

Experimental Infection of Wild Boars with Metacercariae of *Paragonimus miyazakii* (Trematoda: Troglotrematidae)

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(Accepted for publication; May 27, 1992)

Abstract

Six wild boars were orally or intraperitoneally administered with 190 to 2,000 metacercariae of *Paragonimus miyazakii* and then autopsied 25 to 149 days after administration. A small number of worms (recovery rates ranging from 0 to 1.1%) were recovered from the various parts of the wild boars. Most of them were immature or pre-adult except one juvenile worms recovered from the muscle of a wild boar 25 days after administration. Those juvenile and several immature worms were fed to two puppies. Seventy days later, one adult worm with eggs in the uterus was recovered from the pleural cavity of one of the puppies. Thus it was clarified that wild boars could serve as the paratenic host of *P. miyazakii*.

Key words: lung fluke, *Paragonimus miyazakii*, wild boar, experimental infection, paratenic host

Introduction

Two species of lung flukes, *Paragonimus westermani* and *P. miyazakii* are known as the causative agents of human paragonimiasis in Japan. Many cases of human paragonimiasis have been reported in Kagoshima Prefecture, Japan, between 1973 and 1975 (Norimatsu *et al.*, 1975). It was experimentally shown by Miyazaki and Habe (1975) that those cases were infected by eating raw wild boar meat infected with *P. westermani* larvae. Thereafter, similar cases have been reported in Miyazaki and Oita Prefectures (Miyazaki and Hirose, 1976; Ohtsuka *et al.*, 1980; Tashiro *et al.*, 1984; Araki *et al.*, 1985). *P. miyazakii* is also distributed widely from Tohoku to Kyushu districts in Japan, and many cases of human paragonimiasis by *P. miyazakii* have been reported (Hayashi *et al.*, 1974; Yokogawa *et al.*, 1974). Nevertheless, the cases infected by eating

wild boar meat are quite few in numbers, and only one such confirmed case has been reported so far (Ichiki *et al.*, 1986).

This study was conducted in order to clarify the infectivity of *P. miyazakii* to the wild boars and examine whether they serve as the paratenic host of *P. miyazakii*.

Materials and Methods

Parasite

Metacercariae of *P. miyazakii* were collected by the digestion method in artificial gastric juice from freshwater crabs, *Geothelphusa dehaani*, collected from mountain streams in the middle part of Ehime Prefecture, Japan, which are known as endemic areas for *P. miyazakii*.

Animal hosts

Six wild boars (15–24 wks, weighing 6–7 kg, three males and three females) and two puppies (females, not weighing) were used for the experiment. The wild boars were purchased from a wild boar breeder and dogs were brought from a dog-pound. The dogs were checked for previous *Paragonimus* infection by fecal examination prior to the experiment.

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Method of infection

The wild boars were anesthetized using ketamine hydrochloride (Ketalar 50®, Parke-Davis) and were orally administered with gelatin capsules containing 190–2,000 metacercariae. The puppies were orally administered with two or three larvae which were recovered from the tissues and cavities of the wild boars.

Recovery of parasite

The wild boars and puppies were autopsied on days 25–149th after administration with metacercariae and on days 27–70th after administration with larvae, respectively. The flukes were recovered according to the method of Shibahara and Nishida (1986).

Worms

The larger worms recovered were flattened between two slide glasses and were fixed in 70% alcohol, and smaller ones were flattened between a cover and a slide glass and fixed in Bouin's solution. Then, they were stained with borax-carmin, dehydrated, cleared and mounted in balsam according to the standard methods. Morphological observations and measurements were made on these mounted specimens. The worms were classified morphologically into four developmental stages (Shibahara and Nishida, 1986).

Results

Experimental infection of wild boars with metacercariae

Boars Nos. 1 and 2 were orally administered 190 or 200 metacercariae respectively and autopsied on day 75th after administration (Table 1). The organs and tissues of wild boar No. 2 were intact and no worms were recovered. Twelve whitish button-like nodes (about 10mm in diameter) were found on the liver surface of wild boar No. 1. Two immature worms were recovered from the liver node and abdominal muscle (recovery rate: 1.1%) (Fig. 3).

Wild boar No. 3 was administered with 200 metacercariae by intra-peritoneal injection and autopsied on day 78th after administration. A pre-adult and an immature worms were recovered from the cyst in lungs and liver tissues, respectively (recovery rate: 1.0%). No other gross lesion was found both in the organs and cavities except cyst-formation in the lung.

