

Utilization of modified agglutination test and indirect immunofluorescent antibody test for the detection of *Toxoplasma gondii* antibodies in naturally exposed horses

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Abstract

Infection by *Toxoplasma gondii* in equines is usually not apparent, it being characterized by presence of antibody titers and tissue cysts. This study was aimed at verifying the presence of anti-*Toxoplasma* antibodies in equine serum by modified agglutination test (MAT) and reaction to indirect immunofluorescent antibody test (IFAT). 1984 samples of serum were examined, by MAT, using whole formalin fixed tachyzoites of *T. gondii* as antigen. The samples reacting in the MAT test, and 150 other negative samples, chosen at random, were also tested by IFAT, utilizing anti-equine IgG. The association among the test results was verified by the McNemar test. 138 samples were positive in the MAT test, with 60 (46.38%) presenting reaction at a dilution of 1:64; 52 (37.7%) at 1:256; 19 (13.8%) at 1:1024; five (3.6%) at 1:4096; and two (1.45%) at 1:16384. Of 132 positive MAT samples, 14 were negative in the IFAT test, but the statistical analysis indicated general agreement in results of the tests. The results obtained showed agreement among the tests utilized, and the possibility of participation of equines in the transmission of toxoplasmosis to carnivorous animals, and also to humans.

Key-words:

Toxoplasma gondii.
Equine.
Serology.
IFAT.
MAT.

Introduction

Toxoplasma gondii is a coccidian that infects, probably, all homeothermic animals, including man. Thus, it is considered a significant agent with regard to aspects of animal production as it can provoke abortion among different species of economic importance, as well as to public health, due to high prevalence of human infection¹.

Horses are most commonly infected by ingestion of sporulated oocysts found in feces of infected cats². Numerous studies have demonstrated anti-*Toxoplasma* antibodies, as

well as tissue cysts and clinical manifestations including irritability, motor incoordination, nervous or ophthalmologic alterations, and abortion^{3,4,5,6} in the equine species. Dubey, Kerber and Granstrom⁷ affirm that there is no definite evidence that clinical disease occurs in equines, emphasizing that tissue cysts of this parasite can persist in edible tissue for many months⁸, and then, is important to evaluate the clinical significance of equine toxoplasmosis, and to consider aspects of public health regarding consumption of meat infected by *Toxoplasma*⁵.

Indirect fluorescent antibody test (IFAT) has been used as gold standard to

detect *T. gondii* antibodies⁹ however, IFAT requires a species specific conjugate and special equipment. Modified agglutination test (MAT) is simple to perform and does not require species specific conjugate⁹. Serologic exams for the detection of anti-*T. gondii* antibodies in equines were made in several countries including Brazil, where some papers show a prevalence of 12.1% in horses from Parana State¹⁰, and 15.8% in horse sera samples from Rio de Janeiro⁷. The objective of this study was show the presence of anti-*Toxoplasma* antibodies by MAT in equine sera and compare the performance of MAT and IFAT for detecting *T. gondii* antibodies in selected samples of horse sera.

Material and Method

1. Sampling

Samples of equine serum of different ages, sex, origin, and aptitude were tested. These sera were shipped to the laboratory of *Serviço de Diagnóstico de Zoonoses* of *Faculdade de Medicina Veterinária e Zootecnia*, Botucatu, São Paulo State, Brazil, for serologic diagnosis for leptospirosis or equine infectious anemia, these having been stored at -20°C up to the moment of serologic exam for toxoplasmosis.

2. Serology

RH strain of *T. gondii*, weekly propagated by intraperitoneal inoculation in Swiss mice, was used to prepare antigen for MAT and IFAT. For MAT, tachyzoites suspensions were inoculated into mice with TG180 sarcoma cells, and the antigen prepared as described by Desmonts and Remington¹¹, at a final concentration of 10⁸ parasites/ml. For IFAT, tachyzoites were fixed in formalin and diluted to a concentration of 20-30 parasites for microscopic field at 400x magnification.

Initially, the serum was diluted at 1:64 in phosphate buffered solution, and examined by MAT as described by Desmonts and Remington¹¹. The reactant

sera were then tested in fourfold serial dilutions, according the routine procedures of *Serviço de Diagnóstico de Zoonoses*; horses were considered seropositive at e" 64⁸. A number of 132 MAT positive sera as well as 150 randomly selected negative samples were evaluated by IFAT, performed as described by Camargo¹², using anti-equine IgG conjugate (Zoonosis Control Center, City of São Paulo County Authority) diluted at 1:180 – dilution of conjugate as determined by previous test on *Toxoplasma* positive and negative horse sera. Positive control serum was selected from naturally infected horse, with a titer of 16384 in both IFAT and MAT. Equine sera with no reaction to IFAT and MAT was used as negative control serum.

