

***Toxocara* Infection Seroprevalence and Its Relationship with Atopic Features in a General Adult Population**

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Key Words

Atopy · Immunoglobulin E · Skin prick tests · Asthma · *Toxocara*

Abstract

Background: The relationship of helminth infection with atopy is controversial. Toxocariasis is the most common helminth infection in industrialized countries. The study aimed to investigate the association between *Toxocara* exposure and atopic features. **Methods:** The study is based on a cross-sectional survey of 463 subjects, randomly selected (stratified by decades of age) from a general adult population. *Toxocara* exposure was defined by the presence of serum *Toxocara* antibodies. Main outcome measures included total serum IgE levels, skin prick tests (SPT) to a panel of 13 relevant aeroallergens, specific IgE to aeroallergens (Phadiatop test), and respiratory symptoms evaluated by means of a questionnaire. **Results:** A total of 134 subjects (weighted proportion 28.6%, 95% CI 26.5–30.7%) showed *Toxocara* exposure. Pet ownership, rural habitat, farming, and low educational level were associated with *Toxocara* exposure. *Toxocara* exposure was associated with both pos-

itive SPT (particularly to mites) and positive specific IgE (Phadiatop test) after adjusting for potential confounders. The effect of *Toxocara* exposure on total serum IgE levels and blood eosinophil count was different in SPT-positive subjects and SPT-negative individuals. In SPT-negative individuals, *Toxocara* exposure was associated with an increase in both serum IgE levels and eosinophil counts, whereas an opposite trend was observed in SPT-positive individuals. *Toxocara* exposure was not associated with respiratory symptoms. **Conclusions:** In this adult population, *Toxocara* exposure is associated with allergic sensitization, particularly to mites. There is evidence of an intriguing interaction between *Toxocara* exposure and allergic sensitization for both total serum IgE levels and blood eosinophil counts.

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Introduction

Helminth infection shows an intriguing relationship with atopy. Helminths are among the most common infections of humans, particularly in developing countries [1]. Atopic diseases are among the most common im-

mune diseases of humans, particularly in industrialized countries [1]. Both helminth infection and atopy are characterized by enhanced Th2 responses, high serum IgE levels, and eosinophilia [2]. On the one hand, the genetically determined atopic state could confer an advantage against helminth infection [3]. From this evolutionary perspective, the primary function of the allergic response could be part of an antiparasitic protective mechanism, and allergic disease may be the undesirable reaction to otherwise inoffensive environmental substances [4, 5]. On the other hand, helminth infection could influence the development of allergy. First, specific immune response to helminth allergens may elicit allergic manifestations [6, 7]. In addition, the immune modulation induced by chronic helminth infection could modify the development of atopy and related diseases [8]. In this aspect, results are conflicting, either showing a predictive [4, 9–15] or a protective effect [16–22]. Further evidence for a protective effect is given by the ecological observation of how the prevalence of atopic disease has increased over recent years in industrialized countries at the same time when improved sanitary conditions have decreased helminth infection [1, 4]. The latter is in agreement with the ‘hygiene hypothesis’, which proposes that a reduced exposure to infectious agents is the cause of the rise in allergic and autoimmune diseases in industrialized countries [8, 23].

Most studies on this subject have investigated the relationship between atopic disease and infection by intestinal helminths in children from endemic tropical regions [4, 9, 10, 15–20]. Toxocariasis, an infection by a tissue-dwelling helminth which is common in nontropical regions, may provide an insight into the problem. Toxocariasis is the clinical disease in the human host caused by infection of zoonotic roundworms of dogs and cats, *Toxocara canis* and *Toxocara cati* [24, 25]. Both of these are nematodes in the order Ascaridia, family Toxocaridae, genus *Toxocara*. Their definitive hosts are the domestic dog and cat, in which they live as adults within the lumen of the small intestine [24, 25]. *Toxocara* eggs pass in feces to the soil, where they can remain viable for years [24]. Transmission to the human occurs by ingestion of *Toxocara* eggs from contaminated soil [24–26]. After ingestion, the eggs hatch to release larvae that penetrate the small intestine, enter the circulation, and then wander throughout the body, with the possibility of invading organs for months or up to several years [24, 26]. Clinical syndromes are typical in children and include visceral and ocular larva migrans [24–26]. Subclinical forms have been described in adults and include

