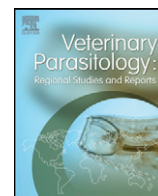


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## *Toxocara* in sandpits of public playgrounds and kindergartens in Flanders (Belgium)

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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).

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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 (Klapek 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.

## 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 ( $\pm 30$  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: ttccctgacaccctacattc, cat: cgatcttctatggacctcaactat) and a universal reverse primer (cctgaagtaggaaccagatg). PCR mixes (25  $\mu$ L per reaction) consisted of 1 $\times$  Hercules II Reaction Buffer, 2% dimethylsulphoxide, 0.1  $\mu$ g/ $\mu$ L bovine serum albumin, 1 unit of Hercules II enzyme (Agilent Technologies), 200  $\mu$ M of each dNTP, 400 nM of each primer, 2  $\mu$ L 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

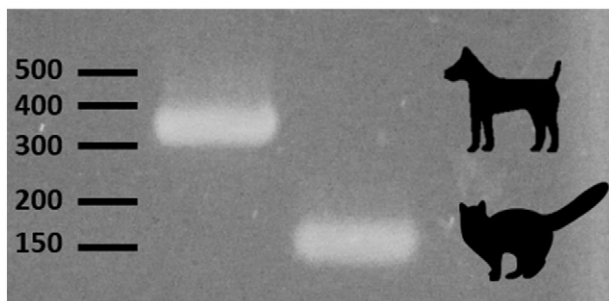


Fig. 1. Example of a duplex PCR result on faecal samples from a dog and a cat.

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 $\times$  magnification for the presence of *Toxocara* eggs by light microscopy.

### 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  $\mu$ m, 125  $\mu$ m and 50  $\mu$ m respectively. The sieve tower was flushed with tap water while shaking during 20 min, after which the fraction residing on the 50  $\mu$ m sieve was collected. After sedimentation for 1 h, this sand fraction, only containing particles with a diameter between 50 and 125  $\mu$ m, 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  $\mu$ m (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,

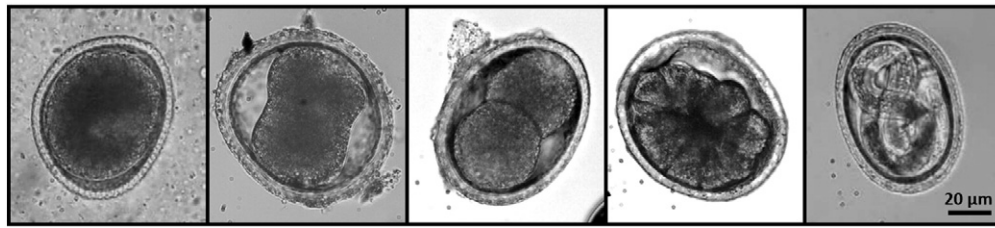


Fig. 2. Different stages of embryonation in *Toxocara* eggs found in sand samples (400×).

and in 2% of samples collected from kindergartens. These data are comparable with similar studies in different European countries, such as Spain (16%) (Dado et al., 2012), Czech Republic (12%) (Dubná et al., 2007), Estonia (4%) (Talvik et al., 2006) and Slovakia (7%) (Papajová et al., 2014). A study in the Netherlands, neighbouring Flanders, demonstrated *Toxocara* contamination in about 50% of public sandpits, but this study goes back to the early nineties (Jansen et al., 1993). Although it is clear from all these studies that environmental *Toxocara* contamination is still common in Europe, care should be taken when comparing different studies since both the sampling procedure and the detection technique are often different. In the current study, a pooled sand sample of about 200 g was collected from each sandpit and subjected to fractionation by sieving. This approach allows flotation on a fraction of 10–20 g in which *Toxocara* eggs have been concentrated. Still, only one or a few eggs were found in most of the positive samples, indicating that *Toxocara* contamination in sand can easily be missed and actual prevalence rates may be higher. This is further supported by the fact that positive faeces were found in 5 sandpits where no eggs were observed in the sand. Nevertheless, the results from this study show that there actually exists contamination of sandpits with *Toxocara* eggs in Flanders, comparable with other European countries.

