

Experimental Studies on Hamster mice as a Paratenic host of *Spirometra* spargana

Comment [V31]:

Abstract

Hamster mice is a good laboratory model for a second intermediate host in the life cycle of *Spirometra* species. A total of 10 hamster mice were experimentally fed orally in the laboratory with *Spirometra* spargana collected from naturally infected frogs (*R. cancrivora*) from the rice fields. A total of 22 spargana were used to infect 10 Hamster mice. On 30th and 120th days post infection. Hamster mice were sacrificed and spargana were recovered. A total of 15 spargana were recovered from 9 hamster mice and one hamster mice was negative. Sites of predilection were neck, axilla and chest. Measurements of the recovered spargana were on average of length 80 -170 mm on the 120th day post infection. *Spirometra* spargana were maintained in the Hamster mice for 120 days, this indicates that Hamster mice is a good laboratory model for a second intermediate host in the life cycle of *Spirometra* species.

Keywords: Hamster mice, paratenic host, *Spirometra*, spargana

Comment [V32]: Please use different word than the ones that already used in the title.

Introduction

The species of the genus *Spirometra*, include: *S. erinaceieuropaei* (*S. erinacei*), *S. decipiens*, *S. mansonii*, *S. ranarum*, and *S. mansonoides* are all intestinal parasites of canine and feline hosts [1]. In the life cycle of *Spirometra* species there are two intermediate hosts involved before the definitive host where the adult stage of the parasite is observed. The first intermediate host is the *Cyclops*. The second intermediate host is as important as the first intermediate host for the survival of the species. The second intermediate host in the case of *Spirometra* infection in the natural habitat are the reptiles, amphibians, birds and mammals [2,3,4,5,6]. The most common second intermediate host is the frog (tadpoles) followed by reptiles (snakes) and sometimes birds and mammals. There is a connection between the frog and snake as the snakes prey on them. However, the birds are also known to feed on tadpoles in the paddy fields and this can be the reason for their infection. This experiment clearly validates the postulation that hamster mice is a good paratenic host which can be used in laboratory experiments of *Spirometra* life cycle and other studies of the parasite. Few studies have been carried out in the laboratory to determine if laboratory animals can be used as intermediate hosts of the parasite. Muller [7] did a study in the laboratory model on *S. mansonoides* spargana growth. In his study he transplanted the spargana into hamster mice. Lee [6] used hamster mice in experimental studies for *S. erinaceid*. Therefore, the aim of this study was to determine experimentally if hamster mice was a paratenic host to infection with *Spirometra* spargana.

Comment [V33]: [2-6]

Materials and method

Laboratory-bred Hamster mice from the Animal House, Faculty of Medicine, University of Malaya, were used for the present experiments and were infection-free. The hamster mice were

about 16 weeks old at the time of experimental infections. A total of 10 hamster mice were used in this study, they were infected with spargana collected initially from naturally infected frogs.



Figure 1. Hamster mice used in the experiment housed in the cage

The Hamster mice used as paratenic hosts in this study are shown in (Figure 1). The spargana collected from frogs were maintained in the hamster mice for further experimentations.

Ten hamster mice were fed orally with spargana of *Spirometra* collected from naturally infected adult frogs. Each hamster was held by the skin at the back of the neck with the three fingers of the left hand and was turned ventro-dorsal, with the back laid on the palm of the hand. Fingers 4 and 5 pressed the hamster on the palm. A 2 ml syringe filled with scoleces of the spargana in water was introduced into the mouth and forced fed. The number of spargana fed to each hamster varied from 1 to 6 (total number of spargana used 22) (Table 1). After infection the animals were housed one per cage, and fed with commercial food pellets and water.

Five experimentally infected hamster mice were sacrificed on the 30th day and the other five on the 120th day, the spargana recovered were as tabulated in (Table 1). Hamster mice were killed with Chloroform. The hamster mice were placed on a dissection board dorso-ventrally, legs stretched and fixed with pins. A midline incision of the skin was made from the perineum extending upwards to the neck. Skin was peeled, organs examined for spargana were: subcutaneous tissue, abdominal wall, trunk, fore and hind legs, chest, neck and head. Sites where spargana were recovered were recorded as shown in (Table1). A total of 15 spargana were recovered from 9 hamster mice and one hamster mice was negative. The spargana recovered from the hamster mice were placed in a petri dish with Normal Saline, left for about 5 minutes to get relaxed from stress before taking morphometric measurements (length and width),

they were stretched on a metal ruler and measurements were recorded. Data as shown in (Table 2, Graph 1, Graph 2).

