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SUSCEPTIBILITY OF *ONCOMELANIA HUPENSIS CHIUI* TO INFECTION WITH *SCHISTOSOMA JAPONICUM* [1]

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ABSTRACT

A small amphibious hydrobiid snail, *Oncomelania hupensis chiui* (originally described as *Tricula chiui*) from the northern tip of Taiwan, known to act as the snail host of *Paragonimus iloktsuenensis,* was found to be able to serve as a good intermediate host for both the zoophilic (Formosan-Changhua) and human (Japanese) strains of *Schistosoma japonicum*. Since the snail host (*O. h. formosana*) of *S. japonicum* in Taiwan from different locations is either refractory or only slightly susceptible to human strains of *S. japonicum* from Japan and the Philippines, this discovery of an efficient potential host brings the establishment of human schistosomiasis within the range of possibility in Taiwan.

The experiments indicated that this snail showed a varying degree of susceptibility to the 2 geographic strains of *S. japonicum* and is, on the whole, a more suitable host for the Changhua strain than for the Japanese strain. As high an infection rate as 100% was obtained for the Changhua strain of *S. japonicum,* when cercariae were shed from the snails 95 days after exposure to 6 miracidia individually. Interesting results were obtained for the Japanese strain. Snails exposed in pairs to 20 miracidiá were 100% infected but did not produce infective cercariae. Only the snails exposed to 5-7 miracidia individually, and infected at the rate of 22.2%, shed cercariae 105 days after infection.

INTRODUCTION

In the course of a study on the trematode genus *Paragonimus* in Taiwan, a small amphibious hydrobiid snail was incriminated as the first intermediate host of *P. iloktsuenensis* (Chiu, 1961, 1965b). This snail was subsequently named *Tricula chiui* by Habe & Miyazaki in 1962. Habe & Miyazaki also stated that the species was allied to *Oncomelania formosana,* the snail host of *Schistosoma japonicum* in Taiwan. This raised the question of whether each of these snails would prove susceptible to the trematode parasite of the other. As a result, it was found that the snail host of the lung fluke could indeed also carry the blood fluke (Chiu, 1965a). While investigations were in process, a systematic malacological study was made on this snail by Dr. G. M. Davis. He came to the conclusion that the so-called *Tricula* was in fact *Oncomelania* (Davis, 1968). Since he considers all so-called "species" of *Oncomelania* as subspecies of *O.*

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hupensis, this new species is now called *O. hupensis chiui*.

The present paper gives further detail on the experimental infection of this snail with the non-human Formosan-Changhua strain of *S. japonicum* and, in addition, reports susceptibility to the human Japanese strain of schistosome.

MATERIALS AND METHODS

Oncomelania hupensis chiui (Habe et Miyazaki) were collected from the type locality, Alilao village, Taipei County (northern tip of Taiwan) where schistosomes have not been found. The eggs of the zoophilic Changhua strain (central part of the western coastal plain of Taiwan) of *Schistosoma japonicum*, used for infecting the snails, were obtained from the liver of a rabbit and a mouse that had been experimentally infected and kept in the laboratory for 40-50 days after infection. Eggs of the human Japanese strain of *S. japonicum* were secured from the liver of a mouse with a 39 day old infection. The infected *Oncomelania nosophora* used for infecting the mouse were obtained from the 406th Medical Laboratory in Japan. These snails were experimentally infected with a strain of *S. japonicum* originally from Yamanashi, Japan.

Mature *Oncomelania hupensis chiui* snails collected from the field were exposed to miracidia either individually or in pairs in small glasses, allowing 3-5 hours for penetration at $26^{\circ} \pm 2^{\circ}C$. After exposure, the snails were kept in clay flower-pots in the laboratory as previously described (Chiu, 1965b) at room temperatures varying between 15° and $32^{\circ}C$. Snails were crushed at different times after exposure to miracidia, to determine infection. Cercarial emergence was checked by isolating snails in a small glass of water for 5 hours in the morning.

EXPERIMENTS

Experiment 1.

A preliminary experiment designed to investigate the susceptibility of *Oncomelania hupensis chiui* to infection with the Changhua strain of *Schistosoma japonicum* was made as follows: 25 snails were exposed individually to 2-3 miracidia for 5 hours on April 9, 1964. The miracidia were hatched from eggs obtained from the liver of a rabbit which had been infected in the laboratory with cercariae of *S. japonicum* shed from naturally infected snails. The results obtained from this experiment have been partly reported before (Chiu, 1965a).

