SUSCEPTIBILITY OF RODENTS TO *STELLANTCHASMUS FALCATUS* INFECTION

Supap Saenphet[1], Chalobol Wongsawad[1], Kanokporn Saenphet[1] and Jong-Yil Chai[2]

[1]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.; [2]Department of Parasitology, Seoul National University College of Medicine, and Institute of Endemic Diseases, Seoul National University Medical Research Center, Seoul, Korea

Abstract. Two rodent hosts, rats (*Rattus norvegicus*) and mice (*Mus musculus*), were examined for their susceptibility to *Stellantchasmus falcatus* infection. The metacercariae were recovered from half-beaked fish, *Dermogenus pusillus,* collected from Hang Dong District, Chiang Mai Province. A single inoculation of three hundred metacercariae was orally conducted with male rats (n=15) and mice (n=15). Uninfected animals were used as controls (n=5). At days 3, 7, 14, 21 and 28, post-infection (PI), three rats and mice, also one from each control group, were sacrificed for adult worm recovery, and the blood was drawn by heart puncture and prepared for white blood cell and eosinophil counts. The results showed that adult worm recovery gradually decreased after day 21 PI in the rats, whereas, reduction in the mice was found after day 7 PI and the adult worms completely disappeared at day 28 PI. Hematologically, total white blood cell counts in two kinds of infected hosts were not statistically different from those of controls. However, eosinophil counts of infected rats slightly increased during days 7-14 PI, and started to decrease at day 21 PI. A similar finding was observed in the infected mice. From these results, it can be concluded that the rat is a more suitable host for *S. falcatus* than the mouse. To understand the mechanisms implicated in determining host susceptibility, intensive studies are required.

INTRODUCTION

Parasitic helminths, includes cestodes, nematodes or trematodes, continue to cause debilitating diseases in both humans and animals throughout the world, and even today they are highly prevalent in the developing countries (Yazdanbakhsh *et al*, 2001). Recently, the infection rate of *Stellantchasmus falcatus* (Trematoda: Heterophyidae) has been high among people and fish intermediate hosts in northern Thailand (Radomyos *et al*, 1998; Wongsawad *et al*, 1998; 2000). Actually, this parasite was found to have a 100% infection rate in its second intermediate host, *Dermogenus pusillus*, which were collected from the Chiang Mai Moat (Wongsawad *et al*, 1998; Saenphet *et al*, 2001). Human infections by *S. falcatus* were reported from patients at Maharaj Hospital, Chiang Mai Province (Kliks and Tantachamrun, 1974; Tantachamrun and Kliks, 1978) and from northeastern Thailand (Radomyos *et al*, 1990). The natural and experimental final hosts recorded were chicks, rats, mice and cats (Tantachamrun and Kliks, 1978; Namue and Wongsawad, 1997; Wongsawad *et al*, 1998). This implies that *S. falcatus* could develop its maturation in various species of vertebrate hosts. The interaction between host and parasite is critically important for parasite development and survival. Within a given host, changes in the parasite's environment can alter the development or the reproductive capabilities of the parasites. Previous reports showed that the rodents served as reservoir hosts for many medically important parasites of humans (Bhaidikul *et al*, 1985). However, there is scarce information on suitable experimental hosts for this heterophyid fluke.

This study was performed to assess the susceptibility of rodent hosts, including rats (*Rattus norvegicus*) and mice (*Mus musculus*) as experimental definitive hosts for *S. falcatus,* by focusing on the worm recovery rate and the alteration of total white blood cell and eosinophil counts in these rodent hosts.

