Proteomic Analysis Of *Trichuris Trichiura* Egg Extract Reveals Potential Immunomodulators And Diagnostic Targets

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Research

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Abstract

Background: *Trichuris trichiura* embryonated eggs are the infectious developmental stage and the first signal to the immune system of the definitive host. Each infective *T. trichiura* egg carries the antigens needed to challenge the immune system with a wide variety of proteins present in the shell, larvae's surface, and the accompanying fluid that contains their excretions/secretions. The parasite eggs constitute the first antigenic stimuli to evoke the host response to this intestinal parasite with direct life cycle and enteric development.

Methods: The soluble egg extract of *T. trichiura* obtained from naturally infected African green monkeys (*Chlorocebus sabaeus)* was investigated using a proteomic approach by mass spectrometry. The antigenic profile of the egg soluble proteins against sera IgG from *C. sabaeus* with trichuriasis was also investigated by Western blot and LC-MS/MS from the corresponding SDS-PAGE gel.

Results: A total of 231 proteins were accurately identified, 168 with known molecular functions. The proteome of the egg lysate revealed common protein families including energy and metabolism; cytoskeleton, motility and muscle; proteolysis; signaling; stress and detoxification; transcription and translation and; lipid binding and transport. Vitellogenin N and VWD and DUF1943 domain containing protein, Poly-cysteine and histidine tailed protein isoform 2, Heat shock protein 70, Glyceraldehyde-3-phosphate dehydrogenase, Actin and Enolase, were among the potential immunoactive proteins.

Conclusions: To our knowledge, this study represents the first attempt to identify the proteome of the *T. trichiura* egg extract as a novel source of immunomodulators and targets for immunodiagnosis able to contribute to the treatment of human autoimmune diseases and to the control of this neglected disease.

Background

Trichuris trichiura causes one of the major soil-transmitted helminth infections, along with the roundworm, *Ascaris lumbricoides*, and the hookworms, *Necator americanus* and *Ancylostoma duodenale*. The intestinal-dwelling *T. trichiura* affects 465 million people worldwide with an estimated global burden of disease of 640,000 DALYs (Disability Adjusted Life Years) [1] and 337,000 YLDs (Years Lost to Disability) [2]. Following accidental ingestion of the embryonated eggs, larvae hatch in the proximal small bowel, and migrate aborally to the colon and cecum, where they remain attached to the mucosa. These nematodes mature to adults in 30–120 days and can survive from 1 to 8 years [3, 4]. After copulation, the females lay eggs that are expelled in feces as the non-infective form. They require appropriate environmental conditions to allow the larvae development to the infectious life-cycle stage. *T. trichiura* eggs measure 50–60 µm in length and 20–30 µm in width; they do not develop in direct sunlight and perish below 9˚C [3, 5].

Trichuriasis is often asymptomatic but it can manifest with abdominal pain, diarrhea and, in severe cases, dysentery syndrome. Infection in children is more common; heavy infections can result in rectal prolapse, severe anemia, stunted growth and poor school performance [5–8] The severity of the symptoms not only depends on the parasite load but also on co-infections, immune-competence and past infections [4, 9].

Diagnosis of infections is usually based on the detection of eggs through coprological analyses, but such techniques lack sensitivity for a variety of reasons [10]. Stool examination does not adequately identify real infection status and parasite loads due to the dynamic events inherited to the life cycle of the nematode [9, 11]. During the prepatent period, lasting from the ingestion of infective eggs to the development of adults to the laying of eggs by the females, the infection may not be identified by coprology, and it cannot be done neither in female nor male single infections [9, 12]. Similar challenges are observed when low-load infections occur, or when timing of stool collection does not match the release of eggs by the female, and these infections remain overlooked [4, 12]. The above reasons highlight the need for an alternative indirect diagnostic test that does not rely on etiological diagnosis.

While *T. trichiura* somatic and excretion/secretion products from the adults have been studied in depth and have been shown to elicit protective immune responses [13–16], other life-cycle stages of the parasite, such as the eggs, have not been fully investigated. Only a few reports indicate that they may be modulators of the immune system, and recognize them as potential sources of diagnostic antigens [17, 18]. Interestingly, the administration of embryonated eggs from the animal species *Trichuris suis* or *Trichuris muris* to humans has been described as potential therapeutic agents for immune-related pathologies such as chronic intestinal inflammatory diseases, emphasizing their capacity to downregulate aberrant intestinal inflammation [6, 19–22]. Their immunomodulatory capacity continues to be investigated with some studies trying to identify the molecules responsible for those effects [14, 22–25].

Each infective *T. trichiura* egg carries the antigens needed to challenge the immune system of the host after the larvae and the accompanying fluids are released. Herein, we carried out the analysis of the soluble egg extract as a stage-specific proteomic approach and the identification of immunomoactive molecules. Detailed information about the specific protein patterns and functional analysis of the molecules provides a first insight into their intricate role within the life cycle and the interactions with the host. The antigenic identification and characterization of these egg-derived proteins will complement the current efforts with the *T. suis* and *T. muris* models focused on the prevention of autoimmune diseases and the development of new immunodiagnostic techniques.

Methods

Sera samples

Sera samples were collected from 10 African green monkeys (AGMs) naturally infected with *T. trichiura* as part of other studies and transferred to 5 mL vacutainers (Covidien Monoject™, Massachusetts, USA). Serum was isolated immediately by centrifugation at 2,000 g for 15 min at 4˚C and stored at -80˚C until analysis.

