Journal of Infection and Public Health xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

# Environmental contamination with *Toxocara* spp. eggs in public parks and playground sandpits of Greater Lisbon, Portugal

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ARTICLE INFO

*Article history:*
Received 20 August 2016
Received in revised form 11 April 2017
Accepted 1 May 2017

*Keywords:*
*Toxocara*
Prevalence
Environmental contamination
Zoonosis
Public health
Portugal

ABSTRACT

Toxocarosis is a zoonotic parasitic disease transmitted from companion animals to humans. Environmental contamination with *Toxocara* eggs is considered to be the main source of human infections. In Portugal, knowledge regarding the current situation, including density, distribution and environmental contamination by *Toxocara* spp., is largely unknown. The present study investigated environmental contamination with *Toxocara* spp. eggs, in soil and faecal samples collected from public parks and playground sandpits in Greater Lisbon, Portugal. A total of 151 soil samples and 135 canine faecal samples were collected from 7 public sandpits and 12 public parks, over a 4 month-period. Soil samples were tested by a modified centrifugation and sedimentation/flotation technique and faecal samples were tested by an adaptation of the Cornell-Wisconsin method. Molecular analysis and sequencing were performed to discriminate *Toxocara* species in the soil. Overall, 85.7% of the sandpits (6/7) and 50.0% of the parks (6/12) were contaminated with *Toxocara* spp. eggs. The molecular analysis of soil samples showed that, 85.5% of the sandpits and 34.4% of the parks were contaminated with *Toxocara cati* eggs. Faecal analysis showed that 12.5% of the sandpits and 3.9% of the parks contained *Toxocara canis* eggs. In total, 53.0% of soil and 5.9% of faecal samples were positive for *Toxocara* spp. Additionally, 56.0% of the eggs recovered from the samples were embryonated after 60 days of incubation, therefore considered viable and infective. The average density was 4.2 eggs per hundred grams of soil. Public parks and playground sandpits in the Lisbon area were found to be heavily contaminated with *T. cati* eggs, representing a serious menace to public health as the studied areas represent common places where people of all ages, particularly children, recreate. This study sounds an alarm bell regarding the necessity to undertake effective measures such as reduction of stray animals, active faecal collection by pet owners, awareness campaigns and control strategies to decrease the high risk to both animal and human health.



## Introduction

Toxocarosis is a zoonotic infectious disease associated with parasites, transmitted from companion animals to man [1]. It can be caused either by *Toxocara canis* or *Toxocara cati*, which are both ubiquitous and prolific monoxenous nematodes. These agents are intestinal parasites of dogs and cats that are present in the majority of new-born puppies and kittens, as well as adult dogs and cats. Several species including humans may serve as paratenic hosts, where larvae migrate and encyst in tissues and organs, surviving for months or even years [2]. Infections with *Toxocara* spp. in humans may cause several clinical syndromes described as Visceral Larva Migrans (VLM), Ocular Larva Migrans (OLM), covert toxocarosis and Neural Larva Migrans (NLM) [3]. However, the vast majority of human *Toxocara* infections are asymptomatic [4].

Although humans may get infected through the ingestion of encysted larvae present in raw or undercooked meat [5,6], most infections are acquired through the ingestion of embryonated eggs by geophagia, in areas where infected cats, dogs or wildlife have defecated. This is particularly common in children [1]. In general,

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http://dx.doi.org/10.1016/j.jiph.2017.05.002

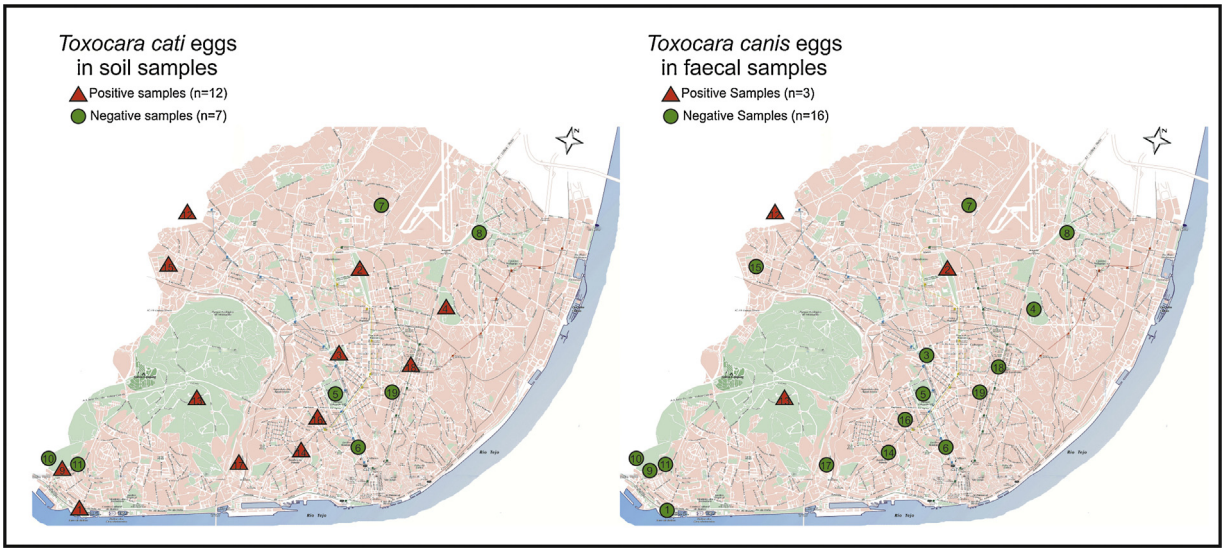


**Fig. 1.** Map of the Lisbon Metropolitan area, highlighting the distribution of positive (triangle) and negative (circle) sites to *Toxocara cati* eggs in soil samples and *Toxocara canis* eggs in faecal samples. *Note*: The numbers inside the triangle or circle correspond to each of the 19 study sites referred on Table 1.

