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Immunodiagnosis of Human Toxocariasis and Prospects for Improved Diagnostics

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Abstract The clinical spectrum of human disease caused by *Toxocara canis* and *Toxocara cati* ranges from visceral and ocular larva migrans to covert and common toxocariasis. Since the parasite is not typically recovered in affected tissues, detection of parasite-specific antibodies is an important and necessary step in establishing a diagnosis of toxocariasis. Most immunodiagnostic methods have historically used and continue to use the *Toxocara* excretory-secretory antigens (TES-Ag) in an ELISA format to detect *Toxocara*-specific antibodies. The TES-Ag ELISA has proven to be specific, robust, and reliable, although questions about specificity and reduced sensitivity leave ample room for improvement in laboratory diagnosis of toxocariasis. Recent advances have focused on development of recombinant protein targets to measure *Toxocara*-specific antibodies. The potential for development of new diagnostics using recombinant proteins is presented herein, as well as possible roles for antigen and molecular detection methods.

Keywords *Toxocara canis* · *Toxocara cati* · Toxocariasis · Visceral larva migrans · Ocular larva migrans · Immunodiagnosis · Serology · Recombinant proteins · Toxocara · Parasitology · Ag, antigen · BPES, *Baylisascaris procyonis* excretory-secretory · ELISA, enzyme-linked immunosorbent assay · IB, immunoblot · Ig, immunoglobulin · ITS, internal transcribed spacer · kDa, kilodaltons · M_r, relative molecular weight · NT, neurotoxocariasis · OT, ocular toxocariasis · TES, *Toxocara* excretory-secretory · VT, visceral toxocariasis

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Abbreviations

Ag	antigen
BpES	*Baylisascaris procyonis* excretory-secretory
ELISA	enzyme-linked immunosorbent assay
IB	immunoblot
Ig	immunoglobulin
ITS	internal transcribed spacer
kDa	kilodaltons
M_r	relative molecular weight
NT	neurotoxocariasis
OT	ocular toxocariasis
TES	*Toxocara* excretory-secretory
VT	visceral toxocariasis

Introduction

Toxocariasis in humans occurs by ingestion of embryonated *Toxocara canis* or *Toxocara cati* eggs. The hatched larvae make their way to the intestine, and subsequently penetrate the intestinal wall and migrate to the liver, where most of the parasites are sequestered in an arrested state of maturation. From the liver, some parasites may migrate to the lungs, muscles, eyes, or central nervous system. The presence of *Toxocara* larvae in tissues results in a wide spectrum of clinical illness, from visceral larva migrans or visceral toxocariasis (VT) [1], to ocular larva migrans or ocular toxocariasis (OT) [2], to neural larva migrans or neurotoxocariasis (NT) [3]. In addition, covert and common toxocariasis, which are predominantly seen in children and adults, respectively, are recognized as asymptomatic forms of infection [4, 5].

Establishing a diagnosis of toxocariasis in humans is difficult, largely because the clinical syndromes can be vague, and

general laboratory findings may be associated with other parasitic and viral infections and immune system abnormalities. *Toxocara* do not develop to the adult stage in the human host, and therefore eggs are not shed in feces. As such, classical ova and parasite examinations are not useful for diagnosis of human toxocariasis. In most cases of toxocariasis, small numbers of tissue-dwelling larvae are widely distributed throughout the body. A definitive diagnosis may be made in rare instances when *Toxocara* larvae are confirmed by histological examination of biopsies of lesions in affected tissue, but biopsies are typically not performed merely for diagnostic purposes, as the probability of obtaining tissue containing a *Toxocara* larva is low. In some patients with ocular infection, viable larvae may be seen with the use of ophthalmoscopy; however, these cases are not the norm. Because of these limitations in observing and identifying *Toxocara*, diagnosis of toxocariasis is almost always accomplished using immunodiagnostic methods.

Parasitological diagnosis

Although rare, parasitological identification of *Toxocara* is possible, especially if larvae are recovered from ocular fluid or cerebrospinal fluid (CSF). Larvae recovered in these specimens are typically viable and are very motile. The arrested *Toxocara canis* larvae found in humans are approximately 400 μm in length and 18–20 μm in diameter in the mid-region. *Toxocara cati* larvae are slightly thinner, about 16 μm in diameter [2]. *T. canis* has spear-shaped cervical alae, while the alae of *T. cati* are described as arrow-shaped [6–8]. Histological examination of tissues may reveal partial or intact larvae [1].

