

Knowledge gaps in the epidemiology of *Toxocara*: the enigma remains

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SUMMARY

Toxocara species infect a wide range of companion, domestic and wild animals as definitive and paratenic hosts, via multiple routes of transmission, producing long-lived tissue-inhabiting larvae and resistant eggs that can survive in the external environment. Therefore *Toxocara* and the disease it causes in humans, toxocariasis, represents an ideal aetiological agent for the development of the one health approach. However, despite increasing awareness of the public health significance of toxocariasis, gaps in our understanding of certain key aspects of the parasite's biology and epidemiology remain. These gaps hinder our ability to integrate research effort within the veterinary, medical and environmental disciplines. This review will highlight key deficits in our understanding of nine dimensions of *Toxocara* epidemiology and discuss a potential scenario to develop a more integrated, one health approach to improve our understanding of the prevention and control of this complex and cryptic zoonosis.

Key words: One health, *Toxocara* spp., toxocariasis, epidemiology, definitive hosts, paratenic hosts, environmental contamination.

INTRODUCTION

In their introduction to a special issue focusing upon zoonoses of people and their pets, Paul *et al.* (2010) defined the one health approach as collaborative work of multiple disciplines to help attain optimal health of people, animals and the environment. The helminth parasite *Toxocara* and the disease it causes, toxocariasis, could not be better placed to exemplify how such an integrated approach is sorely needed – a cosmopolitan zoonotic parasite utilizing dogs, cats and foxes as definitive hosts and one that extensively contaminates the environment with potentially infective eggs. However, a key challenge with respect to the one health approach as it pertains to *Toxocara* is the gaps in our understanding of certain key aspects of the parasite's epidemiology and the lack of integration in terms of research effort between veterinary, medical and environmental disciplines.

It has been over 60 years since *Toxocara* larvae were detected in ocular granulomata from the enucleated eyes of children with suspected retinoblastoma (Wilder, 1950; Nichols, 1956), and since that time a number of other clinical syndromes have been described in humans (Smith *et al.* 2009). However, the significance of human toxocariasis as a disease entity remains enigmatic (Holland and Smith, 2006) partly because of the fact that symptoms can be generalized, multifaceted and cryptic.

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A number of recent publications suggest that awareness of toxocariasis is increasing and highlight some important public health implications of infection. In 2009, Peter Hotez and Patricia Wilkins asked whether *Toxocara* is 'America's most common neglected infection of poverty and a helminthiasis of global importance?' (Hotez and Wilkins, 2009) and in 2013, Calum Macpherson described toxocariasis as 'a zoonosis of global importance' (Macpherson, 2013). In a significant initiative, the Centres for Disease Control and Prevention (CDC) identified five neglected parasitic infections in the USA based upon the following criteria – the number of people infected, the severity of the illnesses associated with such infections and the ability to prevent and treat them. These parasitic infections are considered neglected because of the relatively limited attention that has been devoted to their surveillance, prevention and/or treatment. Among them is toxocariasis.

Furthermore, in a recent opinion piece for JAMA psychiatry, Hotez (2014a) extended his observations on the neglected infections of poverty in the USA to their effects on the human brain, including the possibility that such infections may, at least in part, account for the achievement gap noted among socio-economically disadvantaged students. Some readers may find these observations fanciful, but a large-scale associational study has reported a link between exposure to *Toxocara* and cognitive deficits (Walsh and Haseeb, 2012). Most recently, Fan *et al.* (2015) highlighted the link between cerebral toxocariasis and a number of neurological

dysfunctions and described how mouse models may be useful in understanding the mechanistic basis of Toxocaral brain involvement and pathogenesis.

Despite this increasing awareness, I contend that there remain significant gaps in our knowledge of fundamental aspects of the epidemiology of toxocar-iasis and these gaps hinder our ability to establish a more complete understanding of the parasite and the disease it causes, its relative public health significance and how best to prevent and control parasite transmission, infection and disease. To demonstrate this argument, I will identify some relevant findings but also highlight key deficits in our understanding in nine dimensions of the parasite's epidemiology.

These are

- an overview of the sources of infection and the modes of transmission including their relative importance as a basis for future investigation;
- the role of the veterinarian in the context of a key reservoir of infection – *Toxocara* infection in domestic dogs and cats;
- an evaluation of our current knowledge of the significance of a new potential reservoir of infection – the presence of *Toxocara* eggs on the hair of definitive hosts;
- an improved understanding of variation in exposure to infection in humans and its relationship with disease as a basis for public health education;
- the lack of population-based estimates of ocular toxocariasis (OT);
- the significance of cerebral toxocariasis in humans with a particular focus upon the link between infection and cognitive deficits in children;
- how to improve our understanding of environmental contamination with *Toxocara* eggs including the relative importance of different definitive hosts as sources of ova;
- the significance of non-human paratenic hosts in the transmission of *Toxocara* (including species identification of larvae) to definitive hosts and how best to model paratenesis in the laboratory;
- a relative lack of knowledge of the basic biology and public health significance of *Toxocara cati*; and
- how best to move the research field forward in the context of a one health approach.

I will use selected examples from the literature to illustrate each section.

Complex modes of transmission and sources of infection

One of the most challenging aspects of preventing exposure to *Toxocara* infection is the complexity of the routes of transmission and sources of infection, a number of which we still know remarkably little about.

Adult worms of *Toxocara* spp. reside in a wide range of domestic and feral definitive hosts. For

example, *Toxocara canis* infects domestic dogs, foxes, wolves and coyotes (O'Lorcain, 1994a; Segovia *et al.* 2001; Roddie *et al.* 2008a; Wapenaar *et al.* 2013) and *Toxocara cati* (*syn.* *Toxocara mystax*) infects cats and other felids (Fisher, 2003). In contrast, *Toxascaris leonina* infects both dogs and cats (Miyazaki, 1991). Environmental contamination with *Toxocara* ova is extensive as a consequence of faecal deposition by both domestic and feral definitive hosts and under appropriate conditions of temperature and moisture, such eggs can embryonate and become potentially infective (Traversa *et al.* 2014). Eggs containing infective larvae can be transmitted to humans via contaminated soil, food or utensils. Somatic larvae in the tissues of definitive hosts (particularly dogs – see Coati *et al.* 2004) can re-activate and resume migration and eventually develop into adult worms in the intestine (Schnieder *et al.* 2011).

