INFECTIVITY, GROWTH AND FECUNDITY OF *ECHINOSTOMA MALAYANUM* IN MICE

Tuanchai Srisawangwong[1], Sumalee Chantaluk[1], Paiboon Sithithaworn[1] and Darunee Jintakanon Charoensiri[2]

[1]Department of Parasitology, [2]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract. The population dynamics of *Echinostoma malayanum* infection in mice following a single exposure were investigated. Outbred Swiss albino mice, 6-8 weeks old, were orally infected with 50 metacercariae of *E. malayanum* obtained from naturally infected *Indoplanorbis exustus*. Groups of 5 mice were weekly sacrificed for 12 weeks to determine infectivity, growth and fecundity of the parasites. The infectivities, demonstrated by percentages of worm recoveries, were very high in all groups examined (70-84%). The worms were located only in the anterior part of the small intestine, which was divided into 3 equal parts. The growth of the worms, measured by the aid of Image analyzer software (50 worms/group), rapidly increased during the first three weeks after infection. The length increased from 1.43 mm at week 1 to 5.12 mm at week 3, and reached a peak at week 11. In contrast, the width of the worms increased very slowly and was nearly stable after 5 weeks. The body areas of the worms increased rapidly during the first seven weeks and fluctuated thereafter. The maximum was found at week 11. The worms became sexually mature and produced eggs, which were detected in feces as early as 2 weeks after infection. The number of eggs per gram of feces (epg), determined by modified formalin-ethyl acetate concentration technique, as well as epg per worm, increased at week 3 and remained that high until the end of the observation.

INTRODUCTION

More than 10 species of echinostomes, intestinal trematodes, have been reported to cause echinostomiasis in humans. Among these, *Echinostoma ilocanum* and *E. malayanum* are the common species in Southeast Asia (Waikagul and Radomyos, 1997). In Thailand, most cases of human infections have been reported in the Northeast (Jongsuksantigul *et al*, 1992; Radomyos *et al*, 1994). Humans get infection by ingesting raw or insufficiently cooked 2nd intermediate host, *eg*, *Pila* snails and tadpoles, containing infective stages or metacercariae.

The infectivity and growth of *Echinostoma* spp in the host have been studied in several kinds of experimental animals. *E. revolutum* was studied in golden hamsters (Franco *et al*, 1986, 1988), domestic chicks (Humphries *et al*, 1997), *E. caproni* in mice (Hosier and Fried, 1991; Manger and Fried, 1993; Kaufman and Fried, 1994), and *E. trivolvis* in mice (Gavet and Fried, 1994). The development of *E. malayanum* was studied by some investigators (Lie, 1963; Mohandas and Nadakal, 1978). However, details of the development of this trematode have not been reported in Thailand. To update the knowledge of *E. malayanum* in Thailand, this experiment was designed to determine its infectivity, growth and fecundity, using mice as an experimental host.

Correspondence: Tuanchai Srisawangwong, Department of Parasitology, Faculty of Medicine, Khong Kaen University, Khon Kaen 40002, Thailand.

MATERIALS AND METHODS

Metacercariae of *E. malayanum* were obtained from naturally-infected freshwater snails, *Indoplanorbis exustus*, in Khon Kaen, Thailand. After removal of the shell by pressing snails between two glass plates, the snails were minced and artificially digested with 0.3% pepsin A, in a shaking water bath for one hour. The digested tissues were strained through sieves and sedimented in 0.85% NaCl. The metacercariae were then isolated under a stereomicroscope. Groups of 50 metacercariae were orally introduced to 6-8-week old male Swiss albino mice by gastric intubation. Groups of 5 infected mice were weekly sacrificed for 12 weeks to determine the infectivity, growth and fecundity of the parasites. After they were sacrificed by excess diethyl ether, their intestines were isolated, opened longitudinally with a pair of scissors and placed in 0.85% NaCl for worm recovery. Infectivity was demonstrated by the percentage of worm recovery in each group. The recovered worms were washed in 0.85% NaCl and fixed in alcohol-formalin-acetic acid (AFA) for 24 hours. The fixed worms were then washed and stored in 70% ethanol. The length, width, and body area of worms in each group were measured by the aid of the Image analyzer software (50 worms/group). To determine fecundity, feces of an individual mouse were

collected separately, incubated at 60˚C for 24 hours, weighed and fixed in 10% formalin. A modified formalin-ethyl acetate concentration technique (Elkins *et al*, 1991) was used to determine the number of eggs per gram of feces (epg) and epg/worm.

