A coprological survey of parasitic fauna firstly in Wild Far Eastern Leopard (*Panthera pardus orientalis*)

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Abstract

Background—The Amur leopard, one of nine recently recognized subspecies of leopard, is still most threatened in a stochastic procession of extinction. The potential harmless to the conservation of the Amur leopard originating from the disease is in need of urgent attention. Unfortunately, the research on the potential risk to Amur leopard caused by disease is rare. When the parasites were concerned, even the elementary data, such as parasitic fauna, are absent. Our aim in the study is to accumulate the knowledge of it for a better comprehension. **Results:** There are 7 parasite species, including 3 nematodes (*Toxocara cati*, capillarid-type parasite, and *Metastrongyloidea*-type parasite), 2 cestodes (*Spirometra* sp. and *Taenia* sp.), 1 trematode (*Paragonimus* sp.), and 1 protozoa (*Cystoisospora felis*), were found in this research. The *Toxocara cati* was highest frequent occurrence, followed by *Spirometra* sp. **Conclusion:** The Amur leopard was infected by seven parasites firstly reported.

Background

The leopard (*Panthera pardus*) is a solitary, reclusive species of big feline. It is also the most widespread felid, extending across much of Africa, and Asia from the Middle East to the Pacific Ocean, acting substantial functions for the ecosystems [1]. The Far Eastern leopard (*Panthera pardus orientalis*), also known as the Amur leopard, is one of nine recently recognized subspecies of leopard, whose population and geographic range drastic declining and has lost as much as 98% of their historic range due to the habitat modification, prey depletion, and poaching [2] [3]. Until the two subpopulation of Amur leopard in northern of Primorski Krai disappeared by 1985, the one of the present study becomes only single subpopulation in the planet (Pikunov and Korkishko, 1985) [3]. The subpopulation reside in the region occupying approximately 7,000 km², it is around the borders of the Northeast of China, the extreme southwest of the Primorskii krai (the Far East of Russian), and the extreme north of North Korea [2] [3] [4]. Although the numbers of it is increases in recently [1], however, the Amur leopards on the whole are as few as about 60 individuals[2].As the Amur leopard still is most threatened in a stochastic procession of extinction[5], it is currently classified as Critically Endangered by the International Union for Conservation of Nature, listed in Appendix I of the Convention on International Trade of Endangered Species of Wild Fauna and Flora [2] [1].

Along with the attention paid on the Amur leopard increasing, a few researches are taken on the ecology with population, potential supporting prey, habitat situation, distribution, and genetic diversity[6] [1] [7] [4], however, little research on the disease is made. As the diseases have lethality adverse to protection of the wild Amur tiger [8], it reminds us that the situation of Amur leopard is likely with Amur tiger or even worse because it is a small, single population. Therefore, the potential harmless to the conservation of the Amur leopard originating from the disease is in need of urgent attention. Unfortunately, the research on the potential risk to Amur leopard caused by disease is rare. When the parasites were concerned, even the elementary data, such as parasitic fauna, are absent. Our aim in the study is to accumulate the knowledge of it for a better comprehension.

Results

There are 7 parasite species, including 3 nematodes, 2 cestodes, 1 trematode, and 1 protozoa, were found in this research (see Table 1, Fig. 1). The appearing frequency, infect intensities, and parasite egg sizes were show in the Table 1. The morphology characteristic of parasite eggs was show in Fig. 2. The *Toxocara cati* was higher prevalence, followed by *Spirometra* sp.

Discussion

The *Toxocara cati* is a worldwide parasite of felids, and it is very popular in the both captured and wild Siberian tigers [9-11]. Our research indicates that the *Toxocara cati* also is a common parasite in wild Amur leopard with a high frequency of 61.9%. In general, the Amur tiger, nearly completely sympatric with Amur leopard, could infect two kinds of roundworm, *Toxocara cati* and *Toxascaris leonine*. For the captured tiger, both of them are very popular, whereas *Toxascaris leonine* is rare in the wild Amur tigers [9-11]. The situation of Amur leopard is little difference in Amur leopard as there no *Toxascaris leonine* was found in the wild population in current study.

