

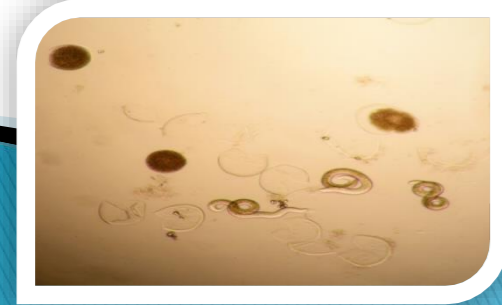
Investigation the presence of specific IgG subclass antibodies (IgG1, IgG2, IgG3 and IgG4) in patients with Toxocarosis and their role in the diagnosis of disease



INTRODUCTION

Toxocarosis is a parasitic zoonosis caused by migration of the animal nematode larvae – *Toxocara canis* and *Toxocara cati* in the human body. Because of nonspecific symptoms diagnosis of the disease is difficult and mainly based on serological methods (ELISA and W. blot) which determine the specific anti-*Toxocara* IgG antibodies against parasite TES antigens. Serological methods however have some disadvantages – presence of cross reactions with antigens of other parasites and long persistence of specific antibodies, which doesn't allow to determine stage of the disease. This requires inclusion of complementary methods to improve diagnosis and assist determination of disease activity.

Purpose – determine the presence of specific IgG subclass antibodies (IgG1-IgG4) in patients with Toxocarosis and their application in the diagnosis of disease.



MATERIALS AND METHODS

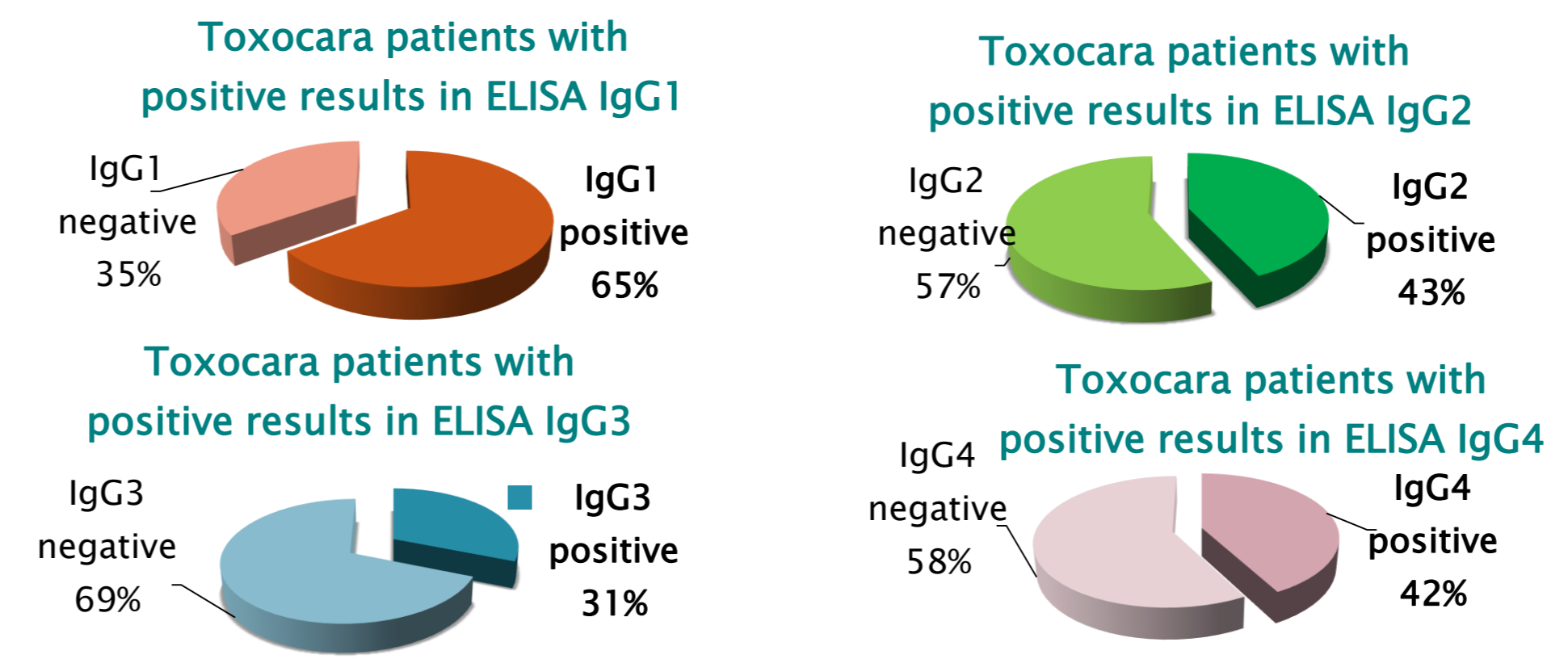
- 130 serum samples from *Toxocara* - patients (positive result in ELISA IgG – R-biopharm and W. blot – LD BIO). 66 male, 64 female; age 1-88 (mean age 36).
- 50 serum samples from patients with other diseases (parasitic – 40 or allergic – 10); 23 male, 27 female; 10-64 age (mean age 37).
- Antigens – TES antigens from in vitro cultivated *T. canis* larvae (de Savigny, 1975).
- ELISA IgG1-4 laboratory methods (specific conjugates – anti-human IgG1-IgG4 peroxidase conjugates (Invitrogen)- Tabl.1.