RESULTS

Infectivity of metacercariae

After orally introduced to mice, the metacercariae of *E. malayanum* excysted, survived and developed into adult worms in the small intestine. The worm recoveries were high until the end of the experiment, *ie* 12 weeks after infection (Fig 1). Approximately 70% of metacercariae were found to survive in the intestine of mice examined one week after infection. The worm recoveries remained high thereafter and as high as 73.6% was found after 12 weeks. All of these worms inhabited the anterior part of the small intestine, which was divided into 3 equal parts.

Growth of parasites

Fig 2 shows that *E. malayanum* metacercariae were able to grow in the intestines of mice. The lengths of the worms rapidly increased during the first 3 weeks, increasing from 1.43 mm at week 1 to 5.12 mm at week 3, increasing slowly thereafter and reaching peak at week 11 (7.73 mm). The widths increased slowly and seemed to be stable from week 5 onwards. The body areas, however, increased markedly during the first 7 weeks and showed a little fluctuation thereafter. The maximum was found at week 11.

Fecundity of the worms

The worms were mature and began to produce eggs after they were 2 weeks old in the mouse intestine. Eggs, detected by modified formalin-ethyl acetate concentration technique, were found in feces after 2 weeks. Egg output increased at week 3 and remained stable until the end of the observation, *ie*, 12 weeks after infection. Epg and epg/worm showed similar patterns (Fig 3) and were correlated with the worm recoveries, depicted in Fig l.

DISCUSSION

It has been pointed out that the establishment, survival, and fecundity of parasites are affected by the species and strain of the host as well as the parasites (Christensen *et al*, 1988). High worm recoveries of *E. caproni* were obtained for many weeks in ICR mice (Hosier and Fried, 1991), while *E. trivolvis* diappeared within 4 weeks from this kind of animal (Weinstein and Fried, 1991). *E. trivolvis* disappeared from BALB/c mice and C3H mice on days 17 and 15, respectively (Fujino *et al*, 1993; 1996). The high worm recoveries of *E. malayanum* in Swiss albino mice demonstrated in this study were similar to those found in white rats studied by Mohandas and Nadakal (1978). This may be due to the low response of these hosts to *E. malayanum* or to the worms being able to tolerate the host response. BALB/c and C3H mice may generate stronger reactions against *E. trivolvis* infection than Swiss albino mice, and white rats against *E. malayanum*.

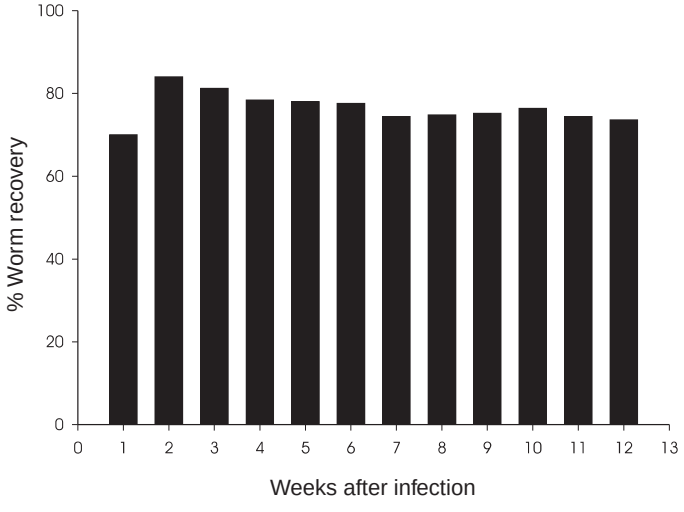

Fig 1- Mean percentages of worm recoveries in mice 1-12 weeks after infection with 50 metacercariae of *E. Malayanum* (5 mice/ group).

