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Short communication

Ascarid infestation in captive Siberian tigers in China

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ABSTRACT

The Siberian tiger is endangered and is listed by the International Union for the Conservation of Nature; the captive environment is utilized to maintain Siberian tiger numbers. Little information regarding the prevalence of parasites in Siberian tigers is available. A total of 277 fecal samples of Siberian tigers were analyzed in this study. The microscopic analysis indicated the presence of ascarid eggs of *Toxascaris leonina* and *Toxocara cati*. The ascarid infection rate was 67.5% in Siberian tigers. The internal transcribed spacer-1 (ITS-1) phylogenetic analysis indicated that *T. leonina* belonged to *Toxascaris* and that *Toxo. cati* belonged to *Toxocara*. The infestation rate and intensity of *T. leonina* were higher than those of *Toxo. cati*. One-way analysis of variance showed that the presence of *T. leonina* was significantly associated with age ($P < 0.05$). Temperature changes also influenced *T. leonina* and *Toxo. cati* infestation, and a rise in temperature caused an increase in the number of *T. leonina* and *Toxo. cati* eggs. This study provides a better understanding of ascarid infestation among the captive Siberian tigers and is helpful for the prevention of the spread of infectious parasitic diseases among other tigers in the zoo.

1. Introduction

The population and geographical distribution of Siberian tigers (*Panthera tigris altaica*), a species of the boreal forest ecosystem in the Russian Far East and northeastern China, has declined dramatically due to habitat fragmentation and poaching over the past century (Tian et al., 2014). The Siberian tiger is listed as an endangered species by the International Union for the Conservation of Nature and is included in the CITES Appendix 1 (Guo et al., 2014). Wild Siberian tiger populations are very small; presently, only approximately 400–500 tigers exist on the planet (Seimon et al., 2013).

Pawar et al. (2012) reported that ascarids could also infest captive Asiatic lions in a zoological garden of India and indicated that the prevalence of *Toxascaris leonina* and *Toxocara cati* was 68.75% (11/16) and 9.09% (1/11). González et al. (2007) reported that the wild Siberian tigers could be infested by *T. leonina* and *Toxo. cati* in the Russian Far East, but no other important epidemiological information could be inferred because only three fecal samples were analyzed in the research. A few other studies were performed on the ascarid infestation of Siberian tigers in the wild or in captivity.

In nature, wild animals live in large areas and consequently have low genetic resistance against parasitic infections because of low exposure. However, when these wild animals are kept in captivity, such as in zoological gardens, the problem of parasitic infections can aggravate and pose a serious threat to endangered species and occasionally cause a sudden and unexpected local decline in abundance (Lim et al., 2008).

Therefore, this study attempted to determine the occurrence and intensity of ascarid infestations in captive Siberian tigers in the Northeast Tiger Zoo of China. The findings of this study provide a better understanding of ascarid infestations among captive Siberian tigers and can help prevent the spread of infectious parasitic diseases among other tigers in the zoo.

2. Materials and methods

There are 526 Siberian tigers of various ages that live in the Northeast Tiger Zoo of China (45°81′N, 126°59′E), and most of them are in a semi-wild condition. A total of 277 fecal samples were collected from tigers of different ages that were sick, injured, or unable to live independently during September 2012 to August 2013. The samples were sorted into six groups based on the temperature

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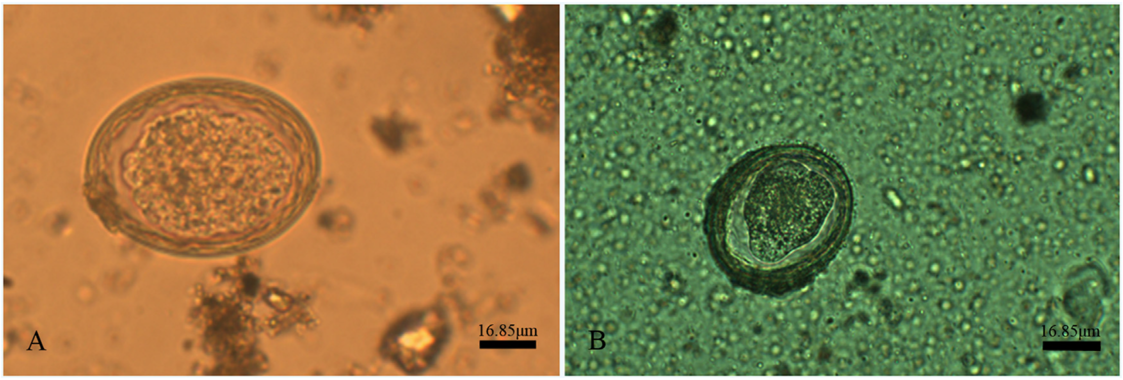

Fig. 1. Morphology of *T. leonina* (A) and *Toxo. cati* (B) eggs recovered from the fecal samples of Siberian tigers (×400).

