EVALUATION OF MODIFIED AGGLUTINATION TEST AND ENZYME-LINKED IMMUNOSORBENT ASSAY IN DETECTION OF *Toxoplasma gondii* ANTIBODY IN HUMAN SERA

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ABSTRACT: Toxoplasmosis is a zoonosis of high prevalence around the world, leading to serious congenital alterations and in immune compromised individuals. Its diagnosis is based in the detection of serum antibodies. This work aimed to compare serum antibody to *Toxoplasma gondii* detection by ELISA for IgG antibodies, and the MAT with the results of IFAT. From 73 samples examined by IgG-IFAT, 53 (72.6%) were positive, IgG-ELISA 55 (75.4%) and MAT, 58 (79.4%). No significative differences among results of tests are found. MAT shows high sensibility (100%) and lower specificity (75%), there were not false-negative results, but showed false-positives (25%). ELISA showed sensibility of 98.1% and specificity of 85,0%, false-negative rate of 1.9% and false-positive rate of 15.0%. MAT performance, when compared with IFAT, suggests the utilization of this method in diagnostic and epidemiological preliminary tests, besides ELISA has been used as a confirmatory test, for its specificity.
KEYWORDS: Toxoplasmosis. Antibodies. Human. Immune fluorescent. ELISA. Agglutination.

AVALIAÇÃO DO MÉTODO DE AGLUTINAÇÃO DIRETA E DO ENSAIO IMUNOENZIMÁTICO NA DETECÇÃO DE ANTICORPOS PARA *Toxoplasma gondii* EM SOROS HUMANOS

RESUMO: A toxoplasmose é uma zoonose de elevada prevalência em todo o mundo, podendo causar sérias alterações congênitas e em pacientes imunodeprimidos. Seu diagnóstico, na maioria das vezes, baseia-se na detecção de anticorpos séricos. Neste trabalho propôs-se comparar a detecção de anticorpos séricos para *Toxoplasma* pelo ELISA para IgG, e o MAD com os resultados da RIFI, em soros de indivíduos provenientes de um assentamento localizado em Eldorado, MS. Das 73 amostras examinadas pela RIFI-IgG, 53 (72,6%) amostras foram positivas, para o ELISA-IgG 55 (75,4%) e o MAD, 58 (79,4%). Não houve diferença significativa entre os resultados dos testes. O MAD apresentou alta sensibilidade (100%) e menor especificidade (75%), 25% de falso-positivos e sem resultados falso-negativos. Já o ELISA apresentou sensibilidade de 98,1% e especificidade de 85,0%, com taxa de falsos-negativos de 1,9% e de falso-positivos de 15,0%. A performance do MAD, quando comparado à RIFI, sugere a utilização deste método em provas de triagem diagnóstica e epidemiológica, enquanto que o ELISA seria utilizado como prova confirmatória, devido a sua especificidade.
PALAVRAS-CHAVE: Toxoplasmose. Anticorpos. Humanos. Imuno fluorescência. ELISA. Aglutinação.

Introduction

Toxoplasmosis is a highly widespread zoonotic disease caused by intracellular protozoan *Toxoplasma gondii*, that affects homeothermic animals, including man (ACHA, SZYFRES, 2003). The infection is very frequent in various animal species. Cats and other Felidae are the definitive hosts, and man, other mammals and birds, intermediate hosts (VERONESI, 2005). Both in man and in animals, *T. gondii* infection can lead to important pathological frames, notably the eye disease, congenital changes and also acute neurologic infection in immunosuppressed patients (DUBEY, BEATIE, 1988; FREYRE, 1989). *T. gondii* has high prevalence, reaching from 60 to 90 of the population in certain countries, and the majority of infected consists of asymptomatic carriers (DUBEY, BEATTIE, 1988).

Even asymptomatic, toxoplasmosis in immunocompetent patients induces a good cellular and humoral immune response, not destroying the parasite but preventing it to cause disease (SUBAUSTE, 2009). The IgM antibodies can be measured from one to two weeks after the start of the infection, reaching a peak in six to eight weeks, when then decline. Titers may persist for more than 12 months. The IgG antibody persists for life in most patients (CANTOS et al, 2000). Thus the determination of antibody titers and class involved in the response are important to identify the stage of infection of individual as well as the need for treatment in specific groups, such as immunocompromised patients and pregnant women (VERONESI, 2005).

