

CAPILLARIA PHILIPPINENSIS IDENTIFIED IN DIARRHEIC PATIENTS FROM BENI-SUEF UNIVERSITY HOSPITALS, EGYPT

By

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Abstract

Capillariasis *philippinensis* is a debilitating nematode zoonotic parasite affecting all ages. Patients usually suffered from borborygmus, abdominal pain, chronic diarrhea, protein loss, anemia, and weight loss, as well as decrease in electrolyte levels of potassium (K), sodium (Na) and albumin due to enteropathy.

The microscopic examinations showed that 39/450 (8.6%) were *C. philippinensis* patients. The different parasitic stages were also examined by SEM. Biochemical estimation of albumin, Na and K levels were assessed as well as blood examination for hemoglobin level, total leucocyte count and eosinophila. Parasitic males, females and egg stages were identified by light microscope and SEM. Only anemia in children was significant as compared to other patients ($P < 0.05$). Most cases were associated with lowered albumin, Na and K levels.

Keywords: *Capillaria philippinensis*, diagnosis, intestinal capillariasis, electrolytes, SEM

Introduction

Capillaria (=Paracapillaria) *philippinensis* initially manifests as abdominal/gastrointestinal disease that can be serious if untreated due to autoinfection. A protein-losing enteropathy can develop, which may result in complications such as cardiomyopathy, severe emaciation, cachexia, and even death (CDC, 2020). Anemia, electrolytes imbalance (low serum levels of Na, & K), low serum level of total protein, albumin, and fat resulted in malabsorption (Abdel-Rahman *et al*, 2019).

C. philippinensis is endemic in the Philippines and Thailand (Chitwood *et al*, 1964) and sporadic cases have been reported from other East and Southeast Asian countries (Austin *et al*, 1999). In Egypt, it was the first reported by Youssef *et al*. (1989) and then some cases sporadically were reported in Middle Egypt (El-Dib *et al*, 1999; Ahmed *et al*, 1999; El-Dib and Doss, 2000; El-Karaksy *et al*, 2004; Attia *et al*, 2012; Amin *et al*, 2014; Khalifa and Othman, 2014; Ali *et al*,

2016; El Dib and Ali, 2020), and in 3% of stray cats (Khalafalla, 2011).

Intestinal capillariasis mostly affects adult females who test fresh fish during food processing (El-Dib *et al*, 2004). Diagnosis is by stool examination for eggs, larva, or adults (Cross, 1992). Charcot-Leyden crystals were detected in stools of some patients, with various number and size (Khalifa *et al*, 2020). Intapen *et al*. (2010) reported that accuracy of stool examination can misdiagnose infection due to a scarcity of worm eggs in the stool specimens Min *et al*. (2013) suggested that scanning electron microscopy (SEM) gave an illustrating approach with clear insights to the ultrastructure of *C. philippinensis*.

This study aimed to evaluate *Capillaria philippinensis* in out-patients from Beni-Suef University's Hospitals suffering from abdominal pain, diarrhea, anemia and suggestive other manifestations, by light microscopy and SEM as well as by the biochemical parameters.

Material and Methods

Study design: This descriptive analytical study included 450 patients complaining of gastro-intestinal troubles mainly abdominal pain and diarrhea attending different outpatient clinics and laboratories in Beni-Suef University's Hospitals. They were males and females with ages ranged between 4 to 70 years, distributed all over the year. Sheets were filled out on each patient.

Morning stool samples were taken in labeled covered carton boxes. Also, blood samples were taken to separate sera for CBC, and biochemical analysis.

Stool examinations: 1- Wet mount microscopy: Two slides were made from each sample. A homogeneous thin film was prepared by placing 4mg on a glass slide and mixed with a drop of normal saline solution or 1% Lugol's iodine, covered and examined by low power or high power if indicated (Garcia, 2001). 2- Formalin-ethyl acetate sedimentation method: One gram of stool was emulsified in 10% formalin, strained via 2 layers of wet gauze, and centrifuged at 1500 rpm for 5 min. The supernatant was discarded, and sediment was shaken in 5ml of 10% formalin followed by addition of 3ml of ether, vigorous shaking for 1min. and centrifugation at 1500rpm for 5min. The plug of debris was loosened, supernatant fluid was decanted, and was microscopically examined and adding suitable cover if indicated (Methanitikorn *et al*, 2013). Other helminths and protozoa were detected were not included in the results.

SEM: Worm external features from fresh samples were followed by isolation of 30 intact whole worms, one larva and eggs stored in 4% glutaraldehyde, pH 6 at 4°C. Then, 1% osmium tetroxide was used to treat of worm samples for 2 hours, washed with phosphate buffer solution, dried, and incubation in a sputter-coating apparatus for coating samples with gold before scanning examination (Attia *et al*, 2012). Sera were examined for hemoglobin, albumin, sodium and potassium levels.

