# Assessment of Crude Antigens Extracted from *Toxocara Canis* Adult Worms for Sero-Diagnosis of Toxocariasis in Animals and Patients

T.T.T. Thai, D.T. Vo, D.H. Duong, and H.H. Nguyen

**Abstract**

Toxocariasis is zoonosis disease caused by infection with *Toxocara canis* and/or *Toxocara cati* that parasitise Canidae and Felidae respectively. In paratenic hosts *Toxocara* larvae are limited in the second stage and remain in tissues. Diagnosis of toxocariasis is therefore mainly depended on detection of specific antibodies in the circulation. Different *T. canis* derived antigens, including excretory/secretory antigens produced by larvae, crude antigens extracted from larvae and crude antigens extracted from adult worms, have been applied in sero-diagnosis. The two formers have been intensively studied but the last is not fully understood. In this study, crude antigens extracted from adult worms and their organs were explored for sero-diagnosis of toxocariasis by a western blot assay. Our data showed that adult worm's crude antigens made several pseudo-positive reactions with IgG antibodies in uninfected mice and it is unable to discriminate infected from *T. canis* uninfected mice. Analysis of isolated reproductive compartments showed that vas deferens and seminal vesicles of male reproductive system contain three low molecular weight proteins which strongly contribute to the formation of pseudo-positive reactions. These observations were also found in rabbits and human. We conclude that crude antigens extracted from adult worms are likely unable to be used in sero-diagnosis of toxocariasis in both animals and human.

**Keywords**

*Toxocara canis* • Sero-diagnosis • Paratenic host • Adult worm • Crude antigens

## 1 Introduction

Toxocariasis is a zoonosis disease caused by infection with *Toxocara canis* and *Toxocara cati* whose definitive hosts belong to the Canidae and Felidae respectively. Human and many other animals is defined as paratenic host where the development cycle of the nematodes is limited to the second larval stage which remains in the tissues for up to years [1] causing different syndromes: visceral larva migrans (VLM), ocular larva migrans (OLM), neural larva migrans (NLM) or covert or common toxocariasis (CT).

Toxocariasis is considered as a neglected disease and the number of afflicted people in worldwide may be currently seriously underestimated. In Brazil 38.8% of school children were infected [2]. In Iran the infection level was 15.8% [3]. In the USA 13.9% of people >6 years old age were infected [4]. In Vietnam infection rate was around 30% in patients who visited hospitals [5].

Because of the restriction in the development cycle and the uncontrolled migration of larvae into different tissues in the paratenic hosts, symptoms of infection are unspecific and this makes the diagnosis of toxocariasis difficult. Immunological approaches, therefore, have been developed to detect

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specific antibodies binding to toxocariasis antigens and different sources of *T. canis* antigens have been studied. Two of those are the excreted/secreted (E/S) antigens and the crude antigens derived from *Toxocara canis* larvae which have been intensively studied and showed >92% sensitivity and >86% specificity [6, 7]. The other comes from the crude antigens extracted from adult worms and has been less understood [8, 9]. By using SDS-PAGE, these crude antigens were analyzed previously [8]. However, whether these antigens can play a role in sero-diagnosis of toxocariasis in largely unknown. By using a western blot assay, this study therefore was aimed to explore this issue.

## 2 Materials and Methods

### 2.1 Animals and Toxocariasis Animal Models

Swiss outbred mice (albino) (20–25 g in weight) and rabbits (2 kg in weight) were purchased from the Pasteur Institute in Ho Chi Minh City, Vietnam and then kept in our laboratory during experiments. Pups (2–3 months old) were purchased in Ninh Thuan Province, Vietnam.

Mice and rabbits were infected with 1000 larvae of *T. canis* (prepared previously in our lab) by intraperitoneal injection [10]. Four weeks later, sera from these animals was collected and kept at −20 °C. Sera obtained from untreated animals were used as negative control. Toxocariasis animals were confirmed by using a dot blot assay with E/S antigens produced by *T. canis* larvae (data not shown).

