

Intensity of Infection and Development of Adult *Clonorchis sinensis* in Hamsters*

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Abstract: In an attempt to determine the susceptibility of inbred golden hamsters (*Mesocricetus auratus*) to the experimental infection with *Clonorchis sinensis*, twenty-five hamsters were divided into 5 groups and administered orally 5, 10, 20, 30 or 50 metacercariae each. The hamsters were killed on the 45th day after infection.

The adult flukes were recovered from all hamsters. The overall recovery rate for the fluke was 57.9% with the range of 48.4% to 92.0%. As the number of the metacercariae given increased, a proportionate decrease in the recovery rate was found. The prepatent period of the fluke in the hamster varied from 15 to 17 days, with the average of 16 days. No significant differences in the development of the flukes were observed. Egg production expressed by EPG, increased step by step as the time elapsed. However, a tendency of decreasing egg laying capacity was observed with the increase of worm burden. It is suggested that the hamster is a suitable final host of *C. sinensis*.

Key words: *Clonorchis sinensis*, hamster, intensity of infection, development

INTRODUCTION

Laboratory animals, rabbits (Kobayashi, 1912), guinea pigs (Kobayashi, 1912; Wykoff, 1958), dogs and cats (Kobayashi, 1912; Mukoyama, 1921; Faust and Khaw, 1927) have been experimentally infected with *Clonorchis sinensis*. Cats, rabbits and guinea pigs are found to be highly susceptible, but rats (Seo, 1958; Chai, 1966) and mice (Komiya and Tajimi, 1953; Rhee and Seo, 1968; Yoshimura *et al.*, 1972) are not satisfactory final hosts.

Wykoff (1958) reported that rabbits and guinea pigs were equally susceptible to the infection. However, the susceptibility of golden hamsters to *C. sinensis* infection has not been

fully determined.

Yoshimura and Ohmori (1972) were the first to report the hamster as an experimental host of *C. sinensis*. Hatsushika and Kawakami (1981) and Hatsushika & Naramoto (1982) have studied the host-parasite relationships of the fluke in the hamster model. More recently, Choi *et al.* (1987) proposed that the golden hamster is a more useful animal than the mouse in the experimental transfer of immunity against *C. sinensis*.

The purpose of this study is to observe the development of adult *C. sinensis* and the susceptibility of golden hamsters to infection with this fluke.

MATERIALS AND METHODS

The metacercariae of *C. sinensis* were obtained from the southern top-mouthed minnow, *Pseudorasbora parva* collected in the River Chungdo,

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Kyungpook Province, Korea. The fish were maintained in the laboratory.

A total of 25 inbred golden hamsters (*Mesocricetus auratus*), 30~40 week old and weighing 150~200g, were used. They were divided into 5 groups of equal number, and given orally 5, 10, 20, 30 or 50 cysts each.

Ten days after the oral administration, the feces of hamsters were examined for the presence of *C. sinensis* eggs by the formalin-ether sedimentation technique (Ritchie, 1948). When the eggs were found, eggs-per-gram of feces (EPG) were counted every 3 or 4 days until the 45th day of infection by the Stoll's egg counting technique (Stoll, 1923).

Forty-five days after infection, the animals, except for those that had died, were killed by deep ether anesthesia. The number of adult worms were counted by the method of Wykoff (1958), *i.e.*, the liver was cut into several pieces and compressed between 2 large glass plates (70 × 120 mm). To detect the small worms, the plates were examined under the microscope.

The flukes recovered from the biliary passage were fixed in 70% alcohol and stained with Semichon's acetocarmine. They were examined for the measurement of body length and width, and diameters of oral and ventral suckers.

Eggs-per-gram-per-fluke (EPGPF) and eggs-per-day-per-fluke (EPDPF) were based upon the number of worms recovered and the weight of feces excreted daily.

RESULTS

The overall recovery rate of *C. sinensis* from golden hamsters was 57.9% (Table 1). It was revealed that the recovery rate varied from 48.4 to 92.0%, depending upon the number of metacercariae administered. The highest recovery rate of 92.0% was encountered when 5 metacercariae each were administered.

