Human toxocariasis

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Parasitic nematodes of the genus *Toxocara* are socioeconomically important zoonotic pathogens. These parasites are usually directly transmitted to the human host via the faecal–oral route and can cause toxocariasis and associated complications, including allergic and neurological disorders. Although tens of millions of people are estimated to be exposed to or infected with *Toxocara* spp, global epidemiological information on the relationship between seropositivity and toxocariasis is limited. Recent findings suggest that the effect of toxocariasis on human health is increasing in some countries. Here we review the salient background on *Toxocara* and biology, summarise key aspects of the pathogenesis, diagnosis, and treatment of toxocariasis, describe what is known about its geographic distribution and prevalence, and make some recommendations for future research towards the prevention and control of this important disease.

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Introduction

The first diagnosis of human toxocariasis was the detection of *Toxocara* larvae in enucleated eyes from children with suspected retinoblastoma more than 60 years ago.[1,2] Histological examination of the retinas of these diseased eyes revealed granulomatous lesions that likely contained *Toxocara canis* larvae.[2] Since then, various clinical forms of human toxocariasis have been recognised: ocular larva migrans, visceral larva migrans, covert or common toxocariasis, and neurotoxocariasis.[3–5] Despite the clinical recognition of these syndromes and the rising awareness that human toxocariasis is causing an increasing burden to public health, particularly in subtropical and tropical regions and in disadvantaged communities in some countries,[6–11] there are still major gaps in the understanding of this disease.[7,12–19] Indeed, evidence from recent investigations suggests that human toxocariasis is seriously neglected because limited attention has been paid to its prevention, treatment, and surveillance and because it is a non-notifiable disease.[20–22]

A major challenge in preventing toxocariasis is the complexity of infection sources of *Toxocara* spp and routes of transmission, about which still relatively little is known. Adult worms of *Toxocara* spp live in the small intestines of various wild or domestic definitive hosts (figure 1). For instance, *T canis* infects canid hosts, including coyotes, dogs, foxes, and wolves, and *Toxocara cati* (previously *Toxocara mystax*) and *Toxocara malaysiensis* infect felids.[9,23–25] Related species, such as *Toxocara vitulorum*, *Toxascaris leonina*, and *Baylisascaris procyonis*, infect various carnivorous or ruminant hosts.[9] Human toxocariasis acquired from cats and *Toxoplasma gondii* co-infection might be more common than previously thought.[24,26] Infected definitive hosts, such as dogs and cats, excrete eggs in the faeces, which then contaminate the environment under suitable conditions (humidity and temperature)[27–29] where they can remain infective for many months or even years. *T canis* larvae can also undergo arrested development in the tissues of female canids and reactivate during pregnancy to infect pups through both transplacental and transmammary routes.[30,31]

Eggs containing infective third-stage larvae are accidentally ingested by human beings through contact with contaminated food, water, soil, or utensils. In the small intestine, the larvae are released from the eggs, penetrate the intestinal wall, and travel via the circulatory system to various organs, including the lungs, liver, muscles, and CNS. Except in the definitive canid host, third-stage larvae do not mature but can arrest in development within tissues for many years. Tissue infection evokes an inflammatory immune response in the body that can lead to (usually non-specific) symptoms of disease, such as fever, headaches, coughing, and pains.[3,8,32] Infective eggs ingested by paratenic or transport hosts (eg, mice, rats, rabbits, pigs, cattle, or chickens, and earthworms or other invertebrates) undergo a similar fate, with third-stage larvae arresting in tissues.[33] Although larvae never develop to adult worms in the intestine of human beings or other paratenic hosts, larvae-infected tissues from paratenic hosts can serve as food for human beings and definitive hosts, leading to infection or disease. For instance, the consumption of undercooked or raw liver from infected animals has been implicated in toxocariasis.[34,35]

Although eliminating or reducing intestinal infections in definitive hosts (dogs and cats) by chemotherapy is probably the most efficient way of decreasing environmental contamination and subsequent transmission to human beings and other paratenic hosts, the high prevalence of anti-*T canis* serum antibodies in human beings in many regions of the world, particularly disadvantaged communities,[20,22] indicates that this approach is not being adequately implemented. In this Review, we introduce salient aspects of human toxocariasis and *Toxocara* infections, emphasise key knowledge gaps, and discuss future prospects and research areas to improve insights into what is still a neglected parasitic disease of major public health importance.

Clinical manifestations and disease

In human beings, *Toxocara* larvae penetrate the intestinal mucosa and migrate to liver, lungs, and other organ systems (eg, skeletal muscle, heart, brain, and eyes) by mechanical means and protease digestion.[4,5,34] Migrating larvae are attacked by host immune responses, resulting in local inflammation associated

