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Immunodiagnostic approaches for the detection of human toxocarosis

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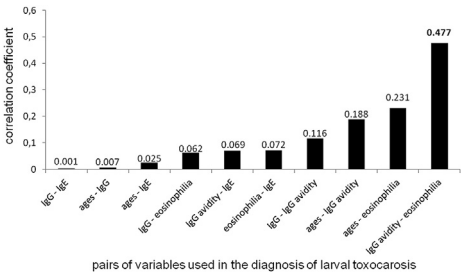
HIGHLIGHTS

- Seroprevalence of toxocarosis in Slovakian population was 15.3%.
- The low-avidity IgG antibodies were frequently found in eosinophilic patients.
- Substantially higher eosinophilia was detected in children than in adults.
- Mild correlation was observed between eosinophil count and the IgG avidity index.

GRAPHICAL ABSTRACT



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ABSTRACT

Human toxocarosis is an important zoonosis caused by larvae of *Toxocara canis/cati*. The objective was to evaluate the role of IgG anti-*Toxocara* antibody detection and the specific IgG avidity in diagnostics of human toxocarosis. Anti-*Toxocara* IgG antibodies and IgG avidity were evaluated by excretory-secretory (ES)-enzyme-linked immunosorbent assay (ELISA). The IgG anti-*Toxocara* seroprevalence in people (n = 7678) from western Slovakia was 15.3% and found to be highest in the oldest age groups. The presence of low- IgG avidity in 179 suspected patients for toxocarosis was evaluated in relation to sex, age, IgG antibody levels, eosinophilia, increased total IgE, domicile, geophagia, dog/cat ownership, anamnesis. Low- IgG avidity index was found in 30.7% of the patients. The low- IgG avidity in eosinophilic group (42.1%) was significantly higher than in non-eosinophilic group (22.0%; $P = 0.043$). Substantially higher eosinophilia was detected in children (under 10 years old; 55.6%) than in adults (aged $\geq$ 41 years; 17.6%; $P = 0.009$). Significant difference between seroprevalence of total IgE in patients coming from towns (48.8%) and patients from villages (21.3%) was established ($P = 0.007$). Mild negative correlation ($r = -0.477$, $P = 0.043$) was observed between the amounts of eosinophils and the values of IgG avidity. The sensitivity and specificity of IgG avidity assay were 43.8% and 83.3%, respectively. Our results suggest that besides anti-*Toxocara* IgG, measurement of IgG avidity may be useful for the determination of acute toxocarosis. Moreover, these tests should be accompanied by other immunological markers and determinants of examined patients such as eosinophilia, increased total IgE and age.

1. Introduction

Human larval toxocarosis, a zoonotic disease caused by larvae of *Toxocara canis* (dog roundworm) and *Toxocara cati* (cat roundworm), is distributed worldwide, and is the most commonly

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diagnosed tissue helminthosis in Slovakia (Elefant et al., 2011; Glickman and Schantz, 1981; Ondriska et al., 2013).

Humans become infected by ingestion of embryonated eggs from soil, dirty hands, raw vegetables or unwashed food (Despommier, 2003). Occasionally, the infection can be transmitted also by passive vectors (synantropic flies) or by consumption of undercooked meat from a paratenic host (Morimatsu et al., 2006; Pegg, 1971). *Toxocara* spp. larvae may invade various organs (liver, lungs, heart, brain and eyes) causing an intense inflammatory response, eosinophilia, higher levels of total IgE; a mechanical damage of the tissues and formation of multiple eosinophilic granulomas (Dattoli et al., 2011; Magnaval et al., 2001; Mirdha and Khokar, 2002). The clinical symptoms vary from asymptomatic forms to those with severe organs injuries (Elefant et al., 2011). In humans, four distinct types of syndromes have been identified: visceral larva migrans (VLM), ocular larva migrans (OLM), covert toxocarosis and neurological toxocarosis (Dziemian et al., 2008).

