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Experimental Infection of Nutrias with the Metacercariae of *Paragonimus miyazakii* Kamo *et al.*, 1961

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The lung fluke, *Paragonimus miyazakii* Kamo, Nishida, Hatsushika et Tomimura, 1961 had long been considered that the adult worms inhabit only in the lung of wild mammals, but this fluke has begun to arouse considerable attention of the parasitologists in Japan, since the patients of paragonimiasis miyazakii were found in Kanto district such as Tokyo, Yokohama City, Kanagawa and Yamanashi Prefectures (Hayashi *et al.*, 1974; Yokogawa *et al.*, 1974). There is no report on the prevalence of *P. miyazakii* outside of Japan.

The wild mammals infected with *P. miyazakii* hitherto reported in literature amounts to seven species such as weasels, martens, dogs, cats, wild boars and badgers (*Meles* and *Nyctereutes*), of which the first one is quite common as the natural definitive host of this fluke. Six species of the laboratory animals, on the other hand, are known as the experimental definitive host for *P. miyazakii*; namely, albino rats, water rats, cats, dogs, rabbits and monkeys. The nutrias inhabiting in Okayama Prefecture have increased in numbers recently, but the nutria-parasite relation is very little known in our knowledge. Recently Nagahana *et al.* (1977) reported that the nutria is a suitable experimental host for *Clonorchis sinensis*. Later the experimental infection of nutrias with *P. miyazakii* was attempted to make clear the host adaptation, the developmental states of worms and the nutria-*Paragonimus* relations by the present authors. In the present paper the results of our experiment was reported.

Materials and Methods

The nutrias, *Myocastor coypus* (Octodontoidea: Capromydae) used for the experimental infection were captured alive in the paddyfield of Okayama Prefecture. The metacercarial cysts used for infecting animals were removed from the fresh water crabs, *Potamon dehaani* collected at Rokuroshi, Iwakuni City in Yamaguchi Prefecture, the type locality of *P. miyazakii*. Six nutrias, weighing 1.5 to 3.6 kg, were orally given to each 15 to 100 metacercarial cysts, and all of the animals were sacrificed for autopsy within a certain period of time except for the accidental death occurring at the experimental period. These animals were fed chiefly on the artificial animal food (MF Chow of Oriental Kobo Co.) through the experimental period. The fecal examinations were made every three days by means of MGL sedimentation until the eggs began to appear in the feces after ingestion of metacercarial cysts. The worms removed from the animals were fixed in 70% alcohol and stained with Delafield's hematoxylin, and examined microscopically for the observation of the developmental states.

Results

As shown in Table 1, all the animals were successfully infected except one, and the recovery rate of the worms in each animal was 10% to 40% with an average of 19.7%. The animal no. 1 orally ingested with 28 metacercarial cysts of *P. miyazakii* did not reveal eggs in the feces till it was sacrificed for autopsy at the end of a 114-day experimental period. The results of the animal dissection were very unfavorable, and no worm or worm cyst was found in the lung of the animal. Typical eggs in the feces were found on the 80th day after ingestion in the animal no. 2, and on the 66th day after ingestion in the animal no. 3, respectively.

In the animal no. 2, four mature worms were found in the lungs (two from a worm cyst in the right upper lobe, and also two from a worm cyst in the left middle lobe) on the 139th day after ingestion. The recovery rate of the worms in the animal no. 2 was 14.1%. The animal no. 3, on the other hand, showed two mature worms and one immature worm in a worm cyst of the right upper lobe of the lung on the 111th day after ingestion, and the recovery rate of worms was 10.7%. The animals nos. 4, 5 and 6 died from some unknown cause were sacrificed for autopsy on the 53th, 72th and 43th days after the ingestion respectively, but no adult worm or worm cyst was found in the lung of these animals. All of the worms removed from three animals were still immature type, and as shown in Table 1, they were found in the pleural cavity (nos. 4 and 5), and in the pleural and the abdominal cavities (no. 6) in free states. The recovery rates in three animals amounted to 40% (no. 4), 10% (no. 5) and 24% (no. 6), respectively.

The morphological features of the mature worms found in the lungs of the animals nos. 2 and 3 were comparable to characteristics of *P. miyazakii* as reported by Kamo *et al.* (1961) and Hatsushika (1967). Seven worms measured 11 to 14 mm (av. 12.2) in length and 4.5 to 7.0 mm (av. 5.8) in width. Thirty eggs in the feces from the animal no. 2 measured 52.9 to 79.3 μm (av. 72.8) in length and 40.8 to 54.3 μm (av. 45.9) in maximum width.

