Original article

https://doi.org/10.31016/1998-8435-2023-17-4-443-452

Helminth zoonoses of wild carnivore mammals in the Primorsky Krai of the Russian Far East

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Abstract

The purpose of the research is to examine the helminthological status of wild carnivore mammals inhabiting the territory of the Primorsky Krai and to give an epidemiological assessment of their role in maintaining the natural foci of zoonotic infections.

Materials and methods. Feces of carnivores were collected in natural habitats of wild animals. Species of animals were identified by the characteristic features of feces and animal tracks. The shape, size, texture and composition of feces were analyzed. The samples were placed in containers with 5% formalin. A part of the material was stored in native form at -12 °C. Muscle tissue samples were obtained from animal carcasses. Feces were examined by flotation techniques with a solution of zinc sulfate, the formalin-ethyl acetate sedimentation technique and using an ammonium nitrate solution. After the study, the samples were disinfected by autoclaving at a pressure of 1.5 atm for 2 hours. Muscle tissue samples were examined by digesting in artificial gastric juice using the Gastros device. The species of *Trichinella* sp. larvae isolated from the positive samples were identified using the nucleotide sequences. In total, 444 feces samples from 13 species of wild carnivore mammals and 449 muscle tissue samples from 13 species were examined.

Results and discussion. Wild carnivore mammals inhabiting the territory of the Russian Far East are often infected with various species of helminths localized in the intestine and tissues, which are causative agents of dangerous parasitic zoonoses. A total of 9 species of helminthes in the Siberian tiger (*Panthera tigris altaica*), 3 species in the Amur leopard (*P. pardus orientalis*), 2 species in the Eurasian lynx (*Lynx lynx*), 4 species in the leopard cat (*Prionailurus bengalensis*), 5 species in the sable (*Martes zibellina*), 2 species in the yellow-throated marten (*M. flavigula*), 5 species in the Siberian weasel (*Mustela sibirica*), 1 species in the American mink (*Neovison vison*), 2 species in the Asian badger (*Meles leucurus*), 8 species in the red fox (*Vulpes vulpes*), 2 species in the raccoon dog (*Nyctereutes procyonoides*), and 9 species in the brown bear and Asiatic black bear (*Ursus arctos* and *U. thibetanus*) were identified at studying of 444 feces samples of wild carnivore mammals in the Primorsky Krai. Among the detected helminths were highly pathogenic for humans: *Toxocara cati*, *Paragonimus westermani* and nematodes of the family Capillariidae. *Trichinella* sp. larvae were detected in 96 samples in the study of 449 samples of muscle tissue from wild carnivore mammals. The above types of helminths are of zoonotic nature. The pathogenic role of accidental infection with helminth species *Baylisascaris transfuga* has not yet been revealed in humans, that makes this type of bear ascaride potentially dangerous for humans. The studies have shown the widespread prevalence of helminth zoonoses in the Primorsky Krai. These data will help to organize properly the work of people whose jobs involve contact with wild animals.

Keywords: helminth zoonoses; carnivores; helminth eggs; prevalence of infection

Financial transparency: none of the authors has financial interest in the submitted materials or methods.

There is no conflict of interests.

For citation: Seryodkin I. V., Kurnosova O. P., Khrustalev A. V., Esaulova N. V., Varlamova A. I., Odoevskaya I. M. Helminth zoonoses of wild carnivore mammals in the Primorsky Krai of the Russian Far East. *Rossiyskiy parazitologicheskiy zhurnal = Russian Journal of Parasitology*. 2023;17(4):443–452. (In Eng.).

https://doi.org/10.31016/1998-8435-2023-17-4-443-452

Научная статья

УДК 619:616.995.1:639.111.7

https://doi.org/10.31016/1998-8435-2023-17-4-443-452

Гельминтозоонозы диких хищных млекопитающих в Приморском крае Дальнего Востока РФ

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Аннотация

Цель исследований – изучить гельминтологический статус диких хищных млекопитающих, обитающих на территории Приморского края, и дать эпидемиологическую оценку их роли в поддержании природных очагов зоонозных инвазий.