T. trichiura adults

Adult worms were obtained at necropsy from the large intestine of naturally infected animals. The large intestine was placed in 0.9% saline solution for approximately 2 h at room temperature (30-32°C). During this time the nematodes were released from the mucosa. Thereafter, the large intestine was opened and washed over a 100 µm sieve, and the sieved content was examined under a stereomicroscope (7x – 10x magnification) for the presence of *T. trichiura* adults, which were isolated, sexed and preserved at - 80˚C.

T. trichiura egg extract

Uteri were removed from *T. trichiura* females using a 30G ½" needle (BD Microlance™, Fraga, Huesca, Spain) (10x – 30x magnification) and placed on phosphate buffered saline (PBS; pH 7.4). The uteri were opened with a longitudinal incision to facilitate the release of eggs which were pooled. The pooled eggs were washed five times in PBS (10,000 g; 1 min), after the final wash the supernatant was removed and PBS containing 1% protease inhibitors cocktail (Complete mini EDTA-free™, Roche, Berlin, Germany) and 1% Triton™ X-100 (Sigma-Aldrich, Steinhiem, Germany) was added to prepare homogenates as described elsewhere [26]. To ensure disruption of *Trichuris* eggshells, the homogenate was frozen at -20˚C and sonicated while frozen using ten cycles of 10x 1-second pulses at maximum intensity with a Microson Ultrasonic Cell Disruptor XL™ (Misonix, Farmingdale, NY, USA). Homogenates were checked under a stereomicroscope, centrifuged (10,000 g; 10 min at 4˚C) and the supernatant containing the soluble egg proteins was recovered as the *T. trichiura* egg extract (EE). The total protein concentration of the extract was determined by a commercial Protein Assay (Bio-Rad®, Hercules, USA), which is based on the Bradford method of quantification of soluble proteins [27]. The EE was stored frozen at -20˚C until further analysis.

T. trichiura female extract

The *T. trichiura* female extract (FE) was analyzed in parallel to make a comparative study of the immunogenic capacity of the egg proteins with respect to those of the whole females. Female adults were obtained from the intestine of infected AGMs. The collected worms were washed several times with PBS and homogenized with a Teflon homogenizer in PBS containing 1% protease inhibitors cocktail (Complete mini EDTA-free™, Roche). After initial centrifugation at a low speed to remove larger particles, the homogenate was centrifuged again (15,000 g; 30 min at 4ºC) and the supernatant collected and stored frozen at -20ºC until further analysis. The protein content was measured in the same way as the EE.

One dimensional SDS-PAGE

T. trichiura EE (10 µg/well) was diluted in Laemmli buffer (4X) (Bio-Rad®) (1:1), denatured at 100˚C for 5 min and separated by one dimensional gel electrophoresis (1-DE) in Mini-Protean® TGX precast acrylamide gels (4-15% gradient, 10 well comb, 50 µL/well) (Bio-Rad®) under reducing conditions with 80 – 120 V in a Mini-PROTEAN Tetra System electrophoresis system (Bio-Rad®) as previously described [28]. Samples were run simultaneously with molecular weight markers (4 µL) (Precision Plus Protein ™ Dual Color Standards, Bio-Rad®).

To analyze the protein patterns obtained after electrophoresis, the gels were stained with Coomassie brilliant blue, which allowed identifying and excising the most prominent bands for proteomic analysis. The procedure for staining was as follows: Fixing solution (50% methanol and 10% glacial acetic acid) overnight with gentle agitation (change solution once at first 1 h). Gels were soaked in staining solution (0.1% Coomassie brilliant blue R-250, 50% methanol and 10% glacial acetic acid) for 20 min, being gently shaken. Destaining solution was used (40% methanol and 10% glacial acetic acid) and the solution was replenished several times until the gel background was fully removed. Finally, gels were stored at 4ºC in 5% glacial acetic acid.

Western Blot

For immunoblotting, following one dimensional electrophoresis, proteins were transferred onto nitrocellulose paper using a Trans-Blot® Turbo™ transfer system (Bio-Rad®) for 7 min. The blotted membrane was blocked with 5% skimmed milk in 0.05% PBS-Tween 20® (PBST) for 2 h at room temperature and, after successive washes in PBST, they were incubated overnight at 4˚C with a pool of serum samples diluted 1:500 in PBST. After three washes for 30 min in PBST, the membranes were incubated for 4 h at room temperature with the secondary antibody (peroxidase-labeled goat anti-primate IgG (Novusbio™, Colorado, USA) (1:5,000 in PBST)). Finally, membranes were washed three times in PBST, for 30 min each and the assay developed using Clarity™ Western ECL substrate (Bio-Rad®) mixed in a 1:1 ratio. The positive reactions were determined by the appearance of clearly defined protein bands detected by chemiluminescence with an Amersham™ Imager 600 (GE Healthcare, New Jersey, USA). The relative molecular masses of the recognized protein fractions were determined by comparison with molecular weight markers (kDa) and data analysis was completed as previously described [29].

Proteomic analysis of the *T. trichiura* egg extract (EE)

Sample preparation

Following electrophoresis and staining, a complete gel strip of egg extract was cut and digested with 500 ng of sequencing grade trypsin (Promega, Wisconsin, USA) in 200 µL of ammonium bicarbonate solution as described elsewhere [30]. The selected bands from other gels (egg and female extracts) were manually excised and digested with 100 ng of sequencing grade trypsin (Promega) in 100 µL of ammonium bicarbonate as described elsewhere [30]. Digestion was stopped with 1% trifluoracetic acid (TFA) and a double extraction with acetonitrile (ACN) was performed. The final peptide solution was vacuum-dried and resuspended with 25 µL of 2% ACN and 0.1% TFA (pH 2.0) for the EE and 9 µL of 2% ACN and 0.1% TFA (pH 2.0) for the individual bands as previously described [28].

