

STUDIES ON AMERICAN PARAGONIMIASIS V. Further observations on the presence of *Paragonimus* in fresh-water crabs from Costa Rica, with notes on susceptibility to cercariae of *P. kellicotti*.*

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ABSTRACT

The fresh-water crabs *Ptychophallus tristani* (Rathbun, 1896) Smalley, 1964, and *Potamocarcinus magnus* (Rathbun, 1896) Pretzmann, 1965, are reported as natural second intermediate hosts for *Paragonimus* from two localities in Costa Rica. The incidences and intensities of infection and their localities by host, sex, and size are reported. The metacercariae of *Paragonimus* were found on the hearts of the infected crabs. Nine specimens of three species of wild-caught fresh-water crabs from Costa Rica were exposed to cercariae of *Paragonimus kellicotti* from Louisiana, but only one specimen was infected, suggesting that the affected specimen had acquired its infection previous to exposure in the laboratory, or that *P. kellicotti* cercariae are not as infective to fresh water crabs as to crayfishes, perhaps reflecting upon the evolutionary biology of this host-parasite relationship. Metacercariae of *Paragonimus* from Costa Rica were fed to an uninfected domestic cat and the infection became patent on 67th day, but was lost after 202 days.

A larval microphallid trematode, possibly the metacercaria of *Maritrema prolium* Caballero and Montero, 1961, was also found in the Costa Rica crabs.

INTRODUCTION

In a previous note (Sogandares and Smalley, 1965) we reported metacercariae of *Paragonimus* from preserved specimens of the Costa Rican fresh-water crab *Ptychophallus tristani* (Rathbun, 1896) Smalley, 1964 (= *Pseudothelphusa tristani*). We also examined preserved immature *Potamocarcinus*

magnus (Rathbun, 1896) Pretzmann, 1965 (= *Pseudothelphusa magna*), but found these to be uninfected. Since our report (*op. cit.*) we have visited different sites in Costa Rica for purposes of examining additional fresh-water crabs for paragonimiasis, and to determine where the metacercariae locate in their host. Some crabs were brought to our laboratories to attempt infections of domestic cats with Costa Rican *Paragonimus*, and to test the susceptibility of these crabs to *Paragonimus kellicotti* Ward, 1908, from Louisiana. Since we do not plan to return to Costa Rica in the near future, we have decided to present our somewhat limited findings.

METHODS

Fresh-water crabs were collected from under rocks in streams from different localities in Costa Rica. These were identified with the aid of Smalley's (1964) keys. The crabs were cut open and carefully dissected with needles under a binocular dissection microscope in our examinations for metacercariae. The cyst membranes of some of the metacercariae were dissected with needles and the metacercariae identified visually under a compound microscope. Thereafter, the cysts of *Paragonimus* metacercariae were easily separated from another species (Microphallidae) found. Crabs from localities where *Paragonimus* had not been found were placed in fingerbowls containing a small amount of dechlorinated tap-water. A crushed *Pomatiopsis lapidaria* (Say) bearing a seventh generation laboratory infection of *P. kellicotti* from Louisiana was placed overnight, at room temperature, in

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each fingerbowl containing the crab to be exposed. This procedure has been reliable in our laboratory to infect Louisiana crayfishes, *Procambarus clarkii* (Girard, 1853), of medium size (about 50 mm) with from 30 to 100 metacercariae. The snail infections were maintained as described by Sogandares (1965). Three metacercariae of *Paragonimus* recovered from one *Ptychoballus tristani* from 3 mi E Atenas, Alajuela Province (580 m elevation) were intubated *per os* into a domestic cat which had been judged to be free of paragonimiasis by several fecal examinations using sedimentation concentration and Telemann's (1908) techniques.

RESULTS

Table I shows that 1 of 5 *Ptychoballus tristani*, all from a locality where *Paragonimus* metacercariae had not been found previously, harbored an infection by *Paragonimus* metacercariae after exposure to cercariae of *Paragonimus kellicotti* from Louisiana. Two specimens each of *Ptychoballus tumimanus* and *P. montanus* were refractory to Louisiana *P. kellicotti*.

Table II shows that *Ptychoballus tumimanus* and *P. montanus* from a swift mountain stream at 1200 m elevation were uninfected with *Paragonimus* metacercariae. One of 15 *Ptychoballus tristani* collected in three different localities, ranging from 580 to 900 m elevation, was naturally infected with three metacercariae of *Paragonimus*. Two of 13 *Potamocarcinus magnus* from two relatively close localities, ranging from 450 to 580 m elevation, were each infected with one *Paragonimus* metacercaria. In all cases the *Paragonimus* metacercariae were found on or in the heart tissues of the affected crabs.

Eggs were first detected in the feces of

the domestic cat exposed to three metacercariae of *Paragonimus* from *Ptychoballus tristani* on the 67th day. Eggs could not be detected, even with Telemann's technique, in the feces of the infected cat by the end of 202 days. The cat was then killed and examined. Post-mortem examination revealed two hemorrhagic spots in the lungs, but no worms were recovered. Further examination of the entire animal, including brain and brain case, sinuses, other organs and muscles, etc., failed to reveal the presence of *Paragonimus* or other trematodes or cestodes.