the so-called covert or common toxocariasis [24–26]. Toxocariasis has a worldwide distribution [24]. Even in industrialized countries, the prevalence of the infection of dogs with adult *Toxocara* worms is about 25% [27], and soil from most parks is contaminated with *Toxocara* eggs [28]. Seroprevalence surveys [reviewed in 26] show that *Toxocara* exposure rates vary widely among different populations, from 2% to more than 90%. Prevalence of *Toxocara* exposure is higher in rural areas, in socioeconomically disadvantaged classes, and in tropical countries [26]. However, toxocariasis is certainly the most prevalent helminthiasis in industrialized countries [26].

Experimental *Toxocara* infection induces airway inflammation in mice [29]. Toxocariasis was associated with the onset of severe asthma in anecdotal case reports [30]. In addition, two seroprevalence studies in Dutch children demonstrated an association between *Toxocara* seropositivity and atopic features (high total serum IgE levels, aeroallergen sensitization, and asthma) [12, 13]. Two case-control studies confirmed an association between asthma and *Toxocara* seropositivity in children from Malaysia [31] and Hawaii (USA) [32]. Two additional studies, however, did not confirm such an association in children from Egypt [33] and the USA [34]. The present study aimed to investigate the relationship between *Toxocara* exposure and atopic features in an adult population-based survey.

Methods

Study Population

The present investigation forms part of the A-Estrada Allergy Study. A detailed description of the study has been reported elsewhere [35]. Briefly, it entails a cross-sectional survey of an age-stratified random sample of the adult population (≥ 18 years) from the municipality of A-Estrada (Spain). The study profile is represented in figure 1. There were no differences in sex, age or habitat (rural/urban) between individuals who participated in the study and those who did not [35].

Main Determinations

Demographic data were assessed by means of an interviewer-administered questionnaire, as previously described [35, 36]. They included: (1) educational level in four categories: none, elementary (1–8 years), secondary (9–13 years), and university; (2) profession (farming/other); (3) smoking habit (yes/no); (4) alcohol consumption in three categories: none, 10–140 g/day, and more than 140 g/day; (5) habitat (rural/urban), and (6) pet (cat or dog) ownership (yes/no). The questionnaire also included items regarding respiratory diseases, as follows: (1) the lifetime history of wheezing explored by means of the question: ‘have you ever had wheezing or whistling in the chest at any time in the past?’ and (2) the current

