ORIGINAL ARTICLE

FREQUENCY OF ANTI-*Toxocara* spp. ANTIBODIES IN INDIVIDUALS ATTENDED BY THE *CENTRO DE SALUD FAMILIAR* AND ENVIRONMENTAL CONTAMINATION WITH *Toxocara canis* EGGS IN DOG FECES, IN THE COASTAL NIEBLA TOWN, CHILE

Catalina VARGAS(1), Patricio TORRES(1), María Isabel JERCIC(2), Marta LOBOS(3), Alan OYARCE(2), Juan Carlos MIRANDA(4) & Salvador AYALA(5)

SUMMARY

The frequency of anti-*Toxocara* spp. antibodies in individuals attended by the *Centro de Salud Familiar* in the coastal Niebla town, Chile, was related to the host and to environmental factors. IgG anti-*Toxocara* antibodies were detected with a commercial ELISA kit (SCIMEDX Corporation, USA). Samples with undetermined absorbance values were subjected to an additional ELISA standardized by the *Instituto de Salud Pública*, Chilean Health Ministry, a commercial ELISA (NOVATEC, Germany), and a commercial Western blot kit (LDBio Diagnostics, France). Hematological exams were performed using an automated blood counter and blood smears. Dog feces were collected from the ground along the main road in Niebla, including rural and urban locations. Ninety (25.4%) of the 355 examined individuals were positive by the ELISA test. The frequency of anti-*Toxocara* antibodies and the infection risk were significantly higher ($p < 0.05$) among those individuals ≥ 40 years old with respect to the 20-39 years old group, in individuals from rural locations, those who did not have a safe drinking water supply in the house or who presented blood eosinophilia. The proportion of positive samples of dog feces and the mean number of *Toxocara canis* eggs/g of feces in urban and rural areas were similar ($p > 0.05$).

KEYWORDS: Seroprevalence; Human toxocariasis; Environmental contamination; Eosinophilia.

INTRODUCTION

Toxocariasis is a neglected worldwide distributed zoonosis caused by the third-stage larva (L3) of *Toxocara canis* and *Toxocara cati* nematodes whose definitive hosts are the domestic dog and cat, respectively. Eggs eliminated by *T. canis* females are expelled with the dog feces. The infective L3 larvae develop in eggs in the soil between 12 °C and 32 °C, with a relative humidity close to 85%. Eggs are viable for years and are found in 6.6% to 95% of soil samples from different countries[1].

Transmission occurs predominantly by ingestion of infective eggs present in soil (onicophagy, geophagy) or less frequently by consumption of contaminated raw vegetables, fruits or water. Other possible route of *Toxocara* transmission is the ingestion of larvae from undercooked tissues of paratenic hosts (birds, ruminants or pigs). Human can also be infected through the direct contact with eggs containing infective larvae present in dog hair[2]. Hatched L3 larvae penetrate the intestinal mucosa and enter the mesenteric vessels to reach the liver, lungs, heart, eyes, and the central nervous system, inducing the formation of granulomas[3].

Diagnosis of human toxocariasis is based on clinical, epidemiological, and serological test data; the latter mainly through the detection of anti-*T. canis*/*T. cati* antibodies by the enzyme linked immunosorbent assay (ELISA)[1]. The combined use of ELISA to detect IgG, with detection of excretion-secretion antigens and Western blot assays constitute a good diagnostic option due to their sensibilities and specificities; even when the application of these tests do not differentiate between an active disease and a past infection[4].

Different clinical forms are described for human toxocariasis: visceral larva migrans (VLM), ocular larva migrans (OLM), neurological toxocariasis (NT) and covert or common toxocariasis[5,6]. In Chile, OLM has been identified in 31 patients with ages ranging from 4 to 45 years old[7]. In addition, one out of 175 children with positive serology also develops retinal granuloma and visual loss[8]. VLM has been described in 129 children with serological evidence associated with lung, hepatic, ocular, neurological or skin problems[9,10]. In addition, a case of NT associated with high eosinophilia and positive serology was diagnosed in a 61 year old man[11].

