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CHARACTERIZATION OF *CAPILLARIA PHILIPPINENSIS* DIFFERENT ANTIGENS AND FISH *CAPILLARIA* SPP. ADULT WORM ANTIGEN BY SDS-PAGE AND WESTERN BLOT

By

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Abstract

Intestinal capillariasis is an emerging zoonotic disease. It can lead to life-threatening protein-losing enteropathy if not diagnosed early and treated promptly. This study clarified the antigenic profiles and immunogenicity of *C. philippinensis* from adult, egg, coproantigen, and fish *Capillaria* spp. adult antigen using SDS-PAGE and Western Blot (WB) techniques against *C. philippinensis* sera. The electrophoretic profiles showed that most proteins were common in all samples at 25.6, 42, 57, & 70 kDa, but some were stage-specific. Immunoblot analysis showed that patient sera recognized and reacted with all examined stages. This indicated that antigenic components of different stages of *C. philippinensis*, and the common components among all life stages improved parasite immunodiagnosis. Also, the results showed that human sera from patients with intestinal capillariasis recognized the antigenic pattern of fish *Capillaria* spp., indicated that potential cross-reactivity could develop more accessible and cost-effective diagnostic tools, mainly in regions where *C. philippinensis*-specific antigens in non-endemic areas.

Keywords: Human *C. philippinensis*, Fish *Capillaria* spp., SDS-PAGE, Immunoblot

Introduction

The first human intestinal capillariasis case was in the Philippines in 1963 (Chitwood *et al*, 1964), in Thailand was in 1973 (Pradatsundarasar *et al*, 1973), and then so numerous cases were identified in the Philippines and Thailand (Cross and Bhaibulaya, 1983). More than 2,000 capillariasis cases (Class Adenophorea, Order Trichocephalida Family Capillariidae) were reported in fish, amphibians, reptiles, birds, and mammals globally (Khalifa and Othman, 2014). But, three species only infect man: *C. hepatica*, *C. aerophila*, and *C. philippinensis* (McCarthy and Moor, 2000). *C. philippinensis* a tiny nematode (Intapan *et al*, 2006) is a highly significant prevalent public health problem, especially in non-endemic areas (Limsrivilai *et al*, 2014). The three main genera infect man are *Capillaria, Trichuris*, and *Trichinella* (Attia *et al*, 2012). The intestinal capillariasis patients usually presented with watery diarrhea weight loss, abdominal pain, borborygmus, muscle wasting, weakness, edema and laboratory examination showed low levels of the potassium and albumin in blood (Tesana *et al*, 1983), and mal-absorption of fats and sugar (Chunlertrith *et al*, 1922)

Nowadays, human capillariasis *philippinensis* outbreaks are increasing globally and marked human cases in Southeast Asia, particularly the Philippines and Thailand, as well as in Taiwan, Indonesia, Korea, Iran, India, and Colombia with a broader geographical spread. *C. philippinensis* has been involved in epidemics and was responsible for many deaths in the Philippines and Thailand (Sichuan *et al*, 2008), Taiwan (Lu *et al*, 2006), Indonesia (Chichino *et al*, 1992), Korea (Hong *et al*, 1994), Iran (Hoghooghi-Rad *et al*, 1987), India (Kang *et al*, 1994), and Colombia (Dronda *et al*, 1993). Therefore, rapid diagnosis of *C. philippinensis* is crucial due to its potential to cause severe manifestaions, and sometimes fatal, outcomes if not well treated (Ziarati *et al*, 2022).

In Egypt, intestinal capillariasis was first identified by Youssef *et al.* (1989). Since th-

en, many cases have been diagnosed in different governorates, mainly in Cairo, El Menia, Beni-Suef, Assiut, and the Greater Cairo (Anis *et al,* 1998; Khalifa *et al,* 2000; El-Dib and Doss, 2002; El-Dib *et al,* 2004; El-Karaksy *et al,* 2004; Abdel-Rahman *et al,* 2005; Amin *et al,* 2011; Attia *et al,* 2012; El-Dib *et al,* 2015a; Ali *et al,* 2016; El-Dib and Ali, 2020), and in Dakhalia G. from wide *Rattus norvigicus* eating fish (El Shazly *et al,* 1994), as well as *C. muris sylvatici* from the Egyptian bank vole *(Clethrionomys glareolus*) eating fish (Ashour *et al,* 1994). Besides, Alsulami *et al.* (2024) in Saudi Arabia repoted *Capillaria* among other parasites and some heavy metals in an aquaculture farmed tilapias in northeast of Jeddah City.