Upon 1st examination, 79 days after infection (Table 1), schistosome sporocysts were found in both snails dissected. These sporocysts contained embryos in various stages. Immature cercariae were seen within some daughter sporocysts. Mature cercariae were found emerging from the snails 95 days after exposure to miracidia, and 2 snails crushed contained sporocysts and cercariae. Similar positive findings were made on the 121st day after infection, in 1 of 3 snails dissected and on the 127th as well as on the 153rd days in 2 of 3 snails crushed. Cercarial emergence was still observed among the 5 surviving snails on the 243rd day after infection. However, on the 282nd day, the 3 surviving snails failed to shed the cercaria and these snails were found dead a month later.

In summary, 9 of 13 snails crushed (69.2%) were found infected with the schistosomes. This experiment showed that *O. h. chiui* is readily infected with the Changhua strain of *Schistosoma japonicum*.

The infectivity of the cercariae shed from *Oncomelania hupensis chiui* was confirmed by means of animal infection.

TABLE 1. Examination of *Oncomelania hupensis chiui* snails exposed to 2 - 3 miracidia each of the Changhua strain of *Schistosoma japonicum*

Date examined 1964-1965	Days after infection	No. snails examined*	No. snails infected	Larval stages found
June 27	79	2	2	Sporocysts
July 13	95	(21)	(Shedding)	Cercariae
		2	2	Sporocysts & Cercariae
Aug. 8	121	3	1	Sporocysts & Cercariae
Aug. 14	127	3	2	Sporocysts & Cercariae
Sept. 9	153	3	2	Sporocysts & Cercariae
Oct. 30	204	(9)	(Shedding only)	Cercariae
Dec. 8	243	(5)	(Shedding only)	Cercariae
Jan. 16	282	(3)	(Shedding only)	(-)
Total dissected		13	9 (69.2%)	

*Numbers in parentheses designate snails that were not dissected.

TABLE 2. Examination of *Oncomelania hupensis chiui* snails exposed to 6 miracidia each of the Changhua strain of *Schistosoma japonicum*

Date examined 1964	Days after infection	No. snails examined	No. snails infected	Larval stages found
Sept. 20	27	1	1	Sporocysts
Oct. 4	41	1	1	Sporocysts
Nov. 27	95	(6)	(Shedding only)	Cercariae
Dec. 7	105	6	6	Sporocysts & Cercariae
Total dissected		8	8 (100%)	

Three mice were exposed to an undetermined number of cercariae from 2 positive snails for 2 hours, and at autopsy, 40 days later, adult *Schistosoma japonicum* were collected.

Experiment 2.

Another experiment, with heavier exposure to miracidia, was made to gain further insight concerning the infectivity of *Oncomelania hupensis chiui* as regards the Changhua strain of *Schistosoma japonicum* in the laboratory. Fifteen snails were exposed individually to 6 miracidia for 4 hours on August 24, 1964. The miracidia were hatched from eggs obtained from the liver of a mouse infected in Experiment 1.

Upon dissection of 1 snail each on the 27th day and the 41st day after infection, sporocysts were encountered in both (Table 2). On the 95th day, cercarial shedding was demonstrated in the 6 surviving snails. Ten days later, these were dissected, and all 6 were found positive for sporocysts and cercariae.

The infection rate among those snails that had survived in this experiment was 100%. This high rate indicates that *Oncomelania hupensis chiui* pos-

TABLE 3. Examination of 2 groups of *Oncomelania hupensis chiui* snails exposed to different number of miracidia of the Japanese strain of *Schistosoma japonicum*

