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ORIGINAL ARTICLE

Comparison of the efficiency of two commercial kits – ELFA and Western blot in estimating the phase of *Toxoplasma gondii* infection in pregnant women

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Abstract

Sera of 89 pregnant women were selected according to the results of ELFA IgM, IgG and avidity IgG, and tested with commercial tests IgM, IgG and avidity IgG Western Blot (WB) to compare the efficacy of both techniques in determining the phase of *T. gondii* infection. In total, 81 of 89 tested sera (91.0%) were classified as positive, both in the ELFA and WB tests for the presence of anti-*Toxoplasma* antibodies of class IgG, indicating a past infection, while the prevalence of anti-*Toxoplasma* positive reactions associated with the antibodies of class IgM indicating a recent infection was much lower – 31.5% and 20.2%, respectively. Sera of 81 women were also tested in the ELFA and WB tests for avidity, e.g. ability of forming high-molecular IgG antibody complexes. Low or medium results in these tests (in this study all classified as low), indicating a recent infection, were detected by ELFA and WB in 22.2% and 45.7% of the total examined samples, respectively. The Spearman's rank test for correlation, performed for recognition of quantitative data of the ELFA and WB tests (index, units or points), revealed a highly significant correlation between the ELFA and WB tests for homologous classes of antibodies, both for IgM and IgG ($p<0.00001$). In contrast, the ELFA and WB tests for detection of anti-*Toxoplasma* IgM antibodies were not correlated with the ELFA and WB tests for detection of anti-*Toxoplasma* IgG antibodies ($p>0.05$), except for the WB test for IgM antibodies, which showed a significant correlation with the ELFA test for IgG antibodies ($p<0.01$). A highly significant negative correlation between the ELFA and WB test for IgM antibodies and ELFA and WB tests for IgG avidity was demonstrated ($p<0.01$), except for a relationship between the WB test for IgM and WB for avidity, which was not significant. Such negative correlations are theoretically expected, as strong complexes with the participation of IgG antibodies are absent in the early phase of toxoplasmosis when early antibodies of IgM class are present. Summarizing, this study indicates the high usefulness of the commercial ELFA and WB tests in serodiagnostics of toxoplasmosis in pregnant women. Special attention should be paid to parallel detection of IgM antibodies and low values in the ELFA and WB tests for IgG avidity, which indicates a recent infection which may be associated with a clinical form of congenital toxoplasmosis and damage to the foetus.

Key words

toxoplasmosis, pregnant women, seroprevalence, antibodies, IgG, IgM, ELFA, Western blot, avidity, correlation of serologic tests

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite of humans and warm-blooded animals. The majority of human infections result from ingestion of raw or undercooked meat containing *T. gondii* tissue cysts, or by ingestion of food or water contaminated with oocysts [1, 2]. Toxoplasmosis may pose a serious public health problem, especially as a congenital infection causes cerebral and ocular damage in newborns [3]. In Poland, the frequency of congenital toxoplasmosis is estimated at 1–2 cases per 1,000 births [4]. In immunocompetent organisms, the most common is an asymptomatic form of *T. gondii* infection, manifested only by a positive serologic response. However, the latent toxoplasmosis may also exert an impact on the infected persons, including the development of behavioural changes and schizophrenia [2]. Serological examination can be of limited usefulness in cases of persons with immunosuppression. In Poland, seroprevalence in people amounts to 40–60%, depending on the examined group [5].

Because of the risk of vertical *Toxoplasma gondii* transmission to the foetus after primary infection in pregnant women, serologic screening should be conducted repeatedly during pregnancy. As the assessment of *T. gondii* phase of infection is necessary to implement the proper treatment, the determining of *T. gondii* immunologic status, based on detection of specific antibodies of IgM, IgA, and IgG classes and avidity of IgG antibody, should be routinely applied. Detection of IgM and IgA antibodies can reveal an acute infection, whereas the presence of IgG without IgM antibody can be a sign of past infection. However, as the specific IgM

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antibodies can persist for months or even years after initial contact, the detection of IgM antibody is not sufficient for the diagnosis of an acute toxoplasmosis; much more important is determination of the avidity of *Toxoplasma*-specific IgG antibodies. Due to possibility of the aberration in immune response (e.g. caused by persistent IgM), determination of the phase of infection might be difficult on the basis of the classical approach and may lead to false conclusions [6]. Thus, using in the Western blot an optimized combination of recombinant antigens for the detection of IgG and IgM antibodies, as well as using different phase-specific avidity antigens, could improve the determination of the *Toxoplasma* infection status.

