



Contents lists available at [SciVerse ScienceDirect](#)

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar



Laboratory diagnosis of human toxocariasis

J. Fillaux^{a,b,1}, J.-F. Magnaval^{c,d,*}

^a Department of Parasitology, Rangueil Hospital, Toulouse University Hospitals, Toulouse, France

^b UMR152, Paul Sabatier University, Toulouse, France

^c Department of Medical Parasitology, Faculty of Medicine Purpan, Toulouse, France

^d CNRS UMR 5288 'Anthropobiology', Paul Sabatier University, Toulouse, France

ARTICLE INFO

Keywords:

Human toxocariasis

Immunodiagnosis

Active infection

Diagnostic strategy

ABSTRACT

Toxocariasis is a helminth zoonosis caused by infection with the larvae of *Toxocara* spp. ascarid worms. Only two species, *Toxocara canis* and *Toxocara cati*, are recognised as causative agents of human disease. The best choice for serodiagnosis of the generalised forms of toxocariasis, visceral larva migrans (VLM) or covert toxocariasis, relies upon the initial use of TES-ELISA, after which any positive result should subsequently be tested by Western blotting (WB). Covert toxocariasis is mostly a benign infection, so a large majority of infected subjects are asymptomatic or have very few symptoms and therefore go undiagnosed. In this form, this helminthosis is often self-limiting, leaving residual specific antibodies. A positive serodiagnosis caused by residual antibodies that do not have any diagnostic significance can be associated with any infectious or non-infectious disease. If separated from the ongoing clinical and laboratory context, such a positive result has no diagnostic value and should be only taken into account after the possible etiologies of any observed syndromes have been ruled out. Unlike the methods used for the immunodiagnosis of bacterial, viral or protozoal (toxoplasmosis) infections, it is not possible with toxocariasis to assess the age of the presence of specific IgG using the levels of specific IgM because IgM antibodies can be found throughout the course of helminthiasis. The detection of other classes of immunoglobulins, namely IgE and IgA, the subclasses, namely IgG4 or circulating Ag was proven to be unable to discriminate between active and self-cured generalised toxocaral infections. Currently, the diagnosis of an active covert toxocariasis relies upon indirect arguments, e.g., the presence of otherwise unexplained symptoms along with blood eosinophilia and/or elevated levels of eosinophil cationic protein (ECP). This situation is far from ideal and more research should be carried out to solve this difficult problem.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Toxocariasis is a helminthic zoonosis caused by infection with the larvae of *Toxocara* spp. ascarid worms. Only two species, *Toxocara canis* and *Toxocara cati*, are

recognised as causative agents of human disease. The adults of both species parasitise the small intestines of their definitive hosts, canids and felids, respectively (Miyazaki, 1991). In the past, it was assumed that *T. canis* was the main cause of *Toxocara* spp. related disease. Although there is evidence to support this position, there is now substantial evidence that humans can also be infected by *T. cati* larvae, and this infection would similarly elicit visceral larva migrans (VLM) and ocular larva migrans (OLM) syndromes (Fisher, 2003).

The first report of *T. canis* larvae in hosts other than dogs was made by Ransom and Foster in 1920 (Barriga,

* Corresponding author at: Service de Parasitologie Médicale, Faculté de Médecine Toulouse-Purpan, 37 allées Jules-Guesde, 31000 Toulouse, France. Tel.: +33 5 61 14 59 81; fax: +33 5 61 14 59 79.

E-mail address: jean-francois.magnaval@univ-tlse3.fr (J.-F. Magnaval).

¹ Service de Parasitologie – Mycologie, CHU Rangueil, 1 avenue du Pr Jean Poulhès, TSA 50032, 31095 Toulouse cedex, France.

1988). In 1952, Beaver et al. published three cases of children presenting with high blood eosinophilia associated with chronic granulomatous lesions of the liver (Beaver et al., 1952). They identified the larvae found in the liver as belonging to *T. canis* species and termed this novel disease “visceral larva migrans”. Beaver later gave a restrictive interpretation of this term in which humans played the role of paratenic host (Beaver, 1956). According to this definition, toxocariasis no longer could be considered as a parasitological dead-end (Harant, 1962) but Beaver’s statement implied that other *Toxocara* species could cause this zoonosis.

2. Epidemiology

Human most frequently become infected by ingesting embryonated eggs present in nearby soil. Toxocariasis is primarily a telluric zoonosis and is a saproozoonosis according to the WHO classification system (WHO, 1979). Along with this telluric transmission route, there is also a foodborne route involving larvae present in the tissues of paratenic hosts that are eaten by humans. The literature reports several cases of toxocariasis resulting from the consumption of raw or undercooked meat or offal from birds or mammals (Akao and Ohta, 2007; Choi et al., 2008; Hoffmeister et al., 2007; Morimatsu et al., 2006; Salem and Schantz, 1992; Sturchler et al., 1990; Taira et al., 2004).

The results of epidemiological surveys have shown that human toxocariasis is one of the most common helminthiasis in the world. In the Western countries, such as those of the G8, the seroprevalence varies between 2% and 5% in urban areas, reaches 15%–20% in semi-rural zones such as residential outskirts of large cities, and peaks at 35%–42% in rural areas (Magnaval et al., 2001b). Factors that have been associated with an increased rate include a low socioeconomic level and poor environmental hygiene (Cilla et al., 1996), which are possibly exacerbated by wet and warm climates, such as the climate found in tropical areas (Magnaval et al., 1994; Thompson et al., 1986).

3. Pathophysiology

Human toxocariasis features an exclusively larval parasitism, since adult worms are never present in the human digestive tract, apart from exceptional cases (Eberhard and Alfano, 1998). In the duodenum, larvae hatched from embryonated eggs (telluric contamination) or released by the digestion of the tissues of paratenic hosts (foodborne contamination) enter the somatic cycle. When the cycle is complete, larvae migrate for a variable period of time in the human body. Once arrested in tissues or organs where immune and/or phagocytic cells are abundant, they can be trapped inside eosinophilic granulomas where they will degenerate (Marty, 2000; Parsons and Grieve, 1990). Larvae continuously release approximately 2 ng/larva/day of soluble glycoprotein antigens termed “excretory-secretory” (ES Ag), which are almost completely absent in the somatic antigenic panel of larvae and adults (Fernando et al., 1970). A part of the ES Ag has an internal origin and is secreted by the larva esophageal gland and excretory column (Page et al., 1992a). The other

part is made up of glycoproteins that are released from the larval outer epicuticular layer (Page et al., 1992b), which is renewed continuously and rapidly: the turnover rate is 50% within 1 h and 80% within 48 h (Maizels et al., 1984). It is likely that ES Ag release is involved in the survival of the larvae inside the paratenic host, which preferentially mounts an immunological response against the soluble Ag rather than against the somatic Ag that are exposed only during larval destruction. ES Ag contain a potent allergenic fraction (Sugane and Oshima, 1983) that was later identified as TBA-1 (Yahiro et al., 1998). This finding would explain the frequency of allergic symptoms in toxocariasis patients (Glickman et al., 1987), especially in atopic subjects (Buijs et al., 1997; Gonzalez-Quintela et al., 2006). The high immunogenicity and allergenicity of ES Ag suggest that most cases of toxocariasis would be caused by tiny larval inocula. In 1959, a volunteer ingested 100 embryonated eggs of *T. canis* following which he presented with a permanent cough that lasted several months. His blood eosinophilia reached 13,500 cells/mm³ on day 30 post-inoculation and still was 6150 cells/mm³ four and a half months later (Chaudhuri and Saha, 1959).

4. Clinical picture

A general agreement now exists concerning the nosology of human toxocariasis, which includes several syndromes (Despommier, 2003; Magnaval et al., 2001b; Schantz, 1989; Smith et al., 2009). However, it should be noted that most infections are clinically asymptomatic.

4.1. Generalised forms

4.1.1. Major syndrome

The so-called VLM syndrome such as defined by Beaver (Beaver, 1956), is caused by ingesting very large amounts of *Toxocara* spp. embryonated eggs and is generally reported in children living in poor socioeconomic conditions or in individuals suffering of mental retardation or severe psychiatric disorders, all of whom present with pica or geophagia. VLM syndrome is uncommon in Western countries. The clinical picture comprises an impairment of the general status that includes weight loss and fever along with asthmatic cough, wheezing, generalised lymphadenopathy, hepatomegaly and often Löfller’s infiltrates on a chest X-ray photographs (Beaver et al., 1952; Ehrhard and Kernbaum, 1979).

