Short Communication

Syrian hamster (*Mesocricetus auratus*) as an animal model for *Haplorchis taichui* infection: biology, morphology, and pathology

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Abstract

Haplorchis taichui is a trematode that can mature in the small intestine of mammals and birds, including humans. Larval stages occur in various invertebrate and cold-blooded vertebrate hosts. The purpose of this study was to investigate parasite growth, including worm recovery rate, the body size of adult worms, eggs per worm and eggs per gram of feces and pathological changes of the small intestine in experimental hamsters. In this study, 6- to 8-week-old male Syrian hamsters (*Mesocricetus auratus)* were each infected with 200 *H.taichui* metacercariae and then sacrificed at weeks 1, 2, 3, 4, 6 and 8 post-infection. The small intestine and feces of each hamster were collected and processed for analysis. Worm recovery was highest at weeks 1, 2 and 3, then continuously decreased at weeks 4, 6 and 8, respectively. Eggs per worm were highest at weeks 2 and 3, and eggs per gram of feces were highest at week 3. Worm size was similar each week. Histopathological changes in the small intestine were observed, including abnormal villi and goblet cells, as evidenced by short villi and an increase in the number and size of goblet cells, compared with the normal control group.

Keywords: animal model, biology, *Haplorchis taichui*, morphology, pathology

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Introduction

Heterophyid intestinal flukes are found at epidemic levels in Southeast and East Asia. *Haplorchis taichui*, a member of the family Heterophyidae, lives in the small intestine of mammals and birds (Toma et al., 1999; Chai et al., 2009; Eom et al., 2014; Abdi et al., 2017). Humans can be infected with *H. taichui* by consuming their metacercariae from infected cyprinoid fish. The eggs of *Opisthorchis viverrini* and minute intestinal flukes, such as *H. taichui,* are similar and difficult to distinguish under a microscope, as such, a more specific technique is required (Kaewkes et al., 1991; Sukontason et al., 1999). Eating cyprinoid fish that have been infected with the parasite causes the body to secrete antibodies, which stimulate the immune system, as evidenced by acute inflammatory cell response to antigens of the parasite in animal models (Toledo et al., 2006). In human cases, several reports have addressed the signs and symptoms of light-to-moderate infections; these include underlying dyspepsia, nausea, vomiting, lassitude, flatulence, and loose fecal excretion (Watthanakulpanich et al., 2010). At present, pathological studies in humans are restricted due to ethical limitations; therefore, animal models have been used to explore the life cycle, biology, and pathology of parasites, including host-parasite interactions. However, in *H. taichui*-infected chicks, worm recovery slowed down after day 18 post-infection (Kumchoo et al., 2003). Syrian hamsters (*Mesocricetus auratus*) are animal models for various parasites such as *Opisthorchis viverrini* (Wonkchalee et al., 2012), *Echinostoma malayanum* (Songsri et al., 2016), *Echinostoma caproni* (Toledo and Fried, 2005) and *Leishmania braziliensis* (Pinto-da-Silva et al., 2002). Moreover, the hamster is an appropriate animal model because it is easy to control and feed, and is available in The Animal Unit, Faculty of Medicine, Khon Kaen University. Therefore, this present study used these hamsters as animal models for *H. taichui* infection, focusing on biology, morphology, and pathology.

Materials and Methods

Preparation of metacercariae: Freshwater cyprinoid fish infected with *H. taichui*, from an endemic area in the northeastern part of Thailand, were blended thoroughly with 0.25% pepsin solution in a ratio of 1:3 by volume, digested in a shaking water bath at 37 °C for 1 h, and then filtered through a sieve with mesh of various sizes (1,000, 300 and 106 µm, respectively), using saline (0.85% NaCl) as a diluent. The sediment was allowed to settle in a sedimentation jar before selecting the metacercariae of minute intestinal flukes under a stereomicroscope (Olympus, Hamburg, Germany).

