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Egg Production of *Clonorchis sinensis* in Different Strains of Inbred Mice

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Abstract: In order to compare the intraspecific variation in host-parasite relationship of *Clonorchis sinensis*, six strains of inbred mice, ICR, DDY, GPC, BALB/c, nude and DS, were infected orally with 20 metacercariae of *C. sinensis*. The biologic incubation period of *C. sinensis* was the shortest in DDY mice, 21.2 days in average, followed by GPC 21.4, BALB/c and DS 23.2, ICR and nude 23.4 days, respectively. The fertile period of the fluke was also the longest in the DDY strain, 164 days on average, followed by GPC 132, BALB/c 97, nude 37, DS 32 and ICR 28 days. The egg-laying capacity of the fluke in DDY and GPC was relatively high and stable compared with the other four strains of mice. It was found that there are intraspecific variations in biologic incubation period, fertile period, and fecundity of *C. sinensis*. The DDY mouse is likely to be the most suitable experimental animal among the six strains of the mice tested.

Key words: Mouse strain, *Clonorchis sinensis*, egg-laying capacity.

INTRODUCTION

Development of *Clonorchis sinensis* in laboratory animals has been studied(Kobayashi, 1912; Kobayashi, 1915; Mukoyama, 1921; Faust and Khaw, 1927; Seo, 1958; Wykoff, 1958; Tasai, 1966; Yoshimura and Ohmori, 1972; Hatsushika and Kawakami, 1981; Hatsushika and Naramoto, 1982; Chung and Choi, 1988). It was Komiya and Tajimi(1941) who were the first to infect mice with *C. sinensis* metacercariae to study the development of the fluke, with special reference to the excretory system. Rhee and Seo(1968) reported that maximal 94 worms were recovered from mice infected with 200 metacercariae.

Yoshimura *et al.*(1972) also infected the DDY strain of inbred mice with the metacercariae to clarify the development and fate of the fluke and demonstrated the fluke from the biliary passage of the mice at 180 days after oral infection. The worm burden and recovery rate of Yoshimura *et al.*(1972) were markedly different from those of Rhee and Seo(1968).

Even among murine strains, variations in host-parasite relationship of certain trematodes (Stirewalt, 1965; Chai *et al.*, 1984; Hata and Kojima, 1989) as well as in induction of acquired immunity after sensitization(Murrel *et al.*, 1979; Choi and Lim, 1986; Kwon *et al.*, 1987; Choi and Park, 1989; Choi and Park, 1990; Choi *et al.*, 1991) have been reported.

However, variations in host-parasite relationship of *C. sinensis* among various strains of inbred mice were not studied thoroughly.

The purpose of present study is to compare the host-parasite relationship of *C. sinensis* based on the length of parasitic life and the chronological egg-laying capacity of *C. sinensis* in six

strains of inbred mice.

MATERIALS AND METHODS

Animals: Female mice of inbred strains of ICR, GPC, DDY, BALB/c, nude and DS mice were used in experimental infection. Ten mice of each strain were used.

Parasites and infection: According to the digestion method (Komiya and Murase, 1951), *C. sinensis* metacercariae were collected from the southern top-mouthed minnow, *Pseudorasbora parva*, and the Korean shiner, *Gnathopogon atromaculatus*, caught in the river Chongdo, Kyungpook Province, Korea.

The metacercariae were washed thoroughly with the physiologic saline and resuspended. Twenty metacercariae per mouse were given orally with a gastric tube under ether anesthesia.

Egg count: Fifteen days after challenge infection, stool examination was performed to demonstrate the eggs of *C. sinensis* by formalin-ether sedimentation technique(Ritchie, 1948). Once the eggs were demonstrated by the sedimentation technique, egg counting method(Stoll, 1923) was employed to determine the number of eggs per gram of feces(EpG) at three or five days' intervals to the 90th day of infection. After that, the count was measured at ten days' interval.

Biologic incubation period: The period from the day of oral infection with *C. sinensis* metacercariae and the day of first demonstration of the eggs by formalin-ether sedimentation technique was defined as the biologic incubation period(prepatent period).

Fertile period: The period between the day of first demonstration of *C. sinensis* eggs and the day of last detection of the eggs was defined as the fertile period.