4.1.2. Minor syndromes

The discrepancy between the high seroprevalence found in most countries and the low number of VLM cases, of which only 970 had been recorded between 1952 and 1978 (Ehrhard and Kernbaum, 1979) suggested the existence of other forms of the disease. In the mid-1980s, two studies were carried out simultaneously in French adults (Glickman et al., 1987) and Irish children (Taylor et al., 1988). They demonstrated that human toxocariasis was not a rare condition and generally assumed the features of a syndrome that included chronic weakness, digestive pain, various allergic signs – often conjunctival or cutaneous, such as itchy rash or pruritus – diffuse myalgias,

angioneurotic edema, irritation cough and, in children, sleeping and behavior disturbances. This clinical form was called “covert toxocariasis” first in Ireland and then in the English-speaking world and “common toxocariasis” in France.

4.2. Compartmentalised forms

4.2.1. Ocular toxocariasis

Ocular toxocariasis was described prior to VLM (Wilder, 1950). Usually the disease occurs in toddlers, young children, teenagers or young adults and is generally unilateral. The presence of a single larva in the eye wall or in close proximity induces chronic inflammation and the immunological response to the ES Ag released in a rather enclosed area plays a crucial role. The pathological outcome is anterior uveitis or, more often, posterior uveitis along with hyalitis and chorioretinitis. The opacification of the vitreous humor can be accompanied by deposits of immune complexes, often in the periphery (“snowballs”), and by the development of fibrous traction bands that result in retinal detachment. If the larva is trapped in the eye membranes by the inflammatory reaction, a retinal granuloma can develop, often at the posterior pole. When large, it can cause a bulging at the posterior pole which resembles a retinoblastoma (Balmer and Munier, 2007). The most common first sign is vision loss in the affected eye that increases quickly within days or a few weeks. Many patients have a subclinical infection and are diagnosed only during a routine eye examination (Good et al., 2004).

4.3. Neurological toxocariasis

Infection of the central nervous system (CNS) by *Toxocara* spp. larvae causes non-specific illnesses such as meningitis, meningoencephalitis or transverse myelitis (Eberhardt et al., 2005; Vidal et al., 2003; Caldera et al., 2012). Thus, frequently the relationship between the observed symptoms and toxocariasis remains unclear (Magnaval et al., 1997). This form of the zoonosis appears to be rare because a rather recent review of the literature reported only 40 cases of neurological toxocariasis (Eberhardt et al., 2005). The involvement of toxocariasis as a primary cause of epilepsy is still controversial.

5. Laboratory diagnosis

5.1. Non-specific tests

5.1.1. Blood count

In VLM syndrome, blood counts always showed the presence of blood eosinophilia, which was usually marked (Altcheh et al., 2003) and was sometimes massive: approximately 10,000 cells/mm³ (Ehrhard and Kernbaum, 1979; Lassmann et al., 2007). In common or covert toxocariasis, blood eosinophilia values are lower on average: approximately 1500 cells/mm³ (Glickman et al., 1987). This abnormality can be absent (Magnaval et al., 1994; Taylor et al., 1988) particularly in patients with chronic skin allergy symptoms (Gavignet et al., 2008). In ocular toxocariasis, blood eosinophilia levels are usually within the

normal range because the disease is usually caused by a single larva (Gillespie et al., 1993b; Glickman and Schantz, 1981). However, when the eye involvement is due to a larval migration occurring during a generalised infection, blood eosinophilia is present (Magnaval et al., 2002). No general conclusions could be reached about neurological toxocariasis given the very low incidence of this form.

5.1.2. Cytological examination of other fluids

The presence of *Toxocara* spp. larvae in the CNS was generally accompanied by eosinophilia and pleocytosis in the cerebrospinal fluid (CSF) (Vidal et al., 2003). The presence of eosinophils was also reported in the aqueous or vitreous humor during ocular toxocariasis (Liotet et al., 1992), but the very small volume of this type of sample makes the test unusable in practice.

5.1.3. Total serum IgE levels

The phenomenon of a chronic increase in total serum IgE levels during a worm infection was first described in 1968 in Ethiopian children with ascariasis (Johansson et al., 1968) and results from a mechanism that remains unclear. This elevation was later found in most helminthiases, including toxocariasis (Hogarth-Scott et al., 1969). In the common form of toxocariasis, 79% of patients presented with an increased level of total serum IgE (Magnaval et al., 1994), and the mean value was approximately 850 kIU/L (Glickman et al., 1987).

5.1.4. Laboratory markers of inflammation

Laboratory hallmarks of inflammation were frequently reported during the course of VLM syndrome (Ehrhard and Kernbaum, 1979). In common/covert toxocariasis, these abnormalities appeared to be rather uncommon because only 11% of patients with this form of the disease exhibited an elevated sedimentation rate (Magnaval et al., 1994).

5.2. Direct optical diagnosis

A diagnosis of toxocariasis is only confirmed by visual methods (the “gold standard”). Gross examination can identify a larva found, e.g., in the CSF (Eberhardt et al., 2005; Vidal et al., 2003) or in ocular fluids (de Souza and Nakashima, 1995). Live larvae are highly motile in liquid media. Microscopic examination of biopsies or organ fragments can discover larval sections or debris (Kirchner and Altmann, 1987; Marty, 2000). *T. canis* larvae, if undamaged, display the morphological characteristics of larval nematodes. On average, they are 400 µm in length and 18–20 µm in breadth. The edges are parallel to the bulk of the body, so the larvae appear squat. The length of the esophagus is about one-third of the total body length (Nichols, 1956). On pathological examinations, the cuticle presents two characteristic lateral alae (Marty, 2000). *T. cati* larvae have a similar morphology (Nichols, 1956).

5.3. Immunodiagnosis

Development of serological methods was prompted by the difficulties and uncertainties of direct visual diagnosis. By the late 1950s, as part of the scientific

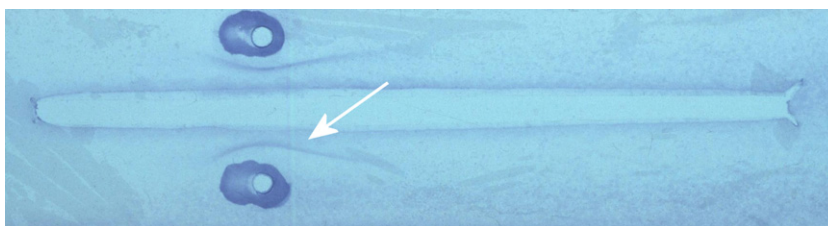


Fig. 1. Immunoelectrophoresis displaying the typical “major” precipitation band.

movement during which novel immunological techniques were applied in parasitology, Kagan reported a test using *Ascaris lumbricoides* or *Ascaris suum* extracts for toxocariasis serodiagnosis (Kagan, 1957, 1958). This first attempt was followed by many others that relied primarily upon the use of sections or soluble extracts from adult worms of *T. canis* (homologous antigen) or *A. lumbricoides* or *A. suum* (heterologous antigens). All of the methods proved to be affected by a severe lack of specificity (Glickman et al., 1986). This shortcoming originated in the high complexity of the antigenic structure of helminths, which elicited a variable level of cross-reactivity between taxonomically distant parasites. This point was analysed and then the “antigenic panel” concept was highlighted by the studies carried out at the Pasteur Institute in Lille, France (Biguet et al., 1965). Moreover, Pasteur’s researchers working with *Schistosoma* spp. demonstrated that the antigenic structure of a given worm contained specific fractions and that some of them were found only at certain developmental stages (Capron et al., 1965). Fernando et al. made similar findings concerning *T. canis* (Fernando et al., 1970). These authors used Ouchterlony’s method (agar double diffusion), and they tested extracts of *T. canis*, *A. lumbricoides* or *Ascaridia galli* embryonated eggs or adults worms against sera from macaques that had been experimentally infected with *T. canis* larvae. The simian sera recognised five fractions in the *T. canis* egg extracts that were absent from the adult worm antigens. Therefore, these results suggested that methods using extracts of *Toxocara* spp. adults, which were already known to display a specificity problem, would also lack sensitivity.