Man is infected with *C. philippinensis* by consumption of raw or undercooked freshwater fish that harbor infective larvae. These larvae reside in the fish's intestines and can cause infection when ingested. Infected patients typically present with abdominal pain, borborygmi, chronic diarrhea, muscle wasting, cachexia, weakness, lower limb edema, hypokalemia, and hypoalbuminemia (Saichua *et al,* 2008; Limsrivilai *et al,* 2014). Certain fish isolates exhibited a closer genetic similarity to human *C. philippinensis* isolates, suggested potential cross-species transmission or shared evolutionary history (Hajipour *et al,* 2023).

Microscopic detection and morphological identification of parasites from clinical specimens are the gold standards for the laboratory diagnosis (Ahmed, 2023). Nevertheless, such diagnostic assays have limitations, including insufficient sensitivity, specificity, and the operating dependence. Moreover, the immunoassays for parasitic antigens are not available for most zoonotic ones and lacked significantly improved the sensitivity of laboratory detection (Dupont *et al,* 2023).

Generally speaking, antigens can be used as diagnostic markers for zoonotic parasites mainly, their excretory-secretory (ES) antigens, released by parasites in-to serum, urine, and tissues such as the kidney, with the good correlations between the antigen levels and parasite burden (Hewitson *et al,* 2009).

But, any delay in diagnosis and proper treatment can lead to serious complications, including death from the heart failure due to electrolyte imbalance (Cross, 1992), or septicemia from secondary bacterial infection (Bair *et al,* 2004). The irregular shedding of parasite in feces and overlapping clinical symptoms with other gastrointestinal parasitic infections often the delay capillariasis diagnosis (Saichua *et al,* 2008; Soukhathammavong *et al,* 2008).

Diagnosis of capillariasis is usually difficult in the non-endemic areas (Vasantha *et al,* 2012). Definitive diagnosis is based on recovery of eggs, larvae, and/or adults from stool, but multiple stool samples may be required (Bair *et al,* 2004). Parasitic coproantigen detection was used By El-Dib *et al.* (2004), along with antibody immunoblotting detection (Intapan *et al,* 2006), ELISA (Intapan *et al,* 2010), and specific nested PCR for *C. philippinensis* in feces based on amplification small ribosomal subunit (El-Dib *et al,* 2015b), as well as immunochromatographic test (ICT) kits using *Trichinella spiralis* larval extract antigen (Intapan *et al,* 2017).

The *C. philippinensis* may secrete bioactive molecules similar to those of *Trichuris*, which releases proteolytic enzymes and pore-forming proteins from their stichosome (Drake *et al,* 1994). But, little was known about the molecular and cellular events associated with *C. philippinensis* invasion (Cappello and Hotez, 1998).

This study aimed to evaluate *Capillaria philippinensis* different stages antigens from both infected patients and fish host in serodiagnosing of human.

Materials and Methods

C. philippinensis adult crude extract antigen was prepared (Lillywhite *et al,* 1995) with some modifications. Adults were collected from patients stool samples after a dose levamsole (2.5mg/kg). They were washed many times with PBS, mixed with RIPA lysis buffer contained a protease inhibitor coc-

ktail (5M NaCl, 0.5 M EDTA pH 8.0, 1 M Tris pH 8.0, NP-40, 10% sodium deoxycholate & 10% SDS), and mixture was homogenized for 30 minutes on ice. After centrifugation at 10,000rpm for 10 minutes at 4°C, protein content in supernatant was estimated by the Nanodrop spectrophotometer, and the antigen was stored at -70°C till needed.

C. philippinensis coproantigen (Skes and McCarthy, 2011): Capillariasis positive stool samples were processed by adding three parts of 0.1 M PBS, pH 7.4, and contained 0.05% Tween 20 to a stool part. The mixture was homogenized by stirring for 3 minutes, sonicated for 5 minutes in an ice, and centrifuged at 10,000rpm for 10 minutes. Supernatant protein was collected, measured and stored at -20°C.

C. philippinensis egg antigen was prepared from immature eggs collected from stool positive samples. They were washed several times with PBS, centrifugated, homogenized and suspended in PBS, and then sonicated on ice at 8mm amplitude using a Soni-prep 150 sonicator (MSE, UK) for 10 minutes. After centrifugation at 10,000rpm for 30 minutes at 4°C, the supernatant was collected and stored at -70°C (Andrade *et al*, 2004).

Crude adult extract antigen from fish *Capillaria* spp. dissected out from stomach and small intestine of Egyptian freshwater fish; *Bagrus bajad* was prepared. The antigen was prepared with same procedure used for *C. philippinensis* adult antigen.