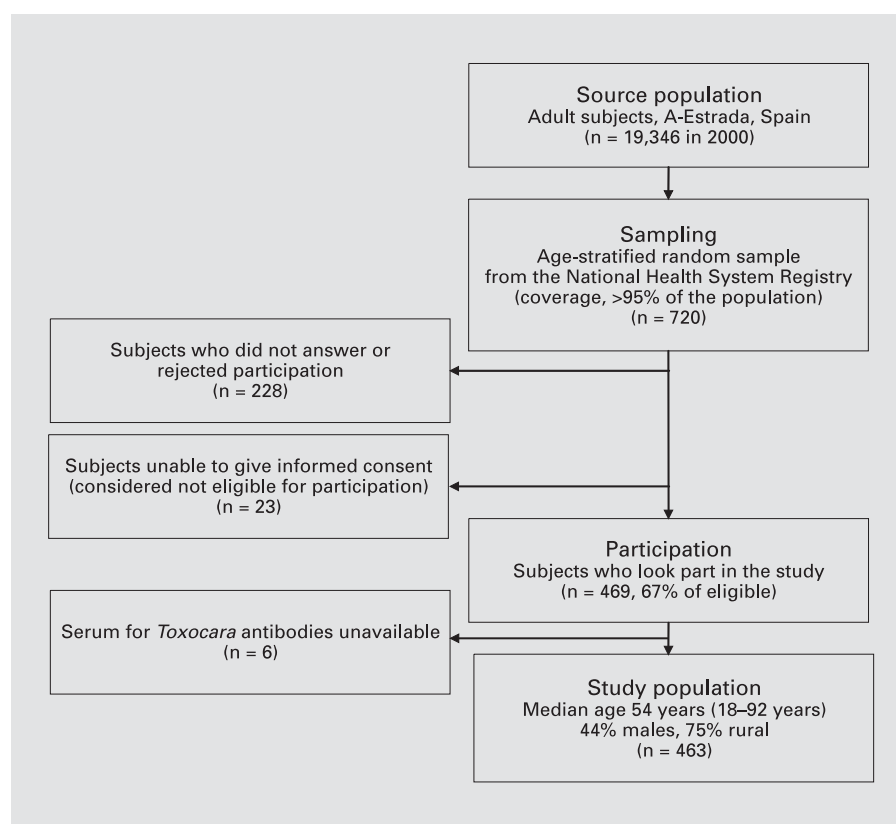


Fig. 1. Study profile.

physician's diagnosis of asthma or chronic bronchitis needing bronchodilator therapy.

Skin prick tests (SPT) to aeroallergens relevant in the area included mites (*Dermatophagoides pteronyssinus*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*), pollens (*Lolium perenne*, *Plantago lanceolata*, *Betula alba*, *Parietaria judaica*), moulds (*Alternaria alternata*, *Aspergillus* spp., *Penicillium notatum*, *Cladosporium herbarum*), and pets (dog and cat; ALK-Abelló, Madrid, Spain). Wheals ≥ 4 mm after 15 min were considered indicative of a positive reaction.

Total serum IgE levels were assayed by chemiluminescent EIA (Immulite, DPC, Los Angeles, Calif., USA). Specific IgE was assayed by means of UniCAP-Phadiatop™ (Pharmacia & Upjohn, Uppsala, Sweden), a qualitative (positive/negative) solid-phase immunoassay that uses a balanced mixture of relevant allergens causing inhalant allergies. The profile of total serum IgE and aeroallergen sensitization (SPT and specific IgE) in the study population have been described previously [35–37]. Blood eosinophil counts (available in 457 out of 463 subjects) were measured with an Olympus Argos/5 Diff (ABX Diagnostics, Hamburg, Germany) automated hematology analyzer.

Serum *Toxocara* IgG antibodies were assayed by means of a commercial ELISA (Bordier Affinity Products, Crissier, Switzerland). The test uses *Toxocara canis* excretory and secretory antigens and has been validated for routine clinical use and seroprevalence studies [38]. Subjects with a positive test were considered to have *Toxocara* exposure, either current or past [26].

Statistical Analyses

A design-based analysis was performed in order to account for the stratified sampling. Compensatory weights were developed to obtain estimates from the original target population. The Stata 7.0 package (Stata, College Station, Tex., USA) was employed. The Pearson χ^2 and the Student t test were employed for comparison of categorical and quantitative variables, respectively. Both logistic and linear regressions were employed for multivariate analyses. In these analyses, age (in years) was entered into the equation as a numerical variable. Binary variables were entered as '1' ('present' or 'yes') or '0' ('absent' or 'no'). Dummy variables were created for nonbinary categorical variables using the lowest category as the reference. Serum IgE values and blood eosinophil counts were $\log_{10}$ -transformed in order to normalize their distribution for some analyses. Stratified analysis was used to evaluate how atopic status and *Toxocara* status influenced serum IgE levels and blood eosinophil counts. The putative interaction between atopic status and *Toxocara* status was then tested by adding the multiplicative term into regression models.

Ethical Considerations

All subjects consented to participate in the study, which conformed to the Helsinki Declaration.

