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SEROLOGICAL, CLINICAL AND EPIDEMIOLOGICAL EVALUATION OF TOXOCARIASIS IN URBAN AREAS OF SOUTH BRAZIL

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SUMMARY

Toxocariasis is a worldwide public-health problem that poses major risks to children who may accidentally ingest embryonated eggs of *Toxocara*. The objectives of this study were to investigate the occurrence of anti-*Toxocara* spp. antibodies in children and adolescents and the variables that may be involved, as well as environmental contamination by *Toxocara* spp. eggs, in urban recreation areas of north central mesoregion, Paraná State, Brazil. From June 2005 to March 2007. a total of 376 blood samples were collected by the Public Health Service from children and adolescents one to 12 years old, of both genders. Samples were analyzed by the indirect ELISA method for detection of anti-*Toxocara* antibodies. Serum samples were previously absorbed with *Ascaris suum* antigens, and considered positive with a reagent reactivity index ≥1. Soil samples from all of the public squares and schools located in the four evaluated municipalities that had sand surfaces (n = 19) or lawns (n = 15) were analyzed. Of the 376 serum samples, 194 (51.6%) were positive. The seroprevalence rate was substantially higher among children aging one to five years (p = 0.001) and six to eight years (p = 0.022). The clinical signs and symptoms investigated did not show a statistical difference between seropositive and seronegative individuals (p > 0.05). In 76.5% of the investigated recreation places, eggs of *Toxocara* were detected in at least one of the five collected samples. Recreation areas from public schools were 2.8 times more contaminated than from public squares. It is important to institute educational programs to inform families and educators, as well as to improve sanitary control of animals and cleaning of the areas intended for recreation in order to prevent toxocariasis.

KEYWORDS: *Toxocara*; Antibodies; Children and adolescents; Soil contamination.

INTRODUCTION

Toxocariasis is a widely distributed zoonosis occurring in developed countries as well as those with poor sanitation, cultural, and social conditions[9]. Human infection occurs by accidental ingestion of embryonated eggs of *Toxocara canis* or *Toxocara cati*, intestinal parasites of dogs and cats, respectively, found in soil and sand contaminated with feces of these animals[3].

The presence of eggs of *Toxocara* spp., especially in public areas of urban centres such as parks and gardens, is an important source of contamination, mainly for children because of their closer contact with sand and soil[19,23]. Parasite contamination of soil in recreation areas has been investigated in different parts of the world [6,15,21,33] with different positivity rates.

Migration of infected larvae through the human tissues and paragenic hosts, in the majority of cases does not result in clinical manifestation[2,20]. However, in parasited individuals with the presence of anti-*Toxocara* spp. antibodies it can cause fever, hepatomegaly, splenomegaly, respiratory symptoms, muscle pain, and ocular damage, and may be accompanied by eosinophilia[14,31].

Seroepidemiological studies to detect these antibodies have revealed high prevalence, particularly in children[11,13,19] and are important in patients with a peripheral eosinophilia above 400 eosinophils/mL[11,23] and patients showing clinical symptoms[17].

Epidemiological studies using the ELISA technique to detect anti-*Toxocara* spp. IgG antibodies have demonstrated prevalence of this zoonosis in Europe ranging from 2% to 44% [10,20]. Studies of seroprevalence in children have reported 67% positivity in the subtropical region of Argentina[19], 12.1% in the Brazilian Northeast[7], and 8.7% to 54.8% in south eastern Brazil[11,32]. In the city of Maringá, in the state of Paraná, south Brazil, seropositivity in children attended at the Municipal Hospital was 28.8%[23] and eggs of *Toxocara* spp. were the most frequently observed parasitic structure distributed among the different locations[33]. These results stimulated the authors to carry out an epidemiological survey in others municipalities of the same region. Then, the objectives of this study were to evaluate the occurrence of anti-*Toxocara* spp.

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antibodies in children and adolescents and the variables that may be involved, as well as environmental contamination by *Toxocara* spp. eggs in urban recreation areas of north central mesoregion, Paraná State, Brazil.

