Saudi Journal of Biological Sciences (2010) **17**, 247–251

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Development and hatching mechanism of *Fasciola* eggs, light and scanning electron microscopic studies

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Received 12 February 2009; accepted 23 September 2009
Available online 13 April 2010

KEYWORDS

Fasciola gigantica;
Fasciola hepatica;
Eggs;
Miracidium;
Hatching mechanism;
Light microscope;
Scanning electron microscope;
Epidermal plates;
Egypt

Abstract Both light microscopy and scanning electron microscopy were used in the description. There were variable measurements of eggs from the same fluke and there was no relationship between the size of the fluke and size of eggs. Light microscopy revealed that the operculum has different shapes in *Fasciola gigantica* and *F. hepatica*. Under normal laboratory conditions of temperature (26 ± 1 °C), miracidia of *F. gigantica* developed within 12–16 days period, but those of *F. hepatica* developed within a period of 13–15 days. The miracidia of *F. gigantica* measured 110 × 70 μm and that of *F. hepatica* measured 136 × 74 μm. The life span of the miracidium in *F. gigantica* ranged between 9 and 12 h, while in *F. hepatica* the life span did not exceed 10 h. Miracidial epidermal plates in miracidium of *F. gigantica* were found to be 20 plates arranged in four tiers of six, four, six and four. Hatching process was recorded using scanning electron microscope, and it indicated partially opened operculum.

1. Introduction

Infection with the liver fluke (*Fasciola* spp.) causes a disease known as fascioliasis in grass-grazing animals and human being, mainly sheep and cattle. In the past, human infections were mentioned as an accidental infection or rarely reported, but human fascioliasis is at present emerging or re-emerging in many countries, including its increase in prevalence, intensity and geographical expansion. In Egypt, animal fascioliasis is a dangerous disease leading to huge economic loss in live stock production and fasciolosis causes severe illness in human liver. Kremer and Chaker (1983) reported no operculum for *F. hepatica* eggs. They considered such anomalous shape to be depending on the way of formation of these eggs. Eggs of *Fasciola* consist of a fertilized ovum with vitelline cells surrounded by proteinous shell (Beaver et al., 1984). Krejci and Fried (1994) reported the presence of a knob above the opercula (aboperular knobs) in their observations of the eggs of *Echinostoma trivolvis* and *E. caproni* eggs. *F. hepatica* eggs are operculated and measured

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doi:10.1016/j.sjbs.2010.04.010

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130–150 μm in length and 63–90 μm in breadth with a characteristic of yellow colour. They were not readily differentiated from *F. gigantica* eggs (Chen et al., 1990). During the hatching mechanism, light stimulates the miracidium development and when fully activated, the miracidium is responsible for altering the permeability of the membrane on the internal concave surface of the viscous cushion. The change in permeability of the enclosing membrane allows the fluid egg contents to permeate into the cushion. The cushion and miracidium are then expelled by hetronicity of the egg contents (Wilson, 1968). Light and dark conditions affect the formation period and the development of miracidium in *E. caproni* eggs. Exposure to light was essentially trigger hatching, with incandescent light providing more consistent stimulation than fluorescent light. The majority of miracidia hatched between 46 and 67 days, indicating a diurnal circadian pattern. (Behrens and Nollen, 1993).

This study aims to investigate the development of miracidium and hatching mechanism of *Fasciola* eggs. Description of the development and hatching mechanism will be clarify using both light microscopy and scanning electron microscopy.

2. Materials and methods

Sexually mature *Fasciola* species were collected from different slaughterhouses in Qena governorate, Egypt. According to Drury and Wallingten (1980), the eggs were fixed in 10% neutral formalin and then they were transferred onto a glass slide. They were mounted in glycerol-jelly (aqueous media), Kaiser's glycerol-jelly. A considerable amount of eggs were put in a small Petri dish under normal laboratory conditions of temperature (26 ± 1 °C) for hatching. The miracidia were studied by light microscope and then a very dilute solution of neutral red (0.1%) was used to detect the epidermal plates. Miracidia were measured using eye piece micrometer. For scanning electron microscope, the eggs were fixed in 5% glutaraldehyde and dropped in sodium cacodylate buffer (pH 7.2) for 48 h. The samples washed three times in the same buffer and postfixed by adding 1% osmium tetroxide for 2 h. The samples were washed again in the same buffer three times. Dehydration was done in ascending concentrations of ethanol. The excess alcohol was withdrawn after passing from water to amylacetate. The specimens were placed in a chamber where liquid carbon dioxide is used to substitute for amylacetate, then heated to 35 °C to separate carbon dioxide from the specimens, then mounted on holders, sputter coating immediately after critical point. Gold was used for coated for about 2–3 min. The specimens were examined using scanning electron microscope (505 LV-Jeol).