Toxocara eggs can infect numerous paratenic hosts including humans. *Toxocara* do not develop to adulthood in such hosts but remain as third-stage larvae in their tissues (Brunaska *et al.* 1995; Strube *et al.* 2013). Paratenic hosts can act as food items for both humans and definitive hosts. Meat-borne transmission via the consumption of raw or undercooked liver has been implicated in human infection (Salem and Schantz, 1992; Yoshikawa *et al.* 2008). In a fascinating recent report, an elderly male patient from France was infected with *Toxocara* after ingestion of live slugs (apparently a long-standing daily intake of slugs as an alternative therapy for gastro-oesophageal reflux) (Fellrath and Magnaval, 2014). Terrestrial molluscs are not recognized as paratenic hosts for *Toxocara*, however the authors hypothesize that the slugs captured embryonated eggs in their mucus thereby playing a phoretic role. More recently, another potential source of infection has been identified with the discovery of the presence of *Toxocara* eggs on the hair of domestic definitive hosts such as dogs and cats.

There are, therefore, four key epidemiological reservoirs of *Toxocara*: intestinal infections in definitive hosts (dogs, cats and foxes), eggs in the environment, larvae in paratenic hosts and somatic larvae in the definitive host (Overgaauw and Van Knapen, 2013). As outlined below, there is also the presence of *Toxocara* eggs on the hair of definitive hosts, but this physical extension of the environmental reservoir of eggs would appear to be less important for transmission. What emerges from this complexity of infection sources is that we still lack knowledge of the relative importance of some sources; but from the point of view of control, it is quite clear that tackling intestinal infections in domestic dogs and cats is by far the easiest way of reducing environmental contamination and therefore exposure of both definitive and paratenic hosts to infection. However, as revealed by high levels of

seroprevalence in humans, especially in tropical and subtropical regions and among the disadvantaged, it is clear that such an approach is not being implemented in a sufficiently rigorous manner to reduce exposure successfully.

The role of the veterinarian with respect to key sources of infection

Reducing infection in domestic animals such as dogs and cats underlines the key front-line role that veterinarians play in the provision of anthelmintic treatment and the education of pet owners and the general public. However, this is a challenge because, for the most part, *Toxocara* infection is not pathogenic in adult dogs and cats and other infections are of greater immediate concern to the clients of veterinarians. Despite this lack of concern, *Toxocara* is a parasite of significant zoonotic potential (Holland and Smith, 2006) and therefore, veterinarians need to extend their role, beyond that of the immediate benefit to their patients, to the education of pet owners and the broader society. This requires communication between medical clinicians and veterinarians in supporting each other with the common goal of reducing overall incidence of this disease. However, toxocarosis is also low on the priorities of most medical clinicians, even ophthalmologists and this is not just a problem restricted to *Toxocara* but also for other neglected diseases (Parise *et al.* 2014).

One recent informative study undertaken by veterinarians in the Netherlands highlights a number of challenges for pet owner education and awareness. A comprehensive survey of the owners of over 900 household dogs over 6 months of age was conducted with epidemiological data collected on prevalence of gastrointestinal parasites and a detailed questionnaire on risk factors. The prevalence of *Toxocara* was 4.6% overall, peaking in 6–12 month-old dogs (7.8%). Risk factors included age, ranging freely, coprophagy and recent kenneling. Only 16% of the dogs were dewormed four times per year (see European Scientific Counsel Companion Animal Parasites (ESCCAP) guidelines, 2010) and only 14% of these were dewormed for public health reasons (other reasons included the dog's health, dogmatic ('because we must'), or a combination of dog's health and public health) (Nijse *et al.* 2015a). Among non-coprophagic, kenneled and leashed dogs that were treated four times per year, no *Toxocara* infection was detected. The authors concluded that owner knowledge was insufficient to expect sound decisions on routine deworming.

In a very useful study that should be emulated in other countries, Palmer *et al.* (2008) undertook a national survey of the gastrointestinal parasites of dogs and cats in Australia. This ambitious study was undertaken in the context of a very high pet

ownership with 53% of all households owning a dog or cat. A total of 2463 faecal samples (1400 canine and 1063 feline) were collected from both urban and rural locations within three climatic zones – tropical, arid and temperate. The prevalence of *T. canis* and *T. cati* in dogs and cats was low compared with previous data collated from the 1970–80s with values of 1.2% (CI 95% 0.6–1.8) and 3.2% (CI 95% 2.1–4.3), respectively. The authors concluded that the frequent administration of anthelmintic treatment had a significant impact on gastrointestinal parasitism in Australia.

Although it is quite clear that puppies and kittens represent the most important source of *Toxocara* infection, there has been some debate about the significance of older dogs as sources of infection and a lack of knowledge of the dynamics of infection in such animals. The role of older animals as a reservoir of infection should not be underestimated, hence the ESCCAP guidelines of average frequency of treatment four times per year for adult dogs and cats (Overgaauw and Van Knapen, 2013). Experimental data has established that low doses of 100 embryonated eggs can induce patency in adult dogs, whereas previous experiments that utilized high doses failed to do so (Fahrion *et al.* 2008). This introduces what Overgaauw and Van Knapen (2013) describe as the therapeutic paradox whereby with declining exposure, there is an increasing probability that older animals will get infected and harbour patent infections.

The epidemiological significance of Toxocara eggs on the hair of definitive hosts

Contact with soil contaminated with infective (embryonated) eggs is considered to be the primary route of transmission of *Toxocara* spp. to humans. This is in part due to the fact that *Toxocara* eggs are not infective on shedding but require a period of time, under appropriate environmental conditions, to develop to infectivity. However, a recent focus of interest has been the epidemiological significance of the presence of *Toxocara* eggs on the hair of domestic pets, particularly dogs, raising the possibility of direct transmission of infection to humans through close contact with their pets.

Some much earlier work by Hasslinger *et al.* (1973) examined the hair of 17 owned and stray cats and found a single animal (6% prevalence) to have eggs of *Toxocara mystax* (= *T. cati*) on its hair. No attempt was made to count the number of eggs or to determine whether the eggs were embryonated or even viable. No further observations were reported until 30 years later, when Wolfe and Wright (2003) detected a higher prevalence of *Toxocara* eggs on the hair of 60 dogs and designated the viability and embryonation status of the eggs. Since then a number of studies from a range of

geographical locations have provided data on dog (and in few cases cat) hair as a potential source of infection. Despite variation in sample size, dog status (stray *vs* owned, breed, coat type, age and sex), the location on the body from where the samples were taken and the methods of detection, a number of clear trends have emerged. The prevalence of *Toxocara* eggs on hair is higher in stray *vs* owned dogs, with a peak prevalence of 67% (rising to 100% in the sampled puppies) among a sample of stray dogs reported by Roddie *et al.* (2008b) (see Table 1). As might be expected, the total numbers of eggs detected on hair vary considerably but the pattern for higher numbers on stray animals remains (Table 1).