MATERIALS AND METHODS

Study area: The urban areas of the municipalities of Astorga (23º13'57''S; 51º 39'56''W), Colorado (22º50'15''S; 51º58'23''W), Mandaguaçu (23º20'50''S; 52º05'43''W), and Nova Esperança (23º11'01'' S; 52º12'17''W) in the north-central mesoregion of Paraná, Brazil, with populations between 17,000 and 30,000 inhabitants and with 4,000 to 6,000 children and adolescents aging from one to 12 years were selected for the study. In these municipalities, approximately 85% of the population live in the urban environment[16].

Blood collection and analysis: A total of 376 blood samples were collected, 91 in Astorga, 104 in Colorado, 67 in Mandaguaçu, and 114 in Nova Esperança, from children and adolescents aging from one to 12 years, of both genders, attended by the Public Health Service (SUS), from June 2005 through March 2007. For the sample size calculation, a 95% confidence level, 5% error, and 29% prevalence for toxocariasis were used[23]. The blood samples were collected by the laboratories associated with the SUS. We used a non-random sampling method and included the available children and adolescents from the population who were referred to us. The serum samples were sent to the Environmental Parasitology Laboratory of the State University of Maringá (UEM), and stored at -20 ºC until they were tested.

At the time of blood collection, the children and adolescents' guardians answered a questionnaire containing epidemiological data such as age, sex, habits of onycophagy and geophagy, presence of a pet animal (dog or cat) in the domicile, dwelling and whether the school backyard had sand or a lawn. Also, signs or symptoms such as persistent wheezing, fever, pain in the lower limbs, and abdominal pain were reported. The data for eosinophilia were compiled from blood count analysis performed by the SUS laboratories. Those children and adolescents that could be contacted and who showed seropositivity for toxocariasis, peripheral eosinophilia (≥ 400 eosinophils/mL), or/and a clinical picture of persistent wheezing were sent to the Pneumology Clinic in Maringá. A medical evaluation, chest X-ray, and tests for allergies, mainly mites, house dust, animal dander, and foods were carried out. Fecal parasite examinations were also performed on these children and adolescents. All of the positive children were treated with 25 mg/Kg/day of thiabendazole for seven days[24,26], and the ELISA test and blood count analysis were repeated one and three months following the treatment.

Anti-*Toxocara* spp. antibodies were detected by the ELISA test, using the *T. canis* excretions and secretions larvae antigen (TES) obtained according to De SAVIGNY *et al.*[8] and modified by RUBINSKY-ELEFANT *et al.* (2006)[28]. All sera were previously absorbed with *Ascaris suum* antigens, and a standard positive (reactive) serum, a standard negative (non-reactive) serum, and a limiar reactive serum (LRS) were used in all tests. The cut-off value was calculated based on mean absorbance of 96 negative serum controls plus three standard deviations[28]. The results were expressed as the Index of Reactivity (IR) which was a relation between the optical density of sample and LRS. Reactive samples were considered as those that showed IR ≥1. All samples were tested in duplicate.

Soil collection and analysis: From June 2005 to March 2007, in the urban area of each municipality, all the public squares and public schools with spaces reserved for recreation that contained sand or grass were investigated. In the city of Astorga, three samples were collected from locations with sand and six with lawns; in Colorado, six with sand and one with lawns; in Mandaguaçu, four with sand and five with lawns; and in Nova Esperança, six with sand and three with lawns.

At each locality containing sand or grass, five samples were collected, one from each corner and another in the central part. For each sample, 100 g of sand was taken at an approximate depth of five centimetres from the soil surface. From the grass lawns, a surface area of 20cm x 10cm was removed at each collection point. The samples were stored in plastic bags, labelled, and sent to the UEM Environmental Parasitology Laboratory where they were processed in the same day.

The techniques of centrifuge-flotation with a zinc sulphate solution (density 1,420) and sedimentation in water were used for each sample. The results were expressed as the analysis of these two techniques.

