EGG PRODUCTION CAPACITY OF ONE-PAIR WORMS OF *SCHISTOSOMA JAPONICUM* IN ALBINO MICE

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Abstract. In the present study, a series of procedures of egg count were carried out to determine the egg production capacity in 21 ICR mice each infected with one-pair of *Schistosoma japonicum*. The egg count began from the first day, they were detected in the feces, which was based on a stool collection over 24 hours, twice a week. Each female *S. japonicum* produced an average of 2,198 eggs/day during the study period of 99 days after infection (ranged 61-147 days). Fourty-seven percent of the eggs were in the feces and 53% in tissues (45% in large intestine, 31% in small intestine, 23% in liver, 0.4% in pancreas, 0.2% in lungs, 0.1% in spleen, 0.1% in lymph nodes, 0.06% in stomach and 0.05% in heart, kidney, diaphragm and brain).

INTRODUCTION

Schistosome eggs have been determined to play an important role in the pathogensis of schisomiasis in animals (Lin, 1963; Warren, 1964) and humans (Lichtenberg, 1955; Lin, 1963). The production, development, distribution, destruction and passage of these eggs have been reported by Faust (1946), Moore and Sandground (1956), Moore and Warren (1967), Cheever and Anderson (1971), Fan (1974) and Cheever *et al* (1994). All of these reports, except Fan (1974), were based on multi-pairs of schistosomes. Moore and Warren (1967) infected mice with one-pair of *S. mansoni* or *S. japonicum* to compare their hepatosplenic pathology, but no attention was paid to the total egg production capacity by a single worm-pair of schistosomes. The present study is continuation of our previous investigation to determine the eggs production capacity of one-pair of *S. japonicum* after counting the total number of schistosome eggs distributed in various organs and passed in the feces of mice. The number of eggs in the ectopic sites were also recorded.

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MATERIALS AND METHODS

Infection of one-pair of *S. japonicum* in mice

Twenty-one Swiss albino mice (18-20 g in weight) were anesthetized by injecting sodium pentobarbital (0.3% solution, 0.1 ml/10 g body weight). The mouse was then fixed in a plastic plate and the hairs on the abdomen were shaved by an animal clipper. The shaved part was wetted with dechlorine tap water. Each of 21 mice was infected with a presexed pair (one male and one female) of *S. japonicum* cercariae (Fan, 1974), which were placed on a coverglass (22 x 22 mm) with the aid of a capillary dropper under microscope. The coverglass was then placed up side down on the shaved area for 30 minutes.

Determination of eggs in feces

Eight weeks after infection, feces in each of the 21 caged mice were collected and weighed daily. One gram of the fresh pellets was put into a 50-ml beaker, which was filled with 30 ml of normal saline and allowed to stand until softened, macerated and disintegrated by a magnetic stirrer. Three fecal samples of 0.2 ml from each 24-hour fecal matter sample were withdrawn with a tuberculin syringe attached with plastic tubing from the beaker being continuously stirred. Each sample was then placed on a ruling engraved micro slide (2 x 3 in) and covered with a cover glass (24 x 40 mm). The eggs were counted under a

microscope. The egg counts of the three samples were averaged and then multiplied by the dilution titer to obtain the 24-hour output.

Determination of eggs in various organs

The mice were sacrificed from 61 to 147 days after infection. The worms were recovered by the perfusion technique from each mouse. After washing the carcass, the whole digestive tract was removed and the thoracic and abdominal cavities were flushed out. The digestive tract was cut into four parts: esophagus, stomach, small and large intestines. All the internal organs were removed and weighed separately.

One gram of tissue was taken from each organ and finely minced with a pair of sharp scissors, then transferred into a 120 ml flask, filled with 40 ml of 0.5% pepsin. The flask was placed on a rotarymotion shaker in a 37ºC incubator and the contents were stirred continuously for 10-12 hours or overnight.

After digestion, the contents of the flasks were transferred to 50 ml graduated conical tubes, centrifuged, and the supernatants were discarded. The sediments were thoroughtly mixed with 0.85% normal saline and centrifuged again. When there were very few eggs in the samples, the sediments were concentrated by centrifugation before a total egg count was made.

RESULTS

Eggs passed in feces of mice

From the 1st to 15th week after detection of eggs in the feces of 21 mice, a mean egg count of 1,071 was found. The egg count ranged from 0 to 3,050 (Table 1).