Table 1. Sociodemographic characteristics in relation to *Toxocara* status

Variable	<i>Toxocara</i> -positive (n = 134)	<i>Toxocara</i> -negative (n = 329)	p value
Age, years	53.0 (50.0–56.0)	49.3 (48.0–50.5)	0.08
Sex (male)	55 (42.5)	150 (44.5)	0.7
Educational level			0.02
None	25 (17.0)	33 (9.0)	
Elementary (1–8 years)	90 (66.2)	219 (62.7)	
Secondary (9–13 years)	13 (11.7)	57 (20.9)	
University	6 (4.9)	20 (7.1)	
Rural habitat	113 (83.6)	234 (70.1)	0.003
Farming occupation	59 (41.6)	76 (20.9)	<0.001
Pet ownership	114 (84.0)	231 (70.2)	0.002

For age, data represent means and 95% CI (in parentheses) and for the other variables they represent absolute numbers and proportions (in parentheses). Estimates (means and proportions) were weighted according to the study design.

Table 2. Aeroallergen sensitization and respiratory symptoms in relation to *Toxocara* status

Variable	<i>Toxocara</i> -positive (n = 134)	<i>Toxocara</i> -negative (n = 329)	p value
Skin prick test positivity			
Overall	43 (34.9)	77 (25.5)	0.04
Mites	41 (33.4)	69 (23.2)	0.02
Pollens	12 (9.4)	35 (11.8)	0.4
Moulds	5 (3.8)	6 (1.8)	0.1
Pets	2 (1.5)	3 (0.9)	0.5
Specific IgE (Phadiatop) positivity	46 (36.2)	70 (22.7)	0.003
Lifetime prevalence of wheezing	43 (31.4)	101 (30.2)	0.8
Current diagnosis of asthma or bronchitis	6 (6.6)	21 (5.5)	0.6

Data are absolute numbers and proportions (in parentheses). Proportions were weighted according to the study design.

Results

A total of 134 out of 463 subjects showed *Toxocara* positivity (weighted prevalence 28.6%, 95% CI 26.5–30.7%). No clear association was observed between age and *Toxocara* seropositivity (table 1). The prevalence of *Toxocara* positivity ranged from 21.7% (in subjects aged 18–29 years) to 34.8% (in subjects aged 60–69 years). Similarly, no association was observed between *Toxocara* seropositivity and sex (table 1). *Toxocara* seropositivity was associated with a low educational level, rural habitat, farming, and pet ownership (table 1).

A comparison of atopic features in *Toxocara*-seropositive and *Toxocara*-seronegative subjects is represented in table 2. Overall SPT positivity (reactivity to at least 1

of the 13 allergens tested) was more prevalent in *Toxocara*-seropositive than in *Toxocara*-seronegative subjects. When groups of allergens were considered separately, the association between *Toxocara* seropositivity and SPT positivity was restricted to mites. Specifically, *Toxocara* seropositivity was not associated with sensitization to pollens, the second cause of aeroallergen sensitization in this population. The number of mould- and pet-sensitized individuals was very small in this series (table 2). The association between *Toxocara* seropositivity and both overall SPT positivity and mite positivity was still present after adjusting for age, sex, educational level, smoking, and alcohol consumption (adjusted OR 1.9, 95% CI 1.2–3.0, $p = 0.007$, and adjusted OR 2.1, 95% CI 1.3–3.4, $p = 0.002$, respectively).

Table 3. Multivariate analysis of factors associated with total serum IgE levels and blood eosinophil counts

