Ultrastructure of eggs of Paracapillaria (Crossicapillaria) philippinensis and evidence related to its life cycle

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Abstract

The varied ultrastructure of the eggshell of Paracapillaria (Crossicapillaria) philippinensis, collected from a human sample, is reported from a scanning electron microscopy study. Two distinct egg shapes were identified: typical peanut-shaped and swollen peanut-shaped. Both thick and thin eggshells were detected. Thick eggshells are either fairly smooth or bear a beam-like network in relation to the pillars in their surface ultrastructure. Thin eggshells are transparent allowing visibility of the coiled larva within. Presence of the thin shell provides supportive evidence of autoinfection involved in the life cycle of this medically important parasite.

Keywords: Paracapillaria (Crossicapillaria) philippinensis; Eggshell; Ultrastructure; Scanning electron microscopy

Paracapillaria (Crossicapillaria) philippinensis is one of the most virulent gastrointestinal parasites of human beings. Capillariasis, or infection with this nematode, produces morbidity and mortality of people primarily in the Philippine Islands and Thailand (Chitwood et al., 1968; Cross, 1992). More cases have been recorded to expand the incidence of this parasite to several other countries in Asia, South America, and northern Africa (e.g. Bhaibulaya et al., 1977; Moravec, 2001). Clinical manifestations of infection usually involve abdominal pain, intermittent diarrhea, borborygmus, and edema. Loss of body weight, dehydration, vomiting, and anorexia are also seen, and untreated cases ultimately lead to death (Cross, 1992). This notorious human parasite was only recently renamed as Paracapillaria (Crossicapillaria) philippinensis from the longstanding, well known name of Capillaria philippinensis (Moravec, 2001). Reassignment of this species of nematode under this new genus and subgenus is due to taxonomic advances that have been made in recent years.

Humans, in particular men between 20 and 50 years of age, become infected with P. philippinensis by ingesting infective larvae present within undercooked or raw small freshwater or brackish water fish that serve as intermediate hosts. The larvae become adults in the lumen and mucosa of the intestine. Adults of this parasite are very fine and filamentous. The length of males ranges from 1.5 to 3.9 mm and females range from 2.3 to 5.3 mm (Chitwood et al., 1968; Cross, 1992). The possibility of autoinfection arises within humans since female parasites are sometimes larviparous, or can produce thin-shelled eggs that hatch within the intestinal tract, thereby allowing reinfection with more individual parasites (Cappelo and Hotez, 1998). Diagnosis of infection with this parasite is made by examination of a stool specimen and confirmed presence of the unique bioperculate and peanut-shaped eggs. Previous investigations have indicated two types of eggs using light microscope: thick-shelled and thin-shelled (Cross, 1992; Sun, 1999; Moravec, 2001). However, there is no previously ultrastructural report of this parasite’s eggs collected from human case. Herein, we present the ultrastructure of the eggshell of P. philippinensis with the aid of SEM of eggs collected from the stool specimen of an infected patient.

A fecal specimen heavily infected with P. philippinensis was obtained from a patient admitted to the Maharaj Nakorn Chiang Mai Hospital, Thailand, with chronic diarrhea. The diagnosis of capillariasis was performed by stool
examination using a formalin-ether concentration method (Ritchie, 1948), the remaining fecal specimen was then processed for the SEM observation.

The fecal specimen was mixed with distilled water in a centrifuge tube and agitated at 1500 rpm in a centrifuge during four separate 5 min intervals. After the last agitation, the sediment was then smeared onto a cover glass (15 mm in diameter) that had been coated with Poly L-Lysine (Sigma®). For the coating process, cover glasses were first placed in a beaker containing 0.1% HCl in 70% ethanol overnight. After being left to air dry, the cover glasses were then placed in a beaker containing 10% Poly L-Lysine diluted with distilled water for 5 min and finally left to air dry overnight before being smeared with a fecal sample.

After the coated cover glasses had been smeared with a fecal specimen, they were left at room temperature for 15 min. Then specimens were prepared for the SEM by fixing in 2.5% glutaraldehyde at 4°C for 24 h. The fixed specimens were placed in 0.1 M phosphate buffer solution (PBS) at a pH of 7.4 for three separate 15 min intervals. They were then treated for 2 h with 1% osmium tetroxide at room temperature for post-fixation, followed by 15 min rinsing with PBS three times. For dehydration, specimens were dehydrated in graded aqueous ethanol (30% v/v to 100%). Smearied cover glasses remained in each concentration of alcohol for 1 h during each step of the dehydration process and concluded with acetone for 15 min. Dehydration of the specimens was followed by critical-point drying, mounting them on stubs, and sputter-coating them with gold in the sputter coating apparatus for a 6 min. period for subsequent study in a JEOL-JSM840A SEM (JEOL, Japan).

To measure the egg dimension and thickness of the eggshell, the fecal specimens after sedimentation was dropped onto a clean glass slide, covered with a cover slip and examined under a light microscope equipped with a calibrated eye piece micrometer. The eggs dimension and thickness were measured from 26 intact eggs using ocular micrometer.