Immunodiagnosis

Early serology tests utilized extracts prepared from adult *T. canis* worms and contained epitopes that were highly cross-reactive with *Ascaris* and other helminths. Research has shown that adult worm preparations are not useful for immunodiagnosis because most of the larval antigens recognized by infected human hosts are not shared with adult worms [9]. In 1975, de Savigny described an in vitro culture system that supported maintenance of *T. canis* larvae in serum-free media [10]. In vitro cultured larvae are the ideal source of material for immunodiagnosis, as the larvae remain motile and metabolically active and secrete antigens for months, ostensibly mimicking human toxocariasis. A detailed procedure for generating the *Toxocara* excretory-secretory antigens (TES-Ag) is presented in a recent review by Fillaux and Magnaval [11]. The availability of TES-Ag led to improved diagnostics for toxocariasis, and in 1979 the TES-Ag was used to develop an ELISA [12]. The TES-Ag ELISA, with a sensitivity of 78 % and a specificity of 92 % [13], represented a significant improvement in diagnostics for toxocariasis, although cross-reactions with other nematode species have been reported in the years since the original report [14–16]. Overall, assay performance has remained consistent over many decades, with variations depending on the cutoffs used. CDC reports a sensitivity of approximately 75 % in patients with clinically diagnosed VT and a specificity greater than 90 % at titers of ≥1:32 (personal communication) for the TES-Ag ELISA. The presence of cross-reactive antibodies is especially problematic in tropical regions, where infection with multiple helminths is common [17, 18]. Some have proposed absorbing the cross-reacting antibodies using antigen extracts from related nematodes, particularly *Ascaris*, prior to testing in the TES-Ag ELISA as a means to improve specificity [17, 19]. TES-Ag ELISA results are often reported as a titer, although results from commercial kits can be semiquantitative or qualitative (positive or negative). As a rule, serology cannot differentiate current from past infection.

Confirmation of positive TES-Ag ELISA results using the TES-Ag immunoblot (IB) improves specificity [20, 21]. Antibody reactivity to the lower-M_r TES antigens (24, 28, 30, and 35 kDa) is more frequently associated with toxocariasis than reactivity to higher-M_r antigens (50, 81, 132, 147, and 200 kDa). Reactivity to the higher M_r antigens is associated with infections caused by other helminths [21–24].

Detection of Toxocara-specific Ig subclasses and IgG isotypes

The classical TES-Ag ELISA detects *Toxocara*-specific IgG. Detection of specific IgE has been shown to improve the specificity of the TES-Ag ELISA and the TES-Ag IB [25, 26], although others have suggested that specific IgE detection is less sensitive than IgG detection [19, 27]. In France, an IgE TES-Ag ELISA is routinely used for clinical diagnosis [25]. Specific IgM is not useful in diagnosing acute infection, however, as Ig antibodies persist throughout the course of infection [27], likely because *Toxocara*-specific glycans on the surface of the parasite continue to induce parasite-specific IgM [28].

TES-Ag-specific IgG isotypes have been evaluated as possible options for improving the sensitivity and specificity of the TES-Ag ELISA. Research has shown that all four human Ig isotypes (IgG1–4) are produced to the TES-Ag. In one study, specific IgG2 was shown to be the most sensitive and IgG3 was shown to be the most specific and accurate, although the sensitivity of total specific IgG was only 50 % in this study [18]. Others have shown that detection of specific IgG4 can increase specificity, especially if used as a confirmatory test for positive TES-Ag IgG ELISA results [18, 29, 30].

Antibody persistence

Arrested larvae are long-lived, surviving in human tissue for months to years and continuing to release antigens that serve as ongoing stimulus for immune response. The length of time that specific antibodies persist after therapeutic cure or natural resolution infection is unknown [31]. As the risk of infection is lower in adults compared to young children, it is not surprising that studies comparing antibody titers in preschool children to adults showed that antibody levels decreased with age [32], which suggests that antibodies gradually decrease after the parasite dies. One study that monitored antibody levels after anthelminthic treatment showed that specific IgG levels eventually decline but remain detectable for 4–5 years. IgE levels decreased but were still measureable one year following treatment [19, 33••]. Other studies reported that antibodies to the TES-Ag, when measured in the TES-Ag IB, persisted for about 1 year [11]. Evaluations of tests that measure the avidity of specific IgG have shown that they are able to differentiate newer from older infections, although this method has not been adopted to date for clinical diagnostic purposes [27, 34].

Antigen detection

Because the arrested *Toxocara* larvae remain metabolically active and produce substantial amounts of antigen [10], tests to detect circulating *Toxocara* antigens would seem to be the method of choice for differentiating active versus resolved infections. Monoclonal antibodies generated against the TES-Ag have been incorporated into antigen capture assays, with mixed results. Some monoclonal antibodies appear to be species-specific, while others detect both *T. canis* and *T. cati* antigens [35–37, 38••]. While some authors report detection limits as low as 5 ng/mL, most studies show that patients with inactive disease are less likely to have detectable serum concentrations of circulating antigen [36]. Questions also remain as to the specificity of some of the monoclonal antibodies used to date, as up to 25 % of sera from patients with other helminth infections also demonstrate reactivity. It is unclear whether this reactivity is from *Toxocara* antigens due to latent undiagnosed toxocariasis in these patients or from the presence of shared antigens between helminths.

Molecular detection

Molecular methods using serum have not proven useful for the general clinical diagnosis of toxocariasis. However, application of PCR-based tools may be of value in the detection of *Toxocara* DNA in human biopsy specimens. PCR assays that amplify sequences of the internal transcribed spacer (ITS) regions ITS-1 and ITS-2 of ribosomal DNA, which can differentiate *T. canis* and *T. cati*, have been used to identify *Toxocara* in animal tissues, bronchial alveolar lavage, and ocular fluids [39, 40•, 41, 42]. In one study, PCR was used to detect parasite DNA in human OT, and although no positive specimens were detected in this study [43], the application of PCR for diagnosis of OT seems reasonable.

