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TOXOCARIASIS IN CHILDREN ATTENDING A PUBLIC HEALTH SERVICE PNEUMOLOGY UNIT IN PARANÁ STATE, BRAZIL

Edson V. GUILHERME(1), Ariella A. MARCHIORO(2), Silvana M. ARAUJO(1), Dina Lúcia Morais FALAVIGNA(3), Carolina ADAMI(4), Gustavo FALAVIGNA-GUILHERME(5), Guita RUBINSKY-ELEFANT(6) & Ana Lucia FALAVIGNA-GUILHERME(1)

SUMMARY

The enzyme-linked immunosorbent assay (ELISA) is the most widely used tool to detect anti-*Toxocara* IgG antibodies for both serodiagnostic and seroepidemiological surveys on human toxocariasis. In the last eight years a high prevalence of toxocariasis (32.2-56.0%) has been reported in children attending public health units from municipalities in the state of Paraná, Brazil. Therefore, the aim of this work was to compare the frequency found among the general child population with that of children attending a public pneumology service in Maringá, Paraná, Brazil and describe the laboratorial, clinical and epidemiological findings. The research was conducted at the Consórcio Público Intermunicipal de Saúde do Setentrião Paranaense (CISAMUSEP) from July 2009 to July 2010 among children aged between one and 15 years. From a total of 167 children studied, only 4.2% (7/167) tested positive for anti-*Toxocara* spp. IgG antibodies and presented mild eosinophilia (2/7), increased serum IgE levels (6/7) and a positive allergy test for mites (5/7). The presence of pets (dogs or cats) at home did not correlate with the seroprevalence. In conclusion, cases of toxocariasis involving the respiratory tract are rare in children attending a public health pneumology unit in the northwestern region of Paraná State, despite the high prevalence of this type of toxocariasis among the infantile population attending Basic Health Units in the same geographical area.

KEYWORDS: *Toxocara* spp.; Children; Respiratory tract; Toxocariasis.

INTRODUCTION

Human toxocariasis is present worldwide and children are more likely to be exposed to the risk of infection[4,16,23]. Transmission occurs by the accidental ingestion of embryonated eggs of *Toxocara canis* or *T. cati*, the roundworms of dogs and cats, respectively, which may be present in soil and contaminated sand[16,20,22] with the feces of these animals[9,13,23,24]. The migration of *Toxocara* infective larvae through human tissues does not result in severe clinical manifestations in most cases[18,20,22]. However, it may cause fever, hepatomegaly, splenomegaly, respiratory symptoms, muscle pain, ocular damage and eosinophilia, and circulating anti-*Toxocara* IgG antibodies are often present[2,11,20].

Human toxocariasis is currently classified into four clinical forms: systemic or visceral larva migrans (VLM), compartmentalized (neurologic and ocular toxocariasis), cover, and asymptomatic[16,22]. In the systemic form, pulmonary involvement is shown through asthmatic episodes and/or acute bronchitis and eosinophilia[12,18,20]. These symptoms are due to an increase in inflammatory cells (macrophages and eosinophils) and a change in the host lymphocyte subpopulation in response to antigens secreted by the migrating larvae of *T. canis*[12,25].

The method of choice for both immunodiagnostic and epidemiological studies is the enzyme-linked immunosorbent assay (ELISA) test using the excretory-secretory antigens of infective larvae of *T. canis* (TES), which possess high sensitivity and specificity[6,20,25]. Many studies concerning the seroprevalence of toxocariasis in children have been conducted in Brazil reporting 12.1-60.0% in the northeast[9,22,26], 3.0-35.5% in the midwest[9,22], 21.5-52.0% in the north[9,22] and 2.8-54.8% in the southeast[9,22,25].

Since 2003, our group has been conducting research to evaluate the prevalence of toxocariasis in children from one to 15 years of age attending public basic health units (BHU) in northwestern Paraná. In the nine cities investigated (Astorga, Colorado, Mandaguaçu, Mandaguari, Marialva, Maringá, Nova Esperança, Paiçandu, and Sarandi), we have observed that the seroprevalence of toxocariasis varies from 32.2% to 56%[1,5,14,15,17,18]. Among the signs and symptoms analyzed, pulmonary ones were the most reported[1,5,14,15,17,18]. Therefore, the aims of this work were to verify whether the rates of toxocariasis frequency found in our previous research was repeated in children referred to the public pneumology service, and describe the laboratorial, clinical and epidemiological findings of the seropositive children.