Regarding the absence of direct parasitological evidence of infection, immunological methods play a relevant role in the diagnosis of toxocarosis. The laboratory diagnosis of infection is usually based on the specific antibody detection against *Toxocara* excretory-secretory (ES) antigens, using an enzyme-linked immunosorbent assay (ELISA) (Demirci et al., 2010; De Savigny et al., 1979). The preferred approaches are the detection of IgG antibodies as a screening test and confirmation of positive sera with an immunoblot test (Smith et al., 2009). In ocular toxocarosis cases, probably due to the low number of infective larvae, serum anti-*Toxocara* antibodies may be present in very low titers or be even undetectable (Magnaval et al., 2002; Sharkey and McKay, 1993). However, titers of specific antibodies in intraocular fluids, such as vitreous or aqueous humor, are usually higher than those in serum, suggesting a local antibody production (Elefant et al., 2010). The human IgG response elicited by *Toxocara* larvae may persist for many years (Elefant et al., 2006) and, therefore, IgG antibody levels cannot be used to distinguish between recent and chronic infections (Roldán and Espinoza, 2009).

The exact determination of current status of *Toxocara* infection is not possible because the incubation period may range from weeks to months, depending on infection intensity, re-infection and patient sensitivity (Dziemian et al., 2008). In case of VLM, some frequent laboratorial findings are leukocytosis with intense eosinophilia, hypergammaglobulinemia and isohemagglutinin titer elevation (Jacob et al., 1994). In cases of OLM and covert toxocarosis, blood eosinophilia can be absent in some patients. Other diagnostic tests should therefore be considered, the most promising of which is determination of the total IgE concentration (Magnaval et al., 2001). There are very few publications on the diagnosis of toxocarosis based on the detection of IgA antibodies. In the study of Elefant et al. (2006), the sensitivity of ELISA IgA was 47.8%. In human toxocarosis, IgM antibodies are also generated and may be detected in both acute and chronic phases, differing from most of unrelated infections, in which they are transient (Smith, 1993).

It is known that avidity of antibodies increases with time after antigen challenge and the measurement of the avidity have been used when differentiation of recent and distant infections is crucial. For example, the determination of IgG avidity in sera is useful in some parasitic diseases including toxoplasmosis (Alvarado-Esquivel et al., 2002), neosporosis (Aguado-Martinez et al., 2005), fascioliasis (Abou-Basha et al., 2000) and *Trypanosoma cruzi* infections (Marcipar et al., 2001).

The study was focused on distinction of acute and chronic *Toxocara* infections in humans following the assessment of specific IgG avidity. We aimed to determine the seroprevalence of toxocarosis in humans coming from western Slovakia, in respect to some determinants such as eosinophilia, increased total IgE, specific IgG levels, sex, age, domicile, geophagia, dog/cat ownership, anamnesis and travel history.

2. Materials and methods

2.1. Biological material

To determine *Toxocara* spp. IgG seroprevalence in humans (aged 0–94 years) serum samples from 7678 patients were examined. All patients originated from western Slovakia (Bratislava Region, Nitra Region, Trnava Region and Trenčín Region). The samples were collected at the health care centers within the catchment area of the HPL (Ltd) Medical Laboratories. Sera were separated and stored at + 4 °C until used. One hundred seventy-nine sera of patients (0–81 years; 90 men and 89 women; different domicile) suspected of toxocarosis (based on the presence of anti-*Toxocara* IgG antibodies) were examined for IgG avidity. Additionally, all participants were interviewed using an individual clinical-epidemiological questionnaire. The questionnaire included anamnesis data (clinical signs, symptoms), demographic features (age, sex, domicile), immunological markers (eosinophilia, increased total IgE) and risk factors (ownership of dogs or/and cats, history of pica and/or geophagia, consumption of raw meat). These mentioned characteristics of patients were obtained from physicians involved in the current treatment of the person. Patient information has been provided in 88 (49.2%) persons of the total study group. Eosinophilia and increased total IgE were detected in 43.2% and 34.1% of patients who have completed clinical-epidemiological questionnaires, respectively.

2.2. Enzyme-linked immunosorbent assay for determination of specific IgG antibody production

Sera of patients were examined by ELISA method (EIA Toxocara IgG diagnostic kit, Test-Line Ltd. Clinical Diagnostics, Czech Republic) for the presence of IgG antibodies against *Toxocara* spp. according to the manufacturer's instructions. The base serum dilution was 1/100. The color intensity of each well was read at 450 nm. The results were expressed as an index, calculated by dividing the specimen absorbance value by the calibrator absorbance value; indices of <0.90 were considered negative, indices from 0.90 to 1.10 were considered ambiguous, and indices of >1.10 were considered positive. Index values from 1.11 to 2.0 were considered low positive, values from 2.01 to 2.6 were considered medium positive and values >2.61 were considered high positive.