A first improvement came from the use of heterologous egg extracts. In 1973, Niel et al. used immunoelectrophoresis (IEP) to test various antigenic extracts from several *Ascaris* and *Toxocara* species against sera from patients presenting with chronic blood eosinophilia (Niel et al., 1973). When extracts from female *A. suum* genital tracts were used, these authors observed a characteristic precipitating line that they designated as “major”. Subsequently, Magnaval et al. (1986) carried out further investigations with a similar extract. They demonstrated that the ovular fraction involved in the major precipitating band (Fig. 1) was present in *T. canis* ES Ag but was absent from *A. suum* coelomic fluid or *T. canis* somatic extracts. This typical band was only observed when sera from a macaque that had been orally infected with *T. canis* embryonated eggs or from eosinophilic patients who displayed a clinical syndrome consistent with toxocaral disease were tested by IEP. Serum samples from patients with known helminthiasis other than toxocariasis did not react (Magnaval et al.,

1986). This IEP technique had an excellent specificity but exhibited a low sensitivity because precipitation tests are known to require high levels of specific antibodies to turn positive. In the mid-1970s, Cypess et al. (1977) reported a test that combined ELISA, a novel assay described in 1971 (Engvall and Perlmann, 1971), with extracts of *T. canis* embryonated eggs as antigen. To avoid the cross-reaction shortcoming, the sera were first adsorbed on a powdered extract of *A. suum* adults. The subsequent assessment of this method found a sensitivity of 78% and a specificity of 92.3% (Glickman et al., 1978). With these results, a convenient solution to the issue of toxocariasis serodiagnosis was offered for the first time. However, the adsorption step using *A. suum* powder made this diagnostic procedure rather cumbersome. Moreover, a sensitivity of 78% suggested that low-burden infections could be missed.

In 1976, a decisive step forward was made when de Savigny published a method for maintaining *T. canis* larvae in culture (de Savigny, 1975) and *T. canis* ES Ag (TES Ag) became available on a large scale. This author went on to describe an ELISA using TES Ag (de Savigny et al., 1979), thus bringing the immunodiagnosis of toxocariasis into the modern era.

5.3.1. Production of TES Ag

All laboratories that carry out the “cultivation” of *T. canis* larvae use variants of de Savigny’s original method. Briefly, the female worms are collected from puppies after deworming with piperazine, an anthelmintic drug that does not kill eggs, or after necropsy. Female worms are washed with tap water and then dissected. The genital tracts undergo a controlled artificial digestion in a pepsin-HCl mixture to remove the surrounding tissues. The collected eggs are distributed in glass tubes that are plugged with cotton wool and contain a 0.9% NaCl solution supplemented with 1% formalin. The tubes are incubated at 37 °C in a stirring water bath for 2–3 weeks. Periodically, the embryonation level is assessed by microscopic inspection. When the rate is approximately 50%, the eggs are dissected in a Potter’s grinder. Further steps require a sterile environment provided by a laminar flow bench. The resulting mixture, which contains living and dead larvae along with eggshell fragments, is diluted in RPMI 1640 medium. Viable larvae are extracted from the crude mixture using a method similar to the Baermann technique employed to retrieve *Strongyloides stercoralis* larvae from stools. The larvae are then distributed in flat vials of the type used in virology for cell cultures that are filled with RPMI 1640 medium supplemented with glutamine (Fig. 2). The final larval concentration is approximately 1000 larvae/mL.

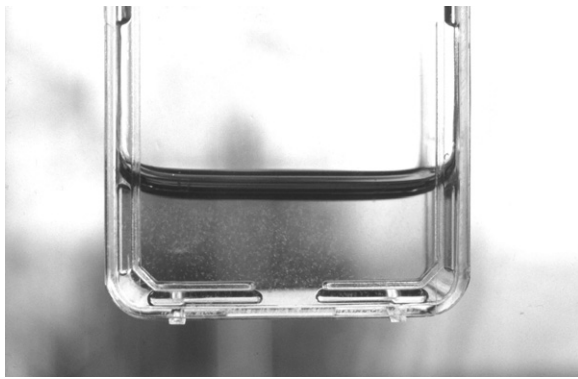


Fig. 2. Virology flask containing *Toxocara canis* larvae in RPMI 1640 medium.

The vials are incubated at 37 °C in a 5% CO₂ atmosphere. Once a week, the vials are tilted at 45 °C to allow the larvae to deposit. The supernatant containing TES Ag is collected, ultracentrifuged and finally dialysed against distilled water. The protein concentration is measured and the mixture is then aliquoted into special vials and freeze-dried under a nitrogen atmosphere. The vials are stored at –80 °C, and continuously repeated quality assessments have demonstrated that this TES Ag stock retained its antigenic properties over a 20-y period.

5.3.2. Routine methods and diagnostic strategies

An ELISA detecting IgG antibodies against TES Ag (IgG TES-ELISA) has become the reference test for toxocarasis immunodiagnosis. Currently, several manufacturers market kits and claim performance levels that are overall quite similar (Smith and Noordin, 2006). However, it should be noted that an assessment of only one kit has been published (Jacquier et al., 1991). The results were a sensitivity of 91% and a specificity of 86%. Examination of the technical leaflets from other kits showed that manufacturers often included a reference to this paper, but the manufacturing process was inevitably different. A concomitant assessment of the marketed ELISA kits is therefore highly desirable, similar to the assessments that are periodically carried out using dipstick kits for malaria diagnosis (Guthmann et al., 2002).

Jacquier et al. (1991) reported that cross-reactions occurred when sera from patients with strongyloidiasis or trichinellosis were tested. These findings were in agreement with Lynch et al. (1988) who created an in-house TES-ELISA in 1988. These authors demonstrated that sera from ascariasis patients induced highly positive results; therefore the value of TES-ELISA in tropical areas was quite questionable. To dampen this cross-reaction phenomenon, Lynch et al. used the method previously described by Cypess et al. (1977): a prior adsorption of the sera on *A. suum* powdered extract (Lynch et al., 1988). The TES-ELISA specificity was drastically enhanced. However, according to previous studies (Magnaval et al., 1986; Niel et al., 1973) indicating that antigenic fractions were shared between TES Ag and *A. suum* egg extracts, it was feared that the sensitivity would be decreased. This became obvious when the results of seroprevalence surveys in children living in



Fig. 3. Positive Western-blot result displaying the typical 7-band pattern.

urban tropical areas were compared. Brazilian researchers found a seroprevalence rate of 8.7% (Teixeira et al., 2006), whereas their Argentina counterparts who used a Western-blot assay on a similar population found that 67.0% of the results were positive (Lopez Mde et al., 2005).

The indisputable specificity problem of TES-ELISA arising when the test was used in possibly multiply parasitised subjects stressed the need for a more specific method, without paying for it through a loss of sensitivity. In the wake of the improvement of immunodiagnosis of HIV infection (Esteban et al., 1985), a Western blotting (WB) procedure that detected IgG specific for TES Ag was established in the late 1980s by Magnaval et al. (Magnaval et al., 1991). Tested by WB, sera from an experimentally infected rabbit and from patients presenting with ocular or generalised toxocarasis displayed a characteristic banding pattern, including a group of 4 bands in the lower molecular weight (LMW) zone at 24, 28, 30 and 35 kDa and a group of 3 bands in the higher molecular weight (HMW) zone at 132, 147 and 200 kDa (Fig. 3). Sera from patients with toxocarasis,

cystic echinococcosis, hookworm infection, pin-worm infection, various types of filariases or schistosomiasis, strongyloidiasis, taeniasis or trichinellosis were negative or showed only an incomplete profile, which was characterised by bands only in the HMW zone. A detailed statistical analysis demonstrated that only the antibodies that reacted with the LMW antigenic fractions were specific for toxocariasis. Subsequent studies that were carried out in animal models, either rabbit (Morales et al., 2002) or mouse (Sarimehmetoglu et al., 2001), or in humans (Roldan and Espinoza, 2009; Zarnowska and Jastrzebska, 1994) also found the previously described banding pattern comprising seven bands distributed between the LMW and HMW zones and thus confirmed the specificity of the antibodies reacting with the LMW fractions of TES Ag. Additionally, a later study (Roldan and Espinoza, 2009) showed that the bands occasionally observed in the zone ranging from 55 to 65 kDa when certain WB commercial kits were used did not correspond to specific reactions. Finally, comparative assessment of commercial kits for TES-ELISA and in-house WB found a 55% higher sensitivity for the latter method (Courtade et al., 1995; Gueglio et al., 1994).