(1) Programa de Pós-graduação em Ciências da Saúde, UEM, Maringá, PR, Brazil.
(2) Departamento de Farmácia, Faculdade Ingá, Uningá, Maringá, PR, Brazil.
(3) Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, UEM, Maringá, PR, Brazil.
(4) Departamento de Farmácia, Universidade Estadual de Maringá, UEM, Maringá, PR, Brazil.
(5) Departamento de Medicina, Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brazil
(6) Laboratório de Soroepidemiologia e Imunobiologia, Instituto de Medicina Tropical de São Paulo, São Paulo, SP, Brazil.
Correspondence to: Dr. Ana Lúcia Falavigna-Guilherme, Programa de Pós-graduação em Ciências da Saúde/Universidade Estadual de Maringá, Av. Colombo 5790, Bloco 126, 87020-900 Maringá, Paraná, Brasil. Tel.: +55.44.3011-8987, Fax: +55.44.3011-4860. E-mail: alfguilherme@uem.br

MATERIALS AND METHODS

Sampling: The study included 167 children aged from one to 15 years attending the Pneumology Unit of the public health service (Consórcio Público Intermunicipal de Saúde do Setentrião Paranaense - CISAMUSEP) which is located in Maringá and is the referral service for the northwest of Paraná, Brazil, from July 2009 to July 2010. The CISAMUSEP receives children referred by BHUs and other health services in 30 cities. The results were compared with the epidemiological data of children from the same region using previous reported studies[1,5,14,15,17,18].

Laboratory methods: Blood samples from the children in the study sample were collected at the Laboratory of Parasitology, Universidade Estadual de Maringá (UEM), and the serum was stored at –20 °C until use. Anti-*Toxocara* spp. IgG antibodies were detected by means of an ELISA test using TES antigens, which were obtained according to the method of DE SAVIGNY *et al.* (1979)[6], modified by ELEFANT *et al.* (2001)[7]. All sera were previously absorbed with *Ascaris suum* antigens[7]. The cut-off value was calculated based on the mean optical density (OD) of 96 negative control sera plus three standard deviations. The results were expressed as the reactivity index (RI), which was calculated by the ratio between the OD of the sample and the OD of the cut-off value. Samples with an RI ≥ 1 were considered reactive. All samples were tested in duplicate. Positive and negative control sera, as well as a threshold control serum (TCS), were used in all runs. The TCS was prepared with a pool of reactive samples diluted in a volume of pooled negative samples to provide an OD equal to the cut-off. All samples were tested in duplicate.

Complete blood counts (CBC) and eosinophilia were compiled. Eosinophilia was considered when the number of eosinophils was ≥ 500 cells/μL and classified as: normal (< 6%), mild (6-10%), moderate (11-15%) and severe (> 15%) eosinophilia[21].

IgE total levels were measured by nephelometry according to the manufacturer's recommendations (NIGE Siemens®). The IgE reference values were: < 1.5 IU/mL for newborns; < 15 IU/mL for children up to one year old; < 60 IU/mL for children aged from one to five years old; < 90 IU/mL for children from six to nine years old and < 200 IU/mL for children from 10 to 15 years old.

Skin-prick tests: Hypersensitivity allergy tests were performed by skin-prick tests with commercial extracts (Prick Test®, FDA-Allergenic), according to the manufacturer's instructions. The allergens, tested on the right forearm, were grass pollen, feathers, fungi, house dust, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, dog hair, cat dander, cockroach, sheep's wool, *Aspergillus* sp., *Penicillium* sp., and pollen (grass extract).

Clinical and epidemiological data: The following data were recorded for each patient: reasons for referral to a Pneumology Unit, signs and symptoms observed by the physician at the time of the examination (rhinitis, bronchitis, asthma, fever, pneumonia, colds or cough), and presence of pets - dogs or cats - at home.

Chest X-rays were performed at baseline and at follow-up when necessary. Treatment with albendazole (10 mg/kg per day)[13] for 10 consecutive days was prescribed when both clinical and laboratory evidence for toxocariasis was found.

This study was approved by the UEM Ethics Committee of Research Involving Human Beings (COPEP/UEM-018/2008) and by the CISAMUSEP.

