Tropical Medicine and International Health doi:10.1111/j.1365-3156.2012.02996.x

VOLUME 17 NO 6 PP 711–714 JUNE 2012

Frequency of antibodies to *Toxocara* in Cuban schoolchildren

I. Sariego[1], K. Kanobana[2], R. Junco[3], K. Vereecken[2], F. A. Núñez[1], K. Polman[2], M. Bonet[3] and L. Rojas[1]

1 *Institute of Tropical Medicine Pedro Kourí, Havana, Cuba*
2 *Institute of Tropical Medicine, Antwerp, Belgium*
3 *National Institute of Hygiene, Epidemiology and Microbiology, Havana, Cuba*

Abstract

OBJECTIVE The aim of the study was to determine the frequency of antibodies to *Toxocara* in Cuban schoolchildren.

METHODS The frequency of antibodies to *Toxocara canis* was assessed with a commercial enzyme-linked immunosorbent assays kit in school-aged children from two municipalities of Cuba. Univariate analysis and a multivariable logistic regression analysis adjusted for age, sex, municipality and co-infection with helminth and/or protozoa were conducted.

RESULTS The percentage of children with antibodies to *Toxocara* was 38.8% (392/1011; 95% CI = 36.8–42.8). Antibody positivity was significantly associated with gender and co-infections with intestinal parasites, but not with age or municipality.

CONCLUSION Cuban children are highly exposed to the *Toxocara* parasite, corresponding well with reported environmental contamination with *Toxocara* parasite eggs and *T. canis* prevalences in dogs in Cuba. Relevant policy makers and the Cuban population need to be better informed about this preventable infection.

keywords *Toxocara*, toxocariasis, antibody, prevalence, enzyme-linked immunosorbent assays, Cuba

Introduction

Human toxocariasis (HT) is mostly attributed to infection with the larvae of *Toxocara canis*, the intestinal roundworm of dogs. Human infection occurs when embryonated eggs are accidentally ingested as a result of geophagia, poor hygiene or consumption of contaminated food (Magnaval *et al.* 2001). The larvae hatch in the small intestine and migrate through the body. Most infections are asymptomatic, but severe clinical signs may occur owing to organ injury by the migrating larvae (Rubinsky-Elefant *et al.* 2010).

As the parasite does not develop nor reproduce in man, demonstrating its presence is hard and traditional diagnosis of toxocariasis has remained unsatisfactory. Enzyme-linked immunosorbent assays (ELISA) and Western blotting measuring anti-*Toxocara* IgG antibodies to excretory–secretory antigens of the larval stage of *T. canis* (TES) are the best laboratory option for diagnosis. These tests have provided valuable insight into human exposure to *Toxocara* (as determined by the presence of antibodies), demonstrating that the parasite is widely distributed (Rubinsky-Elefant *et al.* 2010).

Information on the epidemiology of HT in Cuba is scarce with only one study reporting a frequency of anti-Toxocara antibodies of 5.1% in healthy children from Havana (Montalvo *et al.* 1994). Based on reported environmental contamination levels with *Toxocara* eggs (up to 75% of public areas and parks) (Duménigo & Galvez 1995; Laird *et al.*1995) and infection levels in dogs in Havana (Duménigo *et al.* 1994; Hernández Merlo *et al.* 2007), a higher percentage of infected children would have been anticipated. The aim of the current study was to obtain more recent data on the frequency of anti-*Toxocara* antibodies in Cuban children and to assess whether previous observations could be confirmed.

Methods

A panel of serum samples from schoolchildren was submitted to *Toxocara* antibody testing. These samples were obtained from a previous study on the relationship between intestinal helminth infections and allergic manifestations conducted between December 2003 and May 2004 in the municipalities of San Juan y Martínez (SYM, Pinar del Río, western Cuba) and Fomento (FO, Sancti Spíritus, central Cuba). The age of the study population ranged between 5 and 14 years, with 80% being below the age of 10. Boys and girls were equally represented (Table 1). Details of the original study design have been published elsewhere (Wördemann *et al.* 2006). Briefly, blood and stool samples were collected from the children.