For the serodiagnosis of the generalised forms of toxocariasis, VLM or common/covert toxocariasis, the best choice relies upon the initial use of TES-ELISA, after which any positive result should be subsequently tested by WB. Moreover, it is necessary to use WB testing on sera displaying optical densities (OD) located in the so-called “gray zone”, of which the upper limit is the cut-off value, and the lower limit varies according to the manufacturer of the TES-ELISA kit. There is great interest in this combined diagnostic procedure because TES-ELISA provides fast and relatively inexpensive negative results, whereas WB checking of OD values situated in the “gray zone” or over the cut-off line, dramatically enhances both the sensitivity and specificity.

The only current concern is the diagnostic value of a positive result, which is constantly obscured by the presence of a more or less elevated seroprevalence rate.

Common/covert toxocariasis is mostly a benign infection, so a large majority of infected subjects are asymptomatic or have very few symptoms and go undiagnosed. In this form, this helminthiasis is often self-limiting and leaves residual specific antibodies. On average, the duration of a positive TES-ELISA result would be 2.7 years (Jeanneret, 1991), and over 5 years for WB (Magnaval et al., 2001b). A positive serodiagnostic caused by residual antibodies that do not have any diagnostic significance can therefore be associated with any infectious or non-infectious disease. If separated from the ongoing clinical and laboratory context, such a positive result has no diagnostic value and should be only taken into account after the possible etiologies of the observed syndromes – including blood eosinophilias – have been ruled out. Thus, a request for toxocariasis serology should always be included in an immunodiagnostic panel, the content of which varies according to the collected epidemiological and clinical information (Magnaval, 2004).

Immunodiagnosis of ocular and neurological compartmentalised forms represents a difficult problem. The

parasite burden is always tiny and often reduced to one larva; thus an immunodiagnostic test carried out on serum usually is negative (Eberhardt et al., 2005; Gillespie et al., 1993b; Glickman and Schantz, 1981; Vidal et al., 2003). If positive, interpretation falls into the quandary caused by the possible presence of residual antibodies. For ocular toxocariasis, the solution came from the generalisation of anterior chamber paracentesis (ACP), which made possible immunodiagnosis using aqueous humor (AH) in a TES-ELISA (Benitez del Castillo et al., 1995; de Visser et al., 2008) or WB (Magnaval et al., 2002). Both methods detect specific IgG. For neurological toxocariasis, immunodiagnosis can be carried out on CSF (Magnaval et al., 1997; Vidal et al., 2003). Immunodiagnosis on AH or CSF should be always coupled with serum testing. If the results are discordant, namely negative on serum but positive AH or CSF, this finding indicates intraocular or intrathecal synthesis of specific anti-ES Ag IgG. When serum and any aspirated fluid are simultaneously positive, synthesis of specific IgG in the eye or CNS should be assessed using Reiber's formula (Reiber and Felgenhauer, 1987).

Post-treatment follow-up is not possible using detection of specific IgG, because the kinetics of this class of immunoglobulin is very slow, even after a successful treatment (Bass et al., 1987; Elefant et al., 2006).

6. Diagnostic perspectives

6.1. Detection of current infections

6.1.1. Eosinophil cationic protein (ECP) levels

Because eosinophil cells accumulate preferentially into tissues (Rytomaa, 1960), it was hypothesised that a lack of blood eosinophilia did not necessarily indicate the absence of eosinophilia elsewhere in the body. ECP and other granular cationic proteins are released when eosinophil cells are activated. A study was carried out in our Department, in order to assess the usefulness of ECP dosage to detect active toxocaral infections (Magnaval et al., 2001a). Briefly, ECP dosage was carried out in four groups of subjects. Group 1 comprised 27 apparently healthy subjects in whom allergy and helminthiasis, including toxocariasis, had been ruled out. Group 2 included 23 subjects who displayed a positive serodiagnosis for toxocariasis, but who were fully asymptomatic, or who presented clinical symptoms not consistent with toxocariasis. Group 3 was made of 36 patients presenting with various intestinal and systemic helminthiasis, and Group 4 comprised 110 patients who exhibited clinical and laboratory features of common/covert toxocariasis. Mean level of ECP dosage was found to be 10.8 µg/L in Group 1, 11.2 µg/L in Group 2, 76.6 µg/L in Group 3, and 42.3 µg/L in Group 4. Distribution of the results was studied using Mann–Whitney *U* test. No significant difference was found between Group 1 and Group 2, but these groups differed significantly from both Group 3 and Group 4 ($P=0.00001$). Since then, the levels of ECP were used to assess the presence of a current active toxocariasis in patients who did not display any blood eosinophilia (Magnaval and Glickman, 2006).

6.1.2. Quantification of the avidity of specific IgG

This technique has proven to be useful in toxoplasmosis serology to investigate seroconversions occurring in pregnant women (Bobic et al., 2009; Lachaud et al., 2009). In 11 Polish children suffering from toxocariasis who were followed over a 6–12-month period, the level of specific IgG avidity increased significantly in 10 children (Dziemian et al., 2008). Further studies are needed to validate this concept.

6.2. Improvement of specificity

6.2.1. Levels of specific IgG subclasses: TES-ELISA for IgG3/IgG4

It has been demonstrated in helminthiasis such as schistosomiasis (Li et al., 2001), hydatidosis (Daeki et al., 2000) or particularly lymphatic filariasis (Addiss et al., 1995) that the level of specific IgG4 synthesis was proportional to subject's tolerance to the involved worm and therefore to the chronicity of infection. A study of specific IgG4 levels in toxocariasis found a significant association with the duration of exposure to reinfections (Magnaval and Glickman, 2006). An ELISA detecting the IgG4 subclass specific for TES Ag was found to have a better specificity but a lower sensitivity than the conventional IgG TES-ELISA (Noordin et al., 2005; Watthanakulpanich et al., 2008). Similar results have been already observed with various filariases (Lal and Ottesen, 1988). As far we are aware, the detection of specific IgG4 does not have any current routine use.

6.2.2. Use of recombinant Ag

Although the performance of the TES-ELISA/WB testing pair is satisfactory with respect to both its sensitivity and specificity, this combined diagnostic process is costly if commercial kits are used and is occasionally cumbersome. By the mid-1990s, a recombinant Ag corresponding to the 26 kDa fraction of TES Ag was synthesised and tested using an ELISA that detects specific IgG. The results were disappointing because the sensitivity was very low (Gems et al., 1995). Later, a recombinant 30 kDa Ag was also assessed using an ELISA for specific IgG. A sensitivity of 100% and a specificity of 97.9% were claimed, but only 11 toxocariasis sera had been tested (Yamasaki et al., 2000). More realistic results, namely 92.3% for the sensitivity and 89.6% for the specificity, came from the further assessment of the recombinant 30 kDa Ag after testing 26 positive reference sera using IgG TES-ELISA (Norhaida et al., 2008). In 2009, a comparison of the diagnostic value of three recombinant Ag with MWs of 26, 30 and 120 kDa was published. After ELISA testing for each of the four IgG subclasses, the best results were observed when IgG4 was tested. The sensitivity correlated with increased Ag MW (26 kDa < 30 kDa < 120 kDa), and a strictly inverse result was observed for the specificity (Mohamad et al., 2009).

Further assessments are clearly required, but the use of recombinant Ag appears to be a very interesting method to address the increasing difficulty in obtaining adult worms and would combine the specificity and sensitivity of WB and the low cost of TES-ELISA in a single DOT ELISA test.

