RESEARCH NOTES

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Coprology of *Panthera tigris altaica* and *Felis bengalensis euphilurus* From the Russian Far East

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ABSTRACT: Fecal samples from the Siberian tiger (*Panthera tigris altaica*) and the Amur cat (*Felis bengalensis euphilurus*) from Far Eastern Russia, were examined for parasites. A natural sedimentation methodology was used and a complete examination of all the sediment was performed. This fecal investigation allowed us to isolate and identify several developmental stages of gastrointestinal, hepatic, and respiratory parasites. Five parasites were found from *P. t. altaica*: 11 trematodes (*Platynosomum fastosum*) and 4 nematodes (*Strongyloides* sp., Ancylostomatidae, *Toxascaris leonina*, and *Toxocara cati*). Five parasites were found from *F. b. euphilurus*: 1 cestode (*Diplopylidium* sp.) and 4 nematodes (*Trichuris* sp., Ancylostomatidae, *Toxascaris leonina*, and *Aelurostrongylus abstrusus*). In addition, trophozoites of the amoeba *Acanthamoeba* sp. were detected in tiger feces.

Although there are several postmortem parasitological studies of free-ranging and captive felids in different parts of the world, coprologic investigations are scarce and limited to a few species, such as *Panthera leo* (Bjork et al., 2000), *Panthera pardus* (Patton and Rabinowitz, 1994), wild *Felis catus* (Palumbo et al., 1976), *Felis sylvestris* (Yamaguchi et al., 1996), and *Felis sylvestris* and *Lynx pardinus* (Rodríguez and Carbonell, 1998). Studies on captive tigers have also been done (Garner et al., 1996; Jakob and Wesemeier, 1996); however, little is known about parasites of wild *Panthera tigris* (Mandal and Choudhury, 1985; Patton and Rabinowitz, 1994; Marathe et al., 2002). In the case of the subspecies, *Panthera tigris altaica*, an occurrence of toxoplasmosis was described as a result of a single veterinary examination in a zoo (Dorny and Franssen, 1989). References regarding *Felis bengalensis* are poor (Patton and Rabinowitz, 1994) and those dealing with the subspecies, *Felis bengalensis euphilurus*, are exclusively from a Czech Republic zoo (Lukesova and Literak, 1998) or postmortem studies on wild specimens (Yasuda et al., 1993, 1994).

The understanding of these pathogens found on wild animals allows us to increase the knowledge about the ecology of wild felids and to establish correct programs of prophylaxis if necessary.

During September and October 2001, an expedition to Far Eastern Russia was carried out with the aim of studying the *Panthera tigris altaica* subspecies and its survival perspectives, and to collect data on another under-studied felid: *Felis bengalensis euphilurus*. Fecal samples were collected by a team of Russian guides and biologists as part of this project. The number of fecal samples found was less than expected because of several different factors: the rugged orography, the number and dispersion of these hosts, and the difficulty in correctly identifying these samples (the expedition team only collected samples that were perfectly identifiable). In this study, 3 fecal samples of Siberian tiger (*P. t. altaica*) and 2 of Amur cat (*F. b. euphilurus*) were collected. Samples were from the reserves of Lazovsky and Sukan, from the south-eastern region of Lake Hanka, and zones near Vladivostok (43°09'N, 131°53'E). Each fecal sample, approximately 10 g, was collected and preserved in 2% aqueous (w/v) dichromate potassium. We employed a filtration–sedimentation technique for the diagnosis of the parasites. All samples were incubated at room temperature in order to observe the embryonic development of eggs.

Parasitic forms representing 9 taxa were found in the fecal samples. Each analyzed sample was positive. Table I shows the number and distribution of the parasitic forms. Parasites were identified according to size and morphology of the forms present in feces (Hartwich, 1974; Khalil et al., 1994; Bowman, 1995). In *P. t. altaica*, *Toxocara cati* shows the higher prevalence, followed by *Platynosomum fastosum*,

whereas in *F. b. euphilurus* the Ancylostomatidae eggs have been the most prevalent, followed by *Trichuris* sp.