### 2.2 Patient Sera

Toxocariasis human sera were a gift from Prof. T.K.D. Tran (Medicine and Pharmacy University Ho Chi Minh City, Vietnam). Cord blood sera from volunteer women were used as a negative control. These sera samples were confirmed by using a dot blot assay with E/S antigens produced by *T. canis* larvae in vitro (data not shown).

### 2.3 Preparation of Crude Proteins from *T. canis* Adult Worms and Their Organs

Pup feces were sampled to test for the presence of *Toxocara* spp. eggs by phase contrast microscopy. Infected animals were anesthetized with ketamine (100 mg/kg of body weight). Adult worms found in the small intestine were collected in pre-warmed PBS buffer and then washed several times in 1% Formalin-PBS buffer. The somatic compartments (esophagus, intestine, body wall including muscle layer, hypodermis, nerve cords and cuticle), reproductive compartments from male worms (testis, vas deferens, seminal vesicles) and from female worms (ovary, oviduct and uterus) were isolated [8]. All tissue samples were then used for extraction of crude protein antigens by using a procedure showed in a previous work [11]. Protein concentration was analyzed by Bradford assay.

### 2.4 SDS-PAGE and Western Blotting

Extracted protein antigens (5 μg/well) were electrophoresed by SDS-PAGE under reducing condition on a 15% gel. PageRuler broad range unstained protein ladder (Pierce Biotechnology) was used as molecular weight standards. For protein detection, coomassie blue staining was used. Protein molecular weight was estimated by using Quantity One software v4.6.3 (Bio-Rad Laboratories).

After SDS-PAGE, crude proteins were transferred to a nitrocellulose membrane (0.45 μm of pore size) using a semi-dry blotter system (TE70XP Semi-Dry Transfer Unit, Hoefer Inc). Blotting times of 90 min were applied. The membrane was then blocked with 0.1% casein in TBST buffer (20 mM Tris, 150 mM NaCl, 0.1% Tween 20, and pH 7.5) for 1 h and washed three times in TBST buffer for 10 min. The membrane was incubated for 1 h with *T. canis* infected mouse or rabbit or human sera diluted 1:1000 in TBST and then washed with TBST buffer. The membrane was incubated with a 1:5000 diluted goat anti-mouse or anti-rabbit or anti-human IgG antibodies conjugated with HRP (Santa Cruz Biotechnology) for 1 h and then washed with TBST buffer. For detection of antigen-antibody immune complexes, the membrane was incubated in enhanced chemiluminescence reagent for 5 min and then scanned on C-digit blot scanner (LI-COR Biosciences) to obtain images which were analyzed by ImageStudioLite programme.

In some experiments, western blot membrane strips were prepared as described elsewhere [12] but with modifications. Briefly, for the SDS-PAGE a comb with wide (60 mm width) teeth was used and 150 μg of crude proteins was loaded (2.5 μg/mm). After electrophoresis, proteins in the gel were transferred onto a nitrocellulose membrane which was then blocked with blocking solution, air-dried, cut into strips of 3 mm width and kept at 4 °C until use.

