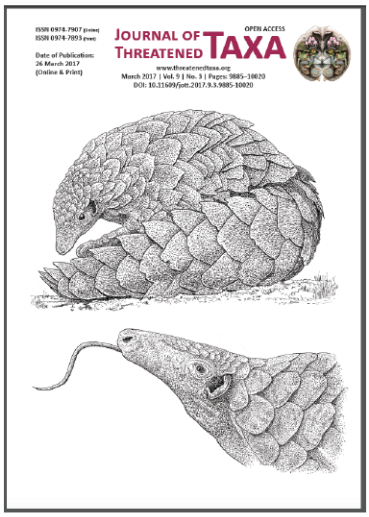

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COMMUNICATION

FIRST RECORD OF TAPEWORM *MONIEZIA* (CESTODA: ANOPLOCEPHALIDAE) INFECTIONS IN LEOPARDS: COPROLOGICAL SURVEY OF GASTROINTESTINAL PARASITES OF WILD AND CAPTIVE CATS IN SRI LANKA

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First record of tapeworm *Moniezia* (Cestoda: Anoplocephalidae) infections in Leopards: Coprological survey of gastrointestinal parasites of wild and captive cats in Sri Lanka

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Abstract: Sri Lanka is home to four species of wildcats: Leopard, Fishing Cat, Rusty-spotted Cat and Jungle Cat. All four, except the Jungle Cat, are listed threatened. A coprological survey was carried out in 2014 to determine the gastrointestinal (GI) parasites of wild and captive cats in Sri Lanka. Parasite eggs and cysts were isolated and morphologically identified using iodine smears and a modified salt flotation. The intensity of infection was quantified using a McMaster counting technique. A total of 45 fecal samples were analyzed. Except for the six captive Rusty-spotted Cats, all cats were infected with one or more GI parasites. The presence of *Moniezia* sp. in Leopards in the Horton Plains National Park with an intensity of 150–1850 EPG (eggs per gram of feces) was unexpected. *Moniezia* is a common GI parasite of ruminants and before our study it had never been recorded in Leopards. Cross species infection with *Moniezia* could be possible due to accidental ingestion of cysticercoid infected oribatid mites, the intermediate host which could have been picked up in the pasture while feeding on carcasses. Among the other parasitic infections in Leopards *Toxocara* was most common (61.9%) followed by strongyle infections (15.4%). Of the fecal samples collected from wild Leopards 80.0% were infected with GI parasites while no GI parasites were found in the captive Leopard samples. The Jungle Cats and the Rusty-spotted Cats sampled were in captivity and only the Jungle Cats were infected with strongyles. *Toxocara* was recorded in Leopards and Fishing Cat both in captivity and in the wild. It is a common GI infection of cats causing morbidity in all age groups and mortality in young animals. Although parasitic infections of cats may not be a direct reason for a species' decline, parasitic infections spreading within a small fragmented population could reduce the vitality and numbers and threaten the population further. This is the first report of GI parasites of wildcats of Sri Lanka and the first record of *Moniezia* infections in Leopards.

Keywords: Gastrointestinal parasites, Leopard, *Moniezia*, Sri Lanka, wildcats.

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INTRODUCTION

Sri Lanka is home to four species of wildcats: Leopard (*Panthera pardus kotiya*), Fishing Cat (*Prionailurus viverrinus*), Rusty-spotted Cat (*Prionailurus rubiginosa*) and Jungle Cat (*Felis chaus*). Of the four species, the Leopard is the largest carnivore and the top predator on the island (Miththapala 2006). All four wildcat species have a wide distribution within the country and the Leopard, Fishing Cat and Rusty-spotted Cat are found in all forest types while Jungle Cats are restricted to the northern monsoon forests of the dry zone (Phillips 1984). The IUCN Red List of Threatened Species (2016) lists the Leopard and Fishing Cat as Vulnerable (VU), Rusty-spotted Cat as Near Threatened (NT) while the Jungle Cat as Least Concern (LC). Habitat fragmentation is the main threat leading to fragmented populations restricted to small forest patches (Baille et al. 2004). Habitat fragmentation alters the distribution and abundance of hosts, vectors and pathogens and hence it is associated with increased disease risks for humans and wildlife (Daszak et al. 2000; Patz et al. 2004). Human encroachment is another problem faced by many of the wildcat species as it results in many Leopards and Fishing Cats being snared or killed in traps set for Wild Boars (Miththapala 2006). Since all four species are carnivores, they play a vitally important role for the stability and the integrity of most ecosystems (Murray et al. 1999). Therefore, the health of wildcats is an important aspect to maintain the health of the ecosystem. Parasitic infections of wild animals alone may not be a direct reason for the decline of species, but infections spreading within small isolated populations could reduce such populations further. Information on the parasites which infect wildcats in Sri Lanka will help to develop measures to prevent that infections spread within such populations.

