, *Spirometra* spp. and hookworm. The eggs were lysed by adding 1.4 ml of ASL buffer and subjected to three cycles of freeze (−80°C for 30 min) and thaw (80°C for 15 min). The DNA was extracted according to the manufacturer's instructions and eluted in 50 µl of elution buffer.

Genotyping of helminths (*Taenia spp.*, hookworm, *D. caninum* and *Spirometra spp.*)

The taeniid lysates were used as a template in a nested PCR targeting part of NADH dehydrogenase subunit 1 as described by Mulinge *et al.* (2018). Briefly, the PCR was carried out in 25 µl reaction containing 1 × DreamTaq™ Green Buffer (Thermo Fisher Scientific, Massachusetts, USA), 0.2 mM deoxyribonucleotide

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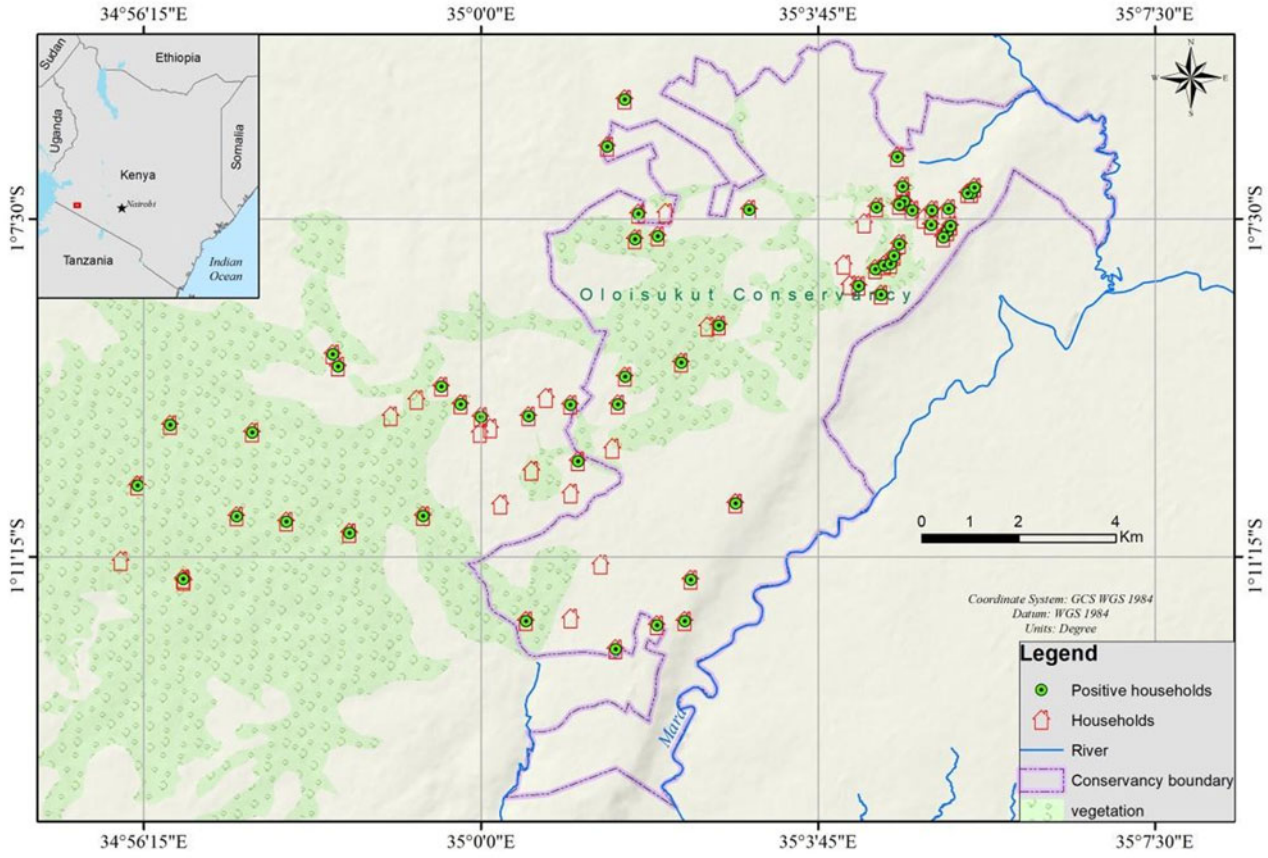