RESULTS

Previous seroepidemiological studies involving children attending regional BHUs in the Brazilian state of Paraná have reported seropositivity rates between 32.2% and 56.0%[1,5,14,15,17,18]. However, in our study, only seven out of 167 children (4.2%) showed anti-*Toxocara* spp. IgG antibodies; two of them (1.2%) had moderate eosinophilia and six showed high IgE levels (Table 1).

The chest X-rays were normal, except one child (case *2) who showed an increased bronchial plot (Table 1). Five of the seven children showed positive reactions to the allergens tested, mostly for mites. In relation to the clinical signs observed, shortness of breath was the main reason for attending the BHU.

Clinical signs of bronchitis and eosinophilia were present in one patient (case *4), which disappeared after being treated with albendazole. Despite having several domestic animals at home, this patient did not show an atopic response to the tested allergens (Table 1). Cases *2,*6 and *7 improved after albendazole treatment, whilst case *5 had no response upon the same treatment. Cases *1 and *3 did not receive specific treatment for toxocariasis because the patients did not return to the public pneumology service after the conclusion of the exams.

DISCUSSION

In contrast to the large number of children presenting seropositivity for *Toxocara* spp. in the area, few cases of toxocariasis were identified in this study among those with pulmonary involvement. Exposure to *Toxocara* spp. in childhood is a very common condition in our region[1,5,15,17] and it is expected that this could result in an increased demand for specialized pulmonary disease services. Nevertheless, this is not the case. Children with minor respiratory involvement are treated by primary care physicians in the BHUs.

Previous studies in this region have reported that eosinophilia is present in 7.8% (30/386)[1,5,14,15,17,18]. In this study, we have observed two cases of moderate eosinophilia in patients with anti-*Toxocara* spp. IgG antibodies. Peripheral eosinophilia is not always specific to toxocariasis, but it has been frequently associated to human toxocariasis, especially in young children who seem to have a more symptomatic course of the disease[16,21]. The increase of IL5, resulting from a dominant type of Th2 response, leads to eosinophilia in *Toxocara* infections[20,22]. PALUDO *et al.*[17] suggested that eosinophilic patients may be associated with toxocariasis 149 times more than seronegative patients. PINELLI and ARAZEMENDI[20] also established that blood eosinophils were significantly higher in the *Toxocara*-seropositive than in the seronegative group. Thus, serodiagnostic tests for toxocariasis should be included in the differential diagnosis of eosinophilia, especially in children and in adults with mild eosinophilia[10,11,21].

Respiratory symptoms as well as increased IgE levels have been

Table 1
Clinical, laboratory and epidemiological data for children who presented with anti-*Toxocara* spp. IgG antibodies at the "Consórcio Público Intermunicipal de Saúde do Setentrião Paranaense" (CISAMUSEP), Maringá, Paraná State, Brazil

Case	Age	Results of laboratory tests					Reason for consultation	Rhinitis, asthma or bronchitis	Number of dogs and cats in the household
		IgE	Eosinophilia (cells/mm^3)	Degree of eosinophilia[d]	Radiograph	Prick test positive[c]			
1	3	258.1	60	Normal	Normal	No	Breathlessness	Yes	1
2	6	628.2	332	Normal	Increased bronchial plot	Yes	Recurrent respiratory infection	Yes	0
3	6	92.6[a]	213	Normal	Normal	Yes	Breathlessness	Yes	1
4	8	361.6	676[b]	Moderate	Normal	No	Breathlessness	Yes	18
5	9	437.9	568[b]	Moderate	Normal	Yes	Allergic asthma	Yes	2
6	4	> 1100	159	Normal	Normal	Yes	Allergic asthma	Yes	0
7	3	1003	452	Normal	Normal	Yes	Breathlessness	Yes	1

[a]Normal value of IgE; [b]Eosinophilia; [c]Skin-prick test positive for dust mites; [d]Normal (< 6%), mild (6-10%), moderate (11-15%) and severe (> 15%) eosinophilia.