Liquid chromatography and tandem mass spectrometry (LC-MS/MS)

Liquid chromatography and tandem mass spectrometry were performed at the Proteomics facility of "*Servei Central de Suport a la Investigació Experimental* (*SCSIE*)" of Universitat de València (Burjassot, Spain).

To initiate the elution process, 5 µL of the final peptide solution was loaded onto a trap column (Nano-LC Column, 3 µm C18-CL, 350 µm x 0.5 mm, Eksigen®, AB SCIEX©, California, USA) and desalted with 0.1% TFA at 3 µL / min for 5 min. The peptides were loaded onto an analytical column (LC Column, 3 µm C18-CL, 75 µm x 12 cm, Nikkyo, Nikkyo Technos Co., Ltd. Tokyo, Japan) equilibrated in 5% acetonitrile, 0.1% formic acid (FA) and eluted using a linear gradient (5-35%) of solvent B (0.1% FA in ACN) in A (0.1% FA) for 120 min for the EE and 30 min for the individual bands at a flow rate of 300 nL/min. The eluted peptides were analyzed with a nanoESI-Q-TOF mass spectrometer (5600 TripleTOF, AB SCIEX©) in an information dependent acquisition mode (IDA). The eluted sample was ionized applying 2.8 kV to the spray emitter and survey MS1 scans were acquired from 350 to 1250 m/z for 250 ms. The quadruple resolution was set to 'UNIT' for MS2 experiments, which were acquired from 100 to 1,500 m/z for 50 ms in 'high sensitivity' mode. The following switch criterion was used: charge 2+ to 5+, minimum intensity, 70 counts per second (cps). Up to 50 ions were selected for fragmentation after each survey scan. Dynamic exclusion was set to 15 s. The system sensitivity was controlled with 2 fmol of 6 proteins (LC Packings, A Dionex Company, Amsterdam, Netherlands).

Bioinformatics

ProteinPilot (version 4.5.1, revision 2768; Paragon™ Algorithm 4.5.1.0, 2765; SCIEX©) Applied Biosystems® / MDS SCIEX©) with default parameters was used to generate a peak list directly from 5600 TripleTof wiff files. All wiff. files from the samples were combined in a single search. The Paragon™ Algorithm included in ProteinPilot™ software was used for searching the NCBI protein

database (version 01-2016) with the following parameters: tryptic specificity, cys-alkylation, Metazoa, Nematoda and *Trichuris trichiura* protein taxonomy restrictions.

Protein grouping was done by Pro Group™ algorithm (a set of proteins that share physical evidence guided by observed peptides only) and identification was considered accurate when the ProteinPilot™ unused score was > 1.3 corresponding to a 96% confidence according to the following equation: ProtScore= -log (1-(percent confidence/100)).

Protein identification was conducted against the *T. trichiura* adult proteome from the Parasite WormBase (version of 2017-262 05 - WormBase - www.parasite.wormbase.org). All identified proteins were subsequently assigned to the UniProt database and classified in Gene Ontology (GO) (https://www.uniprot.org) in accordance to their molecular function and biological process.

Results

In this study we describe for the first time the proteome of the egg extract of *T. trichiura* from AGMs (*C. sabaeus*) and the potential antigens recognized by sera of naturally infected animals.

Proteomic characterization of the *T. trichiura* egg extract (EE)

In a first analysis of the EE, the spectrometric data using ProteinPilot™ software v4.5 identified 246 proteins from which 212 showed significant homologies with known *T. trichiura* adult stage proteins, in addition to 19 novel or uncharacterized proteins with unknown ontology. A total of 231 proteins were accurately identified by ProteinPilot™ and accession numbers from Parasite WormBase. These proteins were categorized by their molecular function according to information obtained from the Gene Ontology (GO); 168 presented known molecular functions (Additional file 1).

Gene ontology (GO)

The different functional groups and biological processes of the most representative proteins of our analysis (with 10 or more distinct peptides) are shown in Table 1 and Figure 1. Only a single annotation was assigned to a given protein. Functional annotation of the identified proteins was assigned using GO, which revealed functionally diverse molecules of the common protein families or groups: energy and metabolism; cytoskeleton, motility and muscle; proteolysis; signaling; stress and detoxification; transcription and translation; and lipid binding and transport (Table 1). Their specific molecular functions range from molecules involved in ATP, actin, carbohydrate, chitin, lipid and magnesium ion binding, as well as molecules that take part in oxidoreductase, aminopeptidase, glycogen phosphorylase and metallopeptidase activity (Table 1). Others include lipid transporter, motor and protein disulfide isomerase activity, together with structural constituents of the ribosome or proteins associated with the elongation phase of protein synthesis. Proteins with kinase and intracellular cholesterol transport functions where also identified (Table 1). The most abundant category for the biological process assigned to the egg proteins were protein folding, translation, gluconeogenesis and glycolytic process all equally represented (13%), followed by cell redox homeostasis (12%) and chitin metabolic function (12%), and to a lesser extent: metabolic process, carbohydrate metabolic process, protein biosynthesis and stress response (Figure 1).

1-DE and immunoblot analysis of Egg and female adult proteins *T. trichiura*

To identify the species-specific parasite antigens, the 1-DE SDS-PAGE and Western blot were performed to investigate the antigenic profile of the EE and FE, and analyze which peptides were recognized by serum IgG from naturally infected AGMs.