Immunodiagnosis of different forms of toxocariasis

Visceral toxocariasis

Visceral toxocariasis occurs when *Toxocara* larvae migrate to the liver and other organs. The severity of symptoms in human toxocariasis is proportional to the number of larvae ingested [44] as well as trapping of larvae in the liver [45]. It is not surprising, therefore, that VT was originally described in children and associated with children who had a history of geophagia. Characteristic manifestations of VT include fever, cough, wheezing, abdominal pain, and hepatomegaly, and in rare instances, pneumonia, myocarditis, and encephalitis. General laboratory findings include leukocytosis, hypereosinophilia, hypergammaglobulinemia, and elevated serum IgE. Asymptomatic or inapparent infection with *Toxocara*, with or without eosinophilia, was also recognized in children [4]. A visceral larva migrans syndrome was later described in adults, which was characterized by symptoms and signs consistent with allergic response: pruritis, rash, urticaria, respiratory problems, cough, eosinophilia, and elevated IgE levels. Hepatomegaly was observed in these patients as well [5]. Since clinical and laboratory findings may be nonspecific, positive serology results from the TES-Ag ELISA are important in establishing a diagnosis of VT [46].

Not only the severity of disease, but the level of antibody response induced in VT is proportional to the infective dose as well [45]. The utility of the TES-Ag ELISA for diagnosis of VT was originally evaluated using 62 sera from cases with presumed clinical VT, which was defined as the presence of 5 of the 6 following criteria: 1) leukocytosis (white blood cell count >10,000/mm3), 2) eosinophilia (>10 %), 3) elevated anti-blood group A isohemagglutinins, 4) elevated anti-blood group B isohemagglutinins, 5) hypergammaglobulinemia; and 6) hepatomegaly [13]. Using these specimens, the sensitivity and specificity of the TES-Ag ELISA were determined to be 78 % and 92 %, respectively. In the study that originally described covert toxocariasis in children, the concentration of specific antibody was higher in children with eosinophilia [4], confirming studies in animal models showing that symptom severity and antibody were proportional to infective dose [44].

Ocular toxocariasis

The occurrence of ocular invasion is almost always unilateral and results in endophthalmitis or retinal granulomas. Signs of

VT are usually not present, and notably, eosinophilia is typically absent. Diagnosis is primarily clinical; larvae are demonstrated only after histopathologic evaluation of an enucleated eye. As with VT, serological testing is useful for diagnosing OT. However, the TES-Ag ELISA is not as sensitive for diagnosis of OT as it is for VT. In one case–control study that evaluated sera from 17 defined cases of OT using the TES-Ag ELISA, only 65 % of cases were seropositive. Sera collected within the first month of infection were more likely to be positive, although some sera were positive even when collected years after infection [47].

Measurement of intraocular antibodies has been shown to improve the sensitivity of OT diagnosis [48–50]. One study compared intraocular antibody levels to serum levels in 49 patients, (37 adults and 12 children) with undetermined posterior uveitis. Five adults were seropositive, but none produced intraocular antibody. In contrast, one-fourth of the children had detectable intraocular IgG but only one child was seropositive. All had higher antibody levels in the vitreous humor than in serum [50]. In another study, serum and aqueous fluid were tested in 14 patients with suspected OT; 12 serum and 12 aqueous humor specimens were positive [48]. Interestingly, 33 % had peripheral blood eosinophilia and 43 % had elevated serum IgE, suggesting that OT was strongly suspected in this group of patients. Ocular fluids typically do not contain an abundance of nonspecific antibodies, so testing can be performed on undiluted or minimally diluted eye fluids, although this has not improved sensitivity [47, 51]. In the clinical setting, testing for intraocular antibody in aqueous or vitreous humor is recommended over serum antibody testing if OT is suspected, but both specimen types should be submitted. Serology is useful for diagnosing OT if TES-Ag results are positive, but OT cannot be ruled out when results are negative.

Neurotoxocariasis

Neurotoxocariasis (NT) is a rare manifestation of toxocariasis characterized by acute eosinophilic meningitis. Serology is almost always necessary to confirm diagnosis, although *Toxocara* larvae may be recovered in the CSF in rare instances. Detection of antibodies in CSF has been shown to be more sensitive than testing serum in patients with NT [52, 53]. In some instances, particularly if the infective dose is high, symptoms may precede antibody development. If a negative serology result is obtained, an additional specimen should be collected one week later and tested. If ocular larva migrans is also suspected, aqueous and vitreous humor should be examined.

When NT is suspected, serology for baylisascariasis should be performed in parallel with serology for toxocariasis to rule out infection with *Baylisascaris*. Patients infected with *Toxocara* produce antibodies that cross-react with *Baylisascaris procyonis* ES antigens (BpES-Ag) (Table 1). This cross-reactivity is seen in both the BpES-Ag ELISA and BpES-Ag IB tests. In general, serum antibodies from the limited number of patients with *Baylisascaris* infections do not cross-react with TES-Ag [54, 55], although one case of baylisascariasis has been reported with high titers to both *Toxocara* and *Baylisascaris* antigens [56]. A patient with positive serology results in both toxocariasis and baylisascariasis tests is usually presumed to have toxocariasis [57]. A diagnosis of baylisascariasis can be established if a patient's serological testing results are negative for toxocariasis and positive for baylisascariasis. Importantly, in recently developed serological tests for baylisascariasis that utilize recombinant protein antigens, antibodies generated in patients with toxocariasis do not cross-react [58•].