Studies carried out on captive cats in zoos in India have recorded common GI parasites including *Paragonimus westermani, Dirofilaria immitis, Toxocara*, *Ancylostoma, Spirurida* and *Diphyllobothrium* spp. (Varadharajan & Kandasamy 2000; Acharjyo 2004; Thawait et al. 2014). Dissanaike & Paramananthan (1961) recorded the lung fluke infections (*Paragonimus westermani)* in Leopards, Fishing Cats and Jungle Cats from the northern parts of Sri Lanka. A recent study carried out in the Dehiwala Zoological Garden in Sri Lanka reported *Toxocara* infections in captive Leopard and Fishing Cat and *Strongyloides* infections in captive Jungle Cat but no GI infections in the Rusty-spotted Cat (Aviruppola et al. 2016). No recent studies are available on parasitic infections of wildcats in Sri Lanka. The present study was carried out to determine types, prevalence and intensity of GI parasites found in the four species of cats in the wild and captivity in Sri Lanka.

MATERIALS AND METHODS

A cross sectional survey of GI parasites was carried out (July–December 2014) by collecting fresh fecal samples from the four cats: Leopard, Fishing Cat, Rusty-spotted Cat and Jungle Cat. Samples were collected from five sites: Wild Cats samples were collected at Horton Plains National Park, Knuckles Forest Reserve and Gannoruwa Forest Reserve. Samples from captive cats were collected at the National Zoological Gardens in Dehiwala and samples from semi-captive cats were collected at Saliya Pura Army camp in the Anuradhapura District (Fig. 1). The semi-wild facility at Saliya Pura Army camp is under the Department of Wildlife Conservation of Sri Lanka and has been established with the aim of protecting wild cats, especially those found outside the protected areas. Injured wild animals are brought to the camp and treated by the camp's veterinarians until they are fit enough to be released back to the wild. While they are at the camp the animals are also vaccinated and treated against parasites. Captive animals at the National Zoological Gardens in Dehiwela receive regular veterinary care with deworming three times a year. All captive samples were collected in the morning soon after defecation. A scientist specializing in scatology was part of the team and identified the samples from wild cats as the animals were not in the vicinity at the time of collection. Samples were stored in a cooler and transported to the parasitology laboratory in the Department of Pathobiology, Faculty of Veterinary Medicine and Animal Science at the University of Peradeniya and were kept at 4^0C until processing. Direct saline smears were prepared for each fecal sample and were then stained with iodine. Then a modified salt flotation was carried out to all of the samples and slides were made to identify parasitic eggs, cysts and oocysts.

Direct saline and iodine mounts

A drop of saline and a drop of Lugol's iodine stain were placed separately on a glass microscope slide. Using a toothpick, a small portion (size of a match head) of the fecal sample was picked up and was mixed with the drop of saline. This was repeated with the drop of iodine. Two smears were covered with separate cover slips and observed under the light microscope. Eggs

