Frequency of antibodies to Toxocara in Cuban schoolchildren

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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 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-Ellefant 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-Ellefant 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 Rio, 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.
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 10ic software (StataCorp., 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 = 0.2–10.7), 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 = 0.8–1.8).

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>(n = 1011)</th>
<th>Number of positives</th>
<th>Proportion of positives (%)</th>
<th>Univariable analysis OR (95% CI)</th>
<th>Multivariable analysis OR (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Municipality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Juan y Martínez</td>
<td>268</td>
<td>101</td>
<td>37.68</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fomento</td>
<td>743</td>
<td>291</td>
<td>39.17</td>
<td>1.06 (0.80–1.42)</td>
<td>1.12 (0.82–1.51)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>525</td>
<td>247</td>
<td>47.05</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>486</td>
<td>145</td>
<td>29.84</td>
<td>0.48 (0.37–0.62)*</td>
<td>0.49 (0.38–0.64)*</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–6</td>
<td>248</td>
<td>97</td>
<td>39.11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7–8</td>
<td>299</td>
<td>110</td>
<td>36.79</td>
<td>0.91 (0.64–1.28)</td>
<td>0.95 (0.66–1.36)</td>
</tr>
<tr>
<td>9–10</td>
<td>270</td>
<td>118</td>
<td>43.70</td>
<td>1.21 (0.85–1.71)</td>
<td>1.15 (0.80–1.67)</td>
</tr>
<tr>
<td>11-max‡</td>
<td>194</td>
<td>67</td>
<td>34.54</td>
<td>0.82 (0.56–1.21)</td>
<td>0.78 (0.52–1.18)</td>
</tr>
<tr>
<td>Parasite infection§</td>
<td>530</td>
<td>245</td>
<td>46.23</td>
<td>1.95 (1.31–2.53)*</td>
<td>–</td>
</tr>
<tr>
<td>Helminth infection</td>
<td>216</td>
<td>116</td>
<td>53.70</td>
<td>2.18 (1.60–2.95)*</td>
<td>2.10 (1.53–2.88)*</td>
</tr>
<tr>
<td>Protozoan infection</td>
<td>401</td>
<td>173</td>
<td>43.14</td>
<td>1.35 (1.05–1.75)*</td>
<td>1.34 (1.03–1.76)*</td>
</tr>
</tbody>
</table>

*\(P < 0.05\).
†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.
Toxocara antibodies in Cuban children

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 toxocarisis (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 toxocarisis. 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

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