6.2.3. Detection of other immunoglobulin isotypes: TES ELISA for specific IgE

As soon as the early 1980s, the presence of IgE specific for TES Ag was established in the sera of toxocariasis patients (Brunello et al., 1983). The results of immunodiagnostic tests detecting this isotype, either by radioimmunoassay (Genchi et al., 1986) or by ELISA (Oliver et al., 1986), were published, but no practical application ensued. By the turn of the 1990s, an immunoenzymatic assay for IgE directed against TES Ag (IgE TES ELISA) was developed and assessed and then finally routinely used on a very large scale (approximately 2500 tests a year) in the Department of Parasitology of Toulouse University Hospitals (Magnaval et al., 1992). Because a part of the Th2 immune response, and not of the Th1 response, was thus quantified, the performance of IgE TES-ELISA differed dramatically from the performance of the conventional IgG TES-ELISA. When a 20 *Toxocara* spp. units (TU)/L cut-off was employed, the specificity was 89.8%, but the sensitivity was only 53.2%. Approximately 20% of patients had a titer below 5 UT/L, which indicates the likely absence of specific IgE. Conversely, 8% of subjects presenting with an atopic status were found to be positive only by IgE TES-ELISA. Moreover, significant titers of specific anti-TES Ag IgE – over the 20 UT/L cut-off were found only in patients having a clinical history and the laboratory hallmarks of allergy (Magnaval et al., 2006). Similarly to specific IgG, specific IgE is long-standing, so the test is not convenient for the detection of active toxocaral infection. However, in patients displaying a significant level of specific IgE, the titer decreased quickly after an effective anthelmintic therapy was administered, and IgE TES-ELISA is currently used in our Department to monitor toxocariasis treatment in atopic subjects (Magnaval, 1995; Magnaval and Glickman, 2006). Anti-TES Ag IgE were also found in the AH of patients who were investigated for ocular toxocariasis. In some cases, the intra-ocular titer was greater than the serum level, which indicated a local synthesis of specific IgE and therefore toxocaral ocular involvement (Genchi et al., 1986; Magnaval et al., 2002).

6.3. Provisional dead-ends

6.3.1. Detection of circulating Ag

Detection of circulating TES Ag with the goal of diagnosing active toxocaral infection was carried out using a sandwich ELISA (Gillespie et al., 1993a; Robertson et al., 1988). Likely because most patients have a very low larval burden and also because larvae can be entrapped and destroyed inside granulomas, the test showed a disappointingly very low sensitivity. This shortcoming was accompanied by a lack of specificity, with 10 positive results among sera from 40 patients with helminthiasis other than toxocariasis (Gillespie et al., 1993a).

6.3.2. Molecular diagnostic methods

Molecular diagnostic methods are hampered by several factors that have thus far made the search for *Toxocara* spp. DNA inconvenient for the routine diagnosis of human infection. The first factor is, once again, that most forms of human toxocariasis are likely caused by tiny inocula and

the probability of obtaining a tissue sample containing a larva appears to be very low. The second point is that the collection of tissue samples is invasive, which is not acceptable for the diagnosis of a usually benign disease from an ethical and technical point of view. Research on this topic did not go beyond experimentation on animal models (Rai et al., 1997). The only prospect could be in the subsequent identification of larval structures found, e.g., in biopsies or in organ fragments from surgery or necropsies and in CSF, but this approach is not currently used in practice (Ishiwata et al., 2004; Caldera et al., 2012).

6.4. Diagnosis of *T. cati* infection

Various serological methods for the diagnosis of *T. cati* infection have been reported, but only Ouchterlony's method has had a published assessment (Petithory and Beddock, 1997). Initial investigations suggested a high degree of homology between ES Ag from both species (Hogarth-Scott et al., 1969). Further studies noted the existence of specific fractions in *T. cati* ES Ag (Kennedy et al., 1987), which could lead to a specific immunodiagnosis for feline toxocariasis. Petithory and Beddock (1997) used Ouchterlony's double-diffusion technique and ES Ag from both *T. canis* and *T. cati* larvae to study 274 sera from patients suspected to have toxocariasis. They reached the conclusion that one third of the patients exhibiting a positive immunodiagnosis might be infected by *T. cati* larvae. Conversely, Western blot using *T. canis* ES Ag seemed unable to discriminate between the two types of infection. Since 1990, more than 60,000 WB tests have been carried out in our department. Although some of the WBs used sample from patients who had circumstantial evidence of feline toxocariasis, a banding pattern different from the pattern described above has not been observed so far.

7. Conclusion

For the immunodiagnosis of human toxocariasis, the combined use of TES-ELISA followed by WB is the current option of choice with respect to sensitivity and specificity. However, the difficulty of diagnosing active infection in common/covert toxocariasis remains a problem. The presence of a more or less important rate of residual antibodies in the general population makes interpretation of a positive test difficult, apart from in cases of compartmentalised infection. Unlike the methods used for the immunodiagnosis of bacterial, viral or protozoal (toxoplasmosis) infections, it is not possible in toxocariasis to assess the age of the presence of specific IgG using the levels of specific IgM because IgM antibodies can be found throughout the course of helminthiasis (Smith, 1993). The detection of other classes of immunoglobulins, namely IgE (Magnaval and Glickman, 2006), IgA (Elefant et al., 2006), the subclasses, namely IgG4 (Smith and Noordin, 2006) or circulating Ag was proven to be unable to discriminate between active and self-cured generalised toxocaral infections. Currently, the diagnosis of an active common/covert toxocariasis relies upon indirect arguments, e.g., the presence of otherwise unexplained symptoms along with blood eosinophilia and/or elevated levels of ECP.

This situation is far from ideal and more research should be carried out to solve this difficult problem.