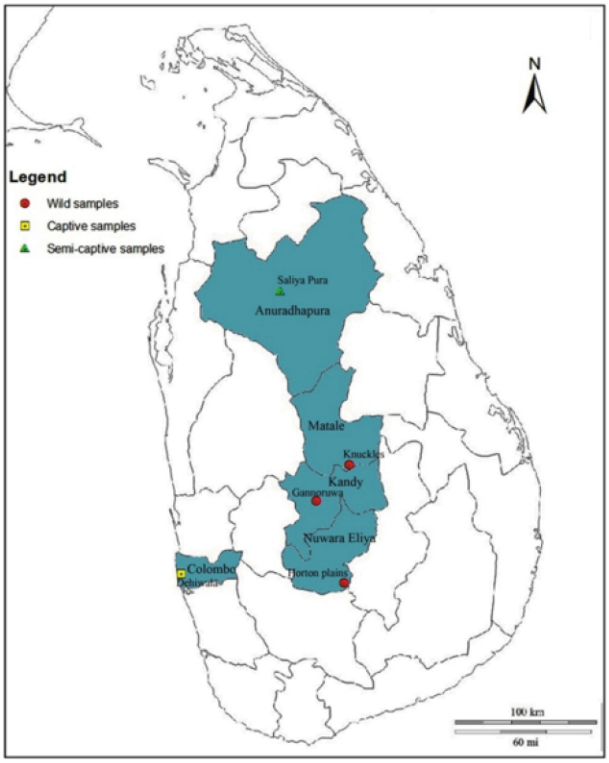

Figure 1. Sample collection sites in Sri Lanka

and cysts were identified and their relative numbers in a sample size of a match head was estimated. Length and the width of eggs and cysts isolated were measured using the calibrated eye piece graticule (10x40).

Modified salt flotation method

Approximately, 3g of feces were measured using an electronic balance and was taken into a 50ml capped centrifuge tube. Then the volume was filled up to 50ml by adding distilled water, and was mixed thoroughly using a wooden applicator. This suspension was centrifuged at 2,045g for 20min. The supernatant was discarded and the pellet was washed twice by re-suspending in distilled water followed by two centrifugations until a clear supernatant was obtained. The pellet was then emulsified with saturated salt and was mixed thoroughly. It was centrifuged again for 20min at 2,045g. Approximately, 5ml of the top meniscus were aspirated and transferred into a 15ml centrifuge tube. The tube was filled with distilled water to a total volume of 15ml and the mixture was centrifuged for 10min at 1,370g. This was repeated and finally 1ml of the suspension with the pellet was mixed and transferred to a 1.5ml microfuge tube (Eppendorf®) using a pasteur pipette. Distilled water was added to bring the volume up to 1.5ml and the mixture was centrifuged for 10min at 1,150g in the micro centrifuge. The supernatant was discarded and the pellet was thoroughly mixed with 0.5ml of the supernatant. Microscope slide smears were prepared using about 0.1ml of the suspension and covered with a cover slip without staining. Five smears were prepared from each sample and observed under the light microscope. Eggs of different species were identified and the number of eggs and cysts in 0.5ml was estimated. Eggs per gram (EPG) of feces were calculated assuming the method had concentrated all the eggs in the 3g of feces into 0.5ml.

We identified parasite eggs and cysts using literature and identification guides (Schmidt 1988; Jones et al. 1994). The number of EPG and cysts per gram (CPG) of feces was determined using a McMaster counting chamber. The prevalence of the GI parasites in the four wildcat species was calculated as a percentage as follows: Prevalence = (infected number Individual examined) 100%.

RESULTS AND DISCUSSION

A total of 45 fecal samples were collected from 20 wild and one captive Leopard, five wild and 10 captive Fishing Cats, six captive Rusty-spotted Cats and three captive Jungle Cats from the five sites (Table 1). Of all the samples 57.8% were positive for one or more GI parasites. The highest prevalence was found in samples from wild Leopards, which were infected to 80.0%. In general the prevalence of GI parasites was higher (68.0%) in samples collected from the wild animals compared to captive animals (45.0%; Table 2).