Table 1 Univariable and multivariable analyses of the frequency of antibodies to *Toxocara* with some demographic and co-infections variables

		Toxocara canis seropositivity 38.8% (95% CI = 36.8–42.8)			
Variable *n* = 1011	*n*	Number of positives	Proportion of positives (%)	Univariable analysis OR (95% CI)	Multivariable analysis OR (95% CI)†
Municipality					
San Juan y Martínez	268	101	37.68	1	1
Fomento	743	291	39.17	1.06 (0.80–1.42)	1.12 (0.82–1.51)
Sex					
Male	525	247	47.05	1	1
Female	486	145	29.84	0.48 (0.37–0.62)*	0.49 (0.38–0.64)*
Age					
5–6	248	97	39.11	1	1
7–8	299	110	36.79	0.91 (0.64–1.28)	0.95 (0.66–1.36)
9–10	270	118	43.70	1.21 (0.85–1.71)	1.15 (0.80–1.67)
11-max‡	194	67	34.54	0.82 (0.56–1.21)	0.78 (0.52–1.18)
Parasite infection§	530	245	46.23	1.95 (1.51–2.53)*	–
Helminth infection	216	116	53.70	2.18 (1.60–2.95)*	2.10 (1.53–2.88)*
Protozoan infection	401	173	43.14	1.35 (1.05–1.75)*	1.34 (1.03–1.76)*

*$P < 0.05$.

†Adjusted for: municipality, gender, age, helminth infection and protozoan infection.

‡Years age categories, except the age category 11-max, which included children from 11 to 14 years because of the low numbers of children of 13 ($n = 3$) and 14 ($n = 1$) years, did not justify a separate category.

§Included infection with either helminth or a protozoan. Variable was not included in multivariable analysis because of colinearity with helminth infection and protozoan infection.

Intestinal parasite infections were determined by direct smear and Kato-Katz examinations. Serum, obtained upon centrifugation of blood, was stored at −20 °C until use. One thousand and eleven serum samples, from the 1320 children initially included in the study, were available for this investigation. Informed consent of the parents or legal guardians of the children was obtained.

TES-specific IgG antibodies were measured with a commercial ELISA kit for diagnosis of HT (Bordier Affinity Products SA, Crissier, Switzerland) following the manufacturer's recommendations. Statistical analyses were conducted with STATA 10 IC software (Stata Corp., College Station, TX, USA). A survey proportion calculation was used to account for clustering effect at school level. Univariate analyses and a stepwise, forward multivariable regression analysis adjusted for age, sex, municipality, helminth and protozoan infection were conducted. The association of antibody positivity with age was tested by adding age either as a continuous variable or as a categorical variable (2-year categories). P values < 0.05 were considered statistically significant.

Results

Of the 1011 samples, 392 (38.8%; 95% CI = 36.8–42.8) were positive in the ELISA. The percentages of children with antibodies to *Toxocara* did not differ significantly between the two municipalities (37.7% and 39.1% for SYM and FO, respectively; OR = 1.1; 95% CI = 0.8–1.5), despite the considerable distance between both. Our data are too limited to assume a homogenous distribution in exposure to *Toxocara* across Cuba, but careful interpretation suggests that these could be comparable in similar ecological settings.

Within our specific population of primary school children, the frequency of anti-*Toxocara* antibodies was independent of age, but significantly lower in girls as compared with boys (29.8% and 47.0% for girls and boys, respectively; OR = 0.5; 95% CI = 0.4–0.6; $P < 0.05$) (Table 1).

Intestinal parasite infections were detected in more than 50% of the children with prevalences of 21.4% and of 39.7% for helminth and protozoan infections, respectively. The predominant helminth species were *Trichuris trichiura* (9.5%, 95% CI = 3.9–15.1), hookworm (8.7%, 95% CI = 1.0–16.4) and *Ascaris lumbricoides* (5.2%, 95% CI = −0.2 to 10.7), whereas *Giardia lamblia* (17.2%, 95% CI = 14.0–20.4), *Endolimax nana* (14.5%, 95% CI = 8.8–20.14) and *Blastocystis hominis* (13.5%, 95% CI = 10.7–16.3) accounted for most protozoan infections. The probability of having antibodies to *Toxocara* was 30% higher in children infected with protozoa (OR = 1.3; 95% CI =

1.0–1.7; $P < 0.05$), and approximately two times higher in children infected with helminths (OR = 2.1; 95% CI = 1.5–2.9; $P < 0.05$) or with any parasite (OR = 1.9; 95% CI = 1.5–2.5; $P < 0.05$), as compared with children with no intestinal parasite infections (Table 1).

Discussion

Reported human anti-*Toxocara* antibody prevalences among healthy individuals range between 2.4% in Denmark (Stensvold *et al.* 2009) and 98% in La Reunion (Magnaval *et al.* 1994) with a tendency to be higher in the tropics. However, studies should be compared with caution because of the lack of standardised methods (regarding both study design and laboratory assays used) and a variety of country-specific factors, which may affect transmission (Rubinsky-Elefant *et al.* 2010).