The immune-complexes identified by Western blot for the EE, were in the range of 37 and 200 kDa, with two marked bands including the most immunogenic ones, band 1W (≈ 170 kDa) and band 2W (≈ 37 kDa) (Figure 2, lane 1). The possible identity of the proteins in those bands was investigated by matching the predicted molecular weight from the EE proteome (Table 2). In addition, the same regions of interest were excised from the SDS-PAGE corresponding gel (labeled as 1G and 2G, Figure 2, lane 2), for new confirmatory proteomic analysis (Table 3).

Regarding the identification of antigens in the gel bands, our proteomic analysis revealed Vitellogenin N and VWD and DUF1943 domain containing protein (VgNVD) and Heat shock protein 70 (HSP-70) among the potential egg immunodominant proteins in band 1W based on the EE proteome (Table 2). The subsequent mass spectrometry of the Coomassie-stained band confirmed that VgNVD is the most representative protein within this area with 241 distinct peptides (Table 3).

The proteomic analysis of the second band (2W) identified Poly-cysteine and histidine-tailed protein isoform 2 (PCHTP-2), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Actin, Enolase in both approximations (Table 2 and 3). Moreover, the analysis of the gel band provided some more identities such as Phosphoglycerate kinase, Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit and Adenosylhomocysteinase (Table 3).

In both samples a highly reactive band, with a mobility corresponding to 37 kDa was detected (2W and 4W, Figure 2). It corresponded to material detected by Coomassie staining, more abundant in the FE; while other two bands (1W and 3W) seemed to be stage-specific (Figure 2). As shown in the Figure 2, the band labeled 1W in EE was barely detected in the stained gel, indicating its low abundance, while band 3W corresponded to a prominent band when stained with Coomassie (3G) (Figure 2).

Regarding the proteomic analysis of reactive areas displayed in FE Western blot, the analysis of the band 3G (≈ 60-70 kDa) (Figure 2, lane 4), which corresponded to band 3W, revealed again PCHTP-2 as one of the proteins identified with the highest number of matching peptides. This protein was also identified in bands, 4.1G and 4.2G (Table 4). The proteomic results of both sections of the band 4W showed some proteins shared between the egg and female extracts, such as PCHTP-2, Actin and GAPDH, suggesting them likely to be the major ones in both samples. This is not surprising, since most of the EE antigens are also present in FE (eggs contained in the uterus).

Despite the fact that both bands (2W and 4W) shared several proteins, around 37 kDa, specific ones appeared for each evolutionary stage. Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48kDa subunit, Adenosylhomocysteinase, Tubulointerstitial nephritis antigen, Calponin domain containing protein, 3 ketoacyl coenzyme A thiolase, Elongation factor 1-alpha and Serpin domain containing protein appeared as typical of EE (Table 3). Meanwhile, Calsequestrin, Epididymal secretory protein E1 and Actin-depolymerizing factor 2-isoform c, were only present in FE (Table 4).

Discussion

Foth and collaborators [15] described the whole-genome sequences of the human-infective *T. trichiura*, as well as the whole-transcriptome in a mouse laboratory model *T. muris* and identified numerous genes that are differentially expressed in a sex- or stage–specific manner. The most abundant transcripts found in this extensive study, included proteins we have also identified in the EE proteome such as two WAP domain containing SLP-like proteins, protease inhibitors such as Cystatin-domain containing protein and nematode cuticle collagen N-terminal domain containing proteins and Chitin binding domain containing proteins such as CBM14 domain containing proteins. Furthermore, with more or less representation, but of particular interest within the context of the present work, we have found trichuris egg proteins with known immunomodulatory properties such as Macrophage migration inhibitory factor homolog (MIF), previously identified in *T. trichiura* adult [14], and 14-3-3 protein which has also been identified in several developmental stages of other nematodes, *Trichinella britovi* [31] and *Trichinella spiralis* [32] and trematodes, *Schistosoma japonicum* [33]. Both proteins are considered as enhancers of humoral and cellular immune responses [34].

Interestingly, two of the proteins identified with the largest numbers of distinct peptides in the EE proteome presented in this study, Vitellogenin N and VWD and DUF1943 domain containing protein (VgNVD) and Poly-cysteine and histidine tailed protein isoform 2 (PCHTP-2), were also found among the top 25 most abundant transcripts found by Foth and collaborators [15]. Vitellogenins are a lipid transfer proteins present in the eggs of most oviparous animals as the major component of yolk. They play a significant role in embryonic development and are extensively conserved amongst insects, nematode and vertebrates [35]. They are produced by extra-ovarian tissues, secreted into the circulatory system and then taken up by the developing oocytes through receptor mediated endocytosis to provide the growing embryo with amino acids [36]. On the other hand, the detection of PCHTP-2 as the second most frequently detected protein is in accordance to Shears and collaborators [37] who found it to be the most abundant protein in the *T. muris* adult secretome; even though a specific function has not been assigned yet. Likewise, Bancroft and collaborators [38] identified PCHTP-2 as the most abundant protein in cecal mucus from chronically infected mice with *T. muris* and confirmed its expression in all developmental stages.

One of the most represented groups of proteins is that of energy and metabolism including proteins related to glycolysis (Enolase and Glyceraldehyde-3-phospate dehydrogenase (GADPH)) and gluconeogenesis (Triosephosphate isomerase and Phoshoenolpyruvate carboxykinase GTP) and other metabolic enzymes such as Alpha-1,4 glucan phosphorylase and Malic enzyme. This fact is consistent with previous studies in which those metabolic enzymes were described in the surface of the helminths,

nematodes and trematodes, participating in parasite invasion and migration processes within the host and in oxidative processes [28,29,39-42].