Материалы и методы. Фекалии хищных млекопитающих собирали в естественных биотопах диких животных. Видовую принадлежность фекалий определяли по характерным видовым признакам и следам животных. Анализировали форму, размер, консистенцию и состав фекалий. Пробы помещали в контейнеры с 5%-ным формалином. Часть материала хранили в нативном виде при –12 °С. Образцы мышечной ткани получали от трупов животных. Фекалии исследовали флотационным методом с раствором сульфата цинка, методом этилацетат-формалинового осаждения и с использованием раствора аммиачной селитры. После исследования образцы материала обеззараживали автоклавированием в течение 2 ч при давлении 1,5 атм. Образцы мышечной ткани исследовали методом переваривания проб в искусственном желудочном соке с помощью аппарата Gastros. Вид личинок *Trichinella* sp., выделенных из положительных проб, определяли на основании нуклеотидных последовательностей. Всего происследовано 444 пробы фекалий 13 видов диких хищных млекопитающих и 449 проб мышечной ткани 13 видов.

Результаты и обсуждение. Дикие хищные млекопитающие, обитающие на территории Дальнего Востока России, часто бывают заражены различными видами гельминтов кишечной и тканевой локализации, являющимися возбудителями опасных антропозоонозов. При исследовании 444 проб фекалий диких хищных млекопитающих в

Приморском крае у амурского тигра (*Panthera tigris altaica*) выявлено 9 видов гельминтов, у амурского леопарда (*P. Pardus orientalis*) – 3, у евразийской рыси (*Lynx lynx*) – 2, у бенгальского кота (*Prionailurus bengalensis*) – 4, у соболя (*Martes zibellina*) – 5, у харзы (*M. flavigula*) – 2, у сибирского колонка (*Mustela sibirica*) – 5, у американской норки (*Neovison vison*) – 1, у азиатского барсука (*Meles leucurus*) – 2, у лисицы (*Vulpes vulpes*) – 8, у енотовидной собаки (*Nyctereutes procyonoides*) – 2 и у бурого и гималайского медведей (*Ursus arctos, U. thibetanus*) – 9 видов. Среди обнаруженных гельминтов были высоко патогенные для человека: *Toxocara cati*, *Paragonimus westermani* и нематоды сем. Capillariidae. При исследовании 449 проб мышечной ткани диких хищных млекопитающих в 96 выявлены личинки *Trichinella* sp. Вышеперечисленные виды гельминтов имеют зоонозную природу. Патогенная роль при случайном заражении гельминтами вида *Baylisascaris transfuga* на сегодняшний день у человека не изучена, что делает этот вид потенциально опасным для людей. Исследования свидетельствуют о широком распространении гельминтозоонозов на территории Приморского края. Полученные результаты помогут правильно организовать работу людей, чья профессиональная деятельность предусматривает контакты с дикими животными.

Ключевые слова: : гельминтозоонозы, хищные млекопитающие, яйца гельминтов, распространение

Прозрачность финансовой деятельности: в представленных материалах или методах авторы не имеют финансовой заинтересованности.

Конфликт интересов отсутствует.

Для цитирования: *Середкин И. В., Курносова О. П., Хрусталев А. В., Есаулова Н. В., Варламова А. И., Одоевская И. М.* Гельминтозоонозы диких хищных млекопитающих в Приморском крае Дальнего Востока РФ // Российский паразитологический журнал. 2023. Т. 17. № 4. С. 443–452.

https://doi.org/10.31016/1998-8435-2023-17-4-443-452

Introduction

Wild animals are an integral part of natural biocenoses. People and pets can come into contact with the natural environment and its inhabitants in varying degrees through forestry, hunting, environmental measures, work in nature reserves, catching, use of common pastures or appearance of forest inhabitants in human settlements [8].