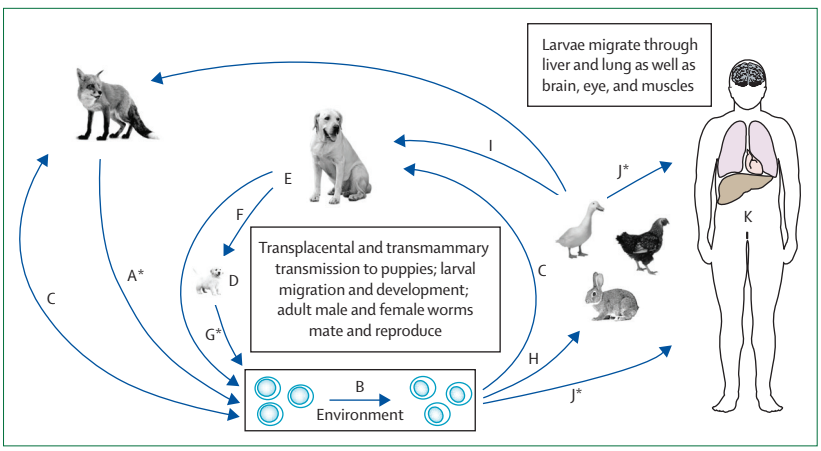

Figure 1: **The life cycle of *Toxocara canis*, the most common species of *Toxocara* infecting human beings**
The life cycle of *T canis* involves canids (including coyotes, dogs, foxes, wolves) as definitive hosts. (A) Eggs of this parasite are released in the faeces of the canid host. (B) Eggs embryonate in the environment and become infective. (C) After ingestion by this host, infective (third-stage) larvae hatch from the eggs, and larvae penetrate the intestinal wall. (D) In young dogs (usually <12 weeks), larvae migrate through the liver and lungs to the airways and then get swallowed and make their way to the small intestine, where fourth-stage larvae develop to adult worms (female and male), mate, and reproduce. (E) In some dogs (usually >12 weeks), oral infection can occur, but larvae tend to encyst in tissues, where they undergo arrested development. (F) In female dogs, encysted larvae are activated, usually in the last trimester of pregnancy, and undergo transplacental (about 99%) transmission to pups in utero and transmammary transmission (about 1%) to newborn pups. (G) Adult worms become established in the small intestine of pups and are a major source of egg contamination. (H) *T canis* can also be transmitted to accidental (paratenic) hosts (eg, mice, rats, and rabbits) via the accidental ingestion of infective eggs; in these hosts, larvae hatch from the eggs and penetrate the intestinal wall to then migrate to various organs and tissues, where they encyst. (I) When a canid eats such infected paratenic hosts, adult worms develop in the small intestine and the lifecycle is completed. (J) Human beings are also paratenic hosts; they can become infected by ingesting larvae in paratenic hosts or infective eggs from contaminated soil, food, or water. (K) After ingestion, such larvae are released and invade the intestinal wall to then be carried via the blood circulation to various tissues (eg, liver, lungs, brain, and muscles); although these larvae undergo arrested development (hypobiosis) in these tissues, they often cause pathogenic effects as a result of inflammatory responses and granuloma formation associated with toxocariasis disease. *Human toxocariasis can be prevented and controlled by disrupting the lifecycle at key points: (1) treatment of pet dogs and cats (<12 weeks of age) and stray carnivores (eg, using baits) with an anthelmintic; and (2) preventing human beings from ingesting infective eggs from the environment or larvae in raw or uncooked tissues from infected paratenic hosts (eg, chicken, duck, and rabbit). Adapted from https://www.cdc.gov/parasites/toxocariasis/biology.html.

with eosinophilia and increased production of cytokines and specific antibodies. Although many *T canis* infections are subclinical in nature, human toxocariasis can manifest itself as syndromes known as visceral larva migrans, ocular larva migrans, neurotoxocariasis, and covert or common toxocariasis.[3–5]

Visceral larva migrans is the most common syndrome in infected people, particularly children, with clinical signs such as coughing, wheezing, myalgia, or cutaneous manifestations (eg, pruritus, rash, eczema, panniculitis, and vasculitis).[36] Although most cases of visceral larva migrans are symptomless, lymphadenopathy, granulomatous hepatitis, nodules myocarditis, nephritis, and arthritis are observed in some people.[32,36,37] Additionally, long-term effects, such as the development of asthma and promotion of pulmonary fibrosis, are also suspected to be associated with visceral larva migrans.[12]

Ocular larva migrans is common and mostly reported in children 3–16 years of age.[5,36] Uniocular visual impairment can occur, accompanied by chronic endophthalmitis, retinitis, or granulomata.[36] The level of impairment associates with migrating or dead larvae and resultant immune reactivity against the worm in the eye.[5] Blindness can result from severe vitritis, cystoid macular oedema, or tractional retinal detachment.[5,38]

Neurotoxocariasis is rare and occurs mainly in middle-aged people.[4,39] This syndrome relates to the migration of *T canis* larvae in CNS and subsequent induction of meningitis, encephalitis, cerebral vasculitis, or myelitis, usually associated with relatively non-specific clinical symptoms (eg, fever and headache).[4,39,40] Possible associations between cerebral toxocariasis and neurodegenerative disorders (eg, seizure, schizophrenia, cognitive deficits, idiopathic Parkinson's disease, dementia) have also been discussed in the scientific literature.[4,15,16] Cognitive or developmental delays among socioeconomically disadvantaged children who become infected is of particular concern.[41] Peripheral nervous system involvement (radiculitis or inflammation of cranial nerves or skeletal muscles) is, however, rarely seen in human beings.[4]

