Research Article

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HELMINTHS OF RARE FELINE SPECIES (*FELIDAE*) IN SIBERIA AND THE RUSSIAN FAR EAST

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ABSTRACT

Parasites diversity in close-related species of hosts may be different depending on habitat use and climatic conditions. The aim of this study was to analyze parasites fauna in four felid species inhabiting Russian Far East and South Siberia (including taiga forest and mountain treeless areas). We have collected 272 feces samples of four felid species: Amur tiger, Amur leopard, snow leopard and Pallas' cat. Helminths (eggs and larvae) in excrements were studied by flotation using a saturated solution of ammonium nitrate. We have described 10 helminths species in Amur tiger feces, 6 – in Amur leopard, 2 – in snow leopard and 3 – in Pallas' cat. Obviously, snow leopard and Palls' cat had lower helminths diversity than two other species. These differences can be explained, to some extent, by climatic parameters. The climate in the snow leopard and Pallas' cat habitats is described by sharp and significant temperature fluctuations - the annual temperature difference can exceed 90°C, which may lead to lower survival of the number of infectious agents in Pallas' cat excrements. In addition, the snow cover that can protect helminth eggs and larvae from the cold temperatures especially in Amur tiger and Amur leopard habitats. Possibly, another important factor is the spatial and social organization of Pallas' cats, with a low frequency of contacts with other individuals. Such way, species-specific differences in helminths were related, probably, with the species evolution in different habitats.

Key words: Helminths, Amur tiger, Amur leopard, Pallas's cat, Snow leopard.

INTRODUCTION

Most populations of feline species *(Felidae)* are reduced due to the decreasing areas of their habitats, primarily under the anthropogenic influence. In such circumstances, the loss of several individuals may cause almost total extinction of isolated groups of rare felines[22,35]. The advanced environmental projects require an integrated approach involving the study of status of rare animals population, availability of food resources, reproductive biology of animals, as well as their veterinarian examination. Tests for invasive diseases are critical when assessing the animals' health status. Improved knowledge of worm infection in wild animals will help elaborate theoretically based measures to protect rare feline species. This information can also be very useful for setting up breeding and rehabilitation centers for animals listed in the Red Book of the Russian Federation.

Siberia and the Russian Far East are inhabited by such rare feline species as, Amur tiger (*Panthera tigris altaica*), Amur leopard (*Panthera pardus orientalis*), snow leopard, (*Panthera uncia*), and Pallas's cat (*Otocolobus manul*). The Amur tiger is one of the biggest tiger sub-species preserved by the beginning of the 21st century mainly in the Russian Far East. Currently its population is 450–500 animals[20]. The Amur leopard is the rarest of all leopard sub-species and is represented by the only population preserved in the south of the Russian Far East, of approximately 70 animals. Snow leopard remains the least studied representative of big cats in the world, which is explained by its habitat inaccessibility and the natural rarity of the species. Russia is inhabited by the northernmost group of snow leopard native to the mountain ranges of southern Siberia (the Altai, the Sayans, the Tuva mountains). Its population is estimated to consist of 70–150 animals[15,34] and tends to decrease[26]. Pallas' cat is a rare and understudied representative of the feline family. Its population in the Russian Federation is estimated at 4,500 individuals, though it was as numerous as 4,500 individuals in the 1940's-1950's just in the Baikal Area[13]. All of these wild cat species are listed in the Red Book of the Russian Federation (2001) and the Red List of the International Union for Conservation of Nature (IUCN). The study of helminth fauna is of great importance to preserve them both in the wild and in zoos.

Data on helminths of tigers living in the wild are scarce[17,18,30]. Most studies on the tiger helminth fauna were performed in zoos[2,4,5,7,10,16,21]. Currently 25 species of tiger helminths are known: *Ancylostoma caninum*; *Ancylostoma ceylanicum*; *Ancylostoma tubaeforme*; *Clonorchis sinensis*; *Cylicospirura felineus*; *Diphyllobothrium latum*; *Dirofilaria immitis*; *Dirofilaria ursi*; *Galonchus perniciosus*; *Gnathostoma spinigerum*; *Hydatigera taeniaeformis*; *Mammomonogamus felis*; *Ollulanus tricuspis*; *Oncicola campanulata*; *Paragonimus westermani*; *Paragonimus pulmonalis*; *Physaloptera brevispiculum*; *Spirocerca lupi*; *Spirometra erinacei*; *Taenia hydatigena*; *Taenia pisiformis*; *Taenia regis*; *Toxascaris leonina*; *Toxocara cati*; *Trichinella nativa*; *Trichinella spiralis*; *Uncinaria stenocephala*[3,8,9,12,17,27,30,37]. Moreover, numerous unidentified species were found, mostly in feces: Dicrocoellidae gen. sp., Ancylostomatidae gen. sp., *Capillaria* sp., *Gnathostoma* sp., *Mammomonogamus* sp., *Molineus* sp., Pseudophyllidaea (*Diphyllobothrium* sp. and *Spirometra* sp.), *Strongyloides* sp., *Trichocephalus* sp., *Paragonimus* sp.