Metacercariae infection and specimen collection: Syrian hamsters (*Mesocricetus auratus*) were studied for their suitability as an *H. taichui* animal model. A total of 35 male, 6-to 8-week-old, 80-90 g, Syrian hamsters were used in the study (The Animal Unit, Faculty of Medicine, Khon Kaen University). Five hamsters were randomly assigned to the normal control group. The remaining thirty hamsters were infected by gastric intubation with 200 metacercariae. Infected hamsters were then separated into 6 groups (5 hamsters/group): i) 1 week post-infection, ii) 2 weeks post-infection, iii) 3 weeks post-infection, iv) 4 weeks post-infection, v) 6 weeks post-infection and vi) 8 weeks post-infection. Hamsters in each experimental groups were observed daily for physical appearance and other objective signs such as cleanliness, behavior, food intake, manure, eye and anus (Qi et al., 2008). All hamsters were maintained at 22±2°C and exposed to 12 h light/dark cycles with free access to food and water. Five hamsters were euthanized at each time point. All experimental protocols were approved by the Animal Ethics Committee of Khon Kaen University (ACUC-KKU-16/2560).

Biology and parasite development

Eggs per gram of feces: To indirectly determine the reproductive organ development, feces from each infected hamster were collected for detection of eggs per gram of feces. Modified formalin technique was performed for the quantitative egg count of minute intestinal flukes. One pellet of feces from the rectum was weighed, fixed and mixed thoroughly with 1,000 µl of 10% formalin. A 100 µl sample with 1% iodine solution was smeared on a glass slide and the number of minute intestinal fluke eggs were counted. This procedure was repeated three times, and the result average was used. The number of eggs per gram was calculated as follow:

Eggs per gram (EPG) = Number of intestinal fluke eggs x 1,000/Feces weight (g) x100

Eggs per worm: Five worms from infected hamsters were individually crushed with 70% ethyl alcohol under a glass slide, then 1% iodine solution was added and the number of minute intestinal fluke eggs were counted under a light microscope (Olympus, Hamburg, Germany). The measurements were repeated three times, and the result average was recorded.

Worm recovery: To compare the worm recovery from infected hamsters each week, the small intestine was dissected in a Petri dish with 0.85% saline and examined under a stereomicroscope. Flukes were collected by pipette and counted. The adults were preserved in 70% ethanol, and feces pellets were collected from the rectum.

Body size of minute intestinal flukes: To determine parasite development in infected hamsters, 6 adult worms were randomly chosen for measurement of body size using carmine staining. Adult worms were fixed in 70% ethyl alcohol, stained with carmine for 30 min, de-stained with 1% acid-alcohol and dehydrated in an ethyl alcohol series (70%, 80%, 90%, 95% and absolute, respectively) for 30 min. Finally, the dehydrated flukes

were mounted with Permount solution on glass slides and the body size was measured using image analysis.

Histopathological study

Small intestine section: The small intestines of infected hamsters were fixed in 10% formalin, then cut and washed with phosphate buffered saline for 2 h and prepared for sectioning. Specimens were dehydrated in an alcohol series – 70% ethyl alcohol for 1 h (two times), 80% ethyl alcohol for 1 h (two times), 95% ethyl alcohol for 1 h (three times) and absolute ethanol for 1 h (three times) – cleared in xylene for 1 h (two times) and paraffinized in an incubator at 60 °C with paraffin solution for 90 min (one time) and 2 h (one time), respectively. Tissues were embedded in paraffin blocks and were cut with a microtome into 4 to 5 µm thick sections, coated on slides, incubated at 60 °C for 24 h and then kept at room temperature.

Hematoxylin and eosin staining: Small intestine tissue sections were deparaffinized with xylene for 3 min (three times) and rehydrated in an alcohol series: absolute ethanol for 2 min (two times), 95% ethyl alcohol for 2 min (two times) and 70% ethyl alcohol for 2 min (two times). The tissue sections were then washed in running tap water and differentiated with acid-alcohol; washed in running tap water to stop the reaction and blued in lithium carbonate for 30 sec; then washed again in running tap water and placed in eosin for 3 min. Finally, the tissue sections were dehydrated in an ethyl alcohol series – 70% ethyl alcohol for 3 min (three times), 95% ethyl alcohol for 3 min (three times) and absolute ethanol for 3 min (three times) – cleared in xylene for 3 min (three times) and mounted on slides with Permount.

