SUSCEPTIBILITY OF SNAIL VECTORS TO ORIENTAL ANTHROPOPHILIC *SCHISTOSOMA*

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INTRODUCTION

Laboratory experiments were carried out to study the susceptibility of snail hosts to various species of Oriental schistosomes. Hunter *et al.*, (1952) attempted to infect *Oncomelania formosana* from Taiwan, with the Japanese strain of *Schistosoma japonicum* but was not successful. They concluded that there must be some differences between the Japanese and Taiwanese strains of parasites. This was later confirmed when it was shown that the Taiwanese strain was zoophilic (Hsü *et al.*, 1955) and did not develop to maturity in man (Hsü *et al.*, 1956). Dewitt (1954) investigated the cross-susceptibility between the four *Oncomelania* races and the Japanese, Taiwanese and Mainland Chinese strains of *S. japonicum*. Hsü *et al.*, (1960) completed the picture by testing the snails with the Philippines strain of parasites. Shao and Xu (1956) infected *O.h. hupensis* from 11 provinces of China with Puzhen and Nanjing strains of *S. japonicum*. The snail infection rates from various provinces were different. Yuan (1958) cross infected snail vectors, *O.h. hupensis* with their *S. japonicum* strains from 6 provinces of China. The results had shown that there might be different geographic strains of snails and their parasites in Mainland China. The susceptibility of *O.h. hupensis* (Taiwanese strain) to the Japanese, Chinese, Philippines and Indonesian strains of *S. japonicum* has been reported (Moose, 1963, 1964; Chiu, 1967; Hsü, 1967; Cross, 1976, 1980; Lee *et al.*, 1982). The results of these tests showed that there were differences in the infectivity of each strain of parasites for the various snail host races. Each geographic strain of *S. japonicum* infected best on the *Oncomelania* race from its own endemic area, and to a lesser degree or not at all on the snails from other endemic areas.

S. mekongi exists in lower Mekong River Basin. In contrast to *Oncomelania*, the amphibious snail hosts of *S. japonicum*, the snail host which transmits *S. mekongi*, *Tricula aperta*, is aquatic. This snail was refractory to all of the strains of *S. japonicum* (Liang, 1980). The geographic strains of *Oncomelania* from the Philippines, Taiwan and Japan, were not susceptible to *S. mekongi* (Sornmani, 1976).

S. japonicum-like eggs have been found in liver tissue of the Orang Asli (aborigines) in Peninsular Malaysia (Murugasu and Dissanaike, 1973; Leong *et al.*, 1975; Murugasu *et al.*, 1978; Kan *et al.*, 1979). Greer (1980a) and Greer *et al.*, (1980b) found small aquatic snail, *Robertsiella kaporensis*, as the intermediate host of *S. japonicum*-like species in Pahang State of Malaysia. The information about artificial infection of *R. kaporensis* and cross-susceptibility with other anthropophilic schistosomes have not yet been reported.

S. sinensium was first discovered in Mianzhu Country, Sichuan Province, China. Their cercariae were found in *Tricula humida* and

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T. gregoriana (Sun, 1959). Recently, the adult worms were discovered in the field rat in Chiangmai Province, Thailand. The snails that harboured cercariae of *S. sinensium* from Chiangmai are *Tricula bollingi*. These snails are aquatic in nature, attached themselves to the roots of the trees along the stream. The cross-susceptibility of *T. bollingi* with other strains of schistosomes have not been reported.

The aim of this study is to determine the susceptibility of snail vectors to Oriental anthropophilic *Schistosoma*, namely, *S. japonicum; S. mekongi; S. japonicum*-like from Peninsular Malaysia and *S. sinesium*.

