Echinostoma revolutum in Domestic Chickens: Developmental Larval Stages and Fecundity of an Intestinal Trematode

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Abstract: This study was conducted to observe the recovery and fecundity of intestinal trematode, *Echinostoma revolutum* in domestic chick (*Gallus gallus domesticus*) and notes on their egg development. Each 60 three days old domestic chicks were fed 50 cysts of *E. revolutum* isolated from *Filopaludina martensi martensi*. Worm recovery, uterine egg counts, numbers of eggs per gram of feces (EPG), egg morphology and development of *E. revolutum* during of experimental infection in chicks were analyzed. The worms survived in chicks for 36 days post infection (PI). The incidence of infection was 60.0% (36/60). Of the 1,800 cysts fed to chicks, 465 (27.1%) worms mainly recovered from the jejunum and ileum, occasionally in caeca. The worms became ovigerous by day 10 and produced eggs, which were detected in feces as early as 10 days PI. The number of EPG, determined by a modified formalin-ether concentration technique, as well as EPG per worm increased slowly during day 10-16 PI and then remaining stable and showed a little fluctuation until day 36 PI. Additionally, egg development was characterized from day 0 to 10 post embryonation. Eggs developed fully formed miracidia from chicks after 10 days and emerged later.

Keywords: *Echinostoma revolutum*, Domestic chicks, Fecundity, Egg development

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Introduction

Echinostoma revolutum (Froelich, 1802) Looss, 1899 (Digenea: Echinostomatidae) is an intestinal parasite of a broad range of vertebrate hosts, such as birds, mammals, including human and occasionally reptiles and fishes (Kanev, 1994; Fried and Graczyk, 2004). *E. revolutum* is cosmopolitan and infect a

large number of different hosts, both in nature and the laboratory. This worm has a wide range of experimental definitive hosts, though the compatibility may differ considerably between species (Fried, 1984; Franco *et al.*, 1986; Humphries *et al.*, 1997). The establishment of *E. revolutum* in experimental animals has been studied in mice (Hosier and Fried, 1986), golden hamsters (Chai *et al.*, 2011; Chantima *et al.*, 2013) and domestic chicks (Fried *et al.*, 1997). Several studies have been reported the fecundity of echinostomes, which had made on the basis of eggs per gram of feces (Odaibo *et al.*, 1988, 1989), uterine egg counts (Christensen *et al.*, 1990) and total amount of eggs in the feces of the host (Toledo *et al.*, 2003; Munoz-Antoli *et al.*, 2007). Detailed information concerning uterine egg counts and egg output to determine fecundity appears limited to infections with *E. revolutum* in experimental animals. However, the fecundity of *E. revolutum* in experimental animals has been studied in detail, but no report for studying in details of the uterine egg counts and egg output of this worm infection in domestic chicks.

Echinostome eggs are unembryonated when laid and require a period of incubation in the environment to form mature miracidia capable of hatching (Huffman and Fried, 1990). A comparative photographic study of the eggs of echinostomes, including *E. paraensei*, *E. trivolvis* and *E. caproni* was done by Krejci and Fried (1994) and Fujino *et al.* (2000). There are not morphologically different from other echinostome eggs, whereas the ultrastructure of *E. revolutum* eggs was not reported previously. Incubation of *E. revolutum* eggs from laboratory infected hosts used to study in avian hosts (Kanev, 1994). In addition, the studied on storage and incubation of this worm eggs recovered from wild Canada geese was done by Davis (2005). Most studies concerned about the hatchability of miracidium in various conditions, without observations on the development of *E. revolutum* egg to form mature miracidia. The developments of *E. revolutum* eggs are not well understood, and there is no report on egg development of this worm recovered from the domestic chicks. Therefore, the present study was aimed to determine the worm recovery and fecundity of *E. revolutum* in an experimental chick. Moreover, the authors have described the ultrastructure and development of eggs to form mature miracidia.

Materials and Methods

Experimental infections and worm recovery

Three days old domestic chicks (*Gullus gallus domesticus*), weighing between 48-60 g were used as the experimental hosts.