After staining with hematoxylin and eosin, small intestine tissue sections were counted for the number of goblet cells per high-power field (HPF) (400×), for over five selected HPFs. Results were presented as the mean number of cells (±SD) per HPF.

Statistical Analysis: All data are expressed as the mean ± standard deviation (SD). Normality test using Shapiro-Wilk test follow by significant differences between groups were analyzed by one-way ANOVA (Post Hoc Multiple Comparisons; Tukey) using SPSS statistical software, version 16.0. Statistical significance was indicated by $^{*}P<0.05$ and $^{***}P<0.001$.

Results and Discussion

The morphology of the adult worm of *H. taichui* as shown in Fig 1 and Fig 2 shows the worm recovery rate from hamsters each week. The average worm recovery at weeks 1, 2 and 3 was 56.2% (112.4 worms), 58% (116 worms) and 68% (136 worms), respectively. The number of worms recovered decreased at weeks 4 38.25% (76.5 worms), 6 36.25% (72.5 worms), and 8 17.4% (34.8 worms). The average length and width of *H. taichui* adults recovered from infected hamsters at weeks 1, 2, 3, 4, 6 and 8 were 376.2 × 216.5, 463.6 × 249.9, 455.6 × 219.9, 397.2 × 210.1, 479.3 × 208.7 and 438 × 211.5 µm, respectively. The average length and width of *H. taichui* adults each week was neither statistically nor significantly different (Fig 3 and Fig 4). *H. taichui* infection in an animal model was reported in rats (*Rattus norvegicus*) (Saenphet et al., 2008) the infectivity rate was 20% on day 3, and no worms could be observed on day 28 post-infection, which was different from the infection in hamsters in this present study. This study is the first report to show that hamsters are also susceptible to *H. taichui* infection, as evidenced by the 100% infectivity rate observed in a hamster model. All hamsters in the experimental groups had normal physical appearance by daily observation, which is similar to human that most infections of heterophyids are asymptomatic (Toledo et al., 2006). Several previous studies have successfully used hamsters as a model for a variety of parasite infections, including *Echinostoma caproni* (Toledo et al., 2006), *Opisthorchis viverrini* (Wonkchalee et al., 2012), *O. felineus* (Lvova et al., 2012), *Trichinella spiralis* (Behnke et al., 1994), *Leishmania infantum* and *Schistosoma mansoni* (Morsy et al., 1998).

The average number of eggs per worm at 1 (127±21.5), 2 (186.2±17.8), 3 (172.6±40.1) and 4 (129.6±20) weeks post-infection was higher than at 6 (82.2±10.3) and 8 (70.2±8.8) weeks, with a statistically significant difference (*P*-value <0.001). The average eggs per gram of feces at 1, 2, 3, 4, 6 and 8 weeks post-infection was 933.3±252.8, 918.1±276.9, 2,703.3±1,148.6, 700.8±245.9, 546±236.5 and 302.9±73.6, respectively (Fig 5). The number of eggs from hamsters at week 3 was highest compared with other weeks, with a statistically significant difference (*P*-value <0.001).

The intestinal villi of hamsters in all infected groups were shorter than those in the normal control group. Goblet cells were observed in all hamsters, but there were differences in the number of cells. Goblet cells exhibited hyperplasia in all infected groups, as evidenced by increase of number and size (Fig 6). The number of goblet cells per high-power field was 11.17±2.14, 19.83±2.71, 20.33±2.94, 20.5±2.43, 26±4.05, 16±1.79 and 11±2.19 at 1, 2, 3, 4, 6 and 8 weeks post-infection and in the uninfected control group, respectively. The number of goblet cells increased at week 2 and decreased at week 8 post-infection. The highest number of goblet cells was observed at week 6 (Fig 7). In this study, worm recovery of *H. taichui* were decreased after 3 weeks post-infection, possibly due to an increase of goblet cells in the small intestine starting at 2 weeks post-infection, similar to infection with *E. malayanum* in a hamster model (Songsri et al., 2016) and other intestinal fluke infections (Fujino and Fried, 1996). Similar to the previous studies, we found that infection with gastrointestinal parasite can increase the number of goblet cells and induction of mucins, causing expulsion of the parasite (Khan, 2008).

