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Soil-Transmitted Helminthic Zoonoses in Humans and Associated Risk Factors

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1. Introduction

The soil is an important route for transmission of numerous human pathogens, including the five major soil-transmitted helminths (STHs), also known as geohelminths, namely: roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), hookworms (*Ancylostoma duodenale* and *Necator americanus*), and threadworm *Strongyloides stercoralis* (Brooker et al., 2006). An estimated one billion people are currently infected with STHs worldwide, particularly in resource-poor settings (WHO, 2011). Although overall mortality due to STH infections is low, morbidity may be significant given the pronounced impact on nutrition, growth, physical fitness, cognitive functions among infected infants, schoolchildren and adults from developing countries (Bethony et al., 2006). In Africa, an estimated 89.9 million children harbor STHs, many of whom are co-infected with two or more STH species (WHO, 2011).

Zoonotic agents, comprising a wide variety of bacteria, viruses, and parasites, account for almost two thirds of all known human infections. Some helminthoses that commonly infect canids and felids are typically soil-transmitted. This chapter focuses on two major groups of STHs that cause disease in humans: (a) the ascarids *Toxocara canis* and *T. cati*, associated with visceral and ocular larva migrans, and (b) the hookworms *Ancylostoma braziliense* and *Anc. caninum*, associated with cutaneous larva migrans. We review the current geographic distribution, laboratory diagnosis and clinical spectrum of these infections, examine the relative contribution of some risk factors for infection and disease, and discuss potential control measures for reducing the burden of disease in companion animals and humans.

A third soil-transmitted ascarid species that can cause human disease is *Baylisascaris procyonis*, commonly found in raccoons in North America. Human infections are characterized by severe neurological disease, leading to death or long-lasting sequelae (Watts et al., 2006). Another nematode species, *Gnathostoma spinigerum*, has occasionally been found in biopsy sample from patients with suspected VLM. More recently species of *Toxocara* including *T. malayensis*, a parasite of the domestic cat, and *T. lyncus*, which infects the caracal, have been identified, but their role in human disease remains unknown (Despommier, 2003). Soil-transmitted larval infection with other common canine and feline hookworms, such as *Anc. ceylanicum*, *Anc. tubaeforme* and *Uncinaria stenocephala*, can also cause occasional dermatological lesions in humans, and *Anc. ceylanicum* can readily develop in adults causing severe enteritis (Bowman

et al., 2010). These zoonotic infections have significant public health implication in specific human populations, but are not reviewed in the present work.

2. *Toxocara canis* and *Toxocara cati*

The causal agents of human toxocariasis are the ascarid nematodes (roundworms) *T. canis* and *T. cati*, whose definitive hosts are dogs and cats, respectively. Although infection with these parasites has been described in their usual hosts for more than 200 years, only in the 1950s were they recognized as important human pathogens. When embryonated eggs are accidentally ingested, larvae hatch in the small intestine, penetrate the intestinal wall and migrate via the bloodstream to the liver, lungs, muscles, eye and the central nervous system. Although most infections are asymptomatic, two well-defined syndromes are classically recognized in human: visceral larva migrans (VLM), a systemic disease caused by larval migration through major organs, and ocular larva migrans (OLM), a disease limited to the eye and optic nerve. Less severe syndromes have been described mainly in children (covert toxocariasis) and in adults (common toxocariasis).

The genus *Toxocara* belongs to the order Ascaridoidea. The life cycle of *T. canis* is complex (Figure 1). Infections are acquired by oral ingestion of infective stages, but also by transplacental or transmammary routes, and may or may not include migration across the viscera of the definitive hosts. Female worms produce up to 200,000 eggs a day, which are shed in dog and cat feces and embryonate in the environment within 2-3 weeks, under ideal humidity and temperature (25-30°C) conditions. Akin to other ascarid eggs, fully embryonated ova contain third-stage larvae (L3). When dogs ingest embryonated eggs, L3 larvae hatch in the small intestine, penetrate the intestinal wall and are carried by the bloodstream to several tissues, particularly the liver and the lungs (entero-hepatic-pulmonar migration). The pulmonary L3 larvae undergo upward tracheal migration and swallowing, to return to the small intestine, where the final two molts take place. Although larvae will remain developmentally arrested in most adult dogs, they usually resume development in pregnant bitches and migrate across the placenta, infecting the foetus. After 4-5 weeks of infection, eggs shed by female worms are detectable in dogs feces, where the prepatent period is slightly longer (8 weeks) in *T. cati* infections of cats. Dogs and other canid species are also infected by transplacentary and transmammary migration of third-stage larvae.

Although *T. canis* is often regarded as the main, or sole, cause of human toxocariasis, the relative contribution of *T. cati* has possibly been underestimated because these two zoonotic species could not be reliably differentiated with the identification methods used in most studies (Fisher, 2003). Serosurvey data from Iceland, however, indicate that *T. canis* may be far more important than *T. cati* as a cause of toxocariasis (Overgaauw, 1997). Dogs have been banned from Iceland since the 1940s, as a measure to prevent human hydatid disease, but cats are allowed as pets. Nevertheless, all adult Icelanders exposed to cats (cat breeders and pet cats owners) tested to date have been seronegative for *Toxocara* (Woodruff et al., 1982).

The age of definitive hosts, particularly dogs, correlates negatively with the burden of infection with adult worms. Adult *T. canis* worms are most commonly observed in puppies up to three months of age. In dogs up to 5-6 months of age, tracheal migration of larvae usually results in the development of adult worms. At six months of age, the number of adult parasites in the intestine decreases drastically, putatively due to acquired immunity against migrating larvae, while L3 larvae typically undergo somatic migration and encapsulate in these hosts. In cats, usually more larvae undergo tracheal migration

following ingestion of embryonated *T. cati* eggs in kittens than in older animals, but even adult animals may be susceptible to infection and disease.