RESULTS

Table 1 shows the biologic incubation period of *C. sinensis* in six strains of inbred mice. The biologic incubation period was the shortest in DDY mice, 21.2 days average, followed by GPC 21.4, BALB/c and DS 23.2, respectively. The period was the longest in ICR and nude mice, 23.4 days.

Table 1. Biologic incubation period of *Clonorchis sinensis* in inbred strains of mice after experimental infection with 20 metacercariae of *C. sinensis*

Strain of mice	Biologic incubation period(days)	
	Range	Average
ICR	23~25	23.4
DDY	21~22	21.2
GPC	21~23	21.4
BALB/c	23~24	23.2
nude	23~24	23.4
DS	23~24	23.2

Table 2. Fertile period of *C. sinensis* in inbred strains of mice infected with 20 metacercariae of *C. sinensis*

Strain of mice	Fertile period(days)	
	Range	Average
ICR	22~ 31	28
DDY	139~179	164
GPC	119~149	132
BALB/c	87~107	97
nude	34~ 43	37
DS	31~ 40	32

Table 2 presents the fertile period of *C. sinensis* in six strains of mice. The period was the longest in DDY strain, 164 days in average, ranging from 139 to 179. The period was the shortest in ICR, 28 days in average. The average period was relatively long in GPC and BALB/c mice, 132 and 97 days, respectively. The period in nude and DS mice was relatively short, 37 and 32 days, respectively.

Table 3 and figures 1, 2 and 3 show the chronologic changes of EpG in six strains of mice during the experimental period.

The eggs were first demonstrated 21th day after infection from the stool specimens of DDY and GPC mice, and 23th day from those of the other strains. The egg-laying capacity of *C. sinensis* in DDY and GPC mice was high and

Table 3. Eggs per gram of mouse feces(EpG) during experimental infection period

Days after experimental infection	Mean EpG by strain (×100)					
	ICR	DDY	GPC	BALB/c	nude	DS
20	—*	—	—	—	—	—
21	—	+**	+	—	—	—
23	+	4	3	+	+	+
24	2	19	11	1	2	1
27	8	11	5	6	6	5
30	7	28	6	1	7	3
33	14	24	4	1	11	10
36	12	23	4	8	10	8
39	11	21	7	3	7	8
42	10	20	4	5	5	5
45	2	16	9	6	5	4
48	0	18	8	2	3	2
51	6	17	12	2	6	1
54	1	13	5	4	2	2
57	0	11	6	3	4	2
60	0	10	5	2	1	7
63		11	6	1	9	1
66		9	6	2	1	0
69		11	5	1	0	0
72		7	6	4	0	
75		9	5	2		
78		12	4	1		
81		12	3	2		
85		7	2	3		
90		8	1	1		
100		17	5	6		
110		12	6	11		
120		14	4	10		
130		10	4	2		
140		11	3	0		
150		6	13	0		
160		5	8			
170		2	4			
180		13	0			
190		19	0			
200		11				
210		0				

*— means negative by formalin-ether sedimentation technique.

**+ means positive by formalin-ether sedimentation technique.

relatively stable from 30th day after infection. No cycle but irregular fluctuations were observed

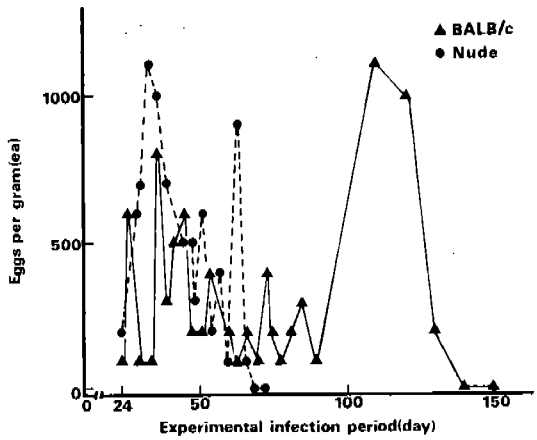

Fig. 1. Eggs per gram of BALB/c and nude feces during the experimental infection period.