(probably *Paragonimus heterotremus*), *Taenia* sp., as well as larvae of nematodes[14].

As concerns Amur tiger, its helminths is understudied. Only fragmentary information derived from exams of dead animals is available[1,8,19,24,25,33]. Amur tiger helminth fauna surveys conducted by J.A. Melnikova and I.V. Voloshina[19] and by V.G. Yudin and E.V. Yudina[40] yielded a list of 12 helminth species identified by autopsies of dead animals. This includes: cestode worms *Taenia bubesei, Taenia pisiformis,* and *Hydatigera taeniaformis,* trematode *Paragonimus westermani*, nematodes *Toxocara mystax, Toxascaris leonina, Gnathostoma spinigerum, Phisaloptera praeputialis, Trichinella spiralis, Forepaws stenocephala* and *Dirophilaria sp*.

According to reference data, 25 helminth species have been registered in leopards: *Ancylostoma caninum*; *Dirofilaria immitis*; *Dracunculus medinensis*; *Echinococcus granulosus*; *Galoncus rammohanii*; *Hydatigera taeniaeformis*; *Mesocestoides lineatus*; *Oncicola campanulata*; *Oncicola dimorpha*; *Oncicola gigas*; *Paragonimus heterotremus*; *Paragonimus westermani*; *Physaloptera praepuriale*; *Spirometra decipiens*; *Spirometra erinacei*; *Taenia acinomyxi*; *Taenia hydatigena*; *Taenia ingwei*; *Taenia pisiformis*; *Toxascaris leonina*; *Trichinella britovi*; *Trichinella nativa*; *Trichinella nelsoni*; *Trichinella spiralis*; *Troglostrongylus subcrenatus*[14].
Among the wild cats living in Russia, the snow leopard is the species with the least studied helminths. 6 species of snow leopard parasitic helminths are only mentioned in literature: *Hydatigera taeniaeformis; Dirofilaria immitis; Spirocerca lupi; Toxascaris leonina; Toxocara cati; Taenia kotlani*[14].

Information on Pallas' cat worms is very scarce and was derived from single animal studies. 10 species of helminths are mentioned in literature: *Cyclospirura subaequlis; Hydatigera taeniaeformis; Macracanthorhynchus catulinus; Metathelazia massino; Physaloptera sibirica; Taenia krepkogorski; Toxascaris leonina; Toxocara cati; Trichinella spiralis; Uncinaria stenocephala*[5,14].
This work was aimed at studying the helminth fauna of rare feline species in the wild.

MATERIALS AND TECHNIQUES

Samples of Amur tiger excrements were collected in 2008-2010 in different parts of its range: southern (123 samples from the Ussury and Lazovsky reserves), south west (22 samples from the territory adjacent to the Kedrovaya Pad reserve), central (3 samples from the Udege Legend National Park), and northern (20 samples from the Anyuiskiy National Park) parts of the Primorskii Krai.
Samples of Amur leopard excrements were collected in 2009–2011 in South Western Primorye - 40 samples.

Snow leopard excrements were collected in 2010–2011 in the Western Sayans (National Sayans Shushensky biosphere reserve) - 12 samples, and in South Western Tyva (Tsagan-Shibetu mountain range) - 19 samples.

Samples of Pallas's cat excrements were collected in 2007 in the vicinity of the village of Tashanta, Kosh-Agach District, Altai Republic: 11 samples, in 2011 in Daurskiy biosphere reserve, Chita Region: 12 samples, and in the Republic of Tyva: 10 samples.
The collected samples were labelled, noting the excreted species, date, and the collection site coordinates, placed in plastic bags, and frozen. The species whose excrements were found was determined by the animal traces near the excrement and by molecular genetic techniques. Samples were classified to a particular species upon receipt of gene fragment sequences of cytochrome b of mitochondrial DNA[36]. Excrements were also taken from the rectum of caught and immobilized tigers. As the individual animals from which excrement samples were taken were not identified, there is a chance that several samples were taken from the same animal. Helminths (eggs and larvae) in excrements were studied by the Department of Parasitology and Parasitic Diseases of Animals in the SBEI HPE the K.I. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology, by flotation using a saturated solution of ammonium nitrate[39]. The proportion of excrement samples with the eggs or larvae of helminths were determined, and dominant helminth species were identified according to [32].