Best practices for diagnosis of toxocariasis

The currently accepted best practice for immunodiagnosis of clinical toxocariasis is to detect *Toxocara*-specific IgG using the TES-Ag ELISA. In Europe, TES-Ag ELISA positive results are confirmed using a TES-Ag IB [21, 25]. Testing in the U.S. is limited to TES-Ag ELISA, and IB is not performed. Commercial TES-Ag ELISA kits available in the U.S. and Europe report improved sensitivities and specificities of approximately 90 % [59, 60]. An IB test that utilizes TES-Ag is manufactured and sold in Europe but cannot be imported into the U.S. for clinical diagnosis. If OT is suspected, serum and vitreous or aqueous humor should both be submitted. Likewise, in NT, both serum and CSF should be tested, and baylisascariasis should be ruled out. A clinically positive result in an asymptomatic patient does not have diagnostic value and should be considered only when all other etiologies have been ruled out [11].

Opportunities for improved immunodiagnosis

Because the TES-Ag ELISA is essentially the sole test used for clinical diagnosis and epidemiological studies, some have suggested that the test is standardized and validated [27]. While it is true that a relatively similar antigen preparation is

Table 1 Reactivity of serum and CSF antibodies produced in NLM syndromes in tests for toxocariasis and baylisascariasis reaction in patients with

Test	Antigen source	*Toxocariasis*	*Baylisascariais*
TES-Ag ELISA	Native parasites	Positive	Negative
BpES-Ag ELISA or BpES-Ag IB	Native parasites	Positive	Positive
Bp rRAG-1 IB	Recombinant protien	Negative	Positive

utilized by virtually all clinical microbiologists and researchers, there is no globally accepted method for establishing a cutoff or even for defining reportable units (titers, ratios, absorbance, or optical density units). Lot-to-lot variation in the TES-Ag preparations is also a recognized drawback. Importantly, the TES-Ag ELISA and IB are dependent on in vitro cultured *Toxocara* larvae to maintain the supply of TES-Ag. In addition to the aforementioned cross-reactivity with other helminths, other limitations include reduced sensitivity (approximately 50 %) for detecting OT, reduced sensitivity for detecting low-level infection, possible reduced sensitivity for detecting *T. cati* infection [61], and lengthy antibody persistence, lasting up to 5 years after treatment. These shortcomings are driving research to identify better diagnostic tools for human toxocariasis.

The development of novel tests utilizing recombinant antigens to detect specific antibodies represents the single greatest opportunity for improved diagnostics of human toxocariasis. Much of the information that can inform the identification of new targets and the development of improved immunodiagnostic tests has come from previous work on the characterization of the TES-Ag. The TES-Ag comprises approximately 10 major proteins based on polyacrylamide gel electrophoresis and approximately 50 proteins when characterized by 2D gel electrophoresis [30]. Histochemical studies and later sequence analysis have suggested that TES-Ag molecules are glycoprotein in nature and are heavily glycosylated, with up to 40 % of the mass attributable to carbohydrate [62]. The TES-Ag IB has helped identify antigens with diagnostic relevance. The lower-molecular-weight TES antigens (24, 28, 30, and 35 kDa) appear to be better candidates for immunodiagnosis than the larger antigens, as the higher-molecular-weight antigens are associated with cross-reactivity [17, 21–24].

The genes for many of the proteins identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) have been cloned and expressed in bacterial or eukaryotic expression systems (reviewed in [63]). The TES-Ag includes mucins, C-type lectins, a phosphatidylethanolamine-binding protein, and a number of enzymatic proteins.

Several of the recombinant forms of the TES-Ag have been analyzed for diagnostic utility (Table 2) [60, 64••, 65–68]. Although these studies are limited in number and scope, findings suggest that recombinant proteins can be used successfully for sensitive and specific immunodiagnosis of toxocariasis. Interestingly, although the native TES-120 is associated with cross-reactivity, when the recombinant protein is expressed in *E. coli* or in *Pichia pastoris*, it appears to perform well as a specific diagnostic antigen [64••, 65, 66]. In another study, a combination of three recombinant proteins

Table 2 Proteins with potential for immunodiagnosis of toxocariasis

Protein	Assay Type[1]	Sensitivity (%)	Specificity (%)	Reference
TES	IgG IB	87 (66/76)[2]	85% (22/148)	[21]
TES High MW cluster		42 (32/76)	89% (16/148)	
TES Low MW cluster		76 (58/76)	93% (10/148)	
TES Low MW only		40 (30/76)	99% (2/148)	
TES 24	IgG IB	94 (47/50)	ND	[21]
TES 28		98 (49/50)	ND	
TES 30		80 (40/50)	ND	
TES 35		46 (23/50)	ND	
TES 132		40 (20/50)	ND	
TES 147		42 (21/50)	ND	
TES 200		34 (17/50)	ND	
TES 24-35	IgG IB	100	97	[20]
TES 56		96	74	
TES 67		22	54	
TES 117-152		100	50	
rTES-26 PEB-1 (*E. coli*)	IgG ELISA	11 (8/136)	88 (1/12)	[67]
rTES-30	IgG ELISA	100 (11/11)	98 (3/142)	[68]
rTES-30USM CTL-2 (*E. coli*)	IgG4 ELISA	92 (24/26)	89 (12/115)	[60]
rTES-120 MUC1,2,3 (*E. coli*)	IgG IB	100 (8/8)	100 (0/32)	[66]
rTES-120 MUC1,2,3 (*Pichia pastoris*)	IgG IB	100 (8/8)	100 (0/45)	[65]
rTES-26 PEB-1 (*E. coli*)	IgG4 ELISA	80 (24/30)	97	[64]
rTES-30 USM CTL-2 (*E. coli*)		93 (28/30)	94	
rTES-120 MUC1,2,3 (*E. coli*)		93 (28/30)	92	
rTES-26 + rTES-30 USM + rTES-120(E.coli)		100 (30/30)	NA	

