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Review Article

Helminths and Helminthiasis in Captive Amphibians and Reptiles: A Brief Overview of Recent Records from the Wild Animal Medical Center of Rakuno Gakuen University, Japan

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Abstract

The corresponding author, a manager at the Wild Animal Medical Center established at Rakuno Gakuen University (Hokkaido, northern Japan), and his students introduce some cases of helminthes and helminthiasis of captive amphibians and reptiles, including the Japanese giant salamanders and variable species of tortoises, terrapins, lizards and snakes, from zoological gardens or exotic pet clinics between 2004 and 2017 based on the research activities performed at the facility. Probably, the present summarization will prove to be a tool for future research.

INTRODUCTION

In 2004, the Wild Animal Medical Center (WAMC) was established at Rakuno Gakuen University (RGU, Hokkaido, northern Japan), to provide facilities for conservation medical research and education. Research activities performed at the WAMC vary, and include veterinary medical studies of wild and/or zoo animals and epidemiological studies of infectious diseases (especially helminthiasis).The present corresponding author is a manager at the WAMC and is a wildlife helminthologist at RGU [1-3]. Additionally, in 2006, the Japanese Society of Zoo and Wildlife Medicine designated the WAMC as a wildlife helminthiasis center of the society, and this designation accelerates studies on helminths and helminthiasis of various vertebrates. The author and his co-workers including his students have published several reviews of the scientific field derived from avian and mammalian hosts [4-10]. Furthermore, several reviews and reports have been published on helminths of free-ranging amphibians and reptiles, including invasive alien species in Japan [1,4,11-13]. However, there have been no comprehensive articles that focused on captive amphibians and reptiles from zoological gardens or exotic pet clinics in Japan; although some short reports have been published in Japanese (references are shown later). Therefore, in this review the authors introduce some cases from between 2004 and 2017 based on the research activities performed at the WAMC.

JAPANESE GIANT SALAMANDERS FOR BREEDING PROJECT

The Japanese giant salamander (*Andrias japonicus*) is an endangered amphibian species kept and bred in Hiroshima City Asa Zoological Park, western Honshu, Japan. To monitor their health, a helminthological survey of carcasses and living salamanders kept in the park was performed at the WAMC. We detected four total nematode taxa, including mature *Amphibiocapillaria tritonispunctati* and immature *Spiroxys* sp., Kathlaniidae gen. sp. and Physalopteroidea fam. gen. sp. worms, and a mature trematode (*Liolope copulans*) from the alimentary tracts of carcasses [14-16] (Figure 1). In addition to the internal parasites, we also detected a leech species (*Hemiclepsis marginata*) from the body surface of a living individual [15], (Figure 2). Overall, except for the leech species, the helminths obtained were previously recorded from free-ranging Japanese giant salamanders, but were not previously found on those in captivity. Consequently, we continue to monitor the relationship between any health problems of the captive salamanders and their diets, because helminth infections are derived from intermediate hosts, such as freshwater fishes. However, this is also the first record of a leech in any Japanese giant salamander, including free-ranging individuals. Because of a leech-caused local lesion on adaptive individual (Figure 3), we speculate that parasitism by leeches is intimately associated with host health.

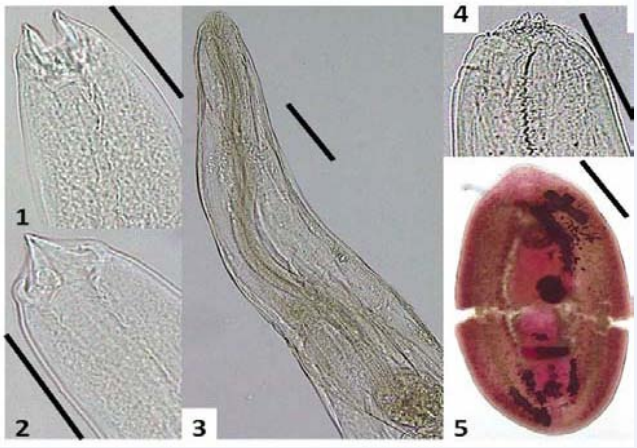

Figure 1 Anterior extremity of nematode larvae (from -1 to -4) and whole body of a trematode (-5) from the alimentary tracts of breeding Japanese giant salamander (*Andrias japonicus*) carcasses [14]. -1 and -2: dorsal (-1) and (-2) lateral side of *Spiroxys* sp.(encysted 3rd stage, bar=50μm); -3: Kathlaniidae gen. sp. (unknown stage, bar=100 μm); -4: Physalopteroidea fam. gen. sp. (unknown stage, bar=100 μm); -5: *Liolope copulans*(bar=500 μm).

Figure 2 A leech (*Hemiclepsis marginata*) from a breeding Japanese giant salamander (*Andrias japonicus*) (upper, bar=2 mm) and skin lesion caused by its parasitism (lower) [15].

