Review on parasites of wild and captive giant pandas (Ailuropoda melanoleuca): Diversity, disease and conservation impact

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ARTICLE INFO

Keywords:
- Giant panda
- Parasite infection
- Diversity
- Disease
- Conservation impact

ABSTRACT

The giant panda (Ailuropoda melanoleuca) is a rare species with a small global population size, and lives in the wild in only a few fragmented mountain ranges of Southwest China. Parasitic infections are among the important causes of death of giant pandas that hamper their group development. We reviewed the parasitic infections prevailing in giant pandas, and the parasitic diversity, diseases and their impact on conservation of this animal. A total of 35 parasitic species were documented in giant pandas, belonging to nematode (n = 6), trematode (n = 1), cestode (n = 2), protozoa (n = 9), and ectozoa (n = 17 (tick = 13, mite = 2, and flea = 2)). Among them, Baylisascaris Schroederi had the highest prevalence and was the leading cause of death for giant pandas. Some parasites caused asymptomatic infections in giant pandas, and their health implications for the pandas remain unknown. As a whole, parasites are reported to be an important threat to the conservation of the giant panda. Regular deworming and environmental disinfection appear to be effective ways to prevent captive giant pandas from parasitoses. In wild panda populations, parasitic control measures are suggested to include detailed examination of the ecology of the host-parasite assembly, with particular attention to density-dependent transmission. The parasitic pathogenesis and detection methods together with their biology, epidemiology, treatment, prevention and control need to be further studied for better protection of giant pandas from parasitoses.

1. Introduction

The giant panda (Ailuropoda melanoleuca) has become an important flagship species of China. However, it is a threatened species, with a small global population (Hu et al., 2017). As reported in 2015, there are only 1864 wild giant pandas inhabit in the fragmented mountain ranges of Southwest China (Zhou et al., 2016). To protect this species, 67 nature reserves have been established in China (Kang and Li, 2018; Wei et al., 2020). Giant pandas have mainly been preserved in natural reserves, breeding bases, and zoological gardens in China (Zhu et al., 2013). Wild giant pandas have only been reported in Minshan, Qionglai, Qinling, Liangshan, Daxiangling, and Xiaoxiangling mountain ranges, mainly in Sichuan, and neighboring Shaanxi and Gansu Provinces in China (Fig. 1).

Low reproductive success may be the main internal reason for the low population number of giant pandas (Hu et al., 2017). Climate change, habitat loss, poaching, and disease may be the main external reasons that have hampered group development (Zhang et al., 2008; Hu et al., 2010; Zhang et al., 2018). Diseases with high mortality in giant pandas include viroses (Feng et al., 2016; Zhang et al., 2017), bacterioses (Zhang et al., 2008), and parasitoses (Zhang et al., 2008). Among the parasitoses, visceral larval migrans (VLM) due to nematodes such as the acute and fatal Baylisascaris Schroederi represents the most important cause of death (Zhang et al., 2008, 2010; Wang et al., 2018).

Many other parasitic infections have been documented in giant pandas (Zhang et al., 2010; Li et al., 2013) that are claimed to hamper their growth and development. Here we reviewed the prevailing parasitic infections in giant pandas, and their diversity, diseases and conservation impact.
2. Literature search strategy

We performed a literature search using PubMed, Web of Science, and the China National Knowledge Infrastructure (CNKI), covering all published papers until December of 2019, using the following keywords: “giant panda” and “parasite.” For each of the parasite species, the keywords of the exact parasite species name (such as “Baylisascaris Schroeder”) and “giant panda” were then used to screening the parasitic infection literature.

Surprisingly, there is limited published information on the parasites of the giant pandas (n = 69 peer-reviewed publications and government compiled books), many of which have been published in the Chinese literature (n = 32 publications). Finally, 56 publications on infections, 13 on treatments, and 13 on conservation of giant pandas were involved in the present study.