Covert toxocariasis in children and common toxocariasis in adults are challenging syndromes to diagnose clinically because of non-specific symptoms.[3] Clinical signs such as fever, anorexia, headache, wheezing, nausea, abdominal pain, vomiting, lethargy, sleepiness and behavioural disorders, pulmonary symptoms, limb pain, cervical lymphadenitis, and hepatomegaly are known to occur in children, whereas weakness, pruritus, rash, pulmonary dysfunction, pulmonary insufficiency, and abdominal pain can be seen mainly in adults.[42] There are also alleged links between common toxocariasis and asthma.[43,44] These clinical features commonly associate with moderate to high anti-*Toxocara* serum antibody titres.[3,8,32]

Diagnosis

The diagnosis of toxocariasis and *Toxocara* infection in paratenic or accidental hosts can be made by histopathological examination, morphometric assessment of larvae (if present), or the specific detection of larval DNA from tissue or body fluid samples.[45] As serological or immunological methods alone do not allow for an unequivocal diagnosis of infection,[18,45] PCR-based tools, using genetic markers in the first and second internal transcribed spacers (ITS-1 and ITS-2) of nuclear ribosomal RNA genes or selected mitochondrial genes, can assist in the specific identification of ascaridoid nematodes (irrespective of developmental stage) and diagnosis, and have been used for epidemiological, population-based genetic, and systematic investigations.[46–59] Some of these molecular tools have allowed *Toxocara* taxa to be unequivocally characterised genetically[46,47] and have led to the discovery of new (cryptic or so-called hidden) species, such as *T malaysiensis*,[58,59] whose zoonotic potential and transmission pattern are unknown.[19] Moreover, *T canis* DNA has been detected by PCR in bronchoalveolar lavage from mice and cerebrospinal fluid from human beings.[40,55]

Sampling tissue biopsies or fluid samples is invasive and can be impractical, so diagnosis of *T canis* infection and toxocariasis in human beings has relied on the use of serological and immunological techniques (table 1),[60–68] sometimes combined with imaging methods.[9,33] ELISA assays have been used to detect *T canis* excretory or secretory (TES) antigens[69] and have been widely used for serodiagnosis and epidemiological studies of *T canis* infection and exposure in human beings.[67,70] Initial serological findings should be confirmed by immunoblotting to avoid false-positive results and to evaluate cross-reactivity with other infective agents.[70] Thus, the combined use of ELISA and immunoblot is preferable,[70] although no unequivocal serological criterion distinguishes active *T canis* infection from past exposure—an area of much discussion in this field of research.[13]

The specificity and sensitivity of serological and immunological assays for toxocariasis diagnosis depend on both the antigens (eg, crude products from *T canis* larvae, native or recombinant TES antigens, or either glycan antigens or deglycosylated TES antigens) and the type of antibodies (eg, total IgG, IgG subclass, or IgM) that are being measured (table 1).[66,68] Modified TES-ELISAs, such as IgG2-TES-ELISA and IgG4-rTES-ELISA, have been developed and assessed.[61,62] Assays for the specific detection of IgG2 and IgG3 anti-*T canis* antibodies appear to have increased sensitivity and specificity.[60] By contrast, an IgG4-ELISA using recombinant TES-120 (rTES-120) and rTES-30 was reported to have 93% sensitivity, and IgG-ELISA using deglycosylated TES was reported to increase both sensitivity and specificity to 100%.[63,66] Other recombinant *T canis* proteins are also under evaluation as potential diagnostic antigens.[71,72] Additionally, measurements of eosinophil cationic protein concentrations or IgG antibody avidity can be used to suggest existing or past *T canis* infection.[67,73–75] However, the performance of each of these assays needs to be comprehensively validated in various countries under different conditions.

Particularly for the diagnosis of ocular larva migrans, anti-*T canis* antibody in serum and vitreous or aqueous humour should be assessed, whereas the diagnosis of neurotoxocariasis should include the detection of specific antibodies and eosinophils in cerebrospinal fluid.[9,40] Imaging techniques can assist clinical diagnosis in patients with ocular larva migrans or visceral larva migrans. Nonetheless, there is a need for improved diagnostic algorithms and clinical definitions for both neurotoxocariasis and covert or common toxocariasis. Various medical imaging techniques, such as ultrasound, CT, and MRI, have been used to scan hepatic lesions relating to visceral larva migrans, and optical coherence tomography, fluorescein angiography, CT, and ocular ultrasound can be used to support the diagnosis of ocular larva migrans .[9,38]