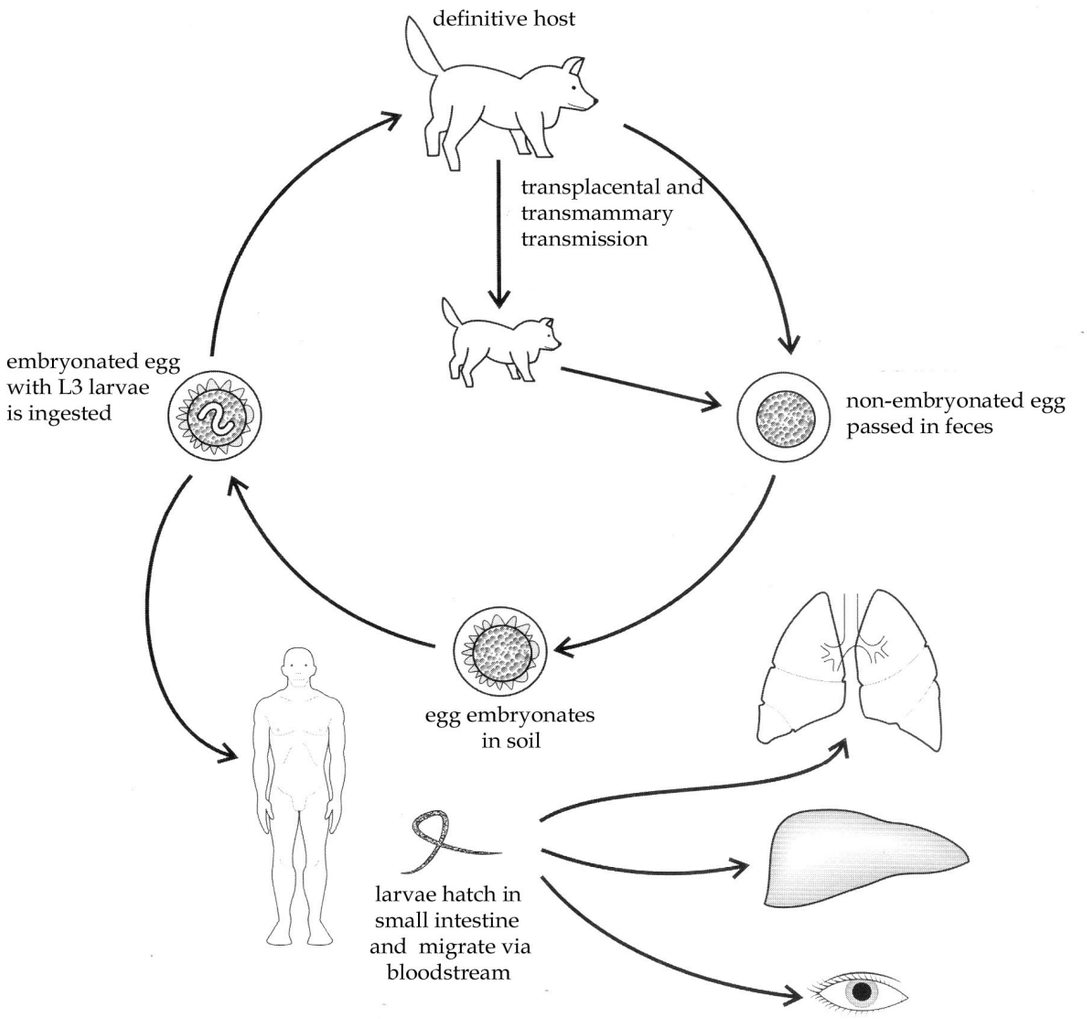

Fig. 1. Simplified life cycle of *Toxocara canis*. *T. cati* has a very similar life cycle, except that cats (instead of dogs) are the definitive hosts. Adapted from Despommier (2003).

T. canis larvae arrested in the tissues may be transmitted from bitches to offspring via the placenta (transplacental transmission). However, this phenomenon is not observed in *T. cati* infections of cats. During the last trimester of pregnancy of the bitch, tissue-arrested larvae become mobile due to hormonal influence. Larvae migrate to the lungs of the fetus. In the newborn puppy, the life cycle is complete when the larva migrates, via trachea, to the intestinal lumen, where the final molts take place. Adult worms can be found at two weeks of age, whereas large numbers of eggs can be detected in the feces after a minimum pre-patent period of 16 days (Barriga, 1988, 1991).

Embryonated eggs present in environment may be ingested by a variety of accidental, paratenic hosts, such as rodents, sheep, pigs, cattle, birds, and humans. When such eggs from the soil or contaminated food are accidentally ingested by humans, L3 larvae hatch into small intestine, reach the lung and the heart and are carried to the systemic circulation.

3. *Ancylostoma caninum* and *Ancylostoma braziliense*

CLM is a relatively common clinical entity in humans caused by larval migration of zoonotic hookworms, mainly *A. braziliense* but also *A. caninum* and a few other species. Infective larvae penetrate the skin and migrate through the epidermis, but are usually confined to the dermis and do not develop into adult worms. Less common clinical manifestations are eosinophilic pneumonitis, localized myositis, folliculitis and erythema multiforme but eye involvement is rarely reported. Larval infection of humans with *Anc. ceylanicum* may occasionally give rise to adult worms that inhabit the small intestine and may cause abdominal discomfort and eosinophilic enteritis. The presence of immature *Anc. caninum* worms in the intestinal lumen of humans has rarely been reported (Bowman et al., 2010).
Infection of both definitive and paratenic hosts with these nematodes is most commonly acquired when third-stage hookworm larvae penetrate in their skin, although these infective larvae may also be ingested. In adult dogs infected with *Anc. caninum*, some larvae may undergo somatic migration and subsequently infect puppies by the transmammary route (Bowman et al., 2010; Soulsby, 1982). These larvae invade the skeletal muscle or gut wall and remain in an arrested state, becoming reactivated during the last two weeks of pregnancy (Barriga, 1988). Adult worms inhabit the small intestine of the definitive hosts (dogs for *Anc. caninum*, *Anc. braziliense*, *Anc. ceylanicum* and *U. stenocephala*; cats for *Anc. tubaeforme*, *Anc. braziliense*, *Anc. ceylanicum* and *U. stenocephala*) and may cause blood loss and anemia. Female worms shed eggs, typically two weeks after ingestion of larvae and about one month after skin penetration, which are passed in the host's feces. Once in the soil, first-stage larvae hatch and develop into infective third-stage larvae (Figure 2).