3. Statistical analysis

Performance of MAT was assessed by measures of sensitivity, specificity and global concordance and their 95% confidence interval (CI95%)¹³ using IFAT as gold test. The association between the MAT and IFAT tests were verified by the McNemar test, calculating also the coefficient of agreement k¹⁴ with $\alpha=0.05\%$.

Results

Of the 1984 equine sera samples tested, 138 (7.0%) were MAT positive. Anti-*T. gondii* antibodies in serum dilution at 1:64 were found in 60 (46.4%) animals; at 1:256 in 52 (37.7%); at 1:1024 in 19 (13.8%); at 1:4096 in 5 (3.6%); and at 1:16384 in 2 (1.4%) horses.

From 138 MAT positive samples, 132 were tested by IFAT, and 14 showed negative at this test, and of the 150 MAT negative samples, all were negative by IFAT. Six MAT positive sera samples were not tested by IFAT because insufficient amount of sera was disponible to perform this test. Statistical analyses showed difference between the tests ($\chi^2=12.07$; $P<0.05$), however the coefficients $k=0.90$ and of global agreement = 0.95 (CI95%: 0.92-0.97),

Table 1 - Comparison of modified agglutination test (MAT) and indirect immunofluorescent antibody test (IFAT) titers in 282 samples of equine serum. Botucatu, Brazil, 2002

Teste		IFAT		Total
		+	-	
MAT	+	118	14	132
	-	0	150	150
Total		118	164	282

$S_r \text{ MAT} = (118/118) \times 100 = 100\%$ (95%IC = 96.9-100); $E_r \text{ MAT} = (150/164) \times 100 = 91.5\%$ (95%IC = 86.1-95.2); global agreement = 0.95 (CI95%: 0.92-0.97)

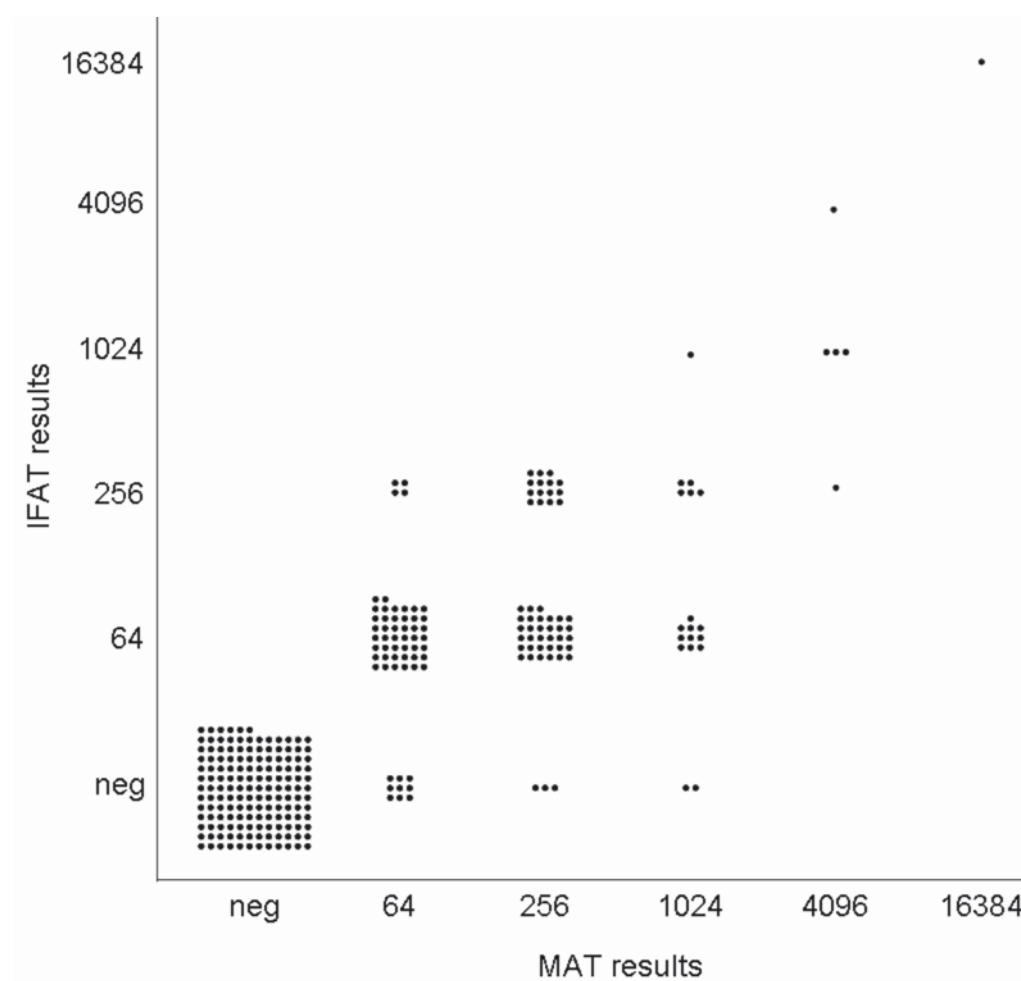


Figure 1 - Comparison of modified agglutination test (MAT) and indirect immunofluorescent antibody test (IFAT) titers in 282 samples of equine serum. Botucatu, Brazil, 2002

indicate great agreement between the tests utilized. Table 1 present the sensibility and specificity values for MAT compared to IFAT. When compared with IFAT, the performance study for the MAT to detect antibody anti-*T. gondii* showed 100% sensibility (CI95% = 96.9 – 100.0) and 91.5% specificity (CI95% = 86.1 – 95.2).

