Seroprevalence of toxocariasis in Lebanon: a pilot study

Z. A. KANAFANI, A. SKOURY, G. F. ARAJ, M. EL-KHOURY, R. A. SAWAYA, S. F. ATWEH *and* S. S. KANJ*

American University of Beirut Medical Center, Hamra, Cairo Street PO Box 113-6044, Beirut 110 32090, Lebanon

(*Received 15 September 2005; revised 25 October 2005; accepted 28 October 2005; first published online 23 January 2006*)

SUMMARY

Toxocariasis is a common helminthic infection that has a worldwide distribution. However, data from Lebanon about the prevalence of this infection are non-existent. We conducted a *Toxocara* seroprevalence study with 150 subjects attending the outpatient clinics at the American University of Beirut Medical Center between May and June 2004. Serum specimens were tested for anti-*Toxocara* antibodies by enzyme-linked immunosorbent assay and confirmed by Western blot. Multivariate analysis was performed to identify risk factors for infection. The seroprevalence rate of toxocariasis was 19%. Male gender and below high school education were significantly associated with a positive serological test (odds ratios = 3·1 and 2·8, respectively). Higher numbers of persons in the household, and low family income during childhood, were significant on bivariate analysis only. Toxocariasis is common in Lebanon. A large population-based survey is needed to confirm these results.

Key words: Toxocara, ELISA, Western blot, seroprevalence, Lebanon.

INTRODUCTION

The agent of toxocariasis is the ascarid canine nematode *Toxocara canis*, although *Toxocara cati* commonly found in cats probably causes a similar infection (Nichols, 1956; Kennedy *et al*. 1987). While the domestic dog represents the definitive host of *T. canis*, aberrant infection in humans is acquired through ingestion of the embryonated eggs, typically via contaminated soil or vegetables. The clinical manifestations are dependent on the organ involved. Although visceral larva migrans and ocular larva migrans are the two clinical syndromes typically described (Despommier, 2003), other manifestations include neurological larva migrans, and covert toxocariasis which reflects a generalized response of the immune system to constant challenge by the parasite antigens (Pawlowski, 2001). First described in 1950 by Wilder (Wilder, 1950) then in 1952 by Beaver *et al*. (1952), and initially regarded as an uncommon paediatric illness, toxocariasis is now believed to be the most prevalent helminthic infestation in industrialized countries (Magnaval *et al*. 2001). Through the use of newly-developed and reliable immunodiagnostic techniques, several studies from various countries around the world have recently investigated the prevalence of human infection and past exposure to the parasite (Herrmann *et al*. 1985; Marmor *et al*. 1987; Buijs *et al*. 1994; Montalvo *et al*. 1994; Holland *et al*. 1995; Rai *et al*. 1996; Jimenez *et al*. 1997; Sadjjadi *et al*. 2000; Hermanowska-Szpakowicz *et al*. 2001; Park *et al*. 2002).

* Corresponding author. Tel: +961 1 350000 ext 5381. Fax: +961 1 370814. E-mail: sk11@aub.edu.lb

The understanding of the epidemiology of *Toxocara* in Lebanon has been very limited. This pilot study addresses unanswered questions about the seroprevalence of toxocariasis in this country and its association with various socioeconomic and environmental factors.

MATERIALS AND METHODS

Study subjects

The study was conducted between 1 May 2004 and 30 June 2004 at the American University of Beirut Medical Center (AUBMC), a major teaching hospital in the country that offers outpatient as well as inpatient healthcare to a wide spectrum of the Lebanese population. Individuals ⩾14 years of age attending the outpatient clinics were included.

Data collection

After obtaining informed consent, a short questionnaire was administered to each subject through a personal interview. Information was collected on demographics, education, life-style, and living conditions. Subjects were also screened for a variety of systemic symptoms.