	Purpose and performance
IgG-TES-WB[60]	Detection of specific total IgG in human sera, with high specificity and minor crossactivity
IgG-TES-ELISA[61]	Detection of specific total IgG in human sera, with a sensitivity of 97% and specificity of 36%
IgG1-TES-ELISA[62]	Detection of specific IgG1 subclass in human sera, with sensitivity of 60% and specificity of 76%
IgG2-TES-ELISA[62]	Detection of specific IgG2 subclass in human sera, with sensitivity of 98% and specificity of 71%
IgG3-TES-ELISA[62]	Detection of specific IgG3 subclass in human sera, with a sensitivity 78% and specificity of 81%
IgG4-TES-ELISA[62]	Detection of specific IgG4 subclass in human sera, with a sensitivity of 64% and specificity of 71%
IgG4-rTES-ELISA[63] (rTES-30USM, rTES-120)	Detection of specific IgG4 subclass in human sera, with a sensitivity of 93% and increased specificity
IgM/G-TES-ELISA[64] (TES-58 and TES-68)	Detection of specific IgG or IgM in human sera, with a sensitivity of 100% and specificity of 100%
IgG-TCLA-ELISA[65]	Detection of specific total IgG in human sera, with a sensitivity of 92% and specificity of 87%
IgG-dTES-ELISA[66]	Detection of specific total IgG in human sera, with a sensitivity of 100% and specificity of 100%
IgG-dTES-WB[66]	Detection of specific total IgG in human sera, with no cross-reactivity with the fractions of 32 kDa, 55 kDa, and 70 kDa of dTES
IgG avidity[67]	Measurement of IgG avidity index of human beings, with a sensitivity of 44% and specificity of 83%
IgG-DiM-BSA-ELISA[68]	Detection of specific total IgG in human sera, with a sensitivity of 92% and specificity of 95%

TES=*Toxocara canis* excretory and secretory antigens. dTES=deglycosylated TES antigens. rTES=recombinant TES antigens. WB=western blot. TCLA=antigens from *T canis* larvae. DiM-BSA=di-O-methylated coupled bovine serum albumin.

Table 1: **Serological techniques for the detection of anti-*Toxocara canis* antibodies**

Epidemiology and public health importance

T canis and *T cati* have a worldwide distribution.[24,76] For this Review, we assessed the prevalence of *T canis* infections or exposure in human beings, as determined using serological assays (figure 2); the studies are summarised in the appendix. The prevalence of *T canis* and *T cati* in dogs and cats, respectively, has been reported as 1·2% and 3·2% in Australia,[77] 4·4% and 4·6% in the Netherlands,[28] and 6·1% and 4·7% in Germany.[78] In some surveys in countries including Nigeria, Portugal, India, and China, the prevalence is found to be as high as 51–100% in puppies, 1–45% in adult dogs, and 3·2–91% in cats.[24,76,79–82] In the USA, some of the more than 77 million dogs and 93 million cats spread toxocariasis by excreting faeces containing eggs into gardens, parks, playgrounds, and sand pits; once the eggs become infective, these spaces pose a major public health risk.[24,83] Human beings acquire toxocariasis through a range of routes, such as accidental ingestion of infective eggs from contaminated soil, water, raw vegetables, or fruit.[84,85] Another risk factor is human contact with dogs or cats,[85] as embryonated eggs are on the hairs of these definitive hosts.[27–29] However, an important observation from work undertaken so far is the very low number of embryonated eggs detected on

See Online for appendix

host hair, particularly among dogs that are well cared for.[86] People can also become infected by ingesting encapsulated third-stage larvae in raw or undercooked meat or organs from paratenic hosts, such as rabbits, sheep, cattle, and chickens.[34,35,87,88] The apparent broad distribution of *T canis* and the various possible transmission routes indicate a relatively high risk of human infection and that toxocariasis is a common zoonosis (figure 2; appendix).

In nationwide surveys of *T canis* infection in human beings in the past 36 years (appendix), the prevalence of anti-*T canis* serum antibody has been estimated at 1·6% in Japan, 2·4% in Denmark, 6·3% in Austria, 7% in Sweden, 14% in the USA, and 19·6% in Malaysia. The apparent seroprevalences are greater than 20% in some ethnic and socioeconomically disadvantaged groups. In Iran, for example, the seroprevalence of *T canis* infection among human beings is 22%, whereas 81% of the human population in Nepal is infected (appendix). Notably, seroprevalence in school children in Manado, Indonesia, was reported to be 85%, and 87% in the Marshall Islands.[20,22] However, the epidemiological information for most countries is still limited in detail.

Although human toxocariasis is expected to be common, particularly in communities where there is a close association between human beings and wild or domestic canids and felids, it is challenging to assess the global effect of this enigmatic parasitic disease[7,13] because of the limitations of existing diagnostic tools.[70,89] The large-scale national survey in the USA by Won and colleagues[21] is exemplary in that representative samples and a defined detection technique (eg, sample dilution, immunoassay, and statistical analysis) were used to obtain seroprevalence data. This study showed that the age-adjusted seroprevalence for toxocariasis was 13·9%, which is lower than in non-Hispanic blacks (21·2%) and both non-Hispanic whites (12·0%) and Mexican Americans (10·7%), suggesting a link to ethnicity. The investigators highlighted that health education messages could be used to target the striking differences in seroprevalence. Nevertheless, even when using serological methods, it is still challenging to distinguish *Toxocara* exposure from infection,[18,24] and thereby advance the epidemiological understanding of human toxocariasis. Overall, there is an urgent need for studies to assess the global burden of human toxocariasis that will better justify investments in large-scale detection and prevention programmes.