Fig. 1. Map of Kenya showing the Oloisukut Conservancy, sampled households and households with dogs infected with intestinal helminths.

triphosphates (dNTPs), 0.25 µM of each primer, 2 mM magnesium chloride ($MgCl_2$), 0.625 units of DreamTaq™ Green DNA Polymerase (Thermo Fisher Scientific, Massachusetts, USA) and 2 µl of taeniid lysate as DNA template. The cycling conditions for both PCRs were: 5 min of initial denaturation at 94°C, 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR amplicons were subjected to a restriction digest using *Hph*I endonuclease in 20 µl reaction containing 1 × CutSmart® Buffer, 0.5 µl of *Hph*I enzyme (New England Biolabs, Massachusetts, USA), 10 µl of PCR product and 7.5 µl of nuclease-free water. The restriction digests were incubated overnight at 37°C and resolved on 3% agarose gel containing ethidium bromide. Known positive controls for *Echinococcus granulosus* sensu stricto, *Echinococcus ortleppi*, *Echinococcus canadensis* (G6/7) and *Echinococcus felidis* were cut with *Hph*I and run alongside test samples. Genotyping of *D. caninum* and *Spirometra* spp. was done through the same nested PCR used for taeniids, but targeted DNA extracted from eggs or lysates of proglottids.

A single PCR targeting the internal transcribed spacer (ITS)-1, 5.8S and ITS 2 regions of *Ancylostoma* spp. and *Uncinaria stenocephala* (Traub *et al.*, 2004) was performed. The 50 µl PCR contained 5 µl genomic DNA, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 × DreamTaq™ Green Buffer (Thermo Fisher Scientific, Massachusetts, USA) and 1.25 units of DreamTaq™ Green DNA Polymerase (Thermo Fisher Scientific, Massachusetts, USA). The cycling conditions were identical to those used for taeniids except the number of cycles (50). A new nested PCR method targeting part of the cytochrome oxidase 1 gene (*cox1*) was developed and applied to genotype canine hookworm. The primary PCR was amplified using primers 5′-TGGTTCCTTTAATGTTGGGTGC-3′ and 5′-TGCTGCAGT AAAATAAGCCCG-3′, resulting in 667 bp amplicons. The nested PCR yielded a product of 606 bp and used primers 5′-GTTG GGTGCACCAGATATAAGT-3′ and 5′-GCTCAAACCACACAA CCAATCA-3′. Identical PCR and cycling conditions stated for taeniids were used for the new *cox1* hookworm PCR. For all the helminths, 10 µl of PCR amplicons were resolved on 2% agarose gel and visualized under ultraviolet following ethidium bromide staining.

Purification and sequencing of PCR amplicons

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR products were sequenced using nested PCR reverse primer depending on the helminth at the International Livestock Research Institute (ILRI), Nairobi, Kenya.

Statistical data analysis

The data were entered in Microsoft Excel 2013 (Microsoft Corporation) and transferred into STATA version 12.0 (STATA Corporation, College Station, Texas, USA) for analysis. The chi-square (χ^2) test was used to determine the potential correlation between age and sex of the dogs with helminth infections. The difference was considered significant when the *P*-value was ≤0.05. DNA sequences were viewed and edited using GENtle

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v. 1.9.4 (http://gentle.magnusmanske.de, Manske M. 2003, University of Cologne, Germany). The sequences were identified by comparing with those available in the National Centre for Biotechnology Information database (NCBI) using the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/; Altschul *et al.*, 1997).

Results

Demographic data

Faecal samples were collected from 100 dogs, 43 females and 57 males. The age of the dogs ranged between three months and 15 years. Faecal samples were collected from 76 out of 109 households (table 1 and fig. 1). Single faecal samples were collected from 57 households, two faecal samples from 15 households, three faecal samples from three households and four faecal samples from a single household (table 1).

Macroscopy examination and coproscopy analysis

Proglottids or whole cestodes were detected in 13 (13%) of the faecal samples. Nine genera were detected in dogs (table 2). Of the 100 faecal samples, 65 (65%) harboured at least one helminth infection and originated from 54/76 (71.05%) households (fig. 1). Hookworm infection was the most common ($n = 39$, 39%), followed by *Spirometra* spp. ($n = 17$, 17%). The other helminths detected included taeniid eggs ($n = 13$, 13%), *Toxocara* and *Trichuris* spp. ($n = 10$, 10%) each, *Spirocerca lupi* ($n = 5$, 5%), *Physaloptera* spp. ($n = 2$, 2%), *D. caninum* and *Strongyloides* spp. ($n = 1$, 1%) each (table 2).