[1]IB, Immunoblot
[2]76 cases were TES-Ag ELISA positive with hypereosinophilia

(rTES-26, rTES-30, and rTES-120) proved to be more sensitive than any single recombinant protein alone [64••]. When the performance of the three recombinant proteins was compared, the antigen that represented the lowest M_r native protein (TES-26) had the lowest sensitivity and the highest specificity, which is the inverse of what is seen with the native proteins. Additional studies are needed to confirm these initial observations in order to determine if or when recombinant proteins can replace the TES-Ag for clinical diagnosis.

Research gaps

In the development of new immunodiagnostic tools, several particulars should be addressed. In addition to determining sensitivity and specificity in more populations with more samples, evaluations of new recombinant protein-based assays must also assess suitability for diagnosis of *T. cati* infections and OT. The incorporation of multiple recombinant proteins into multiplexed particle-based flow cytometric assays (e.g., Luminex®) or multiplexed lateral-flow assays would inform antigen selection for optimal sensitivity and specificity. Measurement of specific Ig subclasses or isotypes may also improve the assay performance of recombinant protein-based assays. More studies are needed that focus on the use of antigen detection or avidity for differentiation of current and resolved infections. Well-characterized sera must be collected and shared for meaningful assay development, and reactivity in the TES-Ag IB should be part of the specimen pedigree.

In addition to the development of improved immunodiagnostic methods, other laboratory studies could inform our understanding of the biology of *Toxocara* and the pathophysiology of the disease. Next-generation sequencing methods should be used to compare the genomes of *T. canis* larvae recovered from cases of VT and OT. The fact that the clinical signs and symptoms of VT are usually absent in OT (e.g., eosinophilia, elevated serum IgE) suggests that the two syndromes may be caused by different organisms or genotypes. If there is no genetic distinction, a comparison of the proteomes of the larvae from VT and OT may suggest the basis for these different clinical syndromes. Molecular or antibody tools should be developed to differentiate *T. canis* and *T. cati* in order to estimate the percentage of toxocariasis caused by *T. cati*. A thorough characterization of the *T. canis* glycome through modern glycomics protocols may enable the identification of novel carbohydrate structures that could be targets for new antigen detection methods or differentiation of *Toxocara* species. Comparative genomics applications could be used to investigate the diversity of *Toxocara* species from various geographic areas and hosts. The degree of inter- and intraspecies polymorphism in *T. canis* and *T. cati* is unknown. Genomic data would appear to be especially valuable to the veterinary pharmaceutical community, which has the resources required to sequence, assemble, and annotate the nuclear genomes of *T. canis* and *T. cati*.

Much work remains to be done. Toxocariasis is increasingly recognized as an important zoonosis [69], and as such, improved laboratory tools will be the basis for better understanding of the parasite and the diseases it causes. Comparative genomics, proteomics, and glycomics will result in the identification of novel diagnostic targets and methods for molecular epidemiological studies. The use of improved immunodiagnostic tools will not only enable greater diagnostic accuracy, but will also generate reliable seroprevalence data. Ultimately, these efforts will lead to better strategies to control this preventable disease.

Compliance with Ethics Guidelines

Conflict of Interest Patricia P. Wilkins declares that she has no conflict of interest

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of significance
- •• Of major significance

1. Beaver PC, Snyder CH, Carrera GM, Dent JH, Lafferty JW. Chronic eosinophilia due to visceral larva migrans; report of three cases. Pediatrics. 1952;9(1):7–19.
2. Nichols RL. The etiology of visceral larva migrans. I. Diagnostic morphology of infective second-stage *Toxocara* larvae. J Parasitol. 1956;42(4 Section 1):349–62.
3. Dent JH, Nichols RL, Beaver PC, Carrera GM, Staggers RJ. Visceral larva migrans; with a case report. Am J Pathol. 1956;32(4):777–803.
4. Bass JL, Mehta KA, Glickman LT, Eppes BM. Clinically inapparent *Toxocara* infection in children. N Engl J Med. 1983;308(12):723–4.
5. Glickman LT, Magnaval JF, Domanski LM, Shofer FS, Lauria SS, Gottstein B, et al. Visceral larva migrans in French adults: a new disease syndrome? Am J Epidemiol. 1987;125(6):1019–34.
6. Sprent JF. The life history and development of *Toxocara cati* (Schrank 1788) in the domestic cat. Parasitology. 1956;46(1–2):54–78.
7. Sprent JF. Observations on the development of *Toxocara canis* (Werner, 1782) in the dog. Parasitology. 1958;48(1–2):184–209.
8. Sprent JFA. Observations on the systematics of ascariid nematodes. In: Stone A, Platt H, Khalil L, editors. Concepts in nematode systematics. London: Academic Press; 1983. p. 303–19.
9. Glickman L, Schantz P, Grieve R. Toxocariasis. In: Walls K, Schantz P, editors. Immunodiagnosis of parasitic diseases. New York: Academic Press; 1986. p. 201–31.