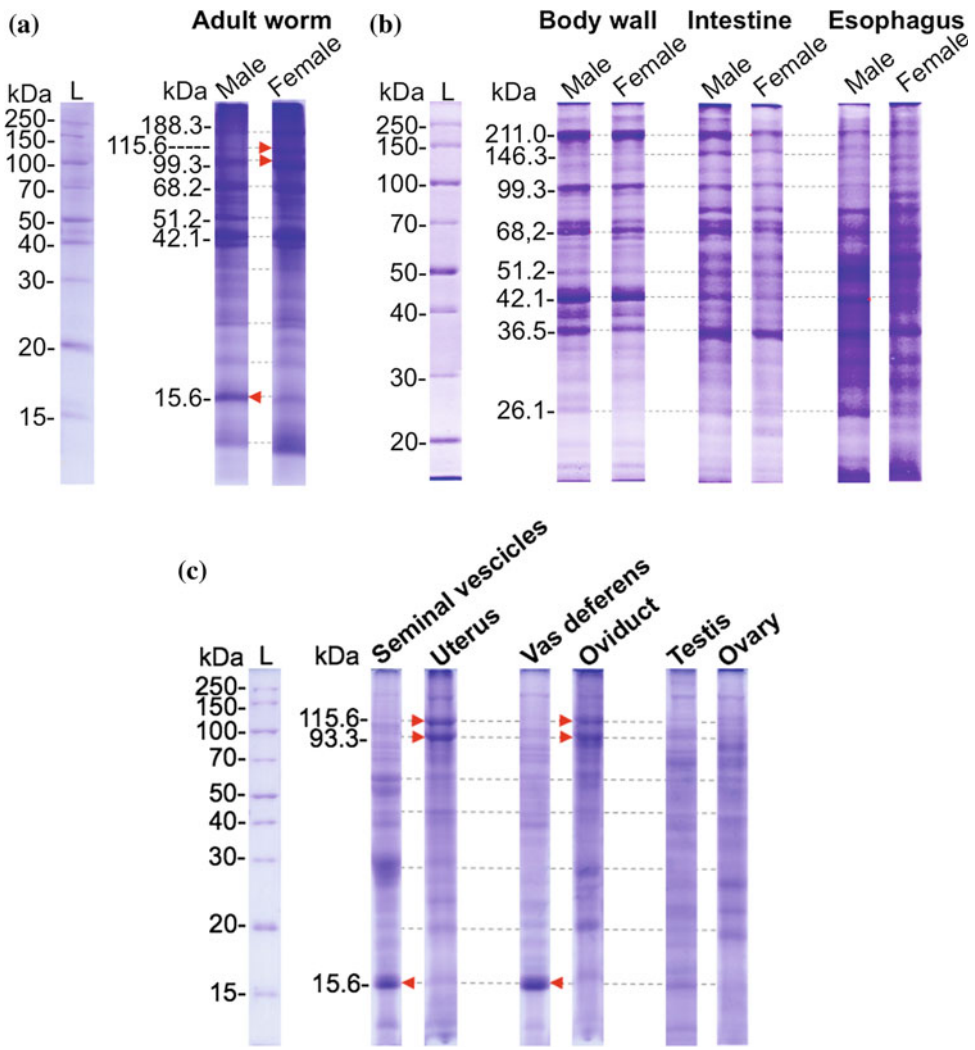


**Fig. 1** Analysis of protein antigens extracted from *T. canis* adult worms and their organs by SDS-PAGE. **a** Antigens from adult worms. **b** Antigens from somatic organs. **c** Antigens from reproductive organs. *Red arrows* show dominant bands found in male or female compartments (color in online)

## 3 Results

### 3.1 Analysis of Protein Antigens Extracted from *T. Canis* Adult Worms and Their Compartments

To understand protein constituent profiles from the adult worms and their organs, SDS-PAGE analysis was applied. Our results showed that protein band patterns are similar between male and female adult worms except that 15.6 kDa band is dominantly expressed in the male while 99.3 and 115.6 kDa bands is apparently up-regulated in the female (Fig. 1a). This observation indicates that these proteins may associate with the reproductive organs of the adult worms. To address this issue, different organs, that were isolated from male and female worms, were analyzed by SDS-PAGE. Analysis of somatic organs (cuticle, intestine and esophagus) showed that two genders express similar protein constituent profiles (Fig. 1b). Medium to high molecular weight (MW) proteins were dominantly found in the cuticle and the intestine but not in the esophagus which gives dominantly medium MW proteins. In contrast to somatic organs, reproductive organs gave distinct protein band profiles in which 15.6 kDa is highly expressed in the vas deferens (TcVD) and the seminal vesicles (TcSV), while 99.3 and 115.6 kDa bands are highly expressed in the oviduct (TcOvi) and uterus (TcUt). However, these three bands are less different between the testis and the ovary.