It is known that wild animals are, being in their natural habitat, susceptible to various diseases not only infectious ones [11]. Throughout their life, such animals are constantly infected with different species of parasites often with lesions of many organs and tissues. Certainly, parasitic diseases cause significant harm to health, and can lead to death in some cases. High pathogenicity of such helminth genera as Paragonimus, Capillaria and Filaria for animals' health has been proven [4, 8, 11, 13, 15].

Carnivorous mammals play an important role in circulation of many infective natural focal diseases of domestic animals and humans [14, 15]. The most dangerous diseases for humans are echinococcosis, trichinellosis, toxocarosis and paragonimosis. The helminths that cause these diseases can make serious harm to human health, and in some circumstances, these parasitic diseases can be life threatening [12]. The rate of infection of some of these helminthoses can reach 97–100% in wild carnivores in some regions of the country [1]. The vast majority of carnivores is of commercial importance and provides fur, meat, fat, etc. [1, 12]. Therefore, first of all, hunters and local residents are at risk of infection with dangerous zoonotic diseases.

Safety measures to prevent from infection with parasitic diseases must be observed by hunters during carcasses processing or collecting of seasonal forest products, by timber procurers, employees in specially protected natural areas and scientists involved in sampling biological material, necropsy [1, 9, 13, 14].

The aim of this study was to examine the helminthological status of wild carnivore mammals inhabiting the territory of the Primorsky Krai and to give an epidemiological assessment of their role in maintaining the natural foci of zoonotic infections, using any available material.

Materials and methods

Feces of carnivores were collected in natural habitats of wild animals. Species of animals were identified by the characteristic features of feces and animal tracks. The shape, size, texture and composition of feces were analyzed. These signs are species-specific and described for the studied

region [5, 7]. In addition, in some cases, animals left paw prints, fur, and characteristic scratches. These tracks were also used in species identification [6]. Bears were not identified to species. The samples were placed in containers with 5% formalin. A part of the material was stored in native form at -12 °C. Muscle tissue samples were obtained from animal carcasses. Most of the muscle tissue samples were provided by hunters.

Feces were examined by flotation techniques with a solution of zinc sulfate (density – 1.38), the formalin-ethyl acetate sedimentation technique and using an ammonium nitrate solution with density of 1.24 [3]. Microscopy was carried out at ×10 and × 40 magnifications using the biological microscope Biolam (LOMO; Russia). After the study, the samples were disinfected by autoclaving at a pressure of 1.5 atm for 2 hours.

Muscle tissue samples were examined by digesting in artificial gastric juice using the Gastros device (Petrolaser, St. Petersburg). The species of *Trichinella* sp. larvae isolated from the positive samples were identified using the nucleotide sequences [20].

In total, 444 feces samples from 13 species of wild carnivore mammals and 449 muscle tissue samples from 13 species (Table 2) were examined.

Table 1 [Таблица 1]

The results of coproscopical examination of feces samples of wild carnivore mammals in the Primorsky Krai
[Результаты копроскопического исследования проб фекалий диких хищных млекопитающих в Приморском крае]

