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ASSESSMENT OF THE PRESENCE OF *Toxocara* EGGS IN SOILS OF AN ARID AREA IN CENTRAL-WESTERN ARGENTINA

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SUMMARY

With the aim of studying the contamination of soils with eggs of *Toxocara* spp. in an arid area in the central-western region of Argentina, 76 soil samples were collected from 18 towns belonging to six provinces of central-western Argentina. They were processed by the centrifugal flotation method. No eggs of *Toxocara* spp. were found. It can be concluded that the negative results are directly related to the characteristics of the environment and climate present in the studied area. The finding of eggs in soils depends on several factors: the presence of canine or feline feces, the hygienic behavior of pet owners, the presence of stray animals without veterinary supervision, the weather and environmental conditions, and laboratory techniques used; and all these circumstances must be considered when comparing the results found in different geographical regions. In order to accurately define the importance of public spaces in the transmission of infection to humans, it is important to consider the role of backyards or green spaces around housing in small towns, where the population is not used to walking pets in public spaces, and in such cases a significant fraction of the population may acquire the infection within households.

KEYWORDS: Toxocariosis; Soil contamination; Zoonotic; Environment; Laboratory method.

INTRODUCTION

Toxocariosis is the most frequently reported zoonotic geohelminthic infection worldwide and is caused by *Toxocara canis* or *Toxocara cati*. Although it has not yet been clearly demonstrated which one of these species is the most relevant in the epidemiology of the infection, due to the inability of routine procedures to distinguish one species from the other^{3,9}.

The life cycle of *Toxocara* shows the importance of soil in the transmission of infection to humans. The eggs expelled in pets' feces complete their maturation in the soil, until full development of infective larvae, and thus, they contaminate the surface of the soil¹⁸. Public places like parks, sidewalks, beaches etc are commonly shared by people and dogs as places for recreation and transit, and, therefore, in order to understand the epidemiological pathways of various zoonoses in relation with the environment, the rate of parasitic intestinal infestation of pets is usually studied and related to soil contamination¹³.

Although *Toxocara* spp. is described as an agent present worldwide, no reports exist regarding the contamination of recreational public areas in the central-western region of Argentina; for this reason, the aim of this work is to assess the contamination of soils with eggs of *Toxocara* spp. in that area.

MATERIALS AND METHODS

Study Area: Soils from the central-western region of Argentina were studied. This is an extensive area located between the parallels 28 and 32 of SL and the meridians 64 and 68 of WL. The climate in this region is dry, arid, with cold winters (an average annual temperature of less than 12 °C) and warm summers, with an important daily temperature amplitude, with little snow and/or rainfall (< 200 mm per year), dry winds and scarce vegetation. Historical records of temperature and precipitation averages for the month of September in this region are: minimum temperature 7 °C, average temperature 15 °C, maximum temperature 23 °C and average rainfall 12 mm¹⁶.

Children's recreational sites were selected in the following locations: the city of Mendoza and the towns of La Paz and Uspallata in the province of Mendoza; the towns of Caucete and St. Lucia in the province of San Juan; the city of Merlo in the province of San Luis; the city of La Rioja and the towns of Patuá, Chepes and Anillaco in the province of La Rioja; the city of San Fernando and the town of Valle Viejo in the province of Catamarca; and the towns San Antonio de Arredondo, El Condor, Nono, Las Rabonas, San Javier and Luyaba in the western region of the province of Córdoba (Fig. 1).

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Fig. 1 - Map of Argentina showing the provinces composing the central-western region.

Sampling: In September 2012, 76 soil samples were collected from 35 different sites in 18 towns belonging to six provinces of the central-western region of Argentina.

Samples were taken from sandboxes and children's playgrounds in squares and parks, and from other sites where recreational use was evident. Samples consisted of 200-250g of dry soil collected from every square meter of the site. Bare ground with little drainage and no evidence of fouling by dogs or cats were selected. Samples were kept in the dark at room temperature until processing².

Methodology: Soil samples were carefully mixed and sieved through a 4 mm² mesh to remove large stones and plant residues and were divided into four aliquots of 5 g each. The aliquots were washed with tap water twice, centrifuged and the supernatants were discarded. Pellets were suspended in two different flotation solutions: two in saturated CINa solution (δ 1.205)¹⁷ and two in saturated sucrose solution (δ 1.27)²³. Each suspension was mixed and centrifuged at 2000 g for 10 min, and then completed with solution to the formation of meniscus and covered with a cover slip. After 20 min they were observed under light microscopy. No egg count was performed and the data were recorded as presence/absence of eggs¹.

RESULTS AND DISCUSSION

No eggs of *Toxocara* spp. were found in any of the 76 samples studied.

The recovery of *T. canis* eggs from soil samples depends on several

factors, such as environmental conditions (including light, temperature, humidity and air quality)⁴, the sampling site, the laboratory method employed etc²¹. *Toxocara* spp. eggs require a period of time under appropriate environmental conditions to become infective to definitive and paratenic hosts. Temperature and humidity are important factors known to affect the levels of development in soil¹⁰. MIZGAJSKA stated that, in arid conditions, with exposure to sunlight and temperatures below 10 °C, *Toxocara* eggs die. It should be noted that sampling of this work was carried out in late winter¹⁵.

In the environment, feces carrying hundreds of thousands of eggs are dispersed by the physical action of: trampling, rain, wind, vectors etc., spreading the eggs' parasitic forms. The presence of *Toxocara* eggs is an indicator of canine fecal contamination above ground, resulting in the exposure of every human in the local population regardless of sex, age or socioeconomic status⁸.