The ensuing functional group with the largest number of representatives is the cytoskeleton and motility and muscle proteins. Actin, Tropomyosin, Paramyosin, Intermediate filament protein IFA 1 and Epididymal secretory protein E1 were found with a high number of distinct peptides. These proteins are essential to enhance the motility of the nematodes and have also been recorded in many helminthic proteomes: somatic extract of adults of *T. spiralis* [43], *T. britovi* [31], *Syphacia muris* [42] and *Echinostoma caproni* [44]; and in egg secretions of *Schistosoma mansoni* [17]. Specifically, Intermediate filament protein IFA1 has been studied in *Caenorhabditis elegans* demonstrating that in nematodes they allow epidermal elongation in the larval stages to grow into adults [45].

In addition to the proteins already mentioned, another group essential for the survival of the nematode within its host is that of the stress and detoxification, including antioxidants and chaperones. The Cu/Zn superoxide dismutase (Cu/Zn-SOD) was found in the EE and it has also been identified on the adult surface and larval extracts (secreted and somatic) of *Toxocara canis* [34], in somatic extract of adults of *Fasciola hepatica,* and in *S. mansoni* egg secretome [17,46]. This essential enzyme antagonizes the inflammatory responses in the host by regulating the free radical balance and reactive oxygen species in cells protecting helminths against cell death [47]. Heat shock proteins (HSP90, HSP70, HSP60) are inducible conserved proteins widely described in parasite proteomes and secretomes, acting as molecular chaperones which fold, assemble and translocate other proteins to ensure the survival of the parasite by defending it against stressful situations being important in stress tolerance [48]. Small heat shock proteins HSP-20 and HSP-20 domain containing protein were also identified in EE, which are known to aid parasite survival under hostile conditions such as heat or nutritional stress [49].

Within the proteins implicated in signaling pathways, we identified galectin, a type of lectin found in different extracts of nematodes such as adults and larvae of *T. canis* [34] and extract of infective larvae (L3) of *Haemonchus contortus* [50] with a role in immune signaling pathways. Nematode galectins are believed to be immunological mediators with implications in survival and interaction with the host [51] and modulate a range of immune responses including the cellular immune response, inflammatory processes and immune regulation [52].

Antigenic profile of *T. trichiura* EE and FE extracts and identification of immunodominant proteins

This type of immunoproteomic approach has been was applied in previous studies to determine both the antigenic proteins of different helminths developmental stages (larvae and adults), and evaluate the serological response to the soluble protein extracts of *Ascaris lumbricoides* [53], *T. britovi* [31], *Schistosoma japonicum* [54] and *Taenia solium* [55].

Parasitic worms have a remarkable ability to modulate the host immune response through several mechanisms; specific parasite-derived proteins can modulate immune functions playing an important role in the parasite-host interaction. Excretion/secretion proteins from larvae and adults of the porcine whipworm*, T. suis*, closely related to the human *T. trichiura*, were investigated by Leroux et al. [21], who identified a subset of proteins that promote specific anti-inflammatory functions and immunomodulatory properties.

Shears and collaborators [37] identified VgNVD in extracellular vesicles (EVs) of *T. muris* as a potential immunogenic candidate. Antigenic homologs have been identified in both free-living nematodes such as *C. elegans*, and adult parasites secretomes of *Ascaris suum*, *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus* and *Litomosoides sigmodontis* [56-59] and also in *H. polygyrus* eggs [60].

HSP-70 and heat shock proteins in general, have caught the attention of researchers for acting typically as immunodominant antigens eliciting strong humoral responses as major targets of host immune responses, suggesting them out as possible candidates for antiparasitic, allergic and autoimmune diseases treatments [61,62]. The HSP70 is amongst the most highly abundant protein identified in egg secretions of *S. mansoni* and *H. polygyrus* [17,60], and also heavily represented in *E. caproni, F. hepatica, H. polygyrus, Schistosoma bovis, T. trichiura, T. britovi* and *Zygocotyle lunata* adult worms extract [14,31,41,44,63,64]. Others have reported on their immunogenicity linked to stimulation of IgG and IgM responses [39,65,66] and they have been suggested as possible vaccine targets [67].

PCHTP-2 was identified as a strong immunogen of *Trichinella pseudospiralis* adult secretome [68]. Another protein of the same family, Poly-cysteine and histidine-tailed metalloprotein, implicated in metal storage and/or transport, was the first member of the nematode poly-cysteine protein family described in *T. spiralis*. Since these proteins are unique for parasites of the Superfamily Trichinelloidea their potential applications in diagnostics and treatment could be exploited in the future [69]. Bancroft [38] hypothesized that the unique structural features of this protein allow binding to IL-13 which is considered the key effector cytokine responsible for *T. muris* expulsion, able to inhibit IL-13 function both *in vitro* and *in vivo*.

Certain glycolytic enzymes, Enolase and GAPDH, have been identified as inmunoactive components of the *Trichuris* egg proteome. Both of them are present in the surface of helminths interacting with the host surface. Furthermore, Enolase plays an important role in fibrinolysis and degradation of the intracellular matrix through the activation of plasminogen, which may induce plasmin-mediated proteolysis and facilitate the invasion, migration and fixation in the host [15,17,29,42]. In *T. spiralis* [39] and *T. britovi* [31] this enzyme has been confirmed as immunodominant suggesting that it may assist in tissue migration of the larvae. Enolase and heat shock proteins have also been classified as exosome markers [37,70]. Likewise, GAPDH has been previously linked to fibronectin, laminin, entactin and collagen binding [71]. Cass and collaborators [17] suggested that in the case of *S. mansoni* this protein could be involved in the attachment of the eggs to host tissues or aid the passage of live eggs across host tissues to the external environment.