A decreasing recovery rate was observed according to the intensity of infection, *i.e.*, the rates were 70.7% (given 10 metacercariae), 68.0% (20 metacercariae), 57.3% (30

and 48.4% (50 metacercariae).

In the present study, two out of five hamsters given 30 metacercariae died on the 37th and 40th days, and two of five given 50 metacercariae died on the 13th and 36th days of infection, respectively.

Almost all of the flukes were found in the intrahepatic duct. A few were found in the common duct or in the gall bladder.

The average prepatent period of *C. sinensis* in golden hamsters was 16 days with the range of 15 to 17 days (Table 2).

Many abnormal eggs were found in the early stages of infection.

Table 3 shows the measurements of the adult flukes recovered from hamsters sacrificed on the 45th day of infection. The average size of the flukes, without regard to the number of metacercariae given, was 7.31 by 2.10 mm ranging from 4.78~9.31 by 1.59~2.65 mm. The ratios of length to width were between 2.1 and 4.9, with a mean of 3.5. The average diameters of oral and ventral suckers were 0.35 and 0.32mm respectively. Some difference was observed in the size of the flukes among animals given an equal number of metacercariae and even among the flukes recovered from an animal. No correlation was seen between the size and number of the flukes recovered. It was discovered that the uterus was filled with eggs and other internal organs were fully developed.

Table 4 shows the egg-laying capacity of adult fluke in hamsters from the 16th to 45th day of infection. The EPG, EPGPF and EPDPF increased step by step with the lapse of time. EPG was found to be directly proportional to the number of flukes recovered, while EPDPF was in inverse proportion. The maximum EPGPF and EPDPF, 3,771 and 8,226 respectively, were observed on the 45th day of infection.

DISCUSSION

Many investigators have determined the suitability of a laboratory animal as an experimental host of *C. sinensis* by the recovery rate

Table 1. Infection and worm recovery rates of *Clonorchis sinensis* from golden hamsters

Animal group	No. of		Total No. of		Mean No. of worms recovered	Recovery rate(%)
	Hamsters used	Metacercariae administered	Metacercariae administered	Worms recovered		
1	5	5	25	23 (5, 5, 5, 4, 4)	4.6	92.0
2	5	10	50	35 (8, 7, 7, 7, 6)	7.0	70.0
3	5	20	100	68 (15, 14, 14, 14, 11)	13.6	68.0
4	5	30	150	86 (21*, 17**, 17, 16, 15)	17.2	57.3
5	5	50	250	121 (28°, 26°°, 24, 22, 21)	24.2	48.4
Total (average)	15		575	333	(13.3)	(57.9)

*, **, ° and °° : The hamsters died on the 37th, 40th, 13th and 36th days of infection respectively.

Table 2. Prepatent periods of *C. sinensis* in golden hamsters

Group	Prepatent period in each hamster	Average prepatent period
1	15, 16, 16, 16, 16	16
2	15, 16, 16, 16, 17	16
3	15, 16, 16, 17, 17	16
4	15, 16, 16, 16, 16	16
5	16, 16, 16, 17	16

and by the development of the fluke in the animal(Wyckoff, 1958; Rhee and Seo, 1968; Yoshimura and Ohmori, 1972; Yoshimura *et al.*, 1972).

They found that the recovery rate of *C. sinensis* is influenced by the biological conditions of the metacercaria such as maturity and activity, and anatomical factors of the biliary passage of the host animal.

In the present study, it was found that the recovery rate for the fluke from 25 hamsters was 57.9%, and significantly higher than the rate(26.4%) reported by Hatsushika and Kawakami(1981). It seems that this divergence resulted from the difference in age of the host; 3~4 weeks in Hatsushika and Kawakami's (1981) experiment, while 30~40 weeks in the present study. In addition, the discrepancy may have resulted from the difference in the method of administration of cysts. Yoshimura and Ohmori(1972) reported the rate to be 21.3%

without comments on the age of the hamsters.