The aim of this study was to assess the usefulness of commercial kits ELFA and Western blot for determining the phase of *T. gondii* infection in a selected group of pregnant women.

MATERIALS AND METHODS

Collected sera. In total, 89 selected sera of pregnant women – patients of private doctor's and persons routinely tested in the Department of Health Biohazards and Parasitology, Institute of Rural Health in Lublin (2012–2014) from Lublin region (eastern Poland), were selected for the study.

All serum samples were analyzed and characterized by immunoenzymatic tests ELFA (Enzyme Linked Fluorescent Assay) for the presence of IgG and IgM antibodies against *Toxoplasma gondii,* and for estimation of the IgG avidity (Vidas Toxo IgG, Vidas Toxo IgM, Vidas Toxo IgG Avidity tests, bioMérieux, Marcy l'Etoile, France). To perform the tests by the ELFA technique, a Mini VIDAS device was used. Tests were used in accordance with the the manufacturer's instructions. The IgG level <4 IU (International Units)/ml was reported as negative, >8 IU/ml was reported as positive, and ranging from 4–8 IU/ml was reported as doubtful. For IgM, the level <0.55 IU/ml was considered negative, >0.65 IU/ml was considered positive, and ranging from 0.55–0.65 IU/ml was considered doubtful. The avidity was determined for these serum samples in which the Vidas Toxo IgG was ≥8 IU/ml. According to the manufacturer, low avidity suggests a recent infection, whereas a high avidity strongly suggests an infection of more than 4 months.

In total, 81 of 89 tested sera (91.0%) were positive in ELFA IgG. The average reactivity of the ELFA IgG was 161.1 UI/ml. Among 89 sera, 28 were positive both in IgM and IgG classes, 50 sera were positive only in IgG class and negative in IgM class, whereas 3 sera were positive in IgG class and doubtful in IgM class. Among 81 sera examined by ELFA IgG avidity, 63 sera had high IgG avidity (time post-infection – tpi >4 m) and 18 sera were with low IgG avidity (tpi < 4 m).

According to the results in ELFA and data from questionnaires, the tested samples included negative sera (8 samples), sera of patients suspected of having acute toxoplasmosis (IgM positive, IgG positive, and IgG low avidity; 14 samples), sera of patients with recently aquired toxoplasmosis (IgM positive, IgG positive and high IgG avidity; 16 sera), sera of patients with latent toxoplasmosis (IgM negative, IgG positive and high IgG avidity; 46 sera), and sera of patients with latent toxoplasmosis after treatment (IgM negative, IgG positive and low IgG avidity, 4 sera) (Tab. 1).

Western blot (WB) examinations. All serum samples were tested for the presence of IgG and IgM antibodies against *Toxoplasma gondii* and for IgG avidity by Western blot technique (commercial sets: recomLine *Toxoplasma* IgG, recomLine *Toxoplasma* IgM and recomLine *Toxoplasma* IgG (Avidity) (Mikrogen, Germany)). All tests were performed according to manufacturer's instructions.

Comparative analysis. Comparative analysis of results achieved in WB (reaction of individual classes of antibodies with specific antigens characteristic for phases of infection: ROP1 (66 kDa), MAG1 (65 kDa), SAG1 (30 kDa), GRA7 (29 kDa), GRA8 (35 kDa), and in ELFA (presence of IgM and IgG antibodies and IgG avidity) was conducted.

Statistical analysis. The results were analyzed by chi-square test and Spearman test for correlation, using STATISTICA v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

In total, 81 of 89 tested sera were positive in WB IgG (similar to ELFA IgG results) with average reactivity – 18.5 sum of points. In total, among sera tested in WB, 18 were positive both in IgM and IgG classes, 41 sera were positive only in IgG class and negative in IgM class, 22 sera were positive in IgG class and doubtful in IgM class, whereas 2 sera were negative in IgM class and doubtful in IgG class (Tab. 1).

