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

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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 pandas. 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.

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## 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 schroederi*") 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* sp., *Strongyloides* sp., *Ogmocotyle* sp., and lungworm (Lai et al., 1993; Yu et al., 1998; Zhang et al., 2010; Li et al., 2013; Hu et al., 2018). However, in the last decade molecular techniques have emerged as important tools for the characterization of some parasites, such as *Baylisascaris schroederi*, *Ancylostoma ailuropodae*, *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/larvae 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*


**Fig. 1.** Distribution of wild giant pandas in six mountain regions (Qinling, Minshan, Qionglai, Liangshan, Daxiangling and Xiaoxiangling) in three Provinces (Gansu, Shaanxi, and Sichuan) of China. Adapted from Wang et al. (2018).

**Table 1**
List of parasites in giant pandas.

| Taxa | Parasite species | Site of infection/infestation | Location of first report | Year of first report | Reference |
|---|---|---|---|---|---|
| Nematode | *Baylisascaris schroederi* | Small intestine | Sichuan; Shaanxi; Gansu | 1939 | McIntosh (1939) |
| | *Toxascaris seleactis* | Small intestine | Sichuan | 1993 | Lai et al. (1993) |
| | *Ancylostoma ailuropodae* | Small intestine | Sichuan Baoxing | 1995 | Li et al. (2013) |
| | *Strongyloides* sp. | Small intestine | Sichuan | 1993 | Lai et al. (1993) |
| | Lungworm | Intestinal tract and lung | Sichuan Quanxing | 1993 | Lai et al. (1993) |
| | *Bunostomum* sp. | Intestinal tract | Shaanxi Foping | 2018 | Hu et al. (2018) |
| Trematode | *Ogmocotyle sikae* | Small intestine | Shaanxi Foping | 1987 | He et al. (1987) |
| Cestode | *Thysaniezia* sp. | Intestinal tract | Shaanxi Foping | 2018 | Hu et al. (2018) |
| | *Stilesia* sp. | Intestinal tract | Shaanxi Foping | 2018 | Hu et al. (2018) |
| Protozoan | *Sarcocystis* sp. | Muscle | Chengdu zoo | – | Zhang et al. (2010) |
| | *Cryptosporidium* giant panda genotype | Intestinal tract | Sichuan Ya'an | 2013 | Liu et al. (2013) |
| | *Cryptosporidium andersoni* | Intestinal tract | Sichuan | 2015 | Wang et al. (2015) |
| | *Enterocytozoon bieneusi* | Intestinal tract | Shaanxi Xi'an | 2015 | Tian et al. (2015) |
| | *Toxoplasma gondii* | Lung | Zhengzhou zoo | 2015 | Ma et al. (2015) |
| | *Eimeria* sp. *Tyzzeria* sp. | Intestinal tract | Shaanxi Foping | 2018 | Hu et al. (2018) |
| | *Blastocystis* sp. | Intestinal tract | Chengdu, Sichuan | 2019 | Deng et al. (2019) |
| | *Hepatozoon* sp. | Blood | USA, UK, and China | 2019 | Yu et al. (2019) |
| Tick | *Ixodex granulatus* | Body surface | Gansu Wenxian | 1984 | Li et al. (2013) |
| | *Ixodex acutitarsus* | Body surface | Sichuan Tianquan; Sichuan Wenchuan; Gansu Wenxian | 1984 | Li et al. (2013) |
| | *Ixodex ovatux* | Body surface | Sichuan Tianquan; Sichuan Wenchuan; Sichuan Baoxing; Gansu Wenxian | 1987 | Ma (1987) |
| | *Haemaphysalis flava* | Body surface | Sichuan Wenchuan; Sichuan Pingwu; Sichuan Beichun; Gansu Wenxian | 1984 | Li et al. (2013) |
| | *Haemaphysalis aponommoides* | Body surface | Sichuan Pingwu | 1985 | Li et al. (2013) |
| | *Haemaphysalis hystricis* | Body surface | Sichuan Tianquan | 1985 | Li et al. (2013) |
| | *Haemaphysalis longicornis* | Body surface | Sichuan Pingwu | 1985 | Li et al. (2013) |
| | *Haemaphysalis kitaotai* | Body surface | Sichuan Pingwu; Sichuan Beichun | 1987 | Qiu (1987) |
| | *Haemaphysalis megaspinosa* | Body surface | Sichuan Baoxing; Sichuan Pingwu; Gansu Wenxian | 1987 | Qiu (1987) |
| | *Haemaphysalis montgomeryi* | Body surface | Gansu Wenxian | 1984 | Li et al. (2013) |
| | *Haemaphysalis warburtoni* | Body surface | Sichuan Tianquan | 1985 | Li et al. (2013) |
| | *Dermacentor taiwanensis* | Body surface | Sichuan Tianquan; Sichuan Baoxing | – | Li et al. (2013) |
| | *Haemaphysalis ailuropodae* | Body surface | Shaanxi | 1998 | Yu et al. (1998) |
| Mite | *Chorioptes panda* | Body surface and limbs | Sichuan; Shaanxi; Gansu and zoos | 1975 | Fain and Leclerc (1975) |
| | *Demodex ailuropodae* | Hair follicles and sebaceous glands | Shanghai zoo; Chongqing zoo | 1986 | Xu et al. (1986) |
| Flea | *Chaetopsylla mikado* | Body surface | Chengdu zoo | 1990 | Lai et al. (1990) |
| | *Chaetopsylla ailuropodae* | Body surface | Sichuan Pingwu | 1991 | Feng and Zhang (1991) |

