The Journal of Veterinary Medical Science

NOTE

Parasitology

Sporadic endemicity of zoonotic *Paragonimus* in raccoon dogs and Japanese badgers from Miyazaki Prefecture, Japan

Mari ISHIDA[1)#], Chiho KANEKO[2)#], Takao IRIE[1,2)*], Yoshino MARUYAMA[1)], Asami TOKUDA[1)] and Ayako YOSHIDA[1,2)]

[1)]Laboratory of Veterinary Parasitic Diseases, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
[2)]Center for Animal Disease Control, University of Miyazaki, Miyazaki, Japan

J. Vet. Med. Sci.
84(3): 454–456, 2022
doi: 10.1292/jvms.21-0573

Received: 26 October 2021
Accepted: 14 January 2022
Advanced Epub:
3 February 2022

ABSTRACT. Paragonimiasis is a zoonotic trematode infection caused by *Paragonimus* spp. To determine the recent status of *Paragonimus* infections in wild animals, this study investigated *Paragonimus* spp. in 39 raccoon dogs and 54 Japanese badgers from March 2019 to January 2021 in Miyazaki Prefecture, and examined metacercariae in freshwater crabs. Triploid *P. westermani* was found in one raccoon dog (2.6%), and metacercariae were recovered from *Eriocheir japonica* captured near the infected animal collected. One Japanese badger (1.9%) harbored *P. skrjabini miyazakii*; this prevalence was lower than the approximately 30% that was reported in the 1970s. Results indicated that zoonotic *Paragonimus* was sporadically prevalent in wild animals. Further investigation in various animals is awaited to elucidate current wildlife reservoirs for those *Paragonimus*.

KEY WORDS: *Eriocheir japonica*, Japanese badger, *Paragonimus skrjabini miyazakii*, *Paragonimus westermani*, raccoon dog

Paragonimiasis is a parasitic infection caused by lung flukes of the genus *Paragonimus*. Approximately 50 annual cases of human paragonimiasis, caused by *P. westermani* and *P. skrjabini miyazakii*, have been reported in Japan [16, 23]. Several species of carnivorous/omnivorous animals have been recorded as being definitive hosts in nature. Raccoon dogs are capable of harboring both triploid and diploid *P. westermani*; however, epidemiological studies that evaluated *P. westermani* endemicity have been limited [18, 20].

Martens, weasels, and Japanese badgers are known to be suitable hosts for *P. skrjabini miyazakii*, and high prevalence of infection in those animals were reported in decades ago [2, 3, 5–7, 11, 21]. Moreover, although human paragonimiasis cases have been sporadically recognized, the current endemicity of zoonotic *Paragonimus* spp. in wild animals is poorly understood. In this study, prevalence of these parasites in two species of wild omnivores, raccoon dogs and Japanese badgers, were evaluated in Miyazaki Prefecture, which is a major endemic area of human paragonimiasis in Japan.

Carcasses of 39 raccoon dogs and 54 Japanese badgers that were found as roadkill or provided by the local government from vermin control in Miyazaki City and surrounding municipalities were collected from March 2019 to January 2021. A necropsy was conducted after determining body weight and length and checking the sex. Whole lungs were collected and macroscopically searched for *Paragonimus* cysts. Then, flukes were recovered from the detected cysts and stored at −30°C until molecular examination.

Genomic DNA was extracted from flukes collected from individual hosts using a QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany) in accordance with the manufacturer's instructions. Species were molecularly identified by PCR sequencing of the second internal transcribed spacer region of nuclear ribosomal DNA and the cytochrome *c* oxidase subunit 1 gene [8]. To determine the chromosomal ploidy (diploid or triploid) of *P. westermani*, the 16S mitochondrial rRNA gene was also analyzed [1].

Lung cysts were found in one raccoon dog (2.6%; 1/39) and one Japanese badger (1.9%; 1/54). Only one fluke was isolated from a single cyst in the raccoon dog, and the fluke was used for molecular identification. In the Japanese badger, 58 flukes were isolated from many cysts, the number of which could not be counted because of adhesion, and two flukes were arbitrarily selected as representatives for species identification. The species of the lung fluke in the raccoon dog was molecularly identified as triploid

*Correspondence to: Irie, T.: irie.takao.r0@cc.miyazaki-u.ac.jp, Laboratory of Veterinary Parasitic Diseases, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen-kibanadai Nishi, Miyazaki 889-2192, Japan
#These authors contributed equally to this work.