Results in IgM class. Showed considerable divergences. In ELFA IgM, 28 sera were positive, 3 were doubtful and 58 were negative, whereas in WB IgM 18 were positive, 22 were doubtful and 49 were negative (Tab. 2). Much less discrepant results were found in comparison of the results of ELFA IgG and WB IgG, where only 2 sera showed discordant IgG results between WB and ELFA (doubtful/negative results, respectively) (Tab. 3).

Results in panels of sera. The comparison of ELFA and WB results (doubtful results were not taken into account) for the sera of patients with acute toxoplasmosis (14 sera) showed a discordant result for one serum in IgM and for other serum in avidity IgG. In the panel of sera from patients with recently aquired infection (16 sera), false negative results in IgM WB were found for 6 sera. The other discrepant results in ELFA and WB were also found for one serum in avidity IgG. In the panel of sera from patients with latent infection and after treatment (4 sera), the discrepant results in WB and ELFA IgM were found for one serum. Among sera from the panel of chronic stage of *T. gondii* infection (47 sera), discordant results were found in IgM ELFA and WB for 2 sera and in ELFA and WB avidity IgG for one sera (Tab. 1).

Among 81 sera examined by WB IgG avidity, 44 sera were determined as having high avidity IgG (15 sera- tpi >12 m and 29 sera – tpi 6–12 m), 22 sera were with medium IgG avidity (tpi 3–6 m) and 15 sera with low IgG avidity (tpi 0–3 m) (Tab. 4).

Considering patients suspected of latent toxoplasmosis (ELFA ≥4 m and WB >6 m), the proportions of high-avidity results were 63/81 (77.8%) in ELFA and 44/81 (54.3%) in WB (Tab. 4).

Table 1. Comparison of the results of ELFA and Western blot (WB)

	No. of serum	IgM				IgG				Avidity			
		ELFA		WB		ELFA		WB		ELFA		WB	
		Result*	Index	Result*	Σ of points	Result*	IU/ml	Result*	Σ of points	High/ Low	Time post infection (months)	High/ Medium/ Low	Time post infection (months)
Negative	1	-	N/A	-	0	-	N/A	-	0	N/A	N/A	N/A	N/A
	2	-	N/A	-	0	-	N/A	+/-	4	N/A	N/A	N/A	N/A
	3	-	N/A	-	0	-	N/A	-	0	N/A	N/A	N/A	N/A
	4	-	N/A	-	0	-	N/A	-	0	N/A	N/A	N/A	N/A
	5	-	N/A	-	0	-	N/A	-	0	N/A	N/A	N/A	N/A
	6	-	N/A	-	0	-	N/A	+/-	4	N/A	N/A	N/A	N/A
	7	-	N/A	-	0	-	N/A	-	0	N/A	N/A	N/A	N/A
	8	-	N/A	-	0	-	N/A	-	0	N/A	N/A	N/A	N/A
Acute infection	9	+/-	0.59	+	6	+	43	+	16	L	<4	L	0-3
	10	+	0.69	+/-	6	+	80	+	21	L	<4	L	0-3
	11	+	7,3	+	16	+	231	+	21	L	<4	L	0-3
	12	+	0.87	+/-	4	+	114	+	24	L	<4	M	3-6
	13	+	0.86	-	0	+	71	+	21	L	<4	L	0-3
	14	+	0.65	+/-	4	+	85	+	25	L	<4	L	0-3
	15	+	2.08	+	14	+	97	+	10	L	<4	L	0-3
	16	+	2.17	+	14	+	99	+	10	L	<4	L	0-3
	17	+	1.9	+	6	+	>1200	+	17	L	<4	H	6-12
	18	+	1.3	+	6	+	52	+	10	L	<4	L	0-3
	19	+	0.8	+	7	+	29	+	8	L	<4	L	0-3
	20	+	1.54	+	6	+	25	+	10	L	<4	L	0-3
	21	+	1.43	+	6	+	96	+	10	L	<4	L	0-3
	22	+	1.2	+	6	+	59	+	16	L	<4	L	0-3
Recent infection (aprox. up to 1 year)	23	+	0.86	+	10	+	167	+	24	H	>4	H	6-12
	24	+	0.85	+/-	4	+	113	+	25	H	>4	H	6-12
	25	+	0.77	+	8	+	134	+	25	H	>4	H	6-12
	26	+	0.75	-	0	+	73	+	21	H	>4	H	6-12
	27	+	1.59	-	0	+	164	+	24	H	>4	M	6-12
	28	+	2.51	-	0	+	88	+	19	H	>4	M	3-6
	29	+	0.95	+/-	4	+	>1200	+	24	H	>4	H	6-12
	30	+/-	0.61	-	0	+	116	+	21	H	>4	M	3-6
	31	+	0.8	-	0	+	74	+	21	H	>4	H	6-12
	32	+	1.59	-	0	+	164	+	24	H	>4	M	3-6
	33	+	2.51	-	0	+	88	+	19	H	>4	M	3-6
	34	+	0.93	+/-	4	+	>1200	+	24	H	>4	H	6-12
	35	+	0.82	+	10	+	68	+	21	H	>4	M	3-6
	36	+	1.02	+/-	4	+	24	+	16	H	>4	L	0-3
	37	+	1.3	+	10	+	151	+	21	H	>4	H	6-12
	38	+	1.7	+	6	+	119	+	21	H	>4	H	6-12
Latent stage, after treatment	39	-	N/A	+/-	5	+	128	+	16	L	<4	M	3-6
	40	-	N/A	+/-	4	+	122	+	16	L	<4	M	3-6
	41	-	N/A	+	0	+	102	+	21	L	<4	M	3-6
	42	-	N/A	+/-	4	+	179	+	16	L	<4	L	0-3

