Serodiagnosis of Human Toxocariasis Using Adult Antigens of Toxocara canis

N. A. Hassanain*, M. S. Mahmoud
Veterinary Research Division, National Research Center, Post Box 12622, El-Tahrir Street, Dokki, Giza, Egypt
*Corresponding author *E-mail: nnawahlah@yahoo.com

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Introduction
Toxocara canis has a worldwide distribution and is regarded as the main cause of human toxocariasis (1, 2). Infection in humans, particularly children, is frequently caused by accidental ingestion of embryonated Toxocara eggs present in soil, water, food, dirty hands, and vegetables or by ingestion of larvae in under-cooked giblets (3). The disease can be asymptomatic or can lead to death. The presentations of visceral larva migrans include fever, abdominal pain, malaise, weight loss, skin rash, hepatomegaly, hypergammaglobulinemia and respiratory symptoms/signs with eosinophilia (4). Toxocariasis has also been proposed as a potential etiology in neurologic disorders when the larvae migrate to the central nervous system (5, 6). In 1975, Savigny (7) described a technique for in vitro maintenance of T canis larvae with concomitant production of excretory/secretory (ES) or exo-antigen (TEX). TEX was used in ELISA for testing patients with visceral toxocariasis. This assay showed a high degree of sensitivity and specificity (8, 9). Experiences during the past few years have shown several shortcomings in the use of adult somatic and adult excretory-secretory antigens of T. canis for diagnosis of toxocariasis. Therefore, the aim of the present study was to evaluate the efficacy of purified adult somatic and adult excretory – secretory antigens for diagnosis of human toxocariasis using ELISA and enzyme linked immunotransfer blot (EITB).

Materials and Methods
1. Human sera: A total of 83 serum samples were tested, comprising 57 from patients with clinical (visceral toxocariasis), hematological and serological evidence of toxocariasis (T. canis L2 larval ES antigen ELISA (TES-ELISA) and 26 non-Toxocara infected serum samples on TES-ELISA used as negative controls. 2. Parasitological studies: T. canis adults were collected from the small intestine of naturally infected stray-dogs; they were identified as previously (10).

3. Preparation of antigens
3.1. Adult somatic antigen: Whole worm extracts from adult T. canis flukes (TeSA) were prepared (11) and protein concentration was measured (12). TeSA was purified by gel filtration and produced three peaks P-F1, P-F2 and P-F3.

3.2. Adult Excretory –secretory antigen: E/S Products were isolated from the live adult worms and processed (13) and designated as TcESA and its protein concentration was measured (12).

4. Preparation of rabbit anti-sera against T. canis somatic antigen: Two white New-Zealand rabbits were immunized subcutaneously (0.05 mg protein antigen/rabbit) with crude extract of TeSA (14). The serum was designated RaTeSA.

5. Analysis of antigens and antibodies
5.1. Enzyme linked immunosorbent assay (ELISA): ELISA was carried out (5). The positive threshold value was determined to be two-fold the mean cut-off value of negative sera.

5.2. Sodium dodecyle sulfate polyacrylamide gel electrophoresis (SDS-PAGE): The gel cast comprised 12% resolving and 4% stacking gels with applied 10 μg/well of the different antigens; TeSA, P-F1, P-F2, P-F3 and TcESA. Mini-protein II Dual slab cell (Bio-Rad Labs, Richmond, CA) was used to conduct electrophoresis using discontinuous system of Laemmli (16).

5.3. Enzyme linked immunotransfer blot (EITB): The fractionated T. canis antigens (TeSA, P-F1, P-F2, P-F3 and TcESA) were electrically transferred onto nitrocellulose membrane (17). Horse radish peroxidase anti-rabbit IgG and anti-human IgG (Sigma Co.) diluted at 1:1000 in PBS-T were added to T. canis antigens NC strips.

Results and Discussion
ELISA results showed that all the toxocariasis patients (n=57) had antibodies against all the used (100% sensitivity) T. canis antigens (TeSA, P-F1, P-F2, P-F3 and TcESA). On the other hand, TeESA, TeSA, P-F1, P-F2 and P-F3 gave specificity level of 92.30, 76.92, 76.92, 76.92 and 80.76%, respectively. Cut off O.D value was 0.3 for all the T. canis antigens. Electrophoretic analysis of TeSA, P-F1, P-F2, P-F3 and TcESA revealed approximately 8 to 13 bands ranging from 250 – 14.05 KDa (Fig. 1). The immunoblotting profile of TeSA, P-F1, P-F2, P-F3 and TcESA reacted with Ra TeSA and positive human sera detected by ELISA showed approximately 10 to 12 and 7 to 10 bands ranging from 117 – 19.29 and 113.50 = 19.9 KDa, respectively (Fig. 2, 3).
Early detection of toxocariasis and the assessment of its public health significance rely heavily upon serology (18, 19).

ELISA results recorded that all the used *T. canis* antigens demonstrated 100% sensitivity. TcESA demonstrated the highest degree of specificity (92.30%). So, we can suggest that TcESA is the most sensitive and specific adult *T. canis* antigen for serodiagnosis of human toxocariasis using ELISA. Abdel Aal et al. (20) used crude adult worm antigen of *T. canis* and its purified fractions (P-F1, P-F2, P-F3, P-F4 and P-F5) in the serodiagnosis of human toxocariasis using ELISA and reported that P-F1 is the antigen of choice for diagnosis of human toxocariasis.

Electrophoretic analysis of TcSA and TcESA revealed 13 and 8 polypeptide bands at 250, 125 117, 90.43, 69.25, 56.76, 42.50, 40.69, 38, 35.70, 27.91, 21.98, 19.29 and 127, 94, 88.89, 50, 40.69, 33.77, 30, 14.05 KDa, respectively. Aida (21) reported that the SDS profiles of TcSA and adult TcES consist of 7 (125.37, 117.73, 90.00, 69.25, 58.36, 47.13 and 46.53 KDa) and 8 (127.66, 94.30, 64, 58.81, 50.01, 46.61, 40.09 and 19.26 KDa) protein bands, respectively. Aida (21) identified the immunoblot profile of TcSA reacted with rabbit hyperimmune serum raised against the parasite to consist of 4 prominent bands with 2 common reactive bands with our result at 117 and 90 KDa. The immunoblotting profile of TcESA with Ra TcSA showed 3 common reactive bands (22). Morales et al. (23) suggested that the 92 and 35 KDa polypeptides of *T. canis* second stage larvae E/S antigen are specific to *Toxocara* infection. That the 57 KDa fraction of the larval *T. canis* E/S antigen (TcES-57) is specific to *T. canis* infection and does not cross react with sera of other related infections (24).

In the present study, the immunoblotting profile of TcESA reacted with positive human and Ra TcSA sera showed 5 common immunoreactive bands at 47.46, 40.69, 30.53, 21.9 & 19.29 KDa. So, we may suggest that 58.739 and 29.19 KDa polypeptides of the adult *T. canis* E/S is specific for *Toxocara* infection in human and these antigens merit further evaluation as candidates for use in the diagnosis of human toxocariasis.
References