### 3.2 Adult Worm Extracted Crude Antigens Do Not Discriminate Normal Mice from *T. Canis* Infected Mice

A model of toxocariasis was established by injection of 1000 *T. canis* larvae into the peritoneum of the mice. 4 week later,

mouse sera were collected. For assessment of the role of adult worm's crude antigens in sero-diagnosis of toxocariasis in mice, a western blot assay was applied. We unexpectedly found that both normal and infected sera gave similar WB band patterns to crude antigens extracted from male and female worms (Fig. 2) indicating that these patterns might be results from unspecific binding of IgG antibodies. In case of applying male worm's antigens, three intensive and shaped bands (15.6, 17.3 and 18.3 kDa) were found indicating for their remarkable contribution to the unspecific binding of IgG antibodies.

### 3.3 Antigens Extracted from the Male Reproductive Compartments Strongly Contribute to Unspecific Recognition of IgG Antibodies in Normal Mice

Data showed in Fig. 2 prompted us to ask whether reproductive organ antigens play a role in unspecific recognition of IgG in mice. To address this hypothesis, crude antigens extracted from TcVD, TcSV, TcOv and TvUt were tested by western blot assay (Fig. 3). As expected, only crude antigens extracted from TcVD and TcSV gave 15.6, 17.3 and 18.3 kDa unspecific bands which are also the strongest bands found in this situation (Fig. 3a). In contrast, crude antigens extracted from TcOv and TvUt mostly gave high MW and faint unspecific bands (Fig. 3b). There was no difference between normal and *T. canis* larvae infected mice. These observations were also apparently documented in rabbits (data not shown).

### 3.4 Antigens Extracted from the Male Reproductive Compartments Unspecifically React to IgG Antibodies in the Cord Blood Sera

Data of unspecific binding of IgG antibodies to the adult worm's crude antigens showed in animals prompted us to test whether it also happens in human. By using western blot, we tested for the reaction of crude antigens extracted from reproductive organs of the adult worms to the sera collected from cord blood as negative control and toxocariasis patients. Our data showed that cord blood sera and patient sera gave comparable WB band patterns (Fig. 4) indicating for unspecific binding of IgG antibodies. Proteins extracted from TcVD and TcSV gave 15.6, 17.3 and 18.3 kDa bands (Fig. 4a) which were observed in animals (Fig. 3). These bands were not found with antigens extracted

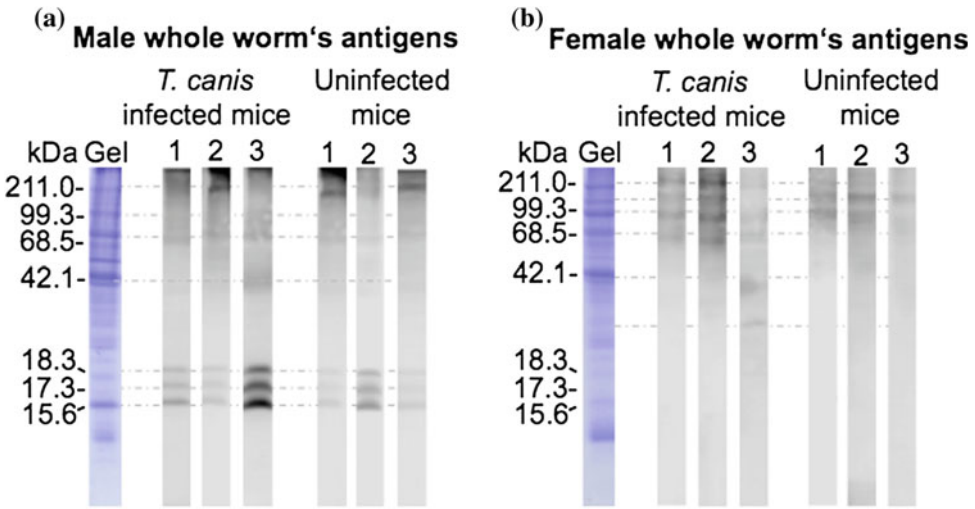


**Fig. 2** Unspecific binding of mouse IgG antibodies to crude antigens extracted from *T. canis* adult worms. **a** Antigens extracted from whole male worms. **b** Antigens extracted from whole female worms

