

Toxocariasis, Intestinal Parasitoses and Eosinophilia in Schoolchildren from Argentina

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ABSTRACT: The purpose was to analyze the relationship between toxocariasis, intestinal parasitosis, eosinophilia, pet ownership and signs and symptoms present in 370 schoolchildren (3-12 years old) of Argentina. The positive serology for toxocariasis was 19.5%, associated with eosinophilia and possession of cats as pets. The stool testing revealed 70.0% of parasitized children. Its frequency was 44.9% helminthes and 53.5% protozoan resulting single significant presence of protozoa in children aged 10-12 years. The species were: *Blastocystis hominis* (45.7%), *Enterobius vermicularis* (43.2%), *Giardia intestinalis* (16.2%), *Hymenolepis nana* (2.4%), *Ascaris lumbricoides* (1.6%) and *Trichuris trichiura* (0.3%). The survey showed 13.0% of children with sporadic abdominal pain and 13.5% with pruritus ani. The results indicate relationship between positive serology and eosinophilia compatible with a clinical aspect of covert toxocariasis self-limiting, associated with cats ownership; in school children from our region who also have high frequency of intestinal parasitoses.

KEYWORDS: toxocariasis, intestinal parasitosis, children, Argentina

Introduction

Many developing countries have experienced a significant decrease in childhood mortality in the last three decades. But there are still some issues such as nutritional disorders, anemia and parasitic infections affecting the physical and intellectual development of children [1]. The so-called “neglected diseases”, parasitoses among them, constitute a challenge for the fulfillment of the Millenium Development Goals [2].

Among chronic diseases prevalent in children in Argentina, parasitoses are a health issue found primarily in preschoolers and schoolchildren in economically challenged populations. The oligosymptomatic process and low morbidity levels of these parasitic infections have caused them to be regarded as inevitable, and they are identified among tropical neglected diseases [3]. Parasites can alter food consumption, digestion, absorption and metabolism [4]. Besides, they can cause chronic immune activation, which also impairs a child's growth and development⁵. The literature published in latest years reveals that the prevalence of intestinal-parasite infections in Argentinean children is 23%-86% [6-11] and zoonotic parasitoses such as toxocariasis are 10.6%-37.9% [6,7].

According to Chinchilla Rojas H [12], eosinophilia as a biological sign of a parasitic disease occurs in helminthoses and miases though it is also possible to observe this phenomenon in some protozoan infections (amebiasis, blastocistosis, dientamebiasis, giardiasis, isosporidiasis, and toxoplasmosis).

But the role it performs in the different infections varies and its presence may also vary in the course of the infection. Thus, in parasitic diseases, especially by helminthes, its role to defend the host is essential and its presence is a hematologic marker of disease. However, eosinophilia may increase or decrease according to factors in the host, parasite development stage, its location inside the patient and the number of parasites [13].

For a decade various projects and parasitosis control programs funded by the University of La Plata have been developed in order to decrease the high prevalences of these infections affecting mostly vulnerable communities. The Program for the Control of Intestinal Parasitoses and Nutrition (PROCOPIN, by its initials in Spanish) has been implemented since 2009 in different communities in the province of Buenos Aires. The program is developed in four stages: 1. Evaluation of the nutritional and parasitic condition of children; 2. Therapeutic intervention in children with nutritional disorders and/or parasites; 3. Educational intervention to avoid their return to the diseased state; 4. Post-intervention control.

This article shows the partial results of the first stage of PROCOPIN, analyzing the relationship between toxocariasis, intestinal parasitoses, eosinophilia, pet ownership and signs and symptoms present in school children from La Plata, Argentina.

Material and methods

Population studied

The survey was carried out in the suburb of La Plata, in the northeast of the province of Buenos Aires. For two decades now, this suburb has been suffering the impact of the different social and economical problems the country is undergoing. This has resulted in at least two well-differentiated regions:

East region, with an area of about 0.65 square kilometers, with residential-neighborhood characteristics, permanent-dwelling, most people have running water and sewer systems and middle-class people living there.

West region (adjoining a highway), of approximately 0.49 square kilometers, with inhabitants experiencing social and environmental problems. This is a settlement consisting of precarious houses with no basic utilities and settlers in social vulnerability and inequality.

The survey was conducted in children residing in the West area attending a local kindergarten and primary school.

The survey was carried out after educational institutions were summoned to perform a one-on-one interview with the students' parents/guardians. Demographic data (sex, age) and socio-cultural data such as pet ownership (dog, cat or other) were registered, as well as reports by the interviewees about presence of signs and symptoms in children during the week prior to the survey.

