



## Can thick-shelled eggs of *Capillaria philippinensis* embryonate within the host?

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Received: 20 March 2020 / Accepted: 13 July 2020  
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**Abstract** Intestinal capillariasis is a newly emerging parasitic disease and its outcome may be fatal if not diagnosed and treated in the proper time. The main diagnosis of intestinal capillariasis is made by identifying eggs, larvae and/or adults in the stools of infected cases. This study aimed to describe a different type of developmental stages, which is the detection of adult females carrying embryonated thick-shelled eggs in their uteri in the fecal samples of infected cases. The study included 40 *Capillaria philippinensis* patients admitted to the hospitals of Kasr Al-Ainy Faculty of Medicine, Cairo University and Beni-Suef University. Stool examination was performed using direct smear and the formalin-ether concentration methods. The adult stages of the parasite were detected in 35 stool samples, eggs were detected in 33 cases, 5 of them were diagnosed by detecting eggs only. We could identify three types of eggs of *C. philippinensis*: non-embryonated thick-shelled eggs in feces of cases, embryonated thin-shelled and embryonated thick-shelled eggs in the uteri of female worms. Only in 2 cases we were able to identify female worms with embryonated thick-shelled eggs inside their uteri. This finding has never been mentioned and may point out to a missing point in the known life cycle.

**Keywords** *Capillaria philippinensis* · Intestinal capillariasis · Oviparous · Larviparous worm · Malabsorption · Fish-borne nematode

### Introduction

*Capillaria philippinensis* is a tiny nematode parasite of fish-eating birds. Human infection occurs accidentally as a result of ingestion of raw or improperly cooked fresh water fish harboring infected larvae in its intestine (Cross and Basaca-Sevilla 1991). Human infection leads to inflammation and atrophy of intestinal villi with subsequent malabsorption of all nutrients (Whalen et al. 1969). Infection can end fatally if the cases are not diagnosed and treated in the proper time (Cross et al. 1970; Cross 1992; El-Dib and Doss 2000).

The first human case was identified in the Philippines in 1964 (Chitwood et al. 1964). Later the disease was reported as endemic in the Philippines with the appearance of some epidemics (Cross 1992; Belizario et al. 2000). The disease was also reported in Egypt (Youssef et al. 1989; El-Dib et al. 1999; Ahmed et al. 1999; El-Dib and Doss 2000; El-Karakasy et al. 2004; Attia et al. 2012; Amin et al. 2014). Diagnosis of infection depends primarily on the detection of eggs, larvae and adult worms of *C. philippinensis* in the stool of patients (Belizario et al. 2000).

The life cycle of *C. philippinensis* was investigated by Cross et al. (1978) and Cross (1992). The authors mentioned that *C. philippinensis* life cycle is unique in having 2 types of females that alternate in generations inside the body of the host. A larviparous female, which produce larvae responsible for internal autoinfection and hyperinfection in the host, and the oviparous female capable of laying unembryonated thick-shelled eggs that are voided in

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feces, develop in water within 5–10 days and infect the fish intermediate host (Cross et al. 1972; Cross 1992).

The aim of this study was to describe the development of mature thick-shelled eggs of *C. Philippinensis* inside the uterus of adult female worm that may provide more details and help in understanding the life cycle of this emerging human infection.

## Materials and methods

This study was conducted over 40 diagnosed *C. Philippinensis* patients admitted to the Departments of Internal Medicine (11 cases), Tropical Diseases (24 cases) and Pediatric Medicine (5 cases) of both Kasr Al-Ainy Faculty of Medicine, Cairo University and Beni-Suef University Hospitals. All cases had chronic diarrhea and other gastrointestinal manifestations (borborygmi, abdominal pain and loss of weight), 35 cases had lower limb edema.