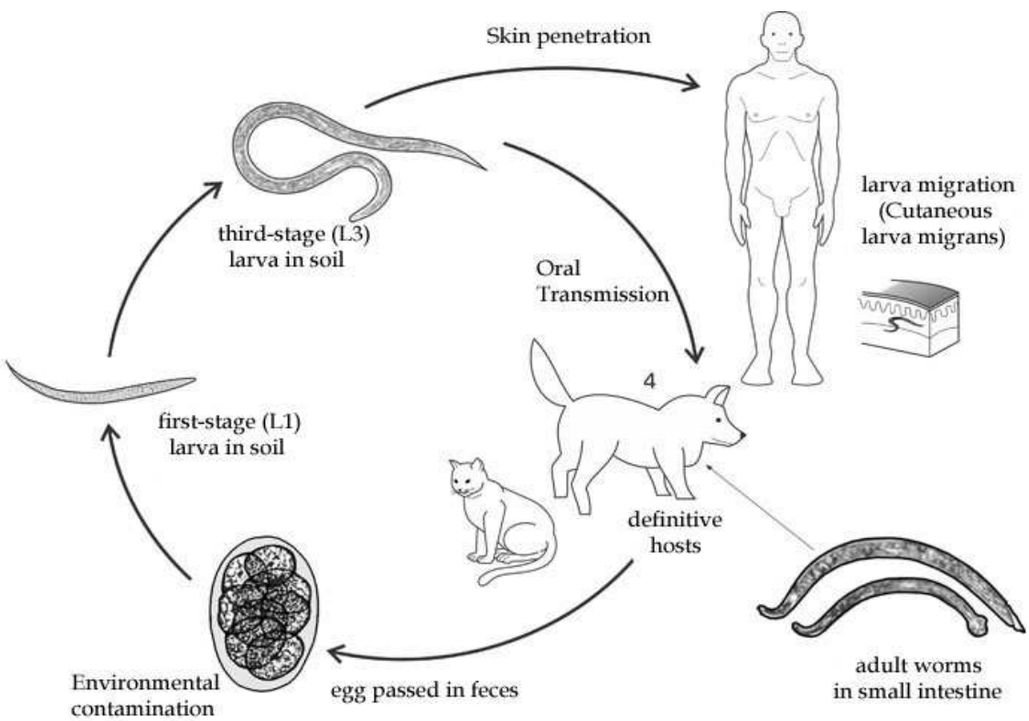

Fig. 2. Simplified life cycle of *Ancylostoma braziliense* and *Anc. caninum*. Adapted from CDC (2011).

4. Soil contamination with infective stages of zoonotic helminths

T. canis is transmitted to humans mainly by incidental ingestion of embryonated eggs present in the soil or soil-contaminated food (Acha & Szyfres, 2003). Since adult female worms produce large number of eggs and nearly all puppies are infected prior to birth, dog populations excrete a huge number of *Toxocara* eggs into the environment (Barriga, 1988).

Under favorable conditions (absence of direct sunlight exposure and appropriate temperature, humidity and oxygenation), particularly in tropical countries, *Toxocara* eggs can survive in the soil for several years. However, heavy environmental contamination has also been found in countries with temperate climate, such as Germany (Düwell, 1984) and Japan (Shimizu, 1993). Most (51-95%) eggs recovered from the soil of temperate countries were fully embryonated and, therefore, infective (Holland et al., 1991; Jarosz et al., 2010).

Humans can also be infected with *Toxocara* by ingestion of raw infected tissues of other paratenic hosts, such as cows, sheep or chicken, containing encapsulated larvae (Finsterer et al., 2010; Nakagura et al., 1989; Salem & Schantz, 1992). Food-borne transmission appears to be relatively common in East Asia (Akao & Ohta, 2007). Larval development progresses no further, but parasites can remain viable for up to seven years after infection (Smith et al., 2009). Although direct contact with infected puppies and kittens is not classically considered a risk factor for human toxocariasis, since the eggs shed by these animals must embryonate in the soil before becoming infective, these pet animals may carry embryonated eggs within their fur (Wolfe &Wright, 2003), in a small numbers (Overgaauw et al., 2009).

The species most commonly involved in human CLM is *Anc. braziliense*. Eggs shed within the feces of infected hosts hatch in the soil and develop into third-stage larvae in the environment. Human infection occurs through contact with contaminated soil of beaches, parks and schools (Bowman et al., 2010).

Eggs of *Toxocara* and zoonotic hookworm larva are found in soils worldwide, especially in public parks, playgrounds, sandpits, and beaches. Reports of soil contamination with infective stages of *Toxocara* and hookworm in public areas are available for several countries (Table 1).

Environmental and technical factors, such as soil type, pre-processing sieving, washing, and re-suspension of sediment, solution employed for washing and flotation, and the specific density of flotation solutions are all presumed to influence the recovery of ascarid eggs (Coelho et al., 2001; Nunes et al., 1994; Oge & Oge, 2000; Ruiz de Ybáñez et al., 2000; Santarém et al., 2009; Santarém et al., 2010).

Other variables, such as climatic conditions (temperature, rainfall, sunlight, etc.) or the amount of herbage and the presence of animals, number amongst other important factors contributing to soil contamination that may influence recovery of eggs.

The presence of dogs and cats may also play an important role on soil contamination by agents of larva migrans. Cassenote et al. (2011) observed that the number of dogs frequenting parks had an impact on soil contamination in public spaces.

The lack of standardisation of techniques as well as the wide range of factors influencing the process of egg recovery can lead to false-negative results and underestimation of the occurrence of contamination, hampering comparison of findings of different reports, and the assessment of their implications for public health (Coelho et al., 2001).

Fahrion et al. (2010) observed that the mean sizes of *T. cati* (62.3 by 72.7 μm) and *T. canis* (74.8 by 86.0 μm) eggs recovered from feces differed statistically. According to Fogt-Wyrwas et al. (2007), the differentiation of *Toxocara* spp. eggs from soil by ocular microscopy is extremely difficult due to the similarity in morphological characteristics of *T. canis* and *T. cati* eggs. As a consequence, studies have been carried out in an effort to provide molecular techniques for amplification of *Toxocara* spp. DNA that can be applied in routine examinations.

