CHARACTERISTICS OF DIFFERENT ISOLATES OF *SCHISTOSOMA JAPONICUM* FROM CHINA IN THE FINAL HOSTS

He Yi-Xun, Hu Ya-Qing, Yu Qi-Fang and Tang Zhi-Jian

Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, WHO Collaborating Centre for Malaria, Schistosomiasis and Filariasis, Shanghai 200025, China.

Abstract. The present paper deals with the susceptibility of common laboratory animals, such as mouse, rat, hamster, jird, rabbit and rhesus monkey, to infection with different isolates of *Schistosoma japonicum* in the mainland of China under laboratory conditions. With the exception of the rat, all the animals under study were permissive hosts for different isolates though their worm recovery rates varied. The mean body length of pair-worms of the Yunnan isolate was considerably smaller than that of the Anhui, Hubei, Guangxi and Sichuan isolates, and the percentage of male specimens with 7 testes in the Yunnan isolate was also significantly less than that in the other 4 isolates. Judging from the egg index (width/length × 100), the eggs of the Sichuan isolate were broad and short in shape, giving a high index; those of Guangxi and Hubei isolates were oblong, giving the lowest index; the other two isolates from Yunnan and Anhui, lay between these two extremes. The mean prepatent periods were longer in mice, hamsters and rhesus monkeys infected with Yunnan and Guangxi isolates, than those with Sichuan isolate. A dendrogram of the 5 isolates of *S. japonicum* was constructed on the basis of similarity coefficients by means of fuzzy cluster analysis on the biological characters mentioned above. Our results provide evidence of the existence of different strains of *S. japonicum* in the mainland of China as shown by comparative studies of their characteristics in the final hosts.

INTRODUCTION

A number of criteria, such as host specificity, size of worm and egg, number of testes, prepatent period, in addition to snail susceptibility have been used to characterize strain differences in *Schistosoma japonicum* (Hsü and Hsü, 1958a, b; 1960; Chiu and Kao, 1973; Chiu and Lu, 1974; Cross 1976, 1978). Little is known about the characteristics in the definitive hosts of schistosomes isolated from various localities in the mainland of China. We have tried, therefore, to tackle the problem by comparing the morphometric and biological aspects of *S. japonicum* isolated from Anhui, Hubei, Guangxi, Sichuan and Yunnan, which were selected on the basis of a previous investigation showing different degree of snail-parasite in compatibility (He *et al,* 1991).

MATERIALS AND METHODS

Source of schistosome cercariae

Naturally infected *Oncomelania hupensis* were collected from the following five localities: (1) Guichi in Anhui (A), at the lower reaches of the Yangtze River in the east; (2) Jianli in Hubei (H), at the middle reaches of the Yangtze River in the middle; (3) Guiping in Guangxi (G), a karst land in the south; (4) Tianquan in Sichuan (S), a mountainous region in the west; (5) Eryuan in Yunnan (Y), a high mountainous region in the southwest. Cercaria shedding was stimulated by fluorescent lights and these cercariae were coded isolate A, H, G, S, and Y respectively.

Infection and autopsy of animals

Six kinds of animal including C57BL inbred mouse, albino rat, hamster, Mongolian jird, rabbit and rhesus monkey were used in this study. Animals were percutaneously exposed to cercariae obtained from pools of naturally infected snails from five localities. The number of cercariae from different isolates of *S. japonicum* used for exposure to each animal was as follows: 40 cercariae for mouse; 80 for rat, hamster and jird, 150 for rabbit and 200 for rhesus monkey. All animals infected with different isolates were sacrificed 42 days post-infection and portal perfusion of infected animals was performed by the conventional method in our Institute. Worms were collected, counted, fixed in 5% neutral formalin and the body length of pair-worms was measured. Subsequently, they were stained with acid carmin for morphological studies.

Stool examination

For the determination of the prepatent period, the animals were separated individually from the 32nd day after exposure onward. Feces passed by each animal were collected daily. The feces were then macerated in 0.9% saline and then concentrated by sedimentation several times until the supernatant fluid became clear. Daily stool examination for eggs or hatching method for miracidia of *S. japonicum* was carried out. The day prior to the detection of eggs in the stools of mice or hatching of miracidia in hamsters, jirds, rabbits and rhesus monkeys was regarded as the last day of the prepatent period.