2.3. Determination of the avidity of the specific IgG anti-Toxocara antibodies

Toxocara-specific IgG avidity was measured using the 'bind and break' method and the calculation of a relative avidity index (AI). The IgG avidity test was performed and interpreted according to directions of the manufacturer (Test-Line Ltd. Clinical Diagnostics, Czech Republic). The sera samples (100 μl per well, 1/100 dilution) were tested on two separate microplates coated with L3 larval ES antigens. After incubation for 30 min at 37 °C, one plate was exposed to urea solution and the second plate to washing solution alone for 20 min at room temperature. Negative and positive reference sera were included in each plate as controls. Each serum was tested in duplicate. The absorbance was read at 450 nm. The relative avidity index was calculated as the ratio of OD values in sera treated with urea and values of nontreated sera, multiplied by 100. Interpretation: AI < 40 was defined by manufacturer as low avidity (indicating recently acquired infection); AI between 41 and 50 as borderline avidity; AI > 51 as high avidity (indicating old infection).

2.4. Statistical analysis

Data were analyzed by OpenEpi statistical software, version 3.03 (http://www.openepi.com/Menu/OE_Menu.htm) and the chi-squared test was used to assess statistical differences. $P < 0.05$ was considered significant.

The correlation coefficient or measurement of association between two random variables was evaluated by Spearman rank correlation analysis, where the r value < 0.3 was considered as no correlation, 0.3–0.5 was considered as mild correlation, 0.5–0.8 was considered as moderate correlation, and if r > 0.8, it was considered as strong correlation (Markechová et al., 2011). The two-dimensional linear regression model was used to specify the nature of the relation between two variables (Microsoft Excel 2010 software).

Analysis of 2 × 2 tables was undertaken using the MedCalc diagnostic test evaluations program version 11.6.1, for Windows (MedCalc Software; http://www.medcalc.org/calc/diagnostic test.php).

3. Results

3.1. Seroprevalence of toxocarosis in Slovakia

Out of 7678 examined patients, anti-*Toxocara* IgG antibodies were detected in 1173 persons (15.3%). Five hundred and fifty-five seropositive males (16.5%) and 618 (14.3%) seropositive females were detected. The difference was statistically significant ($P = 0.007$). Furthermore, there was significant difference in the seropositivity according to the age (≥41 years 22.2%, 11–20 years 13.4%, 21–40 years 12.4% and 0–10 years 9.3%, $P < 0.001$) (Table 1).

3.2. IgG avidity test

Low-avidity anti-*Toxocara* IgG antibodies were detected in 27 (30.7%) cases out of 88 patients who had completed clinical-epidemiological questionnaires. The occurrence of low-avidity IgG antibodies in eosinophilic group (42.1%) was significantly higher than in non-eosinophilic group (22.0%; $P = 0.043$). Gender, age, level of specific IgG antibodies, level of total IgE, residence, geophagia, traveling abroad, consumption of raw meat, owning dogs or cats and anamnesis were not associated ($P > 0.05$) with the presence of low-avidity IgG antibodies in examined patients (Table 2).

Because there is no method for unambiguous confirmation of *Toxocara* infection, the diagnosis (toxocarosis) was based on clinical, epidemiologic and laboratory findings (eosinophilia and increased total IgE). The sensitivity and specificity of IgG avidity assay were 43.8% and 83.3%, respectively. The IgG avidity test had a positive predictive value of 77.8%, and a negative predictive value of 52.6%.

3.3. Eosinophilia

Substantially higher prevalence of eosinophilia was detected in children between 1 and 10 years of age (55.6%) than in adults of 41 and more years (17.6%; $P = 0.009$) although there was no difference between other age groups ($P > 0.05$). No associations ($P > 0.05$) were found between eosinophilia and gender, age, level of specific IgG antibodies, level of total IgE, domicile, geophagia, traveling abroad, consumption of raw meat, dogs/cats ownership or anamnesis (Table 3).