The hepatopancreas of *Potamocarcinus magnus* and *Ptychoballus tristani* from 3 mi E Atenas, Alajuela Province, and also *P. tristani* from 0.5 mi S Cebadilla, Alajuela Province, were not infrequently infected with a microphallid metacercaria which may be *Maritrema prolixum* Caballero and Montero, 1961.

DISCUSSION

The numbers of cercariae of Louisiana *P. kellicotti* to which each crab in Table I was exposed would have been sufficient to produce heavy infections of 30 to 100 metacercariae in medium size (about 50 mm) Louisiana crayfishes such as *Procambarus clarkii*. Only one of five *P. tristani* harbored 3 metacercariae of *Paragonimus* after such a massive exposure, suggesting that the affected specimen had acquired its infection previous to exposure in the laboratory or that *P. kellicotti* cercariae are not as infective to fresh-water crabs as to crayfishes, perhaps reflecting upon the evolutionary biology of this host-parasite relationship.

The Central and South American form (*P. rudis*) of *Paragonimus* and the North American form (*P. kellicotti*) have been synonymized by Caballero and Montero

TABLE I.
Results of exposures of Costa Rican fresh-water crabs to *Paragonimus kellicotti* cercariae from Louisiana.

Species	No. Specimens Exposed	Locality of Origin and Elevation	Incidence	Intensity ¹
<i>Ptychoballus tristani</i> (Rathbun, 1896) Smalley, 1964	5	0.8 mi. W Piedades, San Jose Prov., 900 m	1/5	3
<i>Ptychoballus tumimanus</i> (Rathbun, 1898) Smalley, 1964	2	2 mi. S Cariblanco, Heredia Prov., 1200 m	0/2	0
<i>Ptychoballus montanus</i> (Rathbun, 1898) Smalley, 1964	2	2 mi. S Cariblanco, Heredia Prov., 1200 m	0/2	0

¹ Number of metacercariae recovered after 6 wks.

TABLE II.
Incidence and intensity of natural infections of Costa Rican
fresh-water crabs by *Paragonimus*.

Species	Sex	Numbers/ size ¹	Number infected	Inten- sity ²	Locality and Elevation
<i>Ptychophallus</i> <i>tristani</i> (Rathbun, 1896) Smalley, 1964	♀	1/17	1	3	3 mi. E Atenas, Alajuela Prov., 580 m
	♂	1/27	0	0	
	♂	3/15-38	0	0	0.5 mi. S Cebadilla Alajuela Prov., 600 m
	♂	5/20-31	0	0	0.8 mi. W Piedades, San Jose Prov., 900 m
<i>Ptychophallus</i> <i>tumimanus</i> (Rathbun, 1898) Smalley, 1964	♂	4/30-41	0	0	2 mi. S Cariblanco, Heredia Prov., 1200 m
	♀	2/24-27	0	0	
<i>Ptychophallus</i> <i>montanus</i> (Rathbun, 1898) Smalley, 1964	♂	2/17-24	0	0	
<i>Potamocarcinus</i> <i>magnus</i> (Rathbun, 1896) Pretzmann, 1965	♂	6/60-70	2	1, 1	Río Grande de Tar- coles on Atenas Hwy, Alajuela Prov., Approx. 450 m
	♂	7/24-61	0	0	3 mi. E Atenas, Alajuela Prov., 580 m

¹ Carapace width in mm.

² Numbers of metacercariae recovered from individual crabs.

(1961) and others, yet they seem to possess different biological characteristics insofar as infectivity to the second intermediate host is concerned.

It is yet premature to discuss the distribution of infections of fresh-water crabs by *Paragonimus* in Costa Rica. However, the infections found by us were confined to Alajuela Province at elevations ranging from 450 to 580 m (Table II), and adults and juveniles of *Ptychophallus tumimanus* and adults of *Potamocarcinus magnus* are susceptible to natural infections. The overall incidence and intensity of natural infections in crabs from the localities visited by us is low. Natural infections seem to be discontinuous rather than widespread, but further collections of large series of crabs may prove otherwise.

The loss of the cat infection is indeed puzzling, especially since careful effort was made to examine the host and to recover the worms. A possible explanation is that the worms could have localized in a pedunculated cyst, protruding into the gastroin-

testinal tract, which may have broken off and ruptured, releasing the worms into the lumen of the host gut where they were passed in the feces. There is also a possibility that the worms could have located in the maxillary sinus, as do some other troglotrematids (*Troglotrema acutus* (Leuckart, 1842) in Europe, *Archillurbainia novelli* Dollfus, 1939, from Malaysia, and *A. rec-ondita* Travassos, 1942, from Brazil and the Lesser Antilles), migrated out of their cavity, and then have been passed by the host, though no evidence for this was found.

The adults of the microphallid metacercariae from the crabs, tentatively identified as *Maritrema prolixum*, are known from *Philander opossum fuscogriseus* (Allen) (Caballero and Montero, 1961), the same host from which *Paragonimus rudis* (Diesing, 1850) has been recovered in the general area (Caballero and Montero, 1961) where the infected crabs were collected.

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