Two hundred mature eggs from the feces on 41 days after infection were measured from each species of host infected with one isolate of *S. japonicum.*

The statistical analyses of the results was done by oneway analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Worm recovery

The worm recovery of different isolates of *S. japonicum* in 6 kinds of animal species on 42 days post-infection is shown in Table 1. The mean percentage worm return in rats infected with five isolates was markedly lower than that of the other animals and worms recovered were inmature, indicating that the rat is non-permissive host for different isolates of *S. japonicum* in the mainland of China. With the exception of the rat, all the animals under study were permissive hosts for different isolates of *S. japonicum,* though their worm recovery rates varied with different isolates.

Size of adult worms

Results showed that the mean length of mature pair-worms from Yunnan isolate was considerably smaller than that of the other 4 isolates from the mice, hamsters, jirds, rabbits and rhesus monkeys. On applying the analysis of variance, this difference was significant at $p < 0.05$ (Figs 1, 2).

Number of testes

The testes in male *S. japonicum* varied from 2 to 13, the majority being 7 in number. The percentages of the specimens with 7 testes in the different isolates from the mice, hamsters, jirds, rabbits and rhesus monkeys are shown in Fig 3. The analysis of variance showed that the percentage of specimens with 7 testes in the Yunnan isolate from the five permissive hosts was significantly less than that in other 4 isolates ($p < 0.01$).

Size and shape of the eggs

Comparison was made with mature eggs of *S. japonicum* from the feces of the five permissive hosts. It was found that the size and shape of the eggs varied not only in different host species infected with the same isolate of the parasite, but also among host individuals of the same species infected with the same isolate of parasite. Regarding the size (length × width) of egg in mouse and hamster, large size eggs were found from both Yunnan and Guangxi isolates, while in rabbit and rhesus monkey, large size eggs were found from Sichuan parasites ($p < 0.05$). Judging from the index defined as ratio of egg width/length × 100, the eggs of the Sichuan isolate were broad and short in shape, going a high index. Those of Guangxi and Hubei isolates were oblong, giving the lowest index (Table 2). The other two isolates from Yunnan and Anhui, lay between these two extremes.

Prepatent period

The prepatent period of five isolates of *S. japonicum* has been determined in mice, hamsters, jirds, rabbits and rhesus monkeys (Table 3). The mean prepatent periods in different host species were 35.0 ± 0.8 to 36.4 ± 1.0 days for the Anhui isolate; 34.5 ± 1.2 to 36.4 ± 1.2 days for the Hubei isolate; 34.5 ± 1.3 to 35.8 ± 0.6 days for the Sichuan isolate; 35.1 ± 1.0 to 37.3 ± 1.9 days for the Guangxi isolate and 36.1 ± 1.9 to 37.8 ± 0.8 days for the Yunnan isolate. In general, the prepatent periods were longer in the mice, hamsters and rhesus monkeys infected with Yunnan and Guangxi isolates, than those with Sichuan isolate.

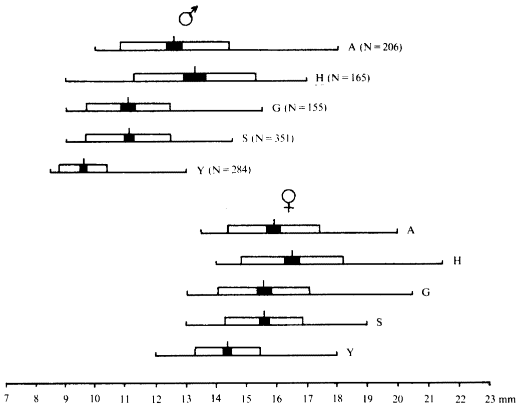

Fig 1—Body length of pair-worms in different isolates of *S. japonicum* in C57BL mice from the mainland of China. N is the number of statistics in each sample. In each sample the horizontal line indicates the range of measurements; the cross-bar, the mean; the hollow rectangle, 1 standard deviation on each side of the mean; the solide rectangle, 2 standard errors on each side of the mean.

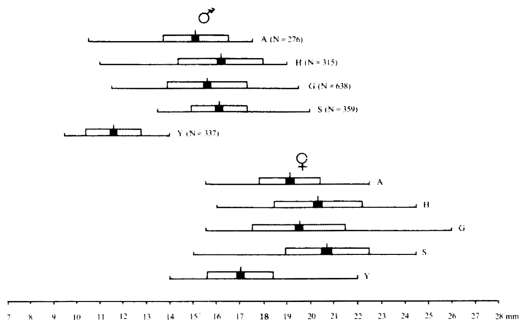

Fig 2—Body length of pair-worms in different isolates of *S. japonicum* in rabbits from the mainland of China. Range, mean, standard deviation and standard error are indicated as in Fig 1.

