

Original

## Occurrence of the Lung Fluke *Paragonimus hueit'ungensis* in Manipur, India

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### Key Words

experimental dog;  
India;  
*Paragonimus hueit'ungensis*

**Background.** *Paragonimus* has emerged as an important parasitic zoonosis in recent years in Manipur, India. However, no scientific data on the systematic and phylogenetic classification of lung fluke in India is available. Therefore, a study was conducted to determine the species of lung fluke prevalent in Manipur by infecting an experimental animal with *Paragonimus* metacercariae obtained from fresh water crabs.

**Methods.** Thirteen *Paragonimus* metacercariae isolated from the fresh water crabs, *Potamiscus manipurensis*, at Churachandpur, Manipur, India were used to infect a puppy orally using a Pasteur pipette. After the infection, fecal specimens were checked for *Paragonimus* eggs by microscopic examination. Autopsy was performed on day 121 post-infection. The worms recovered from the worm cysts were alcohol fixed, flattened and stained with carmine for morphological study.

**Results.** Four adult worms were recovered. The worms presented with singly spaced cuticular spines, and the ventral suckers were slightly larger than the oral suckers. The ovaries consisted of 4 to 5 lobed masses branched like a ginger stem, and the testes were irregularly lobed and elongated. One of the worms showed a different shape and size of the right testis. The morphological features of the worms are similar to the Chinese species, *Paragonimus hueit'ungensis*.

**Conclusions.** The results of this study indicate the possibility of occurrence of *P. hueit'ungensis* as 1 of the important *Paragonimus* species prevalent in Manipur, India after its initial discovery in China.

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In recent years, paragonimiasis has emerged as an important public health problem in Manipur, India and *Paragonimus westermani* was considered to be the only causative agent.<sup>1,2</sup> No published scientific data on systematic and phylogenetic classification of lung flukes in India is available, consequently, *Paragonimus* species and their crustacean and final animal hosts in Manipur have remained undetermined. During 1990-1997, considering the public health importance of paragonimiasis in Manipur and uncertainty regarding prevalent *Paragonimus* species, research studies in lung flukes were conducted jointly

with Japanese medical scientists headed by Dr. Kenjiro Kawashima of the Kyushu University School of Health Sciences, Fukuoka, Japan and also independently in the Department of Microbiology, Regional Institute of Medical Sciences, Manipur, India. Attempts were made to examine as many crabs as possible for *Paragonimus* metacercariae infection and isolate them for morphological and experimental study. The metacercariae so isolated from crabs collected from mountain streams in Churachandpur, Manipur were inoculated orally to 1 puppy to recover adult worms for morphological characterization. In

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In India, occurrence of *P. westermani* in naturally infected cats and dogs was reported by Srivastava and Rao.<sup>3,4</sup> Although the metacercariae used in our study morphologically resembled *P. westermani*, the adult worms recovered from the experimentally infected puppy were morphologically different. Interestingly, the worms have features characteristic of a newly recognized Chinese species, *P. hueit'ungensis*.<sup>5</sup> The detailed morphological features of the adult worms and the eggs recovered from the experimental animal are described.

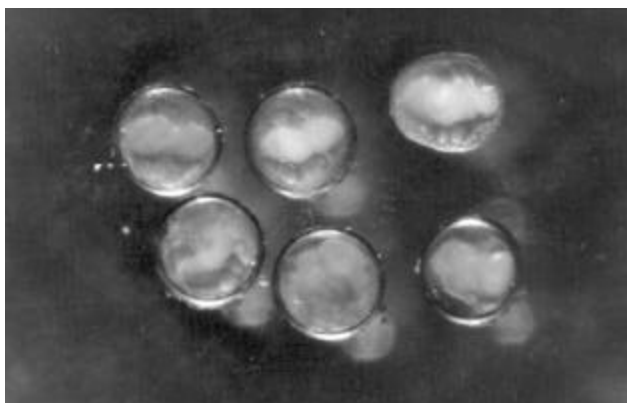
## Methods

### Source of metacercariae

From June 1996 to November 1996, *Paragonimus* metacercariae were collected by digestion with artificial gastric juice for 6 to 12 hours at 37 °C from the fresh water crabs, *Potamiscus manipurensis*, collected from the mountain streams of Churachandpur, Manipur. The metacercariae measuring 400 to 410 µm were spherical or oval in shape and morphologically resembled metacercariae of *P. westermani* (Fig. 1).

### Experimental infection

Thirteen metacercariae in normal saline were fed to a 3-month-old puppy by oral route using a Pasteur pipette. No ethical clearance was required because pup-



**Fig. 1.** Metacercariae isolated from the crab host *Potamiscus manipurensis* collected at Churachandpur.

pies are included among laboratory animals for experimental infection for teaching and research studies in medicine. Before the experimental infection, the animal was checked for previous *Paragonimus* infection by microscopic examination of 3 consecutive fecal samples. No *Paragonimus* egg was detected in the feces.