Continent /Country	Site	Frequency (%)	Reference
Africa			
Niger	Kaduna	9.0[A]	Maikai et al. (2008)
Americas			
U.S.A.	Connecticut	14.4[T]	Chorazy & Richardson (2005)
Argentina	Buenos Aires	13.2[T]	Fonrouge et al. (2000)
Brazil	Fernandopólis	79,36[T] 6,9 [A]	Cassenote et al. (2011)
	Itabuna	47.9[A]	Campos Filho et al. (2008)
	Mirante do Paranapanema	76.9[T]	Santarém et al. (2010)
	Praia Grande	45.9[A]	Castro et al. (2005)
	Ribeirão Preto	20.5[T]	Capuano & Rocha (2005)
	São Paulo	29.7[T]	Muradian et al. (2005)
	Sorocaba	53.3[T]	Coelho et al. (2001)
Chile	Santiago	66.7 [T]	Castillo et al. (2000)
Venezuela	Ciudad Bolívar	61.1[A]	Devera et al. (2008)
Asia			
Japan	Tokushima	63.3[T]	Shimizu (1993)
Thailand	Bangkok	5.71[T]	Wiwanitkit & Waenlor (2004)
Turkey	Ankara	45.0[T]	Avcioglu & Burgu (2008)
	Kirikkale	15.6[T]	Aydenizöz-Ozkayhan (2006)
Europe			
Ireland	Dublin	15.0[T]	O'Lorcain (1994)
Italy	March	33.6[T]	Hábluetzel et al. (2003)

Table 1. Frequency (%) of soil contamination of public areas by *Toxocara* spp. eggs[T] and *Ancylostoma* spp. eggs/larvae[A] in different continents.

Zhu et al. (2001) constructed specific primers for *T. canis* and *T. cati* DNA amplification by the PCR technique, by extracting genomic material from adult worms from dogs and cats. Previously, Jacobs et al. (1997) obtained genomic material from adult worms or from embryonated eggs collected from the uteri of female worm, and devised a polymerase chain reaction-linked to restriction fragment length polymorphism (PCR-linked RFLP) targeting the second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) for *Toxocara* spp. and other zoonotic ascaridoid identification.

Subsequently, further studies based on Jacobs et al. (1997) have been undertaken to detect and differentiate *Toxocara* spp. in soil (Borecka, 2004; Fogt-Wyrwas et al., 2007; Borecka & Gawor, 2008) and in fecal samples (Fahrion et al., 2010).

Fogt-Wyrwas et al. (2007) developed a technique based on a step PCR method for identification of *T. canis* and *T. cati* in soil samples. First, the authors recovered eggs using a flotation technique. Genetic analyses were then carried out after the crushing the eggs by pressing a cover slip on a microscope slide, to produce the embryonic material. Successful results were obtained only when a single or large numbers of eggs were recovered from 40 g soil samples. Both *T. canis* and *T. cati* genetic material were amplified. Borecka & Gawor (2008) verified that the use of proteinase K enabled amplification of genomic DNA from the soil without the need to isolate eggs using flotation or to inactivate PCR inhibitors present in the sample, thus making PCR easier and less laborious for routine use.

Another method developed to amplify DNA is the loop-mediated isothermal amplification (LAMP) assay. Based on a previous review, Parida et al. (2008) stated that LAMP is characterized by the use of six different primers. Amplification and detection of a gene can be completed in a single step, by incubating the mixture of samples, primers, DNA polymerase with strand displacement activity and substrates, at a constant temperature. The result is based on naked eye observations of a white precipitate (magnesium pyrophosphate). Thus, the method does not require expensive equipments, such as thermal cyclers or reagents.

5. Global prevalence of soil-transmitted zoonotic helminth infections in humans and associated risk factors

Human *Toxocara* infection has a cosmopolitan distribution, but reliable prevalence estimates are hard to derive from available serosurvey data, which comprises demographically diverse population samples that may not be representative of the general population of their respective countries, provinces or cities (Table 2).

Continent/ Country	Site(s)	No. of samples	Positive (%)	Reference
Africa				
La Réunion	90 districts	387[C]	92.8	Magnaval et al. (1994)
Americas				
U.S.A.	Various areas	20,395[AC]	13.9	Won et al. (2008)
Argentina	La Plata	156[AC]	46.9	Radman et al. (2000)
Brazil	Resistencia	206[C]	37.9	Alonso et al. (2000)
	Assis Brasil and Acrelândia	606[C]	21.5	Ferreira et al. (2007)
	São Paulo city	399[C]	38.8	Alderete et al. (2003)
	Campinas	138[AC]	27.7	Anaruma et al. (2002)
	Granada	403[AC]	26.8	Rubinsky-Elefant et al. (2008)
	Pres. Prudente	252[C]	11.1	Santarém et al. (2011)
	Teodoro Sampaio (settlement)	79[AC]	21.5	Prestes-Carneiro et al. (2008)
	São Paulo city	338[C]	26.9	Muradian et al. (2005)
	Sorocaba	180[C]	38.3	Coelho et al. (2004)
Peru	Lima	303[AC]	20.5	Espinoza et al. (2010)
Asia				
Iran	Sari City	1,210[C]	25.0	Sharif et al. (2010)
Taiwan	Districts in East	329[C]	76.6	Fan et al. (2004a)
Europe				
Spain	Santiago de Compostela	463[A]	28.6	Gonzalez-Quintela et al. (2006)

Table 3. Seroprevalences for human toxocariasis on different continents. Adapted from Rubinsky-Elefant et al. (2010). (Population studied: adult [A]; children [C]).

Toxocariasis tends to be more prevalent in tropical settings, compared to temperate regions, while rural populations are usually more exposed than urban populations in the same region (Rubinsky-Elefant et al., 2010). Reported seroprevalence rates in apparently healthy

subjects range from 2.4% in Denmark (3247 subjects ≤ 40 years old; Stensvold et al., 2009) to 92.8% in La Réunion (387 subjects> 15 years old; Magnaval et al., 1994).

