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Research Note

First record and molecular identification of *Toxocara cati* in a Pallas' cat *Otocolobus manul* from Kyrgyzstan

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Summary

In the present paper, we the report the first documented occurrence in the wild of *Toxocara cati* in the sole representative of the genus *Otocolobus*, the Pallas' cat. The identity of the parasite was confirmed by morphological characteristics and by genetic barcoding of the second internal transcribed spacer of ribosomal DNA. The morphological measures of the *T. cati* specimens from the Kyrgyz Pallas' cat were comparable to values reported. We discuss the conservation implication of our find.
Keywords: *Toxocara cati*; New host species; Pallas` cat; Kyrgyzstan; barcoding; internal transcribed spacer 2; morphometrics

Introduction

The genus *Toxocara*, which consist of nematode roundworms of the family Ascarididae, is currently considered to contain 27 species (Glickman & Schantz, 1981; Borecka, 2010). Some members of the group are of significant epizootic relevance, with predatory mammals from the family Canidae and Felidae acting as definitive host of the parasite (Okulewicz *et al*., 2012). *Toxocara cati*, for example, is a cosmopolitan parasite of domestic cats (*Felis catus*) whose adults live in the digestive tract of their definitive hosts (Glickman & Schantz, 1981; Mizgajska, 2001; Okulewicz *et al*., 2012). Moreover, the *T. cati* has been reported to occur worldwide in a relatively large number of wild felids (Okulewicz *et al*., 2012).

The Pallas' cat *Otocolobus manul* is a small-sized felid that has a large but fragmented range in Central Asia (Ross *et al*., 2015). It occurs in uplands, hilly areas, grassland steppe and semi desert regions, with its habitat typified by the presence of pikas *Ochotona* spp. and other small rodents that constitute the bulk of its prey. The species distribution coincides with regions characterized by large changes in annual and daily temperature, where deep snow cover does not accumulate (Heptner & Sludskii, 1972). Given its degree of habitat specialisation, the species generally occurs at low densities, even in optimal steppe habitat. However, as a result of habitat loss, hunting for furs and the cat's biological susceptibility to disturbance, populations are in decline and the species is now listed as Near Threatened (Nowell & Jackson, 1996; Ross *et al*., 2015).

Given the decline of wild Pallas' cats, knowledge of the general health status of wild population may be important in a conservation context. For example, while toxoplasmosis leads to high mortality rates of new-borns in captive populations, exposure to *Toxoplasma gondii* appears to be minimal in the wild (Brown *et al*., 2005). Here, we present the first record of a *T. cati* infestation observed in a Pallas' cat sampled in Kyrgyzstan. The identity of the parasite was confirmed both based on morphology and genetic barcoding.

Material and Methods

Sampling

On the 19th of August 2013, a dead female cat was found in a small valley near the Enilchek settlement in eastern Kyrgyzstan (42°0'6.9"N / 79°2'37.8"E; 2560 m.a.s.l.). The animal was identi-

fied as *O. manul* based on its long fluffy coat, short spherical skull, large eyes and small, widely set ears (Heptner & Sludskii 1992). According to a local shepherd, the cat had been killed five days prior by a shepherd dog. While most organs had started to decompose, the digestive tract was well-preserved. While removing and investigating the stomach and the intestinal tract, nine nematodes were found and conserved in 80 % Ethanol. The morphology of eight nematodes (the ninth individual was used for DNA barcoding only) was analysed using a Stemi 2000C (Carl Zeiss Microscopy GmbH, Jena, Germany) stereomicroscope and body measurements were taken with the help of a digital image analysis system (Microimage 4, Windows Corp.). Species identification was performed based on Hartwich (1974). The specimens have been deposited in the collections of the first author (CMH-T.c.7431) as well as of the Musée National d`Histoire Naturelle of Luxembourg (MNHNL 7431MH).