The remaining three wild boars Nos. 4, 5 and 6 were orally administered with 2,000 metacercariae each and autopsied on days 25th, 56th and 149th after administration, respectively. As shown in Table 1, three, four and two worms were recovered from those wild boars, respectively (recovery rates: 0.2, 0.2 and 0.1%). Although more than half of the worms recovered from these animals were immature (Fig. 2), one

Table 1 Results of experimental infection of wild boar (*Sus sucrofa leucomystax*) with metacercariae (Mc) of *Paragonimus miyazakii*

Wild boar No.	Sex	Body weight at autopsy (kg)	No. of Mc fed	Method of infection	Days after infection	No. of worms recovered(%)	No. of worms recovered from				
							muscle	liver	diaphragm	pleural cavity	lung
1	♂	18.3	190	p.o.*	75	2(1.1)	1	1‡			
2	♀	20.2	200	p.o.	75	0					
3	♀	12.0	200	i.p.†	78	2(1.0)		1			
4	♂	10.9	2,000	p.o.	25	3(0.2)	1			1	1
5	♂	12.6	2,000	p.o.	56	4(0.2)	1	1‡	1	1	
6	♀	16.0	2,000	p.o.	149	2(0.1)				2	

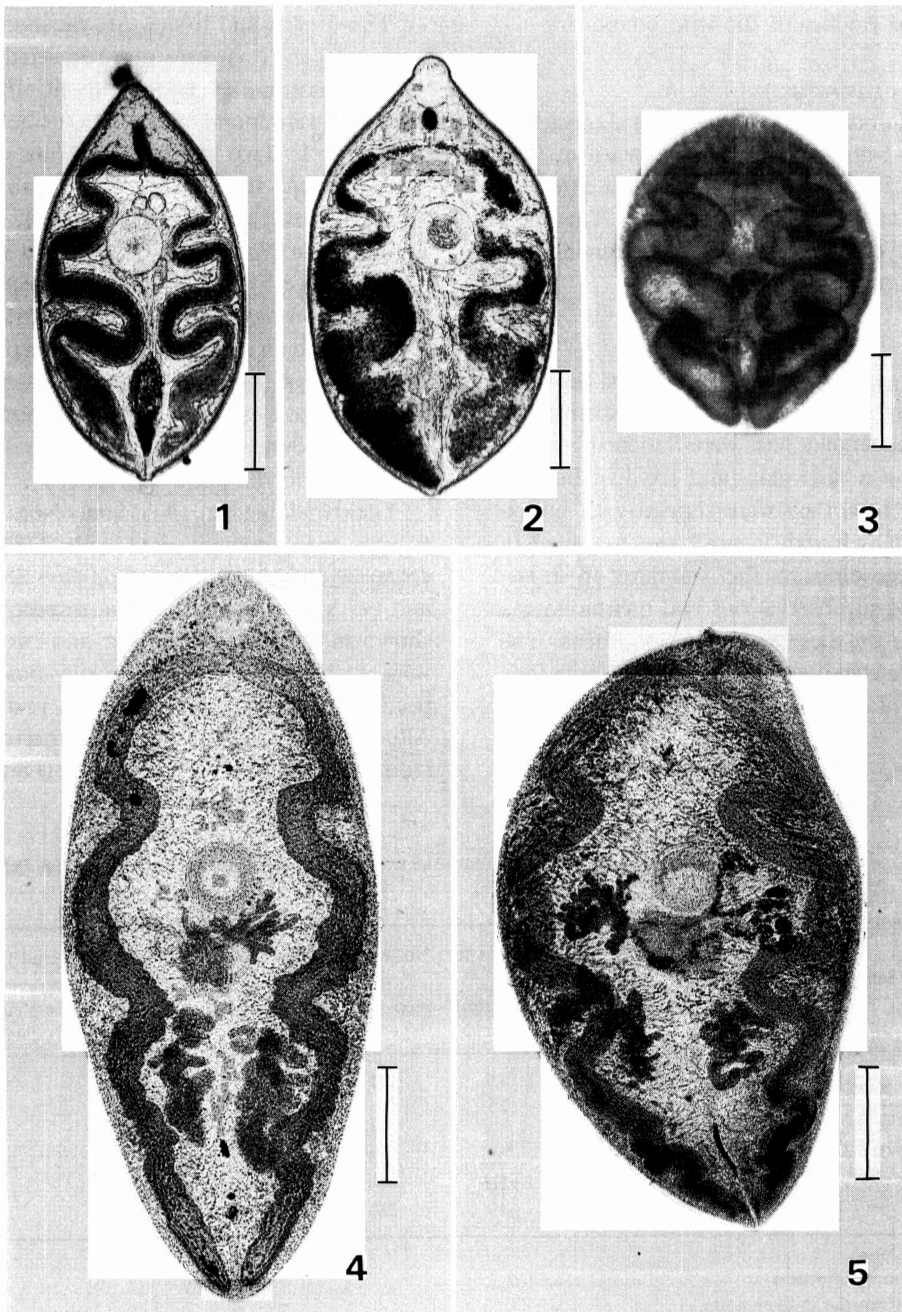
*per os

†intra-peritoneal injection

‡recovered from the cyst or node

juvenile (Fig. 1) and two pre-adult worms (Fig. 4) were recovered from the muscle of wild boar No. 4 and the pleural cavity of wild boar No. 6, respectively (Table 2). The size and morphological features of the juvenile worm were similar

to those of excysted metacercariae. No gross lesion was found both in the organs and cavities except cyst-formation in the liver.