The capillarid type parasites are wildy distributed in the domestic and wild carnivores, and there are four speicies, including *Eucoleus aerophilus* (syn. *Capillaria aerophila*), *Aonchotheca putorii* (syn. *Capillaria putorii*), *Eucoleus boehmi*, and *Calodium hepaticum* (syn. *Calpillaria hepatica*, *Hepaticola hepatica*), have been recorded in felines [12] [13] [14] [15] [16]. The capillarid type parasites in this research were identified as *Eucoleus aerophilus* or *Aonchotheca putorii* by the characteristics of eggs [14] [17]. *Eucoleus aerophilus* is a globally distributed parasite among various wild carnivorous mammals, and resides embedded in host's epithelium of the trachea, bronchi and bronchioles of the lungs. Usually, the pathogenicity of it is considered subclinical with respiratory distress coughing and wheezing, may be the clinical presentations in heavily infected hosts [15]. *Aonchotheca putorii* is a parasitic nematode of the stomach and small intestines of many wild mammals, and causes severe gastric, associated with a gastric ulcer and secondary anemia [16]. What special species the parasite is need a molecular identification in future.

Troglostrongylus brevior (Metastrongyloidea, Crenosomatidae) and *Aelurostrongylus abstrusus* (Metastrongyloidea, Angiostrongylidae) are two important feline lungworms [18] [19]. The *A. abstrusus* is a common nematode of domestic cats, with a widespread and worldwide distribution [18] [20]. While *T. brevior* have been regarded as infected the wild feline only and been neglected for a long time until some domestic cat infestation cases were reported in recently [19] [21] [22], and it was always thought distributed limited around Mediterranean Europe [23]. The adult worms of *A. abstrusus* is localized in the alveolar ducts and the bronchioles and it can cause respiratory signs like cough, dyspnea, pulmonary wheezes, chronic wasting, and a considerable impact on the healthy and welfare [18]. *T. brevior* also localizes in the host's respiratory system , and can cause cough, dsypnea, severe respiratory distress, and a fatal outcome in kitten [19] [21]. The wild felids have been thought was more susceptible host for *T. brevior* than domestic cat, and it occurrences in domestic cats is regarded as atypical, yet it needs more

reliable evidence to confirm [19]. In current study, the larvae was recognized as *T. brevior* based on the larvae morphology such as the tail characteristic and body size, and the epidemiological endemic, such as *T. brevior* was always present in the wild feline, the wildcats may be the natural hosts of *T. brevior*. However, *A. abstrusus* could not be excluded as they overlap the features of L1 (the first stage of larvae, the diagnostic stage of *A. abstrusus* and *T. brevior*) [21] [22] [23] [19].

The *Spirometra* spp., a Pseudophyllidae tapeworm, occupies the intestine of feline and canine definitive hosts. The first intermediate host for the parasite is copepod and the second one always are amphibians, reptiles like frogs (tadpole) and snakes [24] [25]. The domestic cat and dog are frequent with high *Spirometra* spp. infestation [25], while wild carnivores were ones sporadically [26] [27]. As far as the wild felines were concerned, the *Panthera leo* [28], *Lynx lynx* [24], *leopardus pardalis* [26], *Lynx rufus*, *Oncifelis guigna*, *Puma concolor*, *Panthera onca*, [27] and *Panthera tigris* [11] have been found positive with *Spirometra* spp. Some wild carnivores also could be as the paratenic hosts with the stage of spargana, such as *Meles meles*, *Erinaceus europeaus*, *Martes foina*, *Mustela putorius*, *Mus decumanus*, *Mustela lutreola*, *Neovison vison*, *Lutra lutra*, *Nyctereutes procyonoides*, *Mustela ermine*, *Mustela nivalis*, and *Sorex araneus* [29], and most of them were the prey of the leopard. Therefore, it is not strange that the parasite also could found in the Amur leopard with a high frequency of *Spirometra* spp. (38.1%).

