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Evaluation of the performance of the modified direct agglutination test (MAT) for detection of *Toxoplasma gondii* antibodies in dogs

Avaliação da performance do teste de aglutinação modifica (MAT) paa a detecção de anticorpos anti-T*oxoplasma gondii* em cães

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Summary

Toxoplasmosis is a zoonosis that has been the subject of study in Brazil and worldwide. The dogs are sentinels for the infection and can carry *Toxoplasma gondii* in the environment. Seroepidemiological surveys of these animals are an important tool in the surveillance and control of the disease and inform decision-making in health programs. In this study the performance of the *Modified Agglutination Test* (MAT) in the serodiagnosis of canine toxoplasmosis is evaluated and compared to the indirect immunofluorescent-antibody test (IFAT). A sample of 157 dog sera from the county of Monte Negro, Rondônia, with 76.40% positive reactions for *Toxoplasma gondii* (IFAT =16) was analyzed using the MAT (=25), presenting sensitivity of 85.00% (Confidence Interval 95.00%: 79.4 – 90.60%) and specificity of 100.00%.

Key-words
Toxoplasma gondii.
Dog.
Agglutination tests.
Sensitivity and specificity.

Introduction

Whatever a degree of development of a country, toxoplasmosis is an important zoonosis, with high levels of prevalence in dogs, as observed in several parts of the world [1,2,3] including Brazil [4,5]. Most studies use the indirect immunofluorescent-antibody test (IFAT), which has been in use since the 1960s[6] to detect anti-*Toxoplasma gondii* antibodies. This test is used in dogs as a gold-standard owing to its high specificity[7].

Another widely-used technique is the direct agglutination test, described in 1965 by Fulton[8], and modified in 1980, by Desmonts and Remington[9] (*Modified Agglutination Test*–MAT), which has been used in field studies as a screening method, both in domestic and in wild dogs[10], since it does not require species-specific conjugates. However, the sensitivity and specificity of this test have not been clearly described.

The objective of the present study was to assess the performance of MAT in detecting anti-*Toxoplasma gondii* antibodies in dogs, using IFAT as a reference test.

Material and Method

The study was carried out in the municipality of Monte Negro, in the state of Rondônia, in the western region of Brazilian Amazônia (10º 18' South; 63º 14' West).

A census of the dog population of this municipality showed 671 animals, of which 157 were selected in a convenience survey to obtain 5mL of serum in March 2001. The sera were tested by the IFAT with a cutoff point of =16, according to a protocol proposed for serodiagnosis of toxoplasmosis[11]. The antigen that was used was a suspension of tachyzoites of *Toxoplasma gondii* RH strain maintained by successive passages in female albino Swiss mice with ages ranging from 28 to 30 days, in the Department of Preventive Veterinary Medicine and Animal Health of the Faculty of Veterinary Medicine of the University of São Paulo. The conjugate rabbit-IgG anti-dog IgG, tagged with fluorescein isothiocyanate (Sigma F-7884), was prepared in a solution of Evans blue dye, in a dilution of 1:500 in a phosphate-buffered saline solution – PBS 0.01M pH 7.6.

Readings were taken using a fluorescence microscope with a 40x lens, using the following criterion: sera showing fluorescence in the total surface of the tachyzoite were considered positive, and those showing apical (non-specific reactivity) or partial fluorescence were considered negative. Positive sera were titered to the end-point of the reaction.

The MAT was conducted in 96-well U-bottomed microtiter plates. The sera to be tested were diluted in a phosphate buffer (PBS) 0.01M pH 7.2. The PBS buffer was previously filtered through a 0.22 ìm membrane and kept refrigerated according to the protocol proposed by Desmonts and Remington[9]. The positive sera (=25) were titered to the end-point.

The antigen suspension was composed of 2.5 mL of borate buffer pH 8.95 containing 0.40% BSA; 35 ìL of 2-mercaptoethanol, 50 ìL of Evans blue at 2 mg/mL and 140 µL of the suspension of whole tachyzoites of the *Toxoplasma gondii* RH strain inactivated by formalin (kindly supplied by Dr. J.P. Dubey of the *United States Department of Agriculture, Beltsville, Maryland, USA*).

In each well of the microtiter plate 25 ìL of the antigen solution and 25 ìL of the serum to be tested were mixed. Positive and negative control sera were also included. The microtiter plate was covered with transparent adhesive tape and incubated at 37ºC for 12 hours.

The principle underlying this serological reaction is agglutination of *Toxoplasma gondii* tachyzoites fixed in formalin by antibodies present in the serum. The addition of 2-mercaptoethanol to the sera removes the reactivity of IgM antibodies by reducing their dihydrosulfide bonds. The reading of this reaction is based on the sedimentation profile of the tachyzoite suspension, where the formation of a web indicates the presence of antibodies and the formation of a blue dot at the bottom of the well indicates the absence of antibodies.

The results were presented in simple and contingency tables. Performance of the MAT was assessed by measures of sensitivity, specificity and global concordance and their respective confidence intervals (C.I.) of 95.00% [12,13].