Results

The recovery of spargana from these animals was carried out on 30th and 120th days post infection. It was observed on the 30th day, 9 worms (40.9%) were recovered as compared on the 120th day, where only 6 (27.27%) worms were recovered (Table 1). The recovered spargana were also classified under the sites of infection and it was found that the neck region had the highest rate of infection as compared to the axilla and chest areas. All the recovered spargana were found to be healthy and viable. (Figure 2) The average length of the spargana recovered was 80 -170 mm on the 120th day post infection.

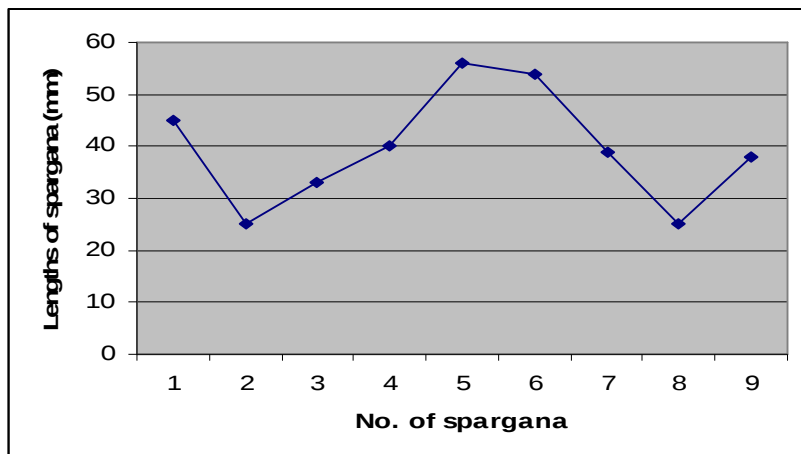
Table 1. Infection and recovery of spargana from hamster mice.

Number	Hamster mice					
	Number of spargana fed	Days and number of spargana recovered		Site recovered		
		30days	120days	Neck	Axilla	Chest
1	1		1	1	0	0
2	2	1		0	0	1
3	4	3		3	0	0
4	1		1	1	0	0
5	1	1		1	0	0
6	1		0	0	0	0
7	2		1	0	1	0
8	2	2		2	0	0
9	2	2		2	0	0
10	6		3	2	1	0
Total	22	9	6	12	2	1
	(100%)	(40.9%)	(27.27%)	(54.54%)	(9.0%)	(4.54%)

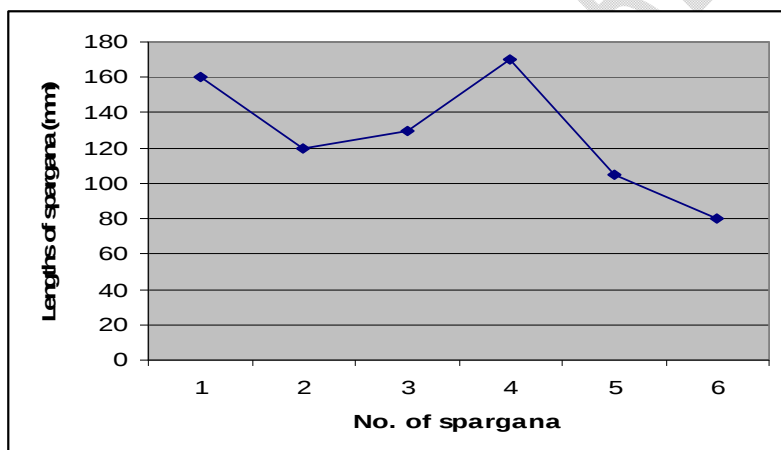
Table 2. Measurements of spargana recovered from experimentally infected hamster mice.

Day	No. of spargana	Length (mm)	Width (mm)
-----	-----------------	-------------	------------

30	1	45	1
	2	25	1
	3	33	2
	4	40	1
	5	56	1
	6	54	1.5
	7	39	1.5
	8	25	2
	9	38	1.5
Average		39.4	1.4
Range		25 to 56	1 to 2
STDEV		11.057	0.416
120	10	160	1
	11	120	1
	12	130	2
	13	170	1.5
	14	105	2
	15	80	2
Average		127.5	1.6
Range		80 to 170	1 to 2
STDEV		33.726	0.491



Graph 1. Lengths of spargana from experimentally infected hamster mice on the 30th day post infection



Graph 2. Lengths of spargana from experimentally infected hamster mice on the 120th day post infection