Metacercariae of *E. revolutum* were collected from naturally infected snails, *Filopaludina martensi martensi* from Chiang Mai province, Thailand. They were collected by the crushing method and isolated using a sharp pin, gently covered with a coverslip, and observed under a light microscope. The presence of a head collar with 37 spines was highly indicative of *E. revolutum*. Fifty encysted metacercariae of *E. revolutum* were orally forced fed to each chick. Each 60 chicks were sacrificed daily at day 1-60 post-infection (PI) by excess diethyl ether for examination of the parasite. All experimental hosts were managed according to the guideline approved by the Animal Ethics Committee of the Faculty of Science, Chiang Mai University. All Chick digestive tracts were roughly divided into the esophagus, crop, stomach (proventriculus and gizzard), small intestine (duodenum, jejunum, ileum), caecum and rectum, longitudinally with a pair of scissors and placed in 0.85% NaCl. Worms were collected and examined under a stereo microscope. The number of recoveries was recorded to determine the infectivity and worm recovery.

Fecundity of the worm

Data on fecundity were determined by uterine egg counts (UEC) and a number of eggs per gram feces (EPG). Fecal samples were examined for determining the EPG. The chick feces were collected separately, incubated at 60°C for 24 hours, weighed and fixed in 10% formalin before an examination. Chick feces were checked daily by a modification of the formalin-ether concentration technique (MFECT) (Elkins *et al.*, 1991). Eggs per gram of feces were determined according to Odaibo *et al.* (1988, 1989). The number of eggs in the uterus of 10 worms from each day during early appeared in uterus (10 days PI) to the end of experimental was determined by dissection (Christensen *et al.*, 1990). The correlation was used to quantify the association between the worm recovery and UEC, and/or EPG. In addition, the correlation of UEC per worm (UEC/worm) and EPG per worm (EPG/worm) was determined.

Egg morphology

The eggs were photographed, measured and illustrated under a compound microscope for morphological study. Morphological traits of eggs (n=30) were studied and measured by an Olympus eye-piece micrometer. Some eggs were studied for the surface ultrastructure with scanning electron microscope (SEM). Briefly, the eggs were rinsed several times in 0.1 M phosphate buffer, pH 7.4, and fixed in 2.5% glutaraldehyde at 4°C for 24 hours, and post-

fixed with 1% osmium tetroxide for 3 hours. Then they were dehydrated in a graded alcohol series, transferred into acetone, and finally dried in a critical-point dryer. The specimens were mounted on stubs and then coated with gold. The specimens were observed and photographed using a JEOL JSM-5400LV SEM.

Observations of egg development

Mature unembryonated eggs were purified from chick feces by filtration through a series of graded sieves, then observed under a stereo microscope. The eggs were washed in 0.85% NaCl several times and then incubated in multi-well plate cultures containing 2 ml of distilled water. The eggs were incubated at room temperature (25-28°C) with ambient room lighting for at least 2 weeks to observe the final hatch to obtain eggs with fully developed miracidia. Egg development was observed under a compound microscope from live eggs prepared as wet mounts. Egg development was monitored daily. Developmental stages were photographed using a compound microscope (OLYMPUS DP20, Olympus).

Results

Worm recovery

After orally introduced to chicks, the metacercariae excysted and developed into adults in the small intestine. From day 1 to day 36 PI, a total of 465 worms were recovered from 36 chicks that had been infected with a total of 1,800 metacercariae. The incidence of infection was 60.0% (36/60) and the average worm recovery per chick was 27.1%, which varied from 2.0 to 74.0%. Metacercariae of *E. revolutum* developed into mature adults after 8 days PI and ovigerous adults developed after 9 days PI. The worms were mostly recovered from the jejunum (75.2%), ileum (23.4%), and some from the caecum (1.4%) while not found the worms in other parts of the digestive tract. The worms were survived until day 36 PI.

Fecundity of worm

The worms vigerous and began to produce eggs on day 10 PI. Based on the eggs in feces (Figure 1), they firstly appeared at 10 days PI and egg was released continuously from the first day until day 36 PI. Egg per gram of feces per worm (EPG/worm) increased slowly and showed a little fluctuation during day 10-22 PI and then slowly decreased until day 36 PI. After that, the worms were expelled from the chicks by day 37 PI. Figure 1 also shows that the uterine egg counts per worm (UEC/worm) during early appeared in uterus (10 days PI) to the end of experimental. The UEC/worm rapidly increased during the first two weeks of