Two studies provided important refinements to the approach. One examined the relationship between eggs on the hair and the worm burden of *Toxocara* at postmortem (Roddie *et al.* 2008b). The relationship varied significantly with age. Puppies demonstrated a highly significant relationship between eggs on the hair and worm burden. In contrast, no such relationship existed for adult dogs. This provides indirect evidence for the likelihood that puppies are deriving hair-borne eggs from their own worm infections, whereas adult dogs may be picking up eggs from the environment. The second study provided an important standardization of the egg detection method (Overgaauw *et al.* 2009).

The most significant observation that emerges from the work undertaken so far is the very low numbers of embryonated eggs detected on host hair. Most studies categorized eggs detected as being non-viable, viable, embryonating or embryonated (Roddie *et al.* 2008b). Proportions of embryonated eggs ranged from the highest value of 8.1% (Aydenizoz-Ozkayhan *et al.* 2008) to 0% (Overgaauw *et al.* 2009; Amaral *et al.* 2010; Keegan and Holland, 2010; Oge *et al.* 2014). It is of interest to note that even in the study by Roddie *et al.* (2008b) – where a very large number of eggs (39 120 in total) were detected – only 0.3% were embryonated (0.312% in puppies, 0.120% in adult dogs). In this context, the proportion of embryonated eggs is a key epidemiological measure and the data suggest that embryonation on hair is a rare phenomenon, particularly among well-cared-for dogs such as those described by Keegan and Holland (2010). Therefore the risk of exposure of humans to such a source of infection is likely to be negligible.

However, an experimental approach undertaken by Keegan and Holland (2013) tested the embryonation rates of *T. canis* eggs in soil *vs* hair under laboratory conditions. *T. canis* eggs were exposed to two temperature (10 and 20 °C) and two moisture regimes (with and without the addition of water) in the contrasting media over an 8-week period.

Embryonation is possible in the medium of hair but the rate of development is significantly lower than that observed in the medium of soil. Temperature is an essential factor determining the rate of embryonation. For example, no embryonation whatsoever occurred at 10 °C with no water added. However, at the higher temperature of 20 °C in the presence of water, embryonation rates were higher in both soil and hair but significantly higher still in soil compared with hair (Keegan and Holland, 2013).

Although the presence of *Toxocara* eggs on the hair of foxes is unlikely to be of any epidemiological significance to humans, comparative data from stray dogs and foxes do provide a possible insight into the epidemiology of *Toxocara* eggs on the hair of dogs. The prevalence and mean eggs per gram of hair on adult dogs are higher than that of adult foxes (prevalence 45 *vs* 25%, mean eggs per gram of faeces (epg) 279 *vs* 1.3). In contrast, higher worm burdens were detected in foxes compared with dogs (prevalence 22.5 *vs* 61%, mean worm burden 3.55 *vs* 0.5) (Roddie *et al.* 2008a, b). The relationship between eggs on the hair and worm burden in foxes was not significant, analogous to that found for adult dogs. This provides indirect evidence that in comparison with foxes, adult dogs are acquiring higher numbers of eggs on their hair. This may be explained by the fact that such dogs inhabit more contaminated environments (due to the presence of other dogs). Lee *et al.* (2010) speculated about the role of scent-rolling among dogs in the acquisition of eggs on the hair. To conclude, it would appear, based upon the available evidence, that there is a low risk of transmission associated with the very low numbers of embryonated eggs found on hair, but the suitability of hair as a medium for oval development should not be ignored.

Variation in exposure to Toxocara infection and the implications for disease

Humans act as paratenic hosts for *Toxocara* infection. On ingestion, embryonated eggs hatch in the small intestine and release larvae that migrate through the tissues and some organs, but do not successfully develop to adulthood in the small intestine as they do in definitive hosts. Definitive diagnosis of *Toxocara* infection is by histopathological examination and morphological and morphometric or molecular identification of larvae in tissue samples (Smith and Noordin, 2006). However, such diagnosis requires biopsy material, the collection of which is invasive and may not even contain larvae of *Toxocara*. It is not a practical approach for routine examination. Therefore, the mainstay of diagnosis of human toxocariasis is serology (Smith *et al.* 2009).

There is significant variation in seroprevalence values worldwide and a number of potentially

Table 1. Summary of studies on the presence of *Toxocara* eggs on the hair of dogs and cats

Study	Geographical location	Pet status (sample size)	Prevalence (%)	Total eggs	Embryonated (total number)	Embryonated (%)
Wolfe and Wright (2003)	UK & Ireland	Owned and stray 60	25	71	3	4.20
Roddie <i>et al.</i> (2008b)	Ireland	Stray 100	67	39 120	120	0.31
Aydenizoz-Ozkayhan <i>et al.</i> (2008)	Turkey	Owned 51	21.6	62	5	8.1
Overgaauw <i>et al.</i> (2009)	Netherlands	Owned 152 (dogs) 60 (cats)	12.2, 3.4	148, 59	0	0
Keegan and Holland (2010)	Ireland	Owned 182	8.8	26	0	0
Amaral <i>et al.</i> (2010)	Brazil	Owned and stray 104	24	881	0	0
El-Tras <i>et al.</i> (2011)	Egypt	Owned and stray 56 (owned) 64 (stray)	10.7, 26.6	584, 2639	16, 53	2.74, 2
Oge <i>et al.</i> (2014)	Turkey	Owned 100 (dogs) 100 (cats)	14, 22	136, 58	0, 2	0, 3.44

confounding factors that may influence the observed values. These include the specificity of the serological test employed, the choice of *Toxocara* antigen, what cut-off titre was employed to designate seropositivity and the composition of the population surveyed (Holland *et al.* 1995). However, despite these caveats, some trends can be observed which include a generally higher seroprevalence in the tropics and among the disadvantaged. For example, a recent seroprevalence survey of primary school children from an urban slum in Lagos state, Nigeria, revealed an 86.1% seropositivity (Western blotting) with risk factors including child age, contact with young dogs, the feeding location of the dogs, consumption of raw vegetables and drinking unboiled water (Gyang *et al.* 2015). However, in the same country, but from Jos, a seroprevalence value of 29.6% (ELISA, enzyme-linked immunosorbent assay) was reported (Ajayi *et al.* 2000).

Even within Europe, where seroprevalence values are much lower than those reported from Nigeria, significant variation is observed. A study of over 3000 serum samples from Denmark reported a seroprevalence of 2.4% (ELISA followed by western blotting) (Stensvold *et al.* 2009). This contrasts markedly with two values reported from Ireland – a large-scale study of schoolchildren (from randomly selected schools) with a value of 31% (ELISA cut-off titre 1:50) (Holland *et al.* 1995), and a more focused (non-random) investigation of asthmatics and their families with a seroprevalence value of 52.1% (ELISA cut-off titre 1:50) (Taylor *et al.* 1988).