The present study seeks to identify and characterize the soluble extracts of *T. trichiura* eggs by proteomic and immunoproteomic approaches. The *T. trichiura* life cycle inside the host starts with the egg hatching and the release of the larva, this period of time remains as an undiagnosed stage, while the proteins described here are directly exposed to the immune system, and as we demonstrate herein, can elicit anti-*Trichuris* antibodies by the host.

Conclusions

This is the first attempt to identify the proteome of the *T. trichiura* eggs as a novel source of potential targets that can be used to develop better strategies for improving diagnosis, treatment and control of this neglected disease.

Eggs as the infective developmental stage of the nematode will signal the host interface with its shell surface antigens and with the release of the larvae and associated fluid posing the first stimuli to the host immune system. This initial list of *T. trichiura* egg proteins (proteome and antigenic profile) can be used in future research aimed at elucidating their specific role in the immunobiology and pathogenesis of this human whipworm, and may find new applications in immunodiagnosis of trichuriasis and in the treatment of human immune-related diseases.

Declarations

Ethics approval and consent to participate

The sample collection procedures from animals enrolled in other studies was approved by the Institutional Animal Care and Use Committees of the Behavioral Science Foundation and the St. Kitts Biomedical Research Foundation (SKBRF) (Study Code S00147).

Consent for publication

Not applicable

Availability of data and materials

The data supporting the conclusions of this study are included within the article and the datasets generated during the present study are included as supplementary information files (Additional file 1).

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

KC performed the experiments, the data curation and analysis, and drafted the manuscript. AM contributed with the conceptualization, methodology, validation, writing review, editing, and funding acquisition. PK and MV contributed with writing review, editing, supervision and resource acquisition. AO contributed with conceptualization, methodology, validation, writing review and editing. MT contributed with the design, conceptualization, methodology and validation of the experiments as well as the writing review and editing, supervision, funding and resource acquisition.

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Tables

Table 1
Main proteins identified in the EE (10 or more distinct peptides) organized by functional annotation. Only a single annotation was assigned to a given protein.

Functional annotation	Molecular function*	Acc. No. Wormbase	% Cov.	Peptides (95%)	MW (kDa)	Signal peptide	Biological process*
Energy and metabolism							
Alpha-1,4 glucan phosphorylase	Glycogen phosphorylase activity	A0A077YWK8	20.29	14	101.447	-	carbohydrate metabolic process
ECH domain containing protein	Catalytic activity	A0A077Z1N9	44.83	17	31.202	-	metabolic process
Enolase	Magnesium ion binding	A0A077YX57	44.49	27	49.513	-	glycolytic process
Glyceraldehyde-3-phosphate dehydrogenase	Oxidoreductase	A0A077ZHV3	56.10	66	37.536	-	glycolytic process
Malic enzyme	Oxidoreductase	A0A077Z5U2	28.04	13	62.847	-	Unknown
Phosphoenolpyruvate carboxykinase GTP	Kinase	A0A077Z7M0	29.04	20	70.975	-	gluconeogenesis
Triosephosphate isomerase	Isomerase	A0A077ZC84	57.26	10	27.399	-	gluconeogenesis
Cytoskeleton, motility and muscle							
Actin	ATP binding	A0A077ZE37	55.59	35	41.838	-	unknown
Actin 5C	ATP binding	A0A077YWW9	53.66	29	41.036	-	unknown
Epididymal secretory protein E1	Intracellular cholesterol transport	A0A077Z0I4	43.44	28	45.783	1 to 23	unknown
Intermediate filament protein IFA 1	Unknown function	A0A077Z6U0	23.39	14	70.711	-	unknown
Moesin-ezrin-radixin 1	Actin binding	A0A077ZIT0	25.97	12	55.989	-	unknown
Paramyosin	Motor activity	A0A077Z8E1	38.61	30	101.488	-	unknown
Tropomyosin	Unknown function	A0A077ZIM1	41.20	38	87.298	-	unknown
Proteolysis							
Cytosol aminopeptidase	Aminopeptidase activity	A0A077Z3I7	23.80	10	54.409	-	unknown
Peptidase M13 and Peptidase M13 N domain containing protein	Metalloendopeptidase activity	A0A077ZJE5	24.05	14	81.361	-	unknown
Signaling							
78 kDa glucose regulated protein	ATP binding	A0A077Z8G8	22.58	12	72.784	1 to 18	unknown
CBM 14 domain containing protein	Chitin binding	A0A077Z111	46.72	38	95.908	-	chitin metabolic function
CBM 14 domain containing protein	Chitin binding	A0A077Z8B3	28.37	18	78.597	-	chitin metabolic process