(Wang et al., 2018). 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., 2011), 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), ATPase subunit 6 (atp6), mitochondrial 12S rRNA (Zhou et al., 2013b), mitochondrial COII (Zhang et al., 2012), mitochondrial cytochrome c oxidase subunit I (Xie et al., 2014), and mitochondrial cytochrome c oxidase subunit II (Wang et al., 2013). The molecular studies reported that the *B. schroederi* isolates in giant pandas exhibit low genetic structure and a rapid evolutionary rate, indicating that there is no geographical separation among the populations (Zhou et al., 2013a; Xie et al., 2014). Other than the microscopic and molecular assays, some progress has been made on developing serological detection methods. For instance, an antibody detection enzyme-linked immunosorbent assay (ELISA) employing a *B. schroederi* glutathione S-transferase antigen was established for the detection of anti-*B. schroederi* serum antibody (IgG) in experimentally infected mice (Xie et al., 2015).

*Baylisascaris schroederi* is the most prevalent parasite in giant pandas, and the infection rate in both wild and captive animals ranges from 7.1 (1/14) to 100% (33/33) (Table 3). The high parasite burdens were widely observed (Ye, 1989; Yang, 1998; Zhang et al., 2010), with the highest documented number of *B. schroederi* up to 3204 in a single giant panda (Zhang et al., 2010). Higher prevalence of the parasite was reported in the wild and/or dead giant pandas (Kong and Yin, 1958; Feng and Zhang, 1991; Yu et al., 1998), while lower prevalence was reported 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 baylisascariasis, 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 baylisascariasis (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.

**Table 2**
The diagnostic stages and detection methods of parasite species in giant pandas.

| Parasite species | Diagnostic stages | Detection methods | References |
|---|---|---|---|
| *Baylisascaris schroederi* | Eggs/ Larva/Adult | Microscopy and PCR | Lai et al. (1993); Wang et al. (2001); Zhao et al. (2012); Wang et al. (2013); Peng et al. (2017); Hu et al. (2018); etc. |
| *Ogmocotyle sikae* | Eggs | Microscopy | Lai et al. (1993); Yu et al. (1998); Zhang et al. (2010); Hu et al. (2018) |
| *Ancylostoma ailuropodae* | Eggs/ Larva/Adult | Microscopy and PCR | Li et al. (2013); Xie et al. (2017) |
| *Toxascaris seleactis* | Eggs/ Larva/Adult | Microscopy | Lai et al. (1993) |
| *Strongyloides* sp. | Eggs/ Larva/Adult | Microscopy | Lai et al. (1993) |
| Lungworn | Eggs/ Larva/Adult | Microscopy | Lai et al. (1993) |
| *Bunostomum* sp. | Eggs/ Larva/Adult | Microscopy | Hu et al. (2018) |
| *Thysaniezia* sp. | Eggs/ Larva/Adult | Microscopy | Hu et al. (2018) |
| *Stilesia* sp. | Eggs/ Larva/Adult | Microscopy | Hu et al. (2018) |
| *Cryptosporidium* giant panda genotype and *C. andersoni* | Oocyst | Microscopy and PCR | Liu et al. (2013); Wang et al. (2015) |
| *Enterocytozoon bieneusi* | Spores | PCR | Tian et al. (2015) |
| *Toxoplasma gondii* | Cyst | Microscopy and PCR | Ma et al. (2015) |
| *Eimeria* sp. | Oocysts | Microscopy | Hu et al. (2018) |
| *Tyzzeria* sp. | Oocysts | Microscopy | Hu et al. (2018) |
| *Blastocystis* sp. | Oocyst | PCR | Deng et al. (2019) |
| *Hepatozoon* sp. | Oocyst | Microscopy and PCR | Yu et al. (2019) |
| Tick | Imago | Microscopy and PCR | Qiu (1987); Yu et al. (1998); Cheng et al. (2013); |
| *Chorioptes panda* | Imago | Microscopy | Ye (1986); Wang et al. (2001) |