DNA extraction, amplification and sequencing

In order to confirm the morphological identification by genetic barcoding, a small part of one nematode (~2 mm) was put into a 1.5 ml Eppendorf with 20 µl of the Dilution Buffer contained in the Phire Animal Tissue Direct PCR Master Mix kit (F-140WH kit, ThermoFisher Scientific) and crushed with a flame-sterilised glass rod. This was followed by a centrifugation step of 1 min at 5000 *g*. Species identification was performed using the second internal transcribed spacer (ITS-2) of ribosomal DNA, using primers NC13 (5'-ATCGATGAAGAACGCAGC-3') and NC2 (5'-TTAGTTTCTTTTCCTCCGCT-3') to amplify a fragment of 527 base pairs (Gasser *et al*., 1997). Polymerase chain reactions were performed in an iCycler (Bio-Rad) in a total volume of 20 µL that contained 1x Phire Plant Direct PCR Master Mix and 0.5 µM of each primer. Finally, 1 µl of a 1 in 10 dilution of the extraction supernatant was added to the reaction tube. Cycling conditions consisted of an initial denaturation at 98 °C for 5 minutes, followed by 40 cycles with denaturation at 98 °C for 7 s, annealing at 60 °C for 8 s and 40s of an elongation at 72 °C, and a final elongation at 72 °C for 3 mins. PCR products were purified with the Agencourt AMPure XP system (Beckman Coulter). Sequencing was performed using the Big Dye Terminator chemistry (Applied Biosystems) on an ABI 3730 capillary DNA automated sequencer (Applied Biosystems). We performed a search for homologous sequences using the nucleotide BLAST algorithm as implemented at the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/).

Results

A total of nine nematodes were recovered from the intestinal tract of the cat and, based on their morphology, identified as *T. cati*. They were characterised by a pair of slightly striated cervical alea that represented 12 % of the total body length and an esophagus with a bulb at the posterior end. The males had terminal appendage and the females a vulva in the body's anterior third. The anterior region of the specimens was curved dorsally and the cuticle striated transversely. The buccal capsules had three lips: a dorsal one characterised by two large papillae its sides, as well as two subventral lips that each had a single large papilla in their centre as well as a small papilla. Denticles ranging in size from 115 to

Table 1. Selected morphometric measurements of *Toxocara cati* from Pallas' cat in Kyrgyzstan

Measurement characteristics	Male[a]			Female[b]		
	Min – max	Mean	±SD	Min – max	Mean	±SD
Body length (mm)	49.5 – 66.8	58.6	8.69	64.90 – 101.50	82.88	16.01
Body width (mm)	0.51 – 0.75	0.66	0.13	0.62 – 1.24	0.87	0.25
Spicules length (mm)	1.47 – 2.19	1.82	0.26			
Dorsal lip length (µm)	131.81 – 185.59	163.87	28.34	198.21 – 324.41	233.87	52.12
Dorsal lip width (µm)	156.57 – 214.54	186.93	29.09	158.54 – 298.65	235.39	56.14
Subventral lips length (µm)	156.56 – 198.61	177.59	29.73	141.59 – 216.86	181.57	30.51
Subventral lips width (µm)	146.81 – 209.61	178.21	44.41	145.26 – 257.61	197.83	40.43
Esophagus length (mm)	3.45 – 4.12	3.75	0.34	2.04 – 4.89	3.53	1.08
Esophagus width from the anterior end (mm)	0.11 – 0.16	0.14	0.03	0.12 – 0.21	0.17	0.03
Bulb length (µm)	254.29 – 571.31	441.28	114.73	301.45 – 754.68	538.72	169.24
Bulb width (µm)	156.51 – 346.50	249.10	95.09	224.49 – 475.11	338.30	91.47
Nerve ring from the anterior end (mm)	0.49 – 1.01	0.73	0.26	0.61 – 1.28	0.49	0.29
Excretory pore from the anterior end (mm)	0.46 – 1.15	0.82	0.35	0.68 – 1.27	0.93	0.27
Cervical alae length (mm)	1.58 – 3.25	2.48	0.70	1.95 – 3.94	2.82	0.72
Phasmids between the ventrolateral pair of papillae width to tail tip (µm)	65.67 – 89.54	75.96	12.27			
Phasmids length to tail tip (µm)				146.57 – 258.25	208.61	40.91
Cloarca to tail lip (µm)	125.57 – 218.79	167.42	47.33			
Anus length to tail lip (µm)				368.54 – 641.5	514.17	102.57

[a] Adults nematodes *n*=3
[b] Adults nematodes *n*=5
±SD Standard deviation

130 µm (mean 119) were located in the margin of each lip, while interlabia were absent.

