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Toxocara in sandpits of public playgrounds and kindergartens in Flanders (Belgium)

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ABSTRACT

Belgium counts more than a million dogs and at least two million cats, of which many are carrying zoonotic nematodes of the genus *Toxocara*. Environmental contamination with worm eggs is considered the key transmission route from animals to humans, and mainly young children are at risk. Contamination of soil with *Toxocara* eggs has been reported from all over the world, but data are lacking for Belgium. In this study, faecal contamination and the presence of *Toxocara* eggs in sand were investigated in sandpits of public playgrounds and kindergartens in Flanders (Northern Belgium). Faeces, of which 85% originated from cats, were found in about one third of the public playgrounds and one fifth of the kindergartens. *Toxocara* eggs were found in 12% of the faecal samples, in 14% of the public sandpits, and in 2% of the sandpits of kindergartens. These data indicate that environmental contamination with *Toxocara* exists in urban areas in Belgium, and that cats are most likely the main source.

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1. Introduction

Toxocara canis and *T. cati* are roundworms of dogs and cats that are generally considered as zoonotic agents. Although humans cannot act as a definitive host for *Toxocara* species, they can accidentally be infected by ingestion of embryonated eggs, after which the larvae can migrate throughout the body and survive for a long time. Ingestion of *Toxocara* eggs can pass by without any symptoms, or can give rise to nonspecific clinical signs like headache and abdominal pain (so-called covert toxocarosis). However, more specific clinical pictures resulting from *Toxocara* infection are described as well, particularly in young children. The so-called visceral larva migrans (VLM) syndrome results from migrating larvae that cause damage to tissues, such as liver and lungs, resulting in local inflammatory responses and extreme eosinophilia. In case of ocular larva migrans (OLM), larvae end up and cause damage to the retina, eventually leading to visual loss. Furthermore, there is growing evidence that *Toxocara* infections contribute to the development of asthma and allergic reactions, although this association has not yet been fully elucidated (Macpherson, 2013; Overgaauw and van Knapen, 2013; Smith et al., 2009).

Belgium counts about 1.3 million dogs, and about 2.0 million cats, stray cats not included (FEDIAF, 2014). A cross-sectional study in Flanders (Northern Belgium) revealed that 4.4% of the household dogs and 26.3% of kennel dogs excreted *T. canis* eggs (Claerebout et al., 2009). In pet shops and breeding facilities, prevalence rates may even be higher (Dupont et al., 2013). In cats, *T. cati* prevalence varies between 8.2% in Belgian household cats and 66.7% in stray cats (Claerebout et al., 2011). These numbers indicate that there exists a large reservoir of *Toxocara* eggs in the Belgian dog and cat population, posing a risk to human health.

Several routes of infection with *Toxocara* in humans have been described, such as direct contact with stray animals carrying embryonated eggs in the fur (Roddie et al., 2008) and consumption of raw unwashed vegetables (Klapec and Borecka, 2012). However, ingestion of soil contaminated with embryonated eggs is considered the main transmission route of *Toxocara* to humans, in particular young children. Indeed, large numbers of eggs are spread into the environment by defecation of dogs and cats, and once embryonated, these eggs remain infective for a long time (Azam et al., 2012). Environmental contamination of soil with *Toxocara* eggs has been reported from around the world, and several studies from the last 10 years indicate that this remains a problem in Europe as well (Dado et al., 2012; Dubná et al., 2007; Ferroglio et al., 2006; Kroten et al., 2015; Papajová et al., 2014; Talvik et al., 2006). In the current study, environmental contamination with *Toxocara* eggs was investigated for the first time in Belgium, with a focus on sandpits of public playgrounds and kindergartens.

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2. Materials and methods

2.1. Study area

This study was performed in two cities in Flanders (Northern Belgium) that want to remain anonymous and are further indicated as 'city A' (about 60,000 inhabitants) and 'city B' (about 250,000 inhabitants). In city A, 16 sandpits were investigated, distributed over 10 public playgrounds. In city B, 61 sandpits were investigated, distributed over 45 public playgrounds. Twelve playgrounds in city B were maintained at least once a week, during which faeces in sandpits were systematically removed. The maintenance frequency of the other playgrounds is not known. In city B, also kindergartens were sampled: 43 sandpits were investigated, distributed over 27 kindergartens for children between 2 and 6 years of age. In 22 of these sandpits, the sand had been replaced between the year 2013 and 2015, while the other sandpits remained untouched during this period.

2.2. Sampling

Every location was visited once (city A: May 2014, city B: April–July 2015), during which all sandpits were inspected for the presence of faeces. If faeces were present, they were collected for further investigation. Subsequently, sand was collected in each sandpit from the top layer (first 5 cm of depth) at 4 to 7 random locations and pooled until a volume of 140 mL was reached. This volume corresponded with 200 g (±30 g) of sand.