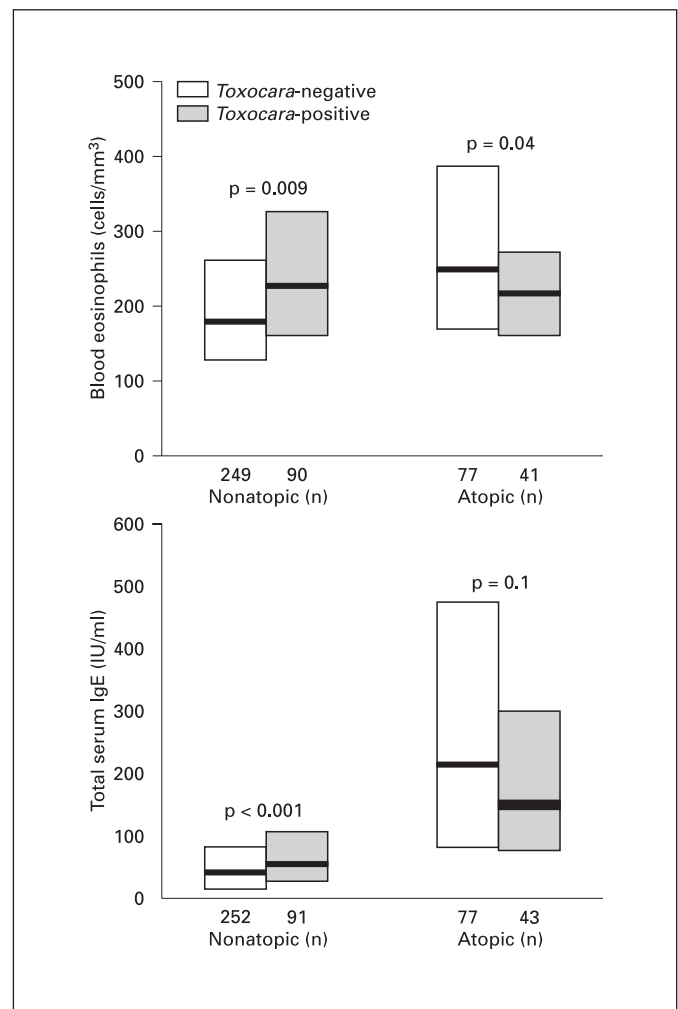
Variable	Blood eosinophil count log ₁₀ (cells/mm ³)			Total serum IgE log ₁₀ (IU/ml)		
	regression coefficient	standard error	p value	regression coefficient	standard error	p value
<i>Toxocara</i> seropositivity	0.076	0.030	0.01	0.244	0.061	<0.001
Skin prick test positivity	0.141	0.036	<0.001	0.767	0.064	<0.001
Interaction term (SPT positivity × <i>Toxocara</i> seropositivity)	−0.182	0.056	0.001	−0.394	0.107	<0.001

Linear regression analysis. Blood eosinophil counts and total serum IgE values were log₁₀-transformed in order to normalize their distribution. Coefficients are adjusted for all listed variables, as well as for age, sex, smoking, and alcohol intake.

The prevalence of positive specific IgE (Phadiatop) was higher in *Toxocara*-seropositive than in *Toxocara*-seronegative subjects (table 2). The association between *Toxocara* seropositivity and Phadiatop positivity was still present after adjusting for age, sex, educational level, smoking, and alcohol consumption (adjusted OR 2.2, 95% CI 1.3–3.4, $p = 0.001$).

The lifetime prevalence of wheezing and the current prevalence of physician-diagnosed asthma or chronic bronchitis were similar in *Toxocara*-seropositive and *Toxocara*-seronegative subjects both in univariate analysis (table 2) and after adjusting for age, sex, educational level, and smoking (data not shown).

Blood eosinophil counts were similar in *Toxocara*-seropositive and *Toxocara*-seronegative subjects as a whole (geometric mean 198 cells/mm³ and 95% CI 186–212 cells/mm³ vs. geometric mean 212 cells/mm³ and 95% CI 194–233 cells/mm³, respectively, $p = 0.2$). Marked blood eosinophilia (>1,000 eosinophils/mm³) was found in only 2 subjects (0.4%), both *Toxocara*-seronegative. Stratified analysis suggested an interaction between *Toxocara* exposure and atopic status (SPT positivity) for eosinophil counts (fig. 2). Among SPT-negative subjects, *Toxocara*-seropositive subjects showed higher blood eosinophil counts than *Toxocara*-seronegative individuals. Among SPT-positive subjects, however, *Toxocara*-seropositive subjects showed lower blood eosinophil counts than *Toxocara*-seronegative individuals (fig. 2). Evidence for an interaction between SPT positivity and *Toxocara* seropositivity for blood eosinophil counts was still present after adjusting for age, sex, smoking, and alcohol consumption (table 3). In the same model, smoking was also positively associated with blood eosinophil counts (data not shown).