3. Results

3.1. Fasciola gigantica eggs

The eggs are large yellowish and operculated with thin shell. Each egg contains one cell stage embryo surrounded by a group of oval body yolk cells. It has a distinct, inner concaved operculum and an umbilicus-like invagination at the posterior end of the shell. The egg contains a fully developed miracidium (Fig. 1). There was a variable size of the eggs from the same fluke.

Figure 1 Photomicrograph of *F. gigantica* egg containing a fully developed miracidium. It has a different shape of operculum (arrowheads) and the umbilicus-like invagination (arrowheads). 400×.

3.2. Development and hatching of F. gigantica eggs

Under normal laboratory conditions of temperature (25–27 °C), maturation of eggs was completed within a period of 13–16 days. The developed miracidia exhibit some movements inside the egg shells before outside escaping from the eggs throughout repeated and strong pushing to the operculum, which became partially opened. The emergence of miracidia occurred within 4 days of their maturation. The miracidia of *F. gigantica* measured 98–119 (110 ± 0.1) × 63–77 (70 ± 0.04) μm (Fig. 2). The life span ranged between 9 and 12 h.

3.3. F. hepatica eggs

The eggs are large, yellowish, oval body with a thin, short and straight/flat opercula. The egg shell contains an umbilicus-like invagination at its posterior end. The egg possesses a full matured miracidium (Fig. 3). The ratio between the size of egg and the size of mother worm was not proportional.

3.4. Development and hatching of F. hepatica eggs

Under room temperature, ranging between 26 ± 1 °C, eggs were kept in distilled water and revealed mature miracidia after 12–15 days. Within 4 days, hatching of eggs occurred during which miracidia pushed the egg's operculum to find their way outside from a narrow and partially opened operculum. The fully extended miracidium of *F. hepatica* (immediately

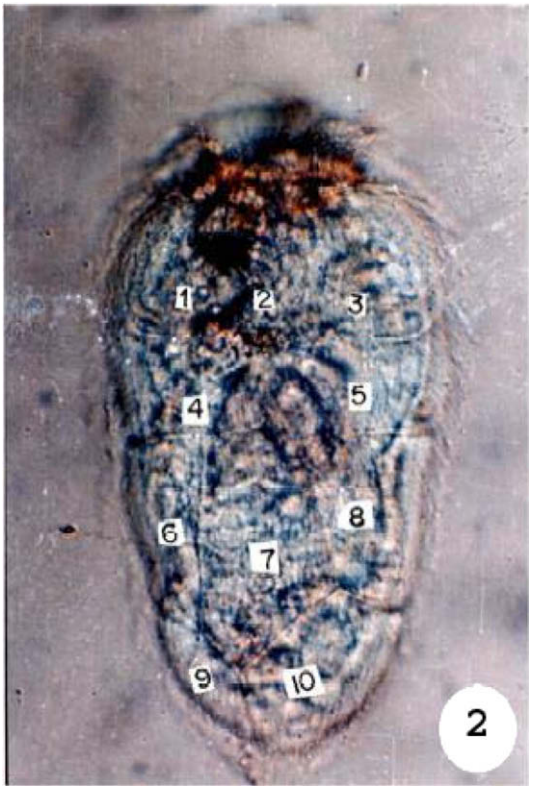

Figure 2 Photomicrograph of *F. gigantica* miracidium showing epidermal plates (numbers referred to position and number of epidermal plates). 400×.

Figure 3 Photomicrograph of *F. hepatica* egg containing a fully developed miracidium. It possesses another shape of operculum (arrowheads) and the umbilicus-like invagination (short arrow). 400×.

after emergence) measured 220–500 (av 304) × 70–80 (av 76) μm. The fixed miracidium measured 110–150 (av 136) × 60–80 (av 74) μm. The life span ranged between 8 and 10 h (Figs. 3–5).

The miracidium of *F. gigantica* was also chosen to detect the number and arrangement of the epidermal plates using diluted neutral red (0.1%) which revealed 20 epidermal plates arranged in four tiers of six, four, six, and four plates (Fig. 2).

The hatching mechanism of *F. gigantica* miracidia was studied using scanning electron microscope, which revealed that the miracidial escape from the egg. The hatching starts by weaking and cracking of one side of the opercular junction (Fig. 6), followed by breaking the shell-opercular attachment at that side. While still attached from the other side, the operculum gradually rises away from the broken side until it allows the miracidium to escape (Fig. 7).