10. de Savigny DH. In vitro maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnostic tests for visceral larva migrans. J Parasitol. 1975;61(4):781–2.
11. Fillaux J, Magnaval JF. Laboratory diagnosis of human toxocariasis. Vet Parasitol. 2013;193(4):327–36. doi:10.1016/j.vetpar.2012.12.028.
12. de Savigny DH, Voller A, Woodruff AW. Toxocariasis: serological diagnosis by enzyme immunoassay. J Clin Pathol. 1979;32(3):284–8.
13. Glickman L, Schantz P, Dombroske R, Cypess R. Evaluation of serodiagnostic tests for visceral larva migrans. Am J Trop Med Hyg. 1978;27(3):492–8.
14. Kennedy MW, Maizels RM, Meghji M, Young L, Qureshi F, Smith HV. Species-specific and common epitopes on the secreted and surface antigens of *Toxocara cati* and *Toxocara canis* infective larvae. Parasite Immunol. 1987;9(4):407–20.
15. Kennedy MW, Qureshi F, Fraser EM, Haswell-Elkins MR, Elkins DB, Smith HV. Antigenic relationships between the surface-exposed, secreted and somatic materials of the nematode parasites *Ascaris lumbricoides*, *Ascaris suum*, and *Toxocara canis*. Clin Exp Immunol. 1989;75(3):493–500.
16. Page AP, Richards DT, Lewis JW, Omar HM, Maizels RM. Comparison of isolates and species of *Toxocara* and *Toxascaris* by biosynthetic labelling of somatic and ES proteins from infective larvae. Parasitology. 1991;103(Pt 3):451–64.
17. Lynch NR, Wilkes LK, Hodgen AN, Turner KJ. Specificity of *Toxocara* ELISA in tropical populations. Parasite Immunol. 1988;10(3):323–37.
18. Watthanakulpanich D, Smith HV, Hobbs G, Whalley AJ, Billington D. Application of *Toxocara canis* excretory-secretory antigens and IgG subclass antibodies (IgG1-4) in serodiagnostic assays of human toxocariasis. Acta Trop. 2008;106(2):90–5. doi:10.1016/j.actatropica.2008.01.008.
19. Elefant GR, Shimizu SH, Sanchez MC, Jacob CM, Ferreira AW. A serological follow-up of toxocariasis patients after chemotherapy based on the detection of IgG, IgA, and IgE antibodies by enzyme-linked immunosorbent assay. J Clin Lab Anal. 2006;20(4):164–72. doi:10.1002/jcla.20126.
20. Roldan WH, Espinoza YA. Evaluation of an enzyme-linked immunoelectrotransfer blot test for the confirmatory serodiagnosis of human toxocariasis. Mem Inst Oswaldo Cruz. 2009;104(3):411–8.
21. Magnaval JF, Fabre R, Maurieres P, Charlet JP, de Larrard B. Application of the western blotting procedure for the immunodiagnosis of human toxocariasis. Parasitol Res. 1991;77(8):697–702.
22. Nunes CM, Tundisi RN, Garcia JF, Heinemann MB, Ogassawara S, Richtzenhain LJ. Cross-reactions between *Toxocara canis* and *Ascaris suum* in the diagnosis of visceral larva migrans by western blotting technique. Rev Inst Med Trop Sao Paulo. 1997;39(5):253–6.
23. Park SP, Park I, Park HY, Lee SU, Huh S, Magnaval JF. Five cases of ocular toxocariasis confirmed by serology. Korean J Parasitol. 2000;38(4):267–73.
24. Speiser F, Gottstein B. A collaborative study on larval excretory/secretory antigens of *Toxocara canis* for the immunodiagnosis of human toxocariasis with ELISA. Acta Trop. 1984;41(4):361–72.
25. Magnaval JF, Fabre R, Maurieres P, Charlet JP, de Larrard B. Evaluation of an immunoenzymatic assay detecting specific anti-*Toxocara* immunoglobulin E for diagnosis and posttreatment follow-up of human toxocariasis. J Clin Microbiol. 1992;30(9):2269–74.
26. Obwaller A, Jensen-Jarolim E, Auer H, Huber A, Kraft D, Aspock H. *Toxocara* infestations in humans: symptomatic course of toxocarosis correlates significantly with levels of IgE/anti-IgE immune complexes. Parasite Immunol. 1998;20(7):311–7.
27. Smith H, Holland C, Taylor M, Magnaval JF, Schantz P, Maizels R. How common is human toxocariasis? Towards standardizing our knowledge. Trends Parasitol. 2009;25(4):182–8. doi:10.1016/j.pt.2009.01.006.
