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IMMUNOPEROXIDASE TEST IN THE DIAGNOSIS OF TOXOPLASMOSIS

Ewa Cisak

Department of Zoonoses, Institute of Agricultural Medicine, Lublin, Poland

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Abstract: The immunoperoxidase test (immunoenzymatic test with whole tachyzoites of *Toxoplasma gondii*) was compared with tests used in the routine diagnosis of toxoplasmosis. This showed a good qualitative and quantitative correlation with the reference tests (immunofluorescence test, direct agglutination test and ELISA). High value of the correlation coefficient for immunofluorescence test (IFT) and immunoperoxidase test (IPT) suggests that IPT can substitute IFT in laboratory diagnostics of toxoplasmosis. The application of immunoperoxidase test for IgM in the case of family toxoplasmosis in the rural area confirmed the diagnosis of acute toxoplasmosis in a 7-year-old child.

Address for correspondence: Ewa Cisak, PhD, Department of Zoonoses, Institute of Agricultural Medicine, P.O. Box 185, 20-950 Lublin, Poland.
E-mail: ewac@galen.imw.lublin.pl

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INTRODUCTION

The infection of toxoplasmosis is caused by the protozoan *Toxoplasma gondii*. It is common worldwide among warm-blooded animals and can be transmitted to humans causing zoonosis. One has to distinguish between the frequent, symptomless (latent) *Toxoplasma* infection and relatively rare symptomatic (acute) disease, the toxoplasmosis [1, 4, 5, 7, 8, 9, 10].

The infection is of particular importance to women who contract it for the first time during pregnancy when an intrauterine transmission of the parasite may occur. This can result in abortion or stillbirth, or lead to serious damage in neonatanes or older children. Latent infection can remain for life and provide immunity. However, if the latter is suppressed (e.g. by immunosuppressive drugs, HIV infection) it may result in a reactivated toxoplasmosis which usually takes a clinical course [1, 3, 4, 5, 11, 13, 15].

There are reports claiming the rates of infection to be higher among the rural population than among town dwellers. Umiński [18] has shown higher titers and more frequent positive reactions with *Toxoplasma* antigen in relation to the control group in people occupationally exposed to *Toxoplasma gondii* antigen, especially in employees in agriculture, meat and poultry processing plants [18].

Clinical symptoms of toxoplasmosis are not specific and therefore laboratory diagnostics plays an important role in the diagnosis of toxoplasmosis. The disadvantages of the custom tests applied in the routine diagnosis of toxoplasmosis (such as animal inoculation, danger of infection, need for additional equipment), led to replacement by others (of high sensitivity and specificity) that only require manufactured reagents.

Only a few reports were found concerning the immunoperoxidase test (IPT) as an immunoenzymatic test with whole tachyzoites of *T. gondii* in the diagnosis of toxoplasmosis [2, 12, 15, 16, 17, 19]. The aim of this study, therefore, was to compare IPT with other serologic tests commonly used in the routine diagnosis of toxoplasmosis: immunofluorescence test (IFT), direct agglutination test (DA), immunoenzymatic test (ELISA).

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Figure 1. High positive result (+++), × 400.

Figure 2. Positive result (++), × 400.

MATERIAL AND METHODS

Patients' sera. 270 human sera (from people suspected of *Toxoplasma* infection) were tested in IPT, IFT and DA, 86 in IPT and ELISA (Organon Teknika, Holland) and 64 in IPT for IgM, Immunosorbent Agglutination Assay for IgM (ISAGA IgM, bioMérieux, France) and ELISA for IgM.

Control sera. Positive control serum for IFT and DA (titer 2000) was obtained from the Pasteur Institute in Paris, while control sera for ELISA and ELISA IgM from Organon Teknika, Holland, and for ISAGA IgM from bioMérieux, France.

