Case report

Tongue worm (Subclass: Pentastomida) infection and treatment in two domesticated reptiles – a case report

Youki Takaki[a,b]*, Takao Irie[b,c]*, Yoshinori Takami[a], Mistuhiko Asakawa[d]** and Ayako Yoshida[b,c]**

[a]Verts Animal Hospital, 4-3-1 Morooka, Hakata-Ku Fukuoka-shi, 812-0894, Fukuoka, Japan

[b]Laboratory of Veterinary Parasitic Diseases, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen-kibanadai-nishi, 889-2192 Miyazaki, Japan

[c]Center for Animal Disease Control, University of Miyazaki, 1-1 Gakuen-kibanadai-nishi, 889-2192 Miyazaki, Japan

[d]Department of Parasitology, Faculty of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu-shi, 069-8501, Hokkaido, Japan

*equal contribution authors

** co-corresponding authors

Co-corresponding authors:

Mistuhiko Asakawa

Address: Department of Parasitology, Faculty of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu-shi, 069-8501, Hokkaido, Japan
Tel +81 11-388-4758, Fax +81 11-387-5890
e-mail: askam@rakuno.ac.jp
Ayako Yoshida
Address: Center for Animal Disease Control, University of Miyazaki, 1-1 Gakuen-kibanadai-nishi, 889-2192 Miyazaki, Japan
Tel & Fax: +81 985-58-7276
e-mail: kukuri@med.miyazaki-u.ac.jp

Abstract

Tongue worms (Subclass: Pentastomida) are endoparasites found in carnivorous reptiles, fish, amphibians, birds, and mammals. Several pentastomids cause pentastomiasis, a zoonotic disease. We encountered tongue worm infection in two reptiles imported into Japan: a Wahlberg's velvet gecko (*Homopholis wahlbergii*) bred in Germany and a wild-caught green tree python (*Morelia viridis*) from the Republic of Indonesia. The infecting tongue worms were identified as *Raillietiella* sp. and *Armillifer* sp. in the gecko and python, respectively, based on morphological and molecular analyses. Gecko is a newly recorded definitive host harboring *Raillietiella* tongue worm. Live tongue worms were first detected in domestic reptiles in Japan, and this indicates a risk that these tongue worms could be spread to other animals, as well as humans. Therefore, the diagnosis and treatment of infected pets are of importance. In the case of the two infected reptiles, the availability of fecal egg examination for diagnosis was shown. In addition, the effectiveness of treatment with 200 µg/kg/day ivermectin orally once weekly was demonstrated, without no side-effects and no re-detection of eggs after the cessation of treatment.

Keywords: *Armillifer*; ivermectin; Pentastomida; *Raillietiella*; reptile

1. Introduction

Tongue worms (Subphylum: Crustacea, Subclass: Pentastomida) are endoparasites found in fishes, amphibians, birds, mammals, and carnivorous/insectivorous reptiles [1, 2]. Several species of pentastomids (*Raillietiella hemidactyli*, *R. orientalis*, *Linguatula serrata*, *Armillifer armillatus*, *A. moniliformis*, *A. grandis*, *A. agkistrodentis*, *Porocephalus crotali*, *P. taiwana*, and *Leiperia cincinnalis*) can cause human pentastomiasis, a zoonotic disease [3-6]. Adult pentastomids yield their eggs from the nasal cavity and pharynx of the definitive host, then the eggs are ingested by the intermediate hosts [7].

In Japan, pentastomids have been recorded in several animals at the time of necropsy [7,9-12,14-17]. Larval pentastomids were detected in mammals as intermediate hosts, including cynomolgus (*Macaca fascicularis*), brown bear (*Ursus arctos*), and potto (*Perodicticus potto*) [7,9,11]. Adult pentastomids have been isolated from several species of reptiles and birds, including a hub (*Protobothrops flavoviridis*), Ryukyu keelback (*Amphiesma pryeri*), Ryukyu odd-tooth (*Dinodon semicarinatum*), tokay gecko (*Gekko gecko*), pinecone lizard (*Tiliqua rugosa*), giant bent-toed gecko (*Cyrtodactylus irianjayaensis*), reticulated python (*Python reticulatus*), black-legged kittiwake (*Rissa tridactyla*), slaty-backed gull (*Larus schistisagus*), and crested auklet (*Aethia cristatella*) [7,10-12,14-17].

