Screening of *Capillaria philippinensis* infection using *Trichuris muris* and *Trichinella spiralis* antigens

Original Article

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ABSTRACT

Background: Intestinal capillariasis is a disease caused by *Capillaria philippinensis*. Human infections became more prevalent in many countries including Egypt. This nematode is related to *Trichinella* and *Trichuris* species, all of them belong to Trichinelloidea superfamily. Diagnosis of intestinal capillariasis may be missed by stool examination.
Objective: This study aimed to use enzyme-linked immunosorbent assay (ELISA) and immunoblotting in screening for intestinal capillariasis as a practical and rapid diagnostic test.
Subjects and Methods: *Trichuris muris* and *Trichinella spiralis* adult worms were isolated from infected mice and crude antigens were prepared. The protein content for both adult worm extracts was determined. Human blood samples were collected from 20 capillariasis patients, 20 control individuals and 10 *W. bancrofti* infected patients. The study evaluated cross-reactivity between *T. spiralis* and *T. muris* antigens and sera from cases infected with *C. philippinensis* using ELISA and immunoblotting.
Results: In ELISA, *T. muris* crude antigen cross reacted with 100% of capillariasis sera with 100% sensitivity and specificity and cross reacted with 10% of sera from bancroftian filariasis. *T. spiralis* crude antigen cross reacted with 50% of capillariasis sera, and 9% of sera from bancroftian filariasis. Neither *T. muris* nor *T. spiralis* crude antigens reacted with sera from control group. Immunoblotting results showed that IgG antibodies from control group didn't recognize specific proteins in *T. muris* antigen, while in *W. bancrofti* group, only one band in one sample appeared (100-135 kDa). IgG antibodies from capillariasis cases recognized multiple common protein bands (35-180 kDa). IgG antibodies from capillariasis, control and *W. bancrofti* sera did not recognize specific proteins in *T. spiralis* crude antigen.
Conclusion: Antigens from *T. muris* and *T. spiralis* can be used successfully to detect infection with *C. philippinensis* using serum samples of cases by ELISA. Crude antigen of *T. muris* gave better results than that of *T. spiralis*. Immunoblotting can be used for diagnosis of capillariasis by using crude antigen of *T. muris*.

Keywords: ELISA, immunodiagnosis, intestinal capillariasis, Trichinelloidea, western blotting.

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INTRODUCTION

The genus *Capillaria* is a member of the superfamily Trichinelloidea. From this group only three major genera: *Trichuris, Trichinella* and *Capillaria* cause human diseases. All Trichinnelloidae members have a unique structure of the esophagus with the presence of stichocytes that differentiates them from other nematodes[1]. *C. philippinensis* is a nematode of fish-eating birds that causes intestinal capillariasis in humans. It first appeared in the Philippines where the disease seems to be endemic[2]. In Thailand, a large number of cases were identified[3]. Subsequent cases were diagnosed in many parts of the world including Korea[4], Taiwan, and China[5,6]. Youssef *et al*.[7] reported the first case in Egypt after which sporadic cases were diagnosed particularly in Upper Egypt[8-13]. Egypt showed the highest number of cases detected outside the endemic areas in the Philippines and Thailand[10].

Infection with *C. philippinensis* is mainly acquired through ingestion of the parasite larva in raw, undercooked, small fresh water or brackish water fish or by contaminated fingers during fish evisceration[10]. Infection leads to inflammation and atrophy of the intestinal villi with subsequent malabsorption of nutrients resulting in extreme protein-deficient enteropathy[14,15]. Patients with intestinal capillariasis usually suffer from chronic diarrhea[16]. The disease can be fatal if diagnosis and treatment are delayed. In non-endemic areas, diagnosis is often skipped due to lack of knowledge of the nature of this disease[17]. Some cases are not easily diagnosed and had to pass

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through many sophisticated, expensive, and sometimes invasive techniques to be diagnosed[10].

This study is based on the concept of similarity between members of the Trichinelloidea superfamily, and the possible cross reactivity between patient sera and antigens from members of the superfamily, which can add a new diagnostic method for the detection of the disease. The aim of the study is to establish a rapid, easy, and accurate diagnostic tool for infection with *C. philippinensis*, as the direct methods are not always positive[18].

SUBJECTS AND METHODS

This descriptive analytical study was conducted at Medical Parasitology Department, Faculty of Medicine, and Department of Zoology, Immunity Division, Faculty of Science, Beni-Suef University from 2018 to 2020.