For the centrifuge-flotation technique, 35g of sand or 50 mL of washings from the grass lawn sample out of a total of 100 mL were used. In the water-sedimentation technique, 35g of sand was diluted in 150 mL of distilled water and 50 mL of lawn washings, filtered through gauze and allowed to settle for between six and eight hours. Two mL of sediment from sand and 1 mL of sediment from grass were inspected with an optical microscope. The results were quantitatively expressed as the number of eggs of *Toxocara* spp. per g of sand or per m^2 of grass. The remainder of each sand sample was sent to the UEM Soils Laboratory for quantification of organic carbon (organic matter) by the Walkley-Black method[18].

Statistical Analysis: To check which variables were associated with the positive serology, multiple logistic regression analysis using two models, one for risk factors, and the other one for the clinical/laboratory spectrum were used for statistical survey. Akaike information criterion in a step wise algorithm (StepAic), available in the free software R 2.8.1, was used to select variables that were included into logistic regression models. However, the chosen model was that with less StepAic that included both age and sex. Adequacy of the model was verified for the identification of possible points of influence, outliers or leverage. To assess which variables affect the frequency of soil sample contamination by *Toxocara* spp. eggs a logistic regression model including all variables (city, location, soil type) was conducted. Results with $p \leq 0.05$ were considered significant.

This study was approved by the UEM Ethics Committee of Research Involving Human Beings (COPEP/UEM-305/2004), and by the Secretariats of Health and Education of the respective municipalities. The study began after the legal guardians of the children and adolescents voluntarily signed the consent term.

RESULTS

Of the 376 serum samples collected in the four cities, 194 (51.6%) were reactive to anti-*Toxocara* spp. antibodies, distributed equally between both genders. Seropositivity was slightly more prevalent in males (51%) than in females (48.9%), but with no statistical significance ($p = 0.72$). The seroprevalence rate was substantially higher among

children aging between one and five years (p = 0.001) and six to eight years of age (p = 0.02) than in older children and adolescents (Table 1). The clinical signs and symptoms investigated did not show a statistical difference between seropositive and seronegative individuals (p > 0.05) (Table 1). In six seropositive cases, one from Astorga and five from Colorado, with eosinophilia and a clinical picture of persistent wheezing, the thiabendazole treatment reduced the pulmonary symptoms, reducing in turn the eosinophil counts. Regarding the allergy tests, all of the children were reactive to the mites *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, and *Blomia tropicalis*. Chest X-rays of these

Table 1
Occurrence of IgG antibodies to *Toxocara canis* excreted-secreted antigens (TES), according to clinical signs and epidemiological factors observed in children resident in urban areas of municipalities of the north-central mesoregion, Paraná State, Brazil. June 2005 to March 2007

Variable	No. of children	Occurrence of IgG antibodies	Odds ratio (95% CI)	p
Cities				
Mandaguaçu	67	53.70%	Ref.	
Astorga	91	53.85%	1.154 (0.636-2.095)	0.637
N. Esperança	114	47.37%	0.724 (0.445-1.180)	0.196
Age				
1 - 5 years	136	58.82%	2.327 (1.392-3.891)	0.001
6 - 8 years	110	54.55%	1.863 (1.094-3.172)	0.022
9 - 12 years	130	41.54%	Ref.	
Gender				
Female	193	25.00%	Ref.	
Male	183	26.60%	0.926 (0.608-1.410)	0.720
Habit of Onichophagy				
Yes	116	30.85%	1.045 (0.647-1.663)	0.853
No	260	69.15%	Ref.	
Habit of Geophagy				
Yes	32	8.51%	0.578 (0.265-1.262)	0.169
No	344	91.49%	Ref.	
Lawn in dwelling and/or at school				
Yes	178	47.34%	0.816 (0.533-1.249)	0.408
No	198	52.66%	Ref.	
Eosinophilia				
Yes	96	25.53%	1.181 (0.727-1.918)	0.505
No	280	74.47%	Ref.	
Abdominal pain				
Yes	79	21.01%	0.847 (0.500-1.435)	0.530
No	297	78.99%	Ref.	
Cephalea				
Yes	33	8.78%	1.000 (0.241-1.119)	0.094
No	343	91.22%	Ref.	
Persistent wheezing				
Yes	33	8.78%	1.891 (0.878-4.071)	0.102
No	343	91.22%	Ref.	

