NOTE *Parasitology*

Experimental Final Hosts of *Metagonimus Hakubaensis* (Trematoda: Heterophyidae) and Their Suitability to the Fluke

Noboru KUDO[1)]*, Chieko OTA[1)], Fumiko SAKA[1)], Yae IKEDA[1)], Yusuke TOMIHISA[1)], Yasunaga ITOI[1)] and Takashi OYAMADA[1)]

[1)]*Department of Veterinary Parasitology, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan*

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ABSTRACT. Seven laboratory mammal and bird species were orally inoculated with 200–1,000 encysted *Metagonimus hakubaensis* metacercariae that had been isolated from naturally infected lampreys (*Lethenteron reissneri*) captured in Aomori Prefecture. At 8 and 15 days post-infection, adult flukes were recovered from all of the laboratory animals tested, and therefore, hamster, rat, mouse, dog, cat, chicken and quail were considered as final hosts of *M. hakubaensis*. Recovery rates of the fluke were higher in dogs and hamsters than in cats, rats, mice, chickens and quails. The flukes recovered from dogs and hamsters showed increased body length and higher fecundity than those recovered from the other hosts. These results indicate that the suitability of dogs and hamsters for *M. hakubaensis* infection is higher than that of the other laboratory animals.

KEY WORDS: experimental infection, final host, *Metagonimus hakubaensis*, suitability

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Trematodes in the genus *Metagonimus* occur naturally in the small intestines of a variety of mammalian and avian hosts. At present, a total of 6 *Metagonimus* species are recognized in Japan: *M. yokogawai* (Katsurada, 1912), *M. takahashii* Suzuki, 1930, *M. katsuradai* Izumi, 1935, *M. otsurui* Saito *et* Shimizu, 1968, *M. miyatai* Saito *et al.*, 1997 and *M. hakubaensis* Shimazu, 1999. Metacercarial infection by *Metagonimus* spp. has been confirmed in a variety of fresh and barackish water fishes [3].

Metagonimus hakubaensis was originally described in the adult flukes obtained from laboratory rats that had been experimentally fed metacercariae isolated from the sand lamprey, *Lethenteron reissneri* (Dybowski), collected in Nagano Prefecture, Japan [11]. No further information on natural and experimental final hosts of the flukes has been reported to date. The present study was therefore conducted to estimate the suitability of several mammals and birds to experimental infection with *M. hakubaensis*, on the basis of recovery rates, development and fecundity of the flukes. Further, since physiological aspects, such as predilection site of adult *Metagonimus* spp. in final hosts, are considered useful for differentiating among members of the genus *Metagonimus* [1, 10], we compared the results from this study with previous studies on other *Metagonimus* spp.

Metagonimus hakubaensis metacercariae were obtained from naturally infected lampreys captured in irrigation ditches in Temmabayashi, Aomori Prefecture, Japan (Fig. 1). The lampreys were cut into small pieces and digested in artificial gastric juice (pepsin, 1:10,000, 7 g; HCl, 7 m*l* in 1,000 m*l* distilled water) at 37°C for 30 min. After digestion, the metacercariae were collected from the digested fluid under a dissecting microscope and used to infect the following laboratory animals: 4 Syrian golden hamsters (4-week-old males), 6 Wistar rats (4-week-old males), 3 ddY mice (4-week-old males), 4 dogs (puppies), 4 cats (kittens), 12 White Leghorn chickens (12- or 15-day-old) and 6 quails (adults). Each animal was orally inoculated with 200–1,000 encysted metacercariae and sacrificed under ether anesthesia at 8 and 15 days post-infection (DPI). The small intestines (divided into upper, middle and lower sections) and large intestines were opened, the contents were removed, and the mucous membrane was exfoliated with pointed forceps. The obtained samples were then washed by repeated sedimentation with saline solution. The flukes recovered by examining the washed sediments under a dissecting microscope were

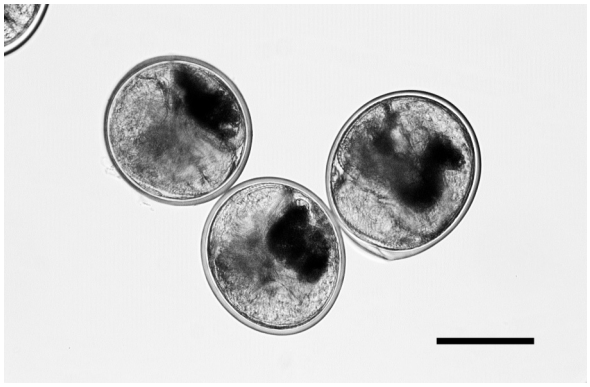

Fig. 1. Encysted metacercariae of *Metagonimus hakubaensis* isolated from *Lethenteron reissneri*. Bar=100 μm.

