Eosinophilic count and seropositivity for IgG antibodies to toxocara spp. in children assisted at the public health service

Avaliação eosinofílica e soropositividade para anticorpos IgG anti-toxocara em crianças atendidas pelo Sistema Único de Saúde

Evaluación eosinófila y suero-positividad para anticuerpos igG anti-toxocara en niños atendidos por el Sistema Único de Saludç

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ABSTRACT

Objective: To study the association between the presence of eosinophilia and IgG antibodies to *Toxocara* spp. in children assisted by the public health service, in the northwestern region of Parana State, in southern Brazil.

Methods: A retrospective study of children aged seven months to 12 years old assisted by the Public Health Service in northwest state of Paraná, Brazil. ELISA test was performed in all children in order to detect IgG antibodies to Toxocara spp. and eosinophil amounts (eosinophilia ≥ 600 cells/mm3).

Results: Among 1,199 screened children, 386 (32.2%) had IgG antibodies to *Toxocara* spp. The seroprevalence of *Toxocara* spp. and the eosinophilia were more common among children of seven months to five years old. Eosinophilia was observed in 7.8% of seropositive patients to *Toxocara* spp.

Conclusions: There was a high prevalence of anti-*Toxocara* spp., mainly in children under five years old. With the exception of some children who had respiratory symptoms and eosinophilia, most of them were asymptomatic and did not present eosinophilia. Eosinophilic count is a secondary laboratory finding in the diagnosis of toxocariasis.

Key-words: toxocariasis/diagnosis; child; eosinophilia.

RESUMO

Objetivo: Verificar a associação entre a presença de eosinofilia e a soropositividade para anticorpos IgG anti-*Toxocara* spp. em crianças atendidas pelo Sistema Único de Saúde no Noroeste do Paraná, Brasil.

Métodos: Estudo retrospectivo com crianças de sete meses a 12 anos, atendidas pelo Sistema Único de Saúde do Noroeste do Paraná, com teste ELISA para a pesquisa de anticorpos IgG anti-*Toxocara* spp. e contagem de eosinófilos (eosinofilia ≥600 células/mm^3).

Resultados: Entre as 1.199 crianças, 386 (32,2%) apresentaram anticorpos IgG anti-*Toxocara* spp. A soroprevalência e a eosinofilia foram mais frequentes em crianças de sete meses a cinco anos. A eosinofilia foi observada em 7,8% dos pacientes soro-reagentes ao *Toxocara* spp..

Conclusões: Foi observada elevada prevalência de anticorpos anti-*Toxocara* spp., principalmente nos menores de cinco anos. Com exceção de algumas crianças que apresentaram sintomas respiratórios e presença de eosinofilia, a maioria foi assintomática e não mostrava eosinofilia. A pesquisa de eosinófilos é ferramenta secundária para o diagnóstico de toxocaríase.

Palavras-chave: toxocaríase/diagnóstico; criança; eosinofilia.

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RESUMEN

Objetivo: Verificar la asociación entre la presencia de eosinofilia y una suero-positividad para anticuerpos IgG anti-*Toxocara* spp. en niños atendidos por el Sistema Único de Salud en el noroeste de Paraná, Brasil.

Métodos: Estudio retrospectivo en niños de siete meses a 12 años de edad, atendidos por el Sistema Único de Salud en el noroeste de Paraná, con prueba de ELISA para la investigación de anticuerpos IgG anti-*Toxocara* spp. y recuento de eosinófilos (eosinofilia ≥ 600 células/mm^3).

Resultados: Entre los 1.199 niños, 386 (32,2%) presentaron anticuerpos IgG anti-*Toxocara* spp. La suero-prevalencia y la eosinofilia fueron más frecuentes en niños de siete meses a cinco años. La eosinofilia fue observada en 7,8% de los pacientes suero-reactivos al *Toxocara* spp.

Conclusión: Se observó elevada prevalencia de anticuerpos anti-*Toxocara* spp., principalmente en niños menores de cinco años. Excepto por algunos niños que presentaron síntomas respiratorios y presencia de eosinofilia, la mayoría de ellas fue asintomática y mostraban eosinofilia. La investigación de eosinófilos es herramienta adicional a los casos con indicios clínicos de toxocariase.

Palabras-clave: toxocariase/diagnóstico; niño; eosinofilia; *Toxocara* spp.

Introduction

With worldwide geographical distribution, toxocariasis is a zoonotic disease prevalent in tropical and developing countries, associated with socio-economic status of the population and affecting mainly children[1-5].

The human infection takes place due to the accidental ingestion of embryonated eggs Toxocara canis or Toxocara cati, intestinal parasites of cats and dogs, respectively, found in soil and sand contaminated with feces of these species[6]. The ingested eggs hatch in the small intestine, larvae penetrate the mucosa and migrate to the liver, passing through the lungs and reach various tissues through the systemic circulation. Migration triggers the production of eosinophilic granuloma, with consequent inflammation[7]. The destruction of parasites by the eosinophils is caused by the toxic action of their cytoplasmatic granules[8,9], which damage non-phagocytic organisms, such as helminths in tissue migration phase[10]. The production of eosinophils is influenced by the helminth species, number of parasites, location in the host and time since infection[11]. Some authors state that helminthiasis that cause tissue invation lead to eosinophilia more often; once eosinophilia is more intense during larval development and migration[12,13].