Treatment

The two major obstacles to the successful treatment of toxocariasis in human beings are: the requirement for drugs to reach larvae across a range of tissues; and the difficulty in verifying drug efficacy in patients. Nevertheless, treatment with anthelmintics is advocated for acute toxocariasis, particularly to prevent *Toxocara* larvae from reaching the brain and eyes.[32,90] Albendazole and mebendazole are commonly used to treat visceral larva migrans, despite their limited efficacy against larvae in tissues;[91] albendazole is preferable because it becomes widely distributed throughout tissues when metabolised, whereas mebendazole is not absorbed outside the gastrointestinal tract. In addition to anthelmintics, anti-inflammatory compounds (eg, corticosteroids or

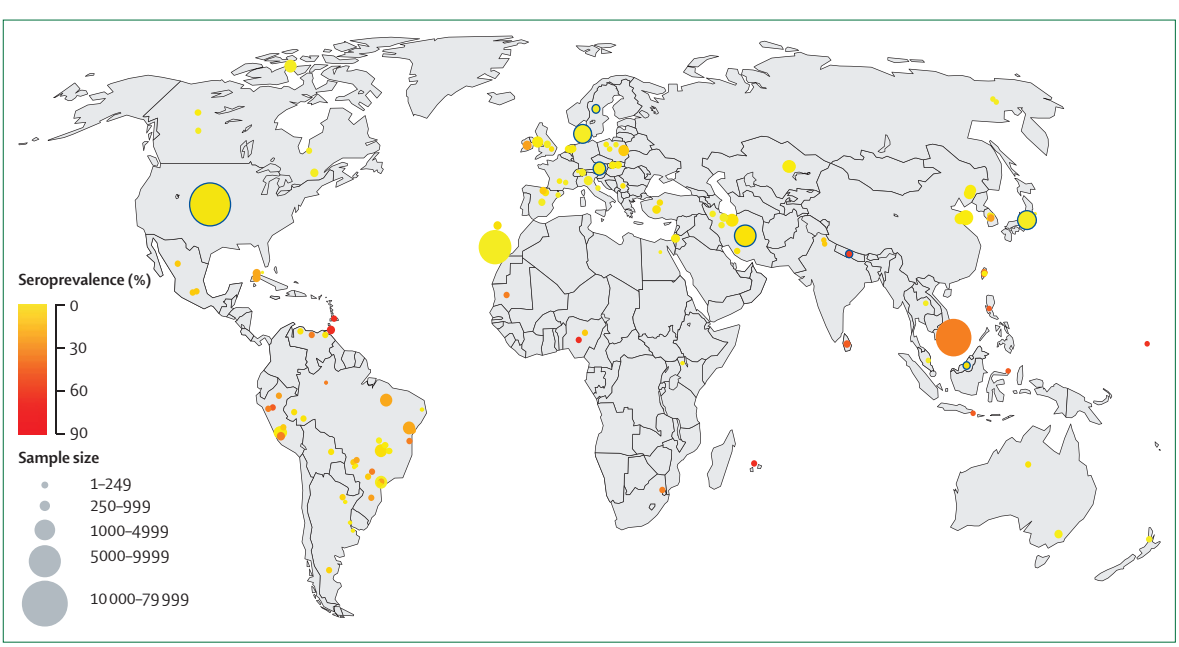

Figure 2: **Estimated seroprevalences and distribution of *Toxocara canis* infection or exposure**
Prevalences and sample-size ranges (number of individuals tested) are summarised from published work (appendix). A yellow dot with a blue halo represents a well-defined national survey or meta-analysis.

non-steroidal anti-inflammatory drugs) can relieve symptoms caused by allergic responses.[36] Specifically, ocular larva migrans can be treated with corticosteroids (eg, prednisolone) and, in selected or severe cases, by ophthalmologic surgery,[36] although the efficiency of treatment is unclear (a combination of albendazole and steroids has resulted in favourable clinical outcomes).[38,92] Albendazole is also recommended for the treatment of neurotoxocariasis because it crosses the blood–brain barrier and is better tolerated than mebendazole and diethylcarbamazine.[14]

Large and well defined clinical studies are urgently needed, particularly in children. Despite being serologically negative, some children with toxocariasis can have symptoms, such as headaches, which persist even after three rounds of treatment with albendazole or mebendazole.[90] Efforts have been made to improve delivery of anthelmintics to tissues, particularly in the brain, by using polyethylene glycol (PEG)-conjugated and liposome-encapsulated compounds.[70,93–95]

Drugs and their formulations for the treatment of toxocariasis are summarised in table 2. In C57BL/6 mice, fenbendazole has been shown to have a relatively high efficacy at killing *T canis* in muscle (89·5%) and brain (66·1%) when delivered by liposomal carrier and administered with an immunostimulatory glucan,[94] and albendazole-PEG has been shown to kill larvae in the brain.[99] Other chemicals and natural products have been tested, revealing the larvacidal or larvistatic activities of nitazoxanide, tribendimidine, phenazines (eg, lapachol, β-lapachone, and β-C-allyl-lawsone), and fatty acid amides (eg, linoleylpyrrolidilamide).[38,101] Some natural products, such as the plant extracts from *Chenopodium ambrosioides* and an aqueous extract of the nutritional supplement Nutridesintox, have some anthelmintic activity against *T canis* larvae in vitro and reduce the inflammatory infiltrates in the liver and lung of CD-1 mice.[100] However, such products need to undergo rigorous assessment in vivo to ensure their efficacy and safety and, if possible, to establish their pharmacodynamics.