Correspondence: Supap Saenphet, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

MATERIALS AND METHODS

Metacercariae of *S. falcatus* were procured from the half-beaked fish, *D. pusillus,* collected from Hang Dong District, Chiang Mai Province. Experimental final hosts, 20 rats (*Rattus norvegicus*) and 20 mice (*Mus musculus)*, aged between 4-6 weeks, were purchased from Mahidol University, Salaya, Nakhon Pathom. Both experimental hosts were left to acclimatize in the animal house at the Department of Biology, Chiang Mai University for 1 week. Thereafter, a single oral inoculation of three hundred *S. falcatus* metacercariae was conducted with each rat (n=15) and mouse (n=15), and the rest served as uninfected control groups (n=5, n=5). Three rats and mice from the infected groups, and one from each control group, were

sacrificed at days 3, 7, 14, 21, and 28 post-infection (PI) for worm recovery. After anesthetization with chloroform, the abdomens of the animals were opened and the small intestines, divided into duodenum, jejunum and ileum, were removed and bathed in cold saline solution for 30 minutes. Thereafter, they were resected and scraped interiorly by a blunt spoon. After several washings of the intestinal contents by normal saline, worms were recovered and counted under a stereo-microscope. Blood was drawn by heart puncture to investigate hematological changes. Twenty μl EDTA blood were mixed with Turk's solution and with Hinkelman's solution for total white blood cell (WBC) and eosinophil counts, respectively. Total WBC and eosinophil counts were measured using an improved Neubauer hemocytometer. The results were compared to the control groups. Statistical analysis was performed by Student's *t*-test.

RESULTS

Worm recovery rate in rats and mice

The recovery rate of the worms from the small intestines of the experimental rats was 20.22% on average, and relatively high, until day 21 PI. After that it decreased rapidly to 0.22% at day 28 PI (Table 1). Among 303.30 worms recovered, almost all worms were collected from the ileum (98.02%) and scarcely found in the jejunum (1.98%). Some adult worms were recovered in the jejunum; however, this was observed only at day 3 PI. Two rats, moreover, were free from infection on day 28 PI. The incidence of this heterophyid fluke in rats was 86.67% (13/15) and the intensity ranged from 1-180.

As shown in Table 2, the recovery rate from mice was 13.56% on average. The recovery rate from mice

Table 1
Worm recovery in rats infected with 300 *S. falcatus* metacercariae.

Days post-infection	No. of infected hosts	No. of metacercariae infected	Mean of worm recovery				Worm recovery rate (%)
			Duodenum	Jejunum	Ileum	Total	
3	3	300	0.00	6.00	122.30	128.30	42.77
7	3	300	0.00	0.00	83.33	83.33	27.78
14	3	300	0.00	0.00	45.00	45.00	15.00
21	3	300	0.00	0.00	46.00	46.00	15.33
28	3	300	0.00	0.00	0.67	0.67	0.22
Total	15	1,500	0.00	6.00	297.30	303.30	20.22

Table 2
The worm recovery in mice infected with 300 *S. falcatus* metacercariae.

Days post-infection	No. of infected hosts	No. of metacercariae infected	Mean of worm recovery				Worm recovery rate (%)
			Duodenum	Jejunum	Ileum	Total	
3	3	300	0.00	15.33[a]	65.00	80.33	26.78
7	3	300	0.00	0.00	118.00	118.00	39.33
14	3	300	0.00	0.00	4.00	4.00	1.33
21	3	300	0.00	0.00	1.00	1.00	0.33
28	3	300	0.00	0.00	0.00	0.00	0.00
Total	15	1,500	0.00	15.33	188.00	203.33	13.56

[a]Including metacercariae

remained relatively high until day 7 PI. After that it rapidly decreased to 0.33% on day 21 PI. Eventually, no worm was recovered on day 28 PI. Among 203.30 worms recovered, the great majority (92.46%) were recovered from the ileum and the rest (7.54%) were collected from the jejunum of the mice. There were some metacercariae and adult worms recovered in the jejunum; however, this was observed only at day 3 PI. The incidence of *S. falcatus* in mice was 73.33% (11/15) and the intensity ranged from 1-182.

