EXPERIMENTAL INFECTION WITH *PARAGONIMUS HETEROTREMUS* METACERCARIAE IN LABORATORY ANIMALS IN MANIPUR, INDIA

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Abstract. This study was aimed to find out the host-parasite relationship between *Paragonimus heterotremus* isolated as metacercariae from mountain crabs, *Indochinamon manipurensis*, in Manipur, India and laboratory animals such as puppies, albino rats, Swiss mice, guinea pigs, and rabbits, as experimental animals. The animals were fed with the metacercariae. Infected animals were sacrificed 35 to 430 days after feeding to recover worms, which were used to determine the developmental stages. Adult worms (n = 14) were recovered from 3 puppies ≥70 days after feeding and immature worms (n = 25) were recovered from 2 other puppies 35 or 43 days after infection. The infection rate in puppies was 100%. Juvenile worms were recovered from 3 of 13 rats: 1 of 11 rats whose viscera and cavities were examined and both of two rats whose muscles were examined. Rats were not a suitable animal model for pulmonary infection with *P. heterotremus*. Mice, guinea pigs, and rabbits were also found to be insusceptible to pulmonary infection with *P. heterotremus*.

Keywords: *Paragonimus heterotremus,* puppy, metacercariae, host-parasite relationship, infectivity, India

INTRODUCTION

In India, *Paragonimus westermani* isolation from tigers, bear cats, mongooses, civet cats and dogs has been reported (Rao, 1935; Dutt and Gupta, 1978; Singh and Somvanshi, 1978; Ravikumar *et al*, 1979; Parihar and Shirivastava, 1988). *Paragonimus* eggs were also detected in fecal samples of civet cats and toddy cats in Manipur, India (Singh *et al*, 1998). A suitable animal model for *Paragonimus* infection is required to study the host-parasite relationship, pathogenesis, host immune response, and for the therapeutic evaluation of drugs. Adult worms are required for morphological and molecular characterization, as well as antigen preparations for the immunodiagnosis of paragonimiasis. Studies have shown that dogs and cats, especially puppies and

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kittens, are susceptible to most *Paragonimus* species prevalent in Asia, while rats and mice served as paratenic hosts when infected naturally or experimentally with some *Paragonimus* species (Shibahara, 1981; Sugiyama *et al*, 1990).

Little is known about experimental animal infection by *Paragonimus* species in India. Therefore, this study was conducted to develop a suitable laboratory animal model for *Paragonimus heterotremus* in a laboratory in Manipur, India. Human infection with this lung fluke species has been confirmed in Manipur (Singh *et al*, 2007) and Nagaland (Singh *et al*, 2009), a state neighboring Manipur.

MATERIALS AND METHODS

Paragonimus heterotremus metacercariae were isolated from freshwater crabs, *Indochinamon manipurensis* (Alcock, 1909), which were formerly referred to as *Potamiscus manipurensis*, at Churachandpur, Pashong Luwangsangbam Matai, and Motbung in the Manipur State of India (Singh *et al*, 2007). The isolation procedure of metacercariae from crabs was described previously (Rangsiruji *et al*, 2006). Using Pasteur pipettes, puppies (n = 5, 8 to 50 metacercariae each), albino rats (Wistar strain, n = 11, 20 metacercariae each), rabbits (n = 4, 20 metacercariae each), Swiss mice (n = 4, 20 metacercariae each), and guinea pigs (n = 4, 18 metacercariae each) were inoculated orally with isolated metacercariae. Pipettes were all heated over a flame to smooth their edges before using for oral inoculation. The metacercariae, 20 each, were also inoculated to the peritoneal cavity of 2 other albino rats under ether anesthesia using the method described previously (Sugiyama *et al*, 1990). The animals were confirmed negative for *Paragonimus* infection by fecal examinations by the formalin-ether sedimentation technique prior to experimental infection. They were autopsied 35 to 430 days after infection and the lungs, liver, and pleural and peritoneal cavities were examined. The skeletal muscles were also examined in some rats (Sugiyama *et al*, 1990). Recovered worms were examined under a microscope before and/or after Borax carmine staining and mounting to determine the developmental stage as adult, pre-adult, immature, and juvenile according to the criteria of Shibahara (1984).

RESULTS

The 5 puppies used in this study were all positive for *P. heterotremus* infection, and 39 flukes were recovered (Table 1). Fourteen flukes recovered 70 or more days after infection were adult (Fig 1), and those (n = 25) recovered 35 and 43 days after infection were at the immature stage. Twenty-eight worms were recovered from 3 of the 13 rats. The flukes from rats were all determined to be at the juvenile stage (Table 2, Fig 2). No worms were recovered and no cysts were identified from the infected rabbits, mice, and guinea pigs.