**General protocols of microscopic diagnosis:** The feces, vomit, intestinal contents (for necropsy), blood or tissue samples, or surface skin samples of giant pandas were obtained, and then subjected to the direct, smear, or stain observation under the microscopy. The parasites were preliminarily identifed based on the morphology, size, coloration, refraction of the eggs/oocysts/cysts/larva, or adult of the parasites, as well as the biological characteristics of the parasitic host.
**General protocols of PCR diagnosis:** The total genomic DNAs of the suspected samples were extracted, and then amplifed in vitro (PCR instrument was usually used) based on the specifc gene sequences (such as, SSU rRNA, ITS). The amplicons were identifed by the electrophoresis, and sequencing if necessary.

### 3.2. Other helminth infections

*Ancylostoma ailuropodae*, *Ogmocotyle sikae*, *Toxascaris seleactis*, *Strongyloides* sp., *Bunostomum* sp., *Thysaniezia* 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* (100%, 5/5) and *A. ailuropodae* (93.3%, 14/15) infections, and lower rates of *Strongyloides* sp. (0.1%, 3/2680) and lungworn (0.04%, 1/2680) infections (Table 3).

*Ancylostoma ailuropodae* parasitizes the small intestine of giant pandas, causing bleeding and inflammation in the intestinal mucosa (Li et al., 2013; Xie et al., 2017). *Ancylostoma ailuropodae*, a previously unrecognized species, was identified in a dead wild giant panda in Sichuan, China, through both morphological and molecular characterization (Xie et al., 2017). In another study, *Bunostomum* sp. showed 4.5% (2/44) infection in giant pandas in Shaanxi, China (Hu et al., 2018).

*Ogmocotyle sikae* parasitizes the small intestine of giant pandas, which can cause multiple bleeding spots on the intestinal mucosa, in addition to digestive function disturbances. Detection of *O. sikae* in giant panda feces showed 0.5% (13/2680) (Lai et al., 1993) and 6.8% (3/44) (Hu et al., 2018) prevalence of the parasite in two separate studies. However, an autopsy report indicated that *O. sikae* widely exists (100%, 5/5) in giant pandas in the Shaanxi Qinling Mountains (Zhang et al., 2010). For *Strongyloides* sp., although the parasitic infection is common in animals, and the parasite possesses a simple life cycle; only 0.1% (3/2680) of giant pandas surveyed were found infected with *Strongyloides* sp. (Lai et al., 1993). Similarly, lungworm was only found in one specimen (0.04%, 1/2680) of the surveyed giant pandas (Lai et al., 1993). However, *Toxascaris seleactis* infection was identified in 4.5% (121/2680) of the giant pandas (Lai et al., 1993).

In case of cestode infection in giant pandas, a recent study reported two species of the parasite, including *Thysaniezia* sp. and *Stilesia* sp. in the animal species (Hu et al., 2018).

### 3.3. Protozoan infections

Protozoan infections are common among giant pandas. The occurrence of *Sarcocystis* sp., *Cryptosporidium* giant panda genotype, *Cryptosporidium andersoni*, *Enterocytozoon bieneusi*, *Toxoplasma gondii*, *Eimeria* sp., *Tyzzeria* sp., *Blastocystis* sp., and *Hepatozoon* sp. have been documented in giant pandas (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). *Sarcocystis* 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, EpbC, CHB1, SC01, D, F, and Peru 6) and five novel genotypes (SC04, SC05, SC06, SC07, and SC08) of the pathogen (Li et al., 2018). 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