No differentiation between *T. canis* and *T. cati* eggs was made in this study. Microscopical differentiation based on differences in egg size was not considered reliable, and molecular differentiation by real-time PCR according to Durant et al. (2012) failed, probably due to the low numbers of eggs in the samples. However, the origin of the faecal samples found in sandpits was successfully identified by PCR in one city, and cat faeces were clearly more present than dog faeces. Given that stray cats in Belgium are far more affected with *Toxocara* than dogs (Claerebout et al., 2009; Claerebout et al., 2011; Dupont et al., 2013), it can tentatively be concluded that cats were the main source of the *Toxocara* contamination observed in the sandpits.

Although *Toxocara* is one of the most widespread zoonotic parasites worldwide, the impact on public health is still elusive, since symptoms are often not pathognomic and the relation with allergies and asthma is yet unclear (Macpherson, 2013; Smith et al., 2009). Furthermore, the zoonotic potential of *T. cati* seems to have been underestimated for a long time compared to *T. canis* (Fisher, 2003). So the fact that fully embryonated eggs – either from *T. canis* or *T. cati* – were found at places exclusively intended for playing children is worrying, but does not allow to speculate on health consequences for these children. Remarkably, a recent study at the Belgian Institute of Tropical Medicine considers toxocarosis as a tropical disease, and showed that 5.5% of the *Toxocara*

Table 1

Faecal contamination in sandpits of public playgrounds and kindergartens. ND = not determined.

	Public playgrounds		Kindergartens
	City A	City B	City B
Locations with faeces	7/10	11/45	6/27
Sandpits with faeces	7/16	13/61	8/43
Faecal samples	7	21	13
Dog faeces	ND	5	0
Cat faeces	ND	16	13
<i>Toxocara</i> positive faeces	2/7	1/21	2/13

tests performed at post-travel consultations were positive, including a number of patients with clear clinical manifestations (Van Den Broucke et al., 2015). Our study shows that those people can as well have been infected at home and raises awareness about human toxocarosis in Belgium.

## 5. Conclusions and recommendations

This study demonstrates *Toxocara* contamination of public and private sandpits for the first time in Belgium, and indicates the need for preventive measures. The effectiveness of such measures largely depends on local circumstances and specific features of the affected location, but appropriate anthelmintic treatment of dogs and cats should be part of an integrated approach. Nijse et al. (2015) estimated that, in the Netherlands, the overall egg output by non-juvenile dogs would be reduced by 29% if 90% of all dog owners would comply to the four times a year deworming advice of the European Scientific Counsel of Companion Animal Parasites (ESCCAP). However, while stimulating commitment of dog and cat owners to anthelmintic treatment schedules is achievable, proper deworming of the stray cat population is much more challenging, if not impossible. Therefore, protecting sandpits against defecating animals is highly recommended, and this can easily be achieved by covering the sand with a canvas or gauze every time it remains unoccupied. Indeed, in this study, all sandpits that were entirely covered at the time of sampling showed no traces of faeces nor *Toxocara* eggs. However, faeces were found in sandpits that were incompletely covered, or where the canvas was blown away by the wind, stressing the need to fix it properly. While systematic coverage is achievable for small sandpits at private locations, this is difficult to organise at larger public places, especially when irremovable playground equipment is present. For such locations, the focus should be shifted towards regular removal of faeces. Both cities involved in this study have a well elaborated dog waste clean-up policy, but still one third of the public locations was polluted with faeces, mainly originating from cats. Therefore, measures that deal with the population of stray cats and feral cats in urban areas are needed to keep the sandpits clean, and free of *Toxocara* contamination.

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Table 2

*Toxocara* eggs in sandpits of public playgrounds and kindergartens.

	Public playgrounds		Kindergartens
	City A	City B	City B
Sandpits positive for <i>Toxocara</i> eggs	1/16	10/61	1/43
Affected locations	1/10	9/45	1/27

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