According to the results reported by Hsü *et al* (1958a,b, 1960) and Chiu *et al* (1973), the mean prepatent period was 42-43 days in albino mice and hamsters infected with Chinese strain of *S. japonicum* provided from Dr Vogel. It indicated that the prepatent period of the strain of *S. japonicum* defined by Dr Vogel as a Chinese strain had been originated from the cercariae in

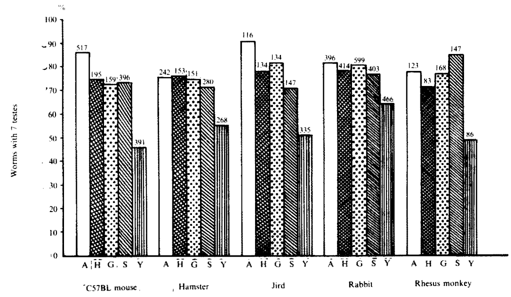

Fig 3—Percentages of male worms with 7 testes in different isolates of *S. japonicum* from the mainland of China. Numbers in each isolate are male worms observed.

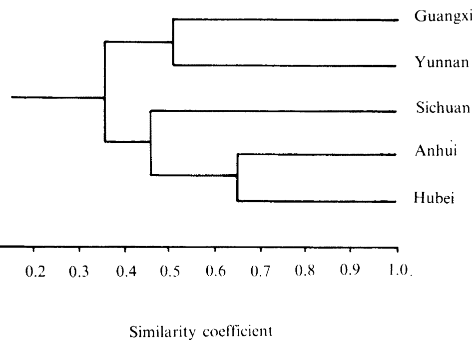

Fig 4—Dendogram showing the relationships of some biological characteristics from 5 different isolates of *S. japonicum* in the mainland of China according to cluster analysis.

infected *Oncomelania hupensis* snails from Jiaxing, Zhejiang (Kashing, Chekiang), China, and established in the Tropeninstitut in Hamburg, since 1937 by repeated passing in dogs and laboratory bred *O. hupensis* snails, was 5-8 days longer than that of all the isolates in our study, possibly due to some behavioral change. We suggest therefore that the stock of *S. japonicum* of Vogel should be defined as "Chinese Vogel strain".

Fuzzy cluster analysis of characteristics of different isolates in the final hosts

Computerized numerical systematics was used to analyse the 5 different isolates of *S. japonicum* in the mainland of China by means of the fuzzy

Table 1

Mean of worms recovered from 6 species of animal infected with different isolates of *S. japonicum* in the mainland of China.

Animal species	Cercariae exposed per animal	Anhui		Hubei		Guangxi		Sichuan		Yunnan	
		$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$	%
C57BL mouse	40	30.3(45)*	75.7	32.9(38)	82.2	23.1(45)	57.8	33.9(51)	84.8	27.9(41)	69.6
Rat	80	22.2(10)	27.8	23.2(5)	29.0	13.0(8)	16.3	11.2(5)	14.0	10.4(24)	13.0
Hamster	80	62.4(11)	78.0	59.6(7)	74.5	64.5(15)	80.6	64.0(9)	80.0	57.4(19)	71.7
Jird	80	59.7(6)	74.6	58.5(8)	73.1	63.7(10)	79.6	65.8(13)	82.3	62.2(14)	77.8
Rabbit	150	120.3(8)	80.2	121.2(5)	80.8	109.6(5)	73.1	110.0(8)	73.3	104.0(10)	69.3
Rhesus monkey	200	148.8(4)	74.4	140.0(3)	70.0	110.2(6)	55.1	156.5(4)	78.3	71.8(6)	35.9

* Numbers in parentheses are animal sacrificed

Table 2

Egg index from different isolates of *S. japonicum* in the mainland of China* ($\bar{X} \pm SD$)

Isolate	C57BL mouse	Hamster	Jird	Rabbit	Rhesus monkey
Anhui	72.5 ± 6.3	73.5 ± 4.7	74.7 ± 6.2	74.9 ± 21.2	74.6 ± 5.9
Hubei	69.8 ± 6.7	72.0 ± 4.8	72.3 ± 5.7	71.9 ± 4.6	75.7 ± 5.2
Guangxi	66.8 ± 8.2	72.8 ± 8.5	72.4 ± 8.3	72.1 ± 8.0	73.1 ± 8.0
Sichuan	75.0 ± 7.0	75.1 ± 6.6	75.5 ± 7.9	73.8 ± 5.6	77.3 ± 7.1
Yunnan	73.6 ± 7.6	74.2 ± 7.7	73.2 ± 9.2	71.7 ± 6.9	75.5 ± 8.3

* Egg index is defined as the ratio of egg width/length × 100

Table 3

Prepatent period of various hosts infected with different isolates of *S. japonicum* in the mainland of China.