**Table 3**
The parasitic infection status of giant pandas.

| Parasite species | Location | Living status of giant pandas | Infection rate (positive number/sample number) | Detection technique | Reference |
|---|---|---|---|---|---|
| Baylisascaris schroederi | | | | | |
| *Baylisascaris schroederi* | Beijing | Zoo (Necropsy) | 100% (1/1) | Microscopy | Kong and Yin (1958) |
| *Baylisascaris schroederi* | Minshan and Qionglai | Wild (Necropsy) | 100% (13/13) | Microscopy | Feng et al. (1985) |
| *Baylisascaris schroederi* | Shanghai | Zoo (captive) | 66.7% (2/3) | Microscopy | Peng et al. (1989) |
| *Baylisascaris schroederi* | Sichuan | Wild (injured) | 26.0% (13/50) | Microscopy | Ye (1989) |
| *Baylisascaris schroederi* | Sichuan | Wild (Necropsy) | 100% (33/33) | Microscopy | Feng and Zhang (1991) |
| *Baylisascaris schroederi* | Sichuan and Gansu | Wild (Nature Reserve) | 56.2% (1505/2680) | Microscopy | Lai et al. (1991) |
| *Baylisascaris schroederi* | Sichuan | Wild (Nature Reserve) | 74.3% (518/679) | Microscopy | Yang (1993) |
| *Baylisascaris schroederi* | Sichuan | Wild | 69.2% (117/169) | Microscopy | Yang (1998) |
| *Baylisascaris schroederi* | Qinling mountain | Wild (Necropsy) | 100% (2/2) | Microscopy | Yu et al. (1998) |
| *Baylisascaris schroederi* | Qinling mountain | Wild | 91.7% (11/12) | Microscopy | Yang (1998) |
| *Baylisascaris schroederi* | Sichuan Chengdu | Zoo (Captive) | 7.1% (1/14) | Microscopy | Wang et al. (2001) |
| *Baylisascaris schroederi* | Sichuan | Wild zoo | 45.5% (5/11) | Microscopy | Qi et al. (2011) |
| *Baylisascaris schroederi* | Sichuan and Gansu | Wild (Nature Reserve) | 55.0% (68/126) | Microscopy | Zhang et al. (2011) |
| *Baylisascaris schroederi* | Minshan | Wild | 31.3% (15/31) | PCR | Zhang et al. (2012) |
| *Baylisascaris schroederi* | Sichuan Ya'an | CCRCGP | 67.3% (37/55) | PCR | Wang et al. (2013) |
| *Baylisascaris schroederi* | Sichuan Ya'an | CCRCGP | 88.0% (44/50) | PCR | Zhou et al. (2013b) |
| *Baylisascaris schroederi* | Sichuan | CCRCGP | 25.7% (54/210) | Microscopy | Li et al. (2014) |
| *Baylisascaris schroederi* | Sichuan and Gansu | Wild | 55.2% (48/87) | Microscopy | Li et al. (2013) |
| *Baylisascaris schroederi* | Shaanxi Foping | Wild (Nature Reserve) | 52.3% (101/193) | Microscopy | Peng et al. (2017) |
| *Baylisascaris schroederi* | Shaanxi Foping | Wild | 56.8% (25/44) | Microscopy | Hu et al. (2018) |
| **Other helminth infections** | | | | | |
| *Ogmocotyle sikae* | Sichuan and Gansu | Wild (Nature Reserve) | 0.5% (13/2680) | Microscopy | Lai et al. (1993) |
| *Ogmocotyle sikae* | Shaanxi Qinling mountain | Wild (Necropsy) | 50.0% (1/2) | Microscopy | Yu et al. (1998) |
| *Ogmocotyle sikae* | Sichuan | Wild (Necropsy) | 14.3% (1/7) | Microscopy | Zhang et al. (2010) |
| *Ogmocotyle sikae* | Qinling mountains | Wild (Necropsy) | 100% (5/5) | Microscopy | Zhang et al. (2010) |
| *Ogmocotyle sikae* | Shaanxi Foping | Wild | 6.8% (3/44) | Microscopy | Hu et al. (2018) |
| *Ancylostoma ailuropodae* | Sichuan | Wild | 93.3% (14/15) | Microscopy | Li et al. (2013) |
| *Bunostomum* sp. | Shaanxi Foping | Wild | 4.5% (2/44) | Microscopy | Hu et al. (2018) |
| *Thysaniezia* sp. *Stilesia* sp. | Shaanxi Foping | Wild | 6.8% (3/44) | Microscopy | Hu et al. (2018) |
| *Toxascaris seleactis* | Sichuan and Gansu | Wild (Nature Reserve) | 4.5% (121/2680) | Microscopy | Lai et al. (1993) |
| *Strongyloides* sp. | Sichuan and Gansu | Wild (Nature Reserve) | 0.1% (3/2680) | Microscopy | Lai et al. (1993) |
| Lungworn | Sichuan and Gansu | Wild (Nature Reserve) | 0.04% (1/2680) | Microscopy | Lai et al. (1993) |
| **Protozoan infections** | | | | | |
| *Cryptosporidium* giant panda genotype | Sichuan Ya'an | CCRCGP | 1.8% (1/57) | PCR | Liu et al. (2013) |
| *Cryptosporidium andersoni* | Sichuan | Captive and Natural reserve | 6.2% (20/322) | PCR | Wang et al. (2015) |
| *Enterocytozoon bieneusi* | Shaanxi Xi'an | RWRBRC and Xi'an QWP | 8.7% (4/46) | PCR | Tian et al. (2015) |
| *Enterocytozoon bieneusi* | China | Captive and zoological gardens | 34.5% (69/200) | PCR | Li et al. (2018) |
| *Toxoplasma gondii* | Henan Zhengzhou | Zoo (Necropsy) | 100% (1/1) | PCR | Ma et al. (2015) |
| *Sarcocystis* sp. | Shaanxi Foping | Wild | 2.3% (1/44) | Microscopy | Hu et al. (2018) |
| *Eimeria* sp. & *Tyzzeria* sp. | Shaanxi Foping | Wild | 15.9% (7/44) | Microscopy | Hu et al. (2018) |
| *Blastocystis* sp. | Chengdu, Sichuan | Giant Panda Breeding center | 12.3% (10/81) | PCR | Deng et al. (2019) |
| *Hepatozoon* sp. | USA, UK, and China | Wild-caught and Captive | 73.9% (17/23) | PCR | Yu et al. (2019) |
| **Ectozoan infestations** | | | | | |
| Tick | Sichuan | Wild (rescued or dead) | 100% (11/11) | Microscopy | Qiu (1987) |
| Tick | Sichuan | Wild (injured) | 2.0% (1/50) | Microscopy | Ye (1989) |
| Tick | Qinling mountains | Wild (Necropsy) | 100% (4/4) | Microscopy and PCR | Cheng et al. (2013) |
| *Haemaphysalis ailuropodae* | Qinling mountains | Wild (Necropsy) | 50.0% (1/2) | Microscopy | Yu et al. (1998) |
| *Chorioptes panda* | Sichuan Chengdu | Zoo (captive) | 66.7% (6/9) | Microscopy | Ye (1986) |
| *Chorioptes panda* | Sichuan Chengdu | Zoo (captive) | 100% (7/7) | Microscopy | Wang et al. (2001) |
| **Subtotal** | | | | | |