Eggs detected in various organs of mice

Table 2 shows the distribution and number of eggs produced by one-pair of *S. japonicum* in 12 organs of 21 mice. The one-pair worm of *S. japonicum* had a mean age of 99 (61-147) days. The mean number of eggs in the 12 organs were in the following order: large intestine (45%) > small intestine (31%) > liver (23%) > pancreas (0.4%) > lungs (0.2%) > spleen (0.12%), lymph nodes (0.10%) > stomach (0.06%) > other organs (heart, kidney, diaphragm and brain) (0.003%) (Table 2).

Table 1
Number of eggs passed in feces of mice infected with one pair of *Schistosoma japonicum.*

Weeks after detection	No. of mice examined	No. of times examined	Number of eggs passed		
			Range	Total	Mean
1	21	42	100-1,400	27,876	664
2	21	33	150-1,866	28,107	852
3	21	33	550-3,050	42,975	1,302
4	20	38	550-2,100	44,700	1,176
5	19	38	50-2,900	49,450	1,301
6	18	19	250-2,250	19,250	1,013
7	16	29	0-2,600	27,641	953
8	13	24	0-2,300	28,150	1,173
9	11	22	300-2,550	24,000	1,091
10	9	16	150-1,650	16,525	1,033
11	6	12	300-2,450	16,725	1,394
12	4	10	150-2,750	15,825	1,583
13	3	7	550-1,600	6,450	921
14	2	5	600-1,500	5,150	1,030
15	2	4	266-1,450	2,866	717
Mean	1	22	0-3,050	23,713	1,080

Table 2
Distribution and number of eggs produced by one-pair of *Schistosoma japonicum* in various organs of mice.

Mouse No.	Age of worms (days)	Number of eggs found in									
		Liver	Small intestine	Large intestine	Spleen	Pancreas	Lungs	Lymph nodes	Stomach	Other organs[a]	Total
1	61	9,700	35,200	27,300	538	1,661	0	46	4	0	74,449
2	63	10,000	8,733	45,400	0	0	0	0	0	2	64,135
3	70	23,266	866	54,333	0	0	976	0	12	0	79,453
4	76	8,350	9,200	42,800	0	29	0	0	56	0	60,435
5	78	17,900	24,650	14,500	0	0	0	0	14	0	57,064
6	83	7,600	1,700	42,100	0	0	0	16	0	8	51,424
7	84	6,600	11,000	34,500	0	0	0	0	0	4	52,104
8	86	14,400	25,200	13,450	105	598	0	98	361	0	54,212
9	87	7,000	3,200	53,200	0	0	0	0	0	0	63,400
10	93	14,600	15,200	55,000	0	135	13	267	0	0	85,215
11	100	18,300	53,200	6,200	245	900	2	0	154	7	79,008
12	100	7,500	62,000	800	0	121	0	0	0	0	70,421
13	103	15,500	16,300	20,700	0	0	0	63	0	0	52,563
14	104	62,400	1,300	69,200	7	290	9	16	0	0	133,222
15	107	7,200	5,400	41,900	0	773	57	293	0	0	55,623
16	114	11,500	37,600	8,200	342	348	1,022	0	73	0	59,085
17	121	10,750	44,600	2,700	8	203	1,065	57	0	12	59,395
18	123	22,800	14,200	64,000	8	267	0	16	117	0	101,408
19	132	42,900	85,400	2,900	361	0	96	412	49	2	132,120
20	140	18,600	1,500	109,000	245	153	289	322	4	0	130,113
21	147	26,567	31,500	267	0	361	8	0	31	5	58,739
Mean	99	17,306	23,236	33,723	87	278	168	76	42	2	74,933
%		23.10	30.99	45.04	1.2	0.37	0.22	1	0.06	0.003	100.0

[a]Including heart (3), kidney (17), diaphragm (17 and brain (3).

Egg producing capacity of one-pair of *S. japonicum* in mice

From 61 to 147 days after infection by one female worm, 53% of eggs were deposited in tissues and 47% passed in feces. The average number of eggs deposited in tissues by one female worm in one day was 1,118 and the corresponding number for the eggs passed in feces was 1,075. Therefore, the average number of eggs produced by one female worm per day was 2,193 (Table 3).