In a clinic-based case control study conducted in the USA to explore the relationship between exposure to *Toxocara* and asthma, investigators found a striking difference in seroprevalence among children, aged 2–15 years, from two towns in Connecticut – 6.1% in New Haven and 28% in Bridgeport (ELISA cut-off titre 1:32) (Shargi *et al.*

2001). Furthermore, children who were seropositive for *Toxocara* were 12 times more likely to be Puerto Rican, eight times more likely to be of other Hispanic origin and seven times more likely to be of Negroid than Caucasian origin.

In a significant departure in terms of scale, a number of investigators have taken advantage of the US National Health and Nutrition Examination Survey (NHANES), which is a nationally representative cross-sectional survey of over 33 000 people. The third such survey took place between 1988 and 1994, and serum samples were analysed for the presence of *Toxocara* antibodies. The age-adjusted seroprevalence for toxocariasis was 13.9% and was higher in non-Hispanic blacks (21.2%) than both non-Hispanic whites (12%) and Mexican Americans (10.7%). Increased *Toxocara* seropositivity was associated with a number of variables: the levels of education of the head of household, poverty, elevated blood lead concentrations and dog ownership (Won *et al.* 2008). This large scale national study was possible because the evaluation of exposure to *Toxocara* was linked to the NHANES survey which was designed to collect health statistics from a large sample of people representative of the civilian, non-institutionalized general US population. The authors highlighted that the striking differences in seroprevalence could be used to target health education messages. Such data from an equivalent European population would be very valuable.

In further work utilizing the NHANES data set, Congdon and Lloyd (2011) evaluated the relative risk of *Toxocara* infection for 20 396 survey subjects using a binary regression model that incorporated demography, family poverty and geographic location. It is of interest that even after allowing for the elevated risk associated with poverty, ethnicity still played an important role in explaining increased

risk of exposure. Prevalence estimates were particularly elevated among non-Hispanic blacks, most notably in the South and Northeast. Hotez (2008) speculated that this elevation might reflect differential contextual exposures linked *inter alia* to ethnic residential clustering and segregation, as distinct from the impact of family poverty *per se*. These observations are particularly potent when linked to the findings of Walsh and Haseeb (2012) as discussed in the section on cerebral toxocariasis.

A major gap in our understanding of the epidemiology of human toxocariasis is our persistent inability to distinguish exposure to *T. canis* vs *T. cati* using serological methods. In many respects our knowledge of *T. cati* is depauperate (Fisher, 2003), but its relative contribution to human exposure remains one of the most pressing issues. In a recent report by Poulsen *et al.* (2015) western blotting was used to attempt to distinguish between sera obtained from pigs infected with *T. canis* and *T. cati*. No proteins were observed that could be used to discriminate between the two ascarid species. The authors emphasized the pressing need to develop species-specific serological methods in order to evaluate the relative significance of *T. canis* vs *T. cati* as aetiological agents of human exposure and consequent disease.

Since the discovery by Wilder (1950) of granulomata in the eyes of children that had been mistakenly diagnosed with retinoblastoma, and the subsequent description of what are now known to be third-stage larvae of *T. canis* in histological sections from such granulomata by Nichols in 1956 that led to the description of OT, the number of syndromes associated with human toxocariasis has expanded. At present there are four distinct clinical entities – visceral larva migrans (VLM), OT, covert toxocariasis (CT) and cerebral toxocariasis or neurotoxocariasis (NT). However, the relationships between these clinical entities and specific symptoms or clinical features are not well understood particularly because of the non-specific nature of most of symptoms (Smith *et al.* 2009).

One of the key difficulties in evaluating the public health significance of toxocariasis is what exposure actually means. In other words, we can say that an individual is exposed to the *Toxocara* parasite as a consequence of detecting a positive titre at a particular cut-off, but what is the significance of such a diagnosis in terms of disease or symptomatology? In a study of 221 individuals (comprising both patients attending an outpatients clinic that had been identified as having high titres and asthmatic patients and their families), the relationship between titre and clinical and laboratory features was explored (Taylor *et al.* 1988). Titres were divided into three categories (I = low or negative titre 0–0.29 $n = 41$; II = moderate, titre 0.30–0.69 $n = 51$; and high titre ≥ 0.7 $n = 129$) and the highest proportion of patients

with 9–16 clinical features was found amongst those with the highest titres (51%). The clinical features most commonly associated with *Toxocara* titres of 0.30 and above were abdominal pain, hepatomegaly, anorexia, nausea, vomiting, lethargy, sleep and behaviour disturbance, pneumonia, cough, wheeze, pharyngitis, cervical adenitis, headache, limb pains and fever. All of these non-specific clinical features are commonly reported in childhood and this study provides support for the view that a proportion of cases of ‘idiopathic abdominal pain of childhood’ is due to toxocariasis (Taylor *et al.* 1987). In the Taylor study, 61% of the subjects that reported abdominal pain had raised *Toxocara* titres.

In an important study providing the kind of data that are sorely lacking, Wisniewska-Ligier *et al.* (2012) evaluated the clinical course of toxocariasis in 103 Polish children who were treated at a hospital’s zoonotic diseases outpatient facility. Among clinically diagnosed children ELISA absorbance values between 32 and 100% were deemed to confirm infection. Children were aged between 1.4 and 14.7 years and lived in towns or villages. The clinical symptoms associated with toxocariasis are shown in Table 2. The vast majority of children were diagnosed with the covert form of the disease (95.1%) with 4.9% with OT. The most common symptom was abdominal pain (Table 2). Children were treated with either albendazole or mebendazole or a combination of both anthelmintics while those children with eye disease were treated in consultation with ophthalmologists and received thiazibendazole, albendazole and/or mebendazole in combination with steroids. Some children required repeated treatment with 45% receiving three courses of anthelmintic treatment. After treatment, the mean titre and the number of children with abdominal pain and lymphatic node enlargement declined, but no decline in headaches was observed after one and two courses of treatment. Even after the third course, the decline did not attain statistical significance (Table 2). In some children, despite the observation of negative titres, symptoms persisted and this was particularly marked for headaches. The authors suggest that this diminishes the value of using headache as a symptom of covert toxocariasis. The authors conclude that due to the risk of eye disease, anti-parasitic treatment should be implemented, but their observations provide important evidence of the long-term persistence of symptoms despite several rounds of treatment.