Table 1. Prevalence of *Paragonimus* spp. in wild animals in Miyazaki Prefecture

		Ashizawa *et al.*† [7] Sampling year: 1974–1975	Ashizawa *et al.* [5] Sampling year: 1974–1976	Ashizawa *et al.*† [6] Sampling year: 1975–1976	Ashizawa *et al.*‡ [3] Sampling year: 1976–1978	Ashizawa *et al.* [2] Sampling year: 1977–1978	This study Sampling year: 2019–2021
Raccoon dogs	*Paragonimus westermani* positive	NA	NA	NA	NA	NA	1
	P. skrjabini miyazakii positive	NA	NA	NA	NA	NA	0
	Paragonimus spp. negative	NA	NA	NA	NA	NA	38
	Prevalence of *Paragonimus* spp. (%)	NA	NA	NA	NA	NA	2.6
Japanese badgers	*P. westermani* positive	NA	0	NA	NA	0	0
	P. skrjabini miyazakii positive	NA	3	NA	NA	2	1
	Paragonimus spp. negative	NA	7	NA	NA	4	53
	Prevalence of *Paragonimus* spp. (%)	NA	30.0	NA	NA	33.3	1.9
Weasels and martens	*Paragonimus* spp. positive	4	NA	10	10*	NA	NA
	Paragonimus spp. negative	22	NA	28	26	NA	NA
	Prevalence of *Paragonimus* spp. (%)	15.4	NA	26.3	27.8	NA	NA

* Cysts or lesions positive for *Paragonimus* spp., † Martens (*Martes melampus melampus*), ‡ Weasels (*Mustela itatsi*/*M. sibirica coreana*), NA stands for "data is not available".

P. westermani and that of the Japanese badger was molecularly identified as *P. s. miyazakii* (Table 1).

Freshwater crabs, *Eriocheir japonica*, were captured in a river near the location where the infected raccoon dog was found (approximately 270 m along the street). A total of 15 crabs were minced and artificially digested, and metacercariae were examined under a stereomicroscope. In total, 63 metacercariae were collected from the crabs and were molecularly identified as triploid *P. westermani*. With regard to the infection source of the positive Japanese badger, a survey targeting freshwater crab (*Geothelphusa dehaani*) was not performed because no part of the river was considered a suspected source of infection around the point where the positive carcass was found.

This study provides a description of the first case of a raccoon dog infected with triploid *P. westermani* in Miyazaki Prefecture. The prevalence was 2.6% in the surveyed raccoon dogs. This was lower than the value reported in previous studies although the surveying conditions were different; the triploid type was detected in 11% (1/9) of raccoon dogs from Yakushima Island in the 2000s [18], and the diploid type was detected in 8.6% (12/140) of raccoon dogs from Hyogo Prefecture in the 1980s [21].

Another zoonotic *Paragonimus* species, *P. s. miyazakii,* was found in 1.9% of Japanese badgers; in comparison, a 30–33% prevalence was recorded in the 1970s in Miyazaki Prefecture [2, 5]. *Moria nipponica* (Mori, 1937) (critically endangered, rare) is a first intermediate host for *P. s. miyazakii* and *Geothelphusa dehaani* (near threatened) is a second intermediate host for *P. s. miyazakii* and *P. westermani*; both of these species are now in the Red Data list (2015 edition) in Miyazaki Prefecture [15]. The current low endemicity of those two *Paragonimus* in wild animals may have been affected by decreasing population sizes and distribution of freshwater snails/crabs that serve as the first/second intermediate hosts in recent decades.

In addition to detecting triploid *P. westermani* in a raccoon dog, metacercariae of the fluke were also detected in freshwater crabs inhabiting a location close to where the infected raccoon dog was collected. Raccoon dogs generally do not have a clearly defined territory, but they have a home range of 8–111 ha depending on surroundings, season, sex, and age class [13, 17]. Therefore, we suppose that the raccoon dog may have used the location where freshwater crabs were investigated as watering places or feeding grounds. Moreover, the raccoon dog is known to sometimes consume crustaceans [9, 12]; therefore, the *P. westermani* lifecycle is suspected to be sporadically maintained via raccoon dogs and freshwater crabs in such riverside environments. Because the other raccoon dogs and Japanese badgers that were collected within a 1-km radius of the points where the positive carcasses were found were negative for *Paragonimus* spp. (data not shown), fluke-positivity could be influenced by individual preference for crustaceans or availability of other preferable food. In addition to the consumption of crustaceans, potential transmission via paratenic hosts of triploid *P. westermani*, e.g., wild boar and deer [21, 24], should be considered because approximately 50% of the carcasses of these animals are known to be visited and scavenged by raccoon dogs [19, 22].