*CORRESPONDENCE TO: KUDO, N., Department of Veterinary Parasitology, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan. e-mail: kudo@vmas.kitasato-u.ac.jp

Table 1. Susceptibility of several mammalian and avian hosts to experimental infection with *Metagonimus hakubaensis*

Host	Days post-infection	No. of animals used	Total no. of larvae inoculated	No. of flukes recovered from				Total no. of flukes recovered	Fluke recovery rate%
				Small intestine			Large intestine		
				Upper portion	Middle portion	Lower portion			
Hamster	8	2	400	0	30	321	0	351	87.8 (86.5, 89.0)*
	15	2	400	0	52	254	1	307	76.8 (73.5, 80.0)
Rat	8	3	600	0	52	270	0	322	53.7 (26.0–73.5)
	15	3	600	0	0	0	0	0	0
Mouse	15	3	900	0	3	34	0	37	4.1 (1.0–10.0)
Dog	8	2	2,000	0	70	1,705	0	1,775	88.8 (88.7, 88.8)
	15	2	2,000	0	26	1,450	0	1,476	73.8 (67.9, 79.7)
Cat	8	2	2,000	0	6	1,120	0	1,126	56.3 (51.6, 61.0)
	15	2	2,000	0	109	366	3	478	23.9 (15.9, 31.9)
Chicken	8	6	1,800	0	17	471	0	488	27.1 (23.0–35.0)
	15	6	1,800	41	24	124	0	189	10.5 (2.7–28.0)
Quail	8	3	900	0	0	18	1	19	2.1 (0–6.3)
	15	3	900	0	0	2	0	2	0.2 (0–0.7)

*Fluke recovery rate in each animal is enclosed in parenthesis.

placed between a glass slide and a cover slip and fixed in 70% alcohol, stained with alum carmine and then mounted in Canada balsam for examination under a camera lucida. All research was conducted according to the Guidelines for the Care and Use of Laboratory Animals of Kitasato University. The experimental protocols were approved by the Animal Care and Use Committee of Kitasato University.

The results of fluke recovery from the experimentally infected animals at 8 and 15 DPI are summarized in Table 1. Flukes were recovered from all of the mammalian and avian hosts. These findings suggest that a variety of mammals and birds could potentially serve as natural final hosts for *M. hakubaensis*, which has remained unknown to date. However, the recovery rates at 8 DPI were highest in dogs (88.8%) and hamsters (87.8%), followed by cats (56.3%), rats (53.7%), chickens (27.1%) and quails (2.1%). Similarly, the recovery rates at 15 DPI were highest in hamsters (76.8%) and dogs (73.8%) and lower in the other animal hosts tested (0–23.9%). The rates of fluke recovery in dogs and hamsters were significantly higher than those in mice, chickens and quails at 8 and 15 DPI ($P<0.01$, z-test). In the previous studies of experimental infections with *M. yokogawai*, the fluke recovery rates at 7 or 14 DPI were highest in dogs (>80%), followed by hamsters (17.8–38.5%), and then ddY mice (3.4%) [5, 6, 13]. In addition, the fluke recovery rate at 8 DPI in chickens experimentally infected with *M. takahashii* was 16% [7]. Thus, with the exception of the high *M. hakubaensis* recovery rates in hamsters, the findings of the present study are similar to those of the previous reports.

At 8 and 15 DPI, most of the flukes in the experimentally infected animals used in this study were found in the lower small intestine, and the remaining flukes were mainly recovered from the middle portion of the small intestine. Several studies examining the distribution of adult *Metagonimus* spp. in definitive hosts have reported differences in the predilection site among *Metagonimus* spp. [4, 5, 8–10, 12]. For example, *M. miyatai*, *M. katsuradai* and *M. otsurui* exhibited a predilection for the lower small intestine, while *M. yokogawai* and *M. takahashii* exhibited a preference for the upper small intestine. The predilection site of *M. hakubaensis* in this study was thus similar to that of *M. miyatai, M. katsuradai* and *M. otsurui*.

All of the approximately 900 flukes that were randomly collected from the different host species for morphological observation were sexually mature with numerous uterine eggs. These flukes were identified as *M. hakubaensis* based on morphological characteristics that are considered typical for the species: the oral sucker is slightly smaller than, as large as, or slightly larger than the acetabulum, the intestinal ceca and vitellaria do not extend posteriorly beyond the right testis, and the uterus hardly enters the post-testicular region.