Specimen analysis

Blood samples were collected into sterile plain collection tubes and centrifuged. The supernatant sera were stored at −70 °C until analysed. A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bordier Affinity Products SA,

Parasitology (2006), **132**, 635–639.
doi:10.1017/S0031182005009637 Printed in the United Kingdom

Table 1. Characteristics of study subjects by *Toxocara* serostatus at AUBMC in 2004

	Seropositive ($n=28$)	Seronegative ($n=122$)
Demographics		
Mean age in years (range)	46 (25–82)	43 (14–87)
Age range in years		
13–30	4	38
31–50	14	43
>50	10	41
Male* (*n*, %)	16 (57)	39 (32)
Life-style (*n*, %)		
Dog/cat ownership (*n*, %)	13 (46)	59 (48)
Frequent contact with soil (*n*, %)	7 (25)	36 (30)
Socioeconomic status (*n*, %)		
Below high school education*	9 (32)	15 (12)
Low family income (*n*, %)		
Child*	7 (25)	10 (8)
Adult	12 (43)	31 (25)
Living conditions (*n*, %)		
<3 rooms in the house		
Child	0	3 (3)
Adult	0	3 (3)
⩾5 persons in the household (*n*, %)		
Child*	27 (96)	98 (80)
Adult	11 (39)	41 (34)
Underground drinking water (*n*, %)	1 (4)	2 (2)
Cesspool sanitary facilities (*n*, %)	5 (18)	14 (11)

* $P \leqslant 0{\cdot}05$.

Crissier, Switzerland) was used to detect IgG antibodies directed against the excretory-secretory antigens (ES) produced by the second-stage *Toxocara* larvae. A Western blot (WB) assay (LDBio Diagnostics, Lyon, France) was used as a confirmatory test on samples that tested positive by ELISA. Specific instructions provided by the manufacturers were followed to perform the testing. The presence of 2 or more bands in the range of 24–35 kDa was indicative of a positive Western blot assay.

Statistical analysis

Data were entered into a database using SPSS 11.0 (SPSS Inc, Chicago, IL). Sample size calculation was not performed as this was intended to be a pilot study. The Mann-Whitney test was used to describe the age distribution in quartiles among seropositive and seronegative subjects. Bivariate analysis to study the association between various sociodemographic variables and the presence of anti-*Toxocara* antibodies was performed using the χ^2 test. Variables that were significant on bivariate analysis were entered into a multivariate backward logistic regression model using Stata 7.0 (Stata Corporation, College Station, TX). The level of significance for all tests was set at 95% (two-sided $P=0{\cdot}05$).

Ethical considerations

All data collected for the purpose of this study were kept confidential and questionnaires were delivered anonymously. All patients read and signed an informed consent form in Arabic prior to enrolment. Individuals under 18 years of age gave their verbal consent and the written consent form was signed by the accompanying adult parent. The study was reviewed and approved by the Institutional Review Board at AUBMC.

RESULTS

A total of 150 study subjects gave their consent to participate in the study. The ELISA test was performed first on all available sera. Eighteen samples tested positive (12%) and another 14 gave borderline results (9%). The remaining 118 samples were negative. The WB assay was next used to confirm the 32 positive and borderline results. All 18 samples positive by ELISA were also positive by WB. Of the 14 sera with borderline results, 10 were confirmed to be positive by WB. Therefore, the total number of positive specimens was 28, giving a seroprevalence rate for toxocariasis of 19%. Three of these 28 specimens reacted only to low molecular weight bands (24–35 kDa).

Table 1 shows the general characteristics of the study subjects categorized according to *Toxocara* serostatus. Variables associated with seropositivity included male gender (57 *vs.* 32%), less than high school education (32 *vs.* 12%), low family income as a child (25 *vs.* 8%), and 5 or more persons residing in the household as a child (96 *vs.* 80%). There were no

https://doi.org/10.1017/S0031182005009637 Published online by Cambridge University Press

significant differences among the two groups with regard to age (comparison of means as well as quartiles through the Mann-Whitney test), frequent contact with soil (farming, gardening, etc.), pet ownership, number of rooms in the house, sanitary facilities (sewer *vs*. cesspool) or source of drinking water (bottled *vs*. pipeline or underground water).

The variables that most strongly predicted a seropositive status were the number of persons in the household and low family income during childhood (odds ratios = 6·6 and 3·7, respectively). However, only low level of education and male gender remained significant (odds ratios = 3·1 and 2·8, respectively) with multivariate analysis.

Study subjects were also questioned about a variety of systemic symptoms including fever, headache, lethargy, abdominal pain, sleep disorders, behavioural problems, allergic symptoms, anorexia, and skin rashes. None were significantly different among seropositive compared to seronegative individuals (data not shown).

DISCUSSION

Four major forms of human toxocariasis are presently recognized: systemic, compartmentalized, covert and asymptomatic. Over the past 4 years, several cases of subacute and chronic myelitis of unclear etiology were encountered at AUBMC. Patients were ultimately found to have positive *Toxocara* serology in both serum and cerebrospinal fluid, and most exhibited clinical and radiological improvement with antihelminthic therapy. The diagnosis of this extremely rare entity of *Toxocara* myelitis aroused interest in the hitherto undetermined distribution of toxocariasis in Lebanon. This pilot study is the first to tackle this problematic parasitic infection and disclose its association with various epidemiological variables.

The diagnosis of toxocariasis relies on the detection of antibodies directed against the larval ES antigens. These immunogenic glycopeptides are released from the epicuticle of migrating larvae as it is shed following the binding of specific antibodies (Page *et al*. 1992). The most commonly utilized immunodiagnostic technique is ELISA (de Savigny *et al*. 1979) with a test sensitivity that ranges between 73% and 90% (Glickman *et al*. 1978; Jacquier *et al*. 1991). However, there has been concern about cross-reactivity with other helminths, such as *Fasciola hepatica* and *Ascaris suum*, giving the ELISA test a specificity of around 93% (Glickman *et al*. 1978; Speiser and Gottstein, 1984; Romasanta *et al*. 2003). The low-cost easy-to-use ELISA method has therefore been recommended as a first-line test, but confirmatory testing by WB should be performed, as the latter offers comparable sensitivity and increased specificity when considering lower molecular weight bands (Magnaval *et al*. 1991; Courtade *et al*. 1995). The WB assay confirmed all of our positive and 10 of the 14 borderline ELISA results. As our knowledge about the time-course of the *Toxocara* antibody titres following infection is limited (how soon they start to rise and how long they remain elevated), caution should be employed when interpreting positive results through population screening. For the most part, these represent past exposure to the parasite rather than recent infection (Magnaval *et al*. 2001).

Toxocara has a worldwide distribution, both in developing and in industrialized countries. In the United States, Herrmann *et al*. (1985) found seropositivity rates between 5 and 7% among children in different geographical areas of the country. Much higher rates approaching 30% were found among African-American children of lower socioeconomic status. Some countries in the Middle East have also published seroprevalence rates for toxocariasis, up to 23% in Egypt (Safar *et al*. 1995), 19·5% in Jordan (Abo-Shehada *et al*. 1992) and 8·5% in Israel in mentally retarded children (Huminer *et al*. 1992). In our study, we calculated a seroprevalence rate of 19% among individuals 14 years of age and older, making Lebanon a country of relatively high endemicity for *Toxocara*. Including younger children in our study population would have probably yielded an even higher rate.

Several epidemiological studies have attempted to identify risk factors for acquiring toxocariasis with inconsistent results. For instance, some studies found a significant association between *Toxocara* seropositivity and variables that include dog ownership (Marmor *et al*. 1987; Holland *et al*. 1995), geophagia (Marmor *et al*. 1987; Holland *et al*. 1995), and rural residence (Herrmann *et al*. 1985; Holland *et al*. 1995; Hermanowska-Szpakowicz *et al*. 2001), while others could not substantiate such a correlation (Glickman *et al*. 1979; Jimenez *et al*. 1997; Sadjjadi *et al*. 2000). Other factors like male gender have exhibited more consistent associations with positive exposure to *Toxocara* (Holland *et al*. 1995; Rai *et al*. 1996; Hermanowska-Szpakowicz *et al*. 2001). Several indicators of low socioeconomic status have also been linked to a diagnosis of toxocariasis. A survey in the United States over a 3-year period found that a positive serological test for *Toxocara* was more common with increased number of persons in the household, decreased income, lower education, and fewer rooms in the house (Herrmann *et al*. 1985). Two other studies reported higher rates of toxocariasis among children with low parental education (Worley *et al*. 1984; Sadjjadi *et al*. 2000). In our study, only below high school education and male gender remained significant with multivariate analysis.

In this first report from Lebanon, we found that toxocariasis is relatively common in this country with a seroprevalence rate of 19%. Males and individuals with less than a high school education constitute the

most at-risk population. This preliminary pilot study provides valuable information and sets the ground for a broader population-based epidemiological survey in the future to validate these results. An environmental study is also being planned where stray dog faeces and soil samples from public parks and gardens will be examined.

REFERENCES

Abo-Shehada, M. N., Sharif, L., El-Sukhon, S. N., Abuharfeil, N. and Atmeh, R. F. (1992). Seroprevalence of *Toxocara canis* antibodies in humans in northern Jordan. *Journal of Helminthology* **66**, 75–78.

Beaver, P. C., Snyder, C. H., Carrera, G. M., Dent, J. H. and Lafferty, J. W. (1952). Chronic eosinophilia due to visceral larva migrans; report of three cases. *Pediatrics* **9**, 7–19.

Buijs, J., Borsboom, G., Van Gemund, J. J., Hazebroek, A., Van Dongen, P. A., Van Knapen, F. and Neijens, H. J. (1994). *Toxocara* seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. *American Journal of Epidemiology* **140**, 839–847.

Courtade, H., Recco, P., Magnaval, J. F., Charlet, J. P. and Seguela, J. P. (1995). Comparative study between two ELISA methods for *Toxocara canis* and the western-blot. *Bulletin de la Société Francaise de Parasitologie* **13**, 37–53.

De Savigny, D. H., Voller, A. and Woodruff, A. W. (1979). Toxocariasis: serological diagnosis by enzyme immunoassay. *Journal of Clinical Pathology* **32**, 284–288.

Despommier, D. (2003). Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clinical Microbiology Reviews* **16**, 265–272.

Glickman, L., Schantz, P., Dombroske, R. and Cypess, R. (1978). Evaluation of serodiagnostic tests for visceral larva migrans. *American Journal of Tropical Medicine and Hygiene* **27**, 492–498.

Glickman, L. T., Schantz, P. M. and Cypess, R. H. (1979). Epidemiological characteristics and clinical findings in patients with serologically proven toxocariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 254–258.

Hermanowska-Szpakowicz, T., Kondrusik, M., Swierzbinska, R., Zajkowska, J. and Pancewicz, S. (2001). Incidence of antibody detection against *Toxocara canis* and clinical symptoms in inhabitants of North-Eastern Poland. *Polski Merkuriusz Lekarski* **10**, 168–170.

Herrmann, N., Glickman, L. T., Schantz, P. M., Weston, M. G. and Domanski, L. M. (1985). Seroprevalence of zoonotic toxocariasis in the United States: 1971–1973. *American Journal of Epidemiology* **122**, 890–896.

Holland, C. V., O'Lorcain, P., Taylor, M. R. and Kelly, A. (1995). Sero-epidemiology of toxocariasis in school children. *Parasitology* **110**, 535–545.

Huminer, D., Symon, K., Groskopf, I., Pietrushka, D., Kremer, I., Schantz, P. M. and Pitlik, S. D. (1992). Seroepidemiologic study of toxocariasis and strongyloidiasis in institutionalized mentally retarded adults. *American Journal of Tropical Medicine and Hygiene* **46**, 278–281.

Jacquier, P., Gottstein, B., Stingelin, Y. and Eckert, J. (1991). Immunodiagnosis of toxocarosis in humans: evaluation of a new enzyme-linked immunosorbent assay kit. *Journal of Clinical Microbiology* **29**, 1831–1835.

Jimenez, J. F., Valladares, B., Fernandez-Palacios, J. M., De Armas, F. and Del Castillo, A. (1997). A serologic study of human toxocariasis in the Canary Islands (Spain): environmental influences. *American Journal of Tropical Medicine and Hygiene* **56**, 113–115.

Kennedy, M. W., Maizels, R. M., Meghji, M., Young, L., Qureshi, F. and Smith, H. V. (1987). Species-specific and common epitopes on the secreted and surface antigens of *Toxocara cati* and *Toxocara canis* infective larvae. *Parasite Immunology* **9**, 407–420.

Magnaval, J. F., Fabre, R., Maurieres, P., Charlet, J. P. and De Larrard, B. (1991). Application of the Western blotting procedure for the immunodiagnosis of human toxocariasis. *Parasitology Research* **77**, 697–702.

Magnaval, J. F., Glickman, L. T., Dorchies, P. and Morassin, B. (2001). Highlights of human toxocariasis. *Korean Journal of Parasitology* **39**, 1–11.

Marmor, M., Glickman, L., Shofer, F., Faich, L. A., Rosenberg, C., Cornblatt, B. and Friedman, S. (1987). *Toxocara canis* infection of children: epidemiologic and neuropsychologic findings. *American Journal of Public Health* **77**, 554–559.

Montalvo, A. M., Espino, A. M., Escalante, G. and Finlay, C. M. (1994). Study of the seroprevalence of toxocariasis in an infantile population in the City of Havana. *Revista Cubana de Medicina Tropical* **46**, 156–158.

Nichols, R. L. (1956). The etiology of visceral larva migrans. I. Diagnostic morphology of infective second-stage *Toxocara* larvae. *Journal of Parasitology* **42**, 349–362.

Page, A. P., Rudin, W., Fluri, E., Blaxter, M. L. and Maizels, R. M. (1992). *Toxocara canis*: a labile antigenic surface coat overlying the epicuticle of infective larvae. *Experimental Parasitology* **75**, 72–86.

Park, H. Y., Lee, S. U., Huh, S., Kong, Y. and Magnaval, J. F. (2002). A seroepidemiological survey for toxocariasis in apparently healthy residents in Gangwon-do, Korea. *Korean Journal of Parasitology* **40**, 113–117.

Pawlowski, Z. (2001). Toxocariasis in humans: clinical expression and treatment dilemma. *Journal of Helminthology* **75**, 299–305.

Rai, S. K., Uga, S., Ono, K., Nakanishi, M., Shrestha, H. G. and Matsumura, T. (1996). Seroepidemiological study of *Toxocara* infection in Nepal. *Southeast Asian Journal of Tropical Medicine and Public Health* **27**, 286–290.

Romasanta, A., Romero, J. L., Arias, M., Sanchez-Andrade, R., Lopez, C., Suarez, J. L., Diaz, P., Diez-Banos, P., Morrondo, P. and Paz-Silva, A. (2003). Diagnosis of parasitic zoonoses by immunoenzymatic assays – analysis of cross-reactivity among the excretory/secretory antigens of *Fasciola hepatica, Toxocara canis*, and *Ascaris suum*. *Immunological Investigations* **32**, 131–142.

Sadjjadi, S. M., Khosravi, M., Mehrabani, D. and Orya, A. (2000). Seroprevalence of *Toxocara* infection

in school children in Shiraz, southern Iran. *Journal of Tropical Pediatrics* **46**, 327–330.

Safar, E. H., Abd-El Ghaffar, F. M., Saffar, S. A., Makled, K. M., Habib, K. S., El Abiad, R. and El Shabrawy, E. (1995). Incidence of *Toxoplasma* and *Toxocara* antibodies among out-patients in the Ophthalmic Research Institute, Egypt. *Journal of the Egyptian Society of Parasitology* **25**, 839–852.

Speiser, F. and Gottstein, B. (1984). A collaborative study on larval excretory/secretory antigens of *Toxocara canis* for the immunodiagnosis of human toxocariasis with ELISA. *Acta Tropica* **41**, 361–372.

Wilder, H. C. (1950). Nematode endophthalmitis. *Transactions-American Academy of Ophthalmology and Otolaryngology* **51**, 99–109.

Worley, G., Green, J. A., Frothingham, T. E., Sturner, R. A., Walls, K. W., Pakalnis, V. A. and Ellis, G. S., Jr (1984). *Toxocara canis* infection: clinical and epidemiological associations with seropositivity in kindergarten children. *Journal of Infectious Diseases* **149**, 591–597.

https://doi.org/10.1017/S0031182005009637 Published online by Cambridge University Press