Host	Cercariae exposed per animal	Anhui(A)	Hubei(H)	Guangxi(G)	Sichuan(S)	Yunnan(Y)
		$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
C57BL mouse	40	36.3 ± 2.3(39)*	36.4 ± 1.0(26)	37.1 ± 1.6(41)	34.8 ± 1.6(42)	36.6 ± 1.6(41)**
Hamster	80	36.4 ± 1.0(7)	36.4 ± 1.2(7)	37.3 ± 1.9(12)	35.2 ± 1.0(10)	37.6 ± 1.4(23)***
Jird	80	36.4 ± 0.9(8)	36.1 ± 1.1(8)	35.1 ± 1.0(10)	35.4 ± 1.6(11)	36.1 ± 1.9(14)
Rabbit	150	35.1 ± 1.8(9)	35.0 ± 1.7(10)	36.3 ± 1.7(10)	35.8 ± 0.6(10)	36.5 ± 2.1(20)
Rhesus monkey	200	35.0 ± 0.8(4)	34.5 ± 1.2(3)	37.0 ± 1.6(6)	34.5 ± 1.3(4)	37.8 ± 0.8(5)****

* Numbers in parentheses are animal examined
** (S) VS. (G), (Y), (A), (H) $p < 0.01$
*** (S) VS. (G), (Y) $p < 0.01$
**** (S), (A), (H) VS. (G), (Y) $p < 0.01$

clustering method with 32 quantitative data of each isolate about the morphometric and biological characters mentioned above. A dendrogram of the 5 isolates of *S. japonicum* was constructed on the basis of similarity coefficients as shown in Fig 4. The phylogenetic relationships revealed by the present study show that the two isolates of Anhui and Hubei come together in one group, then the isolates of Yunnan and Guangxi gather in another group, while the Sichuan isolate is closely related to the group of Anhui-Hubei isolates.

ACKNOWLEDGEMENTS

The authors are grateful to Yu Ping and Zhang Yong-Nian for their participation in a part of the work. This project was supported by the National Natural Science Foundation of China.

REFERENCES

Chiu JK, Kao CT. Studies on host-parasite relationships of Ilan strain of *Schistosoma japonicum* in animals. 1. Prepatent period. *Chin J Microbiol* (Taiwan) 1973; 6 : 72-9.

Chiu JK, Lu SC. Studies on host-parasite relationships of Ilan strain of *Schistosoma japonicum* in animals. 2. Worm recovery rate. *Int J Zoon* 1974; 1 : 75-81.

Cross JH. Preliminary observations on the biology of the Indonesian strain of *Schistosoma japonicum:* Experimental transmission in laboratory animals and oncomelanid snails. *Southeast Asian J Trop Public Health* 1976; 7 : 202-7.

Cross JH. Further observations on the development of the Indonesian strain of *Schistosoma japonicum* in white mice and preliminary studies on the Indonesian, Philippine and Formosian strains in the Taiwan monkey. *Proc Int Conf Schistosomiasis* 1978: 173-9.

He YX, Guo YH, Ni CH. *et al.* Compatibility between *Oncomelania hupensis* and different isolates of *Schistosoma japonicum* in China. *Southeast Asian J Trop Med Public Health* 1991; 21 : 245-8.

Hsü HF, Hsü SYL. Characteristics of geographic strains of *Schistosoma japonicum* in the final host. *Proc 6th Int Congr Trop Med Malaria* 1958a; 2 : 58-66.

Hsü HF, Hsü SYL. The prepatent period of four geographic strains of *Schistosoma japonicum. Trans R Soc Trop Med Hyg* 1958b; 52 : 363-7.

Hsü HF, Hsü SYL. The infectivity of four geographic strains of *Schistosoma japonicum* in the rhesus monkey. *J Parasitol* 1960; 46 : 228.

Hsü HF, Hsü SYL, Tsai CT. Further studies on the prepatent periods of four geographic strains of *Schistosoma japonicum.* Libro Homenaje al Dr Eduardo Caballero y Caballero 1960; 153-60.