Serologic tests help to determine the occurrence of host infection by detecting specific antibodies for *T. gondii*. Since the introduction of the classic test of Sabin-Feldman

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(SF), in 1948, several tests have been suggested to diagnose the infection. Among them we can highlight the immunofluorescent antibody test (IFAT), and the enzyme-linked immunosorbent assay (ELISA), tests with precision comparable to SF, considered the gold-standard test for detecting antibodies against *T. gondii* in man (UCHOA et al, 1999). Both tests allow the detection of IgM and IgG. However, currently, many works have used the modified agglutination test (MAT) mainly in epidemiological research, for its speed, high sensitivity and for being easy (DESMONTS, REMINGTON, 1980).

This work aimed to compare the serum antibodies to *T. gondii* by ELISA and MAT with the results of IFAT in human sera samples from a settlement in the municipality of Eldorado, MS.

Materials and methods

Sample design

Human blood samples were obtained from individuals from properties located in a rural community in the municipality of Eldorado, Mato Grosso do Sul State. This community has 185 properties, a total of 671 inhabitants, with an average of 3.67 per property (median 4), minimum of one and maximum of nine inhabitants, according to the census carried out by the *Instituto de Desenvolvimento Agrário, Assistência Técnica e Extensão Rural* from State of Mato Grosso do Sul (MARQUES et al, 2008). In a study carried out previously (MARQUES, 2008), 20 properties were drawn and all individuals of each property, regardless of gender and age, including-if so children and young people under the age of 18 years, provided with the proper authorization of the responsible, had blood collected for the detection of antibodies to *T. gondii*.

Blood samples were obtained by venipuncture, being placed in test tubes with rubber cover, and identified by a protocol number. After collecting all the samples, they were transported in refrigerated container to the Laboratory of Preventive Veterinary Medicine and Public Health at Universidade do Paraná - UNIPAR Campus, where they were centrifuged at 1650 g for 15 minutes to promote the sera separation, and aliquots of 1 mL of serum were stored in plastic microtubes. The samples were then stored at -20° C until the processing of serologic tests.

Tests for the detection of antibodies

Human serum samples were subjected to the detection of antibodies to *T. gondii* by IFAT for IgG class antibodies (CAMARGO, 1974), by ELISA IgG according to the manufacturer's protocol (Radim ®, Pomezia, Rome) and MAT, with antigens fixed by formalin (DESMONTS, REMINGTON, 1980). To IFAT and MAT all serums have been tested to the initial dilution and 1:25 tested positive in successive dilutions until the extinction of the titer. The titer of the IgG ELISA was given in single dilution of serum (1: 300), in international units (U), obtained by optical density for the sample, interpolated on the results of the curve obtained with standard sera.

Analysis of the results

The frequencies of positivity in each method of detecting antibodies was tabulated and compared with other methods. From 2 x 2 contingency tables obtained from the results of the ELISA and MAT, faced as the results of the test IFAT, regarded as gold-standard, were calculated the sensitivity, specificity and other estimates of the performance of methods for detection of antibodies, using a spreadsheet developed for this purpose (MACKINNON, 2000). The antibody titers were compared between the test methods of nonparametric Spearman correlation test using the BioEstat 5.0 software (AIRES et al, 2007).

All stages of this work were submitted, evaluated and approved by the Ethics Committee on Experimentation involving Human Subjects at the UNIPAR under the Protocol 12535/2008.

Results and discussion

Of the 73, 53 (72.6%), 55 (75.4%) and 58 (79.4%), analysed samples were positive by IgG-IFAT, IgG-ELISA and the MAT, respectively, with no significant difference between the results of the three tests ($p<0.05$).

Performance estimates of the MAT and ELISA against IFAT are show in Table 1. MAT had a high sensitivity (100) and lesser specificity (75), without false-negative results, however with false-positives (25.0). ELISA already presented 98.1 sensitivity and specificity of 85.0, 1.9% of false-negatives and 15.0% of false-positives.