Preparation of adult worm somatic extracts: *T. muris* adult worms were isolated from laboratory infected mice and the homogenate preparation was carried out as previously described[19,20]. Briefly, worms were washed thoroughly in PBS, homogenized in 50 mM Tris HCl (pH 8.8) containing 0.15 M NaCl, 1 mM EDTA, 1 mM 1,4-Dithiothreitol and 50 mM Phenylmethylsulfonyl fluoride using motor driven homogenizer (REMI, Maharashtra, India) at 4°C. The extract was centrifuged at 5000 × g followed by 15,000 x g for 30 min and the clear supernatant was separated.

For *T. spiralis* antigen, adult worms were collected from the small intestine of experimentally infected Wistar rats at 4 days after experimental infection. The collected adult worms were washed several times in PBS and then homogenized in lysis buffer containing 7 M urea, 2 M thiourea, 4% 3-(3-Cholamidopropyl) dimethylammonio-1-propanesulfonate hydrate 65 mM Tris, 2% DTT, and 1% Bio-Lyte (pH 3-10). The adult worms lysates were centrifuged at 20,000 × g at 4°C for 60 min and the supernatant was collected and used as adult extract[21]. The protein content for both adult worm extracts was determined by Bicinchoninic acid assay kit (Sigma)[22].

Human sera: Human blood samples were collected from 20 patients who had been diagnosed by stool samples analysis as having *C. philippinensis* infection by detecting eggs, adults and/or larvae in stool; from 20 control individuals who had not experienced any parasitic diseases; and 10 samples were from patients with *W. bancrofti* infections. Stool examination of the last two groups showed no other nematodes eggs such as *A. lumbricoides, A. duodenale*, and *T. trichuria*.

ELISA: The optional dilutions of various reagents were determined using checkerboard titration. The assay was performed as previously described[23]. Briefly, 96-well ELISA plates (Corning, USA) were coated with adult worm antigens (2.0 µg/ml) in 100 µl of bicarbonate buffer (pH 9.6) overnight at 4°C. After blocking with PBS 0.1% Tween 20 (PBST) containing 5% fetal calf serum at room temperature for 2 h, the following reagents were sequentially added and incubated at 37°C for 1 h: (1) human sera diluted 1:100 in PBST, and (2) horseradish peroxidase-labeled goat anti-human (HRP) IgG antibody (Sigma, USA) diluted 1:5000. The reactions were detected by the addition of the substrate o-phenylenediamine dihydrochloride (OPD; Sigma, USA) plus H2O2 and stopped with 50 µl/well of 2 M H2SO4. Optical density (OD) values at 490 nm were measured with a microplate reader (TECAN, Austria). All samples were run in duplicate. The cut-off value of the ELISA was evaluated for the two antigen preparations based on a calculation of mean control OD + (2 x SD). The recorded ODs above the cut-off value were regarded as positive.

Western blotting: Immunoblotting was carried out as previously described[24]. Adult worm extracts were separated in SDS-10% polyacrylamide gels and transferred to nitrocellulose membranes (0.45 µm; Heidelberg; Serva Electrophoresis GmbH, Germany) by electroblotting. Membranes were washed in PBS/Tween buffer (PBS containing 0.3% Tween-20) and incubated for 1 h at room temperature in blocking buffer containing 5% non-fat milk in PBS/Tween-20, followed by washing and incubation with the human serum (1:100) in washing buffer overnight at 4°C. Formed immunocomplexes were detected by HRP anti-human IgG antibody (1:5000; KPL, Maryland, USA). After 2 h of incubation at room temperature, bands were developed by adding substrate (50 mg 3,3′-Diaminobenzidine tetrahydrochloride and 100 µl H2O2 in 100 ml PBS).

Statistical analysis: SPSS (version 20) statistical program (SPSS Inc., Chicago, IL) was used to carry out one-way analysis of variance (ANOVA) on our data. When significant differences by ANOVA were detected, analysis of differences between the means of the OD values was performed by Dunnett's t-test. The sensitivity and specificity of ELISA were assessed according to the following formulas: Sensitivity = no. of true positives/(no. of true positives + no. of false negatives) × 100; and specificity = no. of true negatives/(no. of false positives + no. of true negatives) × 100[25]. Positive and negative predictive values and diagnostic accuracy were also calculated. *P* values < 0.5 were considered significant.

Ethical approval: The study was approved by the Research Ethical Committee of the Beni-Suef University, Faculty of Medicine, Egypt. Signed informed consent was obtained from all individuals included in the study after explaining the purpose of the study.