Three types of parasites were recorded: *Toxocara* sp., strongyle type (Nematoda) and *Moniezia* sp. (Cestoda; Tables 3 & 4). Wild animals were infected with all three types of parasites, while *Moniezia* infection was absent in the captive animals. Strongyle eggs were found in all the cats except the Rusty-spotted Cat. None of the six Rusty-spotted Cats sampled had any GI parasites. This is consistent with the previous findings where the captive Rusty-spotted Cats didn't harbor any gastrointestinal parasites in the Dehiwala Zoological Gardens (Aviruppola et al. 2016). This could be because the Rusty-spotted Cats were all captive bred animals while the other cats were wild caught. Leopards were infected with *Toxocara, Moniezia* sp and strongyle type parasites.

The occurrence of *Moniezia* sp. in Leopards at Horton Plains National Park was unexpected as this

Table 1. Number of fecal samples collected from wild and captive cat species from the five sampling sites

Status	Collection site	Cat Species	Number of fecal samples
Wild	Horton Plains	Leopards	12
	Knuckles	Leopards	8
	Gannoruwa	Fishing Cat	5
Captive	Dehiwala Zoo	Fishing Cat	8
		Leopard	1
		Rusty-spotted Cat	6
		Jungle Cat	3
	Saliyapura	Fishing Cat	2
Total 5			**45**

Table 2. Prevalence of infection of gastrointestinal parasites in the four cat species

Cat species	Status (n)	Prevalence of GI infections (%)	Total (%)
Leopard *Panthera pardus kotiya*	Wild (20)*	80.0	76.2
	Captive (1)	0.0	
Fishing Cat *Prionailurus viverrinus*	Wild (5)	20.0	60.0
	Captive (10)	80.0	
Rusty-spotted Cat *Prionailurus rubiginosus*	Wild (0)	-	0.0
	Captive (6)	0.0	
Jungle Cat *Felis chaus*	Wild (0)	-	33.3
	Captive (3)	33.3	

Table 3. Information on the intensity of parasite infections in the wild Leopard and Fishing cat positive samples

Nature Reserve	Host	Parasite	Intensity (EPG)
Horton Plains	Leopard 1	*Toxocara* sp.	1300
	Leopard 2	*Toxocara* sp.	50
	Leopard 3	*Toxocara* sp.	200
	Leopard 4	*Toxocara* sp.	250
		Strongyle sp.	200
	Leopard 5	*Moniezia* sp.	800
	Leopard 6	*Moniezia* sp.	1850
	Leopard 7	*Moniezia* sp.	250
	Leopard 8	*Toxocara* sp.	250
		Moniezia sp.	500
	Leopard 9	*Moniezia* sp.	150
		Toxocara sp.	350
	Leopard 10	*Toxocara* sp.	1400
		Strongyle sp.	200
	Leopard 11	*Toxocara* sp.	50
	Leopard 12	*Toxocara* sp.	250
Knuckles	Leopard 13	*Toxocara* sp.	400
		Strongyle sp.	150
	Leopard 14	*Toxocara* sp.	200
		Strongyle sp.	300
	Leopard 15	*Toxocara* sp.	100
	Leopard 16	*Toxocara* sp.	1400
Gannoruwa	Fishing cat	*Toxocara* sp.	100

Table 4. Types of parasites, their prevalence and intensity in the four cat species

Host	Parasite	Prevalence (%)	Mean Intensity (range)
Sri Lankan Leopard (*Panthera pardus kotiya*) All wild	*Toxocara*	61.9	477.6 (50–1400)
	Strongyle	19.0	212.5 (150–300)
	Moniezia	23.8	710.0 (150–1850)
Fishing Cat *Prionailurus viverrinus*	*Toxocara*	53.3	431.3 (50- 80,000)
	Strongyle	6.7	200 (one infected)
Rusty-spotted Cat *Prionailurus rubiginosus* All captive	-	-	-
Jungle Cat *Felis chaus* All captive	Strongyle	33.3	150 (one infected)