Animal species	The number of studied samples	Parasite	The number of positive samples	Parasite prevalence, %
1	2	3	4	5
Siberian tiger	152	*Toxocara cati* *Eucoleus aerophilus* Strongylida gen. sp.[a] *Aonchotheca putorii* *Aelurostrongylus abstrusus* *Eucoleus* sp. *Paragonimus westermani* Trematoda gen. sp.[b] *Taenia* sp. *Spirometra* sp.	99 27 5 26 3 1 37 5 6 5	65.1 17.8 3.3 17.1 2.0 0.7 24.3 3.3 3.9 3.3
Amur leopard	5	*Eucoleus aerophilus* Strongylida gen. sp.[a] *Aonchotheca putorii*	1 1 2	20.0 20.0 40.0
Eurasian lynx	5	*Toxocara cati* *Taenia* sp.	4 2	80.0 40.0
Leopard cat	11	*Toxocara cati* *Aonchotheca putorii* Nematoda gen. sp.[b] Trematoda gen. sp.[b] *Taenia* sp.	8 5 1 5 1	72.7 45.5 9.1 45.5 9.1
Sable	52	*Soboliphyme baturini* *Eucoleus aerophilus* Strongylida gen. sp.[a] *Calodium hepaticum* Capillariidae gen. sp.[c] *Dicrocoelium* sp. Trematoda gen. sp.[b]	9 3 7 1 22 2 11	17.3 5.8 13.5 1.9 42.3 3.8 21.2
Yellow throated marten	1	*Eucoleus aerophilus* *Aonchotheca putorii*	1 1	100 100
Siberian weasel	14	*Soboliphyme baturini* *Eucoleus aerophilus* *Aonchotheca putorii* *Calodium hepaticum* Trematoda gen. sp.[b]	1 1 2 1 1	7.1 7.1 14.3 7.1 7.1
American mink	1	*Soboliphyme baturini*	1	100
Asian badger	5	Strongylida gen. sp.[a] *Aonchotheca putorii*	1 1	20.0 20.0

End of the table 1 [Окончание таблицы 1]

1	2	3	4	5
Red fox	28	*Toxocara* sp.	1	3.6
		Eucoleus aerophilus	4	14.3
		Strongylida gen. sp.[a]	8	28.6
		Aonchotheca putorii	4	14.3
		Calodium hepaticum	2	7.1
		Capillariidae gen. sp.[c]	8	28.6
		Nematoda gen. sp.[b]	2	7.1
		Alaria alata	2	7.1
		Paragonimus westermani	1	3.6
		Trematoda gen. sp.[b]	6	21.4
Racoon dog	6	*Baylisascaris* sp.	2	33.3
		Eucoleus aerophilus	2	33.3
Bears	164	*Baylisascaris transfuga*	44	26.8
		Eucoleus aerophilus	9	5.5
		Strongylida gen. sp.[a]	16	9.8
		Trichurus sp.	2	1.2
		Aonchotheca putorii	3	1.8
		Capillariidae gen. sp.[c]	14	8.3
		Spirurida gen. sp.[a]	1	0.6
		Nematoda gen. sp.[b]	1	0.6
		Dicrocoelium sp.	19	11.6
		Trematoda gen. sp.[b]	1	0.6
		Taeniidae gen. sp.[c]	2	1.2

Note. a – reported as order; b – reported as phylum; c – reported as family.

Table 2 [Таблица 2]

The prevalence of *Trichinella* spp. infection in wild carnivore mammals in the Primorsky Krai [Распространение трихинеллеза у диких хищных млекопитающих в Приморском крае]

Animal species	The number of studied samples	The number of positive samples	Parasite prevalence, %
Eurasian lynx	7	5	71.4
Leopard cat	45	21	46.7
Sable	321	36	11.2
Yellow-throated marten	2	0	0
Siberian weasel	6	3	50.0
American mink	3	1	33.3
Eurasian otter	3	0	0
Asian badger	2	1	50.0
Eurasian wolf	1	0	0
Red fox	8	4	50.0
Racoon dog	25	11	44.0
Brown bear	14	9	64.3
Asiatic black bear	12	5	41.7

Results

Helminth eggs were found in 113 of 152 samples of feces of Siberian tigers (*Panthera tigris altaica*) with parasite prevalence of 74.3% (Table 1). In total, 9 species of helminths were identified in Siberian tigers; among them are *Toxocara cati* (65.1%) (Fig. 1), *Eucoleus aerophilus* (17.8%), nematode eggs of the order Strongylida (3.3%) and *Aonchotheca putorii* (17.1%), *Paragonimus westermani* (24.3%) (Fig. 2) and eggs of *Taenia* sp. (3.9%) (Fig. 3). Tigers were most heavily infected with nematodes *T. cati*.