Conflict of interest statement

None

References

- Addiss, D.G., Dimock, K.A., Eberhard, M.L., Lammie, P.J., 1995. Clinical, parasitologic, and immunologic observations of patients with hydrocele and elephantosis in an area with endemic lymphatic filariasis. *J. Infect. Dis.* 171, 755–758.
- Akao, N., Ohta, N., 2007. Toxocariasis in Japan. *Parasitol. Int.* 56, 87–93.
- Altcheh, J., Nallar, M., Conca, M., Biancardi, M., Freilij, H., 2003. Toxocariasis: aspectos clínicos y de laboratorio en 54 pacientes. *An. Pediatr. (Barc.)* 58, 425–431.
- Balmer, A., Munier, F., 2007. Differential diagnosis of leukocoria and strabismus, first presenting signs of retinoblastoma. *Clin. Ophthalmol.* 1, 431–439.
- Barriga, O.O., 1988. A critical look at the importance, prevalence and control of toxocariasis and the possibilities of immunological control. *Vet. Parasitol.* 29, 195–234.
- Bass, J.L., Mehta, K.A., Glickman, L.T., Blocker, R., Eppes, B.M., 1987. Asymptomatic toxocariasis in children: a prospective study and treatment trial. *Clin. Pediatr. (Phila.)* 26, 441–446.
- Beaver, P.C., 1956. Larva migrans. *Exp. Parasitol.* 5, 587–621.
- Beaver, P.C., Snyder, C.H., Carrera, G.M., Dent, J.H., Lafferty, J.W., 1952. Chronic eosinophilia due to visceral larva migrans; report of three cases. *Pediatrics* 9, 7–19.
- Benitez del Castillo, J.M., Herreros, G., Guillen, J.L., Fenoy, S., Banares, A., Garcia, J., 1995. Bilateral ocular toxocariasis demonstrated by aqueous humor enzyme-linked immunosorbent assay. *Am. J. Ophthalmol.* 119, 514–516.
- Biguet, J., Rose, F., Capron, A., Tanvanky, P., 1965. Contribution de l'analyse immunoélectrophorétique à la connaissance des antigènes vermineux. Incidences pratiques sur leur standardisation, leur purification et le diagnostic des helminthiases par immunoélectrophorèse. *Rev. Immunol. Ther. Antimicrob.* 29, 5–23.
- Bobic, B., Klun, I., Vujanic, M., Nikolic, A., Ivovic, V., Zivkovic, T., Djurkovic-Djakovic, O., 2009. Comparative evaluation of three commercial *Toxoplasma*-specific IgG antibody avidity tests and significance in different clinical settings. *J. Med. Microbiol.* 58, 358–364.
- Brunello, F., Genchi, C., Falagiani, P., 1983. Detection of larva-specific IgE in human toxocariasis. *Trans. R. Soc. Trop. Med. Hyg.* 77, 279–280.
- Buijs, J., Borsboom, G., Renting, M., Hilgersom, W.J., van Wieringen, J.C., Jansen, G., Neijens, J., 1997. Relationship between allergic manifestations and *Toxocara* seropositivity: a cross-sectional study among elementary school children. *Eur. Respir. J.* 10, 1467–1475.
- Caldera, F., Burlone, M.E., Genchi, C., Pirisi, M., Bartoli, E., 2012. Toxocara encephalitis presenting with autonomous nervous system involvement. *Infection*, <http://dx.doi.org/10.1007/s15010-0121-0342-6>.
- Capron, A., Biguet, J., Rose, F., Vernes, A., 1965. Les antigenes de *Schistosoma mansoni*. II. Etude immunoélectrophoretique comparée de divers stades larvaires et des adultes des deux sexes. Aspects immunologiques des relations hôte-parasite de la cervaie et de l'adulte de *S. mansoni*. *Ann. Inst. Pasteur. (Paris)* 109, 798–810.
- Chaudhuri, R.N., Saha, T.K., 1959. Tropical eosinophilia. Experiments with *Toxocara canis*. *Lancet* 2, 493–494.
- Choi, D., Lim, J.H., Choi, D.C., Paik, S.W., Kim, S.H., Huh, S., 2008. Toxocariasis and ingestion of raw cow liver in patients with eosinophilia. *Korean J. Parasitol.* 46, 139–143.
- Cilla, G., Perez-Trallero, E., Gutierrez, C., Part, C., Gomariz, M., 1996. Seroprevalence of *Toxocara* infection in middle-class and disadvantaged children in northern Spain (Gipuzkoa, Basque Country). *Eur. J. Epidemiol.* 12, 541–543.
- Courtade, H., Recco, P., Magnaval, J.F., Charlet, J.P., Seguela, J.P., 1995. Etude comparative de 2 tests ELISA *Toxocara* vis-à-vis du western blot. *Bull. Soc. Fr. Parasitol.* 13, 37–53.
- Cypess, R.H., Karol, M.H., Zidian, J.L., Glickman, L.T., Gitlin, D., 1977. Larva-specific antibodies in patients with visceral larva migrans. *J. Infect. Dis.* 135, 633–640.
- Daeki, A.O., Craig, P.S., Shambesh, M.K., 2000. IgG-subclass antibody responses and the natural history of hepatic cystic echinococcosis in asymptomatic patients. *Ann. Trop. Med. Parasitol.* 94, 319–328.
- de Savigny, D.H., 1975. 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.* 61, 781–782.
- de Savigny, D.H., Voller, A., Woodruff, A.W., 1979. Toxocariasis: serological diagnosis by enzyme immunoassay. *J. Clin. Pathol.* 32, 284–288.
- de Souza, E.C., Nakashima, Y., 1995. Diffuse unilateral subacute neuroretinitis. Report of transvitreal surgical removal of a subretinal nematode. *Ophthalmology* 102, 1183–1186.
- Despommier, D., 2003. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clin. Microbiol. Rev.* 16, 265–272.
- de Visser, L., Rothova, A., de Boer, J.H., van Loon, A.M., Kerkhoff, F.T., Canninga-van Dijk, M.R., Weersink, A.Y., de Groot-Mijnes, J.D., 2008. Diagnosis of ocular toxocariasis by establishing intraocular antibody production. *Am. J. Ophthalmol.* 145, 369–374.
- Dziemian, E., Zarnowska, H., Kolodziej-Sobocinska, M., Machnicka, B., 2008. Determination of the relative avidity of the specific IgG antibodies in human toxocariasis. *Parasite Immunol.* 30, 187–190.
- Eberhard, M.L., Alfano, E., 1998. Adult *Toxocara cati* infections in U.S. children: report of four cases. *Am. J. Trop. Med. Hyg.* 59, 404–406.
- Eberhardt, O., Bialek, R., Nagele, T., Dichgans, J., 2005. Eosinophilic meningomyelitis in toxocariasis: case report and review of the literature. *Clin. Neurol. Neurosurg.* 107, 432–438.
- Ehrhard, T., Kernbaum, S., 1979. *Toxocara canis* et toxocarose humaine. Bulletin de l'Institut Pasteur 77, 225–287.
- Elefant, G.R., Shimizu, S.H., Sanchez, M.C., Jacob, C.M., Ferreira, A.W., 2006. 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.* 20, 164–172.
- Engvall, E., Perlmann, P., 1971. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 8, 871–874.
- Esteban, J.I., Shih, J.W., Tai, C.C., Bodner, A.J., Kay, J.W., Alter, H.J., 1985. Importance of western blot analysis in predicting infectivity of anti-HTLV-III/LAV positive blood. *Lancet* 2, 1083–1086.
- Fernando, S.T., Vasudevan, B., Hamza, M.H., Panditha-Gunawardene, I.K., Samarasinghe, H.T., 1970. Precipitin reactions in monkeys (*Macaca sinica*) experimentally infected with *Toxocara canis* and in children with visceral larva migrans syndrome. *J. Comp. Pathol.* 80, 407–414.
- Fisher, M., 2003. *Toxocara cati*: an underestimated zoonotic agent. *Trends Parasitol.* 19, 167–170.
- Gavignet, B., Piarroux, R., Aubin, F., Millon, L., Humbert, P., 2008. Cutaneous manifestations of human toxocariasis. *J. Am. Acad. Dermatol.* 59, 1031–1042.
- Gems, D., Ferguson, C.J., Robertson, B.D., Nieves, R., Page, A.P., Blaxter, M.L., Maizels, R.M., 1995. An abundant, trans-spliced mRNA from *Toxocara canis* infective larvae encodes a 26-kDa protein with homology to phosphatidylethanolamine-binding proteins. *J. Biol. Chem.* 270, 18517–18522.