### Recovery of worms

Sixty days after the experimental infection, fecal specimens were checked at least twice a week for *Paragonimus* egg by microscopic examination. Each time, formol-ether technique was used to concentrate eggs in the feces. Autopsy was performed on day 121 post-infection following the detection of *Paragonimus* eggs in the fecal specimens. Methods of Kawashima<sup>6</sup> for experimental infection were followed throughout the experiment. First the abdominal cavity was cut open and examined for any lesion and worm cysts in the cavity and viscera. Similarly, the thoracic cavity and lungs were examined for worms and worm cysts. Any suspected lesions, worms and cysts were carefully collected in physiological saline solution in petri dishes or beakers. In the absence of any visible worms or worm cysts the lungs were cut into small slices 3 to 4 mm thick and incubated in Ringer's solution at 37 °C for 6 to 12 hours in order to recover any penetrating worms. On recovery, worms were placed in normal saline for 12 to 24 hours to empty the excretory bladder and the uterus. The infected material, worm cysts and free worms were examined under a dissecting microscope. The worms were pressed between glass slides and fixed in 70% alcohol for 5 to 7 days, each day increasing the pressure by putting on additional weights so that the worms become flattened as thin as 0.5 mm or less. The worms were stained with carmine, dehydrated, cleared with xylene and mounted in Canada balsam. Morphological features of the stained worms were studied under stereodissecting microscope.

## Results

Four cyst-like lesions, 1 in the right lung and 3 in



**Fig. 2.** Three carmine-stained mounted adult worms removed from the lung cyst; a, b: dorsal sides; c: ventral side.

the left lung were detected. Two lesions in the left lung were found to be worm cysts containing a pair of worms and cystic fluid. Others were inflamed and granulomatous lesions. A communicating fibrous tunnel was present between a cyst and granulomatous lesion in the left lung. The worm cysts were 14 to 15 mm in diameter, spherical and firm with fibrous cyst wall. Both cysts were filled with dark brown mucoid fluid containing numerous eggs, a pair of worms, inflammatory cells, erythrocytes, etc. A total of 4 worms were recovered from the 2 worm cysts (recovery rate 31%). The fresh worms were fleshy pale pink and actively contractile and at rest appeared elliptoidal with 2 surfaces, a flat ventral and a convex dorsal. Numerous golden brown eggs were seen expelled through the genital pore and occasionally through oral suckers during their vigorous contractions. The alcohol-fixed, flat tened worms measured 9 to 13 mm in length and 3 to 5 mm in breadth. Several membranous rusty brown haemorrhagic spots containing many eggs were present in the pleurae and thoracic wall. Fig. 2 shows the morphological features of 3 carmine-stained worms. The bodies were elongated and slender. The ventral suckers situated at the anterior third of the body were larger than the oral suckers. The vitelline glands and ducts were well developed and filled with cells. The ovary situated on the right and postero-lateral to the ventral sucker was composed of large 4 to 5 lobed masses, which branched like a ginger stem. The testes were elongated and ir-

regularly lobed masses. The uterus situated opposite to the ovary was a coiled tubular structure containing numerous eggs. The intestine was bifurcated just behind the pharynx. The uterine eggs, measuring on an average  $79.5 \times 48.5 \mu\text{m}$ , were mostly symmetrical with almost uniformly thin egg shells except a few eggs, which appeared, slightly thickened or knobbed at the abopercular regions. One of the worms was smaller ( $9 \times 3 \text{ mm}$ , Fig. 2b) and narrower than the others. The right testis in another worm Fig. 2c presented abnormally as a lumpy mass without appreciable lobes whereas the left testis consisted of elongated lobes, with short rounded lobules.

## Discussion

A puppy was chosen for the experimental infection as dog served as 1 of the natural animal hosts of *Paragonimus* spp. in India.<sup>4</sup> Kawashima<sup>7</sup> use dogs in experimental infection with Chinese *P. westermani* and recovered mature parasites from the lung cysts. In Japan, Raccoon dogs were reported as naturally infected with *P. westermani* and *P. ohirai*.<sup>8</sup> *Paragonimus* metacercariae isolated from the *P. manipurensis* collected from Churachandpur, Manipur were found infective to puppy. The larval worms could grow to adults producing eggs, make pairs and encyst in the lungs indicating that puppy may serve as an ideal experimental animal model for lung flukes in Manipur. The rusty brown membranous patches found on the thoracic wall and pleura suggested that the worms after pairing might have wandered from place to place to find a suitable site for encystation as evident from the finding of communicating tunnel between the two lesions in the lungs.

The morphological features of the worms were similar to *P. hueit'ungensis* recently discovered in Hueitung county of Hunnan province of China and described by Chung *et.al.*<sup>5</sup> The worms like *P. Hueit'ungensis* were encysted in pairs, the cuticular spines were slender, pointed and singly spaced, and the ventral suckers were slightly larger than the oral suckers. The ovary situated below and lateral to the ventral sucker consisted of 4 to 5 stout lobed masses

resembling ginger stems. The testes presented as elongated and irregularly lobed masses. But in 1 of the worms, the ovary was found complexly branched and the right testis presented as a lumpy mass without appreciable lobes, whereas the left testis consisted of an elongated mass. The morphological differences of ovary and the testes found in 1 worm may be considered as a strain variation within the species as found in some of the specimens of *P. hueit'ungensis*. This is the second report on the occurrence of *P. hueit'ungensis* in the world after its first report in China. As in China, this species may be 1 of the important causes of human and animal paragonimiasis in Manipur.

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