Date examined 1965	Days after infection	A exposed to 5-7 miracidia each			B exposed to 10 miracidia each on the average		
		No. exam.	No. infect.	Larval stages found	No. exam.	No. infect.	Larval stages found
March 20	4	-	-		1*	?	(-)
March 22	6	-	-		1*	?	(-)
March 25	9	-	-		1	1	Sporocysts
April 8	23	3	1	Sporocysts	2	2	Sporocysts
April 15	30	1	1	Sporocysts	1	1	Sporocysts
April 22	37	-	-		1	1	Sporocysts
May 4	49	2	1	Sporocysts	-	-	
May 11	56	7	1	Sporocysts	1	1	Sporocysts
May 18	63	2	1	Sporocysts	-	-	
May 27	72	-	-		1	1	Sporocysts
June 19	95	(30)	(Shedding)	(-)	(10)	(Shedding)	(-)
June 29	105	(20)	(Shedding)	Cercariae	(7)	(Shedding)	(-)
July 5	111	5	2	Sporocysts & Cercariae	1	1	Sporocysts
July 12	118	20	3	Sporocysts & Cercariae	1	1	Sporocysts
Sept. 6	174	5	0	(-)	2	2	Sporocysts & degenerating cercariae
Totals dissected		45	10 (22.2%)		11*	11 (100%)	

*The two snails examined as early as the 4th and 6th days after infection are excluded from the total.

sesses a high degree of susceptibility to infection with the Changhua strain of *Schistosoma japonicum*. The snail should be an excellent host for maintaining the life cycle of the Changhua strain of *S. japonicum* in the laboratory unless the schistosome should adapt specifically to *O. h. chiui*.

Experiment 3.

The susceptibility of *Oncomelania hupensis chiui* to the Japanese strain of *Schistosoma japonicum* was tested on 2 groups of snails, A and B, using 2 different doses of miracidial exposure: 75 snails in Group A were exposed individually to 5-7 miracidia, and 42 snails in Group B were exposed in pairs to 20 miracidia, for 3 hours, on March 16, 1965.

Sporocysts were first detected in the internal organs of a snail from Group B 9 days after infection (Table 3), none having been encountered in the 2 snails examined earlier. However, these 2 snails were very possibly infected, since

TABLE 4. Infection percentages of *Oncomelania** from different geographic locations with 4 strains of *Schistosoma japonicum* reported by various workers

Species of *Oncomelania*	*Schistosoma japonicum* strain			
	Chinese	Japanese	Philippine	Formosan (Changhua)
	%	%	%	%
O. h. hupensis (China)	34 (D)	13 (D)	20 (HH)	0 (D)
O. h. nosophora (Japan)	0 (D)	21 (D) 44.4 (HRO) 35.7-43.8 (MW)	9.6 (HH)	21 (D)
O. h. quadrasi (Philippines)	0 (D)	0 (D)	44-75 (P) 28.7-45.0 (MW)	6.4 (D)
O. h. formasana (Taiwan)				
- Changhua	0 (D)	0 (D) 0.8 (HRO)	0 (HH)	35 (D) 18.0-36.2 (MW)
- Ilan	-	5.6 (MW)	5 (MW)	1 (MW)
- Kaohsiung	-	0 (MW)	0 (MW)	0-1.8 (MW)
O. h. chiui (Taiwan)				
- Alilao	-	22.2-100 (C)	-	69.2-100 (C)

*The "species" of *Oncomelania* are here all considered to be subspecies of *O. hupensis*.

Abbreviations:

(D) = DeWitt, 1954;
(HRO) = Hunter, Ritchie & Otori, 1952;
(MW) = Moose & Williams, 1963, 1964;
(P) = Pesigan et al., 1958;
(C) = Chiu, this paper;
(HH) = Hsü & Hsü, 1960.

a 100% infection rate was later shown to prevail in this heavily exposed group. The negative finding suggests that schistosome larvae may stay in the head-foot muscle for a while after penetration. Lower infection rates (22.2% on the average) were observed in Group A, exposed to fewer cercariae. Sporocysts were discovered in 5 out of 15 snails dissected 23-63 days after infection. On the 95th day, shedding failed to occur in both groups. On the 105th day, cercariae were shed by snails of Group A, but not of Group B. Six days later, 2 of 5 snails crushed in Group A harbored sporocysts and cercariae, while 1 snail examined in Group B still harbored nothing but sporocysts. On the 118th day, 3 of 20 snails dissected in Group A were found infected, but only 1 snail harbored cercariae, the other 2 merely sporocysts. In a snail from Group B, again only sporocysts were detected on the 111th and 118th days. The 7 surviving snails were dissected on the 174th day. None of 5 snails crushed in Group A were infected with schistosomes. In contrast, the 2 surviving snails from Group B were parasitized with sporocysts and a few cercariae, but these were degenerated and apparently non-infective.