To conclude, our knowledge to date indicates that seroprevalence values can be high and vary significantly even within the same country, that higher titres are associated with greater symptomatology and that symptoms can persist despite rounds of anthelmintic treatment. However, we still do not know the relative contribution of *T. canis* vs *T. cati* to human exposure and there is a paucity of studies

Table 2. Characteristics and symptomatology of children with toxocariasis (Wisniewska-Ligier *et al.* 2012)

Clinical symptoms	Number of children	% of children
Eosinophilia	45	64.3
Abdominal pain	36	35
Enlargement of lymph nodes	30	29.1
Allergic symptoms (history)	23	22.3
Headache	19	18.4
Loss of appetite	6	5.8
Changes in the eye	5	4.9
Subfebrile conditions	2	1.9
Arthralgia	2	1.9
Mild anaemia	1	1
Sex		
Male	64	62.1
Female	39	37.9
Place of residence		
Town (>200 000)	12	11.7
Town (<100 000)	20	19.4
Village	71	68.9
Effect of treatment		
Abdominal pain	Before	After
First treatment	36 (35%)	29 (29.1%)
Second treatment	17 (23.6%)	9 (12.5%)
Third treatment	11 (23.9%)	4 (8.7%)*
Effect of treatment	Before	After
Headache	19 (18.4%)	18 (18.4%)
First treatment	19 (18.4%)	18 (18.4%)
Second treatment	9 (12.5%)	6 (8.3%)
Third treatment	4 (8.7%)	1 (2.2%)

* Values differ statistically significantly before and after treatment, $P \leq 0.05$.

linking exposure to disease. The exemplary large-scale study by Won *et al.* (2008) indicates that certain high-risk populations can be identified by means of seroprevalence surveys and that public health education could be targeted to such groups.

The extent of ocular toxocariasis

Toxocara larval involvement in the eye, with consequent visual impairment, remains potentially the most devastating of all human sequelae (Good *et al.* 2004). Ocular toxocariasis is generally described as a relatively rare disease primarily observed in children (Taylor, 2006). In this context, one major gap in our knowledge relates to population-based assessments of the prevalence of infection. One of the reasons for this is that toxocariasis is not a reportable disease. A population-based study of 121 156 school children in Ireland reported a prevalence of consultant-diagnosed toxocaral eye disease of 6.6 cases per 100 000 persons that increased to 9.7 cases per 100 000 persons once both definite and strongly suspected cases were included. Geophagia and a history of convulsion were associated with toxocaral eye disease for both school and county-based case control studies

(Good *et al.* 2004). This study provided important data on the prevalence of eye disease among a defined population. It indicates that OT is a rare disease among Irish children (aged 3–19 years) – a country where the previously-recorded seroprevalence rate among a similar schoolchild population (31%) was relatively high compared with other European countries (Holland *et al.* 1995; Smith and Noordin, 2006).

To our knowledge, only one other documented estimate of the prevalence of OT has been published (as an abstract). It reports data collected from eye clinics in Alabama over a 6-month period and yields an estimate of 1 case per 1000 persons (increasing to 11 cases per 1000 persons once ophthalmoscopy had been carried out) (Maetz *et al.* 1987). This value indicates that the prevalence of eye disease is higher in Alabama than in Ireland; indeed it is of interest to note that a more recent web-based survey of American ophthalmologists reported that among 68 patients diagnosed with OLM, 57% emanated from the South (Centres for Disease Control and Prevention, 2011). The most common symptom was vision loss (83%) ($n = 37$) with permanent vision loss among 68% ($n = 25$). The preponderance of neglected parasitic infections, including toxocariasis, in the Southern USA was highlighted by Hotez (2014b).

Most reports of OT tend to focus on children since the disease is more common in those age groups (see Taylor, 2006). However, a recent paper described the clinical features and course of ocular infection in adults in South Korea (Ahn *et al.* 2014). This retrospective cohort study included 101 adult patients diagnosed clinically and serologically with OT. The vast majority of patients (92.1%) were diagnosed by means of the presence of a retinal granuloma and 17 (16.8%) had severe vision loss. Ingestion of raw cow liver and meat was significantly more common among OT cases compared with controls. Combined treatment with albendazole and corticosteroids reduced intraocular inflammation and recurrence.

To conclude, we particularly lack information on the prevalence and pathological implications of OT in populations from the tropics where seroprevalence values can be as high as 93% (Smith and Noordin, 2006).

Cerebral toxocariasis: the most cryptic of all disease syndromes?

The presence of *Toxocara* larvae in the human brain, now described as cerebral toxocariasis or NT, has always been a source of fascination for parasitologists. But of all the disease entities so far described, it is the most cryptic. However, due to a combination of factors – such as improved diagnosis, greater awareness and the use of animal models – our

understanding of the condition is becoming better understood and its public health significance, is improving. Cerebral toxocariasis was first described in an autopsy study of a child, in whom a larva was found in the left thalamus. Initially it was described as a larval *Ascaris*, but after the work of Nichols (1956) it was correctly identified as a larva of *T. canis* (Beautyman and Woolf, 1951, Beautyman *et al.* 1966). In a recent review of the literature, Fan *et al.* (2015) found reports of 86 patients with various neurological manifestations. Nevertheless, given the high levels of exposure to *Toxocara*, the extent of neurological involvement and its manifestations are likely to be significantly underestimated.

One key gap in our knowledge relates to the public health significance of the presence of what are likely to be small numbers of larvae in a human brain. Clearly in some cases the impact can be profound. For example, the symptoms of eosinophilic meningitis were described by Vidal *et al.* (2003) in a 2 year-old boy and included mental confusion, fever, headache, tachycardia, hyperreflexia, dyspnea, lethargy, irritability, motor weakness and nuchal rigidity. *Toxocara* specific immunoglobulin G (IgG) antibodies were detected in both cerebrospinal fluid (CSF) and serum and most symptoms declined in response to treatment with albendazole and corticosteroids. However, many other infections may be asymptomatic or manifest as non-specific behavioural alterations.

There is a paucity of studies exploring the relationship between exposure to *Toxocara* and neuropsychological parameters in young children (Holland and Hamilton, 2013). However in an important contribution, Walsh and Haseeb (2012) measured different components of cognition in a large number of seropositive and seronegative children from the USA, again utilizing the NHANES survey database. Seropositive children ($n = 688$) had significantly lower measures of cognitive function compared with seronegative children ($n = 3261$), after controlling for important potentially confounding variables such as socio-economic status, gender, ethnicity, residence, cytomegalovirus and blood lead levels. Despite being an associational study, these findings are important, particularly as evidence from mouse models has demonstrated clearly reduced learning and memory in infected animals (Hamilton *et al.* 2006).