4. Discussion

Allam in 1992 mentioned a limited measurement to differentiate between the eggs of *Fasciola gigantica* and *F. hepatica*. Abrous et al. (1998) and Srimuzipo et al. (2000) reported that the measurements of *F. hepatica* eggs depend on the host. Valero et al. (2001) also had the same opinion. The present work included light and scanning electron microscopic studies of *Fasciola* eggs. The present study revealed a direct relationship between the length and width of eggs, while the ratio between the size of egg and the size of mother worm was not proportional. Moreover, eggs of both species have nearly the same shape, but have different shaped-opercula. In addition, both types of the eggs possess umbilicus-like invaginations at the posterior end of the egg shells. Statistical analysis of *F. hepatica* egg size in infected rats, egg morphometry did not vary depending on the Altiplanic definitive host species isolated. The study revealed that the definitive host species decisively influenced the size of *F. hepatica* adults and eggs, and these influences did not persist in a rodent definitive host model (Valero et al., 2001). Mendes et al. (2008) reported that eggs of *F. hepatica* released in cattle faeces were significantly bigger than those released in marmoset faeces. Meanwhile, the mammalian origin of *F. hepatica* miracidia had an effect on the number of live rediae, their length, and their redial and cercarial productivity according to Vignoles et al. (2004). Kremer and Chaker (1983) described a *F. hepatica* eggs without operculum, and the formation of the operculum depended on the way of formation of the egg. Krejci and Fried (1994) observed abopercular knobs in the eggs of *E. trivolvis* and *E. caproni* using both light and scanning electron microscopes. The development of miracidia inside the eggs was followed using both light and scanning electron microscopes. Rowan (1956) recorded the process of hatching in *F. hepatica* eggs. The present study reveal that, both *F. hepatica* and *F. gigantica* eggs develop to complete maturation within a period of 12–16 days and the miracidia hatch within 4 days after maturation. The steps of partial opening of the operculum were illustrated through the SEM. In the hatching mechanism of *F. hepatica* eggs, light stimulates the miracidium development and when fully activated, the miracidium is responsible for altering the permeability of the membrane on the internal concave surface of the

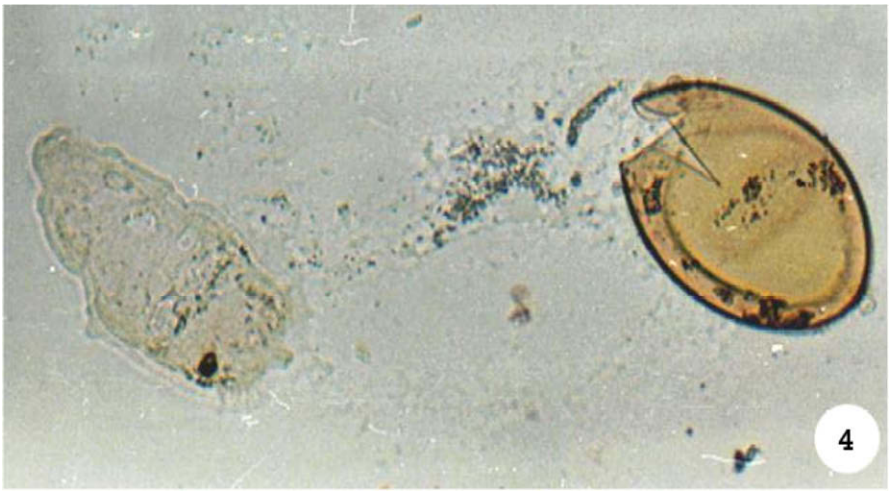

Figure 4 Photomicrograph of egg and miracidium of *F. hepatica* immediately after hatching, showing partially opened operculum and sharply broken egg shell. 200×.

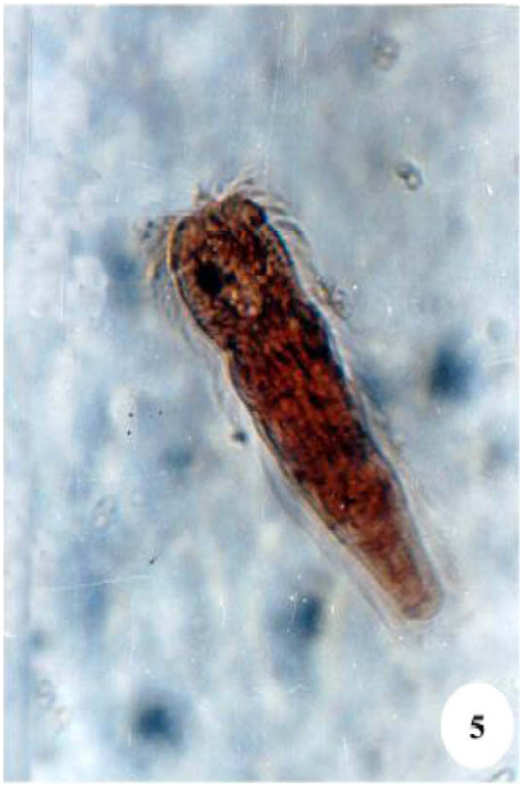

Figure 5 A light micrograph of a fully extended miracidium of *F. hepatica* (after emergence). 200×.