Tabl. 1 Condition of ELISA IgG1, IgG2, IgG3 and IgG4

Parameter	Value
TES antigen concentration	0.05µg/ml (1:10 000)
Sera dilution	1:100
Sera incubation	1 hour/ 37°C
Specific conjugates dilution	
- Ani-human IgG1 peroxidase	1:3000
- Ani-human IgG2 peroxidase	1:2000
- Ani-human IgG3 peroxidase	1:3500
- Ani-human IgG4 peroxidase	1:3500
Conjugate incubation	1 hour/ 37°C
Substrate incubation	20 min. at room temperature in the dark

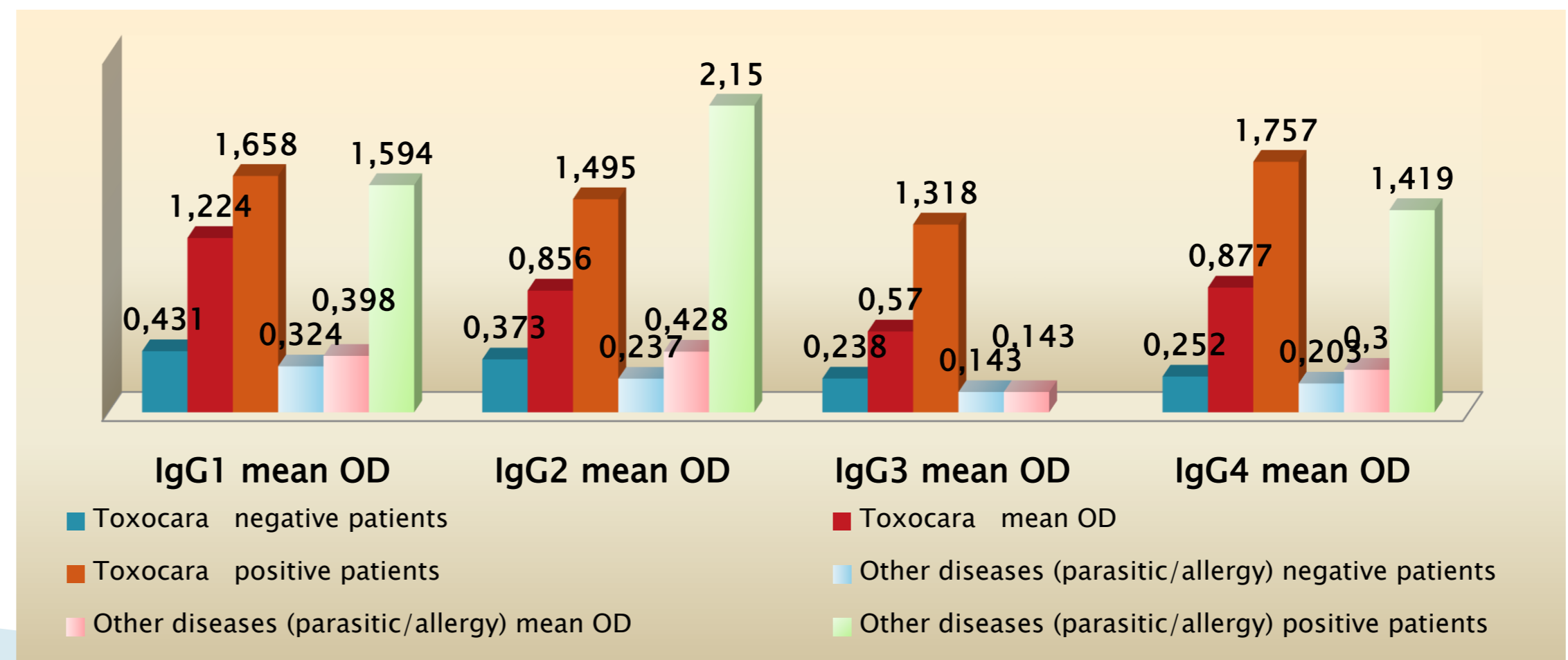
RESULTS

Investigation of patients with Toxocarosis for the presence of specific IgG subclass antibodies (IgG1, IgG2, IgG3, IgG4)



The best sensitivity – ELISA IgG1

Fig.1 Mean OD values obtained in *Toxocara* – patients and in individuals with other diseases (parasitic/ allergies) in ELISA IgG1, ELISA IgG2, ELISA IgG3 and ELISA IgG4.



Tabl. 2 Characteristic of the reactions - sensitivity and specificity

	ELISA IgG	ELISA IgG1	ELISA IgG2	ELISA IgG3	ELISA IgG4
Patients with toxocarosis (130)					
Sensitivity	100%	64,6%	43,1%	30,8%	41,5%
Patients with other parasitic diseases (40) and allergy (10)					
Specificity	82%	94%	90%	100%	92%
Cross-reactions	18%	6%	10%	0%	8%
Positive results (n)	9	3	5	0	4

All reactions for specific subclasses IgG1-4 show higher specificity than ELISA IgG. The best characteristic – ELISA IgG1.

CONCLUSIONS

The synthesis of specific IgG1 subclass antibodies in the majority of patients with toxocarosis and significantly lower percentage of cross reactivity in ELISA IgG1 indicates that determination of this subclass may be used as suitable additional method, confirming the diagnosis of disease.

The authors declare no conflict of interest.