Human toxocariasis, which has a worldwide distribution, is caused by infection with the larvae of the dog ascarid worm *Toxocara canis*. Puppies infected with adult *T. canis* release in the feces large numbers of infectious eggs into the environment. Eggs accidentally ingested by a patient, yield larvae that penetrate the intestinal wall and are carried by the circulation to various organs (Glickman and Schantz, 1981). While the larvae do not undergo any further development in the patient, the larvae can cause local immunological reactions which are the basis of toxocariasis. During their somatic migration through the organs, the larvae shed huge amounts of immunogenic glycoproteins known as *Toxocara* excretory-secretory (TES) antigens. The shedding of prodigious quantities of TES antigens is believed to be a strategy of the larvae to escape the immune attack of the host (Gems and Maizels, 1996).

Most human infections with *T. canis* larvae are asymptomatic. In instances of heavy infection, clinical presentations may be seen. The two main clinical presentations are visceral larva migrans (VLM) and ocular larva migrans (OLM) (Shields, 1984; Schantz, 1989). Diagnosis by identification of the larvae in the tissues is rarely done. Antibody detection is the more common means of confirmation of toxocariasis. The most common serologic test is enzyme-linked immunoassay (ELISA), which uses larval stage antigens extracted from embryonated eggs or TES antigens released by *in vitro* cultured larvae (de Savigny *et al.*, 1979; Jaquier *et al.*, 1991). Assays using such antigens, have drawbacks. First, the assays may yield false positive results because of cross-reactivity with other parasitic nematodes. Second, *in vitro* culture of *T. canis* larvae and harvest of TES antigens are laborious and time consuming. Hence, there have been attempts to produce specific recombinant antigens for use in toxocariasis seroassays (Yamasaki *et al.*, 1998; 2000).

TES-120 glycoproteins of *T. canis* larvae are closely related mucins which form the major constituents of the larval surface coat and ES antigens. Four of the genes encoding these glycopro-
proteins, designated Tc-muc-1 to -4, have been cloned and characterized (Loukas et al., 2000). Among the genes, Tc-muc-1 has been shown to be abundantly expressed and its encoded product is highly antigenic (Gem and Maizels, 1996; Tetteh et al., 1999). We postulated that Tc-muc-1 would be a suitable candidate as a highly specific recombinant antigen for use in toxocariasis seroassays. In our study, we expressed the mature TES-120 peptide in the bacterium Escherichia coli, and evaluated the recombinant TES-120 antigen in immunoblot assays.

Molecular cloning and expression of the Tc-muc-1 gene were carried out using conventional molecular biology approach. First, an in vitro culture of T. canis stage 2 larva (L2) was established using the method described by Maizels et al. (1984). Total RNA was extracted from 200,000 larvae using TRI Reagent (Molecular Research Center, Inc, USA). Reverse-transcription of RNA into cDNA was primed using oligo(dT)_{24} primer, and proceeded with Superscript™ II reverse transcriptase (GibcoBRL®, Life Technologies Inc, USA). PCR amplification on the resulting cDNA was carried out using a primer pair (TES-120F 5’-AGCAGCCGCGGTATTT-3’, and TES-120R 5’-AATCTCTAGTCGGCATT-3’), designed according to the sequence published by Gems and Maizels (1996). The TES-120 PCR fragment of 528 base pairs (Fig 1) was ligated into the intermediate plasmid vector pCR®2.1-TOPO (Invitrogen Corp, USA) and transformed into competent E. coli TOP10 cells. The TES-120 fragment in the recombinant plasmid was then excised with EcoRI and spliced ‘in-frame’ into the EcoRI cloning site of plasmid expression vector.
pTrcHis2C (Invitrogen Corp, USA). Positive recombinant clones harboring the TES-120 fragment were verified via nucleotide sequencing (data not shown).

The expression of the TES-120 antigen in E. coli was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) 3 hours after initiation of culture. Protein was harvested at hourly intervals from pelleted E. coli cells, and analyzed in denaturing SDS-polyacrylamide (SDS-PAGE) gels (Laemmli, 1970) stained with Coomassie blue. Gel analysis showed the expression of recombinant TES-120 antigen, with the expected size of 20.1 kDa (17.6 kDa of TES-120 polypeptide plus 2.5 kDa of tag sequence of the pTrcHis2C vector), 2 hours after induction (Fig 2). This time interval was used as guide when growth and expression was done in a larger volume of culture. The recombinant TES-120 antigen extracted from a large volume culture was purified using the Xpress System™ (Invitrogen Corp, USA). The purification system involved the use of a denaturing agent (urea), and a dialysis step was carried out to remove these agents. SDS-PAGE analysis on the dialyzed recombinant TES-120 antigen showed a major single band (Fig 3). Purified antigen was electroblotted to nitrocellulose membranes and tested with serum samples of patients suffering helminthic and protozoal infections.

Eight of the serum samples were from patients who were clinically and serologically (using a commercial immunoassay kit) confirmed as having toxocariasis. Twenty-two samples were from individuals who were serologically positive for parasitic infections such as cysticercosis (5 samples), filariasis (5), malaria (2), amebiasis (5) and toxoplasmosis (5). In addition, sera from individuals with soil transmitted helminthiasis (STH) were also tested. Five serum samples were from individuals with Ascaris lumbricoides and Trichuris trichiura co-infection, 3 with T. trichiura infection, and 1 each from cases of A. lumbricoides and hookworm infection. In these STH cases, the individuals were confirmed positive by the finding of helminth eggs in their feces. As a control, 5 sera from normal healthy individuals were included in the immunoblot assays. Results from the immunoblot assays showed
that the recombinant TES-120 antigen was detected in all the toxocariasis patients’ sera but none in the normal or non-Toxocara parasitic infection sera (a sample blot is presented in Fig 4). The results indicate that the recombinant antigen was specific for anti-Toxocara antibody. In addition, the recombinant TES-120 antigen did not cross-react with antibodies from patients with other helminthic infections.

Most of the commercial toxocariasis seroassay kits use total TES antigens derived from in vitro larva culture. These antigens are heterogeneous in their composition, and this may increase the risk of cross-reaction with non-Toxocara helminth antibodies. Using a single or homogenous species of T. canis larval antigen, such as those produced by recombinant DNA technology, should be more reliable and specific. One of the main explanations for cross-reactivity among helminth protein antigens is the occurrence of common carbohydrate (glycosyl) moieties in their peptides. Prokaryotic expression systems such as E. coli produce non-glycosylated proteins, therefore, the use of such proteins in seroassays would reduce the possibility of cross-reactivity. Another advantage of utilizing E. coli to produce recombinant antigens is that the technology for large scale culture or fermentation of this bacterium is well established. Furthermore, it is simpler and less expensive to grow E. coli than any other organism in vitro, because E. coli is not a fastidious organism and can be grown in simple and inexpensive growth media.

The findings of our study have laid the foundation for our further endeavor in producing a highly specific recombinant antigen that can be used for the development of an inexpensive seroassay kit for human toxocariasis.

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