In summary, 10 of 45 snails dissected (22.2%) were infected with the parasite in Group A. On the other hand, although presumably a 100% infection rate obtained in Group B, no snail was capable of producing infective cercaria. It was also noted that the snail death rate was significantly higher in Group B (69%) than in Group A (40%). These observations, as compared with those for the Changhua strain of *Schistosoma japonicum*, suggest that *Oncomelania hupensis chiui* is a less suitable host for the Japanese than for the Changhua strain of *S. japonicum*. The infectivity of schistosome cercariae of the Japanese

strain shed from *Oncomelania hupensis chiui* was also confirmed by animal infections. Two mice were exposed to an undetermined number of cercariae for 2 hours, and at autopsy, 42-49 days after infection, adults of *Schistosoma japonicum* were recovered.

DISCUSSION

It is well known that oncomelanid snails from various geographic locations possess a varying degree of susceptibility to infection with different strains of *Schistosoma japonicum* (Hunter et al., 1952; DeWitt, 1954; Pesigan et al., 1958; Hsü & Hsü, 1960; Moose & Williams, 1963, 1964). The knowledge available is summarized in Table 4. It is seen that *Oncomelania hupensis hupensis* from China was susceptible to the Chinese, Japanese and Philippine strains of *S. japonicum*, but refractory to the Formosan-Changhua strain; *O. h. nosophora* from Japan was susceptible to the Japanese, Philippine and Changhua schistosome strains, but not to the Chinese strain; *O. h. quadrasi* from the Philippines to the Philippine and Changhua strains, but not to the other 2 strains; *O. h. formosana* from Changhua, Taiwan, was susceptible to the Changhua strain, was faintly infected by, but an unsuitable host for, the Japanese schistosome strain, and refractory to the other 2 strains. Recent findings by Moose & Williams (1963, 1964) indicate that *O. h. formosana* from Ilan, in north eastern Taiwan, another endemic area for *Schistosoma japonicum*, recently discovered by Kuntz (1965), was relatively susceptible to the Japanese and Philippine strains of schistosome, but exceedingly resistant to the Changhua strain; whereas snails from Kaohsiung, in southern Taiwan, were altogether unsuitable as hosts and resistant to the Changhua, Japanese and Philippine strains.

The author (1965a) has already reported the fact of susceptibility of *"Tricula (=Oncomelania) chiui"* to infection with the Changhua strain of *Schistosoma japonicum*. In the present study, *Oncomelania hupensis chiui* was not only confirmed as a good potential intermediate host for the Changhua strain but also found capable of transmitting the Japanese strain of *S. japonicum*. In other words, the snail can serve as an efficient host for both the non-human and human strains of *S. japonicum*. For the Changhua strain of *S. japonicum* an infection rate of 100% could be obtained. The results for the Japanese strain were interesting, in that moderately heavy exposure (5-7 miracidia per snail) resulted in functional infection at the rate of 22.2%, whereas, with heavier exposure (an average of 10 miracidia per snail), even though the infection rate amounted to 100%, not a single mature infective cercaria could be found throughout the 5 month duration of the experiment. This interrelationship between the cercaria producing capacity of *O. h. chiui* and the number of miracidia to which it was exposed remains to be understood. The results of this study have further demonstrated that *O. h. chiui* also showed a varying degree of susceptibility to 2 geographic strains of *S. japonicum*, as do the other oncomelanid snails, i.e., at approximately equal exposure, infection rates were about 100% for the Changhua strain of *S. japonicum* and 22% for the Japanese schistosome (Tables 2 and 3A). The variety in response now realized to exist in local strains of *O. h. formosana* - from refractive to the indigenous non-human schistosome to slightly susceptible to the foreign human schistosomes - and the discovery of the new subspecies *O. h. chiui*, susceptible to both these schistosomes, already entails a revision of our former views, in particular of the view that Taiwan was safe from the threat of human schistosomiasis, because there was no potential snail host for the human strain of *S. japonicum*. No doubt further investigation will provide further evidence of variation in the snail-parasite relation-

ship.