Statistical analysis: Data were tabulated, computerized and analyzed using Statistical Package for the Social Science (SPSS) version 26. Descriptive data were presented as Mean \pm SD using one-way ANOVA test with p-value considered significant when < 0.05 . Binary logistic regression between variables and *Capillaria* was used.

Ethical considerations: The protocol was approved by Faculty of Medicine Research Ethics Committee which agreed with Helsinki Declaration Guideline on 2008. Signed written informed consent was obtained from all participants or their parents.

Results

Adolescent patients were the most of the patients suffered from flatulence, edema of lower limbs and weight loss and of whom the majority had hypokalemia. All children complained of weight loss, while anemia ($P < 0.05$) and hyponatremia were more dominant in the majority of them than in others.

Of 450 cases, 39 (8.6%) were positives. Morphological features of worms showed that both a females and males were thin anteriorly and slightly thick posteriorly. Body posterior end was rounded with subterminal anus. Esophagus adult had characteristic stichocytes of superfamily Trichinelloidea. Females detected were immature (without eggs in uterus) or mature (with thick-or thin-shelled eggs in uterus). Vulval open located just posterior to esophagus. Males posterior end provided with well-developed terminal protruding spicule covered with very long sheath.

Eggs peanut-shaped in shape with flattened bipolar plugs and striated shell. All egg types detected with thick-or thin shelled embryonate, or unembryonate). Thick shelled eggs contained one or two cell stages embryo filled most of egg. Thin-shelled eggs contained single cell-stage embryos or mature larvae.

Larva transparent with esophagus measured about three quarters of body length. Charcot Leyden crystals slender with tapering both ends. Crystals varied in number and si-

ze. Their number generally higher in more severe clinical manifestations with more eggs in stool.

SEM: Adults slender with envelope-like membrane covering nearly all body surfaces. Different worms covered by patches of detached intestinal villi. Some morphological signs of male and female appeared such as thin anterior portion with a prominent mouth opening with wrinkled and backward ridges radiating from it. Adult body dorsal surface with an apparent long cleft. Adult cuticular surface with transverse and longitudinal stri-

ations. Detected larvae failed, despite the successful isolation of them from the fresh stool of the patients.

Two types of peanut-shaped eggs; thick and thin-shelled both with a swollen appearance. Thick-shelled eggs with a rough irregular orange surface orange peel-like with dispersed numerous openings of various sizes with highly concave lateral polar plugs. Thin-shelled eggs swollen with a smooth surface, but without polar plugs at either end.

Details were given in tables (1, 2, & 3) and figures (1, 2, 3, 4 & 5)

Table 1: Distribution of demography, clinical complaints, and laboratory data among age groups in capillariasis cases.

Variations	Items	Children (6/15.4)	Adolescents (4/10.3)	Adult (29/74.3)	Total 39	P value
Demographic data	Female	(5/83.3)	(4/100)	(25/86.2)	(34/87.2)	
	Male	(1/16.7)	(0/0)	(4/13.8)	(5/12.8)	
	Mean of age	10.5±3.2	17.7±1.5	35.4±11.2	34±2.3	
Physical and Clinical manifestations	Duration of diarrhea (day)	125.0±66.8	157.5±45.0	270.8±210.5	265±204	0.16
	Dysentery	(0/0.0)	(0/0.0)	(3/10.3)	(3/7.6)	0.57
	Flatulence	(5/83.3)	(4/100)	(26/89.7)	(35/89.7)	0.69
	Fever	(2/33.3)	(0/0.0)	(2/6.9)	(4/10.2)	0.11
	Lower limb edema	(5/83.3)	(4/100)	(25/87.2)	(34/87.1)	0.7
	Weight loss	(6/100)	(4/100)	(28/96.6)	(38/97.4)	0.83
Laboratory examinations	Anemia	(5/83.3)	(1/25)	(8/27.6)	(14/35.8)	0.03
	Hypokalemia	(4/66.7)	(3/75)	(21/72.4)	(28/71.7)	0.96
	Hyponatremia	(5/83.3)	(2/50)	(16/55.2)	(23/58.9)	0.42
Stool analysis	Charcot Leyden Crystals	(5/83.3)	(4/100)	(27/93.1)	(36/92.3)	0.59

Table 2: The mean hemoglobin, K, Na and albumin in capillariasis cases

Variations	Children	Adolescents	Adults	P-value
Hemoglobin	11.2±1.15	12.4±0.51	12.3±1.38	0.14
K	3.28±0.87	2.98±0.73	3.2±0.48	0.70
Na	137.4±3.90	133.5±9.43	134.1±6.56	0.51
Albumin	2.22±0.22	1.65±0.74	1.98±0.42	0.14

