



Comparison of IgG-ELISA and IgG4-ELISA for *Toxocara* serodiagnosis

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Abstract

Diagnosis of human toxocariasis, caused by *Toxocara canis* or *Toxocara cati*, normally relies on a combination of the presence of clinical signs and symptoms backed by positive serology. The use of *Toxocara* excretory–secretory antigen (TES) in ELISA assays increases the test specificity. However, in tropical countries where soil-transmitted helminths are endemic, cross-reactivity from antibodies to these intestinal parasites poses a significant limitation for *Toxocara* serodiagnosis. To increase the specificity of serodiagnosis, we compared the use of IgG-ELISA to the use of IgG4-ELISA using commercially manufactured TES-coated plates. The sensitivity of the IgG-ELISA was 97.1%, while that of the IgG4-ELISA was 45.7%; the specificities were 36.0 and 78.6%, respectively. The study shows that employing both assays can improve the serodiagnosis of toxocariasis. An IgG4 immunoassay would also be useful in the secondary screening of antigen clones in the effort to develop improved serological tests for toxocariasis.

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1. Introduction

Toxocariasis is a world-wide zoonotic infection caused by the ascarid nematodes *Toxocara canis* and

Toxocara cati. Commonly, infection is acquired following accidental ingestion of infective ova. Ova, voided in the faeces of infected dogs and cats, develop to infectivity in the environment. Seroprevalence is often lower in developed than in developing countries. In France, 2–5% of apparently healthy adults from urban areas were *Toxocara* seropositive compared to 14.2–37% of

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adults from rural areas (Magnaval et al., 1994), whereas 63.2% *Toxocara* seropositivity was reported in Bali (Chomel et al., 1993) and 20% in Malaysia (Lokman Hakim et al., 1993).

Toxocara cannot complete its life cycle in humans and parasite development is arrested at the second larval (L2) stage. Migrating L2 give rise to the clinical syndromes of visceral larva migrans (VLM), ocular toxocariasis (OT) and covert toxocariasis (CT) (Magnaval et al., 2001). The presentations of VLM include fever, abdominal pain, malaise, weight loss, skin rash, hepatomegaly, hypergammaglobulinaemia and respiratory symptoms/signs with eosinophilia (Magnaval et al., 2001). Childhood bronchial asthma has also been associated with toxocariasis (Oteifa et al., 1998; Chan et al., 2001). OT, caused by L2 migration in the eye, can present as strabismus, pars planitis, endophthalmitis, uveitis, retinal granuloma and retinal detachment leading to loss of visual acuity (Gillespie et al., 1993a). CT presents as a wide range of non-specific signs/symptoms including cough, abdominal pain, headache, sleep and behavioural disturbances (Taylor et al., 1988).

Definitive histopathological evidence of toxocariasis is rarely forthcoming, while clinical diagnosis is hampered by the lack of pathognomonic signs and symptoms. Both early detection of toxocariasis and the assessment of its public health significance rely heavily upon serology (Gillespie et al., 1993b; Ajayi et al., 2000; Alonso et al., 2000). However, serological evidence of widespread infection does not indicate prevalence of clinical disease (Good et al., 2004). *Toxocara* antibodies has been reported to remain positive for years (Ree et al., 1984), and are not very useful for measurement of success of treatment (Gill et al., 1998).

When serological tests are employed, *T. canis* excretory–secretory (TES) antigen derived from in vitro cultured L2 is the antigen of choice, and has been used extensively for the serodiagnosis of human toxocariasis using either commercial or ‘in house’ IgG-ELISA kits (Jacquier, 1991; Akao et al., 1997; Yamasaki et al., 1998). While TES is more specific than crude water-soluble antigens, the level of specificity remains unsatisfactory for its use in countries where other helminth co-infections are prevalent. Sera from patients with ascariasis, filariasis, schistosomiasis and strongyloidiasis react with TES (Maizels et al., 1984; Gillespie et al., 1993b; Nunes et al., 1999; Yamasaki et

al., 2000). Thus, in the tropics, *Toxocara* serodiagnosis remains problematic.

Our previous experiences (unpublished) and those of another investigator (Wiechinger, 1998; dissertation: <http://edoc.ub.uni-muenchen.de/archive/00000178/>) indicated that IgG4-based immunodiagnosis for toxocariasis produced less false positives than IgG-based immunoassay when tested with serum samples from other infections. Since, IgG4 has also been reported as showing higher specificity for parasite antigen in detecting human filariasis (Lal and Ottesen, 1988), and onchocerciasis (Weil et al., 1990), we undertook a comparative study on the usefulness of an IgG-ELISA and an IgG4-ELISA for serodiagnosis of toxocariasis.

2. Materials and methods

A total of 110 serum samples were tested, comprising 35 *Toxocara* sera from patients with clinical, haematological and serological evidence of toxocariasis (mostly VLM); and 75 non-*Toxocara* infection serum samples, 53 were from individuals infected with soil-transmitted helminthiases (STH), 8 from individuals infected with tissue helminths (*Brugia malayi*, *T. canis* and *Gnathostoma spinigerum*), 7 from individuals with invasive amoebiasis and 7 from healthy individuals (see Table 1). At the Scottish Parasite Diagnostic Laboratory (SPDL), an ‘in-house’ ELISA using TES antigen was used to test for antibodies to *Toxocara* using a serum dilution of 1:50. At the University of Malaya (UM), the samples were tested using a commercial TES-ELISA (IgG CELISA; Cellabs, Australia). Serum samples from SPDL and UM were retested at Universiti Sains Malaysia (USM) in this study.