MATERIALS AND METHODS

Laboratory-bred snails (*O.h. hupensis* from Anhui Province, China; *O.h. quadrasi* from Leyte, the Philippines; *R. kaporensis* from Kapor stream of Pahang State, Malaysia) and laboratory-reared snails (*T. aperta*, beta race, from the Mun River, Ubon Ratchathani Province, and *T. bollingi* from Chiangmai Province, Thailand) for more than 6 months were employed in these experiments. The laboratory-reared snails were determined to be free of schistosome cercariae by repeated shedding before exposure to miracidia. *T. aperta* (beta race) was obtained from non-endemic area of Thailand.

Schistosoma strains used in these experiments were as follows: *S. japonicum*, Anhui, China; *S. japonicum*, Leyte, the Philippines; *S. mekongi*, Khong Island, Laos; *S. japonicum*-like species, Pahang State, Malaysia; and *S. sinensium*, Chiangmai, Thailand.

Snail exposures were made with miracidia hatched from ova which were freed from liver and intestinal tissues of infected mice. These mice were sacrificed about 45 days after infection. Sixty snails of each group of the 5 species were infected with various strains of schistosomes. The total was 20 groups. Each snail was exposed individually to 40 freshly hatched miracidia at room temperature (25°C) and under a fluorescent light for 3 hours. After exposure, snails were transferred back to containers or aquaria which were installed in a room with the temperature of 25°-27°C.

Beginning on the 5th week after exposure, snails in the 20 exposed groups were tested for cercarial shedding. On the 6th week after exposure, *T. aperta* was noted to be shedding cercariae. All the other groups were shedding cercariae on the 7th week after exposure. On the 10th week after exposure, all groups of negative snails were crushed and observed for the presence of daughter sporocysts and cercariae. The infection rate of snails was calculated from the number of cercariae-shedding snails or crushed snails with daughter sporocysts and cercariae by total number of snails examined.

For each group of snail experimented, five snails were infected with 50 miracidia/snail for ½ hour and 3 hours, and then fixed in 10% formalin for paraffin section. The other five were infected with 10 miracidia/snail for 3 hours and kept for 14 days, then fixed in 10% formalin. The specimens were dehydrated in graded series of ethanol, infiltrated and embedded in paraffin, and sectioned at 10-12 μm thick with the rotary microtome. Paraffin serial sections of snails infected with miracidia were observed with Olympus compound microscope for the presence of miracidia and daughter sporocysts.

RESULTS

A total of 240 *O.h. hupensis*, 240 *O.h. quadrasi*, 240 *T. aperta* (beta race), 240 *R. kaporensis* and 240 *T. bollingi* were exposed to *S. japonicum* from China, from the Philippines; *S. mekongi* from Laos; *S. japonicum*-like species from Malaysia; and *S. sinensium*

Table 1

Susceptibility of snail vectors to different strains of *Schistosoma.*

Strains of schistosomes	Snail species (60 tested in each group)									
	O.h.hupensis		*O.h.quadrasi*		*R.kaporensis*		*T.aperta*		*T.bollingi*	
	No. surv.	No. infect.	No. surv.	No. infect.	No. surv.	No. infect.	No. surv.	No. infect.	No. surv.	No. infect.
S. japonicum (China)	56 (93.3)	19 (33.9)	58 (96.7)	0	48 (80)	0	51 (85)	0	55 (91.7)	0
S. japonicum (Philippines)	58 (96.7)	4 (6.9)	53 (88.3)	24 (49.1)	49 (81.7)	0	55 (91.7)	0	50 (83.3)	0
S. japonicum-like (Malaysia)	58 (96.7)	0	60 (100)	0	56 (93.3)	24 (24.9)	48 (80)	4 (8.3)	48 (80)	0
S. mekongi (Laos)	59 (98.3)	0	57 (95)	0	55 (91.7)	0	48 (80)	20 (41.7)	54 (90)	9 (16.7)
S. sinensium (Thailand)	58 (96.7)	0	57	0	41 (68.3)	0	43 (71.7)	2 (4.7)	49 (81.7)	28 (57.1)

Percentage in parenthesis.