Table 1. Comparison of the results of ELFA and Western blot (WB) (Continuation)

	No. of serum	IgM				IgG				Avidity			
		ELFA		WB		ELFA		WB		ELFA		WB	
		Result*	Index	Result*	Σ of points	Result*	IU/ml	Result*	Σ of points	High/ Low	Time post infection (months)	High/ Medium/ Low	Time post infection (months)
Chronic stage	43	-	N/A	-	0	+	96	+	10	H	>4	H	6-12
	44	-	N/A	-	0	+	17	+	10	H	>4	H	6-12
	45	-	N/A	-	0	+	84	+	25	H	>4	H	>12
	46	-	N/A	+/-	4	+	86	+	23	H	>4	H	6-12
	47	-	N/A	-	0	+	97	+	25	H	>4	M	3-6
	48	-	N/A	-	2	+	252	+	25	H	>4	H	6-12
	49	-	N/A	-	0	+	19	+	10	H	>4	H	6-12
	50	-	N/A	-	0	+	61	+	24	H	>4	M	3-6
	51	-	N/A	+/-	4	+	232	+	25	H	>4	H	6-12
	52	-	N/A	+/-	4	+	162	+	25	H	>4	H	6-12
	53	-	N/A	-	1	+	65	+	24	H	>4	H	>12
	54	-	N/A	-	0	+	100	+	23	H	>4	M	3-6
	55	-	N/A	-	0	+	17	+	10	H	>4	H	>12
	56	-	N/A	-	0	+	190	+	25	H	>4	H	>12
	57	-	N/A	-	0	+	13	+	14	H	>4	M	3-6
	58	-	N/A	+	10	+	532	+	24	H	>4	H	6-12
	59	+/-	0.61	-	0	+	116	+	21	H	>4	M	3-6
	60	-	N/A	-	0	+	75	+	24	H	>4	H	6-12
	61	-	N/A	-	0	+	43	+	21	H	>4	M	3-6
	62	-	N/A	+/-	4	+	301	+	24	H	>4	H	6-12
	63	-	N/A	+/-	4	+	169	+	25	H	>4	H	6-12
	64	-	N/A	-	0	+	215	+	24	H	>4	H	>12
	65	-	N/A	+/-	4	+	293	+	25	H	>4	H	6-12
	66	-	N/A	+/-	4	+	30	+	20	H	>4	M	3-6
	67	-	N/A	-	0	+	126	+	24	H	>4	H	>12
	68	-	N/A	-	0	+	87	+	25	H	>4	H	>12
	69	-	N/A	-	0	+	147	+	23	H	>4	H	6-12
	70	-	N/A	-	0	+	189	+	24	H	>4	H	>12
	71	-	N/A	-	0	+	13	+	14	H	>4	M	3-6
	72	-	N/A	+	10	+	532	+	24	H	>4	H	6-12
	73	-	N/A	-	0	+	75	+	24	H	>4	H	6-12
	74	-	N/A	-	0	+	44	+	21	H	>4	M	3-6
	75	-	N/A	+/-	4	+	304	+	25	H	>4	H	6-12
	76	-	N/A	+/-	4	+	169	+	25	H	>4	H	6-12
	77	-	N/A	-	0	+	213	+	24	H	>4	H	>12
	78	-	N/A	+/-	4	+	291	+	25	H	>4	H	6-12
	79	-	N/A	+/-	4	+	32	+	21	H	>4	M	3-6
	80	-	N/A	-	0	+	124	+	25	H	>4	H	>12
	81	-	N/A	-	0	+	85	+	24	H	>4	H	>12
	82	-	N/A	-	0	+	145	+	24	H	>4	H	6-12
	83	-	N/A	-	0	+	50	+	12	H	>4	H	>12
	84	-	N/A	-	0	+	136	+	20	H	>4	H	>12
	85	-	N/A	-	0	+	104	+	25	H	>4	H	>12
	86	-	N/A	-	0	+	94	+	22	H	>4	H	>12
	87	-	N/A	+/-	4	+	18	+	14	H	>4	M	3-6
	88	-	N/A	-	0	+	44	+	18	H	>4	L	0-3
	89	-	N/A	-	0	+	9	+	10	H	>4	M	3-6