Antigens. Antigens for IPT, IFT and DA (consisting of whole tachyzoites of *Toxoplasma gondii*) were prepared in the Zoonosis Department of the Institute of Agricultural Medicine (Lublin, Poland) from 3 day peritoneal exudate of mice (Swiss) inoculated with RH strain of *T. gondii*. The formalinized exudate was centrifugated at 3,000 rpm for 15 min, and tachyzoites were suspended in PBS (pH 7.6), dripped on glass slides and stored at -20◦C. Antigens for ELISA and ELISA IgM were obtained from Organon Teknika, Holland and for ISAGA IgM from bioMérieux, France.

Conjugates. Conjugate for IPT (rabbit anti-human IgG labelled with peroxidase) was prepared according to Nakane-Kawoi [14]. In the case of IPT test for IgM, rabbit anti-human IgM labelled with peroxidase (Heintel, Hungary) was used. Conjugates for ELISA and ELISA IgM came from Organon Teknika, Holland, and for ISAGA IgM from bioMérieux, France.

Substrate. Tetrachlordiaminbenzidine (TMB, POCh Gliwice, Poland) was used in IPT. Substrates for ELISA

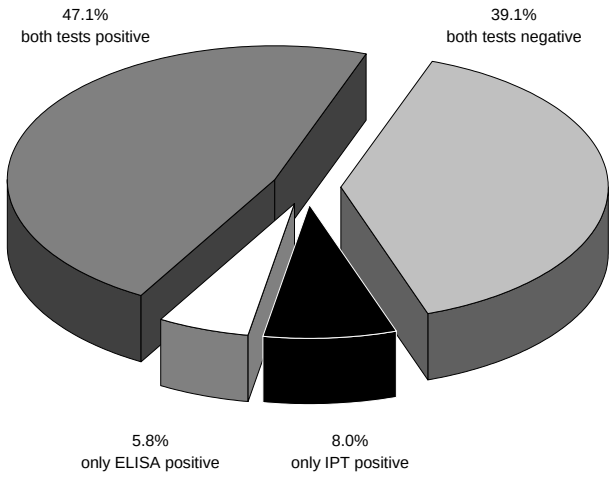

Figure 5. Comparison of qualitative results for IPT IgM and ELISA IgM (N = 87). Compatibility of qualitative results = 86.2%. Sensitivity IPT to ELISA = 85.4%. Specificity IPT to ELISA = 87.2%.

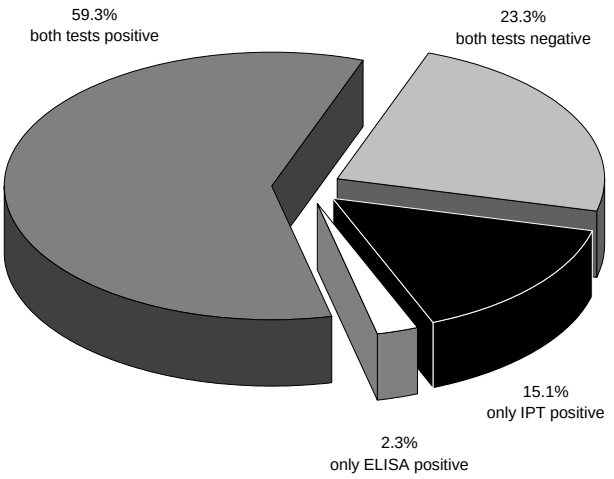

Figure 6. Comparison of qualitative results for IPT IgM and ELISA (N = 86). Compatibility of qualitative results = 82.6%. Sensitivity IPT to ELISA = 79.7%. Specificity IPT to ELISA = 90.9%.

Figure 3. Positive reaction (+), × 400.

Figure 4. Negative result (-), × 400.

and ELISA IgM came from Organon Teknika, Holland and for ISAGA IgM from bioMérieux, France.