2.3. Faecal examination

Faecal samples were subjected to DNA extraction and PCR to determine their origin (dog or cat), according to Nonaka et al. (2009) with slight modifications. Faeces were frozen, the surface was washed to collect intestinal cells, and DNA was extracted using the QiaAmp DNA Stool Mini Kit (Qiagen). Next, a duplex PCR was performed for the mitochondrial D-loop regions of both dog and cat, using species-specific forward primers (dog: ttccctgacacccctacattc, cat: cgatcttctatggacctcaactat) and a universal reverse primer (cctgaagtaggaaccagatg). PCR mixes (25 μL per reaction) consisted of 1× Herculase II Reaction Buffer, 2% dimethylsulphoxide, 0,1 μg/μL bovine serum albumin, 1 unit of Herculase II enzyme (Agilent Technologies), 200 μM of each dNTP, 400 nM of each primer, 2 μL template DNA and an appropriate volume of DNase and RNase free water. 35 cycles of 1′ denaturation at 94 °C, 30″ annealing at 56 °C and 1′ elongation at 72 °C were run and PCR products were separated on 2% agarose and visualised with ethidiumbromide and UV light. Amplicon lengths are 160 bp for cat and 355 bp for dog (Fig. 1).

In addition, faecal samples were investigated for the presence of *Toxocara* eggs by sedimentation-flotation. Approximately 5 g of faeces was mixed with 50 mL of tap water and sieved 3 times through a tea strainer. Sedimentation was allowed for 15 min, and the sediment was subsequently centrifuged for 2 min at 1300 RCF. The supernatant was discarded and the pellet was subjected to flotation at 100 RCF for 2 min in a sucrose solution with specific density 1.27. After another 2 min, the coverslip was transferred to a microscope slide and investigated at 100× magnification for the presence of *Toxocara* eggs by light microscopy.

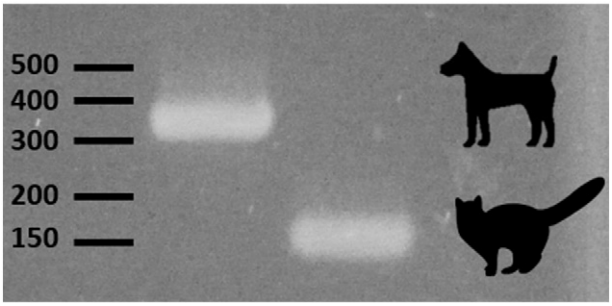

Fig. 1. Example of a duplex PCR result on faecal samples from a dog and a cat.

2.4. Investigation of sand for the presence of Toxocara eggs

Sand samples (200 g) were investigated for the presence of *Toxocara* eggs by flotation on sucrose after enrichment by sieving on a vibratory sieve shaker (Retsch AS200 basic). The sand samples were mixed with an equal volume of 5% Tween 20 and poured onto the first of 3 sieves with mesh diameters of 300 μm, 125 μm and 50 μm respectively. The sieve tower was flushed with tap water while shaking during 20 min, after which the fraction residing on the 50 μm sieve was collected. After sedimentation for 1 h, this sand fraction, only containing particles with a diameter between 50 and 125 μm, was aliquoted into 4 fractions that were each subjected to sedimentation and flotation as described above. *Toxocara* eggs were microscopically differentiated from other structures such as plant pollen, mite eggs and eggs of other nematodes based on the round or slightly oval shape, the typically pitted shell and a diameter of 60–90 μm (Fig. 2).

3. Results

3.1. Faecal contamination of sandpits

Every location in this study was visited once and inspected for the presence of faecal samples (Table 1). Faeces in sand were found at 18 public playgrounds (33%) and 6 kindergartens (22%), and sometimes in numerous sandpits at the same location. At the time of sampling, 12 sandpits in kindergartens were entirely covered with a canvas or finely meshed gauze, and in these sandpits no faeces were found. In city A, the origin of faeces could not be determined since the PCR test was not yet available at that time. In city B, the majority (85%) of faeces originated from cats, although dog faeces were found as well but remained restricted to public playgrounds. Sometimes, several faecal samples were found at the same location, and it is not excluded that these originated from one and the same animal. However, faeces of both dog and cat were found in one of the public playgrounds in city B, indicating that mixed contaminations can occur as well. All faecal samples collected in this study were subjected to sedimentation and flotation, and *Toxocara* eggs were found in 5 out of 41 faecal samples (12%). As far as determined, all *Toxocara* positive faeces originated from cats.

3.2. Toxocara eggs in sand

A total of 200 g of sand was sampled randomly from every sandpit and investigated for the presence of *Toxocara* eggs (Table 2). *Toxocara* was found in 11 sand samples (14%) originating from sandpits distributed over 10 public playgrounds of both cities. Furthermore, eggs were found in 1 sandpit (2%) in a kindergarten in city B. In this kindergarten, the sand had not been replaced for at least 2 years and the sandpit was not covered at the time of sampling. Remarkably, none of the positive sand samples originated from sandpits where *Toxocara* positive faeces were found. The numbers of eggs found per 200 g of sand in the different positive samples varied between 1 and 50. Several developmental stages of *Toxocara* were observed during this study, from non-embryonated eggs over multi-cellular stages to fully larvated eggs (Fig. 2).

4. Discussion

In this study, environmental contamination with *Toxocara* eggs was investigated for the first time in Belgium. *Toxocara* eggs were demonstrated in 14% of the sand samples collected from public playgrounds,

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