	Efficacy
ABZ[96]	47% reduction of clinical symptoms in infected people
DEC[97]	70% reduction of clinical symptoms in infected people
ABZ/PEG-LE[93]	79% larval elimination from brains of infected mice
FBZ/PEG-LE[94]	90% larval elimination from the muscles and 66% from brains of infected mice
ABZ/CH[95]	100% larval elimination from brains, and increased elimination from liver and lungs of infected mice
ABZ/PEG-LE and glucan[98]	92% larval elimination from brains of infected mice
FBZ/PEG-LE and glucan[98]	92% larval elimination from skeletal muscle of infected mice
ABZ/PEG[99]	100% larval elimination in brain, and increased elimination from liver and lungs of infected mice
Natural products[100]	Some efficacy on larvae in vitro and in infected mice with extracts from *Chenopodium ambrosioides* or with Nutridesintox, or both

ABZ=albendazole. DEC=diethylcarbamazine. ABZ/CH=chitosan-encapsulated albendazole. ABZ/PEG=polyethylene glycol-conjugated albendazole. ABZ/PEG-LE=liposome-encapsulated albendazole stabilised with PEG. FBZ/PEG-LE=liposome-encapsulated fenbendazole stabilised with PEG.

Table 2: **Anthelmintic drugs for treatment of toxocariasis in human beings and other host animals**

Immunology and prospect for vaccines

There is increasing interest in vaccines against helminth infections and, specifically, for vaccines to prevent transmission of zoonotic diseases to human beings. A recent example is the development of a vaccine against *Taenia solium* in pigs to prevent human cysticercosis.[102] Like many other helminthic diseases, however, protective immunity is often forestalled by the parasites' ability to block and evade the host immune system.[103] For *T canis*, immune evasion is mediated by surface coat and excretory or secretory molecules,[104] which are also of interest as vaccine targets for protective type 2-like immune responses.[105,106] TES components, first explored by Maizels and colleagues[107] and since characterised in more detail, include the C-type lectins TES-32 and TES-70[105,108,109] and heavily glycosylated mucins, which also make up the surface coat of the larval parasite.[106,109–112] On the basis of genome and transcriptome analyses of *T canis*,[113] a wide range of peptidases, adhesion molecules, SCP/TAPS proteins, and lectins have been proposed to have roles in the host-parasite interplay.[114]

Major features of the host-immune response to *T canis* infection include a dominant CD4+ T-helper type-2 cell (Th2) activity, eosinophilia, and production of specific antibodies.[104,115] TES antigens drive a vigorous production of type-2 cytokines (eg, interleukin 4, interleukin 5, interleukin 10, and interleukin 13) from peripheral T cells of exposed individuals, resulting in eosinophilia and increased production of cytokines and IgE antibodies.[104,115,116] These factors can all contribute to airway hypersensitivity, linking chronic *T canis* infection with allergic diseases such as asthma and allergic rhinitis.[12,117] Wheezing in patients can be associated with visceral larva migrans and covert toxocariasis due to pulmonary migration of *T canis* larvae.[3,43] Whether these people, mostly children, develop asthma later in their childhood remains controversial—there is a possibility that they might be protected from atopic asthma as a result of the immunoregulatory responses induced by infection.[12,104] Although many parasitic helminths can induce immunoregulatory cell populations (eg, regulatory T cells and alternative-activated macrophages),[118] *T canis* has probably evolved mechanisms effective in the dog but not in human beings, which explains its greater immunogenicity in the accidental host. Nevertheless, some anti-inflammatory effects can be mediated by TES, resulting, for example, in the inhibition of Toll-like receptor signalling and nitric oxide production.[118]

Immunomodulation is a feature of *T canis* infections in human beings and other hosts. For example, increases in the concentrations of protective pro-inflammatory cytokines (interleukin 6, interferon γ, and interleukin 13) and anti-inflammatory interleukin 10 has been reported

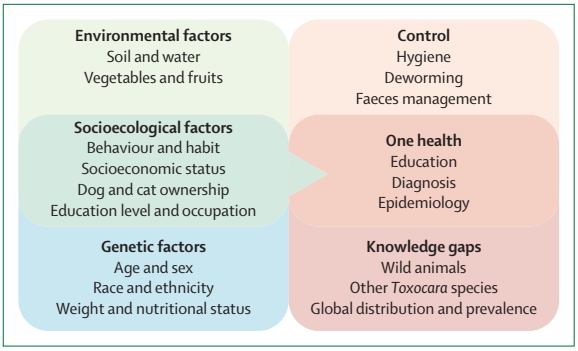

Figure 3: **Epidemiological determinants and risk factors of human toxocariasis**
Disease manifestation of human toxocariasis can be affected by various factors, such as environment contamination with *Toxocara* eggs, human genetics, and socioecological activities. A one-health strategy that includes key components of epidemiology, diagnosis, and treatment should enhance the knowledge base and improve control of human toxocariasis.

in sera from children infected with *T canis*.[119] Macrophages from infected mice show a shift towards increased production of interleukin 10 and transforming growth factor β and reduced production of interleukin 12 and tumour necrosis factor.[120] Simultaneous activation and suppression of immune responses takes place by various mechanisms. First, the surface-coat shedding strategy of the parasite enables migrating larvae to escape from eosinophils that adhere to the parasite surface, thus neutralising the antibody-dependent elimination.[121] Second, TES antigens, a Th2-stimulator, can also modulate local and systematic immune responses.[104] For example, C-type lectins, particularly TES-32 and TES-70, have considerable homology to low-affinity IgE and macrophage mannose receptors in mammals[108] and are capable of targeting host pathways involved in innate immunity.[118] Exosomes and other extracellular vesicles might represent other means by which nematodes modulate immune responses because such vesicles have been shown to transfer excretory and secretory molecules and small RNAs to host cells.[122] However, this new and exciting area of research needs to be explored in much more detail. Clearly, an improved understanding of the immune responses, modulatory mechanisms, and immunogenic molecules that are specific to toxocariasis could assist in developing an effective vaccine against this disease.