Discussion

The experimental infection with the metacercarial cysts of *P. miyazakii* to nutrias was reported for the first time in this paper, and it became clear that *P. miyazakii* can infect the nutria easily and to attain the same degree of the maturity with the other known laboratory animals of *P. miyazakii*. As described above, no adult worm or worm cyst was recovered from the lung of the animal no. 1. One of the reasonable causes of this seems to be the failure in the ingestion methods of the metacercarial cysts to the animal. The animals nos. 4, 5 and 6, on

Table 1 Results of experimental infection of *Paragonimus miyazakii* to nutrias

Animals		No. of metacercariae given	Days from infection to autopsy	Duration of eggs appeared in feces (days)	No. of worm recovered in			
no.	body weight (kg)				lung	pleural cavity	abdominal cavity	total (%)
1	2.0	28	114	—	0	0	0	0
2	3.2	28	139	80	4	0	0	0(14.1)
3	1.5	20	111	66	3	0	0	3(10.7)
4	3.6	15	53*	—	0	6	0	6(40.0)
5	2.0	50	72*	—	0	5	0	5(10.0)
6	1.4	100	43*	—	0	20	4	24(24.0)

* The animals died from some unknown cause.

the other hand, died without any noticeable external cause, but the real reason of death lies in the fact that the animals placed in the laboratory cages for this studies had avoided the artificial food over a long period of time after ingestion of the metacercarial cysts. Therefore, it is hoped that if an investigator uses this animal for the experimental purporse, an effort should be made to discertain that the animal is cuccessfully put a bait on the artificial food before the use.

Although typical eggs in the feces were first found on the 66th and the 80th days after the ingestions of the metacercarial cysts, the period is very close to that of the other known laboratory animal hosts of *P. miyazakii* such as on the 47th to the 57th day (Tomimura *et al.*, 1961), on the 61th to the 84th day (Hatsushika, 1967) and on the 65th day (Hashiguchi, 1973) in albino rats, on the 47th to the 56th day (Tomimura *et al.*, 1961) and on the 46th to the 76th day (Hatsushika, 1967) in cats, on the 49th to the 50th day (Tomimura *et al.*, 1961) and on the 59th to the 64th day (Hatsushika, 1967) in dogs, and on the 58th to the 68th day (Tomimura *et al.*, 1961) and on the 74th day (Hatsushika, 1967) in rabbits. The recovery rats, 10% to 40%, of the worms in nutrias was comparatively low compared with those of dogs and cats, and it closely resembles those of albino rats i. e., 40% from Tomimura *et al.*, (1961), 33.8% from Hatsushika (1967), 27.4% from Yoshida (1970) and 28.3 % from Hashiguchi (1967).

All of the mature worms recovered from the cysts of the lung in two nutrias (no. 2 and no. 3) showed its full development of maturity, and the size of worm body was comparable well with those from the other known definitive hosts for *P. miyazakii*. As for variations in sizes of adult worms of *P. miyazakii* from various hosts, Hatsushika (1967) reported that the body size may be classified into two groups such as dog - cat - wild boar type and albino rat - rabbit type. Moreover, he also suggested that the body length ranged from 9.2 to 15.3 mm in the former type, and from 4.6 to 8.2 mm in the latter. The worms recovered from the nutrias may be included under the former type judging from the body size and the degree of sexual maturity. Threfore, judging from only the body size of the worms it is assumed that the nutria has almost the same susceptibility for *P. miyazakii* as those of the other known definitive hosts.

The nutria, however, may not play the role of the natural definitive host of *Paragonimus*, because they never eat any animal food in its natural life of this animal (Nagahana *et al.*, 1977). The results of the present experiment seem to suggest that the nutria has considerable susceptibility for *P. miyazakii*, though the natural infection should hardly occur.

Summary

Six nutrias were experimentally infected with *P. miyazakii* to know the grade of the host adaptation or host-*Paragonimus* relations. The adult worms were recovered from the lung cysts of two nutrias out of six experimented. Another one gave a negative result, and three died without any discoverable external cause, in which the immature worms were found. The recovery rate of the worms in five nutrias was 10% to 40% (av. 20). The worms recovered from the lung cysts were comparatively big, indicating the full development of the sexual maturity.

The nutria was newly added as the experimental definitive host for *P. miyazakii* in the present paper.

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宮崎肺吸虫のヌートリアに対する感染実験

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さきに，長花ら (1977) は，ヌートリアが実験的に肝吸虫の好適な終宿主となることを報告した．その後，この動物の肺吸虫は対する感受性を調べる目的で，岡山県で生けどつたヌートリア6頭に宮崎肺吸虫のメタセルカリアを10個～100個試食させた．その結果，5頭に感染が成立し，虫体回収率10～40％の成績が得られた．メタセルカリアを20個，28個投与後111日目，139日目に剖検した2頭では，肺臓実質内にそれぞれ肺吸虫に特有の虫嚢腫形成が認められ，成熟虫体各3匹，4匹が回収された．これらの虫体は，体長平均 12.2 mm，体幅平均 5.8 mm で，頗る良好な発育を示しており，性的にも十分成熟していた．また，他の3頭については，メタセルカリア投与後43～72日の間に宿主が死亡したので剖検したが，虫体はいずれも未成熟型で，腹腔内または胸腔内に遊離の状態で見出された．ヌートリアは，その食性が植物質に限られているので，この動物が肺吸虫の流行地に生息しても，肺吸虫の自然感染は起こらないと思われるが，実験的には宮崎肺吸虫の終宿主となることを初めて確認した．