3. Parasitic infections/infestations reported in giant pandas

A total of 35 parasite species were identified in giant pandas, including 6 species of nematode, 1 of trematode, 2 of cestode, 9 of protozoa, and 17 of ectozoa (13 species of tick, 2 of mite, and 2 of flea) (Table 1). Some parasites of giant pandas have only been identified by microscopy, such as Toxascaris spp., Strongyloides spp., Ogmocotyle sp., and lungworm (Lai et al., 1993; Yu et al., 1998; Zhang et al., 2010; Li et al., 2013). The morphology of Baylisascaris Schroederi, Ancylostoma ailuropredae, Toxoplasma gondii, Enterocytozoon bieneusi, Haemaphysalis flava, Cryptosporidium spp., and Blastocystis sp., etc (Lin et al., 2012; Cheng et al., 2013; Liu et al., 2013; Wang et al., 2013, 2015; Ma et al., 2015; Tian et al., 2015; Peng et al., 2017; Xie et al., 2017).

3.1. Baylisascaris Schroederi and baylisascariasis

The first documented roundworm in giant pandas, initially described as Ascaris Schroederi, was discovered in 1939 (McIntosh, 1939). Ascaris Schroederi was renamed as Baylisascaris Schroederi in 1968 (Yang, 1998; Li et al., 2013). The morphology of B. Schroederi has been described by many researchers. The adult B. Schroederi is a thick nematode with white or grayish brown color. The egg of B. Schroederi is characteristic yellow to brown, sub globular (67.5–83.7 μm × 54.0–70.7 μm), and symmetrical (Kong and Yin, 1958; Zhang et al., 2010; Hu et al., 2018).

Baylisascaris Schroederi is a soil-transmitted parasite that mainly infects through the fecal-oral route. Baylisascaris Schroederi eggs are excreted in the stool with strong survival ability in the environment (Li et al., 2013). The egg/larva develops most suitably at 22–28 °C, and the development stops when the temperature is below 4 °C (Li et al., 2013), however maintains infection activity for a long time. Baylisascaris Schroederi developmental stages in vitro have been well described (Wu et al., 1985a, 1985b). The visceral larval migrans stage of B. Schroederi has been observed in mice infection models (Li, 1990).

Baylisascaris Schroederi is a parasite specific to the giant panda, causing baylisascariasis (Zhang et al., 2008). The parasite is found mainly in the small intestine, and has also been found in the pancreatic and bile ducts connected to the intestinal tract (Ye, 1989). The clinical presentation of baylisascariasis comprises some unspecific symptoms, such as weight loss, pale mucous membranes, indigestion, diarrhea or constipation, poor activity, abdominal pain, and disheveled fur (Yang, 1998; Li et al., 2013). Baylisascaris Schroederi larval migration causes mechanical injury, which results in gastroenteritis, cholangitis, pancreatitis, gastrointestinal obstruction, and even secondary infections that may lead to death (Li et al., 2013). In wild and captive giant pandas, the most common and harmful larval migration is VLM, which is responsible for more than half of the deaths reported in China during 2001–2005 (Zhang et al., 2008).

Currently, B. Schroederi detection is mainly based on the morphology of eggs and/or adult worms either at necropsy or in feces or vomit, and some limited molecular tools (Table 2). In case of microscopic examination of B. Schroederi eggs, the undigested bamboo fibers in giant panda’s feces may challenge the detection, sometimes contribute repeated ‘negative’ fecal test results. Hence, test sensitivity appears to be relatively low, in spite of the high reproductive index of B. Schroederi.
PCR-based molecular techniques can overcome this issue. With the research works regarding the molecular detection of *B. schroederi* in giant pandas, the complete mitochondrial genomes (Xie *et al.*, 2013), microRNA sequences (Zhao *et al.*, 2013) and some other genes came out. Subsequently, several sensitive and suitable molecular detection methods have been developed based on specific genes, such as the internal transcribed spacer-1 (ITS-1) (Lin *et al.*, 2012), internal transcribed spacer-2 (ITS-2) (Zhao *et al.*, 2012), enterocytozoon bieneusi (Wang *et al.*, 2018). Usually, multiple (2–8) anthelmintics are given until an individual panda ceases to expel worms and/or eggs in the feces. However, the possibility or likelihood that drug resistance in captive giant pandas in zoos (Wang *et al.*, 2001; He *et al.*, 2012).