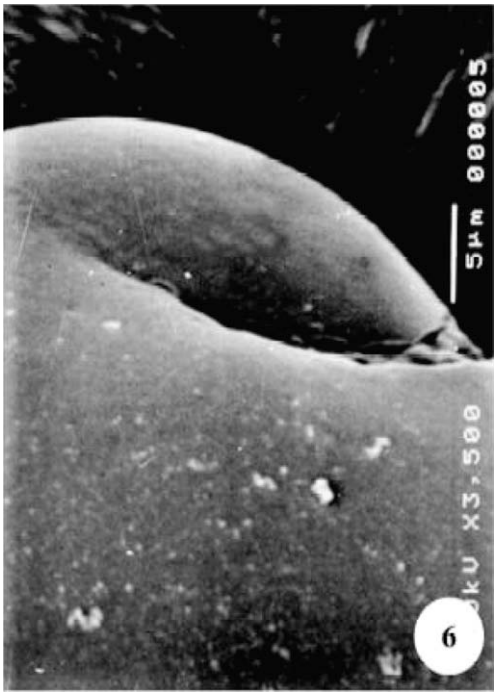

Figure 6 A scanning electron micrograph of *F. gigantica* egg showing the operculum just before opening (notice the beginning of opercular opening at one side). 3.500×.

viscous cushion. Miracidium is then expelled off the egg contents (Wilson, 1968). Shinn and Cloney (2005) had clarified hatching sutures of the egg capsules in *S. franciscanus*. In the present study, miracidia strongly pushing the operculum.

Most of the previous work on miracidia was done on that of *F. hepatica*. Koie et al. (1976) and Malek (1980) described 21 polygonal cells arranged in five rings of six, six, three, four and two cells. Egg capsules of *Syndisyrinx franciscanus*, an intestinal parasite of sea urchins (*Strongylocentrotus* spp.), consisted of a bulb, which contained the embryos, and a stalk-like filament. The end of the bulb opposite to the attachment of the filament bears a reticulum of hatching sutures. Transmission electron microscopy disclosed that hatching sutures traverse the entire thickness of the capsule wall (Shinn and Cloney, 2005). During the present study, without the tedious laboratory use of silver impregnation, the epidermal plates of *F. gigantica* miracidium were shown by supervital staining with weak solution of neutral red to be formed of 20 epidermal plates arranged in four tiers of six, four, six and four plates.

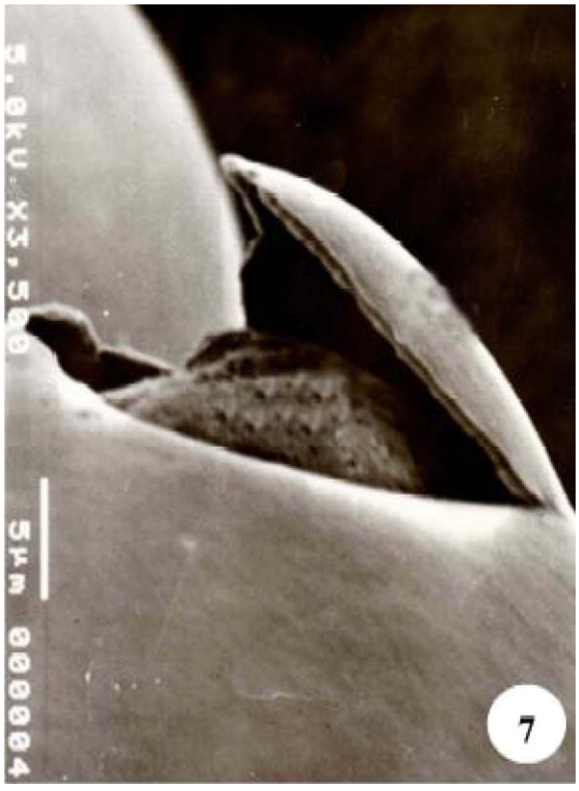

Figure 7 A scanning electron micrograph of *F. gigantica* egg showing miracidium escaping throughout the partially opened operculum. 3.500×.

Acknowledgements

This work is dedicated to the memory of Dr. Ismael Mahmoud Hassan, Zoology Department, Faculty of Science, South Valley University, Qena, Egypt.

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