from Thailand. The results of cross infection from different strains of schistosomes and their snail hosts are shown in Table 1. *O.h. hupensis* became infected with *S. japonicum* from China (33.9%) and the Philippines (6.9%), but were completely resistant to the development of other schistosomes. *O.h. quadrasi* became infected with *S. japonicum* from the Philippines (49.1%), but no evidence of infection was found when they were exposed to the other strains. *T. aperta* (beta race) could be infected with *S. mekongi* (41.7%), *S. japonicum*-like species, Malaysian strain (8.3%) and *S. sinensium* (4.7%). However, they were completely resistant to the development of *S. japonicum* from China and the Philippines. *R. kaporensis* were found to be susceptible only to their local species of schistosome, *S. japonicum*-like species from Malaysia (42.9%). *T. bollingi* were susceptible to *S. sinensium* (57.1%) and *S. mekongi* (16.7%), but no evidence was found when they were exposed to *S. japonicum*-like species from Malaysia.

The results of paraffin section of snails infected with various strains of schistosomes are shown in series of photomicrographs (Fig. 1-3). Fig. 1 is the series of infection and development of *S. japonicum* (Chinese strain) in the snail, *O.h. hupensis* from China. Fig. 1A shows the enter of miracidia into snail host at the head-foot region. At the end of 4 weeks, daughter sporocysts with germ balls have been formed in the hepatopancreas (Fig. 1B). Fig. 1C shows the development of daughter sporocysts after 6 weeks of infection. After 9 weeks, cercariae have been found inside the wall of daughter sporocysts (Fig. 1D).

The infection of *O.h. quadrasi* snails with *S. japonicum* (Philippines strain) is shown in Figs. 2A and 2B. Fig. 2A shows the penetration of miracidia into the head-foot region of snail. After 2 weeks, they migrated to the kidney region and developed into daughter sporocysts (Fig. 2B). Similar development was found in the infection of *T. aperta* snails