Association of age, sex and intestinal helminth infections in dogs

The age groups ≤12, 25–48 and 49–72 months had the highest infections, and this correlated with the number of faecal samples examined (table 3). There was no significant association for helminth infections in general and the age of the dogs ($\chi^2 = 11.60$, $P = 0.07$). In general, helminth infections were more common in the male dogs (42%) compared to the females (24%); however, the difference was not significant ($\chi^2 = 3.49$, $P = 0.06$). Only the infections with hookworms, in which 47% and 28% male and female dogs, respectively, were infected, was significant ($\chi^2 = 3.90$, $P = 0.05$) (table 3).

Genotyping of helminths by PCR, Restriction Fragment Length Polymorphism (RFLP) and sequencing

Taeniids eggs were picked for PCR from nine of the 13-taeniid egg-positive faecal samples. Thirty-one (31) of 168 taeniid eggs tested by nested PCR yielded products and originated from five faecal samples. PCR products from 18 taeniid eggs were sequenced and identified as *Taenia serialis* (17) and *Taenia madoquae* (1). The *T. serialis* eggs originated from four faecal samples and the single egg of *T. madoquae* from one sample. All the *T. serialis* isolates were 100% identical to sequence accession number MH287113 (Mulinge *et al.*, 2020), a representative sequence was deposited in the GenBank under accession number MZ032032. The *T. madoquae* isolate was 99% identical to sequence AB731726 (Nakao *et al.*, 2013) and was deposited in the GenBank under accession number MZ032033. *Spirometra theileri* was detected by nested PCR from three faecal samples out of 17 microscopically positive samples; the other *S. theileri* isolate was from a proglottid since that faecal sample had no eggs on microscopy. The sequences of the *S. theileri* isolates in this study were 100% identical to the sequence accession number MN244299 (Eom *et al.*, 2019), an isolate from a leopard (*Panthera pardus*) in Tanzania. A representative sequence of the *S. theileri* isolates from this study was deposited in the GenBank under accession number MZ032034. One of the faecal samples with proglottids contained *Mesocestoides* sp. that was not identified to the species level. The short sequence (218 bp) was 98% identical to a *Mesocestoides* isolate (accession number MH998127) from a dog in Germany (Hirzmann *et al.*, unpublished data). Three faecal samples contained eggs/DNA for *D. caninum* by PCR and failed to show eggs on microscopy; two representative sequences were deposited in the GenBank with accession numbers MZ209412 and MZ209413. Of the 39 faecal samples containing hookworm eggs, PCR products were obtained from 18 samples: eight by ITS PCR and 15 by *cox1* nested PCR. The ITS PCR–RFLP method identified the eight isolates as *A. caninum*. These results were confirmed by the partial sequencing of the 15 *cox1* gene PCR products. Three sequences (accession numbers MZ021521–MZ021523) out of the four representative sequences were 99%

Table 1. Households and faecal samples collection in a human–animal–environmental interface, Oloisukut Conservancy, Kenya.

Total households	Households sampled	Faecal samples collected per household				
		1[a]	2	3	4	Total
109	76	57	15	3	1	100

[a]1–4 represent the number of faecal samples collected per household.

Table 2. Macroscopic and microscopic examination of dog faecal samples in a human–animal–environmental interface, Oloisukut Conservancy, Kenya.

	Zinc chloride flotation		Formal-ether concentration		Overall (both)	
Intestinal helminths	($n = 100$)	(P%)	($n = 100$)	(P%)	($n = 100$)	(P%)
Hookworm	38	38	4	4	39	39
Spirometra spp.[a]	16	16	10	10	17	17
Cestode proglottids[b]	13	13	13	13	13	13
Taeniid eggs	9	9	13	13	13	13
Toxocara spp.	5	5	6	6	10	10
Trichuris spp.	2	2	9	9	10	10
Spirocerca lupi	0	0	5	5	5	5
Physaloptera spp.	0	0	2	2	2	2
Dipylidium caninum[c]	0	0	1	1	1	1
Strongyloides spp.	1	1	0	0	1	1

P%, prevalence of helminths
[a]*Spirometra* spp. eggs were detected microscopically in 17 faecal samples, from one additional sample the eggs were detected by PCR and sequencing.
[b]Macroscopic examination (cestode proglottids such as *Taenia*, *Spirometra* and *Mesocestoides* spp.).
[c]Three additional samples contained *Dipylidium caninum* DNA/eggs by PCR and sequencing but had no eggs on microscopy.