Transmission and risk factors of toxocariasis vary between different geographical localities, and they have been associated with poverty, low

(1) *Universidad Austral de Chile, Facultad de Medicina, Instituto de Parasitología, Valdivia, Chile.*
(2) *Instituto de Salud Pública de Chile, Departamento Laboratorio Biomédico Nacional y de Referencia, Sección de Parasitología, Santiago, Chile.*
(3) *Universidad Austral de Chile, Facultad de Medicina, Instituto de Medicina, Unidad de Hematología, Valdivia, Chile.*
(4) *Universidad Austral de Chile, Facultad de Ciencias Económicas y Administrativas, Instituto e Estadística, Valdivia, Chile.*
(5) *Instituto de Salud Pública de Chile, Subdepartamento Vigilancia de Laboratorio, Santiago, Chile.*
Correspondence to: Patricio Torres. Instituto de Parasitología, Facultad de Medicina, Edificio de Ciencias Biomédicas, Campus Isla Teja, Casilla 567, Valdivia, Chile. E-mail: ptorres@uach.cl

education level, and lack of control or treatment of definitive hosts. All the above mentioned factors favor contamination which is reinforced by environmental conditions (light, temperature, humidity, pH, vegetation), and also the development, survival and availability of parasite eggs[12]. Seroprevalence of human toxocariasis varies between 1.6% and 92.8% in different countries[1]. Great variability are also described, from 3.7% and 40% among localities of a same country[13]. In Chile, seroprevalence varies between 1.3% and 15.6% in blood donors[14,15]. *Toxocara canis* and *T. cati* prevalences are from 10.7% to 19%[16,17,18] and 65.1% to 70%[16,19] in dogs and cats, respectively, in the region of Valdivia.

Although some risk factors have been identified for toxocariasis, there is still much inconsistent information[1]. For example, there is a wide age range leading to the conclusion that there is a higher prevalence in some age groups[20,21]. In other studies, no association between seroprevalence and age has been found[20,22]. Gender does not seem to be a factor associated with human toxocariasis[23]. However, some authors report the opposite[24]. Toxocariasis tends to be more prevalent in rural areas than in urban ones[23,25]. Higher eroprevalences have been associated with the tenancy of dogs and cats[26], particularly regarding veterinarians and those in charge of pets[27]. Other authors have not found this association[28]. Some reports associate poor sanitation with higher seroprevalences of toxocariasis[20]. Several authors either associate[24,27] or do not associate[22] higher seroprevalences with eosinophilia. In the same way, leukocytosis has been associated[28] or not[20] with higher seroprevalences. Hemoglobin values have not apparently been associated with VLM and OLM[27,10].

The aim of this study is to determine the frequency of anti-*Toxocara* spp. antibodies in individuals attended by the *Centro de salud familiar* in the coastal Niebla town and associate it with clinical, laboratory and environmental data, including the detection of *Toxocara* eggs in dog feces.

MATERIALS AND METHODS

Study area and human participants

The coastal area of Niebla (Fig. 1), located 15 km from the city of Valdivia (39°48'30''S and 73°14'30''W), has a population of 2,202 and 1,376 inhabitants in the urban and rural areas, respectively[29]. The zone has a mean annual temperature of 12 °C and a relative humidity of 88.7%. The study considered individuals who attended the *Centro de Salud Familiar* (CESFAM) in Niebla to perform a complete blood count, between September 2011 and January 2012. The study was approved by the Ethics Committee in Research, Valdivia Health Service, and Chilean Health Ministry. Recruited people consented in performing the ELISA test for toxocariasis. From the total of 490 people invited to participate in the study, 355 agreed and completed a survey containing their names, age, area where they lived (urban or rural), the ownership of pets (dogs or cats), if they had a yard or a garden, vegetables in the garden, and type of water supply in the house. The 355 participants are representative of people living in urban and rural areas of the Niebla coast who have a limited access to laboratory tests such as the ELISA to detect anti-*Toxocara* antibodies due to the high cost. All the participants received the ELISA results. The reasons for requesting the blood count were recorded using the Omega 300 system. The distribution of the study participants according to age, gender, and location is shown in Table 1. The age of participants varied from 9 months to 90 years old.

Laboratory methods in humans

A blood sample from each person was obtained through venous puncture. Samples collected in EDTA tubes were used to prepare blood smears stained with May Grunwald-Giemsa, and analyzed by a hematological counter (Sysmex XT-1800i, Kobe, Japan) at the Central Laboratory of the Hospital Base in Valdivia. Results were obtained using the OMEGA 3000 information system at the Hematological Unit of the School of Medicine, Austral University of Chile. Anemia was determined according to hemoglobin values[30]. Eosinophilia was considered when the number of eosinophils surpassed 4% of the total white cell count, in most cases when it exceeded 500cells/µL[31]. Afterwards, EDTA samples were centrifuged at 1,200 g for 10 min; plasma samples were kept frozen in Eppendorf tubes at -20°C.