Technical details concerning immunoperoxidase test. Glass slides with fixed *T. gondii* tachyzoites were rinsed with distilled water and dried at room temperature. The diluted samples of human sera (including control serum) were then added. The slides with the antigen and sera were incubated at 37◦C for 30 min. After incubation, the slides were washed with PBS at pH 7.6 (3 times for 5 min each), and dried at room temperature, after which the conjugate was added. After the second incubation and washing (as above), substrate with 0.1% hydrogen peroxide was dripped onto the dried slides. The results of histochemical reaction (intensivity of stain) were checked after 15 min of the incubation in ambient temperature under a light microscope at the magnification 400 ×.

Statistical analysis. In comparing the tests, the compatibility of qualitative results, the sensitivity and specificity of IPT to IFT, DA and ELISA were determined.

Compatibility of qualitative results was determined as the percentage of compatible positive and negative results in IPT and a reference test.

Sensitivity was determined as a ratio of compatibile positive results to the number of positive results in the reference test and specificity - as a ratio of compatible negative results to the number of negative results in the reference test.

The coefficient of quantitative correlation for IPT and the reference tests has also been calculated.

RESULTS AND DISCUSSION

The differences between tachyzoites protoplasma's membrane stain were taken into account by the evaluation

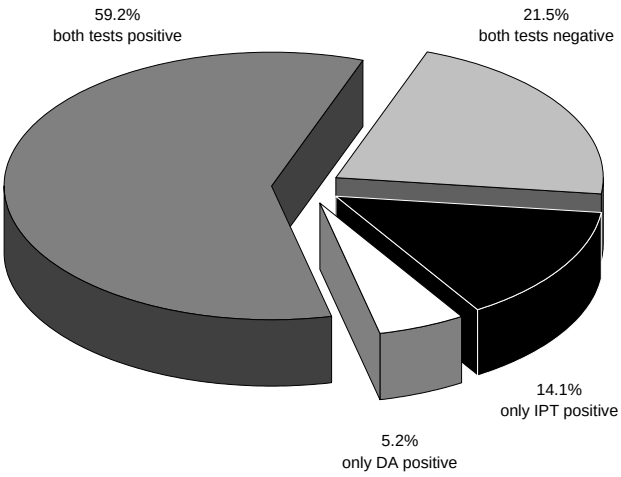

Figure 7. Comparison of qualitative results for IPT and DA (N = 270). Compatibility of qualitative results = 80.7%. Sensitivity IPT to DA = 92.0%. Specificity IPT to DA = 60.4%.

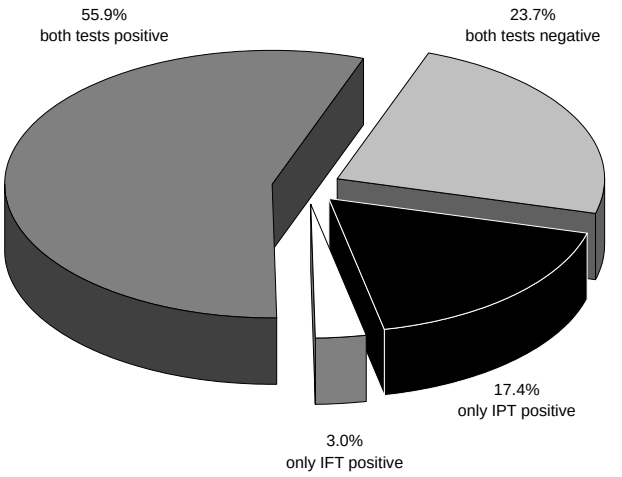

Figure 8. Comparison of qualitative results for IPT and IFT (N = 270). Compatibility of qualitative results = 79.6%. Sensitivity IPT to IFT = 95.0%. Specificity IPT to IFT = 57.7%.

of results of histochemical reaction (Fig. 1-4). By low dilution of sera of high titer, a thick, dark brown tachyzoite's membrane and brown-yellow protoplasma were observed (Fig. 1).

The intensivity of membrane and protoplasma stain decreased with the dilution of sera. A weaker reaction was also observed with sera of low titer (Fig. 2, 3). In the case of negative sera, tachyzoites resembled uniform shapes with barely visible protoplasma (Fig. 4).