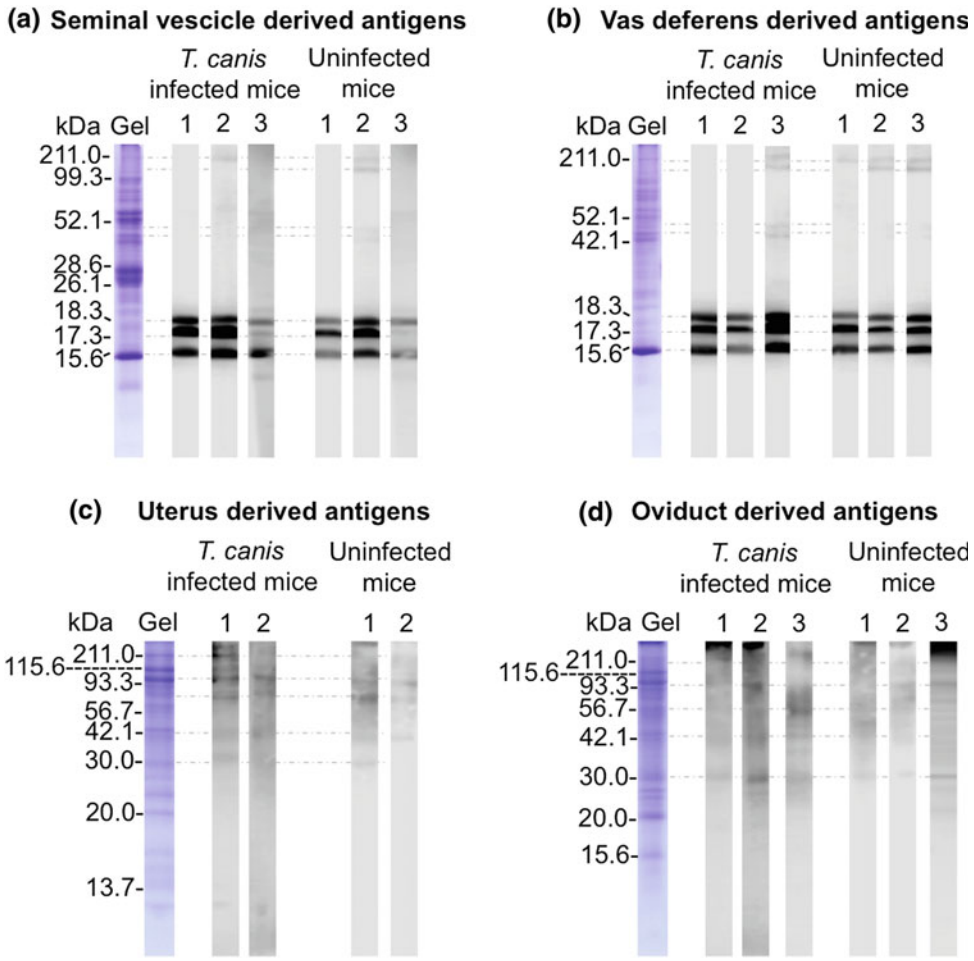


**Fig. 3** Unspecific binding of mouse IgG antibodies to crude antigens extracted from reproductive organs of *T. canis* adult worms. **a** Antigens extracted from TvSV. **b** Antigens extracted from TcVD. **c** Antigens extracted from TcUt. **d** Antigens extracted from TcOv

from TcOv and TvUt which, in contrast, gave mainly high MW and faint unspecific bands (Fig. 4b).

## 4 Discussion

Characterization of protein profiles of *T. canis* worms and their organs may prove useful in diagnosis of infection, the development of vaccines, and in studies on the biology of nematodes [8]. In this study, by a protein approach of SDS-PAGE analysis, we showed that protein profiles is largely similar between two genders in the somatic organs but not in the reproductive systems in which 15.6 kDa is highly expressed in TcSV and TcVD while 99.3 and 115.6 kDa are highly up-regulated in TcUt and TcOv. These proteins may be representative for reproductive systems in *T. canis* nematode.