Hematological and serological analyses: after explaining the aim and procedure of the study to parents/guardians and children and obtaining informed consent from the former, 5ml peripheral blood was extracted from the children. The sample was divided in two tubes, one with EDTAK3 (ethylenediaminetetraacetic acid, tripotassium salt) and the other with no anticoagulant. Two blood smears per individual were performed. The percentage of eosinophils was estimated through white cell count under optical microscope with previous Giemsa stain. Eosinophilia was declared when values were ≥ 5 . Serum was separated within 2 hours of sample collection and preserved at -20°C until processing. Serological determination of anti-toxocara antibodies was performed through the use of ELISA Toxocara Microwell Serum kit (IVD Research, Inc., Carlsbad, USA).

Parasitological analysis: a serial copro-parasitological study and a serial anal scraping were performed to each subject. Instructions for

sample-taking were given orally and in written form to parents/guardians. For the copro-parasitological serial study, each subject (or their parents) daily placed a portion of their stools in one single jar with preservative. The procedure was carried out for five days in a row. For the serial anal scraping, each subject dabbed a folded piece of gauze previously soaked in water around the margins of their anus every morning after waking up. Samples were taken for five consecutive days and gauzes were placed in one jar with preservative. Stools were processed using the modified Telemann technique [11] and the obtained pellets were observed under the optical microscope after being colored with Lugol (three smears per tube). Also, smears were stained with the Kinyoun technique. The serial anal scraping samples were processed by cutting and homogenizing the gauze pieces with the same preservative contained in the jar. After transferring the whole contents to a centrifuge tube, it was concentrated by centrifugation at 1000 xg for 5 minutes. Finally, three smears per tube were observed through the optical microscope.

Ethical aspects: parents were informed orally in detail about the study in group meetings held at the school. Each parent/guardian was requested to give their consent in writing and to be present at the moment of drawing blood. The children with consent from their parents/tutors were informed about the study and the blood-drawing procedure using age-adjusted vocabulary, and their consent to participate was requested. The developed protocols were approved by the Ethics Committee of the entities funding the project. Personal information was obtained in accordance with the Declaration of Helsinki, the Nuremberg Code and National Act #25,326 and remained confidential. The approval of school and municipal authorities in the district was also obtained.

For the statistical analysis, the total and specific prevalences for parasitoses were estimated, as well as the frequencies of the registered variables. The possible associations were analyzed using the Chi-Square Test and Fisher's Exact Test. In the associations that turned out to be significant ($p \leq 0.05$), the odds ratio (OR) and 95% confidence interval (CI) were estimated. The statistical analysis was performed using SPSS 18.0 software.

Results

A total of 450 schoolchildren participated, 370 of which perform blood collection and took samples for parasitological study. The age range was 3-12 years old, 43.5% being female. The children were grouped as follows: 151 children aged 3-5 years old, 160 children aged 6-9 years old and 59 children aged 10-12 years old.

Among the schoolchildren analyzed, 19.5% tested positive for anti-toxocara antibodies (AcTox+); 19.9% of them were female and 19.1% were male ($p=0.859$). The prevalences according to the age groups were as follows: 21.2% in 3- to 5-year-olds; 15.6% in 6- to 9-year-olds and 25.4% in 10- to 12-year olds. The differences were not significant. 48.6% of

schoolchildren AcTox+ showed eosinophilia, while among the seronegative, eosinophilia occurred in 12.4% ($p=0.000$, $OR=6.673$, $CI=3.750$; 11.874). Table 1 shows the results according to each age group. There was no association with dog ownership, since among AcTox+ children, those possessing a dog were 17.2% and those who did not have one were 19.9% ($p=0.614$). On the other hand, AcTox+ schoolchildren were associated with cat ownership: 32 of the 118 children having a cat (27.1%) were AcTox+ and 40 of the 252 children who did not own this pet (15.9%) were AcTox+ ($p=0.011$; $OR=1.972$; $CI=1.163$ - 3.344). The presence of "other animals at home" was not found to be associated.

Table 1. Eosinophilia and serology for toxocariasis in schoolchildren from Argentina.

Age groups (years)	3-5		6-9		10-12	
	N	%	N	%	N	%
+eosinophilia/+serology	19/32	(59.4)	10/25	(40.0)	6/15	(40.0)
+eosinophilia/- serology	13/119	(10.9)	19/135	(14.1)	5/44	(11.2)
<i>p</i>	0.000		0.002		0.005	
OR	11.97		4.070		4.800	
CI	4.793-29.627		1.597-10.375		1.163-19.805	

Copro-parasitological analysis revealed that 70.0% of the children were infected with parasites. 35.9% had only one parasite, 28.1% had two, 4.3% had three, 0.8% had four, and only one child (0.3%) had five intestinal parasites. There were no differences when age groups and sex groups were compared. The frequency of children with helminthes was 44.9% and with protozoa was 53.5%, with no differences as regards sex. However, the age group of 10- to 12-year-old showed a significant prevalence of protozoa when compared to the other groups ($p=0.002$, $OR=2.12$, $CI=1.08$ - 4.19). Parasites found were: *Blastocystis hominis* 45.7%, *Enterobius vermicularis* 43.2%, *Giardia intestinalis* 16.2%, *Hymenolepis nana* 2.4%, *Ascaris lumbricoides* 1.6%, and *Trichuris trichiura* 0.3%.