The logistical and ethical challenges associated with conducting studies on the relationship between cognition and parasitism are manifest (Bundy *et al.* 2009). Consequently, we need good quality laboratory studies in animal models to unravel the relationship between behavioural deficits, neuropathology and larval burden (Fan *et al.* 2015). Mice are useful in this respect because of the ease of manipulation and the availability of inbred murine strains (Holland and Hamilton, 2013).

Early work established the fact that *T. canis* larvae accumulate in the murine brain (Dunsmore *et al.* 1983) and can remain there for long periods of time (Bardon *et al.* 1994). Cox and Holland (2001a) explored the optimum dose of *Toxocara* eggs required in experimental infections and concluded that low dose infections mimic those found in wild mice, whereas higher doses may be selected by the experimenter in order to achieve marked accumulation over time. A wide range of behavioural alterations was observed in both outbred and inbred mice experimentally infected with *Toxocara* (Cox and Holland, 1998; Cox and Holland 2001a, b). However, of particular relevance to human infections was the observation of reduced learning and memory in inbred susceptible BALB/c (inbred strain of mouse) mice infected with *Toxocara* (Hamilton *et al.* 2006).

Among mouse model studies of the link between cerebral toxocariasis and memory impairment, Holland and Hamilton (2013) identified a variety of important host and parasite variables, some of which may be confounders. These include inbred mouse strain, infective dose, duration of infection, method of larval recovery, behavioural tests employed and choice of immunological/neuropathological measures.

To conclude, we now require both data from longitudinal studies in humans to examine the etiological connection between toxocariasis and impaired cognitive function (Walsh and Haseeb 2012), and from animal model work dissecting simultaneously the relationship between learning and memory deficits, neuropathology and immunological responses (Holland and Hamilton, 2013; Fan *et al.* 2015).

Improved integration of our understanding of environmental contamination with eggs of Toxocara spp.

The presence of potentially infective or infective eggs of *Toxocara* spp. in the environment is one of the key routes of transmission to humans. Infected definitive hosts such as dogs, cats and to a lesser extent foxes release their feces, and the helminth eggs within them, into the environment. However, our understanding of the relative importance of the different host sources is incomplete and more hypothesis-driven studies with better integration are urgently required. One of the difficulties is that there are many studies conducted every year on the extent of environmental contamination with *Toxocara* eggs, but because they tend to vary methodologically, valid comparisons between regions, countries and continents are problematic. For example, in a recent review of environmental contamination with helminth eggs, Traversa *et al.* (2014) tabulated 28 different surveys from both

temperate and tropical countries worldwide, with contamination rates for roundworms ranging from a low value of 0.5% to a high of 79.4%. However, when looking at these data it is very difficult to identify any clear trends and considerable variation is exhibited, even within the same geographical location. For example, in Poland soil contamination varied from 3.2 to 26.1% and in Italy from 0.7 to 33.6%. Most studies collected soil samples, but how such samples were collected (site, number and volume, season, representation, depth, soil quality, etc.) and analysed can vary considerably (Mizgajska-Wiktor and Uga, 2006).

Furthermore, the identification of *Toxocara* eggs to species level (i.e. *T. canis* vs *T. cati* vs *T. leonina*) is not always performed and discrimination is not easy (Mizgajska-Wiktor and Uga, 2006). In a survey of public playgrounds in Dublin, Ireland, O’Lorcain (1994b) recorded almost entirely *T. canis* eggs with no *T. cati* and a single *T. leonina* egg. In contrast, from Krakow and two nearby villages, Mizgajska (2000) recorded, 90% of the eggs recovered as *T. cati* with only 10% being *T. canis*. At the light microscope level, *T. canis* and *T. cati* can be distinguished by size (O’Lorcain, 1994b). Recently, a duplex quantitative real-time polymerase chain reaction (PCR) assay has been described that can detect and discriminate between the eggs of *T. canis* and *T. cati* in soil samples (Durant *et al.* 2012).

As is the case for the studies of eggs on the hair of definitive hosts, the proportion of eggs that are embryonated is an important additional piece of information required from soil contamination studies, but such data are not always reported. In a summary of 13 studies of soil contamination that did provide such data, embryonation rates varied considerably from 0.7 to 94.8%. Indeed these two widely diverging figures came from the climatically similar countries of the UK and Ireland (Roddie *et al.* 2008b). Furthermore, the relationship between soil contamination and seropositivity in the human population is not conclusive (Traversa *et al.* 2014 but see Manini *et al.* 2012). This is not particularly surprising, given the wide range of possible sources of infective eggs that a human being might be exposed to (gardens, allotments, public parks, sports fields etc.) Therefore, the question should be asked – how useful are such surveys and what are they telling us?

In a significant advance, an improved quantitative framework for the epidemiology of egg contamination was developed in the city of Bristol, UK (Morgan *et al.* 2013). The authors collected both empirical data (parasitology – prevalence, egg density and host data – faecal output, population density, age and status (owned vs stray)), and utilized a modelling approach. Their key conclusion was that in the absence of a large population of stray dogs and cats, pet dogs (especially those less than 12 weeks of age), dominate total egg output.

This output can be modified by the degree of dog access to public areas and the removal of feces from these areas. However, under certain circumstances foxes can also play a role as contributors to egg contamination. Unsurprisingly, patterns of egg contamination are likely to vary significantly by location and be influenced by the relative proportion of different definitive hosts and their status (stray vs owned), the age structure of such hosts, rates of anthelmintic treatment and habitat use. Interestingly, the authors use their data to emphasize the need to eliminate infection in younger dogs in tandem with improved social responsibility in removing dog feces from public places.

Pertinent to this is the publication of a systematic review of interventions to prevent dog fouling (Atenstaed and Jones, 2011). Of 68 interrogated articles, none fulfilled the authors’ inclusion criteria and the conclusion was that no good-quality studies had been undertaken to assess interventions to prevent dog fouling. Clearly such interventions, in tandem with the approach adopted by Morgan *et al.* (2013) would be extremely beneficial.

Building upon the work of Morgan *et al.* (2013), Nijse *et al.* (2015b) developed a stochastic model to quantify the relative contribution of household dogs, household cats, stray cats and foxes (all older than 6 months of age) to environmental contamination with *Toxocara* spp. eggs in the Netherlands (stray dogs were not included as the Netherlands is free of such animals). The choice of older animals was linked to the debate about whether these animals shed many eggs and require treatment four times per year (Nijse *et al.* 2015a). Both parasite variables (prevalence and intensity of infection) and host factors (density, coprophagic behaviour, feces disposal by owners, cats’ outdoor access) were included in the model. Scenario analyses were performed to evaluate the impact of different deworming strategies and feces clean-up compliances on the expected reduction in dog’s egg output. Dogs were found to be the main contributors to environmental contamination with *Toxocara* ova. However, stray cats, owned cats and foxes also contribute eggs to the environment, and in urban areas egg output is dominated by stray cats. Furthermore, intervention scenarios revealed that only a very high compliance with the four-times-per-year deworming recommendations would yield a reduction in the contribution of dogs to egg output.