Hematological changes in rats and mice

The hematological changes are concentrated on the white blood cells, which are associated with the host's immune response. Total white blood cell and eosinophil counts were therefore evaluated. The eosinophil counts in both rats and mice experimentally infected with *S. falcatus* were significantly increased compared with those of the control groups ($p<0.05$). It increased from the first week of infection and remained relatively high until worm recovery decreased (Figs 1 and 2), whereas, the WBC count from both experimental groups were slightly increased; however, their values were within their normal ranges (Table 3).

DISCUSSION

The susceptibility of animal hosts to parasitic helminths can be demonstrated by observing the worm recovery rate, including the development and fecundity of the worms recovered after the animals have been challenged with those parasites (Chai *et al*, 1984; Ito and Kamiyama, 1987). The present study revealed that *S. falcatus* could complete its maturation in the small intestines of both rats and mice, chiefly in the ileum. However, the number of adult worms recovered

Table 3
Worm recovery and hematological changes in rats and mice infected with 300 *S. falcatus* metacercariae.

Hosts	Changes	Days PI					
		0	3	7	14	21	28
Rats	Eosinophil	75.50	302.32	396.67	311.67	585.65	141.67
	WBC	5,189.00	6,630.00	4,930.00	5,312.93	5,227.5	3,591.68
	Mean worm recovery	0.00	128.30	83.33	45.00	46.00	0.67
Mice	Eosinophil	28.30	141.67	311.67	510.00	566.67	56.60
	WBC	3,177.50	3,177.50	4,462.50	3,570.00	6,948.75	5,355.00
	Mean worm recovery	0.00	80.33	118.00	4.00	1.00	0.00

PI = post-infection

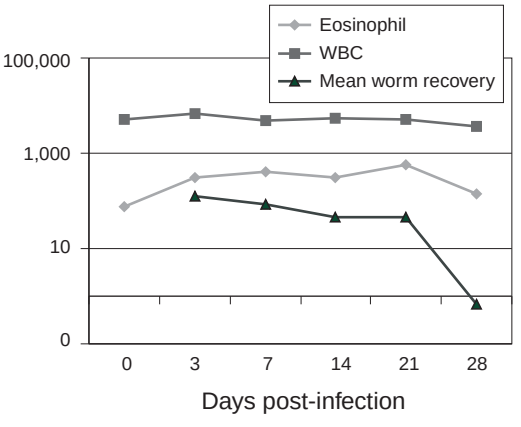

Fig 1- Worm recovery and hematological changes in rats experimentally infected with 300 *S. falcatus* metacercariae (Logarithmic graph).

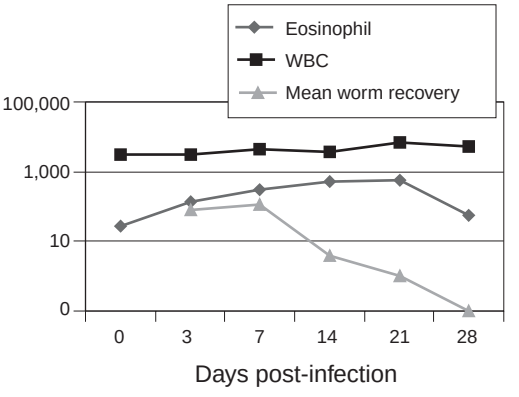

Fig 2- Mean of worm recovery and hematological changes in mice experimentally infected with 300 *S. falcatus* metacercariae (Logarithmic graph).