28. Schabussova I, Amer H, van Die I, Kosma P, Maizels RM. O-methylated glycans from *Toxocara* are specific targets for antibody binding in human and animal infections. Int J Parasitol. 2007;37(1):97–109. doi:10.1016/j.ijpara.2006.09.006.
29. Noordin R, Smith HV, Mohamad S, Maizels RM, Fong MY. Comparison of IgG-ELISA and IgG4-ELISA for *Toxocara* serodiagnosis. Acta Trop. 2005;93(1):57–62. doi:10.1016/j.actatropica.2004.09.009.
30. Smith H, Noordin R. Diagnostic limitations and future trends in the serodiagnosis of human toxocariasis. In: Holland C, Smith H, editors. *Toxocara*: the enigmatic parasite. Cambridge: CABI International; 2006. p. 89–112.
31. Cypess RH, Glickman LT. Serological tests for *Toxocara*. Lancet. 1978;2(8089):579.
32. Rubinsky-Elefant G, da Silva-Nunes M, Malafronte RS, Muniz PT, Ferreira MU. Human toxocariasis in rural Brazilian Amazonia: seroprevalence, risk factors, and spatial distribution. Am J Trop Med Hyg. 2008;79(1):93–8.
33.•• Rubinsky-Elefant G, Hoshino-Shimizu S, Jacob CM, Sanchez MC, Ferreira AW. Potential immunological markers for diagnosis and therapeutic assessment of toxocariasis. Rev Inst Med Trop Sao Paulo. 2011;53(2):61–5. *This research demonstrates that specific IgG is long-lived, persisting for 4–5 years after treatment. Specific IgE may be a better surrogate for cure because it declines in approximately 1 year.*
34. Hubner J, Uhlikova M, Leissova M. Diagnosis of the early phase of larval toxocariasis using IgG avidity. Epidemiol Mikrobiol Imunol: casopis Spolecnosti pro epidemiologii a mikrobiologii Ceske lekarske spolecnosti JE Purkyne. 2001;50(2):67–70.
35. Robertson BD, Burkot TR, Gillespie SH, Kennedy MW, Wambai Z, Maizels RM. Detection of circulating parasite antigen and specific antibody in *Toxocara canis* infections. Clin Exp Immunol. 1988;74(2):236–41.
36. Gillespie SH, Bidwell D, Voller A, Robertson BD, Maizels RM. Diagnosis of human toxocariasis by antigen capture enzyme linked immunosorbent assay. J Clin Pathol. 1993;46(6):551–4.
37. Ishiyamna S, Ono K, Rai SK, Uga S. Method for detecting circulating *Toxocara canis* antigen and its application in human serum samples. Nepal Med Coll J. 2009;11(1):9–13.
38.•• Zibaei M, Sadjjadi SM, Ishiyama S, Sarkari B, Uga S. Production of monoclonal antibody against *Toxocara cati* second-stage larvae and its application for the detection of circulating antigens. Hybridoma. 2010;29(3):217–20. doi:10.1089/hyb.2009.0108. *These authors developed species specific monoclonal antibodies and demonstrated their usefulness to detect circulating antigens in ocular toxocariasis.*
39. Rai SK, Uga S, Wu Z, Takahashi Y, Matsumura T. Use of polymerase chain reaction in the diagnosis of toxocariasis: an experimental study. Southeast Asian J Trop Med Public Health. 1997;28(3):541–4.
40.• Ishiwata K, Shinohara A, Yagi K, Horii Y, Tsuchiya K, Nawa Y. Identification of tissue-embedded ascarid larvae by ribosomal DNA sequencing. Parasitol Res. 2004;92(1):50–2. doi:10.1007/s00436-003-1010-7. *These authors showed that PCR has potential utility as a diagnostic tool for human toxocariasis; these studies showed proof of principle when used to examine ocular fluids from experimentally infected mice.*
41. Pinelli E, Roelfsema JH, Brandes S, Kortbeek T. Detection and identification of *Toxocara canis* DNA in bronchoalveolar lavage of infected mice using a novel real-time PCR. Vet Parasitol. 2013;193(4):337–41. doi:10.1016/j.vetpar.2012.12.029.
42. Zibaei M, Sadjjadi SM, Karamian M, Uga S, Oryan A, Jahadi-Hosseini SH. A Comparative Histopathology, Serology and Molecular Study, on Experimental Ocular Toxocariasis by