Figure 1 shows the agreement between the titers of MAT and IFAT. The data from 282 samples evaluated by IFAT and MAT suggests decrease in titers for IFAT, as compared to MAT. In 66 samples the titer to IFAT where lower than to MAT, and in only 4 samples occurred the opposite.

Discussion

In several countries serologic research show a great variation in prevalence of *T. gondii* infection. The titer considered as positive may also differ among the studies. Furthermore, the possible selection of the samples, the type of serologic methods employed, and differences among the populations tested, make difficult the comparison of results among different studies^{15,16}.

Dubey et al.¹⁷ utilizing the modified agglutination test (MAT), described positive reaction in 10 (13.1%) of 76 samples of serum from horses of Argentine origin, with titers of 25 (n=2), of 50 (n=5), 100 (n=2), and 200 (n=1). Utilizing the same test, in North America¹⁶, of 1788 samples of serum from horses killed for meat evaluated, 124 (6.9%) were positive, with titers of 20 (n=69), 40 (n=37), 80 (n=9), and equal to, or greater than, 160 (n=9).

The antibody titers may frequently be linked to a chronic infection or the presence of tissue cysts¹⁵. Ishizuka, Miguel and Brogliato¹⁸, Larangeira, Ishizuka and Hyakutake¹⁹, and Garcia et al.¹⁰, refer to titer 64 most frequently found by IFAT. Eugster and Joyce²⁰, Costa et al.²¹, Gazêta et al.²², and Vidotto et al.²³ found most prevalent titers of 16 and 64. The most prevalent titer in this study, both for MAT and for IFAT, was

64, which is in agreement with the other authors cited. Dubey and Desmonts²⁴ concluded that titer 64 was specific for MAT, and that equines can develop high titers without, however, showing clinical signs.

The prevalence of equine *Toxoplasma* in the different regions appear to be associated to environmental factors, such as humidity, temperature, and height²². In general, prevalence is greater in warm and humid areas than in arid and semi-arid regions. Also other epidemiological factors must be considered, such as the cat population infected, age, and type of animal management¹⁵. It is, however, can be difficult to determine the origin and time of permanence of the animal in such situations, due to movement of the animals, and therefore to determine the occurrence of the infection, as well as the relation between climatic conditions and occurrence of infection²⁵.

Conclusions

The results obtained demonstrate the agreement between the two tests utilized. Positive reaction of the animals indicates the possible participation of this specie in the epidemiological chain of transmission of toxoplasma to other carnivora, as well as to humans. More sero-epidemiological studies are important to the standardization of tests, and to establish a minimum infectious titer so that results can be compared, and to estimate the zoonotic potential of the animals.

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Utilização do método de aglutinação direta e da reação de imunofluorescência indireta na detecção de anticorpos para *Toxoplasma gondii* em cavalos naturalmente infectados

Resumo

A infecção pelo *Toxoplasma gondii* em eqüinos geralmente é inaparente, sendo esta caracterizada pela manutenção de títulos de anticorpos e presença de cistos teciduais. Este estudo visou verificar a presença de anticorpos anti-*Toxoplasma* em soros de eqüinos pela aglutinação direta modificada (ADM) e reação de imunofluorescência indireta (RIFI). 1984 amostras de soro foram examinadas pela ADM, utilizando-se como antígeno taquizoítos íntegros de *T. gondii* produzidos em células de sarcoma TG-180 e fixados pela formalina, considerando-se como título positivo 64. As amostras reagentes na ADM, e 150 amostras negativas na mesma prova, escolhidas aleatoriamente, foram testadas pela RIFI, utilizando conjugado anti-IgG-eqüina. A associação entre os resultados dos testes foi verificada pelo teste de McNemar. 138 (7%) amostras foram positivas na ADM, com 60 (46,38%) apresentando reação à diluição 1:64; 52 (37,7%) à 1:256; 19 (13,8%) à 1:1024; cinco (3,6%) à 1:4096 e duas (1,45%) à 1:16384. De 132 amostras positivas na ADM, 14 foram negativas à RIFI, mas a análise estatística indicou elevada concordância dos resultados entre os testes utilizados. Os resultados obtidos mostram a concordância entre os testes utilizados e a possibilidade da participação da espécie eqüina na transmissão da toxoplasmose para animais carnívoros, bem como para o homem.

Palavras-chave:

Toxoplasma gondii.
Eqüino.
Sorologia.
RIFI.
ADM.

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