Functional annotation	Molecular function*	Acc. No. Wormbase	% Cov.	Peptides (95%)	MW (kDa)	Signal peptide	Biological process*
Galectin	Carbohydrate binding	A0A077YZM7	50.72	27	31.967	-	unknown
Galectin	Carbohydrate binding	A0A077ZG03	39.64	25	32.25	-	unknown
Stress and detoxification							
Chaperonin protein heat shock protein 60	ATP binding	A0A077ZIE8	28.00	11	62.806	-	protein folding
Heat shock protein 70	L-malate dehydrogenase activity	A0A077Z8E4	20.07	21	130.299	-	stress response
Heat shock protein 90	ATP binding	A0A077Z1F6	17.08	12	82.924	-	protein folding
Protein disulfide-isomerase	Protein disulfide isomerase activity	A0A077ZJZ3	35.03	14	55.125	1 to 18	cell redox homeostasis
Protein disulfide-isomerase	Protein disulfide isomerase activity	A0A077ZLF1	35.95	15	55.73	1 to 16	cell redox homeostasis
Superoxide dismutase [Cu-Zn]	Oxidoreductase	A0A077Z345	69.86	12	15.274	-	unknown
Transcription and Translation							
40S ribosomal protein SA	Structural constituent of ribosome	A0A077YZD4	42.57	13	34.141	-	ribosomal small subunit assembly, translation
Elongation factor 1-alpha	Elongation factor	A0A077YYL7	33.48	12	51.086	-	protein biosynthesis
Mediator of RNA polymerase II transcription subunit 22	Protein disulfide isomerase activity	A0A077Z2H0	69.06	17	15.485	1 to 19	cell redox homeostasis
Ribosomal L18p and L18 c domain containing protein	Structural constituent of ribosome	A0A077ZPB6	42.67	13	35.744	-	translation
Lipid binding and transport							
Vitellogenin N and VWD and DUF1943 domain containing protein	Lipid transporter activity	A0A077ZE83	56.35	205	198.527	1 to 19	unknown
Uncharacterized protein	Lipid binding	A0A077ZMT5	14.14	20	84.314	-	unknown
Others							
DUF290 domain containing protein	Unknown function	A0A077Z8H2	43.67	20	17.876	1 to 19	Unknown
Poly-cysteine and histidine tailed protein isoform 2	Unknown function	A0A077Z5Q5	50.79	109	50.494	-	Unknown
Protein asteroid	Unknown function	A0A077Z2C7	63.64	34	30.674	1 to 23	Unknown
Transthyretin-like protein 46	Unknown function	A0A077Z9N4	42.57	12	16.458	1 to 18	Unknown

Functional annotation	Molecular function*	Acc. No. Wormbase	% Cov.	Peptides (95%)	MW (kDa)	Signal peptide	Biological process*
Uncharacterized protein	Unknown function	A0A077YXT2	16.08	10	69.581	1 to 18	unknown
Uncharacterized protein	Unknown function	A0A077YX18	20.42	10	32.73	1 to 18	unknown
Uncharacterized protein	Unknown function	A0A077Z544	48.83	19	33.553	1 to 23	unknown

* Molecular function and biological process was obtained from the Gene Ontology (GO) database https://www.uniprot.org.

Table 2
Potential identity of the EE proteins targeted by serum IgG based on the MW data of the proteome.

Accession number	Annotation	MW (kDa)	Peptides (95%)
Band 1W (≈ 170 kDa)			
A0A077ZE83	Vitellogenin N and VWD and DUF1943 domain containing protein	198.527	205
A0A077Z8E4	Heat shock protein 70	130.299	21
Band 2W (≈ 37 kDa)			
A0A077Z5Q5	Poly-cysteine and histidine tailed protein isoform 2	50.494	109
A0A077ZHV3	Glyceraldehyde-3-phosphate dehydrogenase	37.536	66
A0A077ZE37	Actin	41.838	35
A0A077YWW9	Actin 5C	41.036	29
A0A077YX57	Enolase	49.513	27
A0A077Z0I4	Epididymal secretory protein E1	45.783	28

Table 3
Identities of proteins within EE excised gel areas with suitable MW and organized by abundance.

Accession number	Annotation	MW (kDa)	Peptides (95%)
Area 1G (≈ 150–200 kDa)			
A0A077ZE83	Vitellogenin N and VWD and DUF1943 domain containing protein	198.527	241
A0A077Z8E4	Heat shock protein 70	130.299	3
Area 2G (≈ 37–45 kDa)			
A0A077Z5Q5	Poly-cysteine and histidine tailed protein isoform 2	50.940	66
A0A077YX57	Enolase	49.513	18
A0A077ZHV3	Glyceraldehyde-3-phosphate dehydrogenase	37.536	15
A0A077ZE37	Actin	41.838	14
A0A077Z3K7	Phosphoglycerate kinase	44.724	12
A0A077Z8 × 2	Dolichyl- diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit	48.787	12
A0A077ZF21	Adenosylhomocysteinase	47.827	10
A0A077Z3H9	Tubulointerstitial nephritis antigen	50.458	9
A0A077YXR0	Calponin domain containing protein	40.694	7
A0A077ZJA3	3 ketoacyl coenzyme A thiolase	43.402	6
A0A077YYL7	Elongation factor 1-alpha	51.086	6
A0A077Z1Z4	Serpin domain containing protein	43.328	6

Table 4
Identities of proteins within FE excised gel areas with suitable MW and organized by abundance.

Accession number	Annotation	MW (kDa)	Peptides (95%)
Area 3G (≈ 60–70 kDa)			
A0A077ZIM1	Tropomyosin	87.298	19
A0A077ZIM7	Papilin	80.804	6
A0A077ZEY0	Calsequestrin	49.211	5
A0A077YX57	Enolase	40.513	5
Area 4.1G (≈ 37–45 kDa)			
A0A077Z5Q5	Poly-cysteine and histidine tailed protein isoform 2	50.494	65
A0A077ZE37	Actin	41.838	7
A0A077ZHV3	Glyceraldehyde-3-phosphate dehydrogenase	37.536	5
A0A077ZEY0	Calsequestrin	49.211	5
Area 4.2G (≈ 33–37 kDa)			
A0A077Z0I4	Epididymal secretory protein E1	45.783	8
A0A077Z0N1	Actin-depolymerizing factor 2, isoform c	35.411	4
A0A077Z5Q5	Poly-cysteine and histidine tailed protein isoform 2	50.494	4