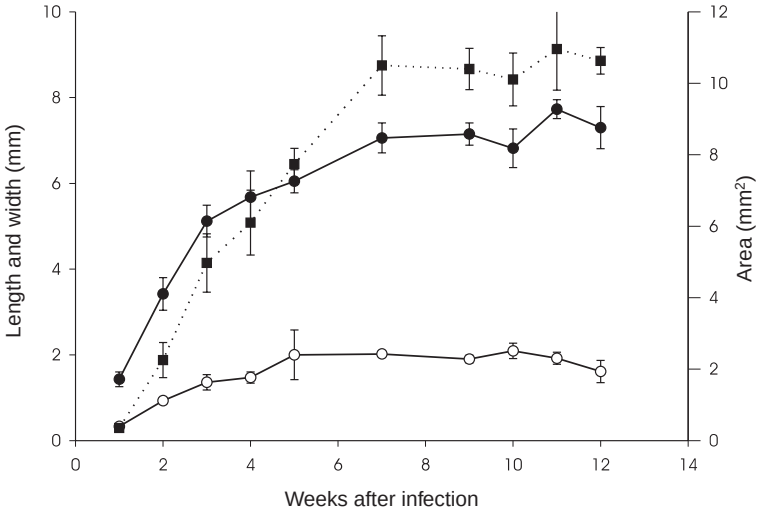

Fig 2- Growth of worms recovered from mice infected with 50 metacercariae of *E. malayanum* 1-12 weeks after infection (50 worms/group).
●— : Length, —○— :Width, ··■·· :Body area

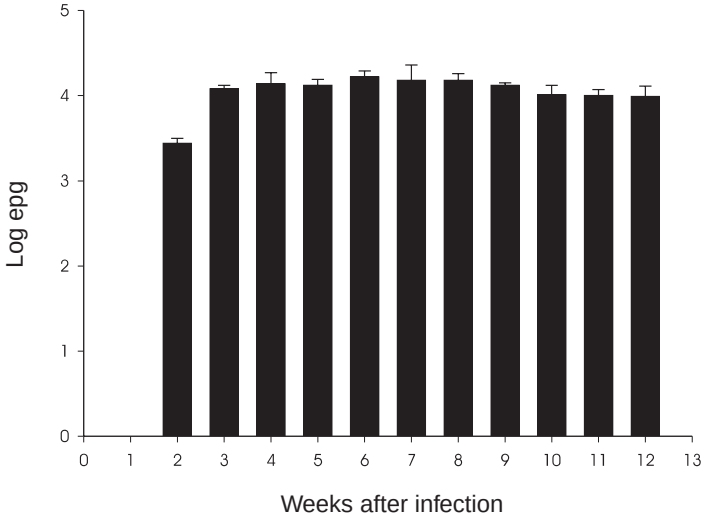

Fig 3- Epg detected from mice 1-12 weeks after infection with 50 metacercariae of *E. malayanum* (5 mice/group).

The burden and longevity of infection can affect survival and distribution of the worms in the intestine of the host. High-level infections of *E. revolutum* remained in mice longer than low-level ones (Christensen *et al*, 1981). *E. trivolvis* were found in the anterior part of the small intestine of ICR mice within 4 days, migrated to the posterior within 8 days, and showed no specific pattern thereafter (Gavet and Fried, 1994). The distribution of *E. malayanum* in this study was similar to that in white rats studied by Mohandas and Nadakal (1978). The worms located in the anterior parts of the small intestines of the animals. However, in heavy infection, the worms distributed over the entire length of the intestine. The prepatent period of *Echinostoma* spp was little different among species. Eggs were nomally detected in host feces on days 11 or 12 after infection (Franco *et al*, 1986) but a little longer in heavily-infected

animals (Franco *et al*, 1988). The normal prepatent period of *E. malayanum* was 13-16 days in white rats (Mohandas and Nadakal, 1978), 14-17 days in rats, mice and hamsters (Lie, 1963). The *E. malayanum* in this study showed a similar rate of worm development. The eggs could be detected in feces within 2 weeks. The growth of the worms increased as the time increased. The flukes continuously released eggs from the uterus, which resulted in the slower increase in the width than the length of the worms. This study provided a basic knowledge of *E. malayanum* in Thailand. More details about this fluke should be investigated.

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