Other carnivorous/omnivorous animals have been reported as definitive hosts of *Paragonimus* spp. in wildlife in Japan. In the early 1950s, *P. westermani* infection in feral dogs was reported at a prevalence of 7.2–21.1% [10, 14]. A Japanese red fox infected with diploid *P. westermani* was also reported [4]. Weasels and martens were also found to be infected with *P. s. miyazakii* with a prevalence of 15–28% in the 1970s [3, 6, 7]. In this study, we could not conclude the main definitive hosts for these *Paragonimus* species in nature. Further investigations into these wild animals could help clarify the current endemicity of these *Paragonimus* species.

In conclusion, our findings clearly show that zoonotic *Paragonimus* is distributed, albeit in low abundance, in raccoon dogs and Japanese badgers from Miyazaki Prefecture, and its lifecycle is sporadically maintained along the water where host animals inhabit and visit. To elucidate potential reservoirs for zoonotic *Paragonimus* in wildlife, further investigations should be conducted that target various animals in different areas.

POTENTIAL CONFLICTS OF INTEREST. The authors have no relationships or support that might be perceived as constituting a conflict of interest.

ACKNOWLEDGMENTS. This work was supported in part by Health and Labor Sciences Research Grants (21KA1003) and JSPS KAKENHI (JP19K15984).