Although some risk factors for toxocariasis have been identified in human populations, results remain largely inconsistent. Male gender, for example, was suggested to be associated with both higher (Alonso et al., 2000; Kanafani et al., 2006; Roldán et al., 2009; Won et al., 2008) and lower (Abo-Shehada et al., 1992; Magnaval & Baixench, 1993) risk of infection, whereas several large studies showed no association between gender and risk (Chieffi et al., 1990; Rubinsky-Elefant et al., 2008. Young age (Fan et al., 2004b; Rubinsky-Elefant et al., 2008), low socioeconomic status (Campos Junior et al., 2003; Lynch et al., 1988a; Santarém et al., 2011; Won et al., 2008), low parental education (González-Quintella et al., 2006; Won et al., 2008), poor sanitation (Alderete et al., 2003; Magnaval et al., 1994) and playing in sandpits (Paludo et al., 2007) are additional factors contributing to *Toxocara* exposure.

Having a dog has been recognized as a risk factor in most (Chiodo et al., 2006; Fan et al., 2004b; González-Quintella et al., 2006; Jarosz et al., 2010; Won et al., 2008), but not all studies of human toxocariasis (Ajayi et al., 2000; Rubinsky-Elefant et al., 2008). Discrepancies are not altogether surprising, especially in tropical settings where dogs roam freely and spread eggs across large areas. As a result, infection may be acquired, especially in sandpits of children's playgrounds, regardless of the presence of pet dogs in the households. The contribution of cat ownership to *Toxocara* seropositivity has been less studied. Having cats as pets has been described, in two recent serosurveys in Brazil, as representing both a risk (Paludo et al., 2007) and a protective factor (Rubinsky-Elefant et al., 2008). A third survey in Poland (Jarosz et al., 2010), but not a large nationwide study in the United States (Won et al., 2008) found cat ownership to be a significant predictor of *Toxocara* seropositivity.

The antigen used in ELISA (Enzyme-linked immunosorbent assay) contains both species-specific epitopes and epitopes that are shared between *T. canis* and *T. cati* (Kennedy et al., 1987). If species-specific epitopes predominate, serology would preferentially diagnose exposure to *T. canis*. Nevertheless, if cross-reactive epitopes predominate and exposure to *T. cati* is frequent, ELISA would be unable to distinguish between exposure to *T. canis* and *T. cati* with both dog and cat ownership emerging as a risk factor for seropositivity.

Positive associations have been described between *Toxocara* seropositivity and current infection with other nematodes, such as whipworm (Cancrini et al., 1998) and hookworm (Rubinsky-Elefant et al., 2008). These results may reflect some degree of cross-reactivity of antibodies to TES with proteins excreted by other tissue- or lumen-dwelling nematodes. Although test sera in most laboratories are pre-incubated with an *Ascaris suum* extract to prevent cross-reactivity with this common human nematode (Elefant et al., 2006), other highly prevalent helminths may still elicit cross-reactive antibodies (Lynch et al., 1988b). Alternatively, *Toxocara* and other soil-transmitted helminths may co-infect the same host due to the similar ways of acquiring these infections.

Prevalence and geographic distribution of infections with zoonotic hookworms in humans and their definitive hosts remain relatively unknown (Bowman et al., 2010). As a rule, human CLM is more prevalent in children living in regions with warm and humid climates. *U. stenocephala* infects dogs and cats in the Americas, Europe, Asia and Oceania, while *Anc. ceylanicum* is commonly found in South and Southeast Asia, Australia, and most parts of South America. *Anc. braziliense* can be found from the southeastern coast of North America (but not on the Pacific Coast of United States and Mexico) down to South America, in African countries and Southeast Asia, but less frequently in Australia.

Human CLM, known or presumed to be caused by *Anc. braziliense,* has been reported in many tropical and subtropical regions, including North and South America, Southern Europe, India, and the Philippines. The distribution of human infection overlaps with the geographic range of *Anc. caninum*. CLM is the most common dermatologic condition that affects North American and European tourists returning from tropical countries. These imported cases are often reported after exposure to beaches in regions where *Anc. caninum* is commonly found in their definitive hosts.

6. Laboratory diagnosis, clinical spectrum and treatment of human toxocariasis

Because larvae do not develop into adult worms in humans, these paratenic hosts do not pass *Toxocara* eggs in their feces. As a consequence, fecal examination does not contribute to the laboratory diagnosis of human toxocariasis. Definitive diagnosis of current infection can only be obtained by histological examination of infected tissue, but biopsies are rarely obtained for diagnostic purposes. Less commonly, ultrasonography, computed tomography, and nuclear resonance imaging are also used to detect and localize lesions suggestive of granulomas (Magnaval et al., 2001; Watthanakulpanich, 2010).

Virtually all *Toxocara* infections in humans are diagnosed serologically. The standard test to diagnose human toxocariasis is the indirect ELISA with antigens excreted-secreted by *T. canis* (TES) L3 larvae (de Savigny, 1975, 1979). The TES-based ELISA for IgG antibodies has been reported to be 78% sensitive and 92% specific (Glickman et al., 1986), although putatively more specific recombinant antigens have been obtained for serology. Since cross-reactive antibodies elicited by exposure to other helminths may reduce the specificity of TES-based serology in tropical populations (Lynch et al., 1988b; Watthanakulpanich et al., 2008), serum samples are usually pre-incubated with antigens of related nematodes, to remove cross-reacting antibodies. Our test samples are routinely pre-incubated with an adult worm extract of *A. suum* (Elefant et al., 2006).

Positive ELISA results can be confirmed by Western blot (Magnaval et al., 1991), but this technique is more expensive and labour-intensive than ELISA. Recombinant *T. canis* antigens, which are species-specific, have been expressed and used in prototype ELISAs for detection of antibodies, with promising results (Yamasaki et al., 2000). Among the four human IgG subclasses, specific IgG2 antibodies to TES antigens yield the highest sensitivity in ELISA (Watthanakulpanich et al., 2008), while detection of IgG4 antibodies contributes to increased specificity (Noordin et al., 2005).

Immunoblotting (IB) techniques, based on TES antigens, have been applied to improve serodiagnosis, and for follow-ups after chemotherapy (Rubinsky-Elefant et al., 2011).