3.4. Increased total IgE antibodies

Significant difference in seroprevalence of total IgE in patients coming from towns (48.8%) and patients from villages (21.3%) was observed ($P = 0.007$). Similarly, significantly higher prevalence of increased total IgE antibodies in patients with negative anamnesis for toxocarosis (43.9%) was recorded in comparison to their presence in patients with positive anamnesis (patients with clinical signs and symptoms of toxocarosis; 16.1%; $P = 0.009$). There were no differences in the level of total IgE according to gender, age, level of specific IgG antibodies, eosinophilia, pica, traveled abroad, consumption of raw meat or owning dogs or cats ($P > 0.05$) (Table 4).

3.5. Correlation analysis of data (Fig. 1)

Correlation analysis between the amounts of eosinophils and the values of IgG avidity revealed mild negative correlation ($r = -0.477$, $P = 0.043$) as depicted in Fig. 2. No correlation was observed between other nine pairs of variables ($0.001 \leq r \leq 0.231$; $0.076 \leq P \leq 0.854$).

4. Discussion

Toxocarosis is a serious zoonosis that occurs worldwide. An increasing number of cats and dogs in urban and rural agglomerations, as well as an increasing contamination of the environment pose the risk of infection (Lee et al., 2014). Also, small rodents play a significant role in the circulation of toxocarosis, and represent an important reservoir of infection for both free-living and domestic carnivores (Reiterová et al., 2013). In Slovakia, larval toxocarosis is the most commonly diagnosed tissue helminthosis (Ondriska et al., 2013).

Table 1
Anti-*Toxocara* IgG antibodies detected in sera of patients from western Slovakia.

Variables		Total	IgG seropositive	P − value
		7678 (100.0%)	1173 (15.3%)	
Sex	male	3357 (43.7%)	555 (16.5%)	0.007
	female	4321 (56.3%)	618 (14.3%)	
Ages	0–10[a,b,c]	1503 (19.6%)	140 (9.3%)	<0.001[overall]
	11–20[a,d,e]	1432 (18.7%)	192 (13.4%)	<0.001[c,e,f]
	21–40[b,d,f]	2166 (28.2%)	269 (12.4%)	<0.001[a]
	≥41[c,e,f]	2577 (33.5%)	572 (22.2%)	0.003[b]
				0.385[d]

[a] Aged 0–10 years and 11–20 years.
[b] Aged 0–10 years and 21–40 years.
[c] Aged 0–10 years and ≥41 years.
[d] Aged 11–20 years and 21–40 years.
[e] Aged 11–20 years and ≥41 years.
[f] Aged 21–40 years and ≥41 years.

Table 2
Low-avidity anti-*Toxocara* IgG seropositivity and socio-demographic, epidemiologic, laboratory characteristics of study subjects.

Variables		Total	Low-avidity IgG	$P-$ value
		88 (100.0%)	27 (30.7%)	
Sex	male	44 (50.0%)	15 (37.5%)	0.488
	female	44 (50.0%)	12 (27.3%)	
Ages	0–10	36 (40.9%)	15 (41.7%)	0.211
	11–20	23 (26.1%)	7 (30.4%)	0.085[a]
	21–40	12 (13.6%)	2 (16.7%)	
	≥41	17 (19.4%)	3 (17.6%)	
Low-positive IgG (index values 1.11–2.0)		16 (18.2%)	6 (37.5%)	0.496
Medium-positive IgG (index values 2.01–2.6)		19 (21.6%)	5 (26.3%)	
High-positive IgG (index values > 2.6)		53 (60.2%)	16 (30.2%)	
Eosinophilia	yes	38 (43.2%)	16 (42.1%)	0.043
	no	50 (56.8%)	11 (22.0%)	
Total IgE	increased level	30 (34.1%)	12 (40.0%)	0.173
	normal level	58 (65.9%)	15 (25.9%)	
Domicile	towns	41 (46.6%)	11 (26.8%)	0.464
	villages	47 (53.4%)	16 (34.0%)	
Risk factors (dog/cat ownership, geophagia, consumption of raw meat)	yes	79 (89.8%)	22 (27.8%)	0.088
	no	9 (10.2%)	5 (55.6%)	
Traveled abroad	yes	11 (12.5%)	3 (27.3%)	0.793
	no	77 (87.5%)	24 (31.2%)	
Anamnesis	positive	31 (35.2%)	7 (22.6%)	0.225
	negative	57 (64.8%)	20 (35.1%)	

[a] Aged 0–10 years and ≥41 years.