In terms of the infection rate and infection intensity of parasitic diseases including baylisiascaris, captive giant pandas and wild giant pandas are quite different. In captivity, giant pandas receive good veterinary care, resulting in minimal rates of infection, and intensity of parasitic diseases, however these rates are substantially higher amongst wild giant pandas. In captive giant panda populations, the transmission of *B. schroederi* depends on various factors, such as housing system, hygiene, management practices and anthelmintic treatment. However, the current short-term control strategies of this parasitic infection are mainly based on monthly coprological examination of the parasitic eggs and a mass anthelmintic treatment. A number of anthelmintics have practically been used, such as pyrantel pamoate, albendazole, fenbendazole, mebendazole; ivermectin, milbemycin oxime, doramectin and selamectin (Wang *et al.*, 2018). Usually, multiple (2–4 times) treatments are given until an individual panda ceases to expel worms and/or eggs in the feces. However, the possibility or likelihood that drug resistance in *Baylisascaris* could emerge as a problem has stimulated the search for alternative methods of prevention and control. One possibility could be to develop a vaccine against baylisiascaris (Wang *et al.*, 2008; Xie *et al.*, 2013). Apart from work directed towards a vaccine against *B. schroederi*, efforts have also been made to understand aspects of the molecular biology and genetics of this parasite.
unrecognized species, was identified in a dead wild giant panda in Shaanxi, China (Hu et al., 2018). Among these helminth infections, the giant pandas had higher rates of lungworm infections have also been reported in giant pandas (Table 1).

3.2. Other helminth infections

Ancylostoma ailuropodae, Ogmocotyle sikae, Toxascaris selectis, Strongyloides sp., Bunostomum sp., Thysanisia sp., Stilesia sp., and lungworm infections have also been reported in giant pandas (Table 1). Among these helminth infections, the giant pandas had higher rates of Ogmocotyle sikae infections (93.3%, 14/15) and A. ailuropodae infections (93.3%, 14/15), and lower rates of Strongyloides sp. infections, and lower rates of Strongyloides sp. (Table 1). The prevalence of the documented protozoan infections ranged from 1.8% (1/57) for Cryptosporidium giant panda genotype to 73.9% (17/23) for Hepatozoon sp. in giant pandas (Table 3). Sarcozystis sp. parasitizes the muscles of giant pandas (Zhang et al., 2010). Cryptosporidium and E. bieneusi parasitize the intestinal tract, mainly causing intestinal tissue damage, diarrhea, and weight loss (Liu et al., 2013; Wang et al., 2015). The coccidian parasites, including Eimeria sp., and Tyzzeria sp. were identified in the fecal specimens of giant pandas by microscopic examination (Hu et al., 2018). However, T. gondii was isolated from the lung of a giant panda (Ma et al., 2015).

As of now, a species and genotype of Cryptosporidium have been documented in giant pandas. The Cryptosporidium giant panda genotype was reported in an 18-year-old male giant panda, with oocysts of an average size of 4.60 μm × 3.99 μm, and a shape index of 1.15 (Liu et al., 2013). Multilocus genetic characterization including the partial 18S rRNA, 70 kDa heat shock protein, Cryptosporidium oocyst wall protein and actin genes confirmed the isolate as a new giant panda genotype (Liu et al., 2013). Cryptosporidium andersoni was reported at a prevalence of 15.6% (19/122) and 0.5% (1/200) in captive and wild giant pandas in Sichuan, respectively, using a PCR and sequencing approach (Wang et al., 2015).

Toxoplasma gondii infection in a giant panda is characterized by acute gastroenteritis and respiratory symptoms, and is confirmed by immunological and molecular methods. A potentially new genotype of T. gondii has been identified by multilocus-nested PCR-RFLP technique that revealed clonal type I at the SAG1 and c29-2 loci, clonal type II at the SAG2, BTUB, GRA6, c22-8, and L358 loci, and clonal type III at the alternative SAG2 and SAG3 loci (Ma et al., 2015).