Eggs of three nematode species were found in feces of Amur leopards (*Panthera pardus orientalis*) with the highest prevalence of infection for *A. putorii*.

One species of nematodes (*T. cati*) and cestodes (*Taenia* sp.) were revealed in feces of Eurasian

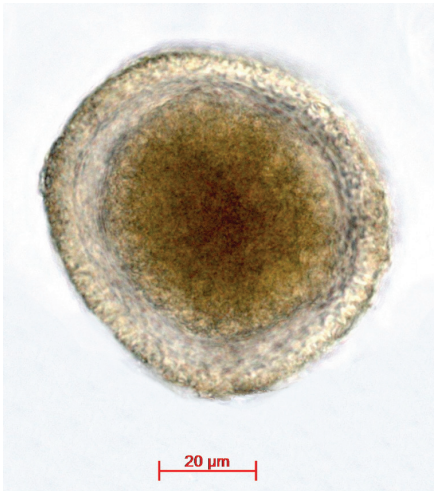

Fig. 1. Egg of *Toxocara cati*
[**Рис. 1.** Яйцо *Toxocara cati*]

Fig. 2. Egg of *Paragonimus westermani*
[**Рис. 2.** Яйцо *Paragonimus westermani*]

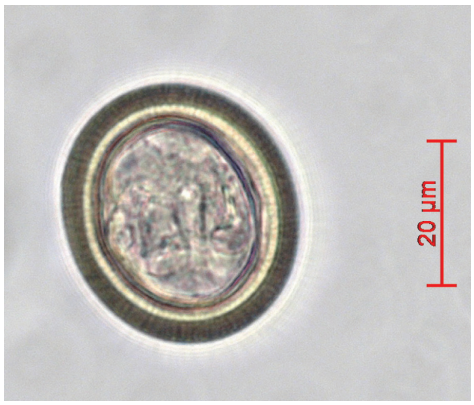

Fig. 3. Egg of *Taenia* sp.
[**Рис. 3.** Яйцо *Taenia* sp.]

lynxes (*Lynx lynx*). The eggs of nematodes were found in 4 of 5 samples.

Eggs of four helminth species were identified in 11 feces samples of leopard cats (*Prionailurus bengalensis*): *T. cati* (72.7%), *A. putori* (45.5%), eggs of the class Trematoda (45.5%), and Taenia sp. (9.1%). The eggs of *T. cati* were most often found in feces of leopard cats as well as in the samples from tigers and lynxes.

Regarding to the results of feces examination, parasite prevalence was 69.2% (36/52) in sables (*Martes zibellina*). A total of 5 helminth species were found: *Soboliphyme baturini* (17.3%), *E. aerophilus* (5.8%), nematode eggs of the order Strongylida (13.5%), nematodes of the family Capillariidae (44.2%) and trematodes (25.0%). Transit eggs of nematodes *Calodium hepaticum* were also identified in sables (data not shown in the table) (Fig. 4). Only feces sample of yellow-throated marten (*M. flavigula*) contained eggs of two nematode species: *E. aerophilus* and *A. putorii*.

Fig. 4. Egg of *Calodium hepaticum*
[**Рис. 4.** Яйцо *Calodium hepaticum*]

Eggs of 4 nematode species (*S. baturini, E. aerophilus, A. putorii* and *C. hepaticum*) and not identified species of trematode were found in feces of Siberian weasels (*Mustela sibirica*). The highest prevalence of *A. putorii* infection was registered. Eggs of *S. baturini* were revealed in the sample of American mink (*Neovison vison*).

Helminths eggs were found in one feces sample out of 5 from Asian badgers (*Meles leucurus*). In this case, infection was caused by two nematode species (the order Strongylida and *A. putorii*).

A total of 28 feces samples of red foxes were studied with 82.1% prevalence of infection. Eight helminth species were identified in foxes: 6 nematode species and 2 trematode species (*Alaria alata* and *P. westermani*). Two nematode species (*Baylisascaris* sp. and *E. aerophilus*) were revealed in raccoon dogs (*Nyctereutes procyonoides*).