Out of a total 97 *P. philippinensis* eggs, two distinct morphological shapes of thick-shelled eggs were detected: the typical peanut-shape (Figs. 1–3) and a swollen peanut-shape (Fig. 4). A typical *Paracapillaria* egg was characterized as being peanut-shaped and bearing slightly concave polar plugs at each end. The surface ultrastructure of the eggshell of the typical peanut-shaped eggs was categorized into three primary groups. The first group, represented most of the eggs observed (80.40%; 78/97), possessed an entirely smooth eggshell (Fig. 1). In the second group the surface of the eggshell was characterized by an entire intricate, beam-like network in relation to the pillars (Fig. 2) and existed in fewer numbers than the smooth eggshell (6.19%; 6/97). An intermediate of these two groups formed the third group and appeared to have a partial beam-like network on the surface of the egg. The number of eggs in this group was minimal (4.15%; 4/97) (Fig. 3). Regarding the swollen peanut-shaped eggs, all of these were observed to have a more or less irregular ‘orange-peeled’ surface (Fig. 4) and was the second most abundant type of egg in our study (8.25%; 8/97). In contrast, eggs with a thin eggshell were scarce (1.03%; 1/97). The thin eggshell revealed the internal coiled larval nematode (Fig. 5).

Our study on the eggshell morphology of *P. philippinensis* by SEM confirms previous information concerning the presence of both thick and thin eggshells in eggs of this parasite (Cross, 1992; Sun, 1999; Anderson, 2000; Moravec, 2001). According to Cross (1992), the uterus of a female parasite contains thick-shelled eggs and thin-shelled eggs, with or without embryos or larvae. A previous study of the eggshell of the closely related species, *Capillaria hepatica*, revealed that it was composed of three layers: an inner lipid layer, a middle chitinous layer, and an outer vitelline layer (Wharton, 1980). The thick eggshell of *P. philippinensis* that was observed by SEM in this study (Figs. 1 and 4) would likely be the vitelline layer. The chitinous layer of
the *P. phillipinensis* eggshell appears as an entire beam-like network connected with the pillars (Fig. 2) or only a partial beam-like network (Fig. 3). The beam-like network resembled that seen in the *C. hepatica* eggshell and was demonstrated by transmission electron microscopy (TEM) in the study of Grigonis and Solomon (1976). According to these authors, the pillars are located between the shell matrix (nonfibrous protein network) and are vertically connected between the outer membrane of the eggshell and non-laminated layer. In the current study, there is a gradual loss of the adhering surface material (Figs. 2 and 3).

Regarding the thin eggshell of *P. phillipinensis* (Fig. 5), this phenomenon was quite rare in our study. Even so, the coiled larva could be easily observed inside the very thin eggshell. The thin eggshell is likely the inner lipid layer enclosing the developing larva based on comparison to the TEM study of another nematode, *Heterodera glycines* (Burgwyn et al., 2003). This very thin layer enables the larva to hatch either in utero or in the lumen of the host’s gut; thereby, facilitating autoinfection.

Autoinfection and hyperinfection have been experimentally clarified as part of the life cycle of *P. phillipinensis* by Cross et al. (1978). Eggs were found to hatch within a few hours after being experimentally fed to freshwater and brackish water fish (Cross, 1992), possibly indicating that the eggs have already contained developed larvae. Once they encountered the appropriate stimulus (e.g. digestive enzymes in the gastrointestinal tract of fish), the larvae rapidly hatched (Cappelo and Hotez, 1998). This would seem to be supported by the present result of the thin *P. phillipinensis* eggshell (Fig. 5).

Observations of eggs of parasites utilizing SEM are usually made from egg specimens obtained from either dissected uteri of adult female parasites or by first experimentally infecting laboratory animals and then collecting eggs from the host’s feces in order to make a definitive diagnosis of the species of parasite (Fujino et al., 2000; Uga et al., 2000; Kaewkes, 2003). In this case, only eggs were found in stool specimen. The identification of the eggs as being those of *P. phillipinensis* was based on their characteristic peanut shape with bipolar plugs and their size. Determined by light microscopy, the means (±SD) of the length and width of the eggs were 41.84 (±1.75) and 20.38 (±4.04) μm, respectively, while the mean thickness of their walls was 1.80 (±0.42) μm (n=26). This information agrees with that previously reported on the egg of *P. phillipinensis* (as *C. phillipinensis*) (Cross, 1992; Moravec, 2001). Moreover, the eggs have been independently confirmed as belonging to this species by Dr J.H. Cross.

In conclusion, this study is the first to describe the thick and thin eggshells of *P. phillipinensis* under scanning electron microscope. This information provides supportive evidence of autoinfection occurring in the life cycle of this species within the host’s intestine. In addition, the fecal
specimens described herein may be useful in future microscopical investigation of eggs of this and other intestinal parasites.

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