Taenie spp., cosmopolitan parasitic tapeworms in animal medicine, including about 45 species, lives on the small intestine of carnivorous mammals, where they complete development to adults and reproduce [30]. Given the *Taenie* species of feline host, fourteen could take feline as the final host, such as *Taenia laticollis*, *T. omissa*, *T. taeniaeformis* (Syn. *H. taeniaeformis*) [30] (Loos-Frank, 2000). The herbivorous and omnivorous always are the intermediate hosts, in which the metacestode inhabits and develops to the stage could infect the final host. A predator-prey relationship between the definitive and intermediate hosts maintains the transmission of *Taenia* spp. The felids, including *Lynx lynx*, *Lynx rufus*, *Lynx Canadensis*, *Puma concolor*, *Felis margarita*, *Panthera leo*, *Panthera tigris*, and *Felis catus* have been found *Taenie* spp. could occurred in them [30] [31] [32] [33] [11] [34]. Amur leopard, a big size cat, Holarctic felid, preys on a wide range of mammals, but it's overwhelming majority of diets were roe deer (up to 66%), wild boars (up to 8%), Siberian musk deer (up to 9%), and sika deer (up to 6%) [7]. Therefore, Amur leopard was involved in the life cycles of *Taenia* sp is not out anticipation. Although it could be concluded that *Taenia* worms infected the Amur leopard based on presenting the distinctly *Taenia* eggs, developed larva with 3 pairs of hooks, and surrounded by a thick and striated shell, however, it is difficult to discriminate the *Taenia* species only based on the morphological characteristics of the eggs as all species of *Taenia* genus share those special characteristics.

The *Paragonimus* spp., lung flukes, are trematodes that parasitize the lungs of mammal animals like Carnivores, the major definitive host. The first intermediate hosts are snails, in which the miracidium lives, and the second intermediate hosts are crustaceans, crabs or crayfishes, where the metacercariae resides. The definitive hosts become infected by eating raw or under-cooked second intermediate hosts or by eating under-cooked meat of paratenic hosts, such as wild boar and sika deer, which contains juvenile

worms[35] [36] [37]. The latter route seems to be more important in Amur leopard, as it always take the wild boar, sika deer and roe deer as food other than crustaceans [7].

There are about fifty nominal *Paragonimus* species, meanwhile, over half of them have been found in China, one of the major endemic foci of paragonimiasis in the world. Two of them, *P. westermani* and *P. skrjabini*, known zoonotic parasites, are more commonly or focused in China. Although most of *Paragonimus* spp. occur in tropical and subtropical regions, however, two species, *P. westermani* and *P. ohirai*, extend far into temperate latitudes of China [35]. The *P. westermani*, the only one, converges the habitats of the Amur leopards. Although twenty of them were found within the cat, while only seven of them, including *P. westermani*, were found in wild felines [35]. For *P. westermani*, it was initially recognized from a Bengal Tiger at the Amsterdam zoological gardens, which migrated from Asia. Thereafter, other seven feline hosts, including *felis catus*, *Catopuma temminckii*, *Neofelis nebulosa*, *Panthera pardus*, *Prionailurus bengalensis*, *Prionailurus planiceps*, and *Prionailurus rubiginosus*, found could be infected by *P. westermani* [35]. The trematode of Amur leopard was easily identified as *paragonimus* with the features of the eggs, the distantly shoulders or opercular ridges which are useful for differentiating *Paragonimus* eggs from the large operculate eggs of other trematodes [38]. For the specific *Paragonimus* species discrimination, with a high degree certainty, it was the *P. westermani* based on the epidemiological data, such as the parasite's endemic region and host species recorded in the past. However, it is not sufficient to deny the possibility of the other *Paragonimus* spp. without molecular evidence.