In this case report, we provided medical treatment for reptilian pentastomiasis verified by the presence of pentastomid eggs in the feces of two pets: a Wahlberg's velvet gecko (*Homopholis wahlbergii*) and a green tree python (*Morelia viridis*).

2. Case description

2.1. Case 1: Wahlberg's velvet gecko

An adult female Wahlberg's velvet gecko was admitted to the animal hospital in Fukuoka, Japan, on June 16, 2018. The gecko was bred in Germany, imported to Japan, and purchased from a pet shop in Fukuoka, Japan. The import and purchase dates are unknown. After being purchased, the gecko was fed crickets, a common food for geckos.

The gecko was taken to the animal hospital for a medical checkup. During the checkup, pentastomid eggs were found in the gecko feces by the saturated salt floatation method using 1 g of feces (Figure 1a) [17]. The detected eggs had a mean size of 127 μm × 95 μm (n=20), and included larva with four biforked hooks, a pyriform mouth, and a biforked tail (Figure 1b). The eggs were molecularly analyzed by 18S rRNA gene sequencing [13], and the obtained sequence (1830 bp) was registered in the GenBank/EMBL/DDBJ databases (accession no. LC695013). The sequence was 99.77% identical to sequences from *Raillietiella* sp. (accession nos. EU370434 and AY744887; 1741 bp/1745 bp with no gaps), and 98.74% identical to sequences from *Reighardia stermae* (AY304521) and *Hispania vulturis* (AY304520) (1722 bp/1745 bp including five gaps).

The gecko was administered 200 µg/kg oral ivermectin once weekly and the number of eggs per gram of feces (EPG) was monitored by the saturated salt flotation technique from day 1 post-administration until eggs were no longer detected (Figure 2). The highest EPG was observed on the day of administration (EPG=400), then the number decreased dramatically, and detection ceased on day 53. Ivermectin administration was stopped on that day. On day 84, the EPG was reevaluated, and no eggs were detected.

2.2. Case 2: Green tree python

An adult male green tree python was admitted to an animal hospital in Fukuoka, Japan, on April 6, 2020. The python was caught in the wild in the Republic of Indonesia, imported to Japan, and purchased from a pet shop in Osaka, Japan. The import and purchase dates are unknown. After being purchased, the snake was fed frozen mice, which were commercially sold.

The python was taken to the animal hospital for a medical checkup. During the checkup, pentastomid eggs were detected in the feces by the saturated salt floatation method using 1 g of feces (Figure 1b).

The python was orally administered 200 µg/kg ivermectin, once weekly. On day 22 post-administration, an adult pentastomid was excreted in the feces of the python, with a length of 4.70 cm (Figure 1c). The pentastomid had 36 clear annuli, a straight-line disposed mouth, and two pairs of hooks (Figure 1d). The 18S rRNA gene sequence of the pentastomid was analyzed [13], and the obtained sequence (1835 bp) was registered in the relevant databases (accession no. LC695012). The sequence had 98.47%–98.69% identity to sequences of *Armillifer* spp. (FJ607339, HM048870, and KX686569), but even higher identity, 98.16%–98.56% to sequences of the genus *Porocephalus* (KC904946 and MT387144).

During the anthelmintics administration period, the EPG was monitored by the saturated salt flotation technique from day 1 post-administration until fecal eggs were no longer detected (Figure 2). The highest EPG was observed on the day of administration (EPG=270), followed by a gradual decrease, and excretion of eggs ceased on day 43. Ivermectin treatment was stopped on that day. The absence of eggs in the feces was reconfirmed on day 57.

3. Discussion

Cases of pentastomid infection in living domesticated reptiles were first recorded in Japan. The detection of live pentastomid eggs indicates a risk of tongue worm infections to other intermediate animals. Several species of pentastomids are zoonotic [3-6], and therefore species identification and effective treatment of the pentastomid definitive host are important.

The morphology of the pentastomid larva in the eggs detected in the Wahlberg's velvet gecko was characteristic of the genus *Raillietiella*, and molecular analysis of the 18S rRNA gene supported this classification. Therefore, the pentastomid detected in the gecko was identified as *Raillietiella* sp., although the species was not determined. This is the first report of Wahlberg's velvet geckos serving as a definitive host of *Raillietiella* sp. Within this genera, *R. hemidactyli* and *R. orientalis* have been reported as zoonotic pentastomids, therefore further evaluation to identify the species of detected worm in this study is needed. For molecular comparison with those zoonotic species, specimens already successfully identified to the species level should be obtained because the currently available sequences cannot differentiate between *Raillietiella* species.