The authors made an important observation that due to the role of stray cats and foxes (and stray dogs in other contexts) control focused upon household pets alone is not sufficient to reduce environmental contamination to very low levels. Models of this kind are a very useful tool in quantifying the sources of *Toxocara* eggs in a given locality so as to prioritize control interventions and to assess the impact of such interventions. Studies such as those of Morgan *et al.*

(2013) and Nijssse *et al.* (2015b) also provide a framework for a more hypothesis-driven approach to the study of environmental contamination.

The role of paratenic hosts in the transmission of Toxocara: the biggest gap of all?

Our knowledge of the significance of paratenic hosts, and in particular feral paratenic hosts, as sources of infection for definitive hosts, both domestic and feral, is probably one of the largest gaps in our understanding of the epidemiology of *Toxocara*. Paratenic hosts are defined as hosts in which development does not occur, but may serve to bridge an ecological, or trophic, gap in a parasite's life cycle (Bush *et al.* 2001). Furthermore, paratenic hosts are likely to disseminate infective stages of the parasite or aid these stages in avoiding unfavourable conditions such as the temporary absence of a definitive host. In the case of *Toxocara*, large numbers of eggs are released into the environment by fecund adult worms inhabiting the intestinal tract of domestic and feral definitive hosts and these eggs can be consumed by paratenic hosts. Our knowledge of the relative infective capacity of a range of vertebrate and invertebrate paratenic hosts is virtually non-existent (Holland and Hamilton, 2006).

There are only a handful of published studies on the seroprevalence and larval burden of *Toxocara* in feral paratenic hosts and these are all confined to small mammal hosts. One of the most comprehensive studies is that of Dubinsky and colleagues (1995) from Slovakia, but it is of concern to note that this was published 20 years ago! Eleven small mammal species were investigated for the presence of parasite-specific antibodies in sera and the presence of *Toxocara* larvae in the brain and the hind leg femoral muscles. Considerable variation between hosts was observed with no detectable seropositivity in *Rattus norvegicus* and the highest level recorded (32%) in the house mouse, *Mus musculus*. The intensity of larval burden was low relative to the proportion of seropositive animals, in the order of one–three larvae per brain, with a higher intensity observed in mammals from suburban locations. These numbers are similar to those recovered from outbred laboratory mice exposed to a dose of 100 eggs under experimental conditions (Cox and Holland, 2001a). Dubinsky *et al.* (1995) concluded that small mammals could act as important foci for the circulation and maintenance of *Toxocara* in the environment. To my knowledge this is the only published study that reported intensity of larval burden in a feral host. More recently, Antolova *et al.* (2004) reported seropositivity from 10 non-commensal rodents from the Slovak Republic, confirming the higher seropositivity from suburban locations but identifying the highest seropositivity in *Apodemus agrarius* (21%).

The role of small mammals as sentinels for the degree of environmental contamination, with *Toxocara* eggs and other important parasitic infections, such as *Echinococcus multilocularis* and *Toxoplasma gondii*, was highlighted by Reperant *et al.* (2009). The highest *Toxocara* seroprevalence (13.2%) was reported in an urban area of Switzerland among four species of non-commensal rodents. In parts of the world where raccoons are present, small mammals could also act as important sentinels for the presence of the highly pathogenic and emerging zoonotic infection, *Baylisascaris procyonis*.

Unfortunately, no data whatsoever exist on the species identity of the *Toxocara* larvae found within the tissues of feral paratenic hosts such as house mice. It would be very interesting and epidemiologically useful to know if the larvae are *T. canis*, *T. cati* or *T. leonina* as this would shed light upon the relative importance of different ascarid species within the tissues of paratenic hosts and therefore, what species are most likely to be disseminated to definitive hosts via this route of transmission. The first and second internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal DNA (rDNA) were first used by Jacobs *et al.* (1997) to identify and differentiate between three species of adult worms – *T. canis*, *T. cati* and *T. leonina*. Such diagnostic PCR could also be used on homogenized tissue to detect and identify *Toxocara* larvae in paratenic hosts (Gasser, 2013). This is particularly interesting given the so-far unproven supposition that infected rodent prey may be a more important source of infection for cats than dogs.

Laboratory models of paratenesis. In contrast to the paucity of data from feral paratenic hosts, our knowledge of experimental infection with (mainly) *T. canis*, and the consequent larval migration, under laboratory conditions is more comprehensive. Paratenic hosts infected under these conditions include mice, rats, guinea pigs, hamsters, gerbils, chickens, quail, pigeons, rabbits, pigs and monkeys (Fenoy *et al.* 2001; Holland and Hamilton, 2006; Strube *et al.* 2013). Some species are more useful than others in terms of reflecting the risk of infection to humans (as food items) or particular aspects of human infection. For example, gerbils are particularly susceptible to eye involvement and are, therefore, good model organisms to explore the pathogenesis of OT (Takayangi *et al.* 1999; Akao *et al.* 2000; Alba-Hurtado *et al.* 2000). Holland and Hamilton (2006) argued that gerbils are less satisfactory model organisms for cerebral toxocariasis, compared with mice, due to the development in gerbils of irreversible brain damage after chronic infection with *T. canis* (Akao *et al.* 2003). In some parts of the world, there is evidence to suggest that humans can become exposed to *Toxocara* larvae as a result of the consumption of raw or undercooked chicken

(Ito *et al.* 1986; Nagakura *et al.* 1989). Experimental infections of chickens with *Toxocara* have demonstrated long-term survival of larvae even at low temperatures thereby underlining the public health risk to humans (Sprent, 1953; Taira *et al.* 2011, 2012).

Following the original work of Done *et al.* (1960), several authors have investigated the pig as a model for human infection (Helwich *et al.* 1999; Taira *et al.* 2003). *Toxocara* larvae were eliminated early in infection and little eye or brain involvement was observed, thereby diminishing the usefulness of the pig as a model system. Furthermore, the large size of the porcine organs makes investigation less tractable. However, consumption of pork may pose a potential zoonotic risk for humans.