- Genchi, C., Tinelli, M., Brunello, F., Falagiani, P., 1986. Serodiagnosis of ocular toxocariasis: a comparison of specific IgE and IgG. *Trans. R. Soc. Trop. Med. Hyg.* 80, 993–994.
- Gillespie, S.H., Bidwell, D., Voller, A., Robertson, B.D., Maizels, R.M., 1993a. Diagnosis of human toxocariasis by antigen capture enzyme linked immunosorbent assay. *J. Clin. Pathol.* 46, 551–554.
- Gillespie, S.H., Dinning, W.J., Voller, A., Crowcroft, N.S., 1993b. The spectrum of ocular toxocariasis. *Eye (Lond.)* 7 (Pt 3), 415–418.
- Glickman, L.T., Schantz, P.M., 1981. Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiol. Rev.* 3, 230–250.
- Glickman, L.T., Schantz, P., Dombroske, R., Cypess, R., 1978. Evaluation of serodiagnostic tests for visceral larva migrans. *Am. J. Trop. Med. Hyg.* 27, 492–498.
- Glickman, L.T., Schantz, P.M., Grieve, R.B., 1986. Toxocariasis. In: Walls, K.W., Schantz, P.M. (Eds.), *Immunodiagnosis of Parasitic Diseases*. Academic Press, New-York, pp. 201–231.
- Glickman, L.T., Magnaval, J.F., Domanski, L.M., Shofer, F.S., Lauria, S.S., Gottstein, B., Brochier, B., 1987. Visceral larva migrans in French adults: a new disease syndrome? *Am. J. Epidemiol.* 125, 1019–1034.
- Gonzalez-Quintela, A., Gude, F., Campos, J., Gare, M.T., Romero, P.A., Rey, J., Meijide, L.M., Fernandez-Merino, M.C., Vidal, C., 2006. *Toxocara* infection seroprevalence and its relationship with atopic features in a general adult population. *Int. Arch. Allergy Immunol.* 139, 317–324.
- Good, B., Holland, C.V., Taylor, M.R., Larragy, J., Moriarty, P., O'Regan, M., 2004. Ocular toxocariasis in schoolchildren. *Clin. Infect. Dis.* 39, 173–178.
- Gueglio, B., de Gentile, L., Nguyen, J.M., Achard, J., Chabasse, D., Marjolet, M., 1994. Epidemiologic approach to human toxocariasis in western France. *Parasitol. Res.* 80, 531–536.
- Guthmann, J.P., Ruiz, A., Priotto, G., Kiguli, J., Bonte, L., Legros, D., 2002. Validity, reliability and ease of use in the field of five rapid tests for the diagnosis of *Plasmodium falciparum* malaria in Uganda. *Trans. R. Soc. Trop. Med. Hyg.* 96, 254–257.
- Harant, H., 1962. La notion d'impasse en parasitologie. Son incidence dans les éosinophilies tissulaires. *Bull. Soc. Pathol. Exot. Filiales* 55, 576–588.
- Hoffmeister, B., Glaeser, S., Flick, H., Pornschlegel, S., Suttorp, N., Bergmann, F., 2007. Cerebral toxocariasis after consumption of raw duck liver. *Am. J. Trop. Med. Hyg.* 76, 600–602.
- Hogarth-Scott, R.S., Johansson, S.G., Bennich, H., 1969. Antibodies to *Toxocara* in the sera of visceral larva migrans patients: the significance of raised levels of IgE. *Clin. Exp. Immunol.* 5, 619–625.
- Ishiwata, K., Shinohara, A., Yagi, K., Horii, Y., Tsuchiya, K., Nawa, Y., 2004. Identification of tissue-embedded ascarid larvae by ribosomal DNA sequencing. *Parasitol. Res.* 92, 50–52.
- Jacquier, P., Gottstein, B., Stingelin, Y., Eckert, J., 1991. Immunodiagnosis of toxocariasis in humans: evaluation of a new enzyme-linked immunosorbent assay kit. *J. Clin. Microbiol.* 29, 1831–1835.
- Jeanneret, J.P., 1991. *Epidémiologie de la toxocarose dans la région jurassienne*. Université de Neuchâtel, Neuchâtel.
- Johansson, S.G., Mellbin, T., Vahlquist, B., 1968. Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E (IgND). *Lancet* 1, 1118–1121.
- Kagan, I.G., 1957. Serum-agar double diffusion studies with *Ascaris* antigens. *J. Infect. Dis.* 101, 11–19.
- Kagan, I.G., 1958. Hemagglutination tests with *Ascaris* antigens. *J. Immunol.* 80, 396–399.
- Kennedy, M.W., Maizels, R.M., Meghji, M., Young, L., Qureshi, F., Smith, H.V., 1987. Species-specific and common epitopes on the secreted and surface antigens of *Toxocara cati* and *Toxocara canis* infective larvae. *Parasite Immunol.* 9, 407–420.
- Kirchner, T., Altmann, H.W., 1987. Parasitenlarven als Ursache umschriebener Leberherde. Morphologie und Differentialdiagnose. *Pathologie* 8, 31–36.
- Lachaud, L., Calas, O., Picot, M.C., Albaba, S., Bourgeois, N., Pratlong, F., 2009. Value of 2 IgG avidity commercial tests used alone or in association to date toxoplasmosis contamination. *Diagn. Microbiol. Infect. Dis.* 64, 267–274.
- Lal, R.B., Ottesen, E.A., 1988. Enhanced diagnostic specificity in human filariasis by IgG4 antibody assessment. *J. Infect. Dis.* 158, 1034–1037.
- Lassmann, B., Tsigrelis, C., Virk, A., 2007. 33-Year-old woman with marked eosinophilia. *Mayo Clin. Proc.* 82, 103–106.
- Li, Y., Sleight, A.C., Ross, A.G., Li, Y., Zhang, X., Williams, G.M., Yu, X., Tanner, M., McManus, D.P., 2001. Human susceptibility to *Schistosoma japonicum* in China correlates with antibody isotypes to native antigens. *Trans. R. Soc. Trop. Med. Hyg.* 95, 441–448.
- Liotet, S., Bloch-Michel, E., Petitthory, J.C., Batellier, L., Chaumeil, C., 1992. Biological modifications of the vitreous in intraocular parasitosis: preliminary study. *Int. Ophthalmol.* 16, 75–80.
- Lopez Mde, L., Martin, G., Chamorro Mdel, C., Mario Alonso, J., 2005. Toxocariasis en niños de una región subtropical. *Medicina (B Aires)* 65, 226–230.
- Lynch, N.R., Wilkes, L.K., Hodgen, A.N., Turner, K.J., 1988. Specificity of *Toxocara* ELISA in tropical populations. *Parasite Immunol.* 10, 323–337.
- Magnaval, J.F., 1995. Comparative efficacy of diethylcarbamazine and mebendazole for the treatment of human toxocariasis. *Parasitology* 110 (Pt 5), 529–533.
- Magnaval, J.F., 2004. Diagnostic des hyperéosinophilies au laboratoire. *Feuill. Biol.* 45, 25–30.
- Magnaval, J.F., Glickman, L.T., 2006. Management and treatment options for human toxocariasis. In: Holland, C., Smith, H.V. (Eds.), *Toxocara. The Enigmatic Parasite*. CABI Publishing, Oxfordshire, UK.
- Magnaval, J.F., Glickman, L.T., Grieve, R.B., Carriere, G., Dubly, E., 1986. Intérêt de l'immunoélectrophorèse utilisant des extraits d'organes génitaux d'*Ascaris suum* dans le diagnostic de la toxocarose. *Bull. Soc. Pathol. Exot. Filiales* 79, 634–641.
- Magnaval, J.F., Fabre, R., Maurières, P., Charlet, J.P., de Larrard, B., 1991. Application of the western blotting procedure for the immunodiagnosis of human toxocariasis. *Parasitol. Res.* 77, 697–702.
- Magnaval, J.F., Fabre, R., Maurières, P., Charlet, J.P., de Larrard, B., 1992. Evaluation of an immunoenzymatic assay detecting specific anti-*Toxocara* immunoglobulin E for diagnosis and posttreatment follow-up of human toxocariasis. *J. Clin. Microbiol.* 30, 2269–2274.
- Magnaval, J.F., Michault, A., Calon, N., Charlet, J.P., 1994. Epidemiology of human toxocariasis in La Reunion. *Trans. R. Soc. Trop. Med. Hyg.* 88, 531–533.
- Magnaval, J.F., Galindo, V., Glickman, L.T., Clanet, M., 1997. Human *Toxocara* infection of the central nervous system and neurological disorders: a case-control study. *Parasitology* 115 (Pt 5), 537–543.