**CCRCGP:** China Conservation and Research Center for the Giant Panda.
**RWRBRC:** Rare Wildlife Rescue Breeding Research Center.
**Xi'an QWP:** Xi'an Qinling Wildlife Park.

strong and significant linkage disequilibrium (LD), indicating a clonal population of the parasite (Li et al., 2017). Subsequently, the STRUCTURE analysis of the isolates proposed three subpopulations of *E. bieneusi* in giant pandas in China (Li et al., 2017).

The first and only report of *Blastocystis* sp. detected the pathogen in 12.3% (10/81) of fecal specimens of giant pandas in Sichuan, China, with identification of subtype ST1 (Deng et al., 2019). Similarly, the first report of *Hepatozoon* sp. revealed a novel *Hepatozoon* sp. with its high prevalence (73.9%, 17/23) in giant pandas (Yu et al., 2019).

### 3.4. Ectozoan infestations

Ectoparasitic infestations are commonly reported on giant pandas. Among the ectoparasites, ticks, mites and fleas cause significant harm to 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 flava* 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., 2010; 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., 2000; Xu and Zhang, 2002).

### 3.4.3. Flea infestations

Flea infestations with *Chaetopsylla mikado* and *Chaetopsylla 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; Feng 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 (Feng 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 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/infection 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 sometimes be 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 pandas. There is no doubt that baylisascariasis, caused by *B. schroederi*, causes serious health problems in the giant pandas and will likely remain one of the biggest challenges for the conservation of this species. Several ectozoan species co-infest the giant pandas 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 pandas remains limited. Regular deworming and environmental disinfection may be effective ways to protect captive giant pandas from parasitoses. However, the development of anti-parasitic drug resistance (specially 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 reintroduction 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.

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