To detect anti-*Toxocara* IgG antibodies at USM, we used a commercial kit (*Toxocara* IgG-ELISA, Cypress Diagnostics, Belgium), consisting of TES precoated Microtiter[®] ELISA plates, secondary reagent (protein A conjugated to HRP), urea peroxide (substrate) and TMB (chromogen) according to the manufacturer’s instructions. Serum samples were diluted to 1:50 prior to use. For each assay run, a cut-off value (COV) was calculated according to the manufacturer’s instructions by dividing the test optical density (OD) by the average OD of two negative controls plus 0.150 OD units.

Overall agreement between IgG-ELISA results with samples tested at both SPDL and USM was 97% (39/30); and overall the agreement between samples tested at both USM and UM was 100% (6/6). No common samples were tested by IgG-ELISA among the three institutions or between UM and SPDL, hence agreements for these could not be ascertained.

The results of the IgG/IgG4 comparison study are presented in Table 1. Using the same TES antigen-coated plates, our results using the IgG- and IgG4-ELISAs differed in terms of sensitivity and specificity. Using the 2×2 tables, the sensitivity of the IgG-ELISA was 97.1%, while that of the IgG4-ELISA was 45.7%; the specificities were 36.0 and 78.6%, respectively. The sensitivity and specificity values of the two assays (IgG-ELISA and IgG4-ELISA) were statistically significant different ($p = 0.00$, McNemar's test). While the IgG-ELISA was more than twice as sensitive as the IgG4-ELISA, the IgG4-ELISA was more than twice as specific as the IgG-ELISA.

Difficulties arose when determining assay specificity. Since, environmental contamination with *Toxocara* and STH are common in the tropics, we cannot exclude the possibility of STH co-infections in the USM and UM *Toxocara* positive samples (Table), nor the possibility of *Toxocara* co-infection in the non-*Toxocara* USM and UM samples. In contrast, these difficulties do not arise for SPDL samples, since diagnostic parasitology for STH infections are usually negative in *Toxocara* positive individuals in United Kingdom. Since, we used the same samples for both IgG-ELISA and IgG4-ELISA, the outcome of our specificity comparison should not be affected.

4. Discussion

While we demonstrated that TES-based IgG-ELISA is sensitive (97.1%) for *Toxocara* serodiagnosis; its specificity is low (36.0%) when tested against our panel of parasitoses-positive sera. This low specificity can be improved significantly by employing IgG4 as the secondary antibody; *albeit* with a significant reduction in sensitivity. The specificity issue may not be a major concern in temperate developed countries where parasite co-infections are uncommon and infection with soil-transmitted helminths are less prevalent, however it is a significant problem for serodiagnosis in the tropics.

Here, commercial IgG-ELISA kits using TES antigen will prove useful for excluding toxocariasis from a differential diagnosis, but when results are positive, interpretation of the test can be problematic.

Efforts should be strengthened to develop highly sensitive and specific assays for human toxocariasis. A number of genes encoding TES proteins have now been cloned (Gems et al., 1995; Gems and Maizels, 1996; Loukas et al., 1999; Maizels et al., 2000) which offer the possibility of a more specific diagnostic reagent. Indeed, Yamasaki et al. (1998, 2000) reported a cDNA clone of 789 bp, corresponding to a gene encoding a 30-kD secreted antigen, which appeared to be a promising candidate for use in diagnostic *Toxocara* serology, however the assay requires further evaluation.

Elevated IgG4 levels have been associated with active infection in other helminth infections such as filariasis and onchocerciasis (Kwan Lim et al., 1990; Lucius et al., 1992) and this IgG subclass shows the sharpest decline following curative chemotherapy (Atmadja et al., 1995). IgG4 responses to TES might reflect ongoing infection with migrating larvae. Longitudinal analyses of *Toxocara* antibodies following treatment are rare in toxocariasis, but would offer one approach to provide supporting evidence regarding the association between anti-*Toxocara* IgG4 antibody levels and active toxocariasis.

Our study shows that using both an isotype-specific (IgG) and IgG subclass-specific (IgG4) ELISA can improve *Toxocara* serodiagnosis. When both tests are positive and clinical/laboratory symptoms are consistent with *Toxocara* infection, there is a higher likelihood that the case is a true positive. Difficulties arise when the IgG4-ELISA result is negative, as the IgG-ELISA result is not as good predictor of seropositivity. In instances when IgG4 is not detectable, another sample should be taken at a later time for IgG4 retesting.

This study also identifies a strategy to immunoscreen *Toxocara* cDNA libraries by employing IgG as the secondary antibody for primary screening of the libraries that will allow selection of all clones, which are reactive to *Toxocara* recombinant proteins. This can then be followed by the use of IgG4 as the secondary antibody for secondary and tertiary screening of the selected clones in order to narrow down the selection to a small number of clones with the greatest diagnostic potential. This approach would be expected to improve the success of identification of diagnostically signifi-

cant antigen clones for further evaluations by Western blot and/or ELISA.

In conclusion, this study highlights the shortcomings of TES-based IgG-ELISA serodiagnosis particularly where infections with multiple migratory helminthiasis are common, and recommends the inclusion of IgG4-ELISA both to attain higher diagnostic specificity in the tropics and for further investigations into developing improved serodiagnostic assays for *Toxocara* infection.

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