Terrapins kept at a primary school

It is a popular tradition for Japanese primary schools to keep terrapins, including *Trachemys scripta* and *Mauremys reevesii* for general educational activities. However, although it looks so strange that there hss no epidemiological survey on the helminth infection. Therefore, we examined some individuals kept at a school in Shizuoka Prefecture, central Japan in 2026. We did not obtain any internal helminths from either terrapin species, but we did obtain a leech species (*Ozobranchus jantseanus*) from *M. reevesii* [17], however, we could not show a risk factor with zoonotic helminthiasis derived from the captive terrapins.

Pet chameleon filarial worms

Recently, chameleons have become one of the most popular pet reptiles in Japan; above all, panther chameleons (*Furcifer pardalis*) are regarded as reasonable ones for the people. Most of these pets are free-ranging individuals captured from the wild; consequently, we found exotic helminths in some of these chameleons. In April 2015, a panther chameleon was sold at a pet shop in Sapporo, capital of Hokkaido, and kept in a private collection. Several weeks later, the chameleon died, and postmortem examination was undertaken at the WAMC.

We confirmed heavy infection by filarial nematodes under the skin (Figure 4). The body length of the male (n=1) was 26 mm, and that of the females (n=3) was 88 mm. Based on morphological observation of nematode anterior and posterior extremities (Figure 5), we identified the nematodes as *Foleyella furcated* [18]. This nematode is a common chameleon parasite but it is not detrimental to public health. Additionally, it did not seem to be pathogenic to the host, so the parasitism may not have

Figure 3 Leech species (*Ozobranchus jantseanus*) obtained from captive terrapins (*Mauremys reevesii*) [17], bar=10 mm).

Figure 4 Heavy infection with filarial worms (Foleyella furcate) in a panther chameleon (Furcifer pardalis) [18].

been associated with the cause of death. However, the pet owner was surprised by the parasitism of the relatively large nematodes. Therefore, the veterinarian had to explain detailed biological and pathological characteristics of the nematodes to the owner.

Chinese crocodile lizard from the illegal pet trade

Because of the increased popularity of owning reptiles, as mentioned above, there has been an increase of illegally collected reptiles in Japan. In Nov. 2007, 13 Chinese crocodile lizards in the illegal pet trade were confiscated at Tokyo International Airport, and the lizards were taken to Yagiyama Zoo, Miyagi Prefecture, in the northeastern part of Japan. Among them, one individual had neurological symptoms, with abnormal neck and leg behaviors (Figure 6, left), observed initially in January 2008. X-ray examination revealed no legions, and the lizard died in February 2008. Postmortem examination showed that a worm was embedded in a posterior part of the brain (Figure 6, middle), and the worm was a nematode larva (4^{th} stage) of the order Ascaridida (Figure 6, right) [19]. The ascarid was a snake parasite (i.e., genus *Ophidascaris*), and infection might have occurred at the zoo. Subsequently, it was concluded that the lizard was killed by larval migration. Hence, the pet-industrial background in the Japanese society has changed; the pet helminthiasis has been changed to more variable as the present case.

Albino snakes co-infected with nematodes and *Chlamydophila*

Dating back to ancient times, Japanese people have believed that white snakes heralded from heaven; even now, white snakes are considered lucky animals. These snakes include albino individuals or populations of Japanese rat snakes (*Elaphe climacophora*), and most of the snakes occur in Yamaguchi Prefecture in the western part of Japan. Because people love them, the local population of the albino Japanese rat snakes is considered important for local tourism.

The prefectural government has conserved this population and instituted a long-standing breeding program. However, many individuals in captivity have died since the end of the last century. Consequently, some of the carcasses were taken to the WAMC, and postmortem examination was performed. We found heavy infection with nematodes (*Kalicephalus sinensis*) from the oral cavity to intestines [20,21], (Figure 7,8) and detected bacteria (*Chlamydophila* sp.) responsible for snake chlamidosis [22]. Furthermore, we also found *Strongyloides* sp. and *Rhabdias horigutii* nematodes. A comparison between host age and parasitism by *K. natiricis* and *R. horigutii* was performed; there were higher intensities of both nematodes per snake in mature snakes that were kept in outdoor breeding facilities covered

Figure 5 Filaria (*Foleyelhas furcata*) from a panther chameleon. Anterior (left) and posterior (middle) male extremities, and a posterior female extremity (right) [18].

Figure 6 A fatal case of nematode larval migration in a Chinese crocodile lizard. Left: neurological symptom of abnormal neck and leg behaviors, middle: a nematode larva embedded in a posterior part of brain, right: anterior extremity of the larva [19].

with natural soil compared with immature snakes that were kept in indoor facilities ($P<0.05$). Therefore, an infection of these nematode species seems to occur on soil of the outdoor because both nematodes have direct life cycles. After diagnosis, a countermeasure against nematode infection was implemented for semi-free-ranging mature snakes; the serial death event is almost stopped now.