In an earlier study, E. bieneusi infection was reported at a rate of 8.7% (4/46) in giant pandas, and all the four isolates were identified as a novel genotype I-like (Tian et al., 2015). In another study, 34.5% (69/200) of the captive giant pandas from conservation bases and zoological gardens were E. bieneusi positive by PCR and sequence analysis of the fecal specimens, having the occurrence of seven known genotypes (SC02, EhpC, CHB1, SC01, D, F, and Peru 6) and five novel genotypes (SC04, SC05, SC06, SC07, and SC08) of the pathogen (Li et al., 2019). Phylogenetic analysis of the ITS (internal transcribed spacer) gene sequences showed that majority of the identified genotypes were clustered into potentially zoonotic group 1, and one genotype (I-like) was clustered into group 2, however a genotype CHB1 did not cluster with any recognized group (Tian et al., 2015; Li et al., 2018). A further study employing multilocus sequence typing of the 69 E. bieneusi isolates identified 24 multilocus genotypes (MLGs), with revealing a
In the first and only report of *Baylisascaris schroederi* in giant pandas (Yu et al., 2019), 73.9% (17/23) of giant pandas showed *Baylisascaris schroederi* infection. Other helminth infections were also detected, including *E. bieneusi* (42% of fecal specimens in Sichuan, China), *Stilesia* (2.3% of fecal specimens in Shaanxi Foping Wild), and *E. bieneusi* (56.2% of fecal specimens in Sichuan and Gansu Wild). Ectoparasitic infestations were also commonly reported, with ticks, mites, and fleas causing significant harm. Ectoparasites were detected in 100% of giant pandas in China Conservation and Research Center for the Giant Panda (CCRCGP) and 25.7% in Sichuan and Gansu Wild (Nature Reserve). Protozoan infections were also reported, with *Cryptosporidium panda* detected in 67.3% of giant pandas in Sichuan and Gansu Wild (Nature Reserve).
the giant pandas. The prevalence of the reported ectoparasitic infestations varied from 2.0% to 100% on giant pandas (Table 3). Co-infestation of the ectozoan species on giant pandas was commonly observed (Yang, 1998; Li et al., 2013).

3.4.1. Tick infestations

Tick infestations with thirteen species have been documented on giant pandas, of which 9 were Haemaphysalis spp., 3 were Ixodes spp., and 1 was Dermacentor sp. (Table 1). Ten taxa of ticks, collected from four Qinling giant pandas in the Qinling mountains, were identified as Haemaphysalis flavus using morphology and molecular markers (nucleotide ITS2 rDNA and mitochondrial 16S) (Cheng et al., 2013). Thus, a combination of morphology and molecular tools is found valuable and efficient for the identification of ticks (Cheng et al., 2013).

The occurrence of tick infestation ranges from 2.0% (1/50) to 100% (11/11) on giant pandas (Qiu, 1987; Ye, 1989; Yu et al., 1998; Cheng et al., 2013). Mixed infestation with tick species is common on giant pandas (Ma, 1987; Qiu, 1987; Cheng et al., 2013). The tick infestations are characterized by anemia, malnutrition, inflammation, and exhaustion in giant pandas (Zhang et al., 2010). More importantly, tick-borne diseases could lead to destructive secondary infections by other pathogens. However, to date, there is no report of any associated tick-borne disease in giant pandas. Ticks are usually controlled by the treatment with ivermectin and selamectin in giant pandas in the breeding centers and zoos (Wang et al., 2018).

3.4.2. Mite infestations

Mite infestations with Demodex ailuropodae and Chorioptes panda cause scabies in giant pandas (Table 1). Demodex ailuropodae mainly infests hair follicles and sebaceous glands of giant pandas, and *C. panda* mainly infests the surface of the body and limbs. The morphology of eggs, larvae, nymphs, and adults of *D. ailuropodae* and *C. panda* as well as their life cycle have been described in details (Fain and Leclerc, 1975; Wang et al., 1985; Xu et al., 1986).

Scabies in giant pandas is characterized by severe skin itching, involuntary scratching, hair that becomes messy and thin, and when the condition is prolonged, skin scabs can form (Yang et al., 2001; Zhang et al., 2016; Li et al., 2013). The occurrence of *C. panda* infestation varies from 66.7% (6/9) to 100% (7/7) on giant pandas (Ye, 1986; Wang et al., 2001). Although *D. ailuropodae* and *C. panda* infestations have occurred on giant pandas throughout the year, more infestations have been reported in the damp, muggy summer and cold winters (Yang, 1998).