Chai(1966), after experimental infection of rats with 5~100 metacercariae of *C. sinensis*, reported that the dose of 10 to 30 metacercariae was found to be optimal for the rat. Our experiment revealed that the dose of 20 metacercariae was most appropriate for the hamster. This result corresponds well with the results reported by Hatsushika and Kawakami (1981). Four out of ten hamsters administered with 30 cysts or more died during the experimental period.

Since the inbred golden hamsters served as the experimental hosts, similar recovery rates were obtained from animals given equal number of metacercariae. The recovery rate decreased, whereas the number of flukes recovered increased when the number of metacercariae administered increased. This result recalls the study of Rhee and Seo(1968) in the mouse model that the most appropriate number of the metacercariae for mouse was 20.

Kobayashi(1912), after a study on the development of *C. sinensis* in various definitive hosts, stated that the size of the fluke recovered is associated with the capacity of the biliary passage as well as the number of parasites in the host. As to the measurements of the flukes in the present study, no significant difference in the size of the fluke among five individual groups was observed. In the present case, the

Table 3. Measurements of adult *C. sinensis* from golden hamsters on the 45th day of infection

Group	Mean and range of body size			Mean diameter (mm)	
	Length (mm)	Width (mm)	Length/Width	Oral sucker	Ventral sucker
1	7.64 (5.64~9.31)	2.11 (1.63~2.33)	3.6 (2.3~4.8)	0.37	0.35
2	7.54 (5.81~8.71)	2.14 (1.76~2.53)	3.5 (2.1~4.7)	0.35	0.32
3	7.49 (6.12~8.15)	2.19 (1.76~2.65)	3.4 (2.3~4.6)	0.36	0.31
4	6.73 (4.78~7.57)	2.12 (1.59~2.45)	3.2 (2.5~4.6)	0.33	0.30
5	7.13 (4.92~7.24)	1.93 (1.63~2.31)	3.7 (2.8~4.9)	0.33	0.31
Mean	7.31 (4.78~9.31)	2.10 (1.59~2.65)	3.5 (2.1~4.9)	0.35	0.32

() : range.

Table 4. EPG, EPGPF and EPDPF from hamsters infected with *C. sinensis* according to days after infection

Group	Egg count	Days after infection								
		16	19	23	26	30	33	37	41	45
1	EPG*	+	1,100	2,600	4,800	7,600	7,500	10,700	13,000	17,200
	EPGPF**		239	565	1,043	1,652	1,630	2,326	2,826	3,739
	EPDPF***		407	961	1,983	3,470	3,261	4,466	5,256	8,226
2	EPG	+	1,400	4,600	9,200	11,800	14,200	16,200	21,400	26,400
	EPGPF		200	657	1,314	1,686	2,029	2,314	3,057	3,771
	EPDPF		400	1,249	2,628	3,541	4,261	4,227	7,031	7,504
3	EPG	+	1,700	3,400	11,200	15,600	16,800	24,300	31,100	37,800
	EPGPF		125	250	824	1,147	1,235	1,787	2,287	2,779
	EPDPF		244	525	1,813	1,156	2,432	3,931	3,659	5,228
4	EPG	+	1,600	6,800	9,800	20,200	22,500	23,200	29,300	37,200
	EPGPF		93	396	570	1,174	1,308	1,349	1,703	2,163
	EPDPF		195	869	1,134	2,616	2,616	2,698	3,576	3,893
5	EPG	+	3,100	17,600	16,400	21,400	28,100	24,700	34,200	37,800
	EPGPF		128	727	678	884	1,161	1,021	1,413	1,562
	EPDPF		269	1,381	1,084	1,679	2,670	2,144	3,109	3,593

* EPG: Eggs per Gram, **EPGPF: Eggs per Gram per Fluke, ***EPDPF: Eggs per Day per fluke

Table 5. Reported prepatent period of *C. sinensis* in experimental animals

Animal	Author (Year)	Prepatent period (days)
Dog	Kobayashi(1915)	23~26
Cat	Kobayashi(1915)	23~26
Rabbit	Wykoff(1958)	22
Nutria	Nagahana <i>et al.</i> (1977)	23
Rat	Chai(1966)	17~26
Hamster	Hatsushika and Kawakami (1981)	22~32
Hamster	the present study(1988)	15~17

45 days of the experimental period was too short to detect the crowding effect on the flukes

in the biliary tract of the hamster. Individual variation was found in the size of flukes recovered from a host. The mean body length and width (7.31 mm/2.10 mm) of *C. sinensis* recovered from hamster 45 days after infection were greater than those (6.43 mm/1.26 mm) from the rats(Chai, 1966) and those (6.26 mm/1.39 mm) from the mice(Rhee and Seo, 1968) 60 days after infection.