was much lower than the number of metacercarial cysts administered, and worm recovery decreased as the experimental period elapsed. These phenomena have also been found in previous reports on other trematodes (Hong *et al*, 1990; Chai *et al*, 1995; Humphries *et al*, 1997; Lee *et al*, 1997; Sukontason *et al*, 1998; Wongsawad *et al*, 1998). As seen in this study, *S. falcatus* has longer survival in rats than in mice. It can survive in a rat's gut as long as 28 days PI, whereas it can live in a mouse's gut for only the first week of infection. In addition, the mean worm recovery in rats (20.22%) was higher than in mice (13.56%). However, the means of worm recovery in both rodent hosts decreased as the experimental period elapsed. This finding suggested that the microenvironment in the small intestines of rats was probably more appropriate for the development of *S. falcatus* than that of mice. Furthermore, there was no worms found in the duodenum of these two experimental hosts. It is possible that duodenal contents seriously impede the development and growth of *S. falcatus*, by the action of several enzymes present in this segment. In addition, some metacercariae and adult worms were recovered in the jejunum of mice, whereas only adult worms were observed in the jejunum of rats. The excystment of this fluke might start in the jejunum due to the optimal environmental and nutritional states. Besides the effects of the host environment or metabolism, Chai *et al* (1999) suggested that the host's immunity also affect the development and infectivity of *Metagonimus yogokawai*, another heterophyid trematode frequently found in Korea. Anyhow, It is also known that the parasites show a tendency to move down rapidly if the suitability of the host is very low.

The numbers of eosinophils were increased and also negatively correlated with the number of adult worms recovered from these rodent hosts. Eosinophil numbers were increased at the first week of infection and remained relatively high until the worms disappeared. Eosinophilia, an increase in the number of eosinophils in the blood or tissues, has historically been recognized as a distinctive feature of helminth infections in mammals. Eosinophils are also responsible for considerable pathology in mammals because they are present in large numbers in inflammatory lesions associated with helminth infections or allergic reactions. Many scientists consider that eosinophils' primary role is protection against parasites, although there is little unequivocal evidence to prove this (Phillips *et al*, 1977; Weller, 1991; Wardlaw *et al*, 1995; Yazdanbakhsh, 1996; Behm and Ovington, 2000). Due to their larger sizes, it is suggested that helminthic parasites are destroyed by the cellular immune system, by mast cells and also by eosinophils (Butterworth, 1984). This mechanism is also associated with the presence of antibodies, chiefly immunoglobulin E (IgE) (Shin *et al*, 1997). However, the precise functions of these cells are still poorly understood and direct evidence for the role of eosinophils in host protection against helminths *in vivo* is lacking, and therefore, the debate continues (Meeusen and Balic, 2000). Nevertheless, the total white blood cells and eosinophil numbers found in this study were not as high as in previous studies. They could, thus, not be used as a major sign for this trematode infection.

From these results, it can be concluded that the rat is a more suitable host for the developement of *S. falcatus* than the mouse, focusing primarily on worm recovery and survival time. However, to understand the mechanisms implicated in determining host susceptibility, intensive studies are required.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Royal Golden Jubilee PhD program (PHD/0013/2545) and sincerely thank Mr Pralongyut Sripalwit, Miss Kanda Kumchoo, and other colleagues for great assistance. Special thanks are extended to the Parasitology Research Laboratory, and also the members of the Animal House Unit, Department of Biology, Faculty of Science, Chiang Mai University.

REFERENCES

Behm CA, Ovington KS. The role of eosinophils in parasitic helminth infections: insights from genetically modified mice. *Parasitol Today* 2000;16:202-9.

Butterworth AE. Cell-mediated damage to helminths. *Adv Parasitol* 1984;23:143-235.

Baidikul V, Upatham ES, Kruatrachue M, *et al*. Study on *Schistosoma sinensium* in Fang District, Chiang Mai Province, Thailand. *Southeast Asian J Trop Med Public Health* 1984;15:141-7.

Chai JY, Seo BS, Lee SH. Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea VII. Susceptibility of various strains of mice to *Metagonimus* infection and effects of prednisolone. *Korean J Parasitol* 1984;22:153-60.

Chai JY, Kim J, Lee SH. Invasion of *Metagonimus yokogawai* into the submucosal layer of the small intestine of immunosuppressed mice. *Korean J*

Parasitol 1995;33:313-21.

Chai JY, Chung WJ, Kook J, *et al*. Growth and development of *Gymnophalloides seoi* in immunocompetent and immunosuppressed C3H/HeN mice. *Korean J Parasitol* 1999;37:21-6.