The model system that has received more attention than any other is that of the mouse. Significant advantages include ease of manipulation, small organ size for easy detection of larvae, availability of inbred and knockout strains and the fact that mice form part of the natural life-cycle of *Toxocara* in the wild (Holland and Hamilton, 2013). Furthermore, larvae are known to accumulate in the murine brain over time (Dunsmore *et al.* 1983) and show significant variation in larval burdens between individual outbred mice (Skerrett and Holland, 1997), thereby suggesting a role for host immunity and genetic resistance/susceptibility to infection (Dold and Holland, 2011). The contribution of factors such as strain (outbred *vs* inbred), dose, days post-infection and larval burden in the brain have been explored both with respect to larval migration and impact on host behaviour (Epe *et al.* 1994; Cox and Holland, 2001*a, b*; Holland and Cox, 2001; Hamilton *et al.* 2006). Interesting differences between the larval migration of *T. canis* *vs* *T. cati* have been observed in mice; *T. canis* larvae accumulate to a greater extent in the murine brain whereas *T. cati* larvae show greater concentration in the muscles (Havasiova-Reiterova *et al.* 1995). Some authors have argued that because *T. cati* larvae are smaller they may be able to exit the arteries more easily whereas *T. canis*, being larger, are more likely to be trapped in the brain tissue (Strube *et al.* 2013). A comparison of the evidence for brain involvement and subsequent accumulation of *Toxocara* larvae was undertaken by Holland and Hamilton (2006) in a range of experimentally-infected animals and it is mice that account for most of the evidence. Such studies underline the important biological differences that exist between the migratory pathway of *Toxocara* spp. and larval involvement in key organs such as the eye and the brain in different paratenic hosts.

Toxocara cati: even more enigmatic?

In 2003, Maggie Fisher described *T. cati* as ‘an underestimated zoonotic agent’. Our understanding

of the role of *T. cati* in exposure to humans, environmental contamination and within paratenic hosts has been hindered by our inability to distinguish between the two species in these various media. Serological differentiation remains a problem but ova and larval identification using molecular methods is now possible (Durant *et al.* 2012; Gasser, 2013). However, as outlined previously, there are no data on the species of third-stage larvae detected in paratenic hosts from the wild. Recently, *T. cati* eggs, identified by PCR, have been detected in dog feces and the explanation for this phenomenon is likely to be coprophagy (Fahrion *et al.* 2011).

Despite receiving less attention than the dog ascarid *T. canis*, a number of interesting biological differences between the two *Toxocara* spp. have emerged. There is evidence to suggest that *T. cati* may be more resistant to cold temperatures. Experimental evidence showed that *T. cati* eggs are more resistant to freezing compared with those of *T. canis* (O’Lorcain, 1995) and *T. cati* infections were used to establish the ability of larvae to withstand cold temperatures in chicken tissue (Taira *et al.* 2012). Furthermore, Akao *et al.* (2000) demonstrated that *T. cati* larvae can migrate to the eye and cause significant pathology in a relatively novel animal model – the Mongolian gerbil. In eight cats and their offspring, the mode of transmission following both natural and experimental infection was explored (Coati *et al.* 2004). Lactogenic transmission of larvae occurred after an acute infection of the queen during late pregnancy but did not occur during chronic natural infection. No evidence of arrested somatic larvae was observed in adult cats.

Differential pathogenesis at the level of the transcriptome was explored for *T. canis* and *T. cati*, respectively (Janecek *et al.* 2015). Major differences between the two species were observed, with *T. canis*-infected brains demonstrating significant disruption of lipid biosynthetic processes. The authors suggest that such disruption may lead to dysfunction in signal transduction and neurodegenerative disease. On day 42 post-infection, *T. canis* mice exhibited partial paralysis of hind limbs as well as ataxia, in contrast to *T. cati*-infected mice that exhibited no neurological lesions at *post-mortem*.

Toxascaris leonina. Experimental infections (from eggs derived from both canine and feline hosts) were originally established in laboratory mice by Sprent (1959) demonstrating that this parasite can infect paratenic hosts. However, the role of *T. leonina* in human disease remains unknown, particularly given our current inability to distinguish *Toxocara* spp. serologically (Gasser *et al.* 2006). It has been suggested that the zoonotic potential of *T. leonina* is limited because somatic migration in definitive hosts does not occur as part of the normal life-cycle and larvae are not vertically transmitted (Overgaaauw and Van Knapen, 2000).

Concluding remarks

How best to move the research field forward in the context of a one health approach. A number of recent observations have increased our awareness of the public health significance of toxocariasis. Evidence that *Toxocara* can be found in the human brain has existed since the 1950s but since then the numbers of cases of cerebral toxocariasis described in the literature has increased due to improved diagnosis and greater awareness among medical clinicians (Fan *et al.* 2015). Despite its associational nature, the link between cognitive deficits and *Toxocara* seropositivity, described by Walsh and Hasseb (2012) in a large and well-controlled sample, is important and requires further investigation. This is particularly so given the very large number of children shown to be exposed to infection, especially in the developing world and in areas of disadvantage within, for example, the USA. However, in order to move *Toxocara* up the public health agenda in organizations such as the WHO, greater scientific integration is required in order to understand the complex epidemiology of this enigmatic parasite and the disease it causes. The one health approach provides an ideal framework as veterinarians, medical clinicians (including ophthalmologists), parasite epidemiologists, environmental health experts and wildlife biologists are all required to participate in such an improved integrated research strategy that is essential if we are to achieve a full understanding of the different strands of the epidemiology of *Toxocara* spp. Furthermore, greater involvement in the funding of such research by drug companies that sell anthelmintic products for roundworms would be desirable. Some indications of the usefulness of this approach can be seen in the work of Won *et al.* (2008) and Lee *et al.* (2010). The former highlighted how the striking differences in seroprevalence observed in the USA could be used to target health education messages. The latter that accurate assessment of seroprevalence and clinical disease in people and companion animals would allow for future targeted interventions and management of zoonotic threat.

Perhaps we should now consider an ideal scenario initially involving a single country or defined region, in which sources of infection in humans, other animals and the environment are comprehensively assessed: *Toxocara* in domestic and feral definitive hosts (dogs, cats and foxes), seropositivity in humans and the extent of significant disease (both OT and CT), detection of eggs in the environment and in paratenic hosts (ideally both feral and domestic). Species identification of eggs and larvae, at the molecular level, would be crucial. Subsequent to this baseline epidemiological investigation and based upon relative seroprevalence values from the human population, an intervention study could be

employed providing systematic anthelmintic treatment of dogs and cats and the instigation of rigorous anti-fouling approaches and appropriate follow-up measurements in humans, definitive hosts and the environment. Such an approach would undoubtedly be ambitious, costly and logistically challenging but for the first time it would place *Toxocara* in a one health context and provide a framework for future prevention and control. This would in turn, flesh out and enrich our understanding of how best a one health approach should be conducted in the context of a complex and cryptic zoonosis.

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