H. taichui infected hamster were sacrificed at weeks 1, 2, 3 and 4 post-infections because this parasite develops completely at 1 week, as evidenced by the observation of eggs in feces. Therefore, we could

observe the development and expulsion of *H. taichui* by assessing worm recovery, body size and egg production (Eggs per worm and eggs per gram of feces) and including pathological change of small intestine. Moreover, hamsters were sacrificed at weeks 6 and 8 post- infection because we want to observe histopathological changes in the small intestine after worm expulsion 28 days post-infection. On day 60 post-infection with *Echinostoma malayanum*, there was acute inflammation observed by the increase in number of goblet cells (Songsri et al., 2016). Therefore, the pathology that occurs in weeks 5 and 7 had no difference in comparison with weeks 6 and 8.

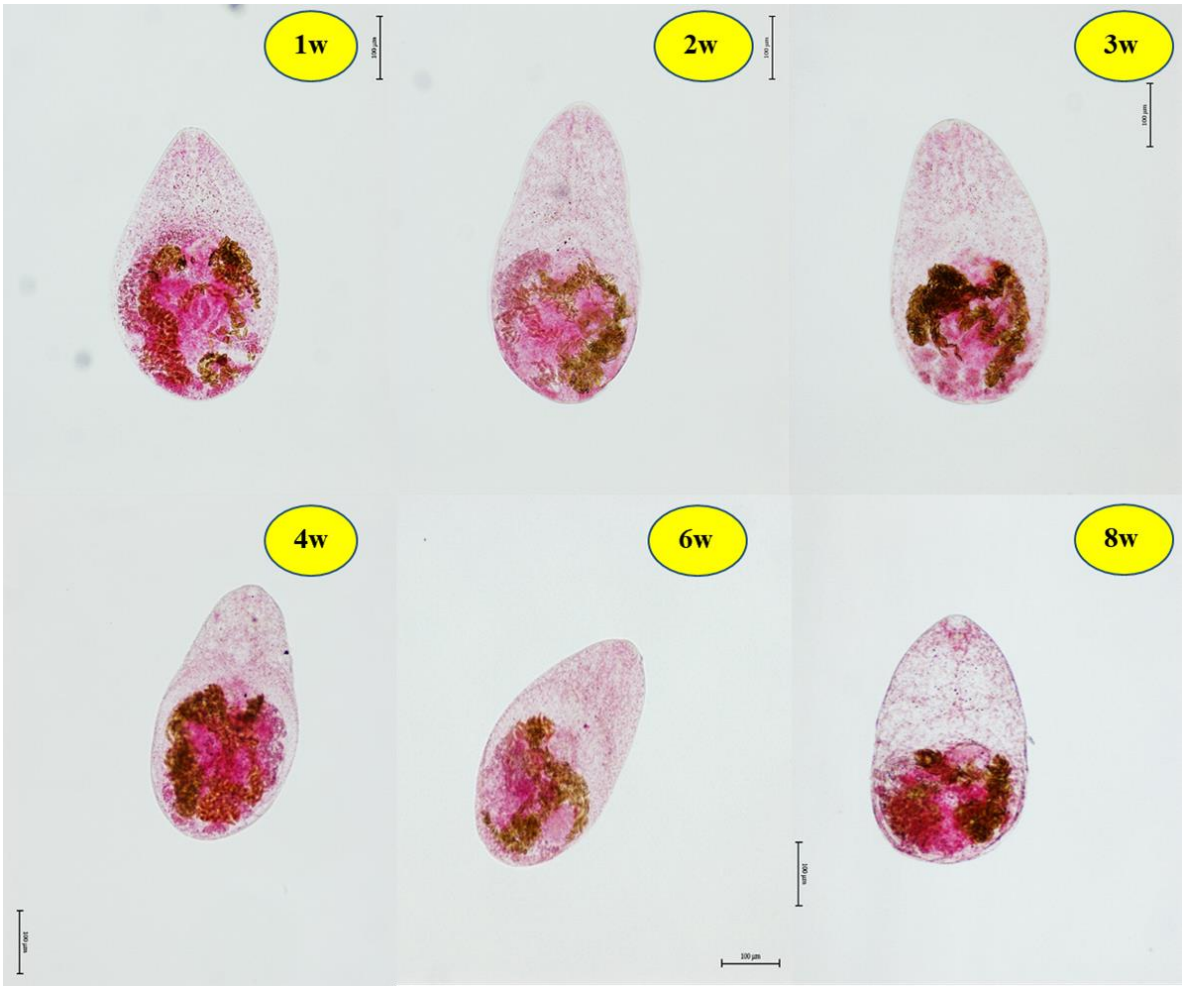

Figure 1 Representative *Haplorchis taichui* adults recovered from infected hamsters each week.

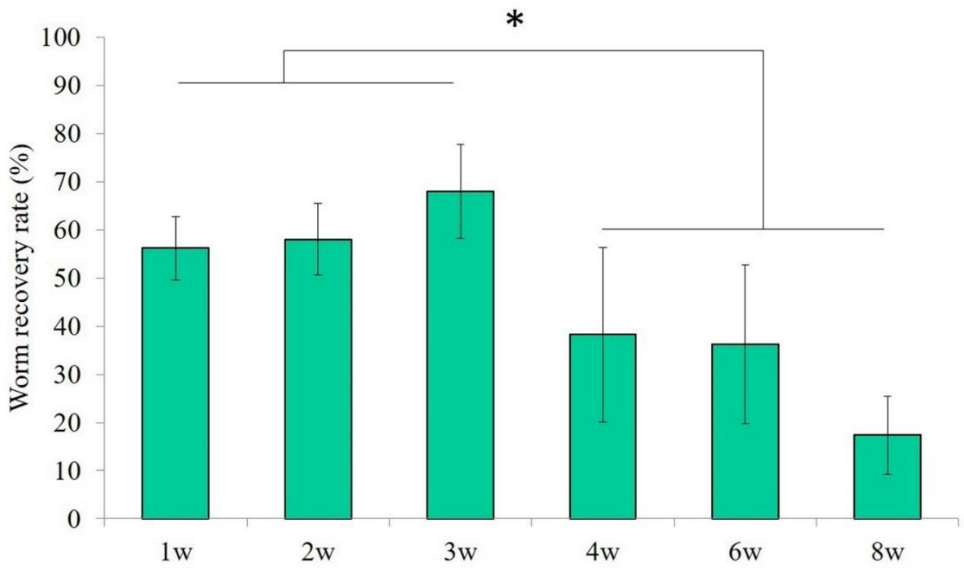

Figure 2 The worms recovered from infected hamsters each week, *P-value <0.05.

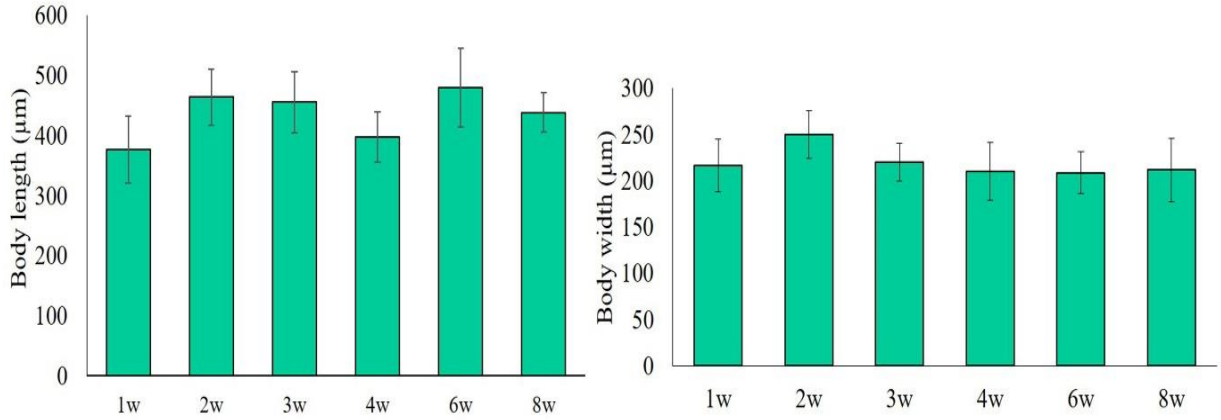

Figure 3 Comparative body size of *Haplorchis taichui* adults collected from infected hamsters each week.