One species of amoeba was found: *Acanthamoeba* sp. We have only observed trophozoites; cysts have not been seen. *Acanthamoeba* sp. (family Hartmannellidae Volkonsky, 1931) was observed in one of the tiger samples. The trophozoites measured 10–25 µm, with hyaline projections as spikelike, star-shaped pseudopodia (acanthopodia). This amoeba belongs to a genus of free-living amoebas inhabiting fresh waters, rivers, pools, lagoons, and ponds. *Acanthamoeba* spp. also has been isolated from vegetation and from animals including fish, amphibians, reptiles, and mammals (Madrigal Sesma, 1988; Dykova et al., 1999). This amoeba occasionally infects man and animals, behaving as a pathogen mainly in immunosuppressed individuals (Bussières and Chermette, 1992). *Acanthamoeba* spp. are the causative agents of granulomatous amebic encephalitis (GAE), a fatal disease of the central nervous system (CNS), and amebic keratitis (AK), a painful sight-threatening disease of the eyes that can also cause cutaneous lesions and sinusitis (Martinez and Janitschke, 1985; Martinez and Visvesvara, 1997). *Acanthamoeba* spp. have been isolated from nasal mucosa, throats, brain, lung tissue, skin lesions, corneal tissue, intestinal contents, and feces (Cerva et al., 1973; De Jonckheere, 1991; Visvesvara et al., 1990). The works about isolation of amoebas from intestinal contents and feces are more common in humans than in animals (Velloso, Alves et al., 1984; Velloso, Manzini et al., 1984; Moura et al., 1985; Echandi et al., 1994; Zaman et al., 1999). Because of the predisposition that tigers show for water, the lack of extended behavior in felids, and the very important role that water plays in the amoeba dispersion, it could be surmised that tigers would be exposed to an infection of this type, particularly considering immunodeficiency states and other factors. It is possible that cysts have not been observed because excystation may have occurred before the samples came to the laboratory.

Eggs of *Platynosomum fastosum* Kossack, 1910, a hepatopancreatic fluke of the family Dicrocoeliidae Odnher, 1911 that occurs in the liver, bile and pancreatic ducts of numerous domestic and wild felids, were isolated from 2 tiger samples. These eggs, 44–60 × 24–38 µm (n = 15), were operculated, brownish, and oval, and contained a fully developed miracidium. The fact that 30% of these eggs hatched could mean that this is a dicrocoelid with an aquatic cycle where miracidia escape from eggs and would infect aquatic snails, but certainly in the known cycle of this species, eggs are ingested by terrestrial snails, such as *Subulina octona*, in which cercariae develops. Therefore, felids only become infected by eating the second hosts (amphibians and reptiles). Because of the size of the second hosts, the infection could occur when tigers are kittens or very young. *Subulina octona* has a worldwide distribution, having been found in Asia, Europe, West Africa, and America, but we cannot affirm the existence in eastern Russia. Genera of snails involved in Dicrocoeliidae cycles in the former USSR are *Helicella*, *Zebrina*, *Helix*, and *Bradybaena* (Boray, 1985). *Dicrocoelium dendriticum* eggs (a parasite of herbivores) are practically equal to those of *Platynosomum* but we discarded such possibility for several reasons. In the first place, *Dicrocoelium* eggs (38–45 × 22–30 µm) are smaller than those of *Platynosomum* and secondly, if they were from an ingested herbivore, we would have found more spurious parasites.

Diplopylidium sp. (family Dilepididae Rillet & Henry, 1909), found in cat feces, is a tapeworm of carnivores with characteristic embryonated eggs. Elliptical egg capsules 55–60 × 20–44 µm (n = 10) contain only 1 oncosphere or hexacanth embryo which is well developed and surrounded by a gelatinous thin layer and an outer fibrous capsule. Therefore each capsule contains 1 egg. *Diplopylidium* species described

TABLE I. Number and distribution of each species of parasite. S. = sample; x/x = number of eggs found in the sample/number of larvae found in the sample.

Parasite name	<i>Panthera tigris altaica</i>			<i>Felis bengalensis euphilurus</i>	
	S.1	S.2	S.3	S.1	S.2
<i>Acanthamoeba</i> sp.			*		
<i>Platynosomum fastosum</i>	10/0	—	105/0	—	—
<i>Diplopylidium</i> sp.	—	—	—	10/0	—
<i>Trichuris</i> sp.	—	—	—	12/0	—
<i>Strongyloides</i> spp.	3/1	—	5/10	—	—
Ancylostomatidae sp.	—	9/4	—	—	43/0
<i>Aelurostrongylus abstrusus</i>	—	—	—	—	0/7
<i>Toxocara cati</i>	95/0	154/0	943/0	—	—
<i>Toxascaris leonina</i>	—	—	23/0	2/0	—

* Presence of amoeba trophozoites.

in felids are *Diplopylidium acanthotetrum* and *Diplopylidium nölleri*. *Diplopylidium acanthotetrum* has been found in wild felids of Europe and the Middle East, having amphibians and reptiles as paratenic hosts. *Diplopylidium nölleri* is a canid and felid parasite found in Asia, Europe, and North Africa. We cannot disregard the possibility that these eggs belong to another dilepidid tapeworm, *Joyeuxiella* sp. Fuhrmann, 1935, in which the uterine capsule also contains only a single egg. To distinguish *Diplopylidium* from *Joyeuxiella* we would need to have the adult forms (Schmidt, 1986). Species described in wild and domestic felids are *Joyeuxiella chyeri* (Ratz, 1897) syn *Joyeuxiella pasqualei* (Diamare, 1893) and occur in Europe, Asia, and Palestine (Schmidt, 1986; Khalil et al., 1994), and *Joyeuxiella rossicum* (Skrjabin, 1923) in Russia. First intermediate hosts known in both dilepidids are coprophagous arthropods, adult or larvae or related to animals (fleas and lice). Reptiles, amphibians, and small mammals are also known as paratenic hosts (Rysavy, 1973; Roca et al., 1987).

The eggs of *Capillaria* sp. (family Trichuridae) (Ransom, 1911) appeared in the cat samples and measured 62–66 × 30–32 μm (n = 10). The eggs differ from those of *Trichuris* only in detail. They are ovoid, lemon-shaped, slightly irregular in symmetry, with a transparent plug at either pole and display a yellowish or orange-brown coloration with a pitted ornamentation. They contain an unsegmented embryo (a cell stage) when laid. First-stage larva developed in about 7 days, then stopped their development. They are widely distributed and can be found in numerous domestic and wild carnivores (Bowman, 1995; Osborne and Delmar, 1995). In dogs and cats, the adult worms have been associated with the intestinal and urinary tract as well as the bronchi. *Capillaria aerophila*, *Capillaria feliscati*, *Capillaria hepatica*, *Capillaria plica*, and *Capillaria putorri* are the species of this genus, which has felids as definitive hosts (Collins and Charleston, 1972; Holmes and Kelly, 1973; Greve and Kung, 1983; Bédard et al., 2002; Foster et al., 2004).

Embryonated eggs and larvae of *Strongyloides* spp. (family Strongyloidea) (Chitwood & McIntosh, 1934) were found in 2 tiger samples. These eggs have a very thin shell and measured 48–50 × 30–32 μm (n = 4); the larvae range was 300–380 μm (n = 8). The eggs hatched in 2 days. It is possible that they were second-stage larvae because of the presence of a rhabditiform esophagus and because the filariform larvae of the third stage are longer (600–620 μm). Four species of *Strongyloides* have been described in felids: *Strongyloides tumefaciens*, *Strongyloides felis*, *Strongyloides stercoralis* (these 3 have a cosmopolitan distribution) and *Strongyloides cati*, from Southeast Asia (Carter, 2001).