Results

It was observed, when using IFAT (=16) that 76.40% of sera reacted positively for *Toxoplasma gondii*.

The performance study for MAT (=25) to detect anti-*Toxoplasma gondii* antibodies, using IFAT (=16) as reference study, showed 85.00% sensitivity (IC 95.00%: 79.40 – 90.60%) and 100.00% specificity. The probability

of the test classifying infected and non-infected animals correctly (global concordance) is 88.50% (Table 1).

A fall in sensitivity of the test, along with a rise in the cutoff point were observed. Specificity did not vary, staying at 100.00% for all cutoff points (Table 2).

Table 1
Detection of anti-*Toxoplasma gondii* antibodies by MAT (= 25) and by IFAT (= 16), in dogs, Monte Negro municipality, São Paulo, 2002

Tests		IFAT		
		+	-	Total
MAT	+	102	0	102
	-	18	37	55
	Total	**120**	**37**	**157**

Table 2
Values for sensitivity and specificity for MAT compared to IFAT (³ 16) by MAT cutoff points, São Paulo, 2002

	MAT	
cutoff point	Sensitivity (%)	Specificity (%)
³25	85.0	100
³50	55.0	100
³100	27.5	100
³200	14.2	100
³400	6.7	100
³800	2.5	100

Discussion

Sensitivity and specificity scores for the MAT in dogs have not been described in the literature. In the present study the MAT showed adequate performance in the serodiagnosis of canine toxoplasmosis, with 85.00% sensitivity and 100.00% specificity.

When a test is performed in a population for screening purposes, animals diagnosed as false negatives take on vital epidemiological importance since their presence ensures the ciculation of the agent in the environment. The occurrence of false negative results (18/120) may be related to factors such as the analytical sensitivity of the test, and the immune response phase (production of antibodies), among others.

Good screening tests are characterized by high sensitivity; that is, positive samples will be correctly detected. Although in this study the sensitivity of MAT was observed to be less than ideal, the use of dilutions less than 1:25 would likely lead to an increase in sensitivity, with values close to 100.00%. Even though this might lead to a loss of specificity, the objectives of screening would be better achieved.

In this case IFAT is characterized by measuring antibodies with a low detection threshold; that is, it generates a signal with small quantities of antigen-antibody interaction. On the other hand, agglutination tests require a greater amount of this interaction to generate a signal, and thus the detection threshold is higher. These factors, in association with the nature of the test and the cutoff point should be adequately discussed when assessing the performance of the MAT, especially in situations where the immune response is reduced or in recent infections.

Cross reactivity with *Neospora caninum* or other apicomplexan parasites is minimal, since the tachyzoites present very specific epitopes on their surface [14]. However, more specific tests enabling demonstration of cross-reactivity were not used in this study.

It should be remembered that levels of circulating antibodies are related to the degree of exposure, to persistent antigenic challenge and/or to control of the parasite by the host. Few studies assess the kinetics of infection of dogs by *Toxoplasma gondii*. However, experimental infection of dogs has shown that IgG antibodies can be measured by the IFAT and by the MAT in the first week post-infection[7]. Although the sensitivity and specificity of these tests have not been related, the present authors recommend the use of the IFAT in the diagnosis of toxoplasmosis as a reference test. Dogs easily acquire immunity, although the

infection remains without clinical signs, in a chronic state, which favors circulation of the agent in the environment and thus its transmission to man and other animals[15].

Another aspect to be borne in mind is the occurrence in agglutination reactions of the prozone phenomenon, which is characterized by inhibition of agglutination because of an excess of the antigen or antibodies[16]. This fact calls for standardization of each batch of antigen, always using the same positive and negative control sera.

Taking the performance of the MAT with the presentation of false negative results and 85.00% sensitivity, for a cutoff point ³ 25, we conclude that it is not suitable for screening canine toxoplasmosis. However, factors such as relatively low cost, the lack of a need to use species-specific antibodies, and the ease and rapidity of execution, make the test potentially useful, especially when it is impossible to use a higher-performing technique such as the IFAT. Enhancement of the MAT so as to improve sensitivity demands further study.

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Resumo

Toxoplasmose é uma zoonose que vem sendo objeto de estudos no Brasil e em todas as partes do mundo. Os cães são considerados sentinelas da infecção, podendo carrear o agente pelo ambiente. Levantamentos soro-epidemiológicos desses animais são importantes ferramentas de vigilância e controle da doença em programas de saúde. Neste estudo a performance do *Teste de Aglutinação Modificada (MAT)* no sorodiagnóstico da toxoplasmose canina foi avaliado e comparado à reação de imunoflorescência indireta (RIFI). Uma amostra de 157 soros de cães do município de Monte Negro, Rondônia, com 76.40% de animais positivos ao *Toxoplasma gondii* (RIFI =16) foi analisado utilizando o MAT (=25) e apresentou sensibilidade de 85,00% (Intervalo de Confiança 95,00%: 79,4-90,60%) e especificidade de 100,00%.

Palavras-chave
Toxoplasma gondii.
Cão.
Testes de aglutinação.
Sensibilidade e especificidade.

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