RESULTS AND DISCUSSION

The summary on the Amur tiger helminth fauna from the excrement analysis is shown in Tables 1 and 2. Helminth eggs and larvae were found in 138 samples of 168 analyzed excrement samples from Amur tiger (82.1% of all samples). 10 helminth species and groups were found, including 2 trematodes, 2 cestodes, and 6 nematodes.

Table 1: Helminth fauna of Amur tiger (n = 168)

Helminth species and groups	Number of samples with pathogen	Percentage of samples, %
Trematodes:		
Clonorchis sinensis	1	0.6
Trematoda spp.	29	17.3
Cestodes:		
Spirometra erinacei	2	1.2
Taenia sp.	4	2.4
Nematodes:		
Toxocara cati	123	73.2
Toxascaris leonina	3	1.8
Aonchoteca putorii	47	28.0
Strongylata sp.	8	4.5
Hepaticola hepatica	2	1.2
Aelurostrongylus abstrusus	5	3.0

n is the number of samples analyzed

Table 2: Helminths in the Amur tiger feces and the nature of their infestation in different parts of its range

Helminth species	Number of samples	Percentage of samples, %
Southern part of the range is the south of the Primorskii Krai (Ussury and Lazovsky reserves), n = 123		
Monoinfestations:	44	35.8
Toxocara cati	33	26.8
Toxascaris leonina	1	0.8
Taenia sp.	2	1.6
Clonorchis sinensis	1	0.8
Trematoda spp.	7	5.7
Mixed infestations by 2 species:	44	35.8
Toxocara cati + *Toxascaris leonina*	2	1.6
Toxocara cati + *Aonchoteca putorii*	30	24.4
Toxocara cati + Strongylata sp.	2	1.6
Aonchoteca putorii + Trematoda spp.	2	1.6
Toxocara cati + *Taenia* sp.	2	1.6
Toxocara cati + Trematoda spp.	6	4.9
Mixed infestations by 3 species:	6	4.9
Toxocara cati + *Aonchoteca putorii* + Trematoda spp.	3	2.4
Toxocara cati + *Aonchoteca putorii* + *Hepaticola hepatica*	1	0.8
Toxocara cati + Strongylata sp. + *Hepaticola hepatica*	1	0.8
Aonchoteca putorii + Strongylata sp. + Trematoda spp.	1	0.8
Mixed infestations by 4 species:	1	0.8
Toxocara cati + *Aonchoteca putorii* + Strongylata sp. + Trematoda spp.	1	0.8
Southern part of the range - Southwest of Primorskii Kray (Kedrovaya Pad reserve), n = 22		
Monoinfestations:	11	50.0
Toxocara cati	11	50.0
Mixed infestations by 2 species:	8	36.4
Toxocara cati + *Aelurostrongylus abstrusus* larvae	3	13.6
Toxocara cati + Strongylata sp.	3	13.6
Toxocara cati + *Aonchoteca putorii*	1	0.8
Toxocara cati + Trematoda spp.	1	0.8
Mixed infestations by 3 species:	3	13.6
Toxocara cati + *Spirometra erinacei* + Trematoda spp.	1	0.8
Toxocara cati + *Spirometra erinacei* + *Aelurostrongylus abstrusus* larvae	1	0.8
Toxocara cati + *Aonchoteca putorii* + *Aelurostrongylus abstrusus* larvae	1	0.8
Central part of the range - Udege Legend National Park, n = 3		
Toxocara cati	2	66.7
Northern part of the range - Anyuiskiy National Park, n = 20		
Monoinfestations:	7	35.0
Toxocara cati	6	30.0
Aonchoteca putorii	1	5
Mixed infestations by 2 species:	11	55.0
Toxocara cati + Trematoda spp.	6	30.0
Toxocara cati + *Aonchoteca putorii*	5	25.0
Mixed infestations by 3 species:	1	5.0
Toxocara cati + *Aonchoteca putorii* + Trematoda spp.	1	5.0

n is the number of samples analyzed

Table 3: Helminths in Amur leopard feces and their infestation type

Helminth species	Number of samples	Percentage of samples, %
Monoinfestations:	19	47.5
Toxocara cati	14	35
Aonchoteca putorii	1	2.5
Trematoda spp.	4	10
Mixed infestations by 2 species:	3	7.5
Toxocara cati + *Aonchoteca putorii*	1	2.5
Aonchoteca putorii + Strongylata sp.	1	2.5
Strongylata sp.+ Trematoda spp.	1	2.5
Mixed infestations by 3 species:	2	5
Aonchoteca putorii + *Aelurostrongylus abstrusus* larvae + *Taenia* sp.	1	2.5
Aonchoteca putorii + Strongylata sp. + Trematoda spp.	1	2.5