Figures

Figure 1

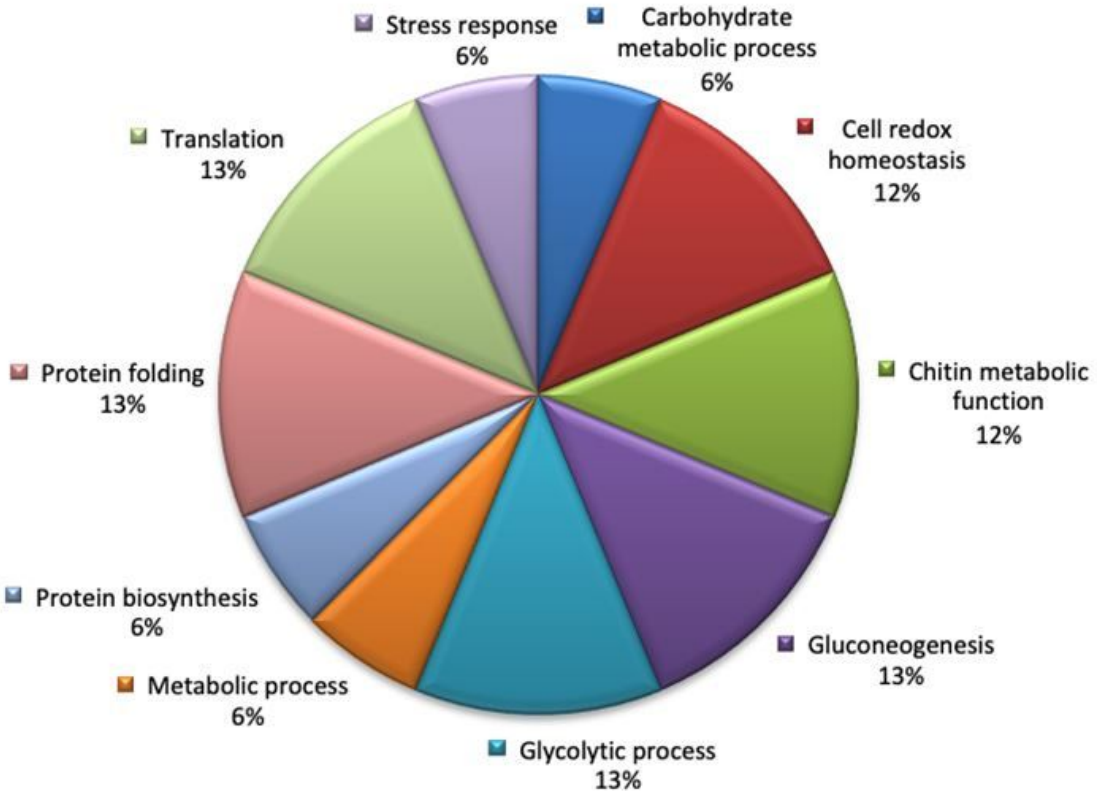

Figure 1

Biological process of the identified proteins in the egg extract (EE) of T. trichiura according to information obtained from the Gene Ontology (GO) database https://www.uniprot.org.

Figure 2

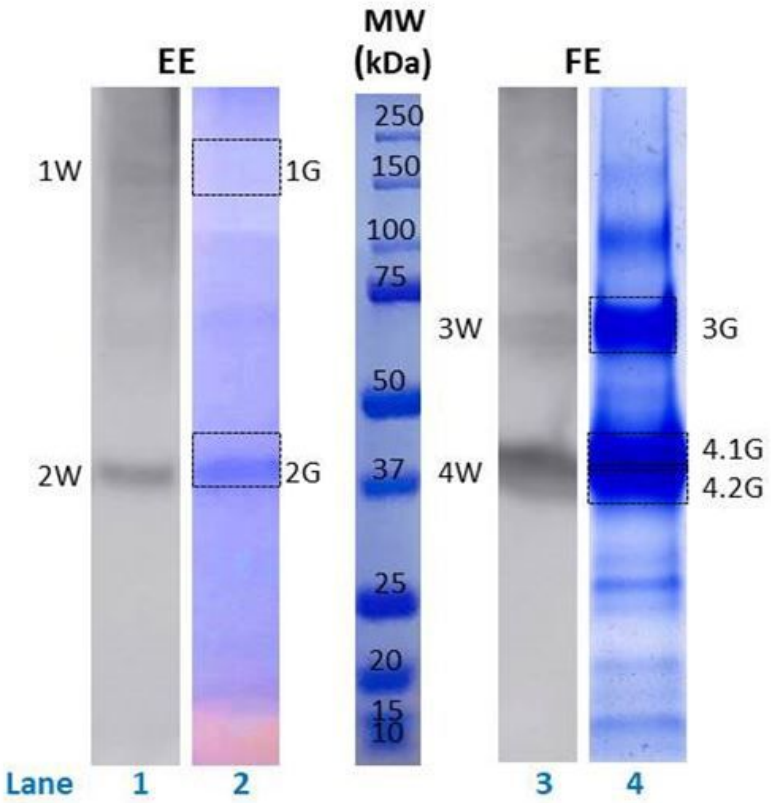

Figure 2

Major immunogenic proteins detected in T. trichiura extracts. Western blot showing AGM serum IgG response to T. trichiura egg extract (EE) (lane 1) and female extract (FE) (lane 3) (10 µg/lane). Bands 1W-2W and 3W-4W indicate the regions containing antigens recognized with more intensity by sera antibodies in EE and FE, respectively. Corresponding SDS-PAGE of EE (lane 2) and FE (lane 4), stained with Coomassie Brilliant Blue R-250 and excised areas of each, 1G-2G and 3G-4.1G-4.2G, containing the most immunogenic peptides for proteomic analysis. Molecular weight in kDa is lane labeled as MW.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx