

Research Note

**Experimental Infection of *Semisulcospira libertina*
with *Paragonimus westermani* (Triploid Type)**

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The snail, *Semisulcospira libertina*, is known to be as the first intermediate host of the lung fluke, *Paragonimus westermani* (Nakagawa, 1915; 1918; Kobayashi, 1918; Ando, 1918; Miyairi, 1918; Yokogawa, 1919). Although none of these workers was able to obtain cercariae from snails experimentally infected with miracidia of the lung fluke in spite of many years of effort, Komiya *et al.* (1961) observed the cercariae in experimentally infected *S. libertina*, collected from an endemic area of paragonimiasis in Shizuoka Prefecture. However, there are as yet many unsettled questions concerning the infection and development of intra-molluscan larvae of the fluke. Therefore, experimental infection was carried out to elucidate the host-parasite relationship between the fluke and the snail.

P. westermani (triploid type) used in the experiments were isolated from *Eriochair japonicus* collected on Tsushima Is. and Amakusa Is., Japan. Eggs collected from the uterus of mature worms removed from dogs. They were incubated at 27 C in water-filled Petri dishes 9 cm in diameter. The water was changed daily. Miracidia were obtained 16 days later. Snails, 5-10 mm in shell width, were collected by random sampling from three different localities, Matsumoto of Nagano Prefecture on June 23, Tokigawa of Saitama Prefecture on

June 7, and Nagaoka of Shizuoka Prefecture on December 6, 1980, in order to provide snails which might have different infectious phases of various cercariae of natural infection. They were used for this experiment, and divided into two groups. One of them was used as control. The other one was exposed to 100 miracidia of the fluke per snail three times. And they were bred at 25 C in separate aquaria (45×25×30 cm) provided with air according to their sampling localities. The water was changed occasionally. Solid food for rabbits, vegetables and tree leaves were given as food once a day. The exposed and control snails were examined at about 20 weeks after the exposure. The intra-molluscan rediae and cercariae were observed under a binocular dissecting microscope. The chi-square test was used to estimate the data of statistical significance. Differences between infection rate with the fluke of snails harboring other cercariae and that of cercaria-free snails were considered to be significant when $P < 0.05$.

As shown by Hamajima *et al.* (1981) and in Table 1, in experimental infection with *P. westermani* from Tsushima Is., snails collected from Matsumoto were infected with cercariae in the combination of *P. westermani* and *Cercaria monostyloides*. Furthermore, snails collected from Tokigawa were infected with cercariae in the combination of *P. westermani* and *C. mono-*

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Table 1 Results of experimental infection of *Semisulcospira libertina* collected from three localities with miracidia of *Paragonimus westermani* (triploid type)

Localities of snails collected	Species of cercariae found	Control			Experiment		
		No. of snails examined	No. of snails infected	% positive infection	No. of snails examined	No. of snails infected	% positive infection
Matsumoto	<i>C. m.</i> *		16	50.0		5	13.2
	<i>P. w.</i> *+ <i>C. m.</i>	32	0	0.0	38	15	39.5 (S)
	Cercaria-free		16	50.0		18	47.4
Tokigawa	<i>C. m.</i>		8	15.7		6	5.5
	<i>C. y.</i> *		3	5.9		3	2.7
	<i>M. y.</i> *		2	3.9		2	1.8
	<i>P. w.</i> + <i>C. m.</i>	51	0	0.0	110	14	12.7 (S)
	<i>P. w.</i> + <i>M. y.</i>		0	0.0		2	1.8
	<i>P. w.</i> + <i>C. y.</i>		0	0.0		1	0.9
	<i>P. w.</i> + <i>C. m.</i> + <i>M. y.</i>		0	0.0		1	0.9
Cercaria-free		38	74.5		81	73.6	
Nagaoka	<i>C. y.</i>		4	2.4		2	2.5
	<i>C. a.</i> *		2	1.2		1	1.3
	<i>C. i.</i> *	170	0	0.0	80	1	1.3 (S)
	<i>P. m.</i> *		0	0.0		1	1.3
	<i>P. w.</i> + <i>P. m.</i>		0	0.0		3	3.8
Cercaria-free		164	96.5		72	90.0	

* *C. m.*=*Cercaria monostyloides*, *P. w.*=*Paragonimus westermani*, *C. y.*=*Cercaria yoshidae*, *M. y.*=*Metagonimus yokogawai*, *C. a.*=*Centrocestus armatus*, *C. i.*=*Cercaria innominatum*, *P. m.*=*Pseudexorchis major*

The abbreviation in parenthesis is significant (S).

styloides, *Metagonimus yokogawai*, and *Cercaria yoshidae*. On the other hand, in experimental infection with *P. westermani* from Amakusa Is., snails collected from Nagaoka were infected with cercariae in the combination of *P. westermani* and *Pseudexorchis major*. As a result, the cercariae of the lung fluke were detected not alone, but with those of other species of natural infection, in infected snails. Similar multiparasitism with *P. westermani* and others has been reported in snails collected in endemic districts of paragonimiasis (Yoshida, 1917; Nakagawa, 1918; Ando, 1918; Yokogawa, 1952; Hamajima *et al.*, 1975). In this experimental infection, the fluke was not found in cercaria-free snails and control snails. And the infection rate of *P. westermani* became higher with an increasing infection rate of other species

(Table 1). A comparison of the results obtained in the snails collected from the three different localities shows that the infection rate of *P. westermani* more or less paralleled those of other cercariae of natural infection. These results suggest that *P. westermani* has a high infectivity to snails which have already been infected with other cercariae. When these snails were experimentally exposed to the lung flukes, the results show that the infection rate and the variety of species of other cercariae was slightly increased as compared to the control. Thus, these data imply that there is some intimate interplay between intramolluscan larvae of the lung fluke and the larvae of other cercariae for the development of the cercariae. And it seems that change of immune response and chemical composition of the snails caused by in-

fection with other cercariae may be a possible factor in determining the development of *P. westermani* cercariae.

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カワニナにおけるウェステルマン肺吸虫 (3n 型) の実験感染

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小宮ら (1961) は、ウェステルマン肺吸虫流行地のカワニナを用いた本虫の感染実験において、初めてセルカリアを得ることに成功した。しかし、カイにおける本虫の感染および発育については不明な点が多い。そこで、われわれは本肺吸虫 (3n 型) の宿主寄生体関係を明らかにするため、環境を異にする 3 地区からカ

ワニナを採集し、それらに対する本虫の感染実験を試みた。その結果、本虫のセルカリアは必ず他種セルカリアと共に見出された。このことは、カワニナに侵入した本肺吸虫が他種セルカリアとの混合感染のもとで、容易にセルカリアにまで発育することを示唆する。