- Magnaval, J.F., Berry, A., Fabre, R., Morassin, B., 2001a. Eosinophil cationic protein as a possible marker of active human *Toxocara* infection. *Allergy* 56, 1096–1099.
- Magnaval, J.F., Glickman, L.T., Dorchie, P., Morassin, B., 2001b. Highlights of human toxocariasis. *Korean J. Parasitol.* 39, 1–11.
- Magnaval, J.F., Malard, L., Morassin, B., Fabre, R., 2002. 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.* 76, 335–339.
- Magnaval, J.F., Faufigue, J.H., Morassin, B., Fabre, R., 2006. Eosinophil cationic protein, specific IgE and IgG4 in human toxocariasis. *J. Helminthol.* 80, 417–423.
- Maizels, R.M., de Savigny, D., Ogilvie, B.M., 1984. Characterization of surface and excretory-secretory antigens of *Toxocara canis* infective larvae. *Parasite Immunol.* 6, 23–37.
- Marty, A.M., 2000. Toxocariasis. In: Meyers, W.M., Neafie, R.C., Marty, A.M., Wear, D.J. (Eds.), *Pathology of Infectious Diseases*. Armed Forces Institute of Pathology, Washington, D.C.
- Miyazaki, I., 1991. *An Illustrated Book Of Helminthic Zoonoses*. International Medical Foundation of Japan, Tokyo.
- Mohamad, S., Azmi, N.C., Noordin, R., 2009. 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.* 47, 1712–1717.
- Morales, O.L., Lopez, M.C., Nicholls, R.S., Agudelo, C., 2002. Identification of *Toxocara canis* antigens by Western blot in experimentally infected rabbits. *Rev. Inst. Med. Trop. Sao Paulo* 44, 213–216.
- Morimatsu, Y., Akao, N., Akiyoshi, H., Kawazu, T., Okabe, Y., Aizawa, H., 2006. A familial case of visceral larva migrans after ingestion of raw chicken livers: appearance of specific antibody in bronchoalveolar lavage fluid of the patients. *Am. J. Trop. Med. Hyg.* 75, 303–306.
- Nichols, R.L., 1956. The etiology of visceral larva migrans. I. Diagnostic morphology of infective second-stage *Toxocara* larvae. *J. Parasitol.* 42, 349–362.
- Niel, G., Pinon, J.M., Couture, J., Rosin, G., Gentilini, M., 1973. Aspect immunologique de syndromes hyperéosinophiliques évoquant une Larva migrans viscérale. *Bull. Soc. Pathol. Exot. Filiales* 66, 324–332.
- Noordin, R., Smith, H.V., Mohamad, S., Maizels, R.M., Fong, M.Y., 2005. Comparison of IgG-ELISA and IgG4-ELISA for *Toxocara* serodiagnosis. *Acta Trop.* 93, 57–62.
- Norhaida, A., Suharni, M., Liza Sharmini, A.T., Tuda, J., Rahmah, N., 2008. rTES-30USM: cloning via assembly PCR, expression, and evaluation of usefulness in the detection of toxocariasis. *Ann. Trop. Med. Parasitol.* 102, 151–160.
- Oliver, S.T., Cousland, H.V., Smith, R.W., Girdwood, R.W.A., 1986. Antibody-isotype reactivity, isotype specific serodiagnostic and IgE antibody specificity in humans infected with *Toxocara canis*. *Trans. R. Soc. Trop. Med. Hyg.* 80, 985.
- Page, A.P., Hamilton, A.J., Maizels, R.M., 1992a. *Toxocara canis*: monoclonal antibodies to carbohydrate epitopes of secreted (TES) antigens localize to different secretion-related structures in infective larvae. *Exp. Parasitol.* 75, 56–71.
- Page, A.P., Rudin, W., Fluri, E., Blaxter, M.L., Maizels, R.M., 1992b. *Toxocara canis*: a labile antigenic surface coat overlying the epicuticle of infective larvae. *Exp. Parasitol.* 75, 72–86.
- Parsons, J.C., Grieve, R.B., 1990. Effect of egg dosage and host genotype on liver trapping in murine larval toxocariasis. *J. Parasitol.* 76, 53–58.
- Petithory, J.C., Beddock, A., 1997. Rôle de *Toxocara cati* dans le syndrome de larva migrans viscéral. *Bull. Soc. Fr. Parasitol.*, 199–211.
- Rai, S.K., Uga, S., Wu, Z., Takahashi, Y., Matsumura, T., 1997. Use of polymerase chain reaction in the diagnosis of toxocariasis: an experimental study. *Southeast Asian J. Trop. Med. Public Health* 28, 541–544.
- Reiber, H., Felgenhauer, K., 1987. Protein transfer at the blood cerebrospinal fluid barrier and the quantitation of the humoral immune response within the central nervous system. *Clin. Chim. Acta* 163, 319–328.
- Robertson, B.D., Burkot, T.R., Gillespie, S.H., Kennedy, M.W., Wambai, Z., Maizels, R.M., 1988. Detection of circulating parasite antigen and specific antibody in *Toxocara canis* infections. *Clin. Exp. Immunol.* 74, 236–241.
- Roldan, W.H., Espinoza, Y.A., 2009. Evaluation of an enzyme-linked immunoelectrotransfer blot test for the confirmatory serodiagnosis of human toxocariasis. *Mem. Inst. Oswaldo Cruz* 104, 411–418.
- Rytomaa, T., 1960. Organ distribution and histochemical properties of eosinophil granulocytes in rat. *Acta Pathol. Microbiol. Scand. Suppl.* 50 (Suppl. 140), 1–118.
- Salem, G., Schantz, P., 1992. Toxocaral visceral larva migrans after ingestion of raw lamb liver. *Clin. Infect. Dis.* 15, 743–744.
- Sarimehmetoglu, H.O., Burgu, A., Aycicek, H., Gonenc, B., Tanyuksel, M., Kara, M., 2001. Application of western blotting procedure for the immunodiagnosis of visceral larva migrans in mice by using excretory/secretory antigens. *Dtsch. Tierarztl. Wochenschr.* 108, 390–392.
- Schantz, P.M., 1989. *Toxocara* larva migrans now. *Am. J. Trop. Med. Hyg.* 41, 21–34.
- Smith, H.V., 1993. Antibody reactivity in toxocariasis. In: Lewis, J., Maizels, R.M. (Eds.), *Toxocara and Toxocarosis: Clinical, Epidemiological and Molecular Perspectives*. Institute of Biology and British Society for Parasitology, London.
- Smith, H.V., Noordin, R., 2006. Diagnostic limitations and future trends in the serodiagnosis of human toxocariasis. In: Holland, C.V., Smith, H.V. (Eds.), *Toxocara. The Enigmatic Parasite*. CABI Publishing, Oxfordshire, UK.
- Smith, H.V., Holland, C., Taylor, M., Magnaval, J.F., Schantz, P., Maizels, R., 2009. How common is human toxocariasis? Towards standardizing our knowledge. *Trends Parasitol.* 25, 182–188.
- Sturchler, D., Weiss, N., Gassner, M., 1990. Transmission of toxocariasis. *J. Infect. Dis.* 162, 571.
- Sugane, K., Oshima, T., 1983. Purification and characterization of excretory and secretory antigen of *Toxocara canis* larvae. *Immunology* 50, 113–120.
- Taira, K., Saeed, I., Permin, A., Kapel, C.M., 2004. Zoonotic risk of *Toxocara canis* infection through consumption of pig or poultry viscera. *Vet. Parasitol.* 121, 115–124.
- Taylor, M.R., Keane, C.T., O'Connor, P., Mulvihill, E., Holland, C., 1988. The expanded spectrum of toxocaral disease. *Lancet* 1, 692–695.
- Teixeira, C.R., Chieffi, P.P., Lescano, S.A., de Melo Silva, E.O., Fux, B., Cury, M.C., 2006. Frequency and risk factors for toxocariasis in children from a pediatric outpatient center in southeastern Brazil. *Rev. Inst. Med. Trop. Sao Paulo* 48, 251–255.
- Thompson, D.E., Bundy, D.A., Cooper, E.S., Schantz, P.M., 1986. Epidemiological characteristics of *Toxocara canis* zoonotic infection of children in a Caribbean community. *Bull. World Health Organ.* 64, 283–290.
- Vidal, J.E., Sztajnbock, J., Seguro, A.C., 2003. Eosinophilic meningoencephalitis due to *Toxocara canis*: case report and review of the literature. *Am. J. Trop. Med. Hyg.* 69, 341–343.
- Wattanakulpanich, D., Smith, H.V., Hobbs, G., Whalley, A.J., Billington, D., 2008. Application of *Toxocara canis* excretory-secretory antigens and IgG subclass antibodies (IgG1–4) in serodiagnostic assays of human toxocarosis. *Acta Trop.* 106, 90–95.
- WHO, 1979. Report of a WHO Expert Committee with the participation of the FAO. Technical Report Series No. 637. WHO Publishing, Genève.
- Wilder, H.C., 1950. Nematode endophthalmitis. *Trans. Am. Acad. Ophthalmol. Otolaryngol.* 55, 99–109.
- Yahiro, S., Cain, G., Butler, J.E., 1998. Identification, characterization and expression of *Toxocara canis* nematode polypeptide allergen TBA-1. *Parasite Immunol.* 20, 351–357.
- Yamasaki, H., Araki, K., Lim, P.K., Zaslavsky, N., Mak, J.W., Taib, R., Aoki, T., 2000. Development of a highly specific recombinant *Toxocara canis* second-stage larva excretory-secretory antigen for immunodiagnosis of human toxocariasis. *J. Clin. Microbiol.* 38, 1409–1413.
- Zarnowska, H., Jastrzebska, M., 1994. Excretory-secretory larval antigens of *Toxocara canis*: physico-chemical characteristics and specificity assayed by Western blot technique. *Acta Parasitol.* 39, 41–45.