Table 1. Estimate ± standard error (EST±EP) and 95% confidence interval (95% CI) of evaluation statistics for modified agglutination teste (MAT) and for IgG enzyme-linked immunosorbent assay (ELISA) for *Toxoplasma gondii* antibody detection compared with antibody immunofluorescent antibody test (IFAT) in human sera samples. Umuarama, 2012.

	MAT		ELISA-IgG	
Statistics	EST±EP	95%-IC	EST±EP	95%-IC
Sensibility	100.0 ± 0.0	93.3 – N.D.	98.1 ± 1.9	89.9 – 99.9
Specificity	75.0 ± 9.7	50.9 – 91.3	85.0 ± 8.0	62.1 – 96.8
Eficiency*	93.2 ± 3.0	84.7 – 97.7	94.5 ± 2.7	86.6 – 98.5
Positive predictive value	91.4 ± 3.7	81.0 – 97.1	94.5 ± 3.1	84.8 – 98.9
Negative predictive value	100.0 ± 0.0	78.2 – N.D.	94.4 ± 5.4	72.7 – 99.9
False--positive	25.0 ± 9.7	8.7 – 49.1	15.0 ± 8.0	3.2 – 37.9
False negative	0.0 ± 0.0	N.D. – 6.7	1.9 ± 1.9	0.0 – 10.1
Kappa	81.3 ± 7.9	65.8 – 96.8	85.8 ± 6.9	72.3 – 99.3
Positive concordance	95.5 ± 2.0	91.6 – 99.4	96.3 ± 1.8	92.7 – 99.9

Negative concordance	85.7 ± 6.3	73.3 – 98.1	89.5 ± 5.2	79.2 – 99.7

*correct classification index; N.D.=not determined.

Camargo et al (1977) showed that was good agreement between ELISA and IFAT, testing these methods in the detection of antibodies to *T. gondii* in sera from patients with acute or chronic infections. Uchoa et al (1999) standardized and compared the ELISA technique with immunofluorescence, ELISA-IgG presented 96.7 sensitivity and specificity of 75 and IFAT, 83.8 and 79.1 respectively, with 88.3 concordance. The author suggests that this difference between ELISA and IFAT can be related to different antigens and the quality of the conjugate used in each test. These authors found some sera with levels of antibodies to *T. gondii* already known, what allowed to evaluate the sensitivity and specificity of the two methods. In this work, the results of performance of ELISA and MAT have been evaluated against results of IFAT, considered similar to SF, used as a definitive diagnosis of human toxoplasmosis (DUBEY, BEATIE, 1988). Thus, these results are different from those reported by literature, and part of this difference is by way of analysis.

Cavalcante et al (2006) compare the results of IFAT and MAT in serum antibodies to *T. gondii* in 266 individuals from rural properties in the State of Rondônia. The prevalence was 73.3 by MAT and IFAT, with 100 of concordance between the two tests and SF.

The antibodies of the IgM class was positive in a sample (1.4%) in IgM-IFAT, the result being corroborated in ELISA test. Uchoa et al. (1999) in his work shows that was more IgG positive samples can be found than IgM positive samples. It is likely that the population studied has a history of continuous exposure to infection by *T. gondii*, hypothesis supported by the results of Marques (2008), that found high rates of infection in dogs, cats and horses living with these individuals, and Galli (2008), that to detect chickens infected by the parasite in properties of this community, showed the contamination of soil by the parasite, and therefore the source of infection for animals and man.

From 53 samples positives to IgG-IFAT, the titers ranged from 25 to 1600 (Figure 1); 79.5 were positive to the MAT, with titers ranging from 50 to 3200 (Figure 1); and 76.7 were positive to ELISA, being 17.8% with titers between 16 and 59 U, 27.4% between 60 and 194 U, and 31.5% with titers above 195U (Figure 2). There was a significant correlation between the tests (P<0.0001) being the largest correlation coefficient between the IFAT and MAT (r=0.82) than among the IFAT and ELISA (r=0.73), correlations higher than those reported by Uchoa et al (1999).