DISCUSSION

The infection rate of puppies was 100%, and the worms recovered 70 or more days after infection with *P. heterotremus* metacercariae were all identified as adults. Employing puppies as an experimental host animal seems to yield sufficient numbers of worms required for morphological identification, antigen preparations, and molecular studies. The study of host immune responses and therapeutic evaluation of antihelminthic drugs can also be carried out in puppies.

The recovery of juvenile worms alone from only 3 of the 13 rats fed with *P. het-*

Table 1
Results of experimental infection of puppies with *P. heterotremus* metacercariae.

Puppy no.	Sex	Route of infection	No. of MC[a]	Autopsy at days after infection	No. (%) of worms recovered	No. of worms recovered from				
						Cyst in lungs	Lungs	Pleural cavity	Liver	Peritoneal cavity
1	F	Oral	20	35	5 (25)	0	1	2	0	2
2	M	Oral	50	43	20 (40)	0	0	20	0	0
3	F	Oral	20	70	5 (25)	0	0	5	0	0
4	F	Oral	25	330	5 (20)	4	1	0	0	0
5	F	Oral	8	430	4 (50)	4	0	0	0	0

[a] MC, metacercariae

Table 2
Results of experimental infection of albino rats with *P. heterotremus* metacercariae.

Rat no.	Sex	Route of infection[a]	Autopsy at days after infection	No. of worms recovered (%)	No. of worms recovered from					
					Cyst in lungs	Lungs	Pleural cavity	Liver	Peritoneal cavity	Muscle[b]
1	F	Oral	50	0	0	0	0	0	0	NE
2	F	Oral	50	1 (5)	0	0	1	0	0	NE
3	F	Oral	90	0	0	0	0	0	0	NE
4	F	Oral	90	0	0	0	0	0	0	NE
5	M	Oral	90	0	0	0	0	0	0	NE
6	M	Oral	112	0	0	0	0	0	0	NE
7	M	Oral	114	0	0	0	0	0	0	NE
8	M	Oral	120	0	0	0	0	0	0	NE
9	F	Oral	142	0	0	0	0	0	0	NE
10	M	Oral	152	0	0	0	0	0	0	NE
11	M	Oral	159	0	0	0	0	0	0	NE
12	M	IP	95	14 (70)	0	0	0	0	0	14
13	M	IP	95	13 (65)	0	0	0	0	0	13

[a] 20 metacercariae were given orally or intraperitoneally (IP); [b] NE, not examined

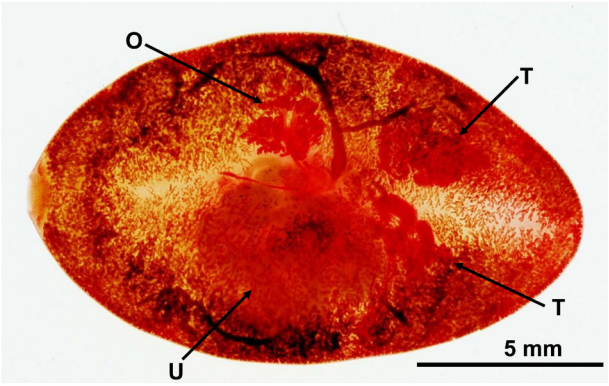

Fig 1–An adult fluke recovered from the lung cyst of tested puppy No. 3, 330 days after oral infection with *P. heterotremus* metacercariae. T, testes; O, ovary; U, uterus.

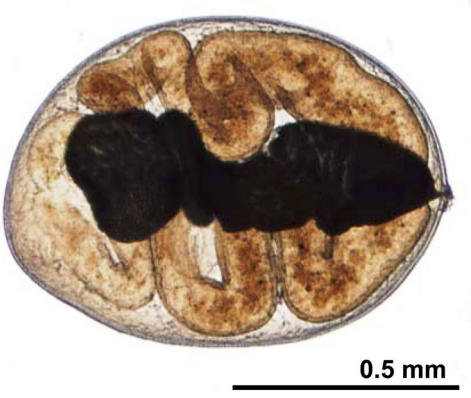

Fig 2–A juvenile fluke recovered from the muscle tissue of tested rat No. 12, 95 days after intraperitoneal infection with *P. heterotremus* metacercariae.

erotremus metacercariae showed that rats are not a suitable final host for this isolate. The rats served as paratenic hosts may be important for natural sources of infection of wild and domestic carnivorous animals.

Adult worms were recovered from rats experimentally infected with *P. heterotremus* experimenting in Thailand (Sugiyama *et al*, 1990) and in Arunachal Pradesh, India (Narain *et al*, 2003). From our observations, together with the previous findings, the host-parasite relationship between *Paragonimus* species and laboratory mammalian hosts might be related to strain variation of the species and the selection of mammalian hosts in different geographical areas (Fan and Chiang, 1970; Habe, 1978; Habe *et al*, 1996). Dogs and cats, especially puppies and kittens, are susceptible to all *Paragonimus* species in Asia; these animals can serve as suitable models for experimental studies which require a greater number of adult worms and a longer host life span.

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