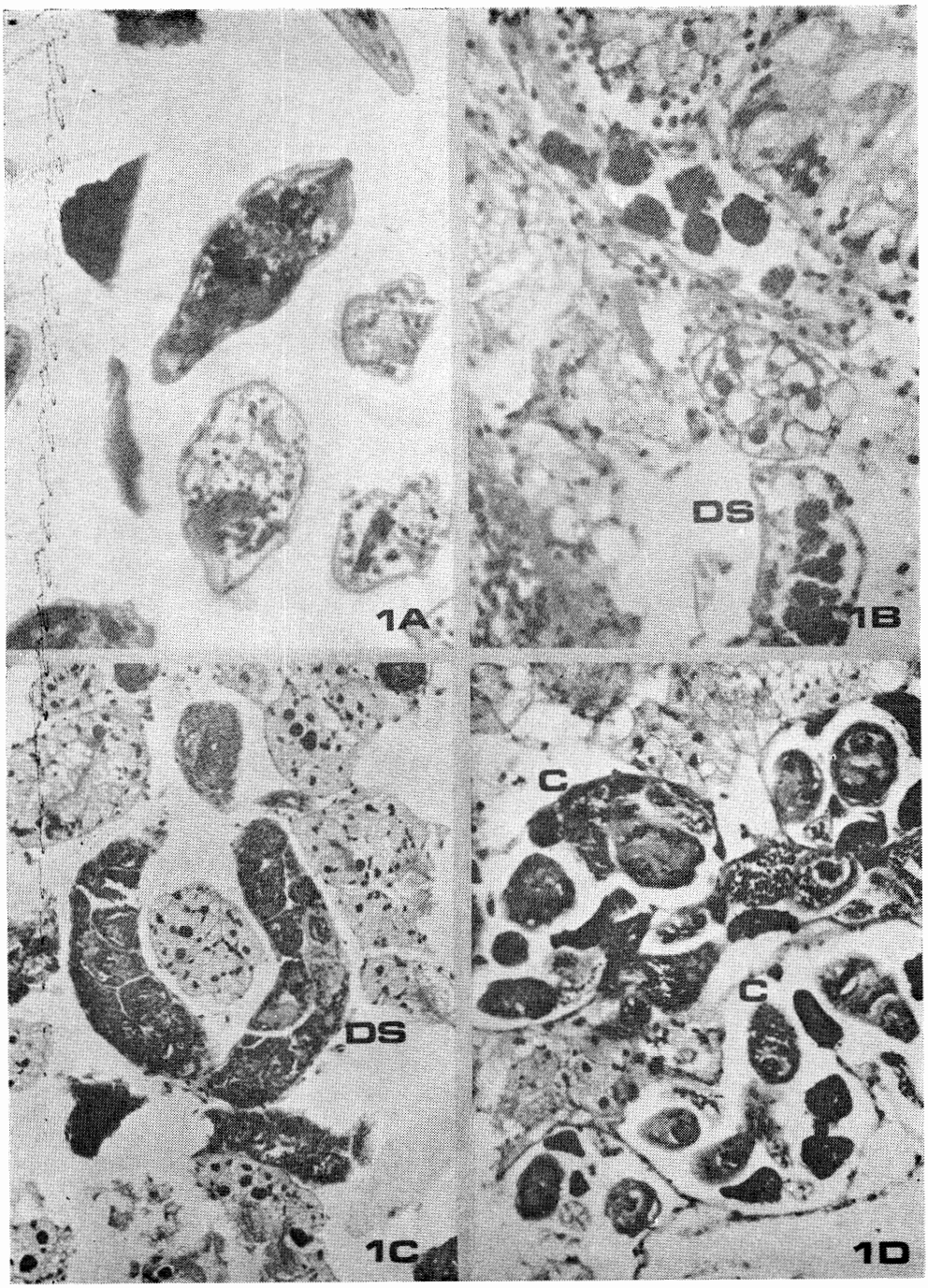