**Fig. 2.** Blood eosinophil counts and total serum IgE levels in *Toxocara*-seropositive and *Toxocara*-seronegative individuals stratified according to their atopic status (SPT positivity). Boxes represent interquartile ranges and median values.

Total serum IgE levels were higher in *Toxocara*-seropositive than in *Toxocara*-seronegative subjects (geometric mean 83.1 IU/ml and 95% CI 67.6–102.3 IU/ml vs. geometric mean 54.9 IU/ml and 95% CI 46.7–63.0 IU/ml, respectively, $p = 0.001$). The proportion of subjects with markedly high serum IgE levels (>500 IU/ml) was similar in *Toxocara*-seropositive and *Toxocara*-seronegative subjects (6.2 vs. 5.6%, $p = 0.80$). Stratified analysis suggested an interaction between *Toxocara* exposure and atopic status (SPT positivity) for serum IgE levels (fig. 2). Among SPT-negative individuals, *Toxocara*-seropositive subjects showed higher serum IgE levels than *Toxocara*-seronegative subjects. Among SPT-positive individuals, conversely, a trend to lower IgE levels was observed in *Toxocara*-seropositive subjects (fig. 2). Evidence for an interaction between SPT positivity and *Toxocara* seropositivity for serum IgE levels was still present after adjusting for age, sex, smoking, and alcohol consumption (table 3). In this model, an alcohol intake >140 g/week was also positively associated with serum IgE levels (data not shown).

Discussion

Toxocara seroprevalence in the studied area (95% CI 26.5–30.7%) is higher than that reported in different regions of Spain [39, 40], possibly reflecting a higher frequency of risk factors such as rural environment, farming habitat and pet ownership in this population. Unlike hepatitis A virus seroprevalence, which is accumulative over age [41], *Toxocara* seroprevalence was not related to age. It should be noted that *Toxocara* seropositivity only reflects *Toxocara* exposure, either current or past [26]. *Toxocara* infection may be self-limited, and positive *Toxocara* IgG antibodies reflect past exposure in these cases [26]. *Toxocara* larvae can also remain for years in host tissues without further growth or differentiation [42]. In these cases, the diagnosis is elusive because the parasite cannot be detected in feces or blood [26]. Subclinical forms (that may be frequent) have been described in adults [24–26]. The relative increase in serum IgE levels and blood eosinophil counts, as observed in some individuals of the present study, can suggest active *Toxocara* infection in some of these cases [26].

To our knowledge, this is the first study reporting a relationship between *Toxocara* exposure and atopic features in adults. Subjects with *Toxocara* exposure showed higher total serum IgE levels and a higher prevalence of aeroallergen sensitization, as revealed by positive specifi-

ic IgE (Phadiatop test) and positive SPT (especially to mites) than did subjects without *Toxocara* exposure. These findings are in agreement with those reported in two consecutive cross-sectional surveys in Dutch children [12, 13]. In these studies, *Toxocara*-seropositive children had higher serum IgE levels and a higher prevalence of allergic sensitization (to any of *D. pteronyssinus*, dog or cat) than *Toxocara*-seronegative individuals [12, 13]. At least two mechanisms, namely helminth-induced immune modulation and cross-reactivity between helminths and aeroallergens, could underlie such an association, as discussed below.