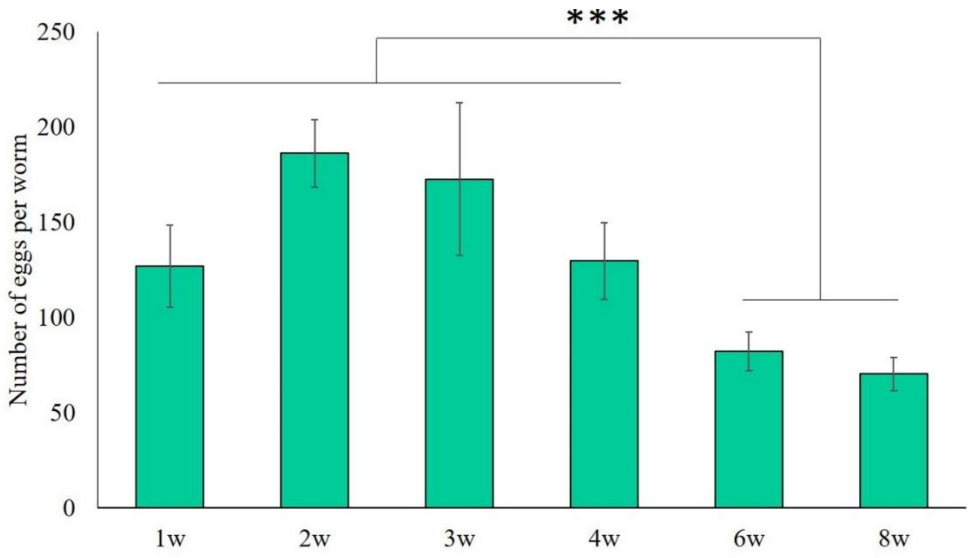

Figure 4 Average number of eggs per worm of *Haplorchis taichui* adults collected from infected hamsters each week, ***P*-value <0.001.

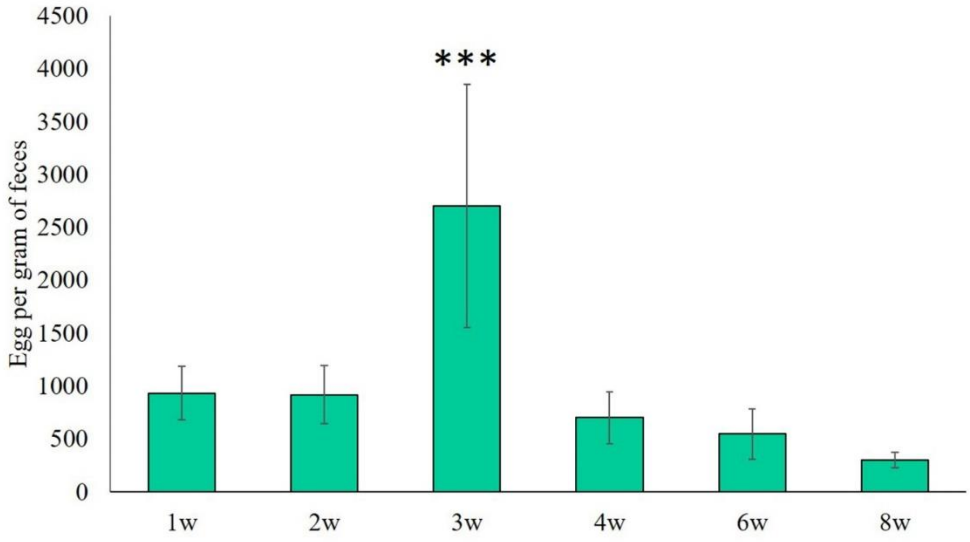

Figure 5 Average eggs per gram of feces collected from infected hamsters each week, ***P*-value <0.001.