Toxocara cati in Mongolian Gerbils and Wistar Rats. BioMed Res Int. 2013;2013:109580. doi:10.1155/2013/109580.
43. Lim SJ, Lee SE, Kim SH, Hong SH, You YS, Kwon OW, et al. Prevalence of Toxoplasma gondii and *Toxocara canis* among Patients with Uveitis. Ocul Immunol Inflamm. 2013. doi:10.3109/09273948.2013.839798.
44. Kayes SG, Oaks JA. Development of the granulomatous response in murine toxocariasis. Initial events. Am J Pathol. 1978;93(2):277–94.
45. Kayes SG, Omholt PE, Grieve RB. Immune responses of CBA/J mice to graded infections with *Toxocara canis*. Infect Immun. 1985;48(3):697–703.
46. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev. 2003;16(2):265–72.
47. Schantz PM, Meyer D, Glickman LT. Clinical, serologic, and epidemiologic characteristics of ocular toxocariasis. Am J Trop Med Hyg. 1979;28(1):24–8.
48. Magnaval JF, Malard L, Morassin B, Fabre R. Immunodiagnosis of ocular toxocariasis using Western-blot for the detection of specific anti-*Toxocara* IgG and CAP for the measurement of specific anti-*Toxocara* IgE. J Helminthol. 2002;76(4):335–9. doi:10.1079/joh2002143.
49. Genchi C, Falagiani P, Riva G, Tinelli M, Brunello F, Boero M, et al. IgE and IgG antibodies in *Toxocara canis* infection. A clinical evaluation. Ann Allergy. 1988;61(1):43–6.
50. de Visser L, Rothova A, de Boer JH, van Loon AM, Kerkhoff FT, Canninga-van Dijk MR, et al. Diagnosis of ocular toxocariasis by establishing intraocular antibody production. Am J Ophthalmol. 2008;145(2):369–74. doi:10.1016/j.ajo.2007.09.020.
51. Glickman LT, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol Rev. 1981;3:230–50.
52. Goffette S, Jeanjean AP, Duprez TP, Bigaignon G, Sindic CJ. Eosinophilic pleocytosis and myelitis related to Toxocara canis infection. Eur J Neurol. 2000;7(6):703–6.
53. Vidal JE, Sztajnbok J, Seguro AC. Eosinophilic meningoencephalitis due to *Toxocara canis*: case report and review of the literature. Am J Trop Med Hyg. 2003;69(3):341–3.
54. Boyce WM, Branstetter BA, Kazacos KR. Comparative analysis of larval excretory-secretory antigens of *Baylisascaris procyonis*, *Toxocara canis* and *Ascaris suum* by Western blotting and enzyme immunoassay. Int J Parasitol. 1988;18(1):109–13.
55. Dangoudoubiyam S, Vemulapalli R, Ndao M, Kazacos KR. A recombinant antigen-based enzyme-linked immunosorbent assay for specific diagnosis of *Baylisascaris procyonis* larva migrans. Clin Vaccine Immunol. 2011. doi:10.1128/CVI.00083-11.
56. Chun CS, Kazacos KR, Glaser C, Bardo D, Dangoudoubiyam S, Nash R. Global neurologic deficits with baylisascaris encephalitis in a previously healthy teenager. Pediatr Infect Dis J. 2009;28(10):925–7. doi:10.1097/INF.0b013e3181a648f1.
57. Wilkins PP. Immunodiagnosis of CNS parasitic infections. Handb Clin Neurol. 2013;114:23–36. doi:10.1016/b978-0-444-53490-3.00003-0.
58.• Rascoe LN, Santamaria C, Handali S, Dangoudoubiyam S, Kazacos KR, Wilkins PP, et al. Inter-laboratory optimization and evaluation of a serological assay for diagnosis of human baylisascariasis. Clin Vaccine Immunol. 2013. doi:10.1128/cvi.00387-13. *This study demonstrates that Toxocara antibodies do not cross react with a recombinant protein from the closely related ascarid, Baylisascaris, resulting in species specific diagnosis for this cause of neural larva migrans.*
59. Jacquier P, Gottstein B, Stingelin Y, Eckert J. Immunodiagnosis of toxocarosis in humans: evaluation of a new enzyme-linked immunosorbent assay kit. J Clin Microbiol. 1991;29(9):1831–5.
60. Norhaida A, Suharni M, Liza Sharmini AT, Tuda J, Rahmah N. rTES-30USM: cloning via assembly PCR, expression, and evaluation of usefulness in the detection of toxocariasis. Ann Trop Med Parasitol. 2008;102(2):151–60. doi:10.1179/136485908x252250.
61. Sakai R, Kawashima H, Shibui H, Kamata K, Kambara C, Matsuoka H. *Toxocara cati*-induced ocular toxocariasis. Arch Ophthalmol. 1998;116(12):1686–7.
62. Meghji M, Maizels RM. Biochemical properties of larval excretory-secretory glycoproteins of the parasitic nematode *Toxocara canis*. Mol Biochem Parasitol. 1986;18(2):155–70.
63. Maizels RM. *Toxocara canis*: molecular basis of immune recognition and evasion. Vet Parasitol. 2013;193(4):365–74. doi:10.1016/j.vetpar.2012.12.032.
64.•• Mohamad S, Azmi NC, Noordin R. Development and evaluation of a sensitive and specific assay for diagnosis of human toxocariasis by use of three recombinant antigens (TES-26, TES-30USM, and TES-120). J Clin Microbiol. 2009;47(6):1712–7. doi:10.1128/jcm.00001-09. *These authors' evaluated 3 different recombinant proteins for immunodiagnosis of toxocariasis and showed that a cocktail of all 3 proteins is more sensitive and specific than the use of any single protein antigen alone.*
65. Fong MY, Lau YL. Recombinant expression of the larval excretory-secretory antigen TES-120 of *Toxocara canis* in the methylotrophic yeast *Pichia pastoris*. Parasitol Res. 2004;92(2):173–6. doi:10.1007/s00436-003-1020-5.
66. Fong MY, Lau YL, Init I, Jamaiah I, Anuar AK, Rahmah N. Recombinant expression of *Toxocara canis* excretory-secretory antigen TES-120 in Escherichia coli. Southeast Asian J Trop Med Public Health. 2003;34(4):723–6.
67. Gems D, Ferguson CJ, Robertson BD, Nieves R, Page AP, Blaxter ML, et al. An abundant, trans-spliced mRNA from *Toxocara canis* infective larvae encodes a 26-kDa protein with homology to phosphatidylethanolamine-binding proteins. J Biol Chem. 1995;270(31):18517–22.
68. Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW, Taib R, et al. Development of a highly specific recombinant *Toxocara canis* second-stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. J Clin Microbiol. 2000;38(4):1409–13.
69. Hotez PJ, Wilkins PP. Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance? PLoS Negl Trop Dis. 2009;3(3):e400. doi:10.1371/journal.pntd.0000400.