A high compatibility was found between the IPT results and the results of other tests (Fig. 5-8). The greatest compatibility of qualitative results was found between IPT IgM and ELISA IgM (86.2% - Fig. 5), next between IPT and ELISA (82.6% - Fig. 6), IPT and DA (80.7% - Fig. 7), and between IPT and IFT (79.6% - Fig. 8). The conformity of qualitative results of or above 80% is defined as significant according to Zemburowa and Roczniak [20]. Comparing the results of IPT and IFT (Fig. 8) with the results of tests obtained by Rosmus *et al.* [15] a high compatibility of the correlation coefficient was found (0.94 in own research and 0.97 by the cited authors).

Thomasi *et al.* [17], by comparison of IPT with IFT, has evaluated the correlation coefficient as 0.93 and for IPT and ELISA as 0.83. Verhofstende *et al.* [19] have tested 31 sera of patients with latent and 12 with acute toxoplasmosis and calculated the coefficients of correlation for IPT anf IFT. In the first case, the coefficient amounted to 0.97, in the second to 0.88. The number of tested sera in own study was twice as high as in the studies of the cited authors and this fact could be a reason for the small divergences in the values of the coefficients of correlation.

Among the few authors applying immunoperoxidase test in the diagnosis of toxoplasmosis, only Gracheva *et al.* [6] have evaluated the compatibility of qualitative results for IPT and IFT, for as high as 80%. In the own research this compatibility was nearly the same and amounted to 79.6%.

Table 1. Quantitative correlation between IPT and other tests.

Compared test	Correlation coefficient (r)	Significance of correlation (p)
IPT - IFT	0.94	$p < 0.001$
IPT - ELISA	0.70	$p < 0.001$
IPT - DA	0.62	$p < 0.001$
IPT IgM - ELISA IgM	0.53	$p < 0.001$

The high values of the coefficients of quantitative correlation for IPT, IFT and ELISA (Tab. 1) in own studies and the significant compatibility of qualitative results for IPT and IFT (79.6%) may suggest that immunofluorescence test and ELISA can be substituted in future by simpler and cheaper immunoperoxidase test. It is noteworthy that in all cases the number of the IPT-positive results was greater by 2.3-14.4% compared to other tests (Fig. 5-8), which suggests a higher sensitivity for this assay.

The IPT was applied in serological studies of a family case of nodal toxoplasmosis in a child from a rural area. The distinct correlation between clinical symptoms and serological reactions before and after treatment was observed (Tab. 2).

The IPT is easy to carry out, it does not require any special technical equipment and moreover, all reagents are easy to obtain. Additionally, the IPT is a safe test which does not require the application of a living strain of *T. gondii* as an antigen.

Table 2. Serologic tests in the case of family toxoplasmosis.

Family members	Age (years)	Date of examination (dd.mm.yy)	Results of serologic examinations (titer)			
			IFT	ELISA IgM	IPT IgM	DA
M.W. Child patient	7	09.07.87	2,000	3,200	NE	1,000
		19.08.87*	2,000	200	128	8,000
		28.12.87**	256	(-)	NE	NE
W.W. Mother	37	18.08.87	512	(-)	(-)	1,000
W.W. Father	39	20.08.87	128	100	256	256
P.W. Brother	16	18.08.87	128	(-)	(-)	256
T.S. Cousin	5	18.08.87	(-)	(-)	(-)	(-)
G.S. Cousin	25	18.08.87	128	(-)	(-)	(-)
M.S. Cousin	10	18.08.87	(-)	(-)	(-)	(-)

NE - not examined, * after the first treatment, ** after the second treatment

Taking into account all these features, one can use the IPT in local, smaller laboratories dealing with diagnostics of toxoplasmosis.

CONCLUSION

Immunoperoxidase test (immunoenzymatic test with whole tachyzoites of *Toxoplasma gondii*) has shown a good compatibility of qualitative results with the reference tests applied in the routine diagnosis of toxoplasmosis.

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