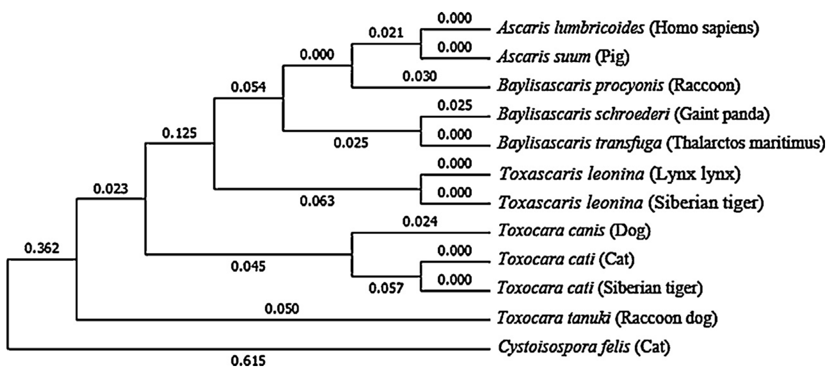

Fig. 2. The inferred phylogenetic relationship among representative ascarids based on ITS-1 utilizing maximum likelihood with *Cystoisospora felis* as the outgroup.

when they were collected, namely, <−20 °C (greater than the naturally lowest temperature in Harbin, China), −20 °C to −10 °C, −10 °C to 0 °C, 0 °C to 10 °C, 10 °C to 20 °C, and >20 °C (less than the naturally highest temperature in Harbin, China), and into four groups based on age, namely, less than 1 year, 1–2 years, 2–3 years, and more than 3 years.

Ascarids, either *T. leonina* or *Toxo. cati*, were identified using standard parasitological criteria based on morphology. Fecal egg counts, an index for the intensity of infestation, were determined by the modified McMaster technique using a saturated solution of sodium chloride as the floating medium (Nwosu et al., 2007). The DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Germany) and according to the manufacturer's instructions. The ITS fragment of *T. leonina* was subjected to polymerase chain reaction (PCR) amplification by forward (NC5:5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and reverse (NC2:5′-TTAGTTTCTTTTCCTCCGCT-3′) primers (Li et al., 2007). The ITS-1 sequences of *Toxo. cati* from several species were aligned to facilitate the design of universal primers: forward (SY:5′-GTGTTGAGGGGAAATGGGTGAC-3′) and reverse (XY:5′-CAATGTGCGTTCGAAATGTTAG-3′). Each PCR reaction yielded a single band. The PCR products were sent to Bios for sequencing (Harbin, China). Using MEGA 4.1 biological software (Liu et al., 2016), the gene sequences of *T. leonina* and *Toxo. cati* were contrasted with other ascarids' ITS-1 sequences available in the GenBank database to establish the phylogenetic tree analysis. A data analysis was conducted using SPSS statistical software (Alemu et al., 2011).

Percent Identity (upper right) / Divergence (lower left)

	1	2	3	4	5	6	7	8	9	10	11	12		
1		100.0	89.9	90.9	79.7	91.2	44.6	66.1	79.5	70.2	80.8	77.4	1	*Ascaris lumbricoides*
2	0.0		89.9	90.9	79.8	91.1	44.6	66.0	79.6	70.1	80.8	77.4	2	*Ascaris suum*
3	10.9	11.0		91.8	78.3	96.9	45.7	65.1	78.0	67.4	79.9	76.0	3	*Baylisascaris schroederi*
4	9.8	9.7	8.7		79.3	93.5	46.5	64.0	79.1	66.2	79.4	76.9	4	*Baylisascaris procyonis*
5	23.9	23.8	25.9	24.3		79.3	43.2	66.5	99.8	69.0	80.7	75.2	5	*Toxascatis leonina* (Lynx lynx)
6	9.4	9.6	3.2	6.9	24.4		45.5	65.6	79.1	68.1	81.1	77.1	6	*Baylisascaris transfuga*
7	104.4	104.4	100.4	98.6	112.0	101.4		45.3	42.9	44.4	50.0	44.0	7	*Cystoisospora felis*
8	46.3	46.5	48.1	50.0	45.2	47.3	100.1		66.5	88.6	100.0	85.5	8	*Toxocara cati* (Cat)
9	24.2	24.1	26.3	24.7	0.2	24.8	113.6	45.2		68.9	80.7	75.5	9	*Toxascatis leonina* (Siberian tiger)
10	38.6	38.7	43.8	45.7	40.5	42.4	104.6	12.4	40.6		93.0	87.8	10	*Toxocara canis*
11	22.4	22.4	23.8	24.5	22.6	22.2	85.8	0.0	22.6	7.4		84.3	11	*Toxocara cati* (Siberian tiger)
12	27.4	27.4	29.6	28.2	30.7	27.9	105.3	16.3	30.2	13.4	17.7		12	*Toxocara tanuki*
	1	2	3	4	5	6	7	8	9	10	11	12		

Fig. 3. ITS-1 sequences homology analysis of partial ascarids.