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Table 3. Association of helminth infections with age and sex in domestic dogs in a human–animal–environmental Interface, Oloisukut Conservancy, Kenya.

Helminth age (months)	All helminths ($n = 65$)	Hookworm ($n = 39$)	*Spirometra* spp. ($n = 18$)	Taeniids ($n = 13$)	*Toxocara* spp. ($n = 10$)	*Trichuris* spp. ($n = 10$)	*Spirocerca lupi* ($n = 5$)	*Physaloptera* spp. ($n = 2$)	*Strongyloides* spp. ($n = 1$)	*Dipylidium caninum* ($n = 1$)
≤12 ($n = 21$)	16	9	5	3	4	2	2	0	1	0
13–24 ($n = 7$)	3	1	0	1	0	1	0	0	0	0
25–48 ($n = 29$)	22	16	5	4	3	3	3	1	0	0
49–72 ($n = 26$)	16	9	7	3	2	1	0	1	0	1
73–96 ($n = 11$)	7	4	1	2	1	1	0	0	0	0
97–120 ($n = 3$)	0	0	0	0	0	0	0	0	0	0
≥121 ($n = 3$)	1	0	0	0	0	1	0	0	0	0
Association with age	$\chi^2 = 11.60$	$\chi^2 = 9.20$	$\chi^2 = 5.34$	$\chi^2 = 1.26$	$\chi^2 = 3.52$	$\chi^2 = 3.62$	$\chi^2 = 5.28$	$\chi^2 = 1.68$	$\chi^2 = 3.80$	$\chi^2 = 2.88$
	$P = 0.07$	$P = 0.16$	$P = 0.50$	$P = 0.97$	$P = 0.74$	$P = 0.73$	$P = 0.51$	$P = 0.95$	$P = 0.70$	$P = 0.82$
Association with sex	$\chi^2 = 3.49$	$\chi^2 = 3.90$	$\chi^2 = 0.84$	$\chi^2 = 0.91$	$\chi^2 = 0.04$	$\chi^2 = 0.38$	$\chi^2 = 1.14$	$\chi^2 = 2.71$	$\chi^2 = 1.34$	$\chi^2 = 1.34$
	$P = 0.06$	$P = 0.05$[a]	$P = 0.36$	$P = 0.34$	$P = 0.84$	$P = 0.54$	$P = 0.29$	$P = 0.10$	$P = 0.25$	$P = 0.25$

[a]*P*-value was significant.

identical to *A. caninum* isolate AP017673 (Kikuchi *et al.*, unpublished data), while the other isolate (accession number MZ021524) was 100% identical to this reference sequence (Kikuchi *et al.*, unpublished data).

Discussion

This study reports nine genera of intestinal helminths detected in dog faecal samples in the Oloisukut Conservancy, a human–animal–environmental interface in Kenya. In a previous study in Turkana, Narok (Maasai Mara), Isiolo and Meru counties, 11 parasite genera/families were detected in dog faecal samples collected from the environment (Mulinge, 2020). The helminths reported in this study are either zoonotic, cause illness in dogs or/and are acquired from wildlife (*T. serialis*, *T. madoquae*, *Mesocestoides* sp., *S. theileri*, *Toxocara* spp., *Physaloptera* spp. and *S. lupi*). The helminths with zoonotic potential which pose a public health problem to humans including *T. canis*, *A. caninum*, *T. serialis*, *Strongyloides* spp., *Mesocestoides* sp. and *Spirometra* spp. On the other hand, *A. caninum*, *S. lupi*, *Mesocestoides* sp. and *Physaloptera* spp. cause illness in dogs and are, therefore, important to animal health. The control of these helminth infections in dogs, humans and wildlife calls for collaborative effort from the human, animal and environment health professionals in the context of the One Health approach.