Antigen-capture ELISAs with monoclonal antibodies have been developed (Gillespie et al., 1993; Robertson et al., 1988) but poor specificity precludes their use in routine diagnosis. Polymerase chain reaction (PCR)-based methods for *Toxocara* identification in clinical and environmental samples have been described (Fogt-Wyrwas et al., 2007; Zhu et al., 2001), but are not widely available.

The clinical spectrum of toxocariasis in humans, ranging from asymptomatic infection to severe organ injury, is determined by parasite load, sites of larval migration, and the host's inflammatory response. Two severe clinical syndromes are classically recognized: VLM (systemic disease caused by larval migration through major organs) and OLM (disease limited to the eye and optic nerve). Half a century ago, Beaver and colleagues described

Toxocara larvae in eosinophilic granulomas in the liver of young children with extreme eosinophilia, hepatomegaly, respiratory symptoms, anemia and geophagia, and introduced the term VLM to describe this clinical syndrome (Beaver et al., 1952). Wilder found nematode larvae in eosinophilic granulomas of enucleated eyes of children with suspected retinoblastoma, providing the first description of the condition currently known as OLM (Wilder, 1950).

Classical VLM occurs typically in children aged 2-7 years, but infections in adults, at least some of which are acquired by ingesting raw organs of paratenic hosts, are relatively frequent in East Asia (Akao & Ohta, 2007). The full-blown VLM syndrome usually includes fever, lower respiratory symptoms such as cough, dyspnea and bronchospasm associated with larval migration, hepatomegaly, abdominal pain and decreased appetite. Laboratory findings include hypergammaglobulinemia, increased isohemagglutinin titres to A and B blood group antigens, anaemia and leukocytosis with marked eosinophilia (Jacob et al., 1994). As a rule, seropositive subjects in population-based surveys are asymptomatic or have rather nonspecific and mild symptoms. A case-control study in Ireland led to the description of a new clinical entity in seropositive children, called "covert toxocariasis", comprising mainly fever, headache, behavioural and sleep disturbances, cough, anorexia, abdominal pain, hepatomegaly, nausea and vomiting (Taylor et al., 1987). Another case-control study, in French adults, led to the definition of "common toxocariasis", a syndrome comprising chronic dyspnea and weakness, cutaneous rash and pruritus, as well as abdominal pain (Glickman et al., 1987).

The liver is the most commonly affected visceral organ. Typical hepatic granulomas have multinucleated giant cells and epithelioid cells surrounding necrotic debris or amorphous eosinophilic material. Eosinophils and mononuclear cells are often seen in the outer layers of the granulomas (Musso et al., 2007). On computed tomography, hepatic lesions are typically ill-defined, low-attenuating nodules (Cameron et al., 1997) that have sometimes been confounded with metastatic cancer (Ota et al., 2009).

The cutaneous manifestations of human toxocariasis have been recently reviewed (Gavignet et al., 2008) and include chronic prurigo, pruritus and urticaria, eczema, exanthema (Bernardeschi et al., 2011), and vasculitis.

Central nervous system involvement in toxocariasis comprises eosinophilic meningitis and encephalitis (Moreira-Silva et al., 2004), myelitis (Lee et al., 2009), cerebral vasculitis (Helbok et al., 2007) and optic neuritis, while manifestations of peripheral nervous system involvement include radiculitis (Moreira-Silva et al., 2004) and cranial nerve palsy (Finsterer & Auer, 2007). Central nervous system involvement in VLM has been associated with epilepsy (Woodruff et al., 1966), behavioral changes and cognitive deficits. *Toxocara* may represent a co-factor in idiopathic seizures (Critchley et al., 1982), and especially in partial epilepsy (Nicoletti et al., 2007). The presence of granulomas in the brain has been suggested to elicit focal seizures (Critchley et al., 1982). Research to verify increased risk of cognitive deficits in infected children has remained inconclusive (Jarosz et al., 2010).

There are very few controlled trials on anthelmintic drugs for VLM in the literature. Since parasitological cure in patients cannot be assessed, the end-point of published trials is a decrease in the severity of clinical signs and symptoms. A dose of 500 mg of albendazole twice a day for 5 days is currently recommended. Albendazole seems to be superior to thiabendazole (50 mg/kg of body weight daily for 3-7 days) (Stürchler et al., 1989). Diethylcarbamazine (3-4 mg/kg of body weight daily for 21 days, starting at 25 mg/day and increasing the dose progressively) is also effective (Magnaval, 1995). Most human

infections with *Toxocara*, however, cause much less severe systemic manifestations, if any, and treatment is not required.

Eosinophilia and elevated levels of IgE are commonly found in toxocariasis, as well as high titres of *Toxocara* antibodies. Covert and common toxocariasis are likely to represent slight variations in the clinical spectrum of mild infections in children and adults, respectively.

Although wheezing is a common presenting feature of VLM, whether or not *Toxocara* infection predisposes to asthma remains uncertain. Some epidemiological studies have shown a positive association between wheezing or asthma and *Toxocara* seropositivity (Desowitz et al., 1981; Ferreira et al., 2007; González-Quintella et al., 2006), while others failed to detect such an effect (Fernando et al., 2009; Sharghi et al., 2001). Asthma symptoms can result from larval migration through the lungs, but a role has also been proposed for parasite-induced atopy (Cooper, 2009).

Compared with systemic disease, ocular toxocariasis or OLM usually affects older children, with an average age at onset of 7.5 years (range, 2-50 years) (Taylor, 2001). About 80% of cases are diagnosed in patients younger than 16 years of age (Brown, 1970). Males tend to be more frequently affected than females (Brown, 1970; Taylor, 2001). The clinical condition currently known as OLM or ocular toxocariasis was first described by Wilder (1950), who found nematode larvae or their residual hyaline capsules during the histological analysis of 24 eyes that had been enucleated from children with suspected retinoblastoma.

The clinical presentation of OLM depends on the primary anatomic site involved and the immune response of the host. A single eye is affected in most patients (Taylor, 2001). The most common symptoms are strabismus, unilateral decreased vision and leukocoria (white eye). Peripheral, posterior pole retinal granuloma and endophthalmitis are the usual presentations on the eye exam.