95% CI = 95% confidence interval.

patients were normal during their evaluations, and parasites were not detected in the fecal analysis. It was not possible to locate and follow up two children and adolescents from Nova Esperança and one from Mandaguaçu municipality.

Neither onycophagy, geophagy, contact with cats and dogs, nor the presence of sand or grass lawn at the home or at school was significantly associated with seropositivity ($p > 0.05$).

In 76.5% (26/34) of the recreational investigated places, eggs of *Toxocara* spp. were detected in at least one of the five samples. The recreational places in public schools were 2.8 times more contaminated than the public squares ($p = 0.05$) (Table 2). The frequency of contamination by *Toxocara* spp. eggs was substantially higher ($p = 0.001$) among recreational places of Nova Esperança than of Astorga (Table 2). There was no significant difference between the number of eggs found in samples with sand or grass lawns ($p > 0.05$) (Table 2). Small amounts of organic matter were found in the sand samples.

DISCUSSION

The study was carried out in urban areas of small municipalities and half of the investigated children and adolescents were seropositive to *Toxocara* spp. In the same region, children from the public-health system of a municipality with more than 300,000 inhabitants (Maringá) showed lower seroprevalence (28.8%)[23] probably because in the smaller cities of our region the spaces of schools and public squares are frequently used for recreation by the entire population. In addition, the users of SUS usually have lower income, and tend to show higher seroprevalence for toxocariasis[5].

Seropositivity was more frequent in children under five years of age, which was also observed by PALUDO *et al.* (2007)[23]. This usually happens because of children's behavioural habits at this age, making them more exposed to contaminated soil[19,23]. In contrast to these findings, MURADIAN *et al.* (2005)[22] observed that seroprevalence of anti-*T. canis* antibodies increased with children's age, possibly because of repeated infection, leading to the persistence of antibodies.

Although we have observed cases suggestive of toxocariasis, the reported signs and symptoms were not related to positive serology, as already mentioned by PALUDO *et al.*(2007)[23]. On the other hand TEIXEIRA *et al.* (2006)[32] found a correlation between positive serology, respiratory symptoms, and eosinophilia. This is explainable, because the clinical aspects of toxocariasis can vary from asymptomatic forms to pulmonary, ocular, and hepatic clinical pictures, among others. All of the six children who received specific treatment, in this study, showed improvement in their clinical picture and disappearance of eosinophilia, in spite of being reactive to the investigated mites and maintaining positive titres for anti-*Toxocara* spp. IgG. Although IgG antibodies are useful for toxocariasis diagnosis, this cannot be considered as a marker for cure of the disease because titres can remain elevated for several years after treatment[19,28]. BUJS *et al.*[4] observed a positive association between asthma, anti-*Toxocara* spp. antibodies and inhaled allergens such as *D. pteronyssinus*. FIGUEIREDO *et al.*(2005)[11] also reported an association between asthma and anti-*Toxocara* spp. IgG antibodies in children.

It should be emphasized that there are innumerable difficulties in laboratory diagnosis of this zoonosis, such as the laborious production of the antigens, and the availability of commercial diagnostic kits with low sensitivity and specificity, or of ones with good quality but high cost[27]. The majority of seropositive children were asymptomatic, and the symptomatic children who received specific treatment showed clinical improvement. These data indicate one more time the need for studies to determine markers for monitoring the cure in toxocariasis.

The absence of correlation between seropositivity to *Toxocara* spp. and the investigated epidemiological factors (onycophagy, geophagy, contact with cats and dogs, the presence of sand or grass lawn at the home or at school) may be attributed to the fact that we evaluated children and

Table 2
Environmental contamination of public recreational sites by eggs of *Toxocara* spp., in urban areas of municipalities of the north-central mesoregion, Paraná State, Brazil. June 2005 to March 2007

Variable	No. of samples	Frequency of contamination	Odds ratio (95% CI)	p
Cities				
Astorga	45	24.44% (11/45)	Ref.	
Mandaguaçu	45	40.00% (18/45)	1.89(0.31-3.47)	0.16
Colorado	35	37.14% (13/35)	1.84(0.15-3.53)	0.24
N. Esperança	45	60.00% (27/45)	4.52(2.79-7.32)	0.001
Locations				
Public squares	25	20.00% (5/25)	Ref.	
Public schools	145	44.14% (64/145)	2.8(1.11-4.55)	0.05
Soil				
Sand	95	41.05% (39/95)	Ref.	
Grass lawn	75	40.00% (30/75)	1.15(-0.28-2.58)	0.67