The pentastomid derived from the green tree python was morphologically similar to the genus *Armillifer* [1, 19], but molecularly almost identical to two genera, namely *Armillifer* and *Porocephalus*. Armilliferinae and Porocephalinae are subfamilies within the Porocephalidae family, and therefore similarity between their 18S rRNA gene sequences would be expected. However, morphologically, these two genera can be clearly differentiated, with adult *Armillifer* having clear abdominal annuli (circular and parietal muscles arranged in thick bands) unlike *Porocephalus* [6]. The 36 annuli observed in the present case were consistent with previous descriptions of *A. arborealis* [21]. Epidemiologically, although adult pentastomids of the Armilliferinae and Porocephalinae subfamilies have been detected in several snake species [1, 20], the genus *Armillifer* has specifically been reported in a green tree python in a previous publication [21]. Taking all of this into consideration, the pentastomid isolated from the python in the present study was identified as *Armillifer* sp.

In this study, two reptiles served as definitive hosts of *Raillietiella* and *Armillifer*, and their eggs were excreted into the feces. Adult *Raillietiella* was previously recorded in zoo reptiles in Japan [10], but adult *Armillifer* have not been detected. Many vertebrates have been recorded as intermediate hosts for larval pentastomids, which infect via oral ingestion of eggs. Humans have also been reported as accidental intermediate hosts for some pentastomids, including several species of *Raillietiella* and *Armillifer* [3-6]. Although human pentastomiasis is usually asymptomatic, in severe cases, patients experience severe fever, ascites, peritonitis, and death [3, 4, 7]. Although the species of pentastomids could not be identified in the present cases, veterinarians should garner public attention to the zoonotic potential of pentastome infection via pet reptiles. In addition, the number of imported reptiles into Japan has increased in recent years, and reached 192,357 in 2016 [24]. In an autopsy case of such an imported reptile, a zoonotic pentastomid, *Armillifer moniliformis*, was reported [17]. Therefore, quarantine of imported reptiles for the detection of infecting parasites, involving examination and/or preventive anthelmintics administration, should be considered.

Regarding the treatment of reptile pentastomiasis, several dosages of ivermectin have been tested in adult pentastomid infection of a reptile. Subcutaneous administration of ivermectin at

200 µg/kg every 10 days was effective in a Bosc's monitor [22], and a single oral treatment of 1000 µg/kg was indefinite in tokay geckos [23]. In the present cases, repeated oral administration of ivermectin at 200 µg/kg successfully resolved the infection, without no side-effects and no re-detection of eggs after the cessation of treatment. To evaluate the effect of residual dead worms in the host’s body, patients should receive ongoing follow-up observation, and such monitoring may contribute to our understanding of reptile pentastomiasis.

Declaration of interest

The authors declare no conflict of interest.

References

[1] G.C.B. Poore, The nomenclature of the recent Pentastomida (Crustacea), with a list of species available names, Syst. Parasitol. 82 (2012) 211-240.

[2] K. Uchida, Systematic Zoology, Vol.6 Articulata, Annelida, Onychophora, Tardigrada and Pentastomida, Nakayama Shoten Co., Ltd. Tokyo (1967) 334-347.

[3] T.A. Morsy, I.M. El-Sharkawy, A.H. Lashin, Human nasopharyngeal linguatuliasis (Pentastomida) caused by *Linguatula serrata*, J. Egyp. Soc. Parasitol. 29 (1999) 1295-1298.

[4] M.H. Yao, F. Wu, F.F. Tang, Human pentastomiasisin China: case report and literature review, J. Parasitol. 94 (2008) 1295-1298.

[5] L.L. Zhang, J.X. Chen, Progress of research on clinical manifestations and diagnosis of human pentastomiasis, ZhongguoXue-Xi-Chong-Bing-Fang-Zhi-Za-Zhi 24 (2012) 222- 227.

[6] M.L. Christoffersen, J.E. De Assis, A systematic monograph of the Recent Pentastomida, with a compilation of their hosts, Zool. Med. 87 (2013) 1-206.

[7] Y. Takaki, M. Asakawa, An overview of pentastomids (Pentastomida: Crustacea) and pentastomiasis with special reference to the related case reports from Wild Animal Medical Center (WAMC), Rakuno Gakuen University. J. Rakuno. Gakuen. Univ. 40 (2015) 11-16.