The 38.8% of children with anti-*Toxocara* antibodies measured in the current study are considerably more than the 5.1% previously reported (Montalvo *et al.* 1994). This is possibly caused by differences in time or study setting. On the other hand, it corresponds better with the high levels of environmental contaminations with *Toxocara* eggs that have been reported in Cuba (Duménigo *et al.* 1994; Laird *et al.* 1995). Comparison with data from other Caribbean countries shows that the frequency of anti-*Toxocara* antibodies in Cuban children is much higher than the 8.3% found in 2–9-year-old children in Puerto Rico (Berrocal 1980), but much less than the 86% reported in 0.5–6-year-old children living in a coastal village of St Lucia (Thompson *et al.* 1986). In two other communities of St Lucia, antibody prevalences of 40% and 60% were reported in children between 5 and 15 years of age (Bundy *et al.* 1987). More recently, an antibody prevalence of 60% was reported among schoolchildren of Trinidad and Tobago (Baboolal & Rawlins 2002). While these studies were all conducted in the same geographic area with similar weather conditions, living habits and socio-economic background, factors known to affect the transmission of toxocariasis (Magnaval *et al.* 2001), may differ substantially between Caribbean islands and partly account for the large differences in reported antibody prevalences.

Male gender was associated with positive serology in several studies (Won *et al.* 2008; Roldán *et al.* 2009), but the association is not consistently present (Alonso *et al.* 2000; Alderete *et al.* 2003). Gender differences are often attributed to the observation that boys tend to spend more time outdoors than girls and are generally less particular about personal hygiene, thus being more at risk of contact with the parasite (Jarosz *et al.* 2010).

The lack of age-related differences observed in this study may be related to the small age range of our study population, which did not include children below the age of 5. Recent evidence indeed suggests that the frequency of anti-*Toxocara* antibodies is higher below the age of 5 (Colli *et al.* 2010; Pinelli *et al.* 2011).

Polyparasitism, and co-infection with other helminths in particular, is known to affect the reliability of TES-based ELISAs owing to cross-reactivity of parasite antigens (Smith *et al.* 2009). According to the manufacturer's information, only minor cross-reactions are expected to occur with the Bordier kit. The kit has been validated for use in a clinical setting, in patients suspected of toxocariasis. It uses a high threshold of positivity, determined by the optical density (OD) value of a low positive sample. This approach reduces the number of false-positives. The cut-off values in our assays ranged between 0.42 and 0.79 and were always at least four times the value of the OD of the negative control sample. Although we cannot prove the absence of cross-reactivity, we expect that the higher level of co-infections with intestinal parasites observed in *Toxocara* seropositive children as compared with *Toxocara*-negative children merely issues from a common mode of exposure shared by the parasites.

This study reports on the frequency of antibodies to *Toxocara* in Cuban schoolchildren, a topic that has been addressed only once, more than a decade ago. The percentage of 38.8% of school-aged children with antibodies to *Toxocara* corresponds well with the few existing data on *Toxocara* prevalences in the environment and in the final dog host (Duménigo *et al.* 1994; Laird *et al.*1995; Hernández Merlo *et al.* 2007). Future studies targeting other age groups and communities are required to obtain a more representative picture of the risk of human exposure to *Toxocara* in the country. Nevertheless, our data clearly demonstrate high levels of exposure to *Toxocara* in a susceptible population, that is, school-aged children, and will hopefully contribute to the advocacy of preventing this infection in Cuba.

References

Alderete JMS, Jacob CMA, Pastorino AC *et al.* (2003) Prevalence of *Toxocara* infection in schoolchildren from the Butantã region, Sao Paulo, Brazil. *Memórias do Instituto Oswaldo Cruz* **98**, 593–597.

Alonso JM, Bojanich MV, Chamorro M & Gorodner JO (2000) *Toxocara* seroprevalence in children from a subtropical city in Argentina. *Memórias do Instituto Oswaldo Cruz* **44**, 303–307.

Baboolal S & Rawlins SC (2002) Seroprevalence of toxocariasis in schoolchildren in Trinidad. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96**, 139–143.

Berrocal J (1980) Prevalence of *Toxocara canis* in babies and in adults as determined by the ELISA test. *Transactions of the American Ophthalmological Society* **LXXCVIII**, 376–413.