Diagnosis of human toxocarosis currently relies on serologic tests using ES antigen to detect anti-*Toxocara* IgG antibodies. In presented work 15.3% prevalence of anti-*Toxocara* IgG antibodies was found. Seroprevalence of toxocarosis in Slovakian population was 5.5–13.7% (Havasiová et al., 1993; Ondriska et al., 2013; Pavlínová et al., 2011). The prevalence of toxocarosis in Europe varies in different geographical regions. Seropositive rates for *T. canis* have been reported to be 6% in Ireland, 7% in Turkey and Sweden, 11% in Netherlands, 20% in the Czech Republic, 22% in France and 28% in Slovenia (Gueglio et al., 1994; Holland et al., 1991; Kaplan et al., 2004; Ljungström and Van Knapen, 1989; Logar et al., 2004; Stejskal, 2005; Van Gemund et al., 1989). The seroprevalence (15.3%) in our study was comparable to seroprevalence data reported in mentioned countries. We detected significantly higher *Toxocara* spp. IgG seroprevalence in adults compared with children, similarly as Korkmaz (1998). On the contrary, more frequent occurrence of toxocarosis was observed among children than adults in studies of Herrmann et al. (1985) and Demirci et al. (2010), possibly due to more frequent contact with contaminated soil and poor hygiene. Gender is not considered as a decisive factor for infection. However, we determined significantly higher seropositivity in males than in females. Similar results were also obtained by Ljungström and Van Knapen (1989) and Kanafani et al. (2006). Conversely, Demirci et al. (2010) found a higher seroprevalence of IgG anti-*Toxocara* antibodies in women (17.9%) compared to men (10.6%).

The disadvantage of IgG-ELISA for diagnosis of toxocarosis is the inability to differentiate between the stages of infection. Consequently, detection of high avidity index in human toxocarosis could be useful for ruling out newly acquired infection. According to Ashburn et al. (1998) and Lappalainen et al. (1993), measurement of IgG avidity serves to recognize recently acquired infection of

Table 3
Socio-demographic, epidemiologic and laboratory characteristics of patients with regard to eosinophilia.

Variables		Total	Eosinophilic patient	$P-$ value
		88 (100.0%)	38 (43.2)	
Sex	male	44 (50.0%)	22 (50.0%)	0.197
	female	44 (50.0%)	16 (36.4%)	
Ages	0–10	36 (40.9%)	20 (55.6%)	0.079
	11–20	23 (26.1%)	10 (43.5%)	0.009[a]
	21–40	12 (13.7%)	5 (41.7%)	
	≥41	17 (19.3%)	3 (17.6%)	
Low-positive IgG (index values 1.11–2.0)		16 (18.2%)	6 (37.5%)	0.854
Medium-positive IgG (index values 2.01–2.6)		19 (21.6%)	8 (42.1%)	
High-positive IgG (index values > 2.6)		53 (60.2%)	24 (45.3%)	
Total IgE	increased level	30 (34.1%)	16 (53.3%)	0.167
	normal level	58 (65.9%)	22 (37.9%)	
Domicile	towns	41 (46.6%)	17 (41.5%)	0.761
	villages	47 (53.4%)	21 (44.7%)	
Risk factors (dog/cat ownership, geophagia, consumption of raw meat)	yes	79 (89.8%)	32 (40.5%)	0.133
	no	9 (10.2%)	6 (66.7%)	
Traveled abroad	yes	11 (12.5%)	2 (18.2%)	0.074
	no	77 (87.5%)	36 (46.8%)	
Anamnesis	positive	31 (35.2%)	10 (32.3%)	0.127
	negative	57 (64.8%)	28 (49.1%)	

[a] Aged 0–10 years and ≥41 years.

Table 4
Socio-demographic, epidemiologic and laboratory characteristics of patients as related to level of total IgE antibodies.