RESULTS

Detection of cross-reactive human IgG using ELISA: When *T. muris* crude antigen was tested for serodiagnostic cross-reacting human IgG, sero-positive reactions were found in 100% from capillariasis and 10% from bancroftian filariasis cases. Calculated sensitivity and specificity for capillariasis were both 100% (Table 1). The difference between the means of OD for control and *Capillaria* or *Capillaria* and *W. bancrofti* were statistically significant ($P<0.01$ and <0.001, respectively) (Fig. 1). In addition, *Trichinella* crude antigen tested for serodiagnostic cross-reacting human IgG recorded positive reactions in 50% from capillariasis and 9% from bancroftian filariasis (Fig. 2). The mean of OD for the reaction with *Capillaria* was significantly higher than reaction with control and *W. bancrofti* infections ($P<0.05$) (Fig. 2). Calculated sensitivity and specificity were 50% and 100% respectively for *T. spiralis* (Table 1).

Table 1. Diagnostic yield and accuracy of ELISA results using *T. muris* Ag and *T. spiralis* Ag among capillariais patients.

		T. muris Ag	*T. spiralis* Ag
ELISA	**Sensitivity**	100%	50%
	Specificity	100%	100%
	PPV	100%	100%
	NPV	100%	66.7%
	Accuracy	100%	75%

Detection of specifically recognized proteins in adult worm antigens using immunoblotting: IgG antibodies from selected controls (n=20) did not recognize specific proteins in *T. muris* crude antigen. In contrast, IgG antibodies from capillariasis cases (n=20) could recognize multiple common protein bands ranging between 35-180 kDa (35, 48, 63, 75, 100, 135,180 kDa). IgG antibodies from *W. bancrofti* antisera (n=10) did not recognize specific proteins in *T. muris* crude antigen except in only one serum sample (No. 8), that recognized a protein band (100-135 kDa) (Fig. 3). IgG antibodies from capillariasis, control and other *W. bancrofti* infections did not recognize specific proteins in *T. spiralis* crude antigen.

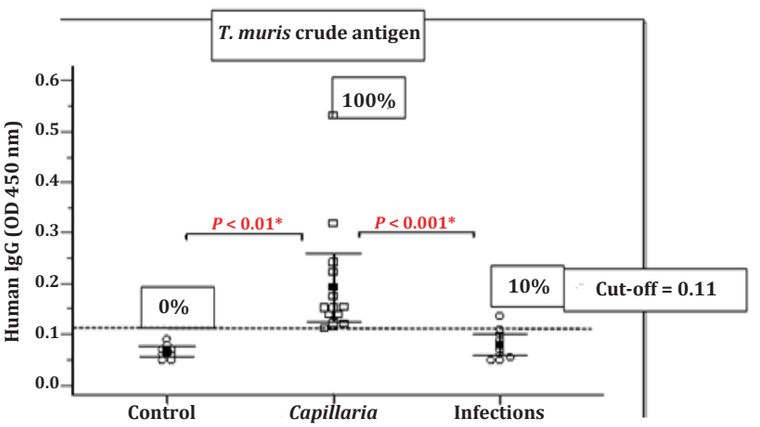

Fig. 1. *T. muris* crude antigen cross reacted with 100% of capillariasis sera, and 10% of other parasitic infections (bancroftian filariasis); ***:** Sifnificant ($P < 0.05$).

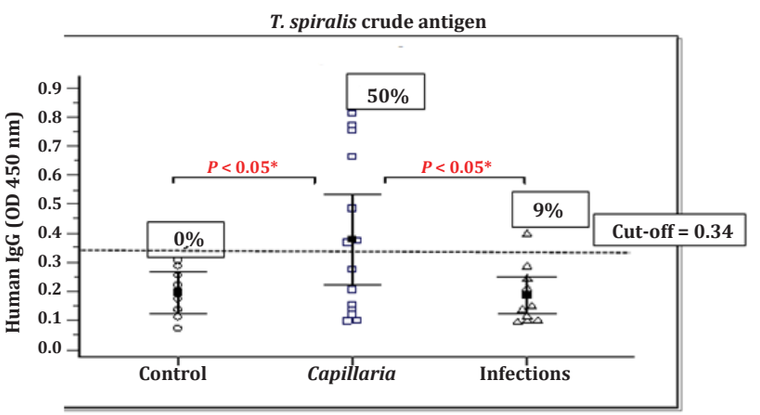

Fig. 2. *T. spiralis* crude antigen cross reacted with 50% of capillariasis and 9% of other parasitic infections (bancroftian filariasis); ***:** Sifnificant ($P < 0.05$)..