In Argentina, urban contamination by *Toxocara* eggs has been extensively described in the central areas of large and medium-size cities due to the poor standards of care and irresponsible pet ownership (Table 1). The following surveys should also be mentioned: MINVIELLE *et al.* on public parks and sidewalks in the city of La Plata¹⁴; FONROUGE *et al.*⁸ and CORDOBA *et al.*⁶, who also studied public walks in La Plata, RUBEL & WISNIVESKY in Buenos Aires¹⁹; MARTIN & DEMONTE in Santa Fe¹¹; SORIANO *et al.* in the city of Neuquén²²; and SÁNCHEZ THEVENET *et al.* on the contamination of canine feces found on sidewalks in the cities of Comodoro Rivadavia (Province of Chubut)²⁰. There are also many reports regarding the environmental pollution in Northeastern Argentina, a subtropical region with high temperatures and high levels of humidity during most of the year. Other noteworthy surveys include those conducted in the city of Corrientes by MILANO *et al.*^{12,13} and by ALONSO *et al.* who evaluated the contamination in Resistencia (Province of Chaco) in 2001¹ and in 2006².

Contrastingly, there are few reports on soil contamination in rural settlements of the country. However, the reports of CHIODO *et al.* on the presence of *Toxocara* spp. eggs in soil samples from General Mansilla, a small rural location in northern Buenos Aires Province⁵, and the findings of FILLAUX *et al.* in some locations in the provinces of Chubut, Neuquén and Río Negro⁷ can be cited.

It may be concluded that the negative results found in this work were directly related to the environmental conditions present in the studied area. Temperature is responsible for the rate of embryonation while moisture is essential for encouraging development and maintaining egg viability in general¹⁰. Regions with high thermal amplitude, low moisture, strong sunlight and soils with little vegetation provide unfavorable conditions for the viability of the eggs of *Toxocara* spp.

Discovery of eggs in the soil of public areas depends on the presence of canine or feline feces, the hygienic behavior of pet owners, the presence of stray animals without veterinary supervision, weather conditions, laboratory techniques employed in the survey etc, and all these circumstances must be considered when comparing the results found in different geographical regions. Moreover, in order to accurately define the importance of public spaces in the transmission of the infection to humans, it is important to consider the role of backyards or green spaces around housing in small towns, where the population is not used to

TABLE 1
Surveys performed on environmental contamination with *Toxocara* eggs in public locations in Argentina

Authors	Location (publication year)	Frequency of positive <i>T. canis</i> eggs in soils and dog feces samples
Alonso <i>et al.</i>	Resistencia (2006)	20.6-33.3% (n=612)
Alonso <i>et al.</i>	Resistencia (2001)	3.4% (n=333)
Córdoba <i>et al.</i>	La Plata (2002)	12.1% (n=140)
Fillaux <i>et al.</i>	Chubut, Neuquén, Río Negro (2007)	35.1% (n=114)
Fonrouge <i>et al.</i>	La Plata (2000)	13.2% (n= 242)
Martin & Demonte.	Santa Fe (2008)	25.7% (n=393)*
Milano & Oscherov	Corrientes (2002)	0.3 (n=324)
Milano & Oscherov	Corrientes (2005)	16% (n=362)*
Minvielle <i>et al.</i>	La Plata (1993)	10.7-13% (n=351)*
Rubel & Wisnivesky	Buenos Aires (2005)	9%-17% (n=2417)*
Sánchez Thevenet <i>et al.</i>	Comodoro Rivadavia (2003)	17.7% (n=163)*
Soriano <i>et al.</i>	Neuquén (2010)	16.35% (n=1944)*

n = total number of soil samples; n)* = total number of dog feces samples.

walking pets in public spaces, and in such cases a significant fraction of the population may acquire the infection within households.

These features justify the need for an international consensus to achieve agreements, in order to standardize methods for soil sampling and laboratory processing, and thus, make results more comparable among the surveys conducted in various regions of the world. This could eventually redefine the epidemiologic importance of public spaces in the transmission of geohelminthic infections to humans.

RESUMEN

Evaluación de la presencia de huevos de *Toxocara* en suelos de una zona árida en la región centro-oeste Argentina

Con el objetivo de estudiar la contaminación de los suelos con huevos de *Toxocara* spp, se obtuvieron 76 muestras de suelo de 18 pueblos pertenecientes a 6 provincias del centro-oeste de Argentina. Las muestras fueron procesadas por el método de centrifugación-flotación. No se encontraron huevos de *Toxocara* spp. en ninguna de las muestras de suelo. Llegamos a la conclusión de que los resultados negativos podrían estar en relación directa con las características ambientales y climáticas presentes en el área estudiada. El hallazgo o no de huevos en el suelo depende de varios factores: la presencia de heces de caninos o felinos, el comportamiento de los dueños de mascotas, la presencia de animales abandonados y sin control veterinario, las condiciones climáticas y ambientales y las técnicas de laboratorio utilizadas. Todas estas circunstancias deben ser consideradas cuando se comparan los resultados encontrados en diferentes regiones geográficas. Con el fin de definir la importancia que tienen los espacios públicos en la transmisión de la infección a los humanos, se debería considerar el papel que tienen los patios y veredas de las viviendas en las ciudades pequeñas, donde la población no acostumbra a pasear mascotas en parques y plazas, y en esos casos, la población puede adquirir la infección dentro de los hogares.

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