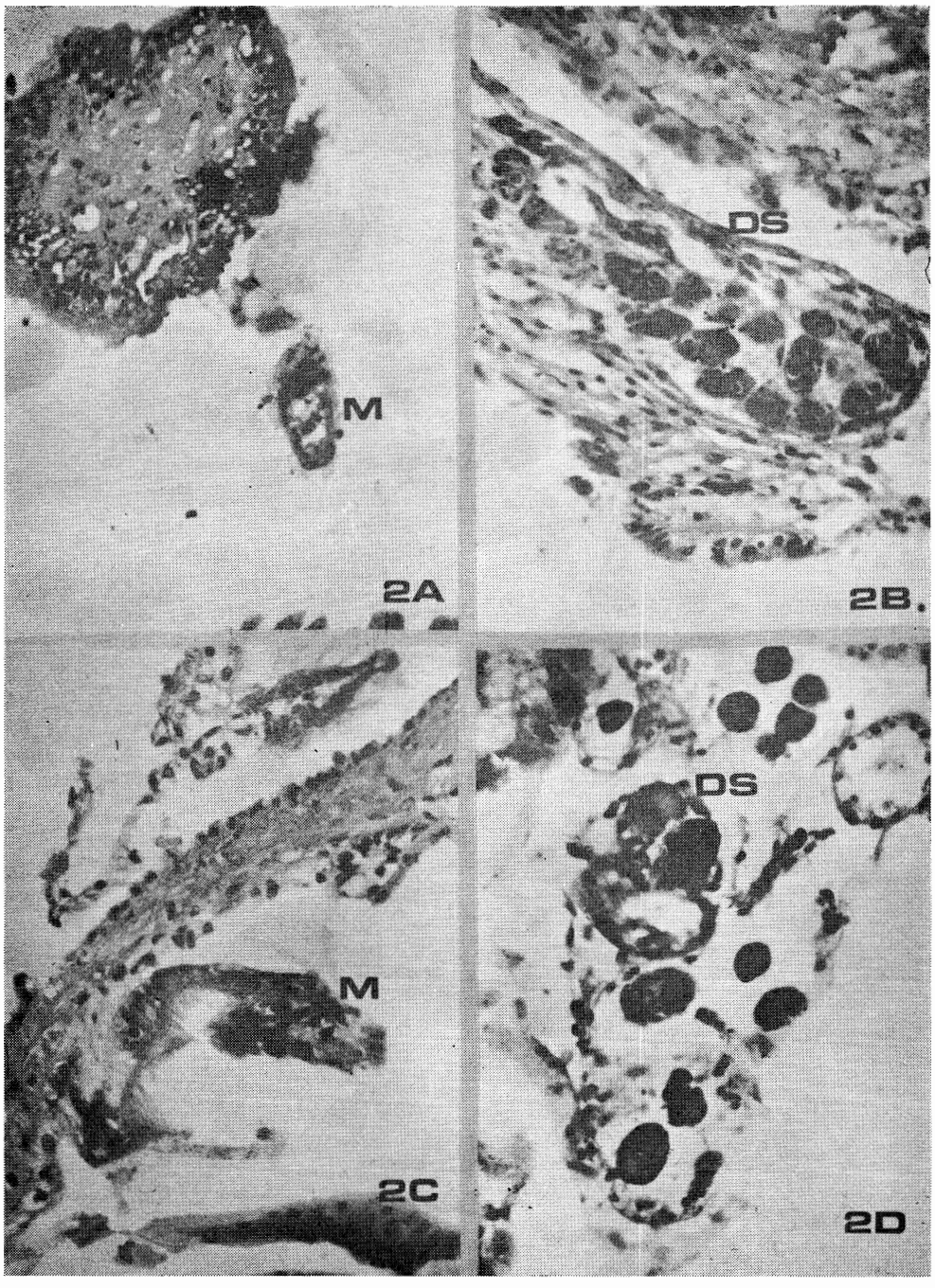