Prevention and control

The remarkably broad geographical distribution of *T canis*, its multiple infection routes (eg, human–dog contact and through neonatal and food-borne transmission), and potential associations of toxocariasis with allergic and neurological disorders have raised considerable public concern. However, community awareness of toxocariasis is still inadequate. Enhancing education will be essential to improve the public's understanding of toxocariasis and its prevention, treatment, and control (figure 3).[17] The American Association of Veterinary Parasitologists and the US Centers for Disease Control and Prevention have created educational websites that provide detailed information on *Toxocara* and toxocariasis. Practicing veterinarians can also educate pet owners about toxocariasis and how to minimise risks of zoonotic transmission.[13] Veterinarians are often infected or exposed to infection because of their occupational contact with small animals.[85,123] The knowledgebase about toxocariasis among doctors and other health-care providers is weak, and efforts to diagnose and test children and adults with asthma-like symptoms and pulmonary dysfunction (covert or common toxocariasis) or cognitive deficits (neurotoxocariasis) are almost non-existent. Hygiene, preventing children from ingesting soil or faeces from carnivores that are contaminated with infective *Toxocara* eggs, and avoiding the ingestion of raw or uncooked meat or liver are central to preventing transmission in endemic countries.[70,124] Moreover, although asymptomatic forms of this disease are usually self-limiting, any case of human toxocariasis should be treated to prevent larvae from invading the brain and eyes.[14,32,90]

The interaction between human beings, animals, and the environment should always be kept in mind. In view of the wide range of reservoirs and various transmission routes available to *Toxocara* spp, a comprehensive one-health approach should be used for effective control of human toxocariasis (figure 3).[17] Prioritising the control of *Toxocara* infections in definitive hosts could substantially reduce the number of infective eggs in the public environment.[77] Pet dogs and cats, particularly puppies younger than 12 weeks and kittens,[125,126] working dogs, and, where possible, stray dogs and cats should be dewormed with effective anthelmintics.[27,29] However, anthelmintic treatment of pregnant dogs and cats is mostly ineffective at preventing transplacental and transmammary transmission.[127] Moreover, interventions to prevent dog fouling have also been suggested.[128] Importantly, the intervention scenarios described by Nijsse and colleagues[129] indicate that deworming strategies (four times per year) and the removal of faeces would reduce egg output and resultant environmental contamination. Nonetheless, due to major knowledge gaps in the epidemiology of *Toxocara* spp, controlling transmission is very challenging when wild animals (eg, foxes) are involved.[17,125] The debate about public health measures that could be implemented in endemic settings (mostly in underprivileged rural communities and in poor communities in tropical cities with poor sanitation and large stray dog and cat populations) needs to be centred not only around understanding parasite biology and epidemiology but also on the feasibility and cost-effectiveness of the measures, as well as a characterisation of populations at increased risk of infection (eg, age groups, rural *vs* urban, and socioeconomic status).

New molecular insights using genomic and transcriptomic tools

The completion of the *T canis* draft genome project was an important step towards advancing the understanding of this parasite and toxocariasis at the molecular and biochemical levels. These data could assist in developing a next generation of diagnostic and intervention methods.[113,114] The draft genome (317 Mb) was predicted to encode 18 596 genes, with 14 583 (78·4%) genes annotated and 5406 (29·1%) genes having homologues in known biological KEGG pathways.[113,114] Specifically, 870 excretory and secretory proteins (including proteases, cell adhesion molecules, lectins, SCP/TAPS proteins, and mucins) were predicted to be involved in host invasion and in parasite–host interactions, such as immune evasion or modulation, whereas 458 protein kinases, 408 phosphatases, and 127 GTPases were proposed to have important roles in reproduction and embryonic and larval development, and 156 G-protein coupled receptors, 268 ion channels, and 530 transporters could potentially serve as drug targets. Additionally, the genome project helped define molecules in the intestine, in particular developmental stages, and in each sex of *T canis* inferred to be essential for survival, development, and reproduction.[113,114] The genomic data form a resource for future investigations of the immunobiology, epidemiology, genetics, and pathogenesis of *T canis* and toxocariasis as well as for the design of improved diagnostic tools and new interventions, including anthelmintics and vaccines.