n is the number of samples analyzed

Comparison of the helminths presence in the Amur tiger excrements collected in the south, center and north of its range suggests that helminth fauna has certain particularities in different parts of the tiger habitat, with about the same proportion of samples with eggs of helminths found. So, in the Amur tiger population in the Southwest of Primorskii Kray that is considered isolated[11,38], two specific parasites were discovered (nematoda *Aelurostrongylus abstrusus* and cestoda *Spirometra erinacei*). Nematoda *Toxocara cati*, found in virtually all excrement samples, is the most common species in all parts of the Amur tiger habitat. The second prevalent species is nematode *Aonchoteca putorii*. The third prevalent helminth is the trematode of not yet defined species. Other helminth species were found in rare Amur tiger excrement samples.

Based on our research findings, helminth fauna of the Far East leopard in Southwest Primorye is represented by 6 helminth species, including 1 trematodes, 1 cestodes and 4 nematodes (Table 3). Helminth eggs and larvae were detected in 24 of 40 analyzed excrement samples from Amur leopard (60% of all samples).

Thus, the Amur leopard helminth fauna is similar to that of Amur tiger, and leopard habitat overlaps the southern part of the Amur tiger range. As with the tiger, the dominant species in Amur leopard are nematodes *Toxocara cati* and *Aonchoteca putorii* and some not identified Trematoda spp. Fewer number of helminth species found in the leopard (6) vs Amur tiger (10) is probably related to fewer samples analyzed (40 for leopard, 168 for tiger).

Analysis of excrement samples from 31 snow leopards yielded the following results: eggs of *Toxascaris leonina* were found in 1 sample (5%) out of 19 studied samples in the South Western Tyva. eggs of *Taenia sp*. were discovered in 2 (16%) of 12 snow leopard samples studied in the National Sayano-Shushensky biosphere reserve.

Helminth eggs were found in 21 of 33 samples (63.6%) of studied Pallas's cat excrements. Helminth fauna of Pallas' cats was represented by 3 helminth species, including 1 cestode, 1 nematode and 1 acanthocephala. Moreover, the percentage (%) of excrement samples where helminth eggs were found varied depending on the region significantly (Table 4).

Table 4: Helminths in the Pallas's cat feces and the nature of their invasion in different parts of its range

Helminth species	Number of samples	Percentage of samples, %
Kosh-Agach District, Altai Republic, n = 11		
Monoinfestations:	10	90.9
Toxascaris leonina	9	81.8
Taenia sp.	1	9.1
Daursky biosphere reserve, n = 12		
Monoinfestations:	2	16.7
Toxascaris leonina	2	16.7
Mixed infestations by 2 species:	1	8.3
T. leonina + Macracanthorhynchus sp.	1	8.3
Republic of Tyva, n=10		
Monoinfestations:	6	60.0
Toxascaris leonina	4	40.0
Macracanthorhynchus sp.	2	20.0
Mixed infestations by 2 species:	2	20.0
T. leonina+ Taenia sp.	2	20.0

n is the number of samples analyzed

Study of Pallas' cat helminth fauna suggested that the highest percentage (%) of excrement samples where helminth eggs were discovered is recorded in the Kosh-Agach District, Altai Republic (90.9%). Pallas' cat carries 3 helminth species in different habitat parts: *Toxascaris leonina*, *Taenia sp.,* and *Macracanthorhynchus sp.*, in monoinfestation as well as in mixed infestations.

CONCLUSION

Thus, according to our research, the helminth fauna of rare feline species in the Russian Far East (Amur tiger and Amur leopard) is entirely different from that of the snow leopard and the Pallas's cat living in southern Siberia, which, to some extent, can be accounted for by climatic parameters. The helminth fauna of Pallas's cat and snow leopard, even given a smaller number of collected samples, is described by a much smaller number of species and mixed infestations. The climate in the snow leopard and Pallas' cat habitats is described by sharp and significant temperature fluctuations - the annual temperature difference can exceed 90°C (low winter and high summer temperatures), which leads to lower contamination of Pallas' cat excrements with a number of infectious agents[23,31]. In addition, the snow cover that can protect helminth eggs and larvae from the cold temperatures lays unevenly, if at all, in the studied areas. Another important factor is the spatial and social organization of Pallas' cats, with quite large habitats and a low frequency of contacts with other animals and their excretions. Snow leopard and Pallas' cat habitats are described by low density of groups, as compared with that of Amur leopard, where other feline species (Amur tiger, leopard cat and lynx) live.

All these factors, taken together, can lead to the differences we identified in the helminth fauna of rare felines of Siberia and the Russian Far East.

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