Finding a biomarker derived from adult worms of *T. canis* for sero-diagnosis of toxocariasis in human has been previously expected [8, 9]. Along with this expectation, our western blot results showed that the binding of IgG antibodies from different paratenic hosts to the adult worm's crude antigens is clearly unspecific. Moreover, we showed that crude antigens extracted from TcSV and TcVD contain three proteins (15.6, 17.3 and 18.3 kDa) which largely contribute to the generation of unspecific interaction. In contrast TcOv and TcUt mainly produce unspecific binding with high MW proteins.

This study demonstrates for the first time the recognizable involvement of 15.6 kDa, 17.3 kDa and 18.3 kDa proteins originated from the male worm's TcSV and TcVD in unspecific binding with IgG antibodies in paratenic animals and human. However, identification of these proteins and a mechanism in which they participate to make this unspecific binding require further investigation.

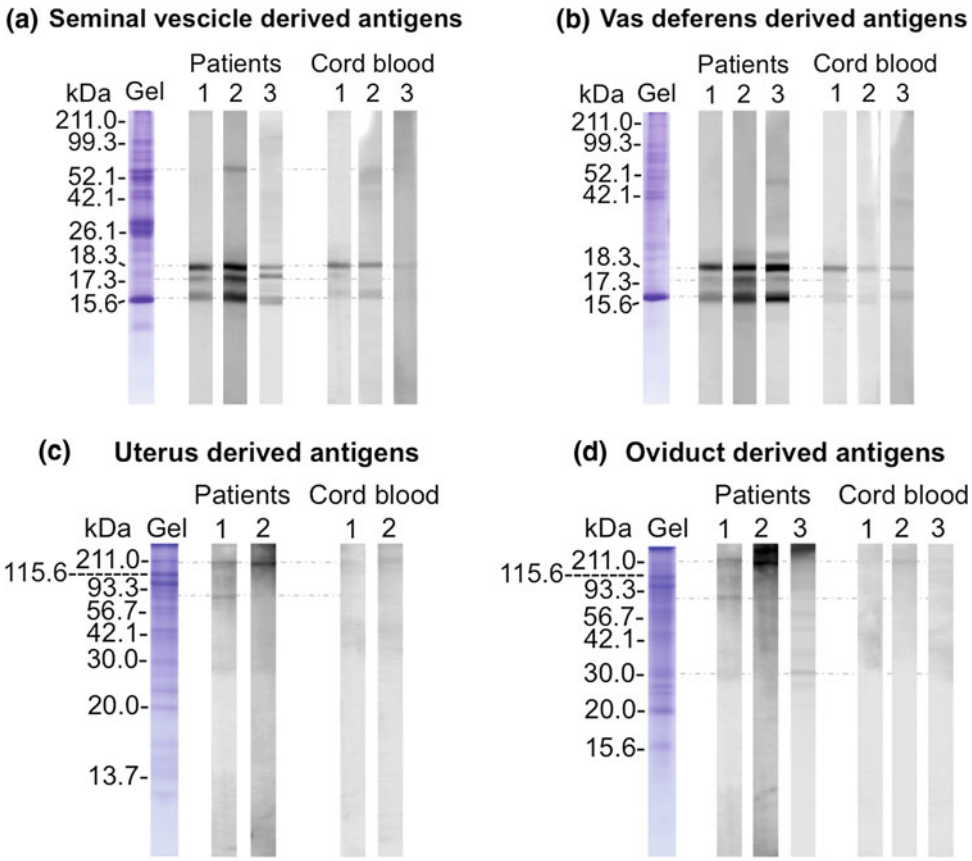


**Fig. 4** Unspecific binding of human IgG antibodies to crude protein extracted from reproductive organs of *T. canis* adult worms. **a** Antigens extracted from TcSV. **b** Antigens extracted from TcVD. **c** Antigens extracted from TcUt. **d** Antigens extracted from TcOv

## 5 Conclusions

We provide in this study the evidences which strongly demonstrate that crude antigens extracted from both *T. canis* adult whole worms and their compartments is likely unable to be used in sero-diagnosis of toxocariasis in both paratenic animals and human.

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