There are only two coccidian species, *Cystoisospora felis* (syn. *Isopora felis*) and *C. rivolta* (syn. *Isopora rivolta*), in cats. The typical symptom of *Cystoisospora* was diarrhea in kittens, occasional mortality for *C. Felis* [39]. The egg size of *C. felis* (32–53×26–43 µm) is much bigger than *C. rivolta* (18–25 x 16–23 µm) [39], so it was certainly identified that the coccidian species from Amur leopard as the *C. felis* based on the eggs sizes. Until now, with the Amur leopard together, those wild felines, including *Panthera tigris*, *Panthera leo*, *Panthera pardus*, *Lynx rufus*, and *Felis silvestris*, have been found infected *C. felis* [39] [40] [41] [42] [43] [44]. Traditionally, the *C. felis* was thought transmitted by oral uptake of oocysts without intermediate host, but this was obviously impracticable for wild Amur leopard as their population density are much lower than domestic felines. Therefore, paratenic host must play an important role in transmission of *C. felis*. Actually, based on the results of bioassay studies, the animals like dog, cattle, pig, mice, and rabbit may act as paratenic host for *Cystoisospora* has been discussed in the past[39].

The wild Amur leopards, a solitary, reclusive species, its life history, living environment, and food have a big difference with the domestic cat. So, the parasite of it, especial the nematodes without intermediate host, must have optional ways instead of the traditional oral-feces route. Considered the paratenic host, which always in the food-chain of the big carnivore, is a necessary actor in parasite distribution among wild Amur leopard will not be too seriously. With the instance of the parasites without intermediate host in the present study, *Toxocara cati*, capillarid-type, *Metastrongyloidea*-type, and *Cystoisospora felis*, all of them are suspected taking paratenic host for their transmission [45].

The non-invasive sampling approach, based on collection of scats in the environment, is extraordinary favorable for epidemiological studies on wild carnivore animals, and necessary when protected species are investigated without interfering in the existing structure of the population. However, the study based on mostly scats collected in the environment did not allow the individual animal identification and some stool samples belonged to the same animal cannot be exclude. Therefore, the term frequency was used instead of prevalence to describe the proportion of parasite infections in this research [46].

Conclusion

The Amur leopard were infected by seven parasites firstly reported.

Methods

The research was carried in the Northeast of China, and most samples were collected in the Wangqing National Nature Reserve (Fig.2). After permission was granted by local government, 42 fecal samples were collected opportunistically from the ground in the areas where Amur leopards resided from 2013-2015. The samples were collected in winter to early spring, in that time the local temperature is absolutely below 0 °C. The samples were stored in -80 °C frozen after back to the laboratory. Parasitic eggs were separate with saturated solution of sodium chloride as the floating medium and identified based on the morphological characteristics with the microscope mechanic, infection intensity was determined by the modified McMaster technique, the detective limitation is 60 n/g [47].

Declaration

Abbreviations

Not applicable

Ethics approval and consent to participate

Collecting the feces samples from the wild Amur leopard was approved by the local government agents.

Consent for publication

Not applicable

Availability of data and materials

All the data and materials used in this report are included in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZH designed the research and wrote the manuscript. ZP and YN collected the samples and isolated parasite eggs of the Amur Leopard. GJ helped in conceived of this work and participated in its design. All authors read and approved the final manuscript.

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Table

Table 1 The fauna, eggs size, frequency and intensity of the parasite in Amur leopard

Parasitic species	Egg sizes µm	frequency %	Infection intensity EPG ^a	Highest infection intensity EPG
<i>Toxocara cati</i>	58×72	61.90	272.24	2790
<i>Eucoleus aerophilus</i> or <i>Aonchotheca putorii</i>	28×55	26.21%	20.33	300
Lungworm (Metastrongyloidea)	312×17 ^b	9.5%	\	\
<i>Spirometra</i> sp.	42×70	38.10%	428.31	3510
<i>Taenia</i> sp.	30×32	2.4%	\	\
<i>Paragonimus</i> sp.	54×76	2.4%	\	\
<i>Cystoisospora felis</i>	38×44	2.4%	\	\

Note^a Eggs Per Gram, EPG; b larva

Figures

Figure 1

The eggs morphology features of the parasites among Amur leopards 2-1 *Toxocara cati*; 2-2 The capillarid type parasite; 2-3 *Metastrongyloidea*-lungworm; 2-4; *Paragonimus* sp. 2-5 *Spirometra* sp.; 2-6 *Taenia* sp.; 2-7 *Cystoisospora felis* The picture showed the morphological characteristic of the targeted parasite eggs.

Figure 2

The picture showed the site of the samples were collected, it was made by one author Dr Zhiwei Peng.