A total of 164 feces samples were examined and 9 species of helminths were found in bears represented by two species (*Ursus arctos* and *U. thibetanus*) in the Primorsky Krai. The bears were most often infected with the nematode *Baylisascaris transfuga* and the trematode *Dicrocoelium* sp. (Fig. 5).

Fig. 5. Egg of *Dicrocoelium* sp.
[**Рис. 5.** Яйцо *Dicrocoelium* sp.]

The examination of 449 samples of muscle tissue from carnivore's mammals was carried out, including 321 samples obtained from sables, 45 from leopard cats, 25 from raccoon dogs, 14 from brown bears, 12 from Asiatic black bears, 8 from red foxes, 7 from Eurasian lynxes, 6 from Siberian weasels, 3 from American minks, 3 from Eurasian otters (*Lutra lutra*), 2 from yellow-throated martens, 2 from Asian badgers and 1 from Eurasian wolf (*Canis lupus*). The data on infection with *Trichinella* spp. are shown in Table 2. The overall prevalence of infection was 21.4%. The nucleotide sequence analysis showed that the larvae of *Trichinella nativa* (Tri102-KU355861, Tri100-KU358874, Tri 108-KU355853) were found in sables, *T. nativa* (Tri107-KU355855) in brown bears and *T. spiralis* (Tri109-KU321696) in red foxes. The larvae of *T. nativa* were also found in other animals [19, 20].

Discussion

A relatively large species diversity of helminths with a high prevalence of infection was identified in carnivorous mammals despite the limited study (only feces and muscle tissue samples). The eggs of the genus Toxocara are the most often found in feces of these feline species (Table 1). Infection with this nematode species occurs trough ingesting helminth eggs, vertical transmission in utero or via breast milk, or by eating reservoirs – small rodents [2]. The high prevalence of eggs of the family Capillariidae and trematodes was registered in felines. The eggs of the family Capillaridae were also often revealed in mustelids (sable, yellow-throated marten and Siberian weasel) and canids (red fox) (Table 1). In addition, mustelids are infected with the nematode *S. baturini*. In bear feces samples, the most frequently found eggs belonged to species *B. transfuga* (26.8%). Infection with the nematode *E. aerophilus* is common to most species of carnivorous mammals (tigers, leopards, sables, martens, weasels, foxes, raccoon dogs and bears). Such pathogenic trematode species as *P. westermani* was found in tigers and foxes. Our data are comparable with the results of other studies [10, 13, 18, 19].

A high level of helminth infection is the most dangerous especially in young and weakened animals. Nematodes of the family Capillariidae have a wide range of localization (nasal cavity, trachea, lungs, esophagus, stomach, intestine, liver and bladder). The species localized in the respiratory organs and liver are the most pathogenic [4]. At high intensity of infections, structural changes in the organs and tissues occur, that can lead to death of animals. Animals become infected by ingestion infective eggs of nematodes of the family Capillariidae.

The trematode *P. westermani* is endemic to the Primorsky Krai. It is a pathogenic species and poses a serious veterinary and medical problem. Infection of tigers and other predators occurs by eating the reservoirs of these trematodes – wild boars and rodents with localization of parasite larvae in muscle tissue [13].

Nematodes of the genus Trichinella circulate widely among many species of wildlife [19, 20]. The species of *T. nativa* is mainly revealed among predators in the Far East. This species is the most resistant to low temperatures. Infection of wild

mammals occurs by eating of killed animals and corpses and participation of birds, mollusks and various corpse-eating insects in the Trichinella spp. circulation can provide the transmission of infection to herbivorous [22].