The presence of a vitreous band, or a membrane extending between the posterior pole and high-reflective peripheral mass, detected by ocular ultrasound, may help in the diagnosis when the ocular medium is opaque (Figure 3).

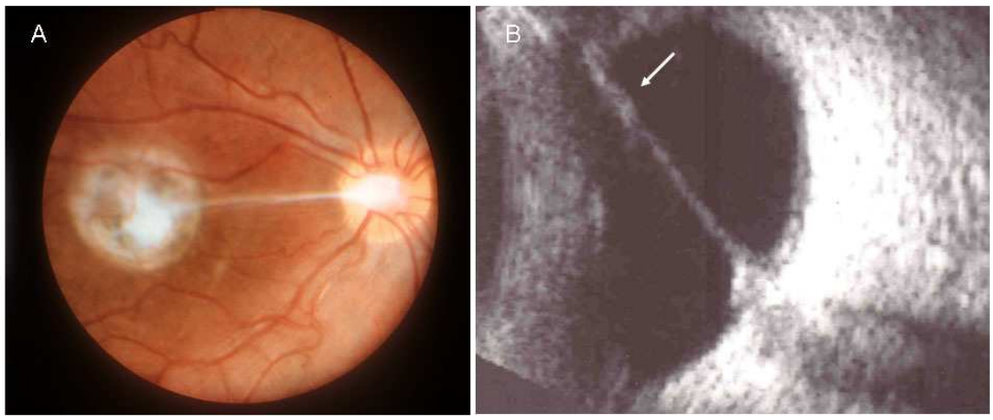

Fig. 3. Clinical presentation of ocular toxocariasis. A. Peripheral retinal and vitreous lesion, a localized mass of whitish tissue involving the retina and peripheral vitreous with a fibro-cellular band running from the periphery toward the optic nerve or posterior retina. B. Ocular ultrasound showing a vitreous band or membrane (arrow) extending between the posterior pole and high-reflective peripheral mass. (Image Courtesy: Ophthalmology Clinic of the University of São Paulo - Medical School General Hospital, HC-FMUSP).

Other uncommon clinical presentations of OLM include optic disc inflammation (papillitis or neuroretinitis), motile intraocular nematode (retina, vitreous body and anterior chamber), keratitis and cataract.

The diagnosis of OLM is usually suggested by the presence of the clinical findings mentioned above, but the detection of specific antibodies is required. However, serum antibodies can often be undetectable (Sharkey & McKay, 1993), possibly due to the relatively low parasite load in these infections (Schantz, 1989). Even low ELISA serum titres may be of diagnostic value in OLM, but there is no consensus on the cut-off titres for diagnosis. Specific antibodies can be also detected in the aqueous humor (AH). The intraocular production of antibodies to *Toxocara* can be assessed by comparing serum and AH samples obtained simultaneously from the same patients and calculating the Goldmann-Witmer (GW) coefficient as: ([levels of specific IgG in AH/levels of specific IgG in serum]/[total IgG in AH/total IgG in serum]). A GW coefficient > 3 indicates intraocular production of specific antibodies (De Visser et al., 2008).

Sight-threatening ocular inflammation secondary to OLM requires aggressive anti-inflammatory therapy, combined with albendazole (800 mg for adults and 400 mg for children daily) over a 2-4 week period. Oral steroids (prednisone 0.5 mg/kg/day) are used to reduce the inflammatory response induced by larvae. Surgical treatment may be required for retinal detachment or intravitreal fibrovascular membrane proliferation.

7. Cutaneous larva migrans

CLM is a clinical entity caused in humans by larval migration of zoonotic hookworms, mainly *Anc. braziliense* but also *Anc. caninum*. Less common clinical manifestations are eosinophilic pneumonitis, localized myositis, folliculitis and erythema multiforme but eye involvement is rarely reported. Human infection occurs when infective L3 larvae penetrate the skin. The infection site may or may not present an erythematous popular or vesicular rash.

The larvae do not undergo further molts in the human host, but as they migrate across in the skin, at a rate of 2.7 mm per day, they leave an erythematous, serpiginous track. The cutaneous lesions can last several weeks and may be severely pruritic, but eventually resolved spontaneously. Secondary bacterial infection may result from scratching.

The most commonly affected sites are those in close contact with the soil (Araújo et al., 2000). A recent case series of CLM in Brazil, for example, showed that cutaneous lesions are more frequently observed on the feet (73.3%), buttocks (14.7%), genital and inguinal areas (8.0%), legs (2.7%), and hands (1.3%) (Jackson et al., 2006). Other sites, such as the face (Bouchad et al., 2000) and the scalp (Guimarães et al., 1999), are rarely affected. Clinical diagnosis is reached based on the typical skin lesions, while biopsies have little diagnostic value, showing an eosinophilic inflammatory infiltrate. Chemotherapy is seldom needed, since larvae die out within a matter of weeks if left untreated, but oral albendazol (400-800 mg/day for 3-5 days), oral ivermectin (200 μg/kg, single dose) or topical thiabendazole (10% aqueous suspension four times a day) may be used.

Larval infection of humans with *Anc. ceylanicum* may occasionally give rise to adult worms that inhabit the small intestine and can cause eosinophilic enteritis (Bowman et al., 2010). In addition to cutaneous lesions, *Anc. caninum* has also been reported to cause eosinophilic enteritis and may be a cause of diffuse unilateral subacute neuroretinitis in humans (Sabrosa & de Souza, 2001).

8. Prevention and control of zoonotic soil-transmitted helminth infections in humans and companion animals

Contaminated soil is the most important route of transmission of zoonotic helminths to humans. Environmental contamination is particularly relevant when public areas (parks, playgrounds, beaches) are affected. Effective preventive measures include covering sandboxes in public parks and playgrounds when not in use, allowing no dogs and cats on bathing beaches, and controlling stray dog and cat populations.

In southeastern Brazil, Santarém et al. (2004) reported a significant decrease in the incidence of LMC after replacement of soil in sandboxes and enclosure of playground areas with fences. Also in southern Brazil, Cassenote et al. (2011) observed that the frequency of geo-helminthes in fenced parks (11.1%) was significantly lower than that verified in non-fenced off areas (45.3). Similarly, Avcioglu & Balkaya (2011) observed in Turkey that fenced parks were free of *Toxocara* eggs, while 64.3% of open areas were contaminated with eggs.