Children with AcTox+ and intestinal parasites were 19.7% and without 18.9% ($p=0.860$). Eosinophilia was present in 20% parasitized children and 18.0% without parasites, with no significant differences. 50% of children with eosinophilia had AcTox+ and intestinal parasites, while those without

eosinophilia frequency was 12.1% ($p=0.000$; $OR=7.542$; $CI=3.781$ - 15.043). Among parasitized children with eosinophilia, 45% of them were AcTox+, while in those without eosinophilia, the frequency of AcTox+ children was 14% ($p=0.001$; $OR=5.035$; $CI=1.748$ - 14.505).

Table 2 includes data from the 12 schoolchildren showing intestinal parasites *H. nana*, *A. lumbricoides* and/or *T. trichuris* with serology and eosinophils count. They were chosen to compare the presence these intestinal helminthes, the presence of AcTox and eosinophilia. As shown in Table 2, three children with *H. nana* parasites and negative serology presented a normal count of eosinophils. One child infected with *A. lumbricoides* parasites and negative serology also had a normal eosinophils count. *H. nana* with AcTox+ and a normal eosinophils count was detected in only one child. Seven children showed one or some of these helminthes with AcTox+ and eosinophilia. No statistical analysis was conducted due to the small number of cases.

Table 2. Presence of *Ascaris lumbricoides*, *Hymenolepis nana*, *Trichuris trichiura*, serology for toxocariasis and eosinophil count in schoolchildren from Argentina.

Age (years)	Eosinophils (%)	<i>Toxocara</i> Serology	<i>Ascaris lumbricoides</i>	<i>Hymenolepis nana</i>	<i>Trichuris trichiura</i>
3	1	-	-	+	-
4	6	+	-	+	-
5	1	+	-	+	-
6	9	+	+	-	-
7	10	+	-	+	-
8	2	-	+	-	-
8	2	-	-	+	-
8	24	+	+	+	+
10	2	-	-	+	-
10	13	+	+	-	-
10	33	+	+	+	-
11	22	+	+	+	-

As regards the presence of clinical signs and symptoms in the week prior to the survey, parents/guardians reported only 13.0% children showed sporadic abdominal pain and 13.5% pruritus ani. No association was shown with the presence of intestinal parasites or positive serology.

Discussion

This study contributes to learning the prevalences of toxocariasis and intestinal parasitoses in schoolchildren living in precarious settlements in the central area in Argentina and their association with peripheral eosinophilia, pet ownership and presence of clinical symptoms. These neighborhoods present a proportion of immigrant adults, both internal and from bordering countries, generally settled on flood lands and lacking utilities such as drinking water and sewers. Most children belong to the first generation born in the neighborhood.

Seroprevalence of toxocariasis was close to 20%, situated in between the results published by Minvielle et al. [14] in blood donors from the central area in Argentina (10.6%) and those published by Alonso et al. [15] in children from the northern area of the country (37.9%). The difference between these results reveals that in the central region children show greater prevalence than adults, thus reflecting an increased risk to contract the infection through their playing habits, with no age or sex differences. Several studies have reported land pollution with *Toxocara* spp. eggs in public areas of our region, strengthening the risk of infection in the children population [5, 16, 17]. Anyway, other forms of infection such as consumption of contaminated vegetables and

direct contact with dog fur should be considered [18, 19]. The difference in prevalence regarding children from the northern areas reveals that those populations have greater probabilities of infection due to the kind of weather existing there, which favors development and permanence of infective eggs. This situation matches data provided by countries north of Argentina: 46.5% in Peru [20], 51.6% in Brazil [21], 47.1% in Colombia [22] and 34% in Venezuela [23].

According to Fillaux and Magnaval [24], covert toxocariasis is mainly a benign infection, so that a large majority of those infected are asymptomatic or have very few symptoms, which are undiagnosed. It is often self-limiting, leaving residual specific antibodies. Therefore, the diagnosis is based on covert toxocariasis indirect arguments, one of which is eosinophilia. Coincidentally, we can consider the children studied who were with AcTox + and eosinophilia support covert toxocariasis self-limiting [25], as previous recorded symptoms were not associated with positive serology and were children considered fit clinically to attend the school.