[8] B. Latif, E. Omar, N. Othman, C. C. Heo, D. Tappe, Human pentastomiasis caused by *Armillifer moniliformis* in Malaysian Borneo, Am. J. Trop. Med. Hyg. 85 (2011) 878–881.

[9] M. Asakawa, Parasitic helminths found from imported crab-eating macaques (*Macaca fascicularis*) in a quarantine station of an experimental animal supplying company and its epidemiological point of view in Japan, J. Vet. Epidemiol. 13 (2010) 29-30.

[10] Y. Takaki, M. Asakawa, Genus *Raillietiella* (Pentastomida) obtained from captive reptiles at a northern Japan zoo, Med. Entomol. Zool. 67 (2016) 35-36.

[11] Y. Yokoyama, T. Inaba, M. Asakawa, Preliminary report on prevalence of the parasitic helminths obtained from pet primates transported into Japan, Jpn. J. Zoo. Wildl. Med. 8 (2003) 83-93.

[12] H. L. Keegan, S. Toshioka, T. Matsui, H. Suzuki, On a collection of pentastomids from East and Southeast Asia, Med. Ent. Zool. 20 (1969) 147–157.

[13] Chen SH, Liu Q, Zhang YN, Chen JX, Li H, Chen Y, Steinmann P, Zhou XN, Multi-host model-based identification of Armillifer agkistrodontis (Pentastomida), a new zoonotic parasite from China. PLoS. Negl. Trop. Dis. 4(4) (2010) 647.

[14] K. Matsuo, G. Sumiya, Y. Oku, M. Kamiya, Parasitic helminths from amphibians and reptiles in Osaka Municipal Tennoji Zoo. Jpn. J. Zoo Wildl. Med. 6 (2011) 35–44.

[15] S. Nakamura, T. Morita, M. Asakawa, New host records of arthropod parasites from sea birds in Hokkaido, Japan, Jpn. J. Zoo. Wildl. Med. 8 (2003) 131-133.

[16] K. Ooi, M. Oobayashi, Tongue worm (Pentastomida) infection in Slaty-backed gull (*Larus schistisagus*), Med. Entomol. Zool. 34 (1983) 139.

[17] J. Yamashita, M. Nakamata, Tongue worm (Pentastomida) infection in the lung of Reticulated python (*Python reticulatus*). Mem. Fac. Agr. Hokkaido. Univ. 1 (1953) 309-311.

[18] M.W. Dryden, P.A. Payne, R. Ridley, V. Smith, Comparison of common fecal flotation techniques for recovery of parasite eggs and oocysts, Vet. Ther. 6 (2005) 15-28.

[19] D. Tappe, M. Meyer, A. Oesterlein, A. Jaye, M. Frosch, C. Schoen, N. Pantchev, Transmission of *Armillifer aemilliatus* ova at snake farm, the Gambia, West Africa, Emer. Inf. Dis. 17 (2011) 251-254.

[20] George H. Penn Jr. The life history of *Porocephalus crotali*, a parasite of the Louisiana muskrat. J. Parasitol. 28(1942)277–283.

[21] Riley, J., Self, J. T. Some observations on the taxonomy and systematics of the pentastomid genus *Armillifer* (Sambon, 1922) in South East Asian and Australian snakes. Systematic Parasitol. 2 (1981)171–179.

[22] J. A. Pare, An overview of pentastomiasis in reptiles and other vertibrates, J. Exo. Pet. Med. 17 (2008) 285-294.

[23] J. Micinilio, Ivermectin for the Treatment of Pentastomids in a Tokay Gecko, *Gekko gecko*, J. Herpetol. Med. Surg. 6 (1996) 5-6.

[24] K. Wakao, J. Janssen, S. Chng, Reptile pet market in Japan, Annu. Rep. Pro Natura. Fund. 27 (2018)1-12.