* + = positive, +/- = doubtful, (-) = negative, N/A – not applicable, H = High, M = Medium, L = Low

Table 2. Correlation between ELFA IgM vs. WB IgM tests with recognition of doubtful results

Results		WB IgM			Sum
		Positive	Negative	Doubtful	
ELFA IgM	Positive	14	7	7	28
	Negative	3	40	15	58
	Doubtful	1	2	0	3
Sum		18	49	22	89

Table 2a. Correlation between ELFA IgM vs. WB IgM in the positive vs. negative category*

Results		WB IgM		Sum
		Positive	Negative	
ELFA IgM	Positive	22	9	31
	Negative	18	40	58
Sum		40	49	89
Assessment of correlation: $\chi^2 = 11.46$, p=0.0007, correlation highly significant				

*Doubtful reactions classified as positive
Tests assessed by chi-square (χ^2) test with Yates correction

Table 3. Correlation between ELFA IgG versus WB IgG with recognition of doubtful results

Results		WB IgG			Sum
		Positive	Negative	Doubtful	
ELFA IgG	Positive	81	0	0	81
	Negative	0	6	2	8
	Doubtful	0	0	0	0
Sum		81	6	2	89

Table 3a. Correlation between ELFA IgG vs. WB IgG in the positive vs. negative category*

Results		WB IgG		Sum
		Positive	Negative	
ELFA IgG	Positive	81	0	81
	Negative	2	6	8
Sum		83	6	89
Assessment of the correlation: $\chi^2 = 53.76$, p=0.0000, correlation highly significant				

*Doubtful reactions classified as positive
Tests assessed by chi-square (χ^2) test with Yates correction.

Table 4. Correlation between ELFA AVI versus WB AVI tests

Results		WB Avidity IgG			Total
		H (>6 m)	M (3–6 m)	L (<3m)	
ELFA Avidity IgG	H (≥4 m)	43	18	2	63
	L(<4 m)	1	4	13	18
Total		44	22	15	81

Table 4a. Correlation between ELFA AVI versus WB AVI tests when reactions MEDIUM and LOW in WB AVI were classified as LOW

		WB AVI		Sum
		H (>6 m)	M+L (<6 m)	
ELFA AVI	H (≥4 m)	43	20	63
	L (<4 m)	1	17	18
Sum		44	37	81
Assessment of the correlation: $\chi^2 = 19.73$, p=0.0000, correlation highly significant				

Tests assessed by chi-square (χ^2) test with Yates correction

The proportion of high-avidity results obtained in ELFA AVI and WB AVI in the group of patients without specific IgM in ELFA IgM was 92% and 68%, respectively. The proportion for sera with positive IgM results in ELFA and low avidity in ELFA and WB was 46.4% vs. 42.9%, respectively.