The control of *Chorioptes* mange is mainly based on chemotherapeutic treatment. Macrocyclic lactones (e.g., ivermectin and selamectin) have been found to be effective when routinely administered on a monthly basis (Wang et al., 2018). Closantel and deltamethrin have also been proposed to be effective against *C. panda* (Wang et al., 2006; Xu and Zhang, 2002).

3.4.3. Flea infestations

Flea infestations with *Chaeotopsylla mikado* and *Chaeotopsylla ailuropodae* have been documented on giant pandas (Table 1). In case of skin infestation, the fleas suck blood and liberate toxins, resulting in anemia and itching in giant pandas. Flea bites can even lead to secondary bacterial infections of the skin resulting in cellulitis and ulcers (Lai et al., 1990; Peng and Zhang, 1991).

4. Impact of parasitism in conservation of giant panda

The giant panda is a global symbol of wildlife conservation. This endangered animal species is threatened by many factors, such as habitat loss, degradation and fragmentation, poor reproduction, climate change and limited resistance to some infectious diseases (Peng et al., 1985; Wei et al., 2015). Of these factors, diseases caused by parasites are reported to be a major threat to the conservation of the giant pandas. Previous reports suggest that parasites of the giant pandas continue to be a persistent and chronic issue, adversely impacting the health and conservation of this iconic animal (Wang et al., 2018).

In this review, the current information on parasites of the giant pandas has been summarized that revealed 35 parasitic species, including nematode, trematode, cestode, protozoa and ectozoa (tick, mite, and flea) in this animal species. High prevalence of the parasitic infections are documented in giant pandas where *B. Schroederi* is the most prevalent parasitic species. At the same time, *B. Schroederi* causes the most harmful parasitosis for giant pandas, which is responsible for more than half of the deaths of the animals reported in China (Zhang et al., 2008). The morbidity and mortality associated with baylisascariasis are observed to be directly related with the intensity of *B. Schroederi* infection; for instance the individual pandas harboring small numbers of worms tend to be asymptomatic. In captive giant panda populations, where there is a focus on controlling *B. Schroederi*, this nematode rarely causes specific clinical symptoms, except for a few instances of acute outcomes due to larval migration through lungs and passing of adult worms in the feces or vomit (Wang et al., 2018). On the other hand, *B. Schroederi* infection is currently recognized as the biggest threat to free-ranging giant panda populations (Qiu and Mainka, 1993). A study analyzed the causes of death in 789 adult wild giant pandas in natural habitats and observed that VLM caused by *B. Schroederi* was the most significant cause of wild giant panda mortality other than food shortage and poaching (Zhang et al., 2008). It was also reported that baylisascariasis caused 50% (12/24) of all deaths in free-ranging giant pandas between 2001 and 2005 (Zhang et al., 2008). Thus, it is obvious that this parasite represents a significant threat to giant panda conservation. The underlying driver of this parasitic disease is related to a relative increase in panda density as the population has been forced to inhabit remnant patches of bamboo forest. Previous data suggest that the population of wild pandas has likely increased (Zhang et al., 2008). Pandas can become infected by *B. Schroederi* through two fecal-oral routes: (1) by walking on fecally contaminated ground, the eggs adhere to the feet and then enter the panda’s mouth when it manipulates bamboo, and (2) pandas communicate territorial boundaries by marking trees with urine and/or feces; when an individual nuzzles or licks the mark, parasites can be transmitted. Increasing density of pandas would likely result in increased transmission of this pathogen via both these pathways (Zhang et al., 2008). Therefore, undoubtedly baylisascariasis continues to cause serious health problems in the giant pandas and will likely remain one of the biggest challenges for the conservation of this animal. Although modern anthelmintics appear to be reasonably effective for the treatment of baylisascariasis, the dissemination of large numbers of eggs into the environment and the resilience of these thick-shelled eggs make this disease/infestation challenging to control *B. Schroederi* without the implementation of an integrated approach, including management components (pen cleaning protocols and housing infrastructure) and regular monitoring for infection in different age groups of pandas, particularly in captivity. Furthermore, to resolve the underlying cause of the emergence of VLM as a threat to panda survival in wild, future panda conservation efforts should include detailed examination of the ecology of this host-parasite assembly, with particular attention to density-dependent transmission.