Many investigators have reported EPGPF and EPDPF in animals infected experimentally with *C. sinensis*; 14 and 1,125 in dogs, 180 and 2,043 in cats(Faust and Khaw, 1927); approximately 100 and 4,000 in rabbits(Wykoff, 1959); 298

and 1,538 in guinea pigs (Faust and Khaw, 1927); 81 and 334 in rats (Seo, 1958); 470 and 11,380 in nutrias (Nagahana *et al.*, 1977).

Hatsushika and Naramoto (1982) reported that the mean EPGPF and EPDPF in hamsters infected with 1 to 20 metacercariae of *C. sinensis* were 4,157 and 7,069 respectively during the period of 50~70th day of infection. They also stated that EPDPF in hamsters increased slowly for the period of 20~30 days after infection (approximately 3,000 in average), while it increased abruptly during the period of 30~40 days after infection (approximately 7,000 in average) and fluctuated between 6,500 and 9,600 after 50 days of infection.

In the present study, the egg production, expressed by EPG, EPGPF and EPDPF, increased step by step as the time elapsed. Maximal EPGPF and EPDPF were 1,562~3,771 and 3,593~8,226 respectively with the number of the metacercariae administered. These counts correspond with those of Hatsushika and Naramoto (1982). It is necessary to observe the egg-production for a long time in order to detect periodic variation of EPGPF and EPDPF. However, a tendency of decreasing egg production was observed with the increase of worm burden. As shown in Table 5, the average prepatent period of 16 days, ranging from 15 to 17 days, is shorter than those in other animals.

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＝國文抄錄＝

햄스터에서의 肝吸蟲 寄生程度와 發育狀

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近交系 골든 햄스터(*Mesocricetus auratus*)의 實驗的 肝吸蟲感染에 대한 感受性を 究明하기 위하여 햄스터 25마리를 5마리씩 5群으로 나누어 各各 5, 10, 20, 30 및 50個의 肝吸蟲 被囊幼蟲을 經口投與하였다. 投與後 第 45日에 햄스터를 屠殺하여 肝吸蟲 成蟲을 回收하였다.

햄스터 25마리에서 肝吸蟲의 蟲體回收率은 48.4~92.0%로 平均 57.9%이었다. 被囊幼蟲 5個씩을 投與한 第 1群에서는 成蟲이 平均 4.6마리 回收되어 蟲體回收率 92.0%로 가장 높았고, 被囊幼蟲 50個를 投與한 第 5群에서는 平均 24.2마리의 成蟲이 回收되어 回收率 48.4%로서 가장 낮았다. 被囊幼蟲 10, 20 및 30個씩을 投與한 第 2, 3 및 4群에서는 各各 그 蟲體回收率이 70.0%, 68.0% 및 57.3%로서 投與한 被囊幼蟲의 數가 많을수록 蟲體回收率은 減少되는 傾向을 나타내었다.

햄스터에서의 肝吸蟲의 蟲卵排出始作 前期間은 15~17日로 平均 16日이었다. 回收한 蟲體의 體長, 體幅, 口吸盤 및 腹吸盤의 크기는 各群間에 有意的 差가 없었다. Eggs-per-gram(EPG)은 感染後 第45日까지 增加되었고 eggs-per-gram-per-fluke(EPGPF)와 eggs-per-day-per-fluke(EPDPF)는 蟲體負荷가 많을수록 減少하였다.

以上の 成績으로 미루어 보아 햄스터는 肝吸蟲의 好適宿主임을 알았다.