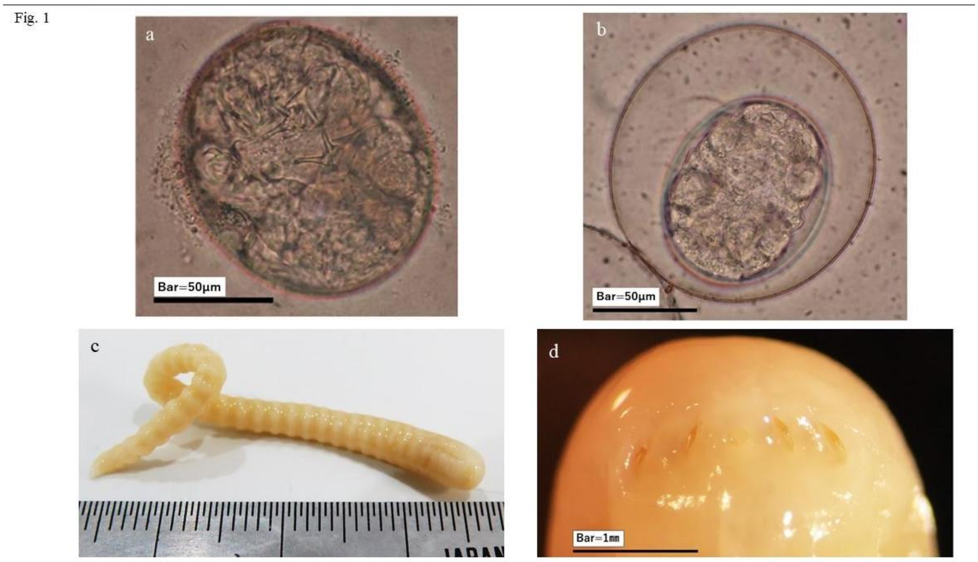

Figure 1 Morphology of pentastomids recovered from two reptiles

(a) Egg of a tongue worm (*Raillietiella* sp.) infecting a Wahlberg's velvet gecko (*Homopholis wahlbergii*); (b) Egg of a tongue worm (*Armillifer* sp.) infecting a green tree python (*Morelia viridis*); (c) Adult tongue worm (*Armillifer* sp.) from a green tree python (*Morelia viridis*); (d) Straight-line disposed oral cadre and two pairs of hooks in the adult tongue worm (*Armillifer* sp.).

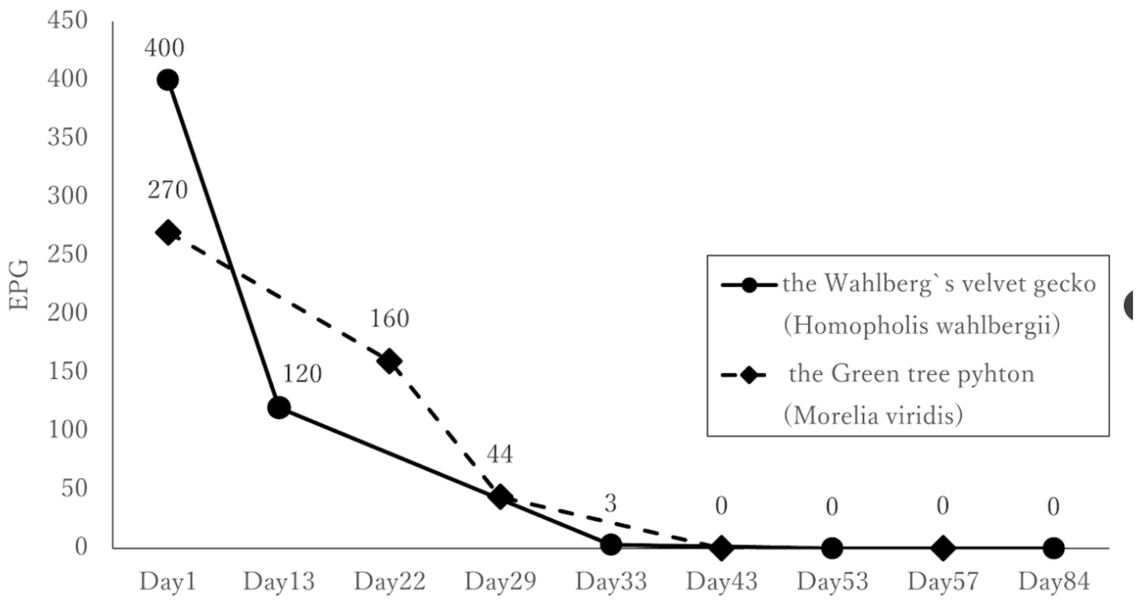

Figure 2 The number of pentastomid eggs detected in the feces during ivermectin treatment of a Walhberg's gecko (*Homopholis wahlbergii*) and green tree python (*Morelia viridis*)