Variables		Total	Increased total IgE	P − value
		88 (100.0%)	30 (34.1%)	
Sex	male	44 (50.0%)	17 (38.6%)	0.368
	female	44 (50.0%)	13 (29.5%)	
Ages	0–10	36 (40.9%)	11 (30.6%)	0.589
	11–20	23 (26.1%)	8 (34.8%)	0.243[a]
	21–40	12 (13.7%)	3 (25.0%)	
	≥41	17 (19.3%)	8 (47.1%)	
Low-positive IgG (index values 1.11–2.0)		16 (18.2%)	3 (18.8%)	0.076
Medium-positive IgG (index values 2.01–2.6)		19 (21.6%)	4 (21.1%)	
High-positive IgG (index values > 2.6)		53 (60.2%)	23 (43.4%)	
Eosinophilia	yes	38 (43.2%)	16 (42.1%)	0.167
	no	50 (56.8%)	14 (28.0%)	
Domicile	towns	41 (46.6%)	20 (48.8%)	0.007
	villages	47 (53.4%)	10 (21.3%)	
Risk factors (dog/cat ownership, geophagia, consumption of raw meat)	yes	79 (89.8%)	27 (34.2%)	0.959
	no	9 (10.2%)	3 (33.3%)	
Traveled abroad	yes	11 (12.5%)	6 (54.5%)	0.126
	no	77 (87.5%)	24 (31.2%)	
Anamnesis	positive	31 (35.2%)	5 (16.1%)	0.009
	negative	57 (64.8%)	25 (43.9%)	

[a] Aged 0–10 years and ≥41 years.

T. gondii. Dziemian et al. (2008) determined high IgG avidity index in 94.1% of patients with long-term *Toxocara* infection. In accordance, we found that 69.3% of the patients had high IgG avidity. In contrast, the study of Elefant (2004) observed high avidity IgG antibodies in 27 children with acute toxocarosis (the diagnosis was based on clinical, epidemiologic and laboratory findings of IgE, IgA and IgG). In our study, the IgG avidity assay reached low sensitivity (43.8%) and specificity (83.3%), therefore the utilization of this test alone will not be useful to distinguish between recent and past *Toxocara* infections. The avidity test should be accompanied by other immunological markers and determinants of examined patients. This claim was also confirmed in our study by detection of substantially higher prevalence of low-avidity IgG antibodies in eosinophilic group (42.1%) than in non-eosinophilic group (22.0%; $P = 0.043$). Roldán et al. (2008) found out that 40% of the eosinophilic children had a significant association to the serology (positivity for anti-*Toxocara* IgG antibodies) in contrast to 19% of eosinophilic children with negative serology ($P = 0.001$). Roldán et al. (2008) confirmed the significant association of *Toxocara* infection with leukocytosis, dry cough, playing on house gardens, playing in public parks, history of pica and geophagia. Possible eosinophilia caused by *Toxocara* infection was reported by other authors (Choi et al., 2003; Demirci et al., 2002; Karadam et al., 2008; Kwon et al., 2006). On the other hand, in our study, the prevalence of high-avidity IgG antibodies in eosinophilic group was 36.8%. Roldán et al. (2008) detected a mild eosinophilia in children with negative serology, which might be due to another tissue parasitic infection or an early *Toxocara* infection without positive serology, or other unknown causes.

In developing countries eosinophilia is usually considered as indicator of a helminthic infection (Leder and Weller, 2000; Wolfe, 1999). Pilger et al. (2011) documented that eosinophilia was strongly associated with the presence of intestinal helminths and accentuated by ectoparasites. We detected substantially higher prevalence of eosinophilia (in association with toxocarosis) in children between 1 and 10 years of age (55.6%) than in adults of 41 and more years of age (17.6%; $P = 0.009$). Eosinophilia was statistically associated with Brazilian and Indian children of 1–10 years, who were seropositive for the IgG antibody to *Toxocara* spp. (Malla et al., 2002; Teixeira et al., 2006). Children are at higher risk of ingesting embryonated eggs from soil and getting infection than those the rest of the population (Despommier, 2003; Espinoza et al., 2008; Magnaval et al., 2001). On the other hand, other factors besides helminthic infection are important causes for eosinophilia. Allergic disorders can cause high eosinophilic counts (Wolfe, 1999). In addition, eosinophil levels are known to be higher during morning hours when corticosteroid levels are low (Löscher and Saathoff, 2008). Knowledge of blood eosinophil count and the presence of low-avidity IgG antibodies tend to carry higher