Fig. 1—The infection of *O.h. hupensis* with *S. japonicum* (China).

Fig. 1A—The penetration of miracidia of *S. japonicum* (China) into the head-foot region of snail, *O.h. hupensis*.
Fig. 1B—The development of daughter sporocyst (DS) in the snail host after 4 weeks of infection.
Fig. 1C—Daughter sporocysts (DS) in the hepatopancreas of snail.
Fig. 1D—Cercariae (C) in the wall of daughter sporocyts.



Figs. 2A, 2B—The infection of *O.h. quadrasi* with *S. japonicum* (Philippines).
Figs. 2C, 2D—The infection of *T. aperta* (beta race) with *S. mekongi.*

Fig. 2A—Miracidial penetration (M) into snail host tissue after ½ hour of infection.
Fig. 2B—Development of daughter sporocyst (DS) in snail after 2 weeks of infection.
Fig. 2C—Miracidial penetration (M) after 3 hours of infection.
Fig. 2D—Daughter sporocysts (DS) in hepatopancreas of snails after 4 weeks of infection.

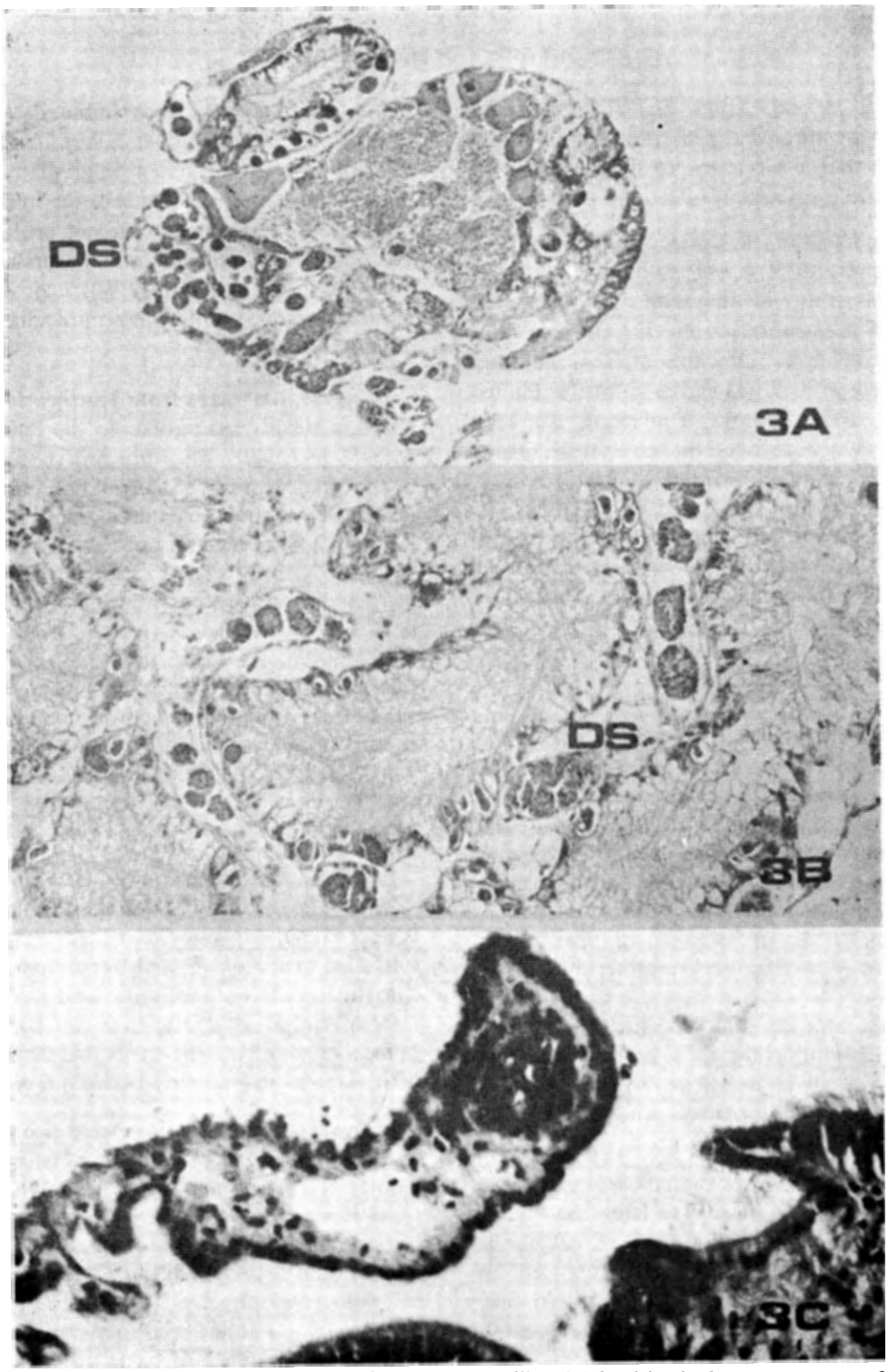


Fig. 3A—Daughter sporocysts (DS) of *S. japonicum*-like (Malaysia) in hepatopancreas of *R. kaporensis* after 4 weeks of infection.
Fig. 3B—Development of *S. japonicum* (Philippines) daughter sporocysts (DS) in snail, *O. h. hupensis* after 4 weeks of infection.
Fig. 3C—Cellular reaction of snail, *T. aperta* to miracidia of *S. japonicum* (China).

with *S. mekongi* (Figs. 2C, 2D). Fig. 2C shows the miracidial penetration and Fig. 2D shows the development of daughter sporocysts after 4 weeks of infection.

Fig. 3A shows the development of daughter sporocysts of *S. japonicum*-like species (Malaysian strain) in the snail, *R. kaporensis* after 4 weeks of infection. Fig. 3B shows the result of cross infection of *O.h. hupensis* infected with *S. japonicum* from the Philippines. After 4 weeks of infection, daughter sporocysts were found in the hepatopancreas. Fig. 3C shows the result of host response when *T. aperta* was infected with *S. japonicum* from China after 3 days of infection. Host tissues were formed around the miracidia of the parasite and no daughter sporocysts developed.

