

COMPARISON BETWEEN DIRECT AGGLUTINATION TEST AND INDIRECT FLUORESCENT ANTIBODY TEST FOR THE DETECTION OF *Neospora caninum* ANTIBODIES IN NATURALLY EXPOSED DOGS

WILLIAN A. CANÓN FRANCO¹, DENISE P. BERGAMASCHI², LAÍS M.A. CAMARGO³, VÂNIA S.O. PAULA¹,
SILVIO L.P. SOUZA¹, SOLANGE M. GENNARI^{1*}

ABSTRACT.- CANÓN FRANCO, W. A., BERGAMASCHI, D. P., CAMARGO, L. M. A., PAULA, V. S. O., SOUZA, S.L.P., GENNARI, S.M. **Comparison of direct agglutination test and indirect fluorescent antibody test for the detection of *Neospora caninum* antibodies in naturally exposed dogs.** [Comparação entre a aglutinação direta e a imunofluorescência indireta para detecção de anticorpos de *Neospora caninum* em cães naturalmente infectados.] *Revista Brasileira de Parasitologia Veterinária*, v. 12, n. 1, p. