EPIDEMIOLOGY OF TOXOCARIASIS IN A STEPPE ENVIRONMENT: THE PATAGONIA STUDY

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Abstract. To investigate the epidemiology of human toxocariasis in a steppe environment, a field survey was carried out in three provinces of Argentina’s Patagonia (Chubut, Neuquen, and Rio Negro) among 114 rural subjects residing in estancias (cattle- or sheep-breeding ranches). Overall seroprevalence was 31.6%, and the contamination rate of soil by *Toxocara* eggs was 35.1%. Bivariate and multivariate analyses were performed for various exposure variables and also for the De Martonne aridity–humidity index. Multivariate analysis revealed that the seroprevalence rate was found to be inversely correlated with age but was positively linked to De Martonne index. These findings suggest that the harsh climatic conditions existing in Argentina’s Patagonia would inhibit embryonation of eggs in the soil, thus lowering the transmission of human toxocariasis.

INTRODUCTION

Toxocariasis is a helminth zoonosis caused by the infection of humans by the larvae of ascarid worms of the genus *Toxocara*. Only two species, *Toxocara canis* and *Toxocara cati*, are recognized as causative agents of human disease.1,2 The adult form of both nematode worms parasitize the small intestine of their definitive hosts, canids and felids, respectively. Eggs passed in host’s feces are not infective and require an incubation period in the soil to embryonate.3 Epidemiology of toxocariasis has been intensively studied, and it is now generally accepted that this zoonosis is primarily soil-transmitted. Concerning the seroepidemiology, a search of the electronically available literature indicated that most part of seroprevalence surveys have been carried out in the Northern hemisphere. Moreover, most of these studies were performed in urban or semi-urban environments, and in temperate or tropical or subtropical humid climates, all conditions that favor the presence of infective *Toxocara* eggs3 in the soil and the survival of these propagules.4 As far we know, only seven seroprevalence surveys concerned regions having an arid or a semi-arid climate (Canary Islands,5 Egypt,6 Greece,6 Northern India,6 Israel,9 Lebanon,10 and Southern Iran11), where heat and drought are suspected to be damaging factors for *Toxocara* eggs.4

The aim of the present study was therefore to assess seroprevalence and risk factors for human toxocariasis in rural areas of Argentina’s Patagonia, a region of the Cono Sur (“Southern Cone”) of South America characterized by a low density of inhabitants and a steppe environment except for a narrow strip along Andes Cordillera. A steppe is a semi-arid plain without trees, usually located in the middle of continents and/or in the lee of high mountains. The climate is characterized by hot summers and cold winters, averaging 250–500 mm of rain per year.12 Argentina’s Patagonia includes five administrative provinces: Chubut (224,686 km² and 413,237 inhabitants), Neuquen (94,378 km² and 474,155 inhabitants), Rio Negro (203,013 km² and 552,822 inhabitants), Santa Cruz (243,943 km² and 196,958 inhabitants), and Isla Grande de la Tierra de Fuego (21,571 km² and 101,079 inhabitants). Human settlements mainly include estancias, namely, cattle- or sheep-breeding ranches, scattered throughout the steppe.

MATERIALS AND METHODS

Subjects. One hundred fourteen subjects living in estancias located in Chubut, Neuquen, and Rio Negro provinces of Argentina Patagonia were included in the study, the design of which was reviewed and approved by the Ethical Committee of Instituto Malbrán, Buenos Aires. In each visited estancia, one inhabitant was randomly selected by drawing. After informed consent was obtained from the subject, or from parents of minors, a survey form was filled in and a venous blood sample was taken. A questionnaire inquired about residence (name and location of the estancia), demography (age and sex), occupation, animal ownership and exposures, deworming of dogs and cats for intestinal roundworms, type of anthelmintic used, diet, water sources, and type of sewage disposal. This field step of the study was carried out by hydatidosis control officers, as part of the “Programa de Control de la Hidatidosis,”13 managed by the respective Ministry of Health of each province. Between January 1, 2002, and December 31, 2004, 29 estancias of 2,990 were visited in Chubut province, 50 out of 821 in Neuquen province, and 35 out of 3,111 in Rio Negro province. Tierra de Fuego province, which has a mostly humid, oceanic climate, was not included in the survey, and health authorities of Santa Cruz province declined to participate in the study.

Immunodiagnosis. In the Department of Parasitology of Toulouse University Hospitals, immunodiagnosis of toxocariasis was based upon the results of a Western blotting (WB) procedure that detected IgG specific for *T. canis* excretory–secretory larval antigens (TES-Ag). TES-Ag was produced by cultivating *T. canis* larvae according to De Savigny’s prototype method,14 as modified by Bowman and others.15 The WB procedure has been described elsewhere.16 Assessment of Western blot specificity had been undertaken using sera from laboratory animals infected by various helminths, including *T. canis*, from French toxocariasis cases, from French...
The presence of Toxocara sp. eggs was subsequently searched in 25 g of this preliminary sample using Sheather’s centrifugal flotation method, at the Department of Parasitology at Instituto Malbrán, Buenos Aires. Results were only qualitative.

Toxocara ELISA test, and from patients with other helminthiasis. By WB, animal and human reference sera from toxocariasis cases showed a typical pattern wherein seven bands were split into two groups. The first group included four low molecular weight bands, while the second group contained three high molecular weight bands. Statistical analysis of the results from all sera demonstrated that this seven-band pattern was significantly correlated with toxocariasis cases. The sensitivity of the WB assay was found to be similar to that of ELISA performed at the Centers for Disease Control in Atlanta.

Soil samples and egg detection. A superficial soil sample was collected by scraping a superficial layer from the ground in the main yard of each estancia. The presence of Toxocara sp. eggs was subsequently searched in 25 g of this preliminary sample using Sheather’s centrifugal flotation method, at the Department of Parasitology at Instituto Malbrán, Buenos Aires. Results were only qualitative.

Climatic data. A climate can be defined according to the De Martonne aridity–humidity index (annual precipitation in mm/annual mean temperature in °C + 10]). Index scale is 0–5 (hyper-arid), 5–10 (arid), 10–20 (semiarid), 20–30 (semi-humid), and 30–55 (humid). The Climate Modeling and Global Change Study Group of the European Center for Research and Advanced Training in Scientific Computation (http://www.cerfacs.fr/globc/), Toulouse, France, had climatic data on Argentina’s Patagonia, as did the Tyndall Center for Climate Change Research, Norwich, Great Britain. From these data, meteorologists calculated De Martonne index for each visited estancia on the basis of its longitude and latitude. The index range for the investigated areas was 6.11–54.3.

Statistical analysis. Data were analyzed using Intercooled Stata 9.1 software (StataCorp, College Station, TX). Bivariate analysis was performed, with WB result (positive or negative) as an outcome variable. Pearson’s χ² test and Fisher’s exact test were used for qualitative variables. Categorical variables (see Table 1) were rated 0 ("no") or 1 ("yes"), and 0 (male) or 1 (female) for sex variable. Water supply was rated from 1 (piped) to 4 (from well). For continuous parameters (age and De Martonne index), the data distribution was checked by Skewness and Kurtosis tests and was found to be non-Gaussian. These data were then analyzed using the non-parametric Mann–Whitney U test, and the distributions are displayed as medians along with interquartile ranges. Hypothesis of colinearity was assessed using a covariance matrix.

Multivariate analysis was then carried out, and a regression logistic model was set up. Only variables that were found to be significant by bivariate analysis, with $P < 0.2$, were tested by backward stepwise logistic regression.

RESULTS

Overall seroprevalence was 31.6% (36 positive subjects out of 114) with a 95% confidence interval (95% CI) of 23.2–40.9. No significant difference was found between the positivity rates observed in investigated Patagonia provinces. Repartition of seroprevalence according to age groups is displayed in Figure 1. The contamination rate of soil by Toxocara eggs was 35.1% (40 samples out of 114) with a 95% CI of (26.4–44.6). Dogs and/or cats were present in 111 estancias, and 98.7% of dogs (75 out of 76) and 100% of cats (76 animals) were said to be dewormed. However, anthelmintics that are active on intestinal roundworms (fenbendazole or pyrantel) were used only for two cats, on two different estancias, so the variable “dewormed or not” was not tested through bivariate analysis. Occupations considered as possibly at risk for acquiring toxocariasis were estancia owner, day laborer, farm employee, peon, sheep shepherd, and veterinarian doctor. Results of the bivariate analysis are shown in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Bivariate analysis of exposure variables (N = 114)</th>
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<tbody>
<tr>
<td></td>
<td>Percentage of positive WB</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male (N = 45)</td>
<td>31.1</td>
</tr>
<tr>
<td>Female (N = 69)</td>
<td>31.8</td>
</tr>
<tr>
<td>Eating rare or raw meat</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Eating raw vegetables</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Occupational exposure</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Ownership of ≥ 2 dogs or cats</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Soil contaminated with Toxocara eggs</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Toilets</td>
<td>Inside</td>
</tr>
<tr>
<td></td>
<td>Outside</td>
</tr>
<tr>
<td>Type of water supply</td>
<td>Piped</td>
</tr>
<tr>
<td></td>
<td>From creek</td>
</tr>
<tr>
<td></td>
<td>From spring</td>
</tr>
<tr>
<td></td>
<td>From well</td>
</tr>
</tbody>
</table>

* WB, immunodiagnosis by Western blot; OR, odds ratio; 95% CI, 95% confidence interval.
† Pearson’s χ² test.
‡ Fisher’s exact test.
§ Mann–Whitney U test.

Overall seroprevalence was 31.6% (36 positive subjects out of 114) with a 95% confidence interval (95% CI) of 23.2–40.9. No significant difference was found between the positivity rates observed in investigated Patagonia provinces. Repartition of seroprevalence according to age groups is displayed in Figure 1. The contamination rate of soil by Toxocara eggs was 35.1% (40 samples out of 114) with a 95% CI of (26.4–44.6). Dogs and/or cats were present in 111 estancias, and 98.7% of dogs (75 out of 76) and 100% of cats (76 animals) were said to be dewormed. However, anthelmintics that are active on intestinal roundworms (fenbendazole or pyrantel) were used only for two cats, on two different estancias, so the variable “dewormed or not” was not tested through bivariate analysis. Occupations considered as possibly at risk for acquiring toxocariasis were estancia owner, day laborer, farm employee, peon, sheep shepherd, and veterinarian doctor. Results of the bivariate analysis are shown in Table 1.

Figure 1. Distribution of seroprevalence according to age group.
A colinear relation was found between the variables of water supply and De Martonne index variables ($p > 0.50$, $P < 0.01$). Adequacy of these variables was assessed using Hosmer–Lemeshow goodness-of-fit test, and De Martonne index variable exhibited a better result ($P = 0.744$) than did water supply ($P = 0.612$). No interaction was detected between age and De Martonne index variables, so these were included in the logistic model. By multivariate analysis, age was found to be negatively correlated with seroprevalence, but De Martonne index positively correlated with the outcome variable (Table 2).

**DISCUSSION**

Epidemiology of human toxocariasis is complex. The main risk factor is the presence, in the close environment of humans, of pets parasitized by adult *Toxocara* worms, a situation that elicits soil contamination by *Toxocara* eggs. However, this major parameter is modulated by other factors. Various studies carried out worldwide, as reviewed by Barriega, have shown the highest soil contamination rates to be observed in urban settlements where playgrounds, public gardens, recreation areas, public squares, and sand boxes were found to be heavily contaminated. Despite these, two studies in the United States and in Southwestern France demonstrated that individuals residing in rural regions were significantly more at risk of toxocariasis than were those living in urban areas. Seroprevalence surveys done in Western (France and Switzerland) and Central (Austria) Europe found positivity rates ranging from 37% to 44.1% in rural adults. Conversely, seroprevalences were 4.5% in French urban adults and 8% in Dutch children living in Amsterdam. This significance of rural residence as a risk factor can be attributed to an increased likelihood of dog ownership, as demonstrated in a French study where the presence of 2 or more dogs was significantly correlated with seropositivity. Moreover, personal hygiene, evaluated through the frequency of hand washing, is lesser in farmers or other rural people who are permanently in contact with soil or dirt, which may explain the seroprevalence peak observed in our population among people of the 25–49 age group (Figure 1). However, the logistic regression demonstrated that the only significant trend was a decrease in seroprevalence according to age (Table 2).

Socioeconomic status, including sanitation level, is certainly another additional exposure parameter that can interact with the infectious power of contaminated soil. For example, in San Sebastian (Gipuzkoa province, Northwestern Spain), antibodies to *T. canis* were detected in 0% of 2- to 5-year-old children from middle class versus 37% in their counterparts from disadvantaged districts of the city. Climatic conditions represent another important issue for transmission of human toxocariasis. Humid and warm climates are probably the best ecosystems for embryonation and survival of *Toxocara* eggs in the soil. The combination of a favorable wet tropical climate and a poor socioeconomic level resulted in the highest degree of contamination for human toxocariasis. The seropositivity rate was found to be 86% in children from Saint-Lucia (West Indies) and peaked at 92.8% in teenagers and adults from La Reunion (Indian Ocean). These data are to be compared with those from hot and dry countries: seroprevalence was 4.1% in children from Northern Greece, 6.4% in adults from Northern India, 6.6% in Egypt, 8.5% in Israeli adults, 19% in Lebanese adults, and 25.6% in children from Southern Iran. The influence of the climate was also demonstrated in the Canary study in this group of subtropical islands, a significant difference ($P < 0.001$) was found between the positivity rates observed in windward, humid parts of the archipelago and those from leeward, drier areas. Moreover, Spanish authors reported a significant correlation ($P < 0.01$) between seroprevalence level and De Martonne index.

The combination of favorable climate and poor sanitation results in a high transmission pressure. When this condition occurs, young age no longer appears to be a prominent risk factor, and the cumulative effect of repeated infections then elicits a seroprevalence increase according to age, as observed in tropical islands.

In Argentina, data from literature suggested that, in urban areas, the above-cited combination was a major feature of toxocariasis transmission. In La Plata city (temperate central part of the country), only 13.2% of soil samples contained *Toxocara* eggs, but 39% of 156 subjects of various ages had specific antibodies; in Resistencia, a subtropical town in the Northeast, soil contamination was only 3.4%, but the seropositivity rate was as high as 37.9% among 206 children and reached 67.0% among those with poor socioeconomic status.

The epidemiologic situation of toxocarasis in three provinces of Argentina Patagonia (Chubut, Neuquen, and Rio Negro) therefore appears to be quite original. Despite an elevated potential of transmission, as indicated by the features of the rural environment in *estancias* (Table 1), along with a high degree of soil contamination, the seroprevalence rate (35.1%) was similar to those reported from Central or Northern Argentina and from rural, temperate areas in Northern hemisphere. Permanent drought that would inhibit embryonation of *Toxocara* eggs could thus lower transmission pressure and could explain this peculiar epidemiologic situation, a hypothesis supported by the positive correlation of seroprevalence with the De Martonne aridity–humidity index.

**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient $\beta$</th>
<th>SE*</th>
<th>$P$</th>
<th>OR†</th>
<th>95% CI‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.03</td>
<td>0.01</td>
<td>0.043</td>
<td>0.98</td>
<td>(0.95–0.99)</td>
</tr>
<tr>
<td>De Martonne index</td>
<td>0.04</td>
<td>0.01</td>
<td>0.011</td>
<td>1.04</td>
<td>(1.02–1.07)</td>
</tr>
<tr>
<td>Constant</td>
<td>-0.8</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard error.
† Odds ratio.
‡ Confidence interval.

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REFERENCES