95% CI = 95% confidence interval.

adolescents from small municipalities with few options for recreation, so children and adolescents are usually exposed to the same areas. In Brazil, these municipalities normally have a predominance of low-income families, such as those attended by the public-health services. This means that children and adolescents of both genders are exposed to the same kind of toys and the same recreational environments. Data on these epidemiological factors are controversial in different study areas[4,23,32]. ANARUMA *et al.* (2002)[1] observed no correlations with soil, presence of dogs or cats while TEIXEIRA *et al.* (2006)[32] observed an association between seropositivity and the presence of a dog or cat, and presence of sand at school. In the present study, the school places were more contaminated than the squares, highlighting the risk to children and adolescents in their school activities. The high contamination rates of recreational areas by *Toxocara* spp. eggs increases the risk of children and adolescents being infected.The difference in the environmental contamination observed between municipalities, e.g. Nova Esperança and Astorga, was not associated with seropositivity in children and adolescents. Under favourable conditions of temperature, soil texture, and humidity, it is possible to find large numbers of infective forms[13], and consequently potential foci of environmental contamination[33]. Eggs of *Toxocara* spp. were the most common parasitic forms found in grass lawns and sand in Maringá[33] a bigger city of the studied region. In Brazil, the growing numbers of dogs and cats, together with the easy access of these animals to recreational areas contributes to soil contamination[29]. In the present study, the small amounts of organic matter found in the sand samples did not reduce the abundance of eggs of *Toxocara* spp. as already observed by PIERANGELI *et al.*(2003)[25] who observed many parasite forms in soil with small amounts of organic matter.

The high seroprevalence rate in children and adolescents under 12 years of age and the high contamination rate in recreational areas, especially in public school areas, pose the need for educational programs for families and educators, besides parasitic control of animals, cleaning and monitoring the environment used for recreation, as measures to improve the control of this zoonosis.

RESUMO

Avaliação sorológica, clínica e epidemiológica da toxocaríase em áreas urbanas do sul do Brasil

A toxocaríase é um problema de saúde pública mundial, com maior risco para crianças que podem, acidentalmente, ingerir ovos embrionados de *Toxocara* spp.. Os objetivos deste estudo foram avaliar a ocorrência de anticorpos anti-*Toxocara* spp. em crianças e adolecentes e as variáveis que podem estar envolvidas, bem como a contaminação ambiental por ovos de *Toxocara* spp., em locais de recreação, em áreas urbanas da mesorregião norte central, Paraná, Brasil. De junho de 2005 a março de 2007 foram coletadas 376 amostras de sangue de crianças e adolescentes de um a doze anos, de ambos os sexos, atendidas pelo Sistema Único de Saúde. As amostras foram analisadas pelo método de ELISA indireto para detecção de IgG anti-*Toxocara e* previamente absorvidas com antígeno de *Ascaris suum.* Foram consideradas reagentes as amostras com índice de reatividade ≥ 1. A análise das amostras de areias (n = 19) e gramados (n = 15) de cada município foi realizada em todas as praças e escolas públicas. Das 376 amostras de soro, 194 (51,6%) foram positivas. A taxa de soroprevalência foi substancialmente mais elevada entre as crianças na faixa etária de até um a cinco (p = 0.001) e de seis a oito anos de idade (p = 0,022). Os sinais e sintomas clínicos investigados não mostraram diferenças estatísticas entre soropositivos e soronegativos (p > 0,05). Em 76,5% dos locais de recreação investigados, ovos de *Toxocara* foram detectados em pelo menos uma das cinco amostras. Os locais de recreação das escolas públicas estavam 2,8 vezes mais contaminados do que as praças. É importante a realização de programas educativos junto às famílias e educadores, o controle sanitário de animais e a higienização dos locais destinados à recreação para prevenção da toxocaríase.

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