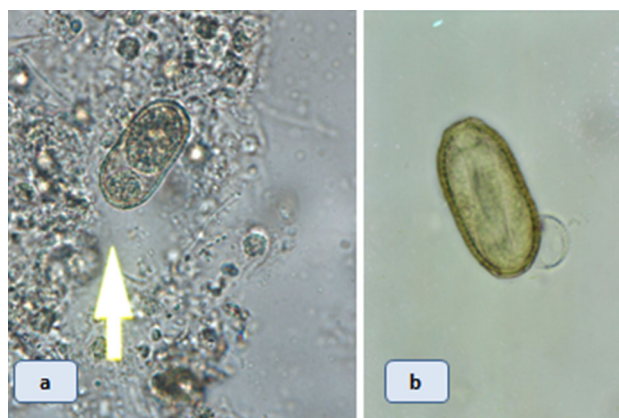
Fresh fecal samples were obtained from each case, transferred to the Medical Parasitology Departments where they were subjected to copro-parasitological examination and diagnosed as intestinal capillariasis. Samples were examined by direct microscopy after the addition of saline and iodine drops. Concentrated fecal samples were done by Formalin-ethyl acetate sedimentation method (Methanitikorn et al. 2003) to help in detection of *C. philippinensis* stages (eggs, larvae and adults).

## Results and discussion

All stages of *C. philippinensis* (eggs, larvae and adults) were detected in stool samples of 7 cases. *Capillaria* adult stages and eggs were found in 21 stool samples, eggs only in 5 cases, and adults only in 7 cases of all diagnosed cases. Thick-shelled eggs of *C. philippinensis* identified in fecal samples of cases were immature with one or 2 cell stages (Fig. 1). Female worms detected in the specimens were either immature (without eggs in their uteri) or mature, with unembryonated thick-shelled eggs in their uteri (Fig. 2a), or females with thin-shelled eggs “containing larvae” in their uteri (Fig. 2b). In a previous experiment carried out by El-Dib et al. (1999), eggs of *C. philippinensis* have been collected, concentrated and cultured at room temperature. They were able to embryonate in about 10 days (Fig. 1b).

We were able to identify that there were 3 adult worms including embryonated thick-shelled eggs “with larvae” in their uteri (Fig. 3a, b).

The first patient that passed this female with embryonated thick-shelled eggs was a typical case of intestinal capillariasis. A 30 years old female with chronic diarrhea (of



**Fig. 1** Thick-shelled eggs of *C. philippinensis* in fecal sample. **a** Unembryonated egg X400 and **b** embryonated egg after culture of stool for 10 days at room temperature X400

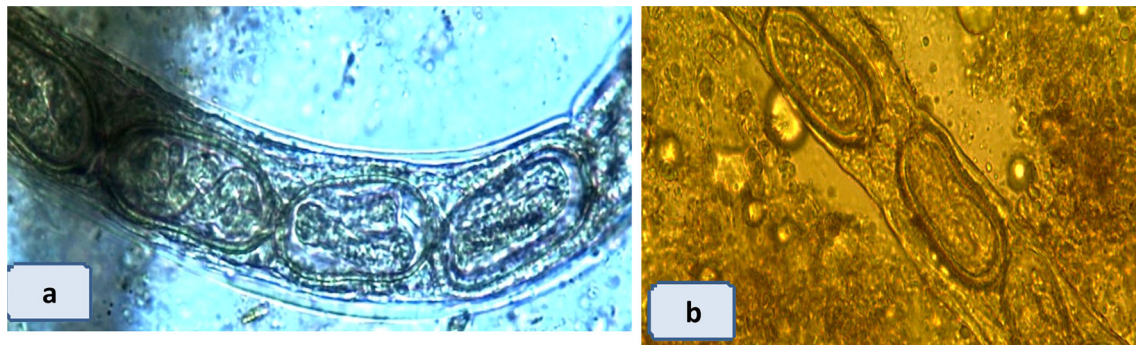
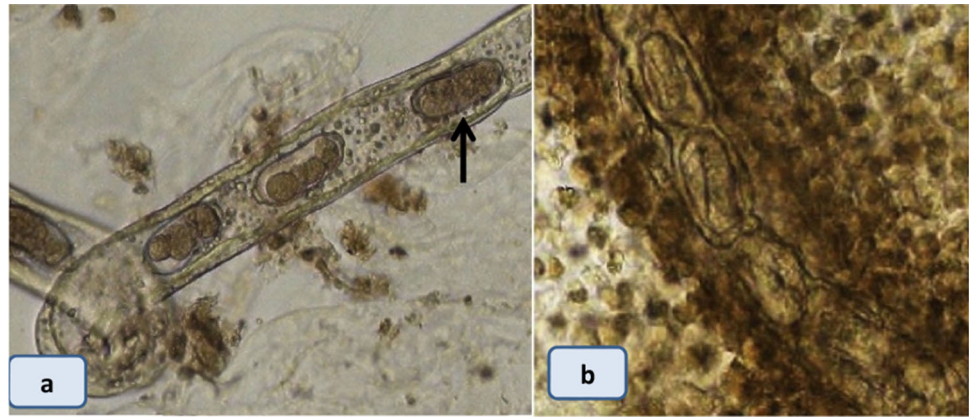
8 months), from Beni-Suef, with loss of weight (about 10 kg), hypokalaemia (2.4 mEq/L), hyponatraemia (130 mEq/L), hypocalcaemia (7.1 mg/dL) and hypoalbuminaemia (2.3/dL). She had a history of preparing fish at home, which could allow her to catch infection in fingernails during fish evisceration; particularly she used to fry fish for the rest of the family, who didn't have similar infection. This mode of infection was suggested by El-Dib and Doss (2000).