reported in toxocariasis by many authors[3,8,25]. In this study six patients had increased levels of IgE, and in five out of seven cases breathlessness was reported as the main complaint. SHAZLY *et al.*[24] observed that patients with bronchial asthma have a high probability of developing clinical symptoms after an infection with *Toxocara* spp. COLLI *et al.*[5] observed similar results in children attending BHUs in three municipalities in this region, with 1.9% of children presenting high rates of eosinophilia, shortness of breath and increased levels of anti-*Toxocara* spp. IgG. MARCHIORO *et al.*[14] reported that young patients with asthma and pulmonary involvement may also have hepatomegaly, eosinophilia and increased levels of IgE. Recently, LOPEZ *et al.*[12] and PINELLI & ARANZAMENDI[20] discovered that infection with *Toxocara* spp. leads to an exacerbation of experimental allergic airway inflammation, since infections with this helminth share common clinical features with allergic asthma such as wheezing, coughs, mucus hyper-secretion and bronchial hyper-reactivity. Likewise, infection with *Toxocara* spp. results in the induction of a dominant T-helper 2 (Th2) type of immune response characterized by the production of cytokines such as Interleukin-4 (IL-4), IL-13 and IL-5, which causes eosinophilia and increased levels of IgE, respectively[3,20]. Larval invasion of, and migration through the lungs might be the cause of wheezing and other asthma-like symptoms[25], but the question of whether *Toxocara* antigens could trigger allergic asthma in *Toxocara* infected individuals is still being evaluated[12,20].

Most patients who were seropositive for *Toxocara* spp. also had increased IgE levels and skin allergenic reactivity associated mainly with clinical manifestations and inhalant aeroallergens[11]. However, BUIJS *et al.*[3] explains that *Toxocara* spp. may stimulate allergic asthma and atopic manifestations due to a hereditary mechanism that distorts type 2 T-helper cell function, promoting parasite host longevity through the increase of an allergen-specific and *Toxocara*-specific IgE. Mice infected with *Toxocara* spp. showed serious respiratory allergic inflammation leading to eosinophilia and respiratory tract hyper reactivity[19]. Some of the symptoms typical to VLM such as bronchial spasms, pneumonia and cutaneous reactions[25] were not observed in our study.

The decrease in the severity of the clinical signs and symptoms has been used in all of the published trials to estimate the end-point of parasitism in VLM patients[22]. In our study, the treatment with albendazole has been evaluated as effective in four out of five children treated, including those who had had contact with dogs and cats. In fact, the prevalence of this zoonosis can be attributed to cultural practices regarding domestic animals[5,20,22,27].

The seroprevalence of *Toxocara* infection was low among children attending a public Pneumonology Unit located in the northwestern region of Paraná. However, pulmonary disease specialists and pediatricians should be aware of the high prevalence of toxocariasis in the general pediatric population in this area.

RESUMO

Toxocaríase em crianças atendidas em Unidade de Pneumologia do Serviço de Saúde Pública, Paraná, Brasil

O teste imunoenzimático (ELISA) constitui a ferramenta mais utilizada no diagnóstico individual da toxocaríase humana e/ou em inquéritos soroepidemiológicos para pesquisa de anticorpos IgG anti-*Toxocara* spp. Tendo em vista a elevada freqüência da toxocaríase (32,2% e 56,0%) observada em crianças atendidas em Unidades Básicas de Saúde de municípios do noroeste do Paraná, durante pesquisas realizadas ao longo dos últimos oito anos, o objetivo deste trabalho foi comparar estas frequências em crianças encaminhadas a uma unidade de Pneumologia do serviço de saúde pública da cidade de Maringá, noroeste do Estado do Paraná, Brasil e descrever os achados laboratoriais, clínicos e epidemiológicos das crianças soropositivas. A pesquisa foi realizada no Centro de Especialidades Regional - CISAMUSEP - entre julho de 2009 a julho de 2010, em crianças com idade entre um e 15 anos. De 167 crianças

investigadas, sete (4,2%) apresentaram anticorpos IgG anti-*Toxocara* spp. Entre elas, duas (1,2%) crianças soropositivas apresentaram eosinofilia (baixos níveis), seis mostraram níveis elevados de IgE e cinco, teste alérgico positivo, principalmente para ácaros. Concluindo, no noroeste do estado do Paraná foram observados raros casos de crianças com toxocaríase envolvendo o trato respiratório e que foram atendidas por serviços públicos de Pneumologia ainda que na região estudada tenha sido observada elevada prevalência de toxocaríase em crianças assistidas pelas Unidades Básicas de Saúde (BHU).

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