Ancylostoma caninum was the only species from the genus identified in this study and these findings are similar to previous ones in Maasai Mara (Mulinge *et al.*, 2019). However, in that study, *Ancylostoma braziliense* and *Ancylostoma duodenale* were reported from other counties in Kenya. *Ancylostoma caninum* cause haemorrhagic enteritis in young puppies and chronic iron-deficiency anaemia in older dogs (Georgi *et al.*, 1969), while in humans it causes cutaneous larva migrans and eosinophilic enteritis (Bowman *et al.*, 2010). A few studies have reported the presence of *A. caninum* eggs/DNA in human faeces; however, it remains uncertain if these were natural infections or inadvertent passage, as non-patent worms of this species were detected in human intestines previously (George *et al.*, 2016; Ngcamphalala *et al.*, 2019; Furtado *et al.*, 2020).

The dogs could have acquired infection with *Toxocara* spp. by ingesting the embryonated eggs or paratenic hosts (rodents, lagomorphs, birds) infected with third-stage larvae or by transmammary or transplacental transmission (Macpherson, 2013). *Toxocara* spp. cause a range of conditions in humans such as ocular and visceral larva migrans, covert or common toxocariasis and neurotoxocariasis. *Toxocara* spp. eggs survive in the environment for a long time, increasing the risk of zoonotic transmission to humans. This is especially true for children, due to their habit of ingesting/playing with soil or being in close contact with domestic dogs (Ma *et al.*, 2018). The actual burden of human toxocariasis worldwide is likely to be underestimated due to the lack of diagnosis, resulting in underreporting (Holland, 2017). Similarly in Kenya, human infections with *Toxocara* spp. are not documented and, therefore, the relative contribution of domestic dogs and cats to human toxocariasis is unknown. The only available literature has shown that 7.5% of nomads from Turkana tested positive for antibodies against *T. canis* excretory–secretory antigens (Kenny *et al.*, 1995).

This study reports for the first time the detection of *Physaloptera* spp. in dogs in Kenya. The only previous account of *Physaloptera* spp. in Kenya was in baboons (Hahn *et al.*, 2003). The dogs could have acquired the infection with *Physaloptera* spp. after ingesting arthropod intermediate hosts (cockroach, crickets or beetles) or feeding on infected paratenic hosts such as rodents. Although *Physaloptera* spp. has no zoonotic potential, it causes gastritis, enteritis and excessive mucous secretion in dogs after the adult worm invades the stomach and duodenal walls (Anderson *et al.*, 2009).

Spirocerca lupi has serious effects in dogs, causing nodules in the oesophagus, aortic lesions and weight loss. *Spirocerca lupi* also infects wild canids such as foxes, wolves and raccoons (Anderson *et al.*, 2009) and infections in dogs were previously detected in Kenya (Murray, 1968; Wandera, 1976; Brodey *et al.*, 1977). Spirocercosis was also reported as the second common cause of death in dogs in Kenya among parasitic diseases after ancylostomiasis (Kagira & Kanyari, 2000).

Spirometra spp. are found frequently in dogs and wild carnivores in the Maasai Mara ecosystem (Engh *et al.*, 2003;

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Mulinge, 2020). This observation supports the earlier suggestion that a sylvatic cycle involving wild carnivores and herbivores as the second intermediate hosts could be responsible for the transmission of *Spirometra* spp. in Maasai Mara (Nelson *et al.*, 1965). The dog infections in this area could be due to the consumption of second intermediate hosts such as frogs. In Kenya, few human sparganosis case reports have been reported in Maasai and Pokot communities (Schmid & Watschinger, 1972). A recent case report was also identified in Maasai Mara during the Guinea worm eradication campaigns (MOH, 2016). These human infections could be due to consumption of water containing infected copepods originating from dog or wild carnivore faeces.

The two *Taenia* species (*T. serialis* and *T. madoquae*) reported in this study are transmitted mainly in sylvatic cycles. This observation is not surprising considering dogs live in a conservancy where they are free to hunt wild intermediate hosts such as hares and small antelopes. *Taenia serialis* uses the domestic dog as a definitive host and lagomorphs as intermediate hosts and is rarely found in squirrels, other rodents, cats and humans. The detection of *T. serialis* as the most common species in this study is contrary to previous findings that reported *Taenia hydatigena* and *Taenia multiceps* as the most common species in dogs in Maasai Mara (Mulinge *et al.*, 2020). Although *T. multiceps* was not reported in this study, a case of coenurosis in a goat was detected after necroscopy from one of the households in the conservancy. In addition, several clinical cases of coenurosis were encountered in small ruminants during this study and farmers reported that it is a common disease in the community. *Taenia serialis* was reported to be frequent in dogs from Meru and Isiolo counties (Mulinge *et al.*, 2020). So far there are no recorded human infections with *T. serialis* in Kenya and only 30 human coenurosis cases (African type coenurosis) are reported in Africa (Deplazes *et al.*, 2019). The dog infection with *T. madoquae* could have been acquired through feeding of the skeletal muscles of small antelopes such as dik-dik (*Madoqua* spp.). This species has been reported in dogs in Turkana, Isiolo and Maasai Mara (Mulinge *et al.*, 2020) and from silver-backed jackal (Jones *et al.*, 1988; Zhang *et al.*, 2007).