eggs require an incubation period of 3–6 weeks in the environment before becoming infective. Therefore, public parks and playground sandpits may play a crucial role in the perpetuation of this infection. Indeed, *Toxocara* spp. eggs have been recovered worldwide in sand or soil samples from playgrounds and/or public parks [7]. In Europe high levels of environmental contamination with *Toxocara* spp. have been reported, ranging from 16.4% in Spain [8], 5.0–45.0% in the Czech Republic [9] to 10.7–81.8% in Poland [10,11]. Furthermore, studies performed in humans to assess antibodies against *Toxocara* revealed high seroprevalence levels [12].

In companion animals, particularly puppies, heavy prenatal infections may cause severe disease with alternating diarrhoea and constipation, vomiting, respiratory signs due to pneumonia, reduced growth, cachexia, typical 'pot belly', poor coat and even death [13]. In older dogs less severe clinical manifestations are observed and most infections pass unnoticed. In kittens, clinical signs are similar to puppies, although generally less severe [1].

In Portugal, knowledge about the current situation, distribution and contamination by *Toxocara* spp. is largely unknown, particularly regarding environmental contamination. However, the few existent studies indicate that *Toxocara* spp. are widespread in Portugal, occurring in wild species [14], rural animals [15–17] and urban dogs and cats [18,19]. Toxocarosis was also reported in children from Portugal, causing acute pericarditis [20] and panuveitis [21]. Therefore, a large-scale study was designed to assess the environmental contamination by *Toxocara* spp. eggs in soil and faecal samples, collected from public parks and playground sandpits in Lisbon. In addition, egg density of contaminated soil was calculated and viability of the collected eggs was assessed after incubation.

## Material and methods

### *Study design and sampling area*

This survey was conducted in 7 playgrounds sandpits (PS) and in 12 main public parks (PP) of Lisbon, totalizing 19 distinct study sites (numbered from 1 to 19) (Fig. 1). The sites were chosen in order to cover all public playgrounds in Lisbon that still have sandpits, as well as the public parks located in the most populated areas of the city.

The study comprised a total of 151 soil samples and 135 canine faecal samples, undertaken from February to May 2015. Soil samples were collected at a depth of 0 cm to 15 cm and only fresh faecal samples were collected. In order to obtain a representative sample, soil and faeces were collected on three different occasions, with a minimum two months' interval, and at different periods of the day (morning, midday and afternoon). All samples were refrigerated at 4 °C until analysis (maximum of three days).

The authors were unaware of any cleaning or disinfection activities that may have been performed on the surveyed playground sandpits.

### *Soil samples analysis*

Soil samples were analysed using a modified centrifugation and sedimentation/flotation technique [22–24]. For each soil sample, one hundred grams were weighed and mixed with 100 mL of 5% Tween20 solution, homogenized for 10 min and allowed to stand for 12 h. The contents were then sieved (diameters 1.000 mm; 0.300 mm; 0.150 mm and 0.063 mm) and samples washed in running water for 30 min. The sediment present in the sieve of 0.063 mm was transferred to a sedimentation cup, to which was added distilled water up to 2/3 of the top and leaved to rest for 12 h. The supernatant was then discarded and the superficial layer of the sediment was transferred to the centrifuge tubes with a Pasteur pipette until 1/4 of them were filled. Distilled water was added to half of each tube, vortexed, centrifuged at $200 \times g$ for 10 min, after which the supernatant was discarded. Sucrose solution ($\rho \sim 1.3\ g/cm^3$) was added to half of each tube, vortexed and centrifuged at $200 \times g$ for 10 min, after which each of them was filled with the same saturated solution to form a positive meniscus. A cover slip was added to the top of each tube and after 30 min was observed with an optical microscope at a magnification of 100×.

### *Faecal samples analysis*

An adaptation of the Cornell-Wisconsin method was used [25,26], in which 10 g of faeces of each sample were homogenised in 100 mL of distilled water. The suspension was transferred into 4 centrifuge tubes which were vortexed, centrifuged at $200 \times g$ for 10 min, and the supernatant discarded. Sucrose solution with a density of approximately $1.3\ g/cm^3$ was added until half of each tube, vortexed, centrifuged at $200 \times g$ for 10 min, and later each tube was filled with the same saturated solution to form a positive meniscus.

Please cite this article in press as: Otero D, et al. Environmental contamination with *Toxocara* spp. eggs in public parks and playground sandpits of Greater Lisbon, Portugal. J Infect Public Health (2017), http://dx.doi.org/10.1016/j.jiph.2017.05.002