For many individuals from the middle and high social classes, dog and/or cat ownership originates in a close emotional connection between people and pets, resulting in a beneficial relationship known as human-animal bond. However, individuals living in economically disadvantaged neighborhoods, with precarious housings made of metal sheet and wood, own pets because they provide warmth during winter months (animals and children sleep together) and chase away rodents in warmer months (garbage heaps are normally

found close to their houses). Both dogs and cats can play a significant role in communicating zoonotic agents such as *Toxocara*, contaminating the human environment. For Lee et al. [26], *Toxocara canis* is known more frequently as an etiological agent of human toxocariasis, while Fisher [27] reports *T. cati* as an underestimated zoonotic agent. In this study, while no parasitic infection in pets was assessed, an association was detected between positive serology and cat ownership. In our country, Sommerfelt et al [28] collected street-cat feces from open spaces in public institutions in the city of Buenos Aires. From the 465 samples obtained, 58.3% were parasitized; *T. cati* had a frequency of 61.2%, demonstrating thus the contamination of the environment, which turned these dangerous spaces risky for the human population spending time in those places. The results from our study reinforce the role of the parasite-infected cat as carrier of toxocariasis.

In this study, seven out of 10 children showed intestinal parasites, a similar frequency to that reported by the research group in low-class children in a town near La Plata [8]. These results have been overtaken by studies carried out by Zonta et al. [29] in natives from the Argentinean NE (95.0%) and by Menghi et al. [30] in natives from the NW (94.6%). On the other hand, in a study conducted by Soriano et al. [31] in children from the south of Argentina, the prevalence reached 50.7%. These results show that intestinal parasites are spread all over Argentina, and while the studied populations are similar as regards economic characteristics, the different frequencies are probably associated to climate conditions varying so widely in the country as well as different cultural standards of natives as compared to individuals living in slums.

The children infected with helminthes and protozoa did not present differences as regards sex, but they did in age group. The significantly higher frequency of protozoa in schoolchildren aged 10-12 years old can be related to their activities. Children in this age group often move in a wider territory, increasing the possibility of infection from other contaminated environments or from other people they socialize with. There are also differences among the species found. While in the studies from northern areas from Argentina there were high frequencies of *B. hominis* and *Uncinarias* [29, 30], in our study *B. hominis*, *E. vermicularis* and *G. intestinalis* prevailed, matching results in studies from central areas [15, 32]. According to WHO

estimations, approximately 10% of the 14-year-old Argentinean population (about 950,000 children) need preventive chemotherapy for their geohelminthiasis [33]. However, this scenery probably matches the situation in the Northern provinces, where these helminthes infections are frequent. Our results, as those from other field studies in the central region, indicate that geohelminthiasis are less frequent and less important in relation to the actual number of infected children [9-11].

Eosinophilia was not related to the presence of intestinal parasites but it was associated with residual infection with *Toxocara* spp.; coinciding with Alonso et al [15] and Chiodo et al. [5] in Argentina and Seo M and Yoon S [34] in Korea. As shown in table 1, eosinophilia and AcTox+ also associated with in each age group. When analyzing children with intestinal parasitoses with eosinophilia, we also found an association with toxocariasis respect of parasitized without eosinophilia. These results indicate that in this population, eosinophilia is associated with toxocariasis because most species of found intestinal parasites not generate this response.

But, Table 2 shows that most children up to 7 years with toxocariasis and some of these intestinal helminthes presented eosinophilia up to 10%, whereas in older school values exceeded this percentage. Further, percentages of eosinophils were above 20% in those with two or three helminthes and positive serology; indicating the age and species diversity helminthes associated to infection with *Toxocara* spp. produces a higher eosinophilic response. Studies need to be expanded with a higher number of cases.

From the survey to parents and guardians about signs and symptoms in the week previous to the sample taking, the concept is reinforced that oligosymptomatic process and low levels of morbidity in these parasitic infections have led to their not being considered as priority diseases within the context of Public Health in developing countries. Parasites may affect food consumption, digestion, absorption, and metabolism. They have a negative effect on the socio-economic progress and it is the primary chronic disease with negative effects over the nutritional status and intellectual condition primarily of the children.

As a second stage of PROCOPIN, all the children with intestinal parasites and their family groups were treated with antiparasitic drugs. Besides, with the contribution of students of the

Medicine and Natural Sciences Departments at the University of La Plata, educational workshops on basic preventive measures to be taken by the people living in the neighborhood to avoid re-infection are conducted with children and their families.

Conclusion

This study reveals the association of positive serology and eosinophilia compatible with the symptoms of self-limiting covert toxocariasis associated to cat ownership in schoolchildren from our region that also have an increased frequency of intestinal parasites. The results in this study contribute to a comprehensive approach that allows appropriately designing and implementing efforts in prevention and control of parasitoses in children.

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