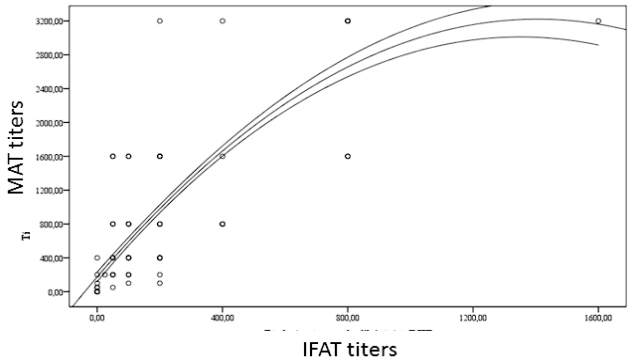

Figure 1: Correlation of titers in immunofluorescent antibody test (IFAT) and modified agglutination test (MAT) in human sera. Umuarama, 2012.

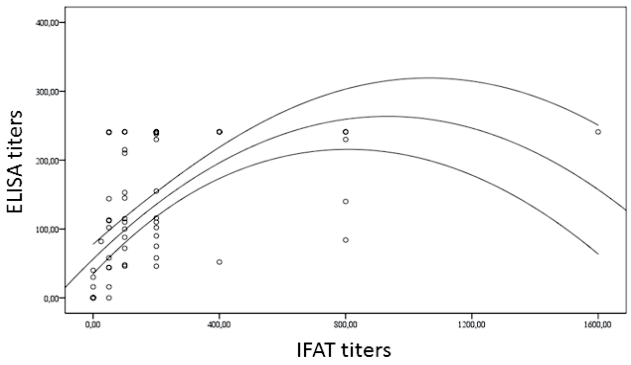

Figure 2: Correlation of titers in immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) in human sera. Umuarama, 2012.

Despite the gathering of significant correlation between the titers to IgG-IFAT and the MAT, there was a significant difference (P<0.0001) among the titers determined by two methods. The use of a specific IgG conjugate, conditions that the reaction of the indirect immunofluorescence antibody detects only this class. Already in MAT, the use of 2-mercaptoethanol prevents the IgM and dimeric IgA promote agglutination of the antigen. On the other hand, there are studies that investigate possible loose connections due to the presence of IgE antibody that may be high in infection with *T.* gondii (KAHI et al, 1999). This is a hypothesis that could explain the difference in titers by two methods, which need to be investigated.

The distribution of titers to IFAT was similar to that found by Amendoeira et al (2003), where the majority of IFAT-positive individuals had titers below 256.

Cavalcante et al (2006), researching 266 individuals by MAT, found positive 73.3%, similar result obtained in this work, including antibody titers above 400 in 66.7% of samples evaluated. These authors showed still high concordance with the MAT and IFAT that corroborates the results obtained in this study. The authors have no comparison data between the titles of IFAT and MAT.

Fulton and Turk in 1959 were the first to describe an agglutination test, which failed success due to low specificity and the need for a large number of tachyzoites in each test. Later Couzineau and Baufine-Ducrocq in 1970, and Desmonts and Remington in 1980, substantially improved the reproducibility and sensitivity of this method (DA SILVA et al, 2002). The agglutination test is a test of practical, feasible

to test a small number of samples, fast and has the ease of reading without the need of special instruments as in the case of immunofluorescence and ELISA. Also has the advantage of the possibility of carrying out the test for any animal species, since it does not require specific reagents, and even the possibility of test samples recently hemolisated, unlike in immunofluorescence (VERONESI, 2005). However reading the results is more time consuming, since the test plate requires 12 to 24 hours of incubation and antigens are difficult to availability. The agglutination test presents titles slightly higher, when compared with the immunofluorescence.

The obtained results allow you to indicate the MAD as an alternative test, or even complementary one, for the diagnosis of human toxoplasmosis. Once the antigen production limitations for this method are outdated, it may become a widely used screening method given the ease of implementation and by the fact of not using equipment for its execution and reading, as in the cases of immunofluorescence and ELISA (DA SILVA et al, 2002).

Conclusion

The performance of MAT, when compared to IFAT, suggested using this method in epidemiological and diagnostic screening tests, while ELISA would be used as a confirmatory one, due to its specificity.

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