Miscellaneous cases of pentastomidiasis from zoos and commercially available reptiles

Overall, it is confirmed that helminth infections were quite common in the captive reptiles mentioned above; even in Hokkaido in the northernmost part of Japan. We confirmed heavy helminth infections based on comprehensive survey of 113 fresh feces from 28 species (Testudines, Squamata, and Crocodylia) and five and 22 carcasses of12 species from zoo collections in Sapporo City [23]. Therefore, from a public health perspective, monitoring surveys were needed of pet reptiles sold in the capital city and surrounding area of Hokkaido, neighboring districts to WAMC. The first survey materials were tortoise and terrapin carcasses, because they are the most popular pet reptiles in Japan. From the 11 tortoise species surveyed, only nematode parasites were obtained; from the hosts *Chelus fimbriatus* (Cf), *Chelodina siebenrocki* (Cs), *Testudo horsfieldi* (Th), and *Kinixys* sp. (K), 6 nematodes were found, including *Thaparia* sp. (host: Th), *Mehdiella* sp. (host: Th), *Tachygonetria* sp. (hosts: Cs, Th), *Labiduris* sp. (host: K), Capillariidae gen. sp. (host: Cf), and encysted larvae (host: Cf) [24]. Subsequently, 5 families, 21 genera, and 29 species and subspecies of terrapins were examined. Among them, an acanthocephalan parasite, *Neoechinorhynchus* sp. (host: *Trachemys scripta* sub sp.) and nematode parasites including *Serpinema trispinosus* (hosts: *Graptemys geographica*, *T. scripta* sub sp., *Chelydra serpentina osceola*), *S. microcephalus* (hosts: *Siebenrockiella creassicollis, Ocadia sinensis*), *Atractis* cf. *dactyluris* (hosts: *Rhinoclemmys pulcherrima* subsp., *R. p. rogerbarbouri*) and *Cissophyllus* sp. (hosts: *G. barbouri, Sacalia bealei*) were obtained [5]. Adding to the records, zoogeographical discussion of the genus *Serpinema* was given [25,26]

Second, we investigated snakes and lizards sold from three pet shops in the area mentioned above. Several parasites were

Figure 7 Parasitism with nematodes (Kalicephalus sinensis) from the oral cavity (left and middle) to intestines (right) of a captive albino Japanese rat snake (Elaphe climacophora) [20].

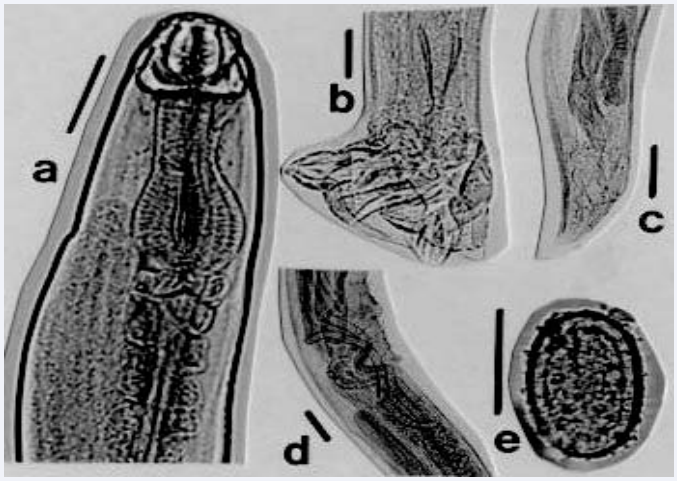

Figure 8 *Kalicephalus sinensis* obtained from captive albino Japanese rat snakes (*Elaphe climacophora*) [20]. -a: male anterior extremity (bar=200 µm), -b: male posterior extremity (bar=100 µm), -c: female posterior extremity (bar=200 µm), -d: vulva of a female (bar=200 µm), -e: egg (bar=50 µm).

Figure 9 Adult pentastomid worms (left, scale=10 mm) and encysted nymphs (right) of the genus *Armillifer* [2].

obtained from pet snakes of the families Colubridae and Boidae, including the nematode parasites *Kalicephalus* spp. (hosts: *Lampropeltis getula californiae*, *L. triangulum* subsp., and *Python regius*) and Heterakidae gen. sp. (*Python regius*), and encysted larvae of acanthocephalan parasites (hosts: *Python molurus bivittatus* and *Chondropython viridis*) [27]. Alternatively, from 28 species of pet lizards that belong to six families and 23 genera, we obtained the nematode parasites *Meteterakis* sp., *Gireterakis* sp., *Strongyluris* sp., *Alaeuris* sp., Pharyngodonidae gen. sp., *Strongyloides* sp., and *Physaloptera* sp., and the trematode parasite *Zeylanurotrema* sp. [28]. Adding to the helminthes sensu stricto mentioned, we could find a pentastomid parasite from the survey.

Pentastomids are crustaceans (phylum Arthropoda), but their general shape resembles helminths (Figure 9, left). Consequently, they are discussed here. Pentastomids are responsible for pentastomidiasis in humans caused by the nymphs inside an entire of the body (Figure 9, right). Therefore, we published a checklist of the pentastomid species in Japan with special reference to related case reports, including those of captive reptiles and mammals, free-ranging sea birds, and wild simian species for using experiments or pet's monkey imported from foreign countries etc from the WAMC [29]. In addition to the review, we recently two pentastomid species of the genus *Raillietiella* from *Gekko gecko* and *Trachydosaurus rugosus* individuals [30], but they are not generally considered zoonotic agents.

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