The diagnosis of toxariasis is based on clinical manifestations and laboratory tests, especially the ELISA IgG antibodies to *Toxocara* spp. test and blood test[14,15], and eosinophilia can be established, in children, as an absolute number of eosinophils ≥600/mm^3[16,17]. Thus, the objective of this work was to verify the association between the presence of eosinophilia and seropositivity for IgG antibodies to *Toxocara* spp., in children assisted by pelo Unified Health System (SUS).

Methods

From February 2004 to November 2007, children aged seven months to 12 years old, assisted by SUS and residing in urban areas within the northwest of the state of Paraná[18], Brazil, within the coverage are of the 15th Regional Health System of the state of Paraná, Brazil. This random research was prompted by the shortage of data in the literature about the prevalence of toxocariasis in the state of Paraná, as well as by the large number of dogs, and by the frequent epidemiologic factors that contribute to the dissemination of a zoonotic disease that is highly neglected at public health care services. Thus, after spontaneously signing the informed consent form of the guardian (when there was indication of a blood test), serological investigation to detect the presence of IgG antibodies to *Toxocara* spp. was conducted randomly, and eosinophilia data was collected in the blood test.

Approximately 85% of the population in investigated municipalities (Astorga, Colorado, Mandaguaçu, Mandaguari, Marialva, Maringá, Nova Esperança, Paiçandu and Sarandi) reside in an urban area[18]. Based on the annual statistical survey, the size of the sample was calculated with an expected prevalence of 25% and 5%[19] accuracy for IgG antibodies to *Toxocara* spp. For each listed municipality, a sample of children was calculated, with a 95% confidence interval, using the Statistica 7.1 software application. A total of 1,199 children were included in the research.

The municipalities of Astorga, Colorado, Mandaguaçu and Nova Esperança have populations ranging from 17,000 to 30,000 people, with 4,000 to 6,000 children aged seven months to 12 years old[18]. The municipalities of Marialva, Mandaguari and Paiçandu have a population ranging from 31,000 to 45,000 people, with 6,000 to 8,000 children in this age group[18]. The city of Sarandi,

with 79,686 people, has 16,824 children in the seven months to 12 years old age group[18]. In the city of Maringá, with 325,968 people, we chose to investigate children assisted at Hospital Municipal de Maringá, which serves SUS patients exclusively; an average of 13,000 children are attended annually.

The results of the serology were then associated with the hematologic values obtained in the leukogram (absolute count of eosinophils). The adopted criterion to establish eosinophilia was a value equal or greater than 600 eosinophils/mm^3 (0.60 x 10^9/L), the reference limit for age and sex[16,17]. The degree of eosinophilia was classified according to Naveira[11]: non-eosinophilic (≥1% and ≤4%), Degree I eosinophilia (>4% and ≤10%), Degree II (>10% and ≤20%), Degree III (>20% to ≤50%) and Degree IV (over 50%).

The IgG antibodies for *Toxocara* spp. were detected by the immunoenzymatic test ELISA (Enzyme Linked Immunosorbent Assay), using *T. canis* excretion-secretion antigen (TES)[19,20]. The sera were previously absorbed with *Ascaris suum* antigens, and all tests used positive (reagent) serum, negative (non-reagent) serum, and Reactivity Threshold Serum (RTS). The results were expressed in Reactivity Index (RI), relative to the optical density of the sample/optical density of the *RTS*. Reagent samples were considered to be those that presented RI ≥1. All samples were tested twice.

The data were compared by descriptive statistic and chi-squared test, using the BioEstat 3.0 software application. The test of proportions was carried out with the Statistica 7.1 software application. The research was approved by the Research Ethics Committee (COPEP/UEM), according to report nº305/2004, as well as by the municipal departments of education and health at the investigated municipalities.

Results

From a total of 1,199 patients whose serologies were carried out during the period of the proposed study, 92 were from Astorga, 82 from Colorado, 59 from Mandaguaçu, 92 from Mandaguari, 123 from Marialva, 260 from Maringá,

Table 1 – Frequency of IgG antibodies to Toxocara spp. and eosinophilia in children of different age groups.

Eosinophilia	Age (years)	IgG Antibody to Toxocara spp.	
		Reagent	Non-reagent
		% (N/T)	% (N/T)
Yes	0.7-5	56.7 (17/30)	36.5 (35/96)
	6-8	23.3 (7/30)	35.4 (34/96)
	9-12	20.0 (6/30)	28.1 (27/96)
No	0.7-5	46.0 (164/356)	46.7 (335/717)
	6-8	34.3 (122/356)	23.3 (167/717)
	9-12	19.7 (70/356)	30.0 (215/717)
Total		32.2 (386/1199)	67.8 (813/1199)

N= Number of cases; T= Total number of samples.