The present results suggest that hamsters are one of the optimal hosts for *H .taichui*, and these hamsters would be suitable for future animal studies on various aspects of *H .taichui* infection.

Conflict of interest: The authors declare no conflict of interests in this study.

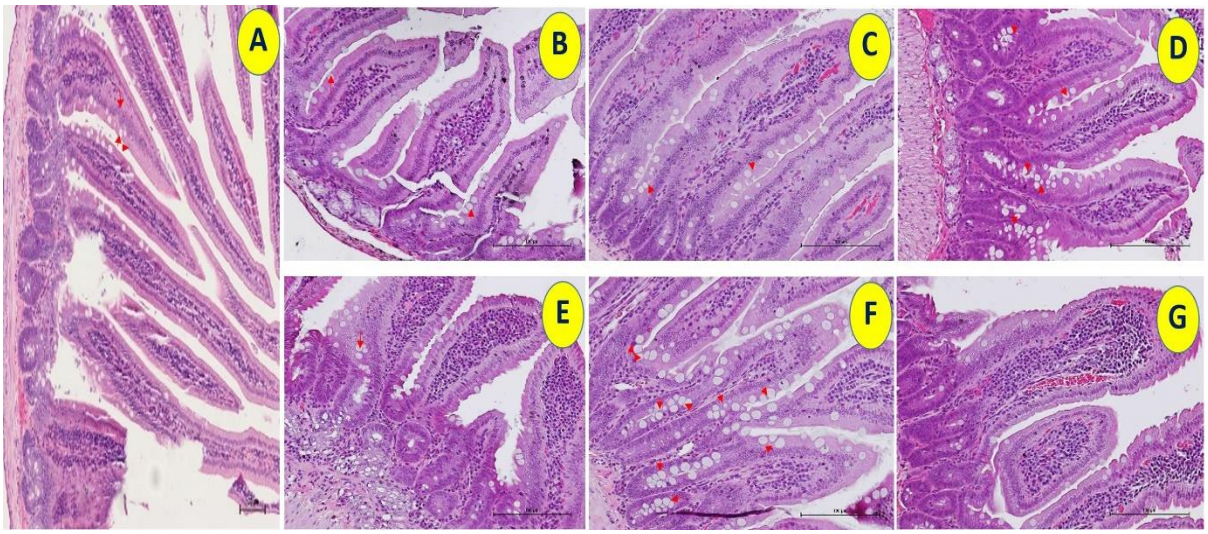

Figure 6 Histopathological changes of the small intestine of *Haplorchis taichui*-infected hamsters. A) Uninfected, B) 1 week post-infection, C) 2 weeks post-infection, D) 3 weeks post-infection, E) 4 weeks post-infection, F) 6 weeks post-infection and G) 8 weeks post-infection.