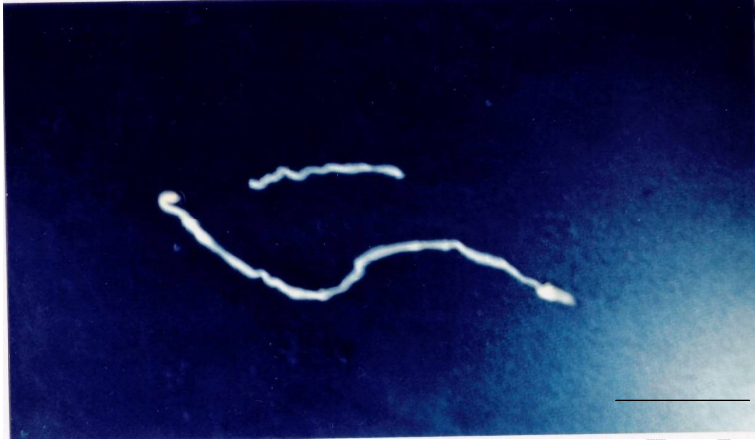


Figure 2. Spargana from experimentally infected hamster mice

Discussion

In the present study the site of predilection of spargana in Hamster mice experimentally infected with spargana were neck, axilla and chest. Previous studies showed that in hamster mice, the spargana were distributed almost to every organ including the pancreas, liver, urogenital and myocardium [6]. The choice of sites of predilection by the larvae (spargana) in hamster mice has not been thoroughly investigated. However, some workers content that the posture of the animal may have an impact on the location [5]. We tend to agree with Opuni, in some of his observation especially in relation to the distribution of the larvae in the infected animals. However, there was no any significant relationship to posture and location of larvae. According to the observations in the present study, it was found that the posture of resting animals (hamster mice) have some relationship to the site of predilection of the larvae. When the Hamster mice were resting, they curled their bodies like balls with the necks flexed and the heads facing down. Most of the larvae from these hamster mice infected with spargana from naturally infected frogs in the laboratory were recovered from the neck region. Therefore, I find it difficult to conclude that posture has a definite impact on the location of the *Spirometra* spargana in the infected animal (Hamster mice). Therefore, the host-parasite relationship displays a degree of sustainability for survival of the *Spirometra* spargana. The second intermediate host is as important as the first intermediate host for the survival of the species. The second intermediate host in the case of *Spirometra* infection in the natural habitat are the reptiles, amphibians, birds and mammals [2,3,4,5,6]. The most common second intermediate host is the frog (tadpoles) followed by reptiles (snakes) and sometimes birds and mammals. There is a visible connection between the frog and snake as the snakes prey on them. However the birds are also known to feed in the paddy fields where the tadpoles are found in abundance and this can be the reason for their infection. This experiment clearly validates the postulation that hamster mice is a good paratenic host which can be used in laboratory experiments to keep spargana recovered from naturally infected frogs viable for later use.

Previous studies have shown that there is an increase in the measurements of the length of spargana, that were transplanted from a naturally infected host to an experimental host. This indicates that there is growth of spargana in the laboratory model. Muller (1966) has reported these findings in his studies

Comment [V34]: Please write {2-6}

on *S. mansonioides* spargana transplanted into hamster mice which measured 100 - 800 mm. In the present study the length of spargana recovered from hamster mice fed orally with spargana from *R. cancrivora* was 80 -170 mm on the 120th day post infection. These results do not conform with the findings of Muller [7], the reason could be that the growth of the parasite is influenced probably by differences in the simulated laboratory conditions and the species of laboratory models.

Conclusion

This study clearly validates the postulation that hamster mice is a good paratenic host which can be used in laboratory experiments to keep spargana recovered from naturally infected frogs viable for later use. In this study it can be concluded that, the site of predilection of spargana in Hamster mice is neck region. It was found that the posture of resting animals (Hamster mice) have some relationship to the site of predilection of the spargana. When the Hamster mice were resting, they curled their bodies like balls with the necks flexed and the heads facing down.

Ethical issue

There is no ethical issue in this manuscript because no human was used.

References

1. Mueller JF. The biology of *Spirometra*. *J Parasitol* 1974; 60: 3-14.
2. Okumura, T. An experimental study of the life history of *Sparganum mansoni* .Cobbold (a preliminary report). *Kitasaro Arch. Exp. Med.*1919; (3): 190 -197.
3. Li, H. C. The life histories of *Diphyllbothrium decipiens* and *S. erinacei*. *Am. J. Hyg.* 1929; 10 (3): 527 - 550.
4. Mueller, J. F. The laboratory propagation of *Spirometra mansonioides* as an experimental tool.1. Collecting incubating and hatching of the eggs. *Journal of Parasitology*.1959; 45: 353 - 361.
5. Opuni, E. K., and Muller, R. Absence of sparganum growth factor in African *Spirometra* spp. *J. Parasitol.*1974; 60 (2): 375 -376.
6. Lee SH, We SJ, Sohn WM, Hong ST, Chai JY. Experimental life history of *Spirometra erinacei*. *Korean J Parasitol* 1990; 28: 161-173.
- 7 Mueller, J.F.The laboratory propagation of *Spirometra mansonioides* (Mueller, 1935) as an experimental tool. VII. Improved techniques and additional notes on the biology of the cestode. *J. Parasit.* 1966; 52: 437-443.

Comment [V35]: Please add more references