Periodic prophylactic deworming of companion animals and educational measures aimed at pet owners are also critical for controlling infections by soil-transmitted helminths (Stull et al., 2007). A strategic program for decreasing soil contamination with zoonotic helminths should include elimination of intestinal parasites from puppies and kittens. Since puppies and kittens harbor adult *Toxocara* and hookworm due to infections via placenta and/or milk, treatment must target newborn animals, before eggs are first shed in the feces.

The WHO (2011), based on considerations by Barriga (1988, 1991), currently recommends treatment for puppies at two weeks of age to eliminate larvae acquired through transmammary or transplacentary transmission. Treatment is repeated at 4, 6 and 8 weeks. For kittens, treatment must be done at third, fifth, seventh and ninth weeks of life to eliminate the larva passed through milk. A single dose of anthelmintic for queens, 10 days after delivery, is also recommended (Barriga, 1991). Laboratory confirmation of infection, with stool examination using concentration methods (most often based on flotation procedures), is normally required prior to treatment of animals older than six months of age, to prevent uncontrolled use of anthelminthic drugs and the emergence of resistant parasites.

Veterinarians are thought to be on the 'front line' of prevention of pet-associated zoonotic parasitic infections (Smith et al., 2009). However, recent surveys have revealed that veterinarians often misinterpret and misuse the available protocols for deworming newborn pets. In Canada, 80-90% of the protocols recommended for puppies and kittens were inappropriate (Stull et al., 2007), and in the USA only 16% of the veterinarians interviewed knew how to deworm puppies (Harvey et al., 1991). In addition, veterinarians' perception concerning small animal-derived zoonoses should be improved, with emphasis on their role in disseminating information about these diseases to their clients (Stull et al., 2007).

Human infections with canine and feline helminths ranks among the most common zoonotic infections worldwide, yet remain relatively unknown to the public and pet owners. Katagiri & Oliveira-Siqueira (2008), in São Paulo, Brazil, observed a low level of risk perception of zoonotic infection by dog owners in Brazil. Pet owners should know how to prevent environmental contamination and to reduce the risk of human infection with zoonotic helminths. This requires a clear understanding of zoonoses acquired from small animals, of the need for appropriate deworming strategies for pets, and the need for removing feces from the environment where their dogs evacuate.

The public must also be informed about the risks of exposing children to public parks and beaches frequented by animals and of eating soil or biting nails, as well as about the benefits of washing hands after handling fecal material or playing with pets.

The American Veterinary Medical Association (AVMA, 2008) considers that the convergence of people, animals, and our environment has created a new dynamic in which the health of each group is inextricably interconnected. The Association proposed a holistic, collaborative approach aimed at improving animal and human health globally through collaboration among all the health sciences, especially between the veterinary and human medical professions to address critical needs.

9. Conclusion

Based on the findings in this review, it can be asserted that the lack of standardisation of techniques coupled with the host of factors influencing the process of egg/larvae recovery can lead to false-negative results and underestimation of the occurrence of soil contamination. Thus, the development of new methods is necessary to provide more reliable data under field conditions. Molecular analyses, based on amplification of genetic material extracted from eggs/larvae present in soil, are promising techniques both for identifying and characterizing of helminths present in soil.

It was also observed that soil contamination in public areas can be reduced by adopting a number of measures including: restriction of uncontrolled dogs and cats, cleaning up dog feces from soil and pavements by their owners, preventing access of dogs and cats to public spaces (especially children's playgrounds) and by use of strategic anthelmintic treatment of dogs and cats with emphasis on puppies, kittens, nursing bitches and queens.

Programs designed through collaborative efforts of both human and veterinary doctors/researchers are essential to create fresh tools for diagnosis and new strategies for controlling the transmission of soil-transmitted helminthic zoonoses to humans, until new technologies become available.

According to the WHO (2011), one of the main strategies for controlling zoonotic diseases is to promote advocacy so as to emphasize their burden on society and create demand at all levels of society to control them. The American Veterinary Medical Association (AVMA, 2008) has considered that the convergence of people, animals, and our environment has created a new dynamic in which the health of each group is inextricably interconnected. The Association has proposed a holistic, collaborative approach aimed at improving animal and human health globally through collaboration among all the health sciences, particularly between the veterinary and human medical professions to address critical needs.

Many diseases are considered neglected zoonotic diseases, including soil-transmitted helminths. Thus, efforts to design public educational programs raising awareness of agents of larva migrans are fundamental to prevent the burden of diseases in companion animals and humans. Further, improvements in diagnostic testing and expansion of epidemiologic surveillance should be promoted in parallel with control and prevention efforts.

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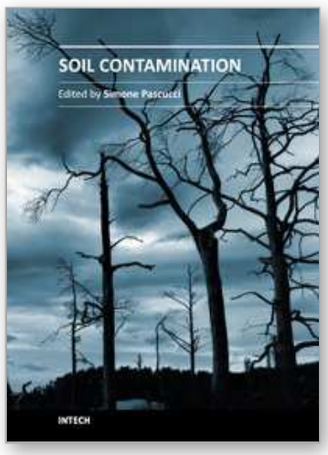

Soil Contamination

Edited by MSc Simone Pascucci

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Soil contamination has severely increased over the last decades, mainly due to petroleum hydrocarbons, solvents, pesticides, lead and other heavy metals from industrial wastes and human activities. The critical point regarding contaminated soil monitoring is the intrinsic difficulty in defining fixed monitoring variables and indicators as the establishment of any a priori criterion and threshold for soil quality can be still considered subjective. This book is organized into eight chapters and presents the state-of-the art and new research highlights in the context of contaminated soil monitoring and remediation strategies, including examples from South America, Europe and Asia. The chapters deal with the following topics: - monitoring of dioxin, furan, hydrocarbons and heavy metals level in soils - bioindicators and biomarkers for the assessment of soil toxicity - use of reflectance spectroscopy for soil contaminants and waste material detection - remediation technologies and strategies.

How to reference

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