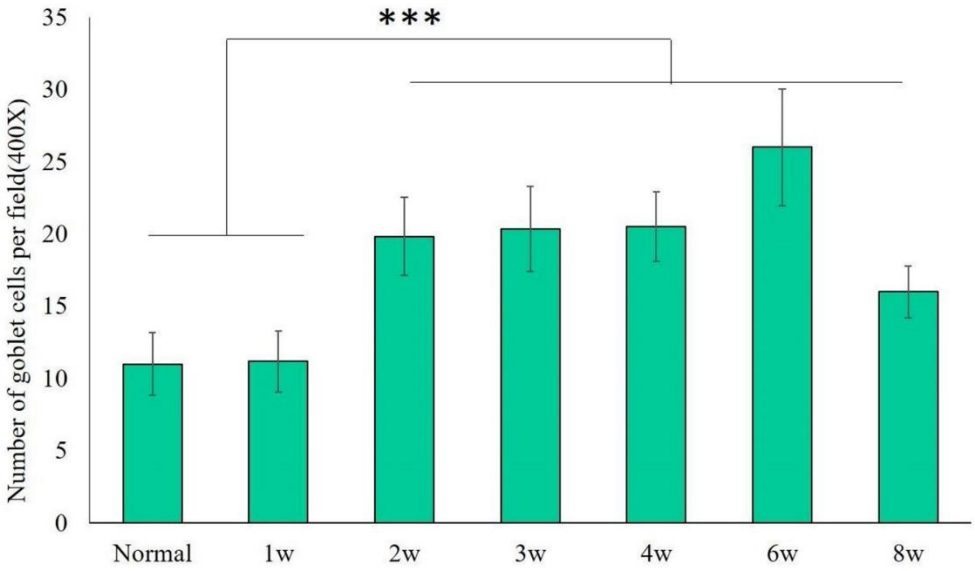

Figure 7 Average number of goblet cells per high-power field in non-infected and infected hamsters each week,*** *P*-value <0.001.

Acknowledgements

This study was supported by TRF Senior Research Scholar Grant no. RTA5880001.

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บทคัดย่อ

การใช้หนูไซเรียนแฮมสเตอร์ (*Mesocricetus auratus*) เป็นสัตว์ทดลองตัวแบบสำหรับการติดเชื้อพยาธิ *Haplorchis taichui*: รูปร่างลักษณะ ชีววิทยา และพยาธิวิทยา

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Haplorchis taichui เป็นพยาธิใบไม้ลำไส้ขนาดเล็กซึ่งสามารถเจริญเป็นตัวเต็มวัยในสัตว์เลี้ยงลูกด้วยนม สัตว์ปีก รวมทั้งในคน และสามารถพบระยะตัวอ่อนในสัตว์ไม่มีกระดูกสันหลังและสัตว์เลือดเย็น วัตถุประสงค์ในการศึกษาในครั้งนี้เพื่อศึกษาการเจริญของพยาธิใบไม้ลำไส้ขนาดเล็ก ซึ่งได้แก่ จำนวนพยาธิ (worm recovery) หลังจากการติดเชื้อ ขนาดลำตัวตัวเต็มวัย (body size) จำนวนไข่ต่อพยาธิ 1 ตัว (eggs per worm) จำนวนไข่ต่ออุจจาระของหนู 1 กรัม (eggs per gram) และพยาธิสภาพของลำไส้เล็ก ในการศึกษาครั้งนี้ใช้ หนูไซเรียนแฮมสเตอร์ (*Mesocricetus auratus*) อายุ 6-8 สัปดาห์ เพศผู้ โดยทำให้ติดเชื้อเมตาเซอร์คาเรียของพยาธิ *H. taichui* จำนวน 200 เมตาเซอร์คาเรียต่อหนู แฮมสเตอร์ และเก็บตัวอย่างหลังจากติดเชื้อไปแล้ว 1 2 3 4 6 และ 8 สัปดาห์เพื่อนำมาวิเคราะห์ผล ผลการศึกษาพบว่าจำนวนพยาธิสูงที่สุดที่ 1 2 และ 3 สัปดาห์หลังจากติดเชื้อ หลังจากนั้นจำนวนพยาธิค่อยๆลดลงที่สัปดาห์ที่ 4 6 และ 8 ตามลำดับ จำนวนไข่ต่อพยาธิ 1 ตัวพบสูงที่สุดที่ 2 และ 3 สัปดาห์หลังจากติดเชื้อ จำนวนไข่ต่ออุจจาระของหนู 1 กรัมพบสูงที่สุดในสัปดาห์ที่ 3 ขนาดลำตัวของพยาธิในทุกสัปดาห์ไม่แตกต่างกัน และพบพยาธิสภาพของลำไส้เล็กที่ villi และ goblet cell โดยพบ villi ที่สั้นลง และ พบว่า goblet cell เพิ่มขึ้นทั้งจำนวนและขนาดเมื่อเทียบกับหนูที่ไม่ติดเชื้อ

คำสำคัญ: การจำลองในสัตว์ ชีววิทยา *Haplorchis taichui* รูปร่างลักษณะ พยาธิสภาพ

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