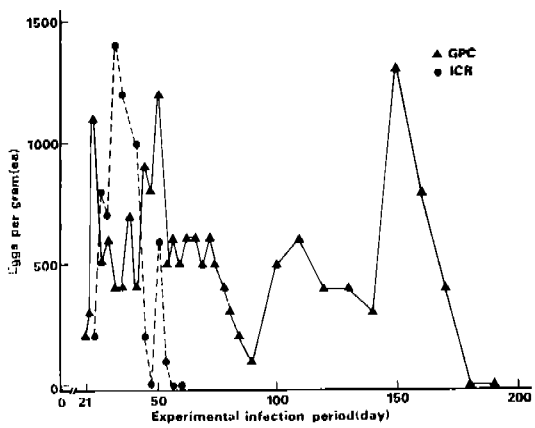

Fig. 2. Eggs per gram of GPC and ICR feces during experimental infection.

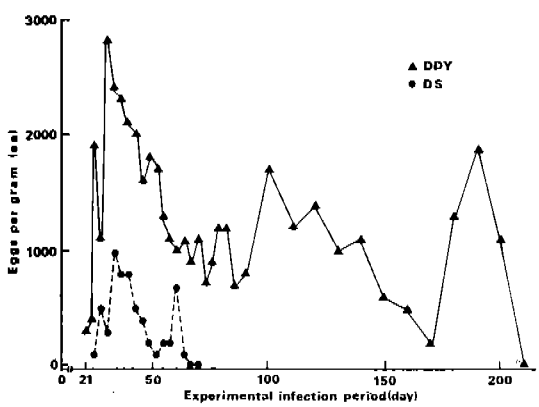

Fig. 3. Eggs per gram of DDY and DS feces during the experimental infection period.

in the other four strains. The highest EpG was observed at 30th day of infection in DDY strain, at 150th day in GPC, at 33th day in ICR, nude and DS, and at 110th day in BALB/c.

DISCUSSION

As Spent (1969) indicated the susceptibility or resistance of a host species to parasitic infection to be the product of a variety of factors which influence the host-parasite relationship, the susceptibility can be regarded as one side of the coin, host-parasite relationship, and the resistance as the other side. An animal species may be susceptible or refractory to infection by a certain parasitic helminth. On the other hand, a relationship, in which a host is refractory to infection by a parasite, may also be expressed, showing, that the host is not specific for the parasite. Host specificity is, in general, determined by the extent of the relation during evolution of both the host and parasite.

Experimental infection of laboratory animals with the liver fluke, *C. sinensis*, has been carried out to evaluate the suitability of the animals as experimental hosts (Kobayashi, 1912; Mukoyama, 1921; Faust and Khaw, 1927; Seo, 1958; Tasai, 1966; Wykoff, 1958; Yoshimura and Ohmori, 1972; Hatsushika and Kawakami, 1981; Hatsushika and Naramoto, 1982; Chung and Choi, 1988). In study of host-parasite relationship of *C. sinensis*, the followings are observed or measured: (1) infection rates which refer to the rates of infected individuals among animals challenged with the infective larvae, metacercariae; (2) recovery rates or worm burdens; (3) development and growth expressed into prepatent period or longevity of infection and measurement of worms, respectively, and (4) fecundity or egg-laying capacity.

The infection rate in rabbits given 10 or more metacercariae of the fluke was 76.9% whereas that in guinea pigs given 10 or more metacercariae was 91.7%. Tasai (1966) reported that the rate in rats given 10 or more metacercariae was 100% while the rate in rats given 5 metacercariae was 80%. Nagahana *et al.* (1977) found that the rate in nutrias given 20 or more metacercariae was 100%. Yoshimura *et al.* (1972) revealed that the rate in mice varied from 26.4 to 100% when 30~50 metacercariae were given.

Wykoff (1958) found that the recovery rate of the fluke, in rabbits given 10 or more metacercariae of the fluke was 35.0% whereas that in guinea pigs given 10 or more metacercariae was 32.0%. Tasai (1966) reported the rate in rats given with 5 or more metacercariae ranged from 12.1 to 34.0%. Chung and Choi (1988) reported the rate 57.9% when 5 to 50 metacercariae were given to golden hamsters.

Yoshimura and Ohmori (1972) reported the size of the fluke observed 160 to 190 days after infection was the largest in guinea pigs and hamsters, followed by rabbits, rats, dogs and mice. The size of the fluke 180 days after infection (Yoshimura and Ohmori, 1972) was not larger than that in 60 days reported by Rhee and Seo (1968).