Eggs of Ancylostomatidae Looss, 1905, were found in both hosts' samples, being smaller in the tigers, 60–80 × 36–60 μm (n = 15), ovoid-shaped, with a thin and transparent shell and containing 8–16 blastomers. Embryonic development happened before the seventh day and allowed us to see the first-stage larvae inside the egg. We could also observe them hatch as larvae of 310–350 μm with rhabditiform

esophagus and without sheath. Eggs found in the cats were bigger: 80 × 60 μm. It was not possible to distinguish the different genera of this family via examination of these eggs. Focusing on egg size, the eggs of Ancylostomatidae found in the tigers are more similar to those of *A. caninum* and *A. tubaeforme* (Melhorn et al., 1992), whereas those recovered from the cats are more similar to *A. ceylanicum*. According to the geographical distribution, each of the cited parasites is cosmopolitan in distribution, although *A. ceylanicum* has been cited from Asian felids, including the tiger (Biocca, 1951).

Larvae of the parasite *Aelurostrongylus abstrusus* (Railliet, 1898) (family Angiostrongylidae Boehm and Gebauer, 1934) were found in 1 of the cat samples; their measurements varied between 340 and 360 μm (n = 5) in length by 15 μm wide, which is the reason we think they are first-stage larvae. We observed the characteristic kinked tail with a spine at the terminal end of these larvae. *Aelurostrongylus abstrusus* displays an indirect cycle and requires different snails and slugs (*Helix*, *Deroceras*, *Arion*, and *Agriolimax*) as intermediate hosts, where the third-stage infective larva is developed. Amphibians, reptiles, birds, insectivorous mammals, and rodents could act as paratenic hosts. Presumably, the main route of infection is the ingestion of these paratenic hosts (Hobmaier and Hobmaier, 1935).

Toxocara cati (Schrunk, 1788) (family Ascarididae Baird, 1853) is a worldwide parasite that occurs in the small intestine of felids and appears also in humans, causing the disease referred as visceral or ocular larva migrans. Eggs of this ascarid have been found in tiger samples and were slightly ovoid or subspherical, 70–75 × 56–72 μm (n = 20), with a finely pitted outer layer, 2–4 μm, reminiscent of a golf ball and also containing a single cell. In both cases, embryonic development happened in 10–15 days at room temperature and allowed us to observe the different segmentation stages until the formation of an embryo, the differentiation of the first and second-stage larvae, and the hatch of this last stage, which took place in 40% of the observed eggs. Bowman (1995) mentions that second-stage larvae of *Toxocara cati* hatch when eggs come in contact with gastrointestinal and bile fluids of the host. We have detected that a considerable number of larvae have hatched under laboratory conditions. No explanation has been found for this behavior. The low intensity of this parasite seems to indicate that tigers have acquired the parasite by ingestion of L₃ larvae in paratenic hosts.

Toxascaris leonina (v. Linstow, 1902) is also a member of the family Ascarididae. It occurs in the small intestine and sometimes in the bile ducts of wild and domestic canids and felids in most parts of the world. Eggs were found in 1 sample of each host. These eggs measured 60–75 × 75–85 μm (n = 20), were colorless and slightly oval with a smooth shell surface. The outer layer was about 2 μm, without striations or albuminous coat. These eggs contained 1 or 2 spherical cells, which did not occupy the entire interior of the egg. In this case, we were also able to observe every phase of the embryonic development until the second-stage larvae, which developed in 4–6 days. However, hatching of these second-stage larvae was never observed. The egg of *Toxascaris leonina* containing a second-stage larva is also the infective stage.

All parasites are cited for the first time on these hosts with the exception of *Toxocara cati* which has been described on *P. t. altaica*.

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