Among other related hydrobiid snails, DeWitt (1954) reported that the North American *Pomatiopsis lapidaria* was capable of infection with the Chinese (1%) and the Changhua (3%) strains of *Schistosoma japonicum*.

It is also of interest to note that Hunter & Abbott (1949) reported on an infection experiment with *Tricula minima* from Japan and the Japanese strain of *Schistosoma japonicum*. They found that the miracidia could not develop to the cercarial stage in that snail; only a few degenerating mother sporocysts were discovered 7-71 days after infection.

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ADDENDUM

Further experiments have indicated that *Oncomelania hupensis chiui* is also susceptible to infection with the Formosan-Ilan, Japanese-Kurume, Philippine and Chinese strains of *Schistosoma japonicum*, at the rates of 56.5-100%, 87.5-100%, 43.8-100% and 66.7-87.0% respectively. The exposure per snail ranged from 1 to 15 miracidia.

RESUMEN

SUSCEPTIBILIDAD DE *ONCOMELANIA HUPENSIS CHIUI* A LA INFECCION POR *SCHISTOSOMA JAPONICUM*

Jui-Kuang Chiu

Se ha descubierto que el pequeño caracol hydróbido, anfibio, *Oncomelania hupensis chiui* (descripto originalmente como *Tricula chiui*) del extremo norte de Taiwan (Formosa), conocido como huésped de *Paragonimus iloktsuenensis*, es también capaz de servir de intermediario tanto para la raza zoofílica (Formosa-Changhua), como para la raza que ataca al hombre (japonesa) de *Schistosoma japonicum*. Desde que el caracol huésped (*O. h. formosana*) de *S. japonicum* en Taiwan de diferentes localidades, es refractario o muy ligeramente susceptible a las razas de infección humana de *S. japonicum*, este descubrimiento de un huésped potencialmente eficiente, trae la esquistosomiasis humana dentro del área de posible establecimiento en Taiwan.

Los experimentos indicaron para este caracol un grado variable de susceptibilidad a las dos razas geográficas de *S. japonicum*, y es, en general, un huésped más adaptable a la raza Changhua que a la japonesa. El linaje Changhua mostró una proporción infecciosa del 100%, y los caracoles libraron cercarias 95 dias después de ser expuestos a 6 miracidios por individuo. Resultados interesantes se obtuvieron del linaje japonés. Caracoles expuestos en parejas a 20 miracidios, fueron 100% infectados pero no produjeron cercarias infecciosas. Sólo aquellos expuestos individualmente a 5-7 miracidios, infectados en una proporción del 22.2% libraron cercarias 105 dias después de la infección.

АБСТРАКТ

ВОСПРИИМЧИВОСТЬ *ONCOMELANIA HUPENSIS CHIUI* К ЗАРАЖЕНИЮ *SCHISTOSOMA JAPONICUM*

ДЖУ-КУАНГ-ШИУ

Мелкая амфибийная гидробия *Oncomelania hupensis chiui* (первоначально описанная как *Tricula chiui*) с северной оконечности Тайваня известна как промежуточный хозяин *Paragonimus iloktsuenensis;* было найдено также, что она может служить хорошим промежуточным хозяином как для зоофильного штамма (из формозы-Чангуа), так и для человеческого (японского) *Schistosoma japonicum.* Поскольку улитка-хозяин (*O. h. formosana*) паразита *S. japonicum*б из различных мест Тайваня являесся устойчивой или лишь слабо-восприимчивой к человеческой форме *S. japonicum* из Японии и филиппин, открытие весьма эффективного потенциального хозяина *Schistosoma* позволяет ожидать развития человеческого шистозомиазиса на Тайване.

Эксперименты показывают, что этот моллюск имеет различную степень восприимчивости в двум географическим штаммам *S. japonicum* и, в целом, является более подходящим хозяином для штамма из Чангуа, чем для японского штамма. 100% заражение было получено для штамма *S. japonicum* из Чангуа, когда церкарии выходили из моллюсков через 95 дней после индивидуального заражения их 6 мирацидиями каждый. Интересные результаты были получены для японского штамма. Улитки заражались попарно 20 мирацидиями и давали 100% заражение, но не давали заражающих церкарий. Улитки, зараженные 5-7 мирацидиями индивидуально и давшие только 22.2% заражения, давали церкарии через 105 дней после заражения.