Kobayashi (1915) reported the biologic incubation period of the fluke in dogs and cats ranged from 23 to 26 days. Wykoff (1958) revealed the period in rabbits to be 22 days in average. Tasai (1966) found the period in rats to vary from 17 to 26 days whereas Nagahana *et al.* (1977) found the period in nutrias to be 23 days. Chung and Choi (1988) reported that in hamsters to range from 15 to 17 days. In the present study, the period in mice ranged from 21 to 25 days.

Therefore, it is apparent that there are intraspecific differences in host-parasite relationship of *C. sinensis* by species, and mice are relatively unsuitable for the experimental hosts.

In spite of those reports in which mice were regarded as relatively unfavorable hosts for experimental infection with *C. sinensis*, mice have sometimes been used as the experimental host because of their small size and ease of control.

Moreover, the variations in host-parasite relationship of certain trematodes (Stirewalt,

1965; Chai *et al.*, 1984; Hata and Kojima, 1989) have been reported.

Stirewalt (1965) found that the best penetration and greatest consistency of *Schistosoma mansoni* cercariae were encountered in mice of C3H strain among four strains tested. Beige and hairless mice were comparatively resistant to penetration by the cercariae. He suggested that the establishment of reproducible differences in infections of hosts at the subspecific level, provides basic information for investigation of factors which influence penetration and maturation and which are thus involved in host specificity and innate resistance in closely related animals.

Chai *et al.* (1984) reported that the infection rate of mice with *Metagonimus yokogawai* ranged from 25.0 to 83.3% by strains. They also revealed that the worm recovery rate was the highest in KK strain. According to them, the susceptibility of ICR strain mice to *M. yokogawai* could be elevated by prednisolone injection and that is thought to derive from the suppression of immune responses of the mice.

Hata and Kojima (1989) who infected mice with the metacercariae of *Paragonimus miyazakii*, however, reported somewhat different results. According to them, BALB/c-nu/nu mice, lack of thymus, showed relatively lower worm burdens as compared with BALB/c mice. They suggested that the susceptibility may not be determined solely by immunological mechanisms.

Choi and his associates also revealed that the recovery rate of *C. sinensis* from athymic nude mice was lower than that in BALB/c mice.

The results in the present study coincided with those of Hata and Kojima (1989).

It could be speculated that the susceptibility of mice which are unfavorable hosts for certain trematodes, *P. miyazakii* and *C. sinensis*, to the flukes is more dependent on the physiological factors other than on the immune system. Size or capacity of the biliary passage could be possible factors in the case of mice–*C. sinensis* model.

In the present study, the prepatent period, longevity of infection and chronological fecundity of *C. sinensis* in 6 strains of inbred mice was determined. The average prepatent period or biologic incubation period of the fluke ranged from 21.2 to 23.4 days varying by strain of the mice. The average fertile period also varied from 28 days to 164 days.

However, the DDY mouse is likely to be the most suitable experimental animal among the six strains of mice tested. Further studies are necessary to determine the factors from which the variations resulted in.

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＝國文抄錄＝

近交系 마우스에서 肝吸蟲 寄生期間과 產卵力의 變動

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여섯 系統의 近交系 마우스間에 肝吸蟲의 寄生期間과 經時的 產卵力을 根據로 하여 宿主 寄生蟲 關係의 系統別 差異를 究明하기 위해 마우스當 20個의 肝吸蟲 被囊幼蟲을 經口的으로 感染시켰다. 感染 후 肝吸蟲의 蟲卵이 처음 檢出될 때까지의 期間은 DDY 마우스에서 平均 21.2日로 가장 짧았고, GPC 21.4日, BALB/c 및 DS 23.2日, ICR 및 nude 23.4日의 順이었다. 總產卵期間 역시 DDY 마우스에서 164日로 가장 길었으며, GPC 132日, BALB/c 97日, nude 37日, DS 32日 및 ICR 28日의 順이었다. DDY 및 GPC 마우스에서는 肝吸蟲의 產卵數가 比較的 높고 安定的이었으나 나머지 4系統의 마우스에서는 觀察期間 동안 不規則하였다. 以上의 成績으로 미루어 보아 마우스의 系統에 따라 肝吸蟲의 宿主 寄生蟲 關係의 差異를 確認하였고 DDY 마우스가 6系統中 가장 好適宿主임을 알았다.

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