The ectoparasitic infestations constitute the second highest prevalence among parasitic infestations in giant pandas. Although ectoparasitic infestations are usually associated with non-specific clinical features, they may cause anemia, skin disease and most importantly induce secondary bacterial and fungal infections that may result in the life threatening to giant pandas (Yang, 1998; Li et al., 2013). On the other hand, there are some other parasites, especially the protozoa that cause asymptomatic infections in giant pandas (Liu et al., 2013; Tian et al., 2015; Deng et al., 2019; Yu et al., 2019). The health implications of the protozoa in giant pandas remain unknown. However, there is one report of an acute and fatal toxoplasmosis case, characterized by serious respiratory and gastroenteritis symptoms in a captive giant panda (Ma et al., 2015). Thus, it is obvious that some parasitic infections can cause
serious health problems in giant pandas and likely remain as one of the big challenges for the conservation of this animal. Therefore, concerted research efforts are needed to understand the biology, epidemiology, diagnosis, treatment, prevention and control of these parasites, to guide conservation decisions.

5. Conclusions
In this review, we have summarized the reported parasitic infections in giant pandas, along with their diversity, disease and conservation impact. A total of 35 parasitic species are found to infect the giant panda. There is no doubt that biliaryascariasis, caused by B. Schroederi, causes serious health problems in the giant panda and will likely remain one of the biggest challenges for the conservation of this species. Several ectozoon species co-infest the giant panda are commonly documented with non-specific clinical features. However, some parasitic species are associated with asymptomatic parasitism without any evidence for health implications in giant pandas. On the other hand, the direct evidence for pathogenesis of many parasites in giant panda remains limited. Regular deworming and environmental disinfection may be effective ways to protect captive giant pandas from parasites. However, the development of anti-parasitic drug resistance (especially for the anthelmintics commonly used against B. Schroederi), due to routine and excessive use of the drugs in captive giant pandas and spreading of drug resistance genes carried by the parasite through reproduction of carrier captive giant pandas to the wild, is an obstacle that demands an integrated approach for parasitic control in this animal species (Wang et al., 2018). Such approach might include the use of effective disinfectants to block transmission, new drugs with different modes of action and/or vaccination and the investigation of the ecology of host-parasite assembly, with particular attention to curtail the density-dependent transmission (Zhang et al., 2008; Xie et al., 2013). On the other hand, the development of sensitive and convenient detection methods of giant panda parasites is another important issue to assess the prevalence and distribution of parasites in captive and wild populations. Such limitation could be overcome by the development of a PCR-based diagnostic approach for the simultaneous genetic ‘fingerprinting’ of individual pandas and the detection of their parasites in fecal samples, which could be used for field studies, in order to explore the distribution and dynamics of parasitic diseases. Additionally, the PCR-based or high-throughput DNA sequencing technology might detect the emerging parasite species in giant pandas (Wang et al., 2018). Despite the significance of parasitic diseases in giant pandas, it is found that the parasitological researches are limited in this animal species. Therefore, it is recommended to pay more attention to the parasitic diseases that are likely to threaten the conservation of this critically endangered species.

Ethical standards
Not applicable.

Declaration of competing interest
The authors declare that they have no competing interests.

Acknowledgement
This study was supported by the Key Program of the National Natural Science Foundation of China (31330079), the National Science Foundation of Henan Province (162300410129), the Key National Science and Technology Specific Projects (2012ZX10004220-001), the National Natural Science Foundation of China (U1404327), and the PhD research startup foundation of Henan University of Chinese Medicine (KYQD2021). We are grateful to Dr. Abu Nasar Md. Aminor Rahman, Professor, Wildlife Reproduction & Conservation Laboratory, Department of Gynecology, Obstetrics & Reproductive Health, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh for his critical review and language editing of this manuscript.

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