Host immunomodulation forms part of the sophisticated mechanisms that helminths promote in order to establish chronic infections evading the host immune system [2, 8]. On the one hand, helminth-induced immunomodulatory mechanisms could prevent the development of atopic diseases [8, 21, 23, 43]. This may explain the inverse association between helminthiasis and allergen skin test reactivity [16, 20, 21] or asthma symptoms [17–19]. These and additional experimental animal studies have offered a rationale for the investigation of helminths as a potential alternative therapy for asthma and other immunological diseases [44]. On the other hand, enhanced Th2 responses to helminths and subsequent polyclonal IgE synthesis could foster the allergic reactivity toward environmental allergens [4, 10]. In fact, some other studies found a positive association between helminthiasis and allergen skin reactivity [9, 11] or asthma symptoms [11–13]. These controversial results have raised the question of whether helminth infection is predictive of or protective for atopy and asthma [1, 45]. Indeed, antihelminthic treatment of chronically infected children resulted in decreased allergen skin test reactivity in some studies [10], and in increased allergen skin test reactivity and improvement of asthma symptoms in others [22]. Furthermore, the interaction of helminth molecules with the host could favor allergic sensitization while inhibiting inflammatory allergic disease [2]. Much of the apparent controversy in the association of helminthiasis with atopic features may be due to (1) the extrapolation of data derived from animal models (which frequently use human parasites in nonpermissive rodent hosts), (2) the lack of distinction between natural and zoonotic infections (which frequently induce a powerful destructive response that often results in elimination of parasites), (3) the nature of helminths (able or unable to develop tissue-dwelling stages), and (4) the burden of infection [2]. Low-grade/occasional helminth infections (typical of industrialized countries) are stimulatory of al-

lergic reactivity, whereas high-grade/frequent infections (typical of developing countries) are suppressive [10, 45, 46]. In the present population from a theoretically industrialized country, we found a positive association between some atopic features and exposure to *Toxocara*, a zoonotic tissue-dwelling parasite which, as an exception, is able to establish long-lasting infections after a fine interaction with the host's immune system [2, 24–26, 42].

In connection with immune modulation, the observed interaction between *Toxocara* exposure and atopic status (as defined by SPT positivity) for both total serum IgE levels and blood eosinophil count may be of significance. Among nonatopic patients, *Toxocara* exposure was associated with increased serum IgE levels and increased blood eosinophil counts, whereas an opposite effect was observed in atopic subjects. This indicates that atopic status modifies the effect of *Toxocara* exposure on these two variables. These results are in keeping with the hypothesis that the atopic state may modulate the immune response to helminth infection [3, 4].

A second explanation for the association between *Toxocara* exposure and allergic sensitization is helminth-aeroallergen cross-reactivity. There is some degree of cross-reactivity between *Toxocara* and other nematodes [47] and, interestingly, between nematodes (such as *Anisakis*) and mites [48]. Allergy to *Anisakis* is rather common in other parts of Spain [6], but not in the studied area [49]. Although never tested, cross-reactivity between *Toxocara* and mite antigens could be suspected. Note that the association between *Toxocara* exposure and allergic sensitization was restricted to mites in the present series. Perhaps, combined exposure to common antigens from

mites and *Toxocara* could favor mite sensitization. This intriguing hypothesis should be tested in future studies.

Methodological issues of this and similar studies should be considered. First, the confounding effect of unknown variables that can be associated with both exposure and atopic features could explain the contradictory results observed among studies. Second, temporal ambiguity is an obvious drawback of cross-sectional studies for inferring causality. Third, participation rate was only 67%, but there is little support for a selection issue that could have biased the prevalence of *Toxocara* exposure. Finally, nondifferential misclassification due to poorly defined outcomes (such as wheezing or asthma/bronchitis) could contribute to the null results observed in the present as well as in some previous studies.

Studying the IgE system in its natural state (i.e. in response to helminthic parasites) may give insights into the reasons why it malfunctions causing atopic disease [4]. The present study shows a link between *Toxocara* exposure and atopic features, but the mechanisms of this link remain intriguing. *Toxocara* infection deserves further studies because it is the most prevalent helminthiasis in industrialized countries [26]. Studies investigating the association of atopy with pet ownership, rural environment, farming or educational level should consider *Toxocara* exposure as a potential confounder.

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