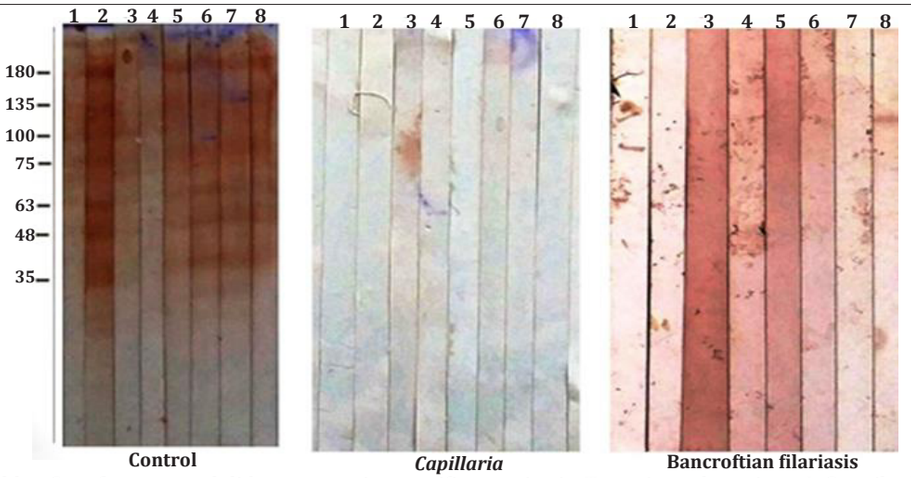

Fig. 3. Western blot. Sera from control did not recognize specific proteins in *T. muris* crude antigen. IgG antibodies from *Capillaria* positive cases could recognize multiple common protein bands ranging between 35-180 kDa. There is an apparent band (100-135 kDa) in serum no. (8) in bancroftian filariasis group.

DISCUSSION

The real prevalence of *C. philippinensis* may be higher than predicted, but the lack of awareness of the parasite and the outcome of infection by physicians and laboratory technicians is delaying the diagnosis[3]. Immunodiagnosis may allow early detection of the parasite. Using it as an extra diagnostic tool helps to detect *C. philippinensis* infection[18]. In our study, the reactivity of serum samples from 20 cases diagnosed as human *C. philippinensis* infection was examined by ELISA and immunoblotting against *T. muris* and *T. spiralis* antigens. By ELISA, 100% of the *C. philippinensis* cases were reactive with *T. muris* antigen and 50% of cases reacted with *T. spiralis* antigens with statistical significance. Only 10% of bancroftian filariasis antisera reacted with *T. muris* antigen while 9% reacted with *T. spiralis* antigens, indicating the superiority of the former as a diagnostic antigen. The similarity of stichocytes between the members of the family Trichinelloidae, allows for the use of their antigens in immunological reactions[26].

These cells are rich in antigens, that can be used for the serodiagnosis of capillariasis[27]. Similar studies consistent with our results were conducted worldwide. Intapan *et al.*,[27] diagnosed intestinal capillariasis using *T. spiralis* antigen by ELISA that reacted positively with 75% of trichinellosis. Another study conducted by Banzon *et al.*[28] proved that *C. philippinensis* sera were also reactive with *T. spiralis* and *T. vulpis* antigens.

To avoid the false negative diagnosis of infection with *C. philippinensis* in the absence of eggs in stool, an immunological study was carried by El-Dib *et al.*[18] in which coproantigen was extracted from the stools of infected patients. Hyperimmune serum was prepared against this antigen and used successfully to detect the copro antigen of *C. philippinensis* in stool samples of suspected cases by sandwich ELISA.

By Immunoblotting, IgG antibodies from capillariasis cases could recognize multiple common protein bands in *T. muris* crude antigen ranging between 35-180 kDa (35, 48, 63, 75, 100, 135,180 kDa). No bands were detected in the control group. Similarly, Abdel-Rahman *et al.*[29] used *C. philippinensis* coproantigen and egg antigen for diagnosis of intestinal capillariasis utilizing the immunoblotting method. Results showed no bands in the control group, while multiple protein bands were recognized for both antigens in capillariasis cases. These bands ranged from 10 to 148 KDa for *C. philippinensis* coproantigen and from 60 to 62 for egg antigen. On the other hand, in our study IgG antibodies from *W. bancrofti* antisera did not recognize specific proteins in *T. muris* crude antigen except in only serum sample that recognized a single protein band (100-135 kDa). There may be cross reactivity between filarial infections and other nematodes including *Capillaria* as tested by Joekel *et al.*[30] who proved immunological cross-reactions between *Dirofilaria* and *Capillaria aerophila* in expermintally infected dogs.

In conclusion, this study showed that antigen from *T. muris* and *T. spiralis* can be used successfully to detect infection with *C. philippinensis* using serum samples of cases by ELISA technique. Crude antigen of *T. muris* gave better results than crude antigens of *T. spiralis*. Western blot technique also can be used for diagnosis of capillariasis by utilizing crude antigen of *T. muris*.

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Conflict of interest: All authors declare that they have no conflict of interest.

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