Distribution of eggs in ectopic organs

There were three *S. japonicum* eggs in the heart of one mouse, 17 eggs in the kidney of three mice, 17 eggs in the diaphragm of five mice, and three in the brain of one mouse.

DISCUSSION

Moore and Sandground (1956) reported that *S. mansoni* produced 300 eggs/days/female and *S. japonicum* deposited 3,500 eggs/day/female in average. However, these estimations were based on multi-pair worm infections. In my previous study, I determined an egg production capacity of 322 eggs/day/female for *S. mansoni* (Fan, 1974). More recently, Cheever and his coworkers (1994) reported that *S. mansoni* worm pairs laid approximately 350 eggs per day, with no change in the apparent rate of egg laying between 8 and 52 weeks after infection and approximately one-third of the eggs were passed in the feces. *S. japonicum* worm pairs laid approximately 2,200 eggs per day initially and this decreased to 1,000

Table 3
Egg producing capacity of one pair of *Schistosoma japonicum* in 21 mice.

Category	Mice (N=21)	
	Mean	Range
No. of eggs deposited in tissue/female	74,933 (53%)	51,424 - 133,222
No. of eggs deposited in tissue/day/female	1,118	516 - 1,850
No. of eggs passed in feces/female	16,203 (47%)	14,830 - 131,700
No. of eggs passed in feces/day/female	1,080	664 - 1,583
Total no. of eggs produced/female	141,373	78,230 - 261,813
Mean no. of eggs produced/day/female	2,193	1,469 - 3,599

eggs per day by the end of the experiment, 54 weeks after infection, with one-third to one half of the eggs being passed in the feces. In the present study, we obtained an egg produciton capacity of 2,193 eggs/day/female for *S. japonicum.* These figures were quite similar with Cheever's report (Cheever *et al*, 1994). There was marked variability in the fecundity of individual worm pairs, but the number of eggs passed in the feces of individual mice correlated with the number of eggs in the intestines at all time points in *S. japonicum*-infected mice and at 7 and 10 weeks of *S. japonicum* infection (Cheever *et al*, 1994).

Moore and Warren (1967) reported that the total number of eggs in the livers of mice infected with one-pair of *S. japonicum* were 14,300, 17,000, and 22,800 in 8, 10, and 12 weeks after exposure respectively. In the present study, the number of eggs in the liver of the mice infected with one-pair of *S. japonicum* had a tendency of increasing with the duration after the exposure. This finding is similar to that of Moore and Warren (1967).

According to Moore and Sandground (1956), the distribution of *S. mansoni* eggs in hamsters was as follows: 22% execreted in the feces, 18% in the large intestine (including appendix), 32% in the small intestine, 26% in the liver, and >2% in other tissues. The distribution of *S. japonicum* eggs in hamsters was as follows: 16% in the feces, 50% in large intestine, 10% in small intestine, 23% in liver, and 1% in other parts. In my previous study, I found that 43.5% of *S. mansoni* eggs were passed in the feces and 56.5% in various tissues (Fan, 1974). In the present study, 53% *S. japonicum* eggs were passed in the feces and 47% were deposited in various tissues. These findings did not agree with those of Moore and Sandground (1956). This may be due to a difference in the strain of the parasites, the experimental host and/or the duration of infection. The intensity of infection may also be an important factor. This needs further clarification.

Previous workers reported that the eggs of *S. mansoni*, *S. japonicum* or *S. haematobium* were readily detected in the liver, small and large intestines, stomach, spleen and mesenteric lymph nodes in experimental animals (Kuntz and Malakatis, 1955; Moore and Sandground 1956; Hsu and Hsu, 1960; Cheever and Powers, 1969; Chiu and Lu, 1969; Myers *et al*, 1970; Sadun *et al*, 1970) and in human subjects (Alves, 1958; Lin, 1963; Khaw and Liu, 1969). In my previous study, I found that *S. mansoni* eggs were widely distributed in 14 organs of the mouse (liver, small intestine, large intestine, spleen, lungs, pancreas, kidney, lymph nodes, stomach, brain, heart, esophagus, diaphragm, testicle/ovary) (Fan, 1974). In the present study, *S. japonicum* eggs were recovered from 12 organs of mice. This is the first record of detecting *S. japonicum* eggs from the tissues of heart, kidney, diaphragm and brain in mice.

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