The second patient was a 3 years old female child with chronic diarrhea (of one year), from Beni-Suef city. Her father was a fisherman. She had loss of weight (about 4 kg), lower limb edema and facial edema. The blood picture showed (Hb: 10.8 mg/dL, WBCs: 13,000/mcL and eosinophilia: 1%), hypokalaemia (2.9 mEq/L), hyponatraemia (135 mEq/L), hypocalcaemia (7.3 mg/dL) and hypoalbuminaemia (1.6/dL). The mother gave a history that her child used to play with fish and may taste it, when she was preparing it for cooking. Upper gastrointestinal endoscopic examinations was done and showed nodular lymphoid hyperplasia of the duodenum. Abdominal ultrasonographic examination, showed a rim of fluid collection in the spleno-renal pouch, and the bowel loops were dilated and distended.

According to the experimental life cycle developed by Cross et al. (1978), adult females either carry larvae in thin-shelled eggs (embryonic membrane) (Fig. 2b), or just carry unembryonated thick-shelled eggs (Fig. 2a).

This study confirmed these observations concerning the presence of two types of eggs. However; we were able to detect embryonated thick-shelled eggs containing larvae in the uterus of female worms in the body of the host. This finding has never been mentioned before. It is not the usual development of thick-shelled eggs, which should embryonate in water within 5–10 days. We are unaware if any other researcher had come across such a finding?

**Fig. 2** Parts of the uteri of *C. philippinensis* adult female worms. **a** The uterus of an oviparous female worm with unembryonated thick shelled eggs X400. The arrow shows an egg with early division, **b** the uterus of a laviparous female worm, containing larvae in thin-shelled eggs X400



**Fig. 3** Parts of the uteri of *C. philippinensis* adult female worms. **a, b** Females with embryonated thick-shelled eggs inside their uteri X400

We don't know about the nature of triggers that caused such an odd development of eggs in the uteri of worms inside the host. Also we don't know if such finding could be missed in other studies due to their rarity.

This finding may have great importance for a better understanding of the life cycle of *C. philippinensis*. Thus, more research and investigations are warranted in this respect.

The importance of this finding may change some information related to the life cycle and more studies on the topic should be considered.

**Author's contribution** NED and MIA contribute to every activity of the manuscript.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in the study involving human participants were in accordance with the ethical standards of the Research Ethical Committee of Beni-Suef University, Faculty of Medicine, Egypt. Informed consent was obtained from all individual participants included in the study after explaining the purpose of the study.

**Informed consent** Signed Informed consent (in Arabic) was obtained from the '30 years old female' and from the mother of 'the 3 years old girl' after telling them the intention of publication.

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