Table 2 – Differest degrees of eosinophilia in children who were serum-reagent and non-reagent to *Toxocara* spp., assisted in public health care services.

IgG Antibody to Toxocara spp.	Age group (years)	Eosinophilia degree				*Total*
		I	II	III	IV	
Reagent	0.7-5	12	3	1	1	17
	6-8	4	3	0	0	7
	9-12	2	3	1	0	6
	Total:	18*	9	2	1	30
Non-reagent	0.7-5	19	14	2	0	35
	6-8	17	14	3	0	34
	9-12	13	12	2	0	27
	Total:	49	40	7	0	96

* = Majority degree I p=0.0001

98 from Nova Esperança, 82 from Paiçandu and 311 from Sarandi.

The research showed that 386/1,199 (32.2%) of children had the presence IgG antibodies to *Toxocara* spp. (Table 1). Most of the children in the study did not present eosinophilia (Table 1). Eosinophilia was observed in 126/1,199 (19.6%) of patients investigated. From the 386 patients serum-reagent to *Toxocara* spp., 30 (7.8%) showed concomintant eosinophilia. There was no association between eosinophilia and the presence of antibodies to *Toxocara* spp.; on the contrary, children who were not carriers of antibodies to *Toxocara* spp. presented, with greater frequency, an eosinophilic count over 600/mm^3 (p=0.0332).

The seroprevalence of antibodies to *Toxocara* spp. and eosinophilia were more frequent in children aged seven months to five years old than in children aged six to eight years old and nine to 12 years old (p=0.011and p= 0.005, respectively).

Among the 30 individuals who were reagent to *Toxocara* spp. and with the increase of eosinophils (Table 2), most presented with degree I eosinophilia (p=0.0001). The distribution of eosinophilia degrees among the three age groups (Table 2) did not show statistica difference (p=0.51). From the 30 children, ten (33.3%) reported respiratory symptoms and, from these, seven presented degree I eosinophilia, with one child showing each of the remaining degrees of eosinophilia (II, III, and IV). Most reported contact with dogs and/or cats and with sand/dirt or lawn. The distribution of the eosinophilia degrees among the three age groups for children non-reagent to *Toxocara* spp. was not significant either (Table 2) (p=0.98).

Discussion

Most of the children in the study presented a normal eosinophil count, including those who were serum-reagent to *Toxocara* spp. It is known that the eosinophils commonly increase with allergic and parasitic processes[21,22] and, mainly, in helminthiasis with tissue invasion, more intense during the larval migratory process[12,13]. Some factors are relevant for the development of symptoms, such as the number of migrating larvae, the hypersensitivity and immunological state of individuals.

Although toxocariasis is characterized by the presence of larvae in human tissues, the present study found no association between eosinophilia and seropositivity to *Toxocara* spp. These results are in agreement with the findings of Radman[23], Anaruma[24] and Paludo[5]. Other authors, however, have found an association, but using the relative count of eosinophiles[25,26] or values under 600 eosinophiles/mm^3[3,27] as criteria for eosinophilia. If the relative count of these leukocytes was considered, there would be an increase from 30 (7.8%) to 133 (34.5%) eosinophilic children among the 328 serum reagent children. It is crucial, however, that the clinical aspects are considered, because from 30 seropositive children, only 33% presented toxocariasis symptomatology suggesting toxocariasis, all with eosinophilia. Due to the fact that most individuals remain asymptomatic, eosinophilia is an additional tool for the diagnostic conclusion. However useful for the diagnosis of toxocariasis[1,3,5], the dosage of IgG antibodies does not work as a marker of cure for the disease, because their titles could remain high for years after treatment[4,28]. It should be undescored, however, that in the ten cases that were seropositive, eosinophilic and with a picture of asthma or bronchitis, treatments with tiabendazol 25mg/kg/day for seven days[20,15] reduced the pulmonary symptoms and normalized the number of eosinophils. The *clinical signs of toxocariasis, including the pulmonary clinical picture, are variable and depend on the number of ingested eggs, tissue involved and allergic response induced by the host*[29,30].

Seropositivity was most frequent in younger children, which is in agreement with the results of *Paludo*[5] and Karadam[27]. *These findings are explained by the fact that children in this age group, due to their behavioral habits, are more exposed to contaminated sand and soil*[4,5].

In the present study, we observed a high prevalence of antibodies to *Toxocara* spp., especially in children under five years old. Various works in other Brazilian regions have also observed a similar prevalence(1,3-5,31,32) . In the present research, with the exception of some children who presented respiratory symptoms and the presence of eosinophilia, most children were asymptomatic, and it was not possible to establish an association of the zoonotic disease and their symptomatollgy with eosinophilia. It should be underscored that the parasitosis evolves in an asymptomatic way(15,33,34) and the titles of IgG antibodies to Toxocara spp. may remain high for years after treatment(4,28). Thus the research of eosinophils becomes an additional, but secondary, tool to diagnose patients with clinical signs of toxocariasis.

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