Hong SJ, Lee SH, Chai JY, Seo BS. Recovery rate, growth and development of *Heterophyopsis continua* in experimental chicks. *Kisaengchunghak Chapchi* 1990;28:53-62.

Humphries JE, Reddy A, Fried B. Infectivity and growth of *Echinostoma revolutum* (Froelich, 1802) in the domestic chick. *Int J Parasitol* 1997;27:129-30.

Ito A, Kamiyama T. Cortisone-sensitive, innate resistance to *Hymenolepis nana* infection in congenitally athymic nude rats. *J Helminthol* 1987;61:124-8.

Kliks M, Tantachamrun T. Heterophyid (trematoda) parasites of cats in North Thailand, with notes on a human case found at necropsy. *Southeast Asian J Trop Med Public Health* 1974;5:547-55.

Lee SH, Park SK, Seo M, Guk SM, Choi MH, Chai JY. Susceptibility of various species of animals and strains of mice to *Gymnophalloides seoi* infection and the effects of immunosuppression in C3H/HeN mice. *J Parasitol* 1997;83:883-6.

Meeusen EN, Balic A. Do eosinophils have a role in the killing of helminth parasites? *Parasitol Today* 2000;16:95-101.

Namue C, Wongsawad C. A survey of helminth infection in rats (*Rattus* spp) from Chiang Mai Moat. *Southeast Asian J Trop Med Public Health* 1997;28(suppl 1):179-83.

Phillips SM, DiConza JJ, Gold JA, Reid WA. Schistosomiasis in the congenitally athymic (nude) mouse. I. Thymic dependency of eosinophilia, granuloma formation, and host morbidity. *J Immunol* 1977;118:594-9.

Radomyos P, Chaloenlarp P, Radomyos B, Tangtrongjitr A. Two human cases of *Stellantchasmus falcatus* (Trematoda: Heterophyidae) infection in northeastern Thailand. *Jpn J Parasitol* 1990;39:7-11.

Radomyos B, Wongsaroj T, Wilairatana P, *et al*. Opisthorchiasis and intestinal fluke infections in northern Thailand. *Southeast Asian J Trop Med Public Health* 1998;29:123-7.

Saenphet S, Wongsawad C, Saenphet K. A survey of helminths in freshwater animals from some areas in Chiang Mai. *Southeast Asian J Trop Med Public Health* 2001;32(suppl 2):210-3.

Shin EH, Osada Y, Chai JY, *et al*. Protective roles of eosinophils in *Nippostrongylus brasiliensis* infection. *Int Arch Allergy Immunol* 1997;114 (suppl 1):45-50.

Sukontason K, Sukontason KL, Boonsriwong N, *et al*. Development of *Haplorchis taichui* (Trematoda: Heterophyidae) in *Mus musculus* mice. *Southeast Asian J Trop Med Public Health* 1998;32(suppl 2):43-7.

Tantachamrun T, Kliks M. Heterophyid infection in human ileum: report of three cases. *Southeast Asian J Trop Med Public Health* 1978;9:228-31.

Wardlaw AJ, Moqbel R, Kay AB. Eosinophils: biology and role in disease. *Adv Immunol* 1995;60:151-266.

Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991;324:1110-8.

Wongsawad C, Chariyapongpun P, Namue C. Experimental host of *Stellantchasmus falcatus*. *Southeast Asian J Trop Med Public Health* 1998;29:406-9.

Wongsawad C, Rojanapaibul A, Mhad-arehin N, *et al*. Metacercaria from freshwater fishes of Mae Sa stream, Chiang Mai, Thailand. *Southeast Asian J Trop Med Public Health* 2000;31(suppl 1):54-7.

Yazdanbakhsh M. IgE, eosinophils and mast cells in helminth infections. *Ned Tijdschr Klin Chem* 1996;21:213-6.

Yazdanbakhsh M, van den Biggelaar A, Maizels RM. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. *Trends Immunol* 2001;22:372-7.