IgG anti-*Toxocara canis/cati* antibodies were detected by means of an ELISA kit (SCIMEDX Corporation, New Jersey, USA) at the *Instituto de Salud Pública* (ISP), Chilean Health Ministry. The ISP validated the kit using the Clinical and Laboratory Standard Institute guides N° EP12-A2[32] and EP15-A2[33]. The obtained sensitivity of 91.7% and the specificity of 80% fulfilled the verification requirements recommended by the manufacturer (sensitivity of 93.3% and specificity of 87.5%). To determine the specificity, the commercial kit was applied to serum samples with confirmed diagnosis of hydatidosis, fasciolosis, trichinellosis, cisticercosis, and autoimmune diseases. Absorbance readings, $A_{462} > 0.350$ OD units were considered as positive reactions, while $A_{462} < 0.350$ OD units and $A_{462} = 0.250$-0.350 OD units were considered as negative and undetermined reactions, respectively. At ISP, undetermined samples underwent additional serological testing: an "in house" standardized anti-IgG ELISA (ISP) and a commercial anti-IgG ELISA (NOVATEC, Dietzenbach, Germany). The positive samples had the results confirmed by a commercial Western blot kit (LDBio Diagnostics, Lyon, France). Samples presenting with two or more polypeptide bands of lower molecular weight (40, 30, 25, and 14 kDA), were considered as positive. The "in house" IgG ELISA assay was evaluated with respect to sensitivity, specificity and precision parameters using the Clinical and Laboratory Standard Institute Guides (CLSI), EP12-A2[32] and EP15-A2[33].