The morphometric measurement results for flukes recovered from different hosts at 8 and 15 DPI are summarized in Table 2. The body size of *M. hakubaensis* differed between hosts (Fig. 2); for example, at 8 DPI, the body length of flukes recovered from hamsters and dogs was significantly larger than that of flukes from the other hosts ($P<0.01$, Welch's t-test). Similarly, significantly larger flukes were recovered from hamsters, dogs and cats at 15 DPI, compared with flukes recovered from mice and chickens ($P<0.01$). The smallest flukes at 8 and 15 DPI were found in quails. In experimental infections with *M. yokogawai*, fully developed flukes recovered from several hosts were largest in dogs, followed by hamsters, and then mice and chickens [2, 5]. However, the flukes recovered from mice and chickens were only approximately half the size of flukes recovered from dogs. The recovery of very large flukes from dogs and hamsters in the present study suggests that dogs and hamsters are suitable definitive hosts for *M. hakubaensis*, like in *M. yokogawai*.

The fecundity of flukes varied among the different hosts examined (Table 3). At 8 and 15 DPI, the number of uterine eggs in *M. hakubaensis* recovered from hamsters was similar to that from dogs and markedly higher than that in

Table 2. Measurements of *Metagonimus hakubaensis* recovered from experimentally infected mammals and birds at 8 and 15 days post-infection*

Host	Days post-infection	No. of specimen	Body length	Body width	Oral sucker length × width	Acetabulum length × width
Hamster	8	20	757 ± 26.3†	336 ± 15.9	68 ± 4.3 × 56 ± 4.7	76 ± 3.5 × 46 ± 2.9
	15	20	843 ± 45.5	366 ± 23.2	73 ± 4.3 × 62 ± 3.9	82 ± 4.3 × 50 ± 3.2
Rat	8	20	577 ± 52.7	235 ± 23.3	66 ± 4.0 × 56 ± 4.3	61 ± 8.9 × 40 ± 3.7
Mouse	15	20	624 ± 87.8	272 ± 45.9	65 ± 3.9 × 54 ± 4.5	68 ± 8.1 × 42 ± 3.9
Dog	8	20	770 ± 51.7	302 ± 28.9	67 ± 3.8 × 57 ± 5.4	76 ± 5.1 × 47 ± 2.9
	15	20	834 ± 37.7	357 ± 25.2	70 ± 3.8 × 57 ± 4.9	76 ± 5.0 × 53 ± 5.2
Cat	8	20	652 ± 40.9	258 ± 20.5	67 ± 4.0 × 57 ± 3.8	63 ± 4.8 × 43 ± 2.3
	15	20	806 ± 48.1	317 ± 22.8	70 ± 4.3 × 62 ± 4.5	76 ± 6.2 × 48 ± 2.3
Chicken	8	20	645 ± 39.9	281 ± 19.1	68 ± 1.8 × 58 ± 3.9	68 ± 4.4 × 43 ± 2.3
	15	20	622 ± 40.8	266 ± 22.3	67 ± 3.4 × 57 ± 2.8	66 ± 6.0 × 43 ± 2.9
Quail	8	16	515 ± 37.2	225 ± 17.2	66 ± 3.4 × 55 ± 5.4	59 ± 3.3 × 39 ± 1.8
	15	2	520, 632	220, 240	64 × 52, 68 × 56	60 × 46, 62 × 44

*All measurements are given in μm, †Mean ± standard deviation.

Fig. 2. Adult flukes of *Metagonimus hakubaensis* recovered from experimentally infected (A) dog, (B) hamster, (C) mouse and (D) quail at 15 days post-infection. Bar=100 μm.

Table 3. Fecundity of *Metagonimus hakubaensis* recovered from experimentally infected mammals and birds at 8 and 15 days post-infection

Host	Days post-infection	No. of specimens	No. of flukes with uterine egg counts of				
			0	1–100	101–200	201–300	300<
Hamster	8	20	0	0	0	1	19
	15	20	0	0	0	0	20
Rat	8	20	0	6	13	1	0
Mouse	15	20	0	4	5	7	4
Dog	8	20	0	0	0	2	18
	15	20	0	0	0	2	18
Cat	8	20	0	1	18	1	0
	15	20	0	0	1	14	5
Chicken	8	20	0	1	18	1	0
	15	20	0	14	6	0	0
Quail	8	16	0	15	1	0	0
	15	2	0	2	0	0	0

M. hakubaensis recovered from the other hosts. The lowest fecundity was observed in quails. The present results for fluke fecundity in experimentally infected hamsters and dogs support the suggestion that these animals may be suitable definitive hosts for *M. hakubaensis*. Conversely, quails are considered to be less susceptible to *M. hakubaensis* infection than the other animals tested.

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