REFERENCES

1. Agatsuma, T., Iwagami, M., Sato, Y., Iwashita, J., Hong, S. J., Kang, S. Y., Ho, L. Y., Su, K. E., Kawashima, K. and Abe, T. 2003. The origin of the triploid in *Paragonimus westermani* on the basis of variable regions in the mitochondrial DNA. *J. Helminthol.* **77**: 279–285. [Medline] [CrossRef]
2. Ashizawa, H., Habe, S., Marukami, T., Nosaka, D., Tateyama, S. and Morimoto, Y. 1980. Cases of Paragonimus infection in the Japanese badger. *Bull. Fac. Agric. Miyazaki Univ* **27**: 55–62 (in Japanese with English Summary).
3. Ashizawa, H., Habe, S., Marukami, T., Nosaka, D., Tateyama, S. and Morimoto, Y. 1980. Paragonimus infection in weasels produced in Miyazaki Prefecture, Japan. *Bull. Fac. Agric. Miyazaki Univ* **27**: 251–259 (in Japanese with English Summary).
4. Ashizawa, H., Kugi, G., Nosaka, D. and Tateyama, S. 1980. A case of paragonimiasis in Japanese fox, with special reference to the pathological features of the lungs. *Bull. Fac. Agric. Miyazaki Univ* **27**: 47–53 (in Japanese with English Summary).
5. Ashizawa, H., Marukami, T., Habe, S., Nosaka, D. and Tateyama, S. 1977. Pathological findings on the lungs of Japanese badger infected with *Paragonimus miyazakii*. *Bull. Fac. Agric. Miyazaki Univ* **24**: 255–264 (in Japanese with English Summary).
6. Ashizawa, H., Marukami, T., Kiriki, Y., Nosaka, D., Tateyama, S. and Habe, S. 1976. Spontaneous Paragonimus infection in Japanese martens inhabiting Miyazaki prefecture. *Bull. Fac. Agric. Miyazaki Univ* **23**: 395–401 (in Japanese with English Summary).
7. Ashizawa, H., Marukami, T., Nosaka, D. and Habe, S. 1975. *Paragonimus miyazakii* infection in Japanese martens living in Miyazaki prefecture. *Bull. Fac. Agric. Miyazaki Univ* **22**: 203–209 (in Japanese with English Summary).
8. Doanh, P. N., Shinohara, A., Horii, Y., Habe, S., Nawa, Y., The, D. T. and Le, N. T. 2007. Morphological and molecular identification of two *Paragonimus* spp., of which metacercariae concurrently found in a land crab, *Potamiscus tannanti*, collected in Yenbai Province, Vietnam. *Parasitol. Res.* **100**: 1075–1082. [Medline] [CrossRef]
9. Ikeda, H., Eguchi, K. and Ono, Y. 1979. Home range utilization of a raccoon dog, *Nyctereutes procyonoide*s *viverrinus*, in a small islet in western Kyushu. *Jap. J. Ecol.* **29**: 35–48.
10. Isobe, H. and Kurata, Y. 1954. Studies on *Paragonimus westermanii* in Ehime Pref. Part 1. Natural infection of dogs in Minamiuwa district. *Jpn. J. Parasitol.* **3**: 116–117 (in Japanese).
11. Katamine, D. and Motomura, K. 1962. The spontaneous Infection with *Paragonimus miyazakii* of the weasels from Nagasaki Prefecture. *Endemic dis*. *Bull. Nagasaki Univ* **4**: 120–124 (in Japanese with English Summary).
12. Matsuo, R. and Ochiai, K. 2009. Dietary overlap among two introduced and one native sympatric carnivore species, the raccoon, the masked palm civet, and the raccoon dog, in Chiba Prefecture, Japan. *Mammal Study* **34**: 187–194. [CrossRef]
13. Mitsuhashi, I., Sako, T., Teduka, M., Koizumi, R., Saito, M. U. and Kaneko, Y. 2018. Home range of raccoon dogs in an urban green area of Tokyo, Japan. 2018. *J. Mammal.* **99**: 732–740. [CrossRef]
14. Miura, Y. 1952. Studies on paragonimiasis. 1. Distribution of lung fluke in dogs in Kochi Prefecture. *J. Osaka Med. Coll* **12**: 144–151 (in Japanese).
15. Miyazaki Prefecture. 2015. About a Miyazaki Prefecture edition red list and a red data book. https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fwww.pref.miyazaki.lg.jp%2Fdocuments%2F9795%2F9795_20160424151758-1.xlsx&wdOrigin=BROWSELINK [accessed on July 28, 2021].
16. Nagayasu, E., Yoshida, A., Hombu, A., Horii, Y. and Maruyama, H. 2015. Paragonimiasis in Japan: a twelve-year retrospective case review (2001–2012). *Intern. Med.* **54**: 179–186. [Medline] [CrossRef]
17. Saeki, M., Johnson, P. J. and Macdonald, D. W. 2007. Movements and habitat selection of raccoon dogs (*Nyctereutes procyonoides*) in a mosaic landscape. *J. Mammal.* **88**: 1098–1111. [CrossRef]
18. Sato, H., Suzuki, K., Osanai, A. and Aoki, M. 2006. *Paragonimus westermani* and some rare intestinal trematodes recovered from raccoon dogs (Nyctereutes procyonoides viverrinus) introduced recently on Yakushima Island, Japan. *J. Vet. Med. Sci.* **68**: 681–687. [Medline] [CrossRef]
19. Selva, N., Jędrzejewska, B., Jędrzejewski, W. and Wajrak, A. 2005. Factors affecting carcass use by a guild of scavengers in European temperate woodland. *Can. J. Zool.* **83**: 1590–1601. [CrossRef]
20. Shibahara, T. 1985. Experimental infection of raccoon dogs with two types (Diploid and Triploid) of the lung fluke, *Paragonimus westermani*. *Jpn. J. Parasitol.* **34**: 165–171.
21. Shibahara, T. and Nishida, H. 1985. An epidemiological survey of the lung fluke, *Paragonimus* spp. in wild mammals of the northern part of Hyogo Prefecture, Japan. *Nippon Juigaku Zasshi* **47**: 911–919. [Medline] [CrossRef]
22. Sidorovich, V. E., Polozov, A. G., Lauzhel, G. O. and Krasko, D. A. 2000. Dietary overlap among generalist carnivores in relation to the impact of the introduced raccoon dog *Nyctereutes procyonoides* on native predators in northern Belarus. *Z. Säugetierkd.* **65**: 271–285.
23. Sugiyama, H. 2010. Food-borne parasitic infection as food poisoning. *Jpn. J. Food Microbiol.* **27**: 1–7 (in Japanese). [CrossRef]
24. Yoshida, A., Doanh, P. N. and Maruyama, H. 2019. *Paragonimus* and paragonimiasis in Asia: An update. *Acta Trop.* **199**: 105074. [Medline] [CrossRef]