Fecal dog samples collection and laboratory methods

In March 2012, samples of dog feces were collected from the ground along the main road in Niebla, including about 9 km of rural and urban locations (Fig.1). Samples were collected, manually, using double polyethylene bags by two people through a visual inspection of the main road path. At the laboratory, each sample was weighed, homogenized and kept at 4 °C. Five grams were taken from each sample and fixed in 10 mL of formol-saline to perform the qualitative Telemann modified method (TMM) in combination with a floating method (FM) which uses 70 g of zinc sulphate in 100 mL of distilled water[19]. The combination of TPM and FM offers a greater efficacy in the detection of *Toxocara* spp. eggs, in comparison with the techniques applied independently[18]. Regarding the Knight *et al*. quantitative method (KM)[34], feces from homogenized samples were added to a conical calibrated centrifuge tube containing 7 mL of formol-saline to complete 8 mL. To find *T. canis* eggs using the combined TMM and FM method, two microscopic preparations of the sediment and one preparation of FM were examined. For the KM, all the sediment was examined in microscopic preparations[34]. All preparations
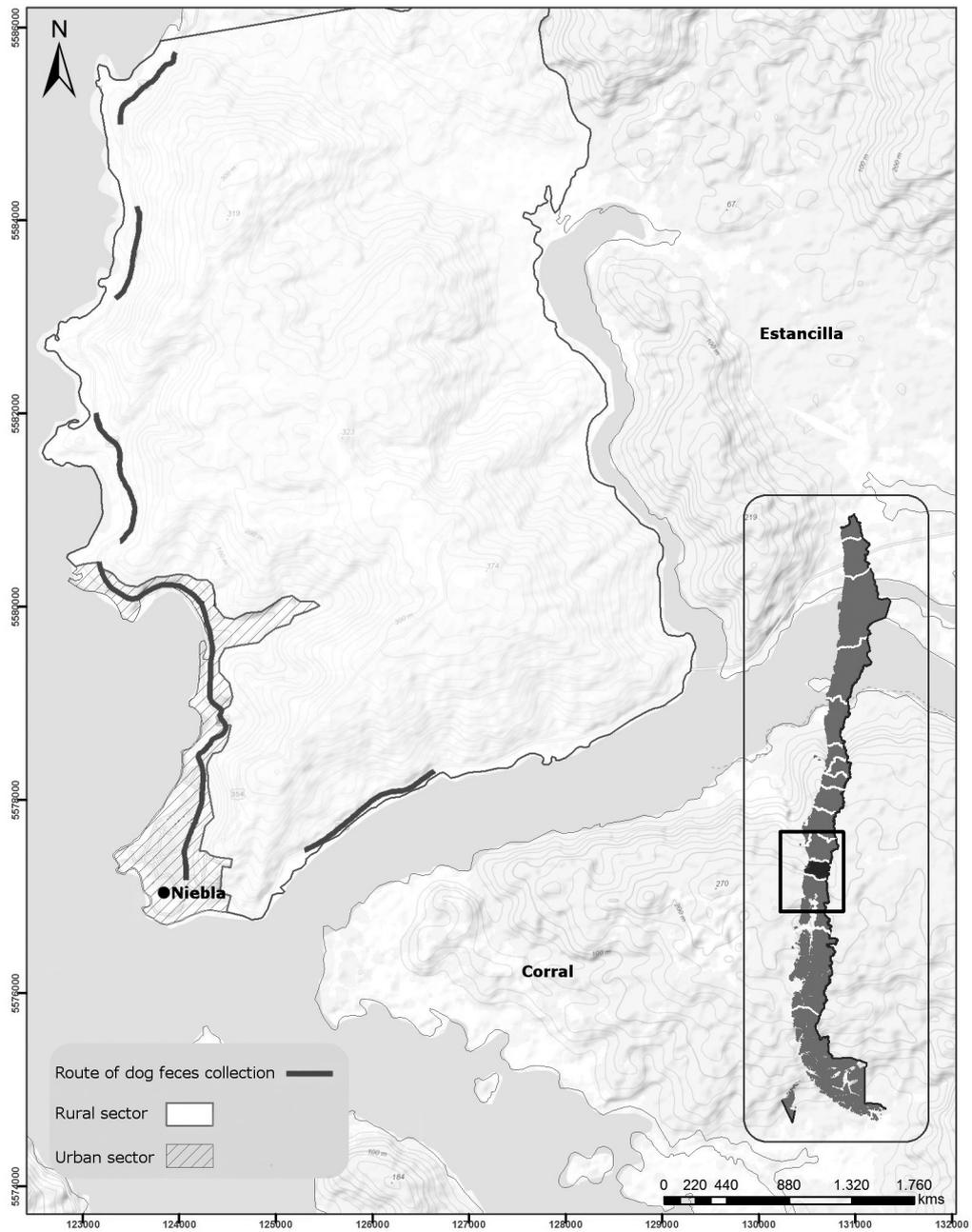
Fig. 1 - Route of dog feces collection from the ground and rural and urban sectors in the Niebla coastal locality, Chile.