tapeworm had never been recorded from a carnivore before. *Moniezia* spp. have an indirect life cycle with ruminants such as sheep, goats or cattle as final hosts and oribatid mites (also called "moss mites" and "beetle mites") as intermediate hosts (Daubney 1932). Adult *Moniezia* live in the small intestine of the definitive host, usually a ruminant, where they lay their eggs, which are shed into the outside environment with the feces (Daubney 1932). These eggs are sticky and adhere to the vegetation or soil particles and can survive for months in the environment. The eggs are very sensitive to desiccation but some eggs may survive cold winters. The intermediate host, oribatid mite, ingests the eggs and the eggs hatch in the gut and develop to cysticercoids in the body cavity of the mite. Cysticercoids have a livespan of up to 18 months inside the mite. The ruminant becomes infected after ingesting the infected mites in the pasture while grazing. The cysticercoids inside the mites are released in the small intestine. They attach themselves to the wall of the small intestine and develop into adult tapeworms within several weeks. It is likely that the Leopards have accidentally ingested infected oribatid mites while feeding on dead carcasses on the grasslands. The natural hosts, ruminants are infected during the principal transmission cycle of the parasite while the cross species transmission or spill

over infections to the alternative host as alternative host is accidental. Parasites rarely have the ability to spread efficiently within a new, alternative host that was not previously exposed or susceptible. Such cross species transfers indicate either increased exposure of the new host to the parasite or that the parasites have acquired variations that allow them to overcome barriers to infection of the new host. In our case, *Moniezia,* which normally infects ungulates, acquired the ability to mature into the adult inside the carnivore host and produce eggs possibly due increased contact between the parasite and the alternative host; however, the viability of the eggs produced inside the carnivore and their ability to develop to cysticercoids inside the oribatid mite is unknown.

High intensities of *Toxocara* infection were found in Leopards and Fishing Cats. Toxocariasis is one of the most common parasitic infections of cats around the world debilitating all age groups and causing mortality in young animals. Currently, 23 species within the genus *Toxocara* are known (Gibbons et al. 2001). Among them, *T. cati* is a parasite of carnivores known from different geographic areas in the world and the species was reported from Asia (Sadighian 1970; Yasuda et al. 1993, 1994; Esfandiari et al. 2010; Somesh et al. 2010; Ghaemi et al. 2011), Europe (Torres et al. 1998; Bagrade et al. 2003; Valdmann et al. 2004) and Africa (Radwan et al. 2009). Postmortem examination of a young female Persian Leopard *Panthera pardus saxicolor* in Damghan city in Iran also found *Toxocara cati* infection as well as *Taenia taeniaeformes* and *Ancylostoma tubaeforme* (Youssefi et al. 2010). Another study from the Golestan National Park and Biosphere Reserve in Iran also reported *T. cati* infections in male and female Leopards (Ghaemi et al. 2011).

The habitats of wild cats are becoming smaller and more fragmented (Miththapala 2006). Habitat degradation, loss, and fragmentation are considered the primary causes for biodiversity loss (Wu 2013) and are key research topics in landscape ecology (Wilson 2016). Although infectious diseases are seldom considered actual drivers of species extinctions, for threatened populations in fragmented areas they can have an additive effect. Several threatened mammal species are being further negatively impacted by infectious diseases. Examples are the Serengeti lions and black footed ferrets threatened by canine distemper (Williams et al. 1988; Roelke-Parker et al. 1996), species of central African non-human primates threatened by Ebola virus and Marburg hemorrhagic diseases (Leroy et al. 2004) and Tasmanian devils by transmissible facial disease (Pearse & Swift 2006), although none of these species have become extinct yet. Only one study carried out in Christmas Island at the beginning of the 20th century identified diseases as the primary cause of extinction when investigating the disappearance of two endemic murid species (Wyatt et al. 2008). The effect of infectious diseases on biodiversity loss has not been widely studied, and the contribution of diseases to population declines and species extinctions is most likely being underestimated. Therefore information on disease prevalence in already threatened taxa is important to understand the role of disease in endangerment of species. Additional studies as the one we have presented here as well as postmortem examinations of dead wild cats to determine whether the death is due to GI parasites are necessary.

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Author Contribution: VK and CF: collected samples in the field, carried out the fecal analysis in the lab; AT Identified the scat samples and assisted in sample collection; RPVJR Supervised the parasite egg identification; RSR Designed the study, supervised and wrote the manuscript.

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