Dipylidium caninum rarely causes dipylidiasis in humans and only a few cases are reported globally (Sarvi *et al.*, 2018). *Dipylidium caninum* has been detected in dogs in Kenya during post-mortem examination of intestinal contents of dogs in Nairobi and Turkana (Wachira *et al.*, 1993; Buishi *et al.*, 2006) and also after faecal examination of domestic cats in Thika (Nyambura Njuguna *et al.*, 2017). Proglottids of *D. caninum* are not regularly voided in faeces and, therefore, coprodiagnosis techniques are likely to miss up to 95% of infections (Lillis, 1967) and this explains why *D. caninum* DNA was detected in faecal samples in the absence of eggs by microscopy.

Mesocestoides sp. is reported for the first time in dogs in Kenya, as jackals and red foxes are known to be the main definitive hosts (Karamon *et al.*, 2018). The dogs could have acquired infection with *Mesocestoides* by feeding on the tetrathyridium larva in the second intermediate hosts, which are rodents, amphibians, reptiles or birds (Loos-Frank, 1991). *Mesocestoides* spp. are potentially zoonotic, causing human infections after consumption of raw or undercooked snake, chicken and wild game viscera (Szell *et al.*, 2015). *Mesocestoides* spp. also causes a life-threatening illness in dogs. The tetrathyridium larvae can multiply asexually by longitudinal fission, penetrate the intestinal wall and invade the peritoneal cavity leading to peritonitis and pleuritis (Boyce *et al.*, 2011).

This study had a few limitations. First, the detection of taeniid eggs in dog faecal samples by microscopy is likely to underestimate the prevalence because eggs are not shed regularly and, therefore, would be missed during prepatent phase of infection. However, the gold standard method necroscopy and purgation are no longer applied due to ethical issues, side effects to dogs and environmental contamination (Craig *et al.*, 2015). Second, this study characterized individual taeniid eggs instead of extracting genomic DNA from taeniid-positive eggs. This approach is preferred because it has the ability to detect mixed infections of either *Echinococcus* or *Taenia* species or both. Extraction of genomic DNA from pooled eggs could increase the PCR sensitivity at the expense of missing the detection of mixed infections, especially if species-specific primers are not used (Stefanić *et al.*, 2004; Boufana *et al.*, 2008) or multiplex PCR is not applied (Trachsel *et al.*, 2007; Boubaker *et al.*, 2013; Liu *et al.*, 2015). Going forward, more sensitive methods such as real-time PCR are recommended for the characterization of taeniid eggs and DNA extraction from faecal samples whose taeniid eggs fail to yield amplicons.

In conclusion, domestic dogs living in a human–domestic–environmental interface are frequently infected with helminths of both public and animal health importance. This study reports for the first time in domestic dogs the molecular detection of *S. theileri*, *D. caninum* and *Mesocestoides* sp. The presence of some helminths confirms the interaction of domestic dogs and wildlife. This study provides baseline data and paves the way for further studies on humans, livestock and wildlife to better understand the epidemiology of infectious diseases at the human–animal–environmental interface. Addressing potential zoonotic disease threats requires the application of the One Health approach in the collection of data and the design and implementation of control and prevention strategies. Our observations indicate that the existing local intersectoral collaboration will support and promote such an approach.

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Conflicts of interest. None.

Ethical standards. This study was carried out according to the requirements of the Animal Care and Use Committee of Kenya Medical Research Institute, Kenya, the Institutional Animal Care and Use Committee (IACUC, R19IACUC004) and the Institutional Review Board (IRB, 14116363) of Western University of Health Sciences, Pomona, California, USA.

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