using 22 x 22 mm cover glass were examined under a light microscope (100 x). Results of the KM method were expressed as the number of eggs/g of feces.

Statistical analysis

Seroprevalence corresponds to the number of seropositive people/ number of people examined x 100.To compare the seroprevalences, the Pearson´s chi-square with the Yates´s correction was applied. When pairs of samples presented expected frequencies ≤ 5, the Fisher´s exact test was used[35]. The U Mann-Whitney test was used to compare the eggs mean/g

of feces[35]. For all the tests, results were considered significant at $p < 0.05$. Odds ratios (OR) and 95% confidence intervals (CI) were calculated with the free software "VassarStat available at http://vassarstats.net/textbook/ index.html. When odds ratios and confidence intervals values were >1 they were considered significant.

RESULTS

Ninety (25.4%) of the 355 participants were positive for IgG anti-*Toxocara* spp. antibodies by the ELISA test; their location is shown in Figure 1. Twenty serum samples were undetermined but 15 of them

Table 1
Seropositivity of IgG anti-*Toxocara* spp. antibodies according to host and environmental factors, in 355 people attended at
Centro de la Salud Familiar in the coastal locality of Niebla, Chile

Factors	Seropositivity		
	N° of positive samples/examined (percentage)	Chi-square or Fisher test *p*-value	Odds ratios (95% CI)
Age groups (years)			
≤19 (a)	17/77 (22.1)	0.0266	a with b 0.71 (0.33-1.53)
20-39 (b)	15/90 (16.7)		a with c 1.57 (0.85-2.93)
≥40 (c)	58/188 (30.9)		b with c 2.23 (1.18-4.21)
Gender			
Male	33/110 (30.0)	0.2236	0.71 (0.43-1.17)
Female	57/245 (23.3)		
Origin			
Rural	44/140 (31.4)	0.0456	1.68 (1.04-2.73)
Urban	46/215 (21.4)		
Presence of yard/ garden			
Yes	86/342 (25.1)	0.7454	1.32 (0.40-4.41)
No	4/13 (30.8)		
Presence of vegetable in the garden			
Yes	35/114 (30.7)	0.1435	0.67 (0.41-1.09)
No	55/241(22.8)		
Ownership of dogs/ cats			
Yes	80/289 (27.7)	0.0506	0.47 (0.23-0.96)
No	10/66 (15.1)		
Presence of safe drinking water			
Yes	61/272 (22.4)	0.0316	1.86 (1.09-3.17)
No[1]	29/83 (35)		
Anemia			
Yes	2/9 (22.2)	1.0000	1.19 (0.24-5.85)
No	88/346 (25.4)		
Leukocytosis			
Yes	5/17 (29.4)	0.7753	0.81 (0.28-2.36)
No	85/338 (25.1)		
Absolute eosinophilia			
Yes	15/30 (50.0)	0.0025	0.3 (0.14-0.64)
No	75/325 (23.1)		
Relative eosinophilia			
Yes	31/75 (41.3)	0.0006	2.64 (1.53-4.54)
No	59/280 (21.1)		
Most frequent reasons for requesting the blood count			
Health control (a)	27/81 (33.3)	0.7523	
Diabetes (b)	11/38 (28.9)		a with b 1.23 (0.53-2.84)
Dyslipidemia (c)	14/54 (25.9)		a with c 1.43 (0.67-3.1)
High blood pressure (d)	27/80 (33.8)		a with d 0.98 (0.51-1.89)

[1] Included water from spring, borehole or stream

proved definitively to be positive when the three additional tests were performed. The proportion of individuals with anti-*Toxocara* spp. antibodies was significantly higher ($p < 0.05$) in people of different age groups, in all the individuals living in rural areas and among those who did not have a safe drinking water supply in the house (Table 1).

Younger age groups (≤ 40 years old) did not show statistical significant differences ($p > 0.05$) (Table 1). However, the proportion of individuals with anti-*Toxocara* spp. antibodies was significantly lower ($p < 0.05$) in the age group of 20-39 years old with respect to the age group ≥ 40 years old (Table 2). The risk for those ≥ 40 years old was significantly increased (OR 2.23; 95% CI: 1.18 - 4.21) with respect to those with 20-39 years old (Table 1). The infection risk was higher for individuals living in rural areas (OR 1.68; 95% CI: 1.04 - 2.73) and among those who did not have safe drinking water supplies (OR 1.86; 95% CI: 1.09 - 3.17). The proportion of people with anti-*Toxocara* spp. antibodies of different genders did not show significant differences ($p > 0.05$) and the risk did not show significant association (OR 0.71; 95% CI: 0.43 - 1.17) (Table 1).

The existence of a yard, garden or vegetables in the garden of the house of the people surveyed as well as the ownership of dogs or cats was not associated with a significantly higher seroprevalence or risk (Table 2). The same was true for the proportion of environmental samples of dog feces with *Toxocara* eggs and their eggs mean/g feces from rural and urban areas (Table 2).

The frequency of anti-*Toxocara* spp. antibodies was significantly higher ($p < 0.05$) among individuals with relative or absolute eosinophilia. However, in individuals with or without anemia or leukocytosis it was similar ($p > 0.05$) (Table 1). The risk was significantly higher for those individuals with relative eosinophilia (OR 2.64; 95% CI: 1.53 - 4.54).

There was no significant difference between the most frequent reasons (health control, diabetes, dyslipidemia and high blood pressure) for requesting a blood count and the *Toxocara* positivity and negativity ($p > 0.05$) (Table 1). Also, the infection risk did not show a significant association among individuals who performed the blood exam for health control due to diabetes, dyslipidemia and high blood pressure (Table 1).

DISCUSSION

The frequency of anti-*Toxocara* spp. antibodies in individuals attended by the *Centro de Salud Familiar* in Niebla surpassed the values estimated by Herskovic & Astorga[14] in blood donors from three regions in Chile (1.3% to 15.6%); in the population of Robinson Crusoe Island (3.8%)[36], and in blood donors (5.3%) from the city of Valdivia[15].

In a review of toxocariasis in Latin America[37], the seroprevalence in the human populations of different age groups varied between 22.1% and 31.6% in Argentina, 27% and 42% in Bolivia, 47.5% in Colombia, 5.2% in Cuba, 7.3% and 27.9% in Peru, 6.5% in Puerto Rico, and 34.9% in indigenous populations in Venezuela.