In the group of patients without specific IgM in WB IgM the proportion of high-avidity in ELFA and WB was 98% and 61%, respectively. The proportion for sera with positive IgM results in WB and low avidity in ELFA and WB was – 61.1% vs. 50%.

A highly significant correlation (p<0.001) was found between the ELFA and Western blot tests for avidity of the IgG anti-*Toxoplasma* antibodies when reactions MEDIUM and LOW in WB AVI were classified as LOW, both with the use of chi-square and Spearman tests (Tabs. 4a, 5). The significance level appeared to be greater when the test results were assessed by Spearman test in the units or points scale, compared to chi-square test assessed in the category 'positive versus negative' for LOW or HIGH avidity. The results indicate a high reliability of the assessed serological tests, the more so as they are produced by different companies.

Table 5 presents the results of the statistical assessment of the correlations between all the serologic tests applied in this study, performed by the Spearman's rank test with recognition of ELFA or WB potency expressed by number of units or points. The correlations between the ELFA and WB tests proved highly significant, both for IgM and IgG classes of antibody (p<0.00001). The ELFA test for anti-*Toxoplasma* IgM antibodies was not correlated with the ELFA and Western blot tests for detection of anti-*Toxoplasma* antibodies of IgG class. The Western blot test for IgM anti-*Toxoplasma* antibodies had different properties which did not show a significant correlation with the Western blot test for IgG anti-*Toxoplasma* antibodies, but showed a significant correlation with ELFA test for detection of anti-*Toxoplasma* antibodies of IgG class (p<0.01). This result may suggest the possibility of parallel occurrence of anti-*Toxoplasma* antibodies of different classes detected by different tests (Tab. 5).

Table 5. Correlation between serologic tests assessed by Spearman test

Correlation tested	Correlation coefficient (r)	Probability (p)	Evaluation of the correlation
ELFA_IgM versus WB_IgM	0.479	0.000002	Highly significant
ELFA_IgG versus WB_IgG	0.678	0.000000	Highly significant
ELFA_AVI versus WB_AVI*	0.523	0.000001	Highly significant
ELFA_IgM versus ELFA_IgG	0.119	0.264	Not significant
ELFA_IgM versus WB_IgG	– 0.120	0.259	Not significant
ELFA_IgM versus ELFA_AVI	– 0.437	0.000044	Highly significant
ELFA_IgM versus WB_AVI	– 0.352	0.00126	Significant
WB_IgM versus ELFA_IgG	0.315	0.002593	Significant
WB_IgM versus WB IgG	0.058	0.587	Not significant
WB_IgM versus ELFA_AVI	– 0.492	0.000003	Highly significant
WB_IgM versus WB_AVI	– 0.122	0.2754	Not significant
ELFA_IgG versus ELFA_AVI	0.039	0.727	Not significant
ELFA_IgG versus WB_AVI	0.421	0.000089	Highly significant
WB_IgG versus ELFA_AVI	0.417	0.000104	Highly significant
WB_IgG versus WB-AVI	0.506	0.000001	Highly significant

*AVI = IgG avidity

Interesting dependencies were revealed by comparison of the ELFA and Western blot tests for detection of anti-*Toxoplasma* antibodies of IgM class with the ELFA and Western blot tests for avidity of IgG anti-*Toxoplasma* antibodies. It showed a highly significant negative correlation between these 2 pairs of tests ($p<0.01$), except for a relationship between the Western blot test for IgM antibodies and Western blot test for avidity, which was not significant. The observed negative correlations confirm the theoretically expected dependence that strong complexes with the participation of IgG antibodies are formed in the late phase of toxoplasmosis, when early antibodies of IgM class are totally absent.