Table 3: Distribution of infection over the seasons

Season	Children	Adolescents	Adults	P-value
Winter	0 (0.0%)	2 (50%)	4 (13.8%)	0.12
Spring	0 (0.0%)	0 (0.0%)	4 (13.8%)	
Summer	2 (33.3%)	2 (50%)	14 (48.3%)	
Autumn	4 (66.7%)	0 (0.0%)	7 (24.1%)	

Discussion

In the present study, characterized *C. philippinensis* by noninvasive diagnostic tools among all patients was adopted. Capillariasis was more prevalent among adults (74.3%) than children adolescents (10.3%), and children 15.4%, with overall rate of 8.6% among all the diarrheic patients. This rate was more or less higher than 5% (Amin *et al*, 2014) or 5.8% (Ali *et al*, 2016). Increased cases may clarify the more awareness to capillariasis in this community.

In the present study, clinical manifestations in all adolescents were flatulence, edema of lower limbs, weight loss, and majority of them had hypokalemia. All children complained from weight loss, anemia and hyponatremia were more dominant than in others. This could be attributed to the long duration of protein-losing diarrhea and electrolytes imbalance with consequence of dehydration and malnutrition. But, only anemia children were significantly linked to infection with capillariasis infection ($P < 0.05$) as compared

the other patients. The higher rate of anemia among capillariasis infected children may be the type of dietary they have or another medical cause.

In the present study, biochemical profiles showed low albumin, potassium, and sodium. This agreed with Saichua *et al.* (2008); Attia *et al.* (2012) and Ali *et al.* (2016), they reported diarrhea, lower limb edema, anemia, hypoalbuminemia, low sera levels of Na & K and Charcot Leyden crystals in stool samples of their intestinal capillariasis patients. But, Charcot Leyden crystals in stool were proportional to disease intensity as the great number crystals were parallel to the great number eggs the severe symptoms in patients (Intapan *et al.*, 2017). Eosinophilia is characteristic feature in helminthic infections, but, eosinophil count was more or less normal in intestinal capillariasis patients (Amin *et al.*, 2014).

In the present study, isolation of *C. philippinensis* stages from fresh stool was not a simple technique also examination of the intact ultrastructure of worms and eggs under SEM was harder. Min *et al.* (2013) reported that the inflammatory changes the infection caused and its slender shape this more worse since worm being embedded in the intestinal mucosa and passage in stool was accompanied by detachment of villi attached to the outer cuticle showing a difficult surface structure as if worm invasion of mucosa.

In the present study, SEM showed different worm stages covered by patches of detached intestinal villi indicating that the worm disturbed the junction epithelium causing leakage of fluids and electrolytes via the lumen and subsequent chronic protein losing diarrhea (Attia *et al.*, 2012). Rausch *et al.* (2012) reported that the disturbance of microbiota features by the subsequent anti-nematodes immune defense and inflammatory cells infiltration pave the way to bacterial infection and growth, unfortunately increasing the patient's morbidity and mortality.

In the present study, the capillariasis infe-

cted children and adults complaints were the commonest during summer and autumn, but adolescents' complaints were mostly in both summer and winter. El-Dib and Doss (2000) in Egypt reported that the highest infection rate was in the Spring Day Celebration when the great majority the Egyptian must have fish meals.

Conclusion

Capillariasis is a disease suspected in patients with chronic diarrhea, low albuminemia and low electrolytes. On dealing with patients with *Capillaria*, anemia is a complaint of all age groups but when dealing with children it can be multi factor. SEM processing was difficult with *C. philippinensis* in stool but gave descriptive ultrastructures.

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Explanation of figures

Fig.1: a- Adult female *C. philippinensis*, b & c- Adult male with apparent terminal protruding spicule covered with sheath posteriorly, d- larva (x10, iodine stain).

Fig.2: a- Thin-shelled eggs. B & c- Peanut shape thick-shelled (X40) d: Peanut shape (red arrow) with multiple Charcot Leyden crystals in stool samples (white arrows) (X10).

Fig.3: SEM for adult *C. philippinensis*; a- complete adult worm, b- Part of adult with dorsal view showed long cleft under worm (red arrow), c: Envelope like membrane attached to adult cuticle dorsal cleft (green arrows)

Fig. 4: SEM a- adult anterior thin portion, b- anterior part with a prominent mouth opening (red arrow), c-Part of worm covered with intestinal debris (white arrows) and transverse irregular wrinkled striations, d: Part of worm covered with huge intestinal debris.

Fig. 5: SEM of eggs; a-swollen with apparent one lateral mucoid plug and rough orange peel like outer surface, b & c- swollen with smooth surface covered with adherent debris.