Human sera: Sera were collected from ten infected patients attended Assiut University Hospitals, but negative sera were kindly collected from cross matched apparently healthy volunteers, and stored at -70°C until use.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblot: All crude extracted antigens (16µg of protein in 6x sample buffer) were separated under reduced conditions on 12% polyacrylamide gels (Laemmli, 1970). After electrophoresis, gels were either silver-stained to visualize protein bands or left unstained for immunoblotting. The immunoblotting proteins were transferred onto PVDF membranes (Bio-Rad), incubated with patients sera, and then with goat anti-human IgG conjugated with alkaline phosphatase 1:1000 (Sigma) for 1hr at room temperature on a shaker. The blots were visualized by BCIP/NBT substrate (ThermoFisher Scientific).

Results

C. philippinensis protein profiles life stages and fish spp. crude worm antigens were analyzed by SDS-PAGE and visualized with silver staining.

Electrophoretic profiles showed many protein bands common to all samples, but with some stage-specific bands. The crude antigen of *C. philippinensis* adults exhibited 11 bands at molecular weights of 13, 17, 22, 25.6, 36.5, 40, 42, 57, 67, 70, &95.5 kDa. Egg antigen showed bands at 17, 25.6, 42, 54-57, 59-63, 70-73, & 77 kDa. The coproantigen presented bands at 18, 20, 25.6, 34, 40, 42, 48, 57, 67, 70, & 95.5 kDa. The fish *Capillaria* spp. bands in crude antigens were found at molecular weights of 13, 14, 17, 22, 25.6, 32, 36.5, 42, 45, 48, 52, 57, 67, 70, 77, & 95.5 kDa.

Silver staining showed negatively stained bands in several antigens. These appeared at 25.6, 36.5, 40, & 67 kDa, in crude adult antigen at 59-63 kDa, in egg antigen at 34, 36.5, 40-42, 57, 67, & 70 kDa, and in coproantigen at 25.6 & 67 kDa in fish crude antigen.

Common protein bands were at 25.6, 42, 57, & 70 kDa in all antigens. Adult, egg, and coproantigens of *C. philippinensis* shared common bands at 25.6, 42, & 70 kDa. Also, adult crude antigen of *C. philippinensis* and fish spp. crude antigens had common bands at 13, 17, 22, 25.6, 36.5, 42, 57, 67, 70, & 95.5 kDa.

Western blot analysis showed that sera of intestinal capillariasis patients with three polypeptide bands in adult antigen at molecular weights of 36.5, 57, & 67 kDa. Also, sera reacted with egg and coproantigens at molecular weights of 18, 22, 27, 33, & 40 kDa for egg antigen, and at 18, 27, 36.5, 40.5, 42,

& 48-50 kDa for coproantigen. Fish crude antigens showed three polypeptide bands at molecular weights of 42, 48, &52 kDa**.** Details were in table (1) and figures (1 & 2).

Table 1: Antigenic profiles of different antigens resolved by SDS-PAGE and immune reactivity to anti-*Capillaria* sera using Western Blot.

C. philippinensis crude worm Ag		*C. philippinensis* egg Ag		*C. philippinensis* CoproAg		Fish *Capillaria* crude worm Ag	
Silver stain	WB	Silver stain	WB	Silver stain	WB	Silver stain	WB
95.5				95.5		95.4	
		77				77	
70		70		70 -ve		70	
67-ve	67			67-ve		67-ve	
		59-63-ve					
57	57	54-57		57 -ve		57	
						52	52
				48	48-50	48	48
						45	
42		42		42 -ve	42	42	42
40-ve			40	40.5	40.5		
36.5	36.5			36.5 -ve	36.5	36.5	
			33	34 -ve		32	
25.6 –ve		25.6	27	25.6	27	25.6 -ve	
22			22			22	
				20			
				18	18		
17		17	17			17	
						14	
13						13	

Discussion

In the present study, *Capillaria philippinensis* at different human and fish developmental stages showed a range of polypeptides with both shared and unique molecular weights. This agreed with Malacco *et al.* (2024), who reported that some gastrointestinal helminthes-derived excretory-secretory products as macromolecules, proteins, and polysaccharides modulated the antigenic function of dendritic cells with down-stream effects on effector $CD4^+$ T cells.

In the present study, the immunoreactive polypeptides recognized by sera from *C. philippinensis*-infected patients gave insights into diagnostic and epidemiological applications, especially in endemic areas. This agreed with Mitra *et al.* (2023), they reported that the early disease monitoring was crucial, and the detection of miRNAs gave a promising avenue.

In the present study, *C. philippinensis* showed four protein bands, with molecular weights ranged from 13 kDa to 95.5 kDa (25.6, 42, 57, & 70 kDa), which were common in all parasitic stages in man and fish. This agreed with Basano *et al.* (2015), who Brazil studied hepatic capillariosis in man and rodents in reported that its proteins were conserved structural or enzymatic proteins with immunological importance in the developing broad-stage diagnostic markers.