DISCUSSION

The experiments reported in this study contribute information on the compatibility of parasites and snail-host relationship of Oriental anthropophilic *Schistosoma* and their snail vectors. Among the 20 groups of snails exposed to the 5 strains of schistosomes, only 9 groups were found to have infections. Among them, five species of snails were infected with their local strains of schistosomes. The cross infection was successful in *O.h. hupensis* infected with *S. japonicum* from the Philippines, in *T. aperta* (beta race) infected with *S. japonicum*-like species from Malaysia and *S. sinensium*, and in *T. bollingi* infected with *S. mekongi*.

O.h. hupensis from China was found to be susceptible to *S. japonicum* from the Philippines with the infection rate of 6.9%. It was lower than that of 20% reported by Hsü and Hsü (1960). It is possible that *O.h. hupensis* employed in these two studies came from different provinces of China. Hsü and Hsü (1960) obtained their snails from Canton (Guangdong) Province, but in this study, the snails were obtained from Anhui Province. It could be explained that *Schistosoma* and its snail host also exhibited geographic strain differences (Yuan, 1958), hence the infection rates are not the same in different provinces. The number of miracidia for exposure is also different. Hsü and Hsü (1960) exposed 10 miracidia to one snail, while 4 miracidia were used in this study.

T. aperta, the snails from Northeast Thailand are readily susceptible to the infection with *S. mekongi*. They are not susceptible to any classical strain of *S. japonicum* complex except *S. japonicum*-like species from Malaysia (8.3%). The beta race of *T. aperta* is also susceptible to the infection with *S. sinensium* (4.7%). This further confirms that *S. mekongi* is a new species of Oriental *Schistosoma* (Voge, 1978) and *T. aperta* is quite different from *Oncomelania* species. *T. bollingi*, the snails from North Thailand are readily susceptible to *S. sinensium* (57.1%) and *S. mekongi* (16.7%). *R. kaporensis* was only susceptible to *S. japonicum*-like species from Malaysia.

Discontinuous distribution of mollusca provides the necessary geographical isolation. Wright (1960) suggested that there has been a parallel evolution of schistosome parasites with their intermediate hosts, and that this mechanism has given rise to a vast complex of local races of both the snails and the flukes with varying compatibility between them.

In this study, observations were also made on the fate of the five strains of schistosomes namely, *S. japonicum* (Chinese and Philippines strains), *S. japonicum*-like species (Malaysian strain), *S. mekongi* and *S. sinensium* in their intermediate snail hosts: *O.h. hupensis*, *O.h. quadrasi*, *R. kaporensis*, *T. aperta* and *T. bollingi*. It has been observed that the developmental stages of parasites could not be found in the nonsusceptible snail hosts, while daughter sporocysts have been found

in their susceptible snail hosts after exposure for 2-4 weeks. In some strains, destroyed parasite larvae have been observed in the nonsusceptible snail tissues with cellular reaction, but in the susceptible snails, normal developing parasites without cellular reaction were found.

SUMMARY

Laboratory experiments were carried out to study the susceptibility of snail vectors to Oriental anthropophilic *Schistosoma*. *Oncomelania hupensis hupensis* was readily infected with the local strain of *Schistosoma japonicum* (Chinese strain), and also infected with *S. japonicum* (Philippines strain). *O.h. quadrasi* was only susceptible to its *S. japonicum* (Philippines strain). The *Oncomelania* races were refractory to *S. mekongi*, *S. japonicum*-like species (Malaysian strain). *Tricula aperta* (beta race) was readily infected with *S. mekongi*, *S. sinensium* and *S. japonicum*-like species from Malaysia, but not *S. japonicum*. *T. bollingi* was susceptible to *S. sinensium* and *S. mekongi*. *Robertsiella kaporensis* was only susceptible to the local strain, *S. japonicum*-like species from Malaysia. Geographical isolation may be the cause of these differences in compatibility between the snail vectors and the schistosome parasites.

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