In contrast, highly significant positive correlations were found between the ELFA and Western blot tests for detection of anti-*Toxoplasma* antibodies of IgG class with the ELFA and Western blot tests for avidity of IgG anti-*Toxoplasma* antibodies ($p<0.001$), except for a relationship between the ELFA test for IgG antibodies and ELFA test for avidity, which was not significant. These correlations, also theoretically expected, could be explained by the fact that the strong IgG-dependent reactions, as expressed by high numbers of units or points, may result in forming strong complexes, evidenced by the high grade of avidity.

DISCUSSION

Because congenital toxoplasmosis is usually the result of primary infection acquired during pregnancy, it is important to determine whether such an infection during pregnancy has actually occurred. The serologic examinations help to establish the immunological status and to determine the phase of *T. gondii* infection, which has an important meaning in therapy. Specific antibodies of IgM class are detected initially and, in most cases, IgM disappears within a few months. However, in some cases, specific IgM antibodies can still be observed during the chronic phase of infection – one year or longer. Thus, a positive IgM test result in a single serum sample can indicate not only a recently acquired infection, but also an infection acquired in the distant past, or can be considered as a false-positive result. The persistence of IgM antibodies (approximately one year and more) does not appear to have any clinical relevance, and these patients should be considered chronically infected. The interpretation of a positive IgM test result can be complicated by using several methods for its detection. Despite the wide distribution of commercial test kits to measure IgM antibodies, these tests often have low specificity, and the reported results are frequently misinterpreted [7]. Thus, it is recommended that a positive IgM test result should always be confirmed by testing in a reference laboratory.

IgG antibodies usually appear within 1–2 weeks post-infection and usually persist for life. The dye (Sabin-Feldman) test is regarded as the reference method for serodiagnosis of human toxoplasmosis; however, its application is limited by high cost and the need to use live tachyzoites, which creates the risk of laboratory infection. The most commonly used tests for the measurement of IgG antibody are: ELISA, IFA or Modified Agglutination Test (MAT). Recently, a number of tests for estimation of the avidity of *Toxoplasma* IgG antibodies have been introduced to help discriminate between recently acquired and distant infection. The functional affinity of IgG antibodies is low after primary contact with *Toxoplasma* antigen and increases during subsequent phases of infection. Protein-denaturing reagents, including urea, are used to dissociate the antibody-antigen complex. The avidity result is determined using the ratios of antibody titration curves of urea-treated and untreated samples. Diagnostic parameters, such as IgG avidity, are assessed in order to identify the time of infection [8, 9]. The use of recombinant antigens for the avidity test could improve determining of the phase of infection [10]. The antigens ROP1, GRA7, GRA8 are recognized by specific IgG in the early phase of infection, whereas other antigens, i.e., SAG1 and MAG1, are recognize later by mature IgG [11]. The avidity test can provide a rapid identification of the latent *Toxoplasma* infection in pregnant women who have both IgG and IgM anti-*Toxoplasma* antibodies on initial testing during pregnancy. However, there are some limitations in the use of this method. Avidity assays are not conclusive in some immunocompromised patients and those treated for toxoplasmosis.

The presented study compared 2 different assays to detect *T. gondii*-specific IgG, IgM and IgG avidity. The results showed similar percentages of positive results for detection of IgG in ELFA and WB assays (81/89 (91%); however, for a few sera the results in both techniques were discrepant.

IgG avidity was measured in 81 samples and was low in 46% (ELFA) and 43% (WB) of ELFA IgM-positive samples, and in 61% (ELFA) and 50% (WB) of WB IgM-positive samples. These results indicate a slightly more usefulness of ELFA IgM in recognizing a recent phase of *Toxoplasma* infection.

Comparison of the results for IgM negative and high-avidity IgG sera can indicate the greater usefulness of the WB AVI test than ELFA AVI test in the recognition of latent *Toxoplasma* infection (mean reactivity for high avidity: 58.9% versus 87.9%, respectively). The number of IgM positive sera with low IgG avidity was more frequently detected in WB than in ELFA (mean percentages: 53.5% versus 46.5), which may indirectly suggest the greater usefulness of WB AVI test in the recognition also of the recent phase of *Toxoplasma* infection. The use of WB-IgG avidity testing was therefore found to be a highly valuable diagnostic tool for determining the time of post-infection.

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