The identification of the nematodes as *T. cati* was confirmed by genetic barcoding of the ITS-2 region in one individual. It was possible to generate 471 base pairs of clean sequence (GenBank® accession no KT873462). The two sequences producing the best match (both 97 % query cover, 99 % identical) originated from *T. cati* specimens sampled from domestic cats in India (GenBank® no KJ777179) and Japan (GenBank® no AB571303).

The nine recovered *T. cati* consisted of three males and six females. One male was located in the stomach and one female in the colon, while the remaining parasites were found in the small intestine. In the case of the three males, the body length and the body width at the end of the esophagus varied from 49.5-66.8 mm (58.6 ± 8.69 mm) and 0.51 – 0.75 mm (0.66 ± 0.13 mm), respectively. The corresponding values for the females were 64.9-101.5 mm (82.88 ± 16.01 mm) and 0.62 – 1.24 mm (0.87 ± 0.25 mm), respectively. The length of the male spicules varied between 1.47 and 2.19 mm (1.85 ± 0.26 mm). Further morphological measurements can be found in Table 1.

Discussion

In the present paper, we the report the first documented occurrence in the wild of *T. cati* in the sole representative of the genus *Otocolobus*, the Pallas' cat. The nematode does appear to be widespread among the Felidae (Okulewicz *et al*., 2012). In Asia, for example, *T. cati* was identified both in members of the Pantherinae (*Panthera tigris altaica*, Gonzalez *et al*., 2007; *Panthera pardus saxicolor,*Youssefi *et al*., 2010, Esfandiari *et al*., 2010, Ghaemi *et al*., 2011) and other Felinae (*Prionailurus bengalensis euptilurus*, Machida 1970; Yasuda *et al*., 1993, 1994; *Prionailurus bengalensis iriomotensis*, Yasuda *et al*., 1994; *Felis chaus* Sadighian 1970). The morphological measures of the *T. cati* specimens from the Kyrgyz Pallas' cat were comparable to values reported by Warren (1971), Vicente *et al*., (1997), Beldomenico *et al*., (2005), Radwan *et al*., (2009), Esfandiari *et al*., (2010) and Gallas and Silveira (2013). The range and average number of denticles per lip counted in the present study was slightly lower than value reported in Baruš *et al*. (1979) and Gallas and da Silveira (2013).

The conservation implications of the discovery of *T. cati* in the Pallas' cat are not clear. The pathology of the infestation in domestic cats suggests that its effects might be comparatively mild. While pre-natal infection with *T. cati* does not occur in domestic cats, the roundworm can be transmitted to kittens via the trans-mammary route. While kittens up to several months of age may show clinical symptoms, infestation with does not usually result in death. However, ill thrift and death may occur in kittens that are heavily infected as a result of being reared in unhygienic communal environments (Jubb *et al*., 1992).

Another aspect that needs further clarification is how widespread the infestation is in the natural range of the Pallas's cat. The main source of *T. cati* infection in humans is widespread contamination of the environment with infective eggs by domestic cats (Fisher 2003). Given the Pallas' cat's habitat of uplands, steppe and semi-desert regions, human population density in its distribution range is very low. While in the Pallas' cats Russian distribution range, sheepherders and cattle farms keep domestic cats as companion animals, this is not the case in other parts of its range (in Mongolia for example; Brown *et al*., 2005). Given that the Pallas' cats themselves only occur at low densities, it appears unlikely that the parasite generally has a high prevalence in this newly discovered definitive host. Nevertheless, this question needs to be investigated further – especially in regions where people keep domestic cats – via the analysis of the intestinal tracts or faeces of legally obtained carcasses from different parts of the cat's range.

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