The similarity of *Toxocara* spp. infection in the two younger age groups and the significant higher frequency of anti- *Toxocara* spp. antibodies and the higher risk for individuals ≥ 40 years old with respect to the 20-39 years old group suggests that L3 larvae remain in the tissues, and that recurrent reinfections maintain the level of circulating antibodies in the affected population. The survival of the parasite in the tissues is also favored by the mechanisms that L3 larvae use to evade the immunological response[38]. In Valdivia, Navarrete & Rojas[15] did not find significant differences in the seroprevalence of different age groups of blood donors. In Colombia, Agudelo *et al.*[39] found higher seroprevalences in age groups from 10-39 in a population of age varying from months to 70 years old. Rubinsky-Elefant *et al.*[40] found a higher seroprevalence in people from 5 to 14 years old in comparison with those from 15 to 90 years old in the Amazonian region of Brazil. In Bolivia[21] and Brazil[20] seroprevalence was similar in different age groups, in populations from two to 85 and from 3 months to 80 years old, respectively.

In Niebla, the frequency of anti-*Toxocara* spp. antibodies and the infection risk was similar in different gender groups. These data is in agreement with those from other Chilean authors[15] and in other countries[21,23,41]. However, some other studies reported higher seroprevalences in males[1,24] than in females[39]. The latter has been associated with a more intense exposure due to working activities[24] in those with more contact with soil, working in agriculture or cattle rising.

Results suggest that the level of contamination and the risk of transmission of human toxocariasis in rural and urban areas would be similar since the proportion of positive dog feces samples, taken directly from the soil, and the eggs mean/g of feces did not present significant differences between these areas. Nevertheless, the frequency of anti-*Toxocara* spp. antibodies and the risk of infection were higher in individuals from rural areas. Gawor *et al.*[42] concluded that in Poland, the proportion of soil samples from rural and urban homes contaminated with *Toxocara* eggs was similar. The proportion of dog feces samples with *Toxocara* eggs was similar (13.5%) to previous data from the city of Valdivia[16,17,18,43]. In Brazil, Negri *et al.*[44] did not find significant differences in the seroprevalence of people from rural or urban areas. Studies in the

Table 2
Proportion of dog feces positive for *Toxocara* eggs collected from the ground and the mean of the number/g of sample in rural and urban areas in the coastal locality of Niebla, Chile

Descriptors	Areas		*p*-value
	Rural	Urban	
Positive/examined samples (percentage)	12/78 (15.4)	12/77 (15.6)	0.8536[1]
Mean of eggs/g in examined samples (minimum-maximum)	11.3 (0-646)	8.4 (0-513)	0.9840[2]

[1] Chi-square test. [2] U Mann-Whitney test

U.S.A.[25], and Turkey[23] showed that seroprevalences of human toxocariasis were significantly higher in rural areas.

The existence of a yard, garden or vegetable in the garden, in the house, as well as having pets was not associated with seropositivity and infection risk in individuals of Niebla. Roldán *et al*.[28] reported an association between individuals that were seropositive for *Toxocara* and the existence of gardens. Other studies in Brazil[20] and Colombia[39] have shown that there is no association between seroprevalence and having pets. However, there are also studies that have shown the opposite in Argentina[22], Brazil[26], Chile[45], Colombia[46], and Peru[24].

The prevalence of toxocariasis and the infection risk in Niebla were higher among individuals that did not have safe drinking water supplies in their homes. This suggests that the water consumed from springs, boreholes or streams are potentially contaminated with *Toxocara* eggs or that food washed with these water sources could represent a transmission risk, coinciding with a study performed in Colombia[46]. Anaruma-Filho *et al*.[20] concluded that individuals who filtered drinking water before consumption showed a significantly lower frequency of toxocariasis, compared with those who did not treat drinking water.

The frequency of anti-*Toxocara* spp. antibodies in Niebla was significantly higher in people with eosinophilia, confirming the results obtained in Argentina[22], Brazil[41] and Peru[24]. However, the frequency of antibodies was not associated with the presence of anemia or leukocytosis, corroborating other studies[20,24,27,41].

As a conclusion, the frequency of anti-*Toxocara* spp. antibodies and the infection risk was significantly higher among individuals ≥ 40 years old with respect to the 29-39 years group, in those living in rural areas, and also among those that did not have safe drinking water supplies at home or who presented blood eosinophilia.

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