MicroRNAs are increasingly recognised for their roles in parasite development, reproduction, and host–parasite interactions as well as in drug resistance in parasitic helminths. The transcription profiles of small RNAs in adult *T canis* have been analysed with the newly established genomic and transcriptomic resources.[130] The findings suggest that microRNAs *Tc*-miR-2305 and *Tc*-miR-6090 are involved in reproduction and embryonic and larval development in *T canis*, whereas *Tc*-let-7-5p, *Tc*-miR-34, and *Tc*-miR-100 appear to be associated with host–parasite interactions. Other miRNAs, including *Tc*-miR-2861, *Tc*-miR-2881, and *Tc*-miR-5126, might be suitable drug targets or associated with drug resistance. Such study outcomes are beginning to broaden and deepen the fundamental knowledge of *T canis* molecular biology, should provide a basis for experimental investigations of the developmental biology, parasite–host interactions, and disease, and might assist in the design of novel diagnostic and therapeutic approaches.[131–133]

Future prospects and conclusions

Although branded as "America's most common neglected infection of poverty",[7,134] the global importance of toxocariasis remains to be critically assessed.[7,9] No estimate of the global disease burden of toxocariasis exists, and although epidemiological studies and reports of toxocariasis have been done all over the world, major knowledge gaps remain in the epidemiology of *T canis* and *T cati* infections and of *T malaysiensis*, the zoonotic importance of which is unknown, and considerable limitations hamper evaluations of the burden of toxocariasis to public health. For example, the integration of seroprevalence data is challenging for many reasons, such as variations in the way populations were sampled and detection methods (such as the quality of antigens and the cut-off values).[17] The inadequate sensitivity and specificity (cross-reactivity) of some serodiagnostic tools are likely to lead to false-positive results, particularly in tropical areas, where polyparasitism is common, and different serological methods can produce discrepant results.[70,89] Moreover, it is not possible to unequivocally differentiate *T canis* from *T cati* (or *T malaysiensis*) infections using existing serological methods.[18,135] Therefore, optimised, species-specific diagnostic methods are needed to support future epidemiological investigations.

Equally needed are studies to understand the pathogenesis and natural history of covert or common toxocariasis and neurotoxocariasis. Covert or common toxocariasis and neuro-toxocariasis are potentially linked to major causes of pulmonary dysfunction and cognitive delays, respectively, especially in children and adults living in poor and underserved areas, but the evidence base is still missing. Without the evidence base in hand, it is difficult to secure research funds to investigate these disorders, and yet, very little research funding is available to support investigations of human toxocariasis.

Expanding our understanding of the fundamental biology, particularly immune recognition and evasion of *T canis* larvae, should be a priority, and could lead to new diagnostic tools and methods of intervention. Although the identification of genes expressed by the arrested infective larvae have provided insight into larval survival and immune evasion, there is still a paucity of knowledge as to how *T canis* suppresses host immunity at the molecular level and how protective immunity is achieved in definitive and paratenic hosts. The draft genome will assist investigations of the immunobiology of *T canis* as well as the genetics and epidemiology of this parasite.[113,114] Genomic and transcriptomic resources

Search strategy and selection criteria

We searched PubMed and Scopus databases for articles published between Jan 1, 1950, and April 16, 2016, with the terms "toxocar*" AND ("clinical manifestation" OR "syndrome"); "toxocar*" AND "diagnosis"; "toxocar*" AND ("epidemiology" OR "public health"); "toxocar*" AND ("treatment" OR "chemotherapy"); "toxocar*" AND ("immunol*" OR "vaccine"); "toxocar*" AND ("prevention" OR "control"); "toxocar*" AND ("genom*" OR "transcriptom*"). We screened the titles and abstracts and identified articles with relevant content and context. The full texts of these articles were read to verify their relevance.

will facilitate studies of this pathogen's biology, biochemistry, physiology, and processes or mechanisms involved in blocking or evading host immune attack.[114] Also novel diagnostic and therapeutic approaches could be facilitated using these resources. Since a broad range of TES antigens was predicted from transcriptomic data,[113,114] it should be possible to classify and explore their functions in much more detail. Since microRNAs have been assessed for the diagnosis of *Brugia pahangi*, *Dirofilaria immitis*, *Onchocerca volvulus*, and *Onchocerca ochengi* infections,[132,136,137] the identification of specific biomarkers for the diagnosis of toxocariasis seems a promising direction for future investigations.

Genomics-guided methods might complement conventional approaches for drug and drug target discovery.[113,114] For example, eight recognised targets (eg, SLO-1 calcium-activated potassium channels, acetylcholine receptors) and 101 potential targets (eg, kinases and phosphatases) were predicted from the *T canis* genome, all of which might be functionally tested by gene silencing experiments. The gene silencing machinery of *T canis* has been characterised, with 43 RNAi effector genes predicted to date.[113] Although the uptake of extracellular dsRNAs might be limited because *T canis* does not carry the *sid-2* gene, previous gene knock-down analyses in *Ascaris suum* and *T canis* suggest promise for functional genomic investigations of parasite-specific genes in *T canis*.[138,139] Small RNA technology might be useful for the development of novel therapeutics and vaccines.[133] In conclusion, human toxocariasis is a common, neglected parasitic disease of global importance, but remains enigmatic in many respects. Increased public awareness is essential for the treatment and control of this disease. New molecular resources for *T canis* and advanced molecular tools now underpin toxocariasis research and should facilitate the development of radically new diagnostic and intervention strategies.

Contributors

RBG conceived the idea for this review. GM completed the literature search and prepared tables and figures. RBG and GM drafted the manuscript and integrated input from coauthors. All authors viewed the final, submitted version of the manuscript.

Declaration of interests

We declare no competing interests.

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