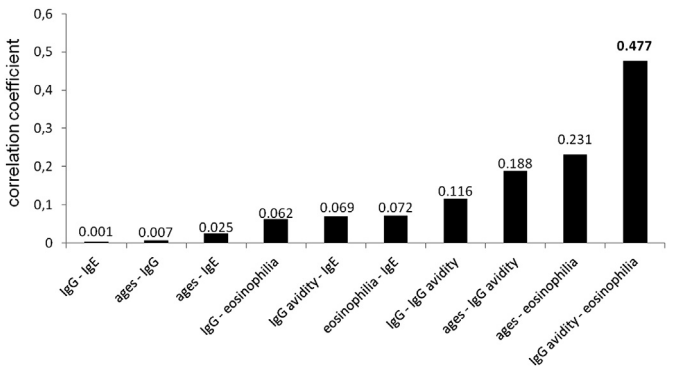

Fig. 1. The correlation coefficients for all pairs of variables used in the diagnosis of larval toxocarosis.

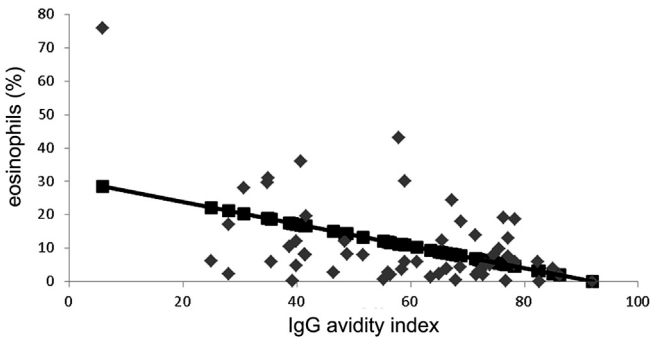

Fig. 2. The regression analysis between the amounts of eosinophils and the avidity index of IgG ($r = -0.477$; $P = 0.043$).

information value of current status of infection. In our study, the mild correlation between eosinophilia and avidity of IgG antibodies was confirmed.

The level of total IgE was further confirmatory marker for determination of current status of infection. Coffman et al. (1993) and Pinelli et al. (2007) showed that parasite-derived antigens stimulate Th0 cells to become Th2 cells, leading to the production of IL-4 and IL-5, which stimulate IgE production and eosinophil proliferation and maturation, respectively. In industrialised countries, high levels of total IgE and eosinophilia are frequently associated with allergy diseases; in developing countries, they are highly associated with parasitic infection (Cooper, 2008). In this paper, significantly higher prevalence of increased total IgE antibodies in patients coming from towns (48.8%) was recorded in comparison to their presence in patients coming from villages (21.3%). Contamination of the environment by *Toxocara* eggs is probably higher in urban areas than in rural areas. Ondriska et al. (2013) recorded *Toxocara* spp. eggs in up to 27% of children playgrounds in selected urban areas of Bratislava, whereas in the rural areas outside of Bratislava eggs were found only in 6.8% of sandpits. An increasing number of cats and dogs in urban agglomerations, the majority of them not being in the care of a veterinarian may pose the risk of infection. On the contrary, many authors indicated higher seroprevalence of toxocarosis in the rural areas in comparison to towns (Demirci et al., 2010; Ondriska et al., 2013). Knowledge of the levels of IgE antibodies has to be accompanied by other immunological markers and determinants of examined patients because of increased levels of eosinophils and low-avidity IgG antibodies were detected only in 53.3% and 40.0% of patients with increased total IgE, respectively.

In conclusion, our results suggest that measurement of the specific IgG avidity, eosinophils, total IgE antibody levels and knowledge of the demographic characteristics of the patients (children might be at greater risk for *Toxocara* infection) may assist in the discrimination between recent and distant toxocarosis.

Conflicts of interest

The authors declare that they have no conflict of interest.

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