Transfer of *P. miyazakii* larvae into puppies

One puppy was orally administered with two immature worms from the muscle of wild boar No. 5. However, the puppy died on day 27th after administration, producing no worms both in the organs and cavities by gross examination.

The other one was orally administered with three worms (one juvenile and two immature worms) from wild boar No. 4 and autopsied on day 70th after administration. Only one adult worm with eggs in the uterus was recovered from the pleural cavity (Fig. 5). The size of the worm was 5.1×3.2 mm.

Discussion

In this study, several doses of metacercariae ranging from 190 to 2,000 were orally administered or intraperitoneally injected to a total of 6 wild boars, and extremely small numbers of worms were recovered (ranging from 0 to 4). The recovery rates were also very low (0 to 1.1%) (Table 1). In the experimental infection of triploid *P. westermani* to wild boars, Miyazaki and Habe (1975) reported that about half (52.3% in average) of the administered flukes were recovered and most (98.8% in average) of them were juvenile worms recovered from the muscle. We have previously recovered the juvenile worms

Table 2 Types and sizes of *Paragonimus miyazakii* larvae recovered from various parts of wild boars

Days after administration	Wild boar No.	No. of worms recovered	Recovery part	Type* of worms recovered (No.)		Body size (mm)	
						length	width
25	[4]	3	muscle	Juv	(1)	-†	-
			pleural cavity	Im	(1)	-	-
			lung tissue	Im	(1)	-	-
56	[5]	4	muscle	Im	(2)	-	-
			pleural cavity	Im	(1)	2.5	1.3
			node (cyst) in liver	Im	(1)	2.6	1.4
75	[1]	2	muscle	Im	(1)	1.1	0.9
			node (cyst) in liver	Im	(1)	1.6	1.0
78	[3]	2	liver	Im	(1)	2.7	1.5
			cyst in lung	Pre-A	(1)	4.2	2.0
149	[6]	2	pleural cavity	Pre-A	(2)	6.2‡	2.8‡

* Types of worms were classified according to the criteria of Shibahara and Nishida (1986); Juv: juvenile worm, Im: immature worm, Pre-A: pre-adult worm, †: not measured, ‡: mean

- Fig. 1 Juvenile worm recovered from the thoracic muscle of a wild boar 25 days after administration with metacercariae. (Living specimen. Scale: $300 \mu\text{m}$)
- Fig. 2 Immature worm recovered from the pleural cavity of a wild boar 25 days after administration with metacercariae. (Living specimen. Scale: $300 \mu\text{m}$)
- Fig. 3 Immature worm recovered from the abdominal muscle of a wild boar 75 days after administration with metacercariae. (Mounted specimen. Scale: $300 \mu\text{m}$)
- Fig. 4 Pre-adult worm recovered from the pleural cavity of a wild boar 149 days after administration with metacercariae. (Mounted specimen. Scale: 1mm)
- Fig. 5 Adult worm recovered from the pleural cavity of a puppy 70 days after administration with larvae recovered from a wild boar 25 days after administration with metacercariae. (Mounted specimen. Scale: 1mm)

of diploid *P. westermani* from the muscles of wild boars at an average rate of 11.3% (range 7.0 to 17.0%) (Shibahara and Nishida, 1986). The recovery rates of *P. miyazakii* is extremely low compared to those of two types of *P. westermani*, therefore it is presumed that wild boars are rather refractory to *P. miyazakii*.

Several pre-adult worms were recovered from pleural cavity or cyst in lung on days 78th and 149th after administration (Table 2). A longer duration of time after infection might have produced fully mature adult worms.

Some investigators have found *P. miyazakii* adult worms in naturally infected wild boars and concluded that wild boars serve as the definitive host of *P. miyazakii* (Hatsushika, 1967; Hamajima and Miyazaki, 1968; Hirai *et al.*, 1978). Recently, Ichiki *et al.* (1986) reported a case of human paragonimiasis miyazakii which was infected by eating raw meat of wild boar, and suggested that wild boars may serve as the paratenic host of *P. miyazakii*. In the present experiment, one adult fluke was recovered from the pleural cavity of the puppy as the result of transfer infection of three larvae from wild boar No. 4. As immature-type worms do not have infectivity to animals such as dogs and cats (Torii *et al.*, unpublished data), a juvenile worm administered might mature to the adult stage. Hence, wild boar may sometimes serve as a paratenic host of *P. miyazakii* in nature.

From the view point of public health, it is needed to pay special attention to eating raw meat of wild boars from endemic areas of *P. miyazakii*, as they may be infested with the infective juvenile worms.

Acknowledgments

The authors wish to thank Dr. Y. Aohagi, Laboratory Animal Research Center, Faculty of Medicine, Tottori University for his kind help in preparing the manuscript and also thank to Dr. S. M. Fazle Akbar, 3rd Department of Internal Medicine, Ehime University School of Medicine, for his critical reading of the manuscript.

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