Bundy DA, Thompson DE, Robertson BD & Cooper ES (1987) Age-relationships of *Toxocara canis* seropositivity and geohelminth infection prevalence in two communities in St. Lucia, West Indies. *Tropical Medicine and Parasitology* **38**, 309–312.

Colli CM, Rubinsky-Elefant G, Paludo ML *et al.* (2010) Serological, clinical and epidemiological evaluation of toxocariasis in urban areas of south Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* **52**, 69–74.

Duménigo B & Galvez D (1995) Contaminación de suelos en Ciudad de La Habana con huevos de *Toxocara canis*. *Revista Cubana de Medicina Tropical* **47**, 178–180.

Duménigo B, Lau N & Bravo JR (1994) Prevalencia de *Toxocara canis* en perros caseros de Ciudad de La Habana. *Revista Cubana de Medicina Tropical* **46**, 99–102.

Hernández Merlo R, Núñez FA & Pelayo Durán L (2007) Potencial zoonótico de las infecciones por helmintos intestinales en perros callejeros de Ciudad de La Habana. *Revista Cubana de Medicina Tropical* **59**, 234–240.

Jarosz W, Mizgajska-Wiktor H, Kirwan P, Konarski J, Rychlicki W & Wawrzyniak G (2010) Developmental age, physical fitness and *Toxocara* seroprevalence amongst lower-secondary students living in rural areas contaminated with *Toxocara* eggs. *Parasitology* **137**, 53–63.

Laird RM, Carballo D, Reyes EM, García T & Prieto V (1995) *Toxocara* sp. en parques y zonas públicas de Ciudad de La Habana. *Revista Cubana de Higiene y Epidemiología* **58**, 116–118.

Magnaval JF, Michault A, Calon N & Charlet JP (1994) Epidemiology of human toxocariasis in La Reunion. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 531–533.

Magnaval JF, Glickman LT, Dorchies P & Morassin B (2001) Highlights of human toxocariasis. *Korean Journal of Parasitology* **39**, 1–11.

Montalvo AM, Espino AM, Escalante G & Finlay CM (1994) Estudio de seroprevalencia de toxocariasis en una población infantil de Ciudad de La Habana. *Revista Cubana de Medicina Tropical* **46**, 156–158.

Pinelli E, Herremans T, Harms MG, Hoek D & Kortbeek LM (2011) *Toxocara* and *Ascaris* seropositivity among patients suspected of visceral and ocular larva migrans in the Netherlands: trends from 1998 to 2009. *European Journal of Clinical Microbiology & Infectious Diseases* **28**, 1327–1334.

Roldán WH, Espinoza YA, Huapaya PE, Huiza AF, Sevilla CR & Jiménez S (2009) Frequency of human toxocariasis in a rural population from Cajamarca, Peru determined by DOT-ELISA test. *Revista do Instituto de Medicina Tropical de São Paulo* **51**, 67–71.

Rubinsky-Elefant G, Hirata CE, Yamamoto JH & Ferreira MU (2010) Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Annals of Tropical Medicine and Parasitology* **104**, 3–23.

Smith H, Holland C, Taylor M, Magnaval JF, Schantz P & Maizels R (2009) How common is human toxocariasis? Towards standardizing our knowledge. *Trends in Parasitology* **25**, 182–188.

Stensvold CR, Skov J, Moller LN *et al.* (2009) Seroprevalence of human toxocariasis in Denmark. *Clinical and Vaccine Immunology* **16**, 1372–1373.

Thompson DE, Bundy DA, Cooper ES & Schantz PM (1986) Epidemiological characteristics of *Toxocara canis* zoonotic infection of children in a Caribbean community. *Bulletin of the World Health Organization* **64**, 283–290.

Won KY, Kruszon-Moran D, Schantz PM & Jones JL (2008) National seroprevalence and risk factors for zoonosis *Toxocara* spp. Infection. *American Journal of Tropical Medicine and Hygiene* **79**, 552–557.

Wördemann M, Polman K, Menocal LT *et al.* (2006) Prevalence and risk factors of intestinal parasites in Cuban children. *Tropical Medicine & International Health* **11**, 1813–1820.

Corresponding Author **Idalia Sariego,** Departamento de Parasitología, Instituto de Medicina Tropical Pedro Kouri, Apartado Postal 601, Marianao 13, La Habana, Cuba. Tel.: +53 7 255 3640; Fax: +53 7 204 6051; E-mail: idalia@ipk.sld.cu