In the present study, cross-reactive bands between human *C. philippinensis* and fish *Capillaria* spp. was particularly relevant in the zoonotic transmission context reflecting antigenic conservation among species. This agreed with Zhang *et al.* (2007), they used two-dimensional electrophoresis and Western blotting with *Trichinella spiralis*, and with Anuracpreeda *et al.* (2016), who reported that *Trichuris trichiura* somatic proteins were identified as immunoreactive targets

In the present study, *C. philippinensis*-infected patients' sera by Western blot analysis showed several immunodominant in adult antigen bands; three polypeptides at 36.5, 57, & 67 kDa were strongly reactive. This agreed with Intapan *et al.* (2006), who reported that these bands were dependable in the serodiagnostic assays. Previously, this agreed with Cappello and Hotez (1998), they reported that the immune response to nematode excretory-secretory (ES) products included mid- to high-MW glycoproteins; strong IgG responses in infected patients. Besides, Maizels and Yazdanbakhsh (2003) reported that molecular weights were associated with

nematode (ES) antigens and tegumental proteins in immune evasion and modulation.

In the present study, egg and co-proantigen identified bands, especially at 27-42 kDa showed that early-stage and non-invasive diagnostic markers were derived by immature stages. This agreed with Cross (1992), who reported that coproantigen-based ELISAs showed significant sensitivity, even with the low or intermittent stool egg output.

In the present study, fish *Capillaria* spp. antigens diagnosed infection human sera showed potential zoonotic antigenic similarity and that fish are important zoonotic reservoirs. This agreed with Khalil *et al.* (2014), who in Saudi Arabia found that fish were the main capillariasis zoonotic host. But, El Shazly *et al.* (2008) in the Nile Delta reported that rodents were the zoonotic reservoir of capillariasis, and Khalafalla (2011) in northern Nile Delta reported that stray cat was the zoonotic reservoir of capillariasis. Abdel-Rahman *et al.* (2024) reported that the genetic similarity analysis provided valuable insights into the relatedness among human and fish *Capillaria* isolates, suggesting potential evolutionary relationships between them.

In the present study, coproantigen and egg antigen showed that bands at 18, 22, 27, 33, 40-42, & 48-50 kDa reacted with patients' sera. This agreed with Mountford *et al.* (2011), who reported that significant antigens released in feces or associated with *Schistosoma mansoni* eggs has non-invasive diagnostic targets.

In the present study, antigenic similarities between human *C. philippinensis* and fish-*Capillaria* shared immunoreactive bands at 42, 48, & 52 kDa showed conserved antigenic epitopes between man and fish species. This agreed with Chitwood and Lichtenfels (1972), who reported that fish act as zoonotic reservoirs or intermediate hosts that must be considered to avoid false positives or species misidentification.

In the present study, detection of negatively stained protein bands at 25.6, 36.5, & 67 kDa in adult extracts, and at 59-63 kDa in egg antigens showed the post-translational modifications (e.g., glycosylation) that affected protein charge or proved the impacting stain uptake. This agreed with Khoo *et al.* (1997), who reported that in the helminthic proteins modifications commonly affected antigenicity and immune recognition.

In the present study, the antigenic bands at 36.5, 42, & 57 kDa recognized across multiple antigens and stages represented core diagnostic targets. This agreed with Yoshikawa *et al.* (1991), who reported that human *Trpichinella spiralis* and rat *T. muris* homologous proteins were specific used in immunodiagnosis with high specificity & sensitivity.

Conclusion

The study characterized human *Capillaria philippinensis* antigenic profiles at different developmental stages, with fish-*Capillaria* spp., which showed several conserved and immunodominant protein bands. The recognition of 42, 48, & 52 kDa bands in fish antigen by patient sera showed that serologic cross-reactivity aided in epidemiology surveys using local parasite sources. The consistent recognized antigens by patients' sera underscored their diagnostic potentially. These shared bands indicated using the fish antigens as accessible, low-cost diagnostic alternative tests in capillariasis endemic areas.

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Recommendations

This outcome result not only supports development of dependable immunodiagnostic tools, but also to help in specific capillariasis vaccination development.

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Explanation of figures

Fig. 1: SDS-PAGE analysis of stages of *C. philippinensis* and fish *Capillaria* spp. antigens, visualized with silver staining. Lane L: BLUelf Prestained Protein Marker; Lane 1: *C. philippinensis* adult antigen; Lane 2: *C. philippinensis* egg antigen; Lane 3: *C. philippinensis* coproantigen; Lane 4: Fish *Capillaria* spp. adult antigen.

Fig. 2: Immunoblot analysis showed recognition of different antigens by sera from intestinal capillariasis patients. Lane L: BLUelf Prestained Protein Marker; Lane 1: *C. philippinensis* adult antigen; Lane 2: *C. philippinensis* egg antigen; Lane 3: *C. philippinensis* coproantigen; Lane 4: Fish *Capillaria* spp. adult antigen.