Wild animals are a source of helminth infection that is dangerous to humans. Many of them circulate almost all over Russia, and paragonimosis found in tigers and foxes in the Primorsky Krai has a natural focus in this region. Paragonimosis, trichinellosis, toxocarosis and group of diseases rarely found in humans called capillariosis pose a serious danger to human health, and can be life threatening in some cases. People become infected with paragonimosis by eating contaminated raw or poorly heat-treated crustaceans, and through water. Adult trematodes affect lungs and cause various clinical symptoms and complications such as secondary infection and pneumonia, as well as migration of parasites to the central nervous system. People become infected with trichinellosis by eating poorly cooked meat from bears, badgers or wild boars infected with larvae. *Trichinella* spp. larvae penetrate the striated muscle tissues of the host, where they gradually become encapsulated. The severity of the disease depends on the intensity of infection and the response to antigenic exposure. Allergic reactions develop in the process of larvae migration and allergic myocarditis is considered to be especially dangerous for human health. Outbreaks of trichinellosis are often of a group nature, when the whole family and relatives become infected after eating the meat of a wild animal [19]. In this case it is quite simple to suspect the disease during collecting an anamnesis, and timely treatment promotes recovery with a minimal risk of complications. Infection with nematodes of the genus Toxocara and nematodes of the family Capillariidae occurs by using raw water or food contaminated with eggs of these helminths. Eggs of *Toxocara* sp. can be transmitted with berries in the forests. Human toxocarosis is caused by migration of *Toxocara* sp. larvae in organs. The disease can have long and recurrent course with different clinical signs with lesion of internal organs and eyes. A complication of the disease is damage to vital internal organs. Toxocarosis is a widespread disease of animals not only in the wild, but today it is a serious veterinary and medical problem. Capillariosis are a group of rare parasitic diseases of humans. For example, hepatic capillariosis can occur by ingesting infective eggs with food or water. Helminths localized in the liver die after laying eggs and then eosinophilic granules are formed in the organ. People can be infected with pulmonary capillariosis through contaminated hands from accidentally crushed earthworms [12].

The genus Baylisascaris includes several species considered relatively specific to many species of animals that are the definitive hosts. Today the high pathogenicity of migrating *B. procyonis* larvae of this genus has been proven; its definitive host is raccoons. The most dangerous and threatening to human health are larvae localized in the brain. One eye is usually affected in the ocular form of the disease. Clinical signs may be nonspecific in the visceral form of infection [16]. *B. transfuga* is found both in wild and captive bears all over the world and animals are significantly infected and intensively release eggs into the environment [21]. This is especially true for captive held bears. The life cycle of *B. transfuga* is not fully studied; it is considered that the intestinal stage is little pathogenic for bears. To date, there are no reports of the possibility of migration of bear ascaride in the human body. The larvae migration has different influence on the body of animals experimentally infected with *B. transfuga*. Thus, the researchers revealed a symptomatic migration of larvae in a limited extent in mice, while larvae migrated to the brain and led to death in Mongolian gerbils (Meriones unguiculatus). However, changes were not observed in the brain tissue [17]. Therefore, *B. transfuga* can be potentially dangerous parasite for humans.

The clinical signs of many of the above diseases are often nonspecific. Various allergic reactions develop in animals with helminth infection that complicates the correct diagnosis. Some helminth zoonoses (capillariosis, paragonimosis) are very rarely recorded in Russia, and it may take a long time for a patient with such diseases to get the final diagnosis. In many cases, delayed medical care is dangerous. Incomplete information about hobbies (hunting, dressing of skins) can also contribute to untimely diagnosis, and as a result, to various complications [12].

Conclusions

The study has showed that wild carnivore mammals inhabiting the Primorsky Krai have a high prevalence of intestinal helminths with wide species diversity.

The following helminth zoonoses were revealed in wild carnivores: trichinellosis, toxocarosis, capillariosis and paragonimosis.

These studies are not only of veterinary importance. They are also relevant for medicine, as they expand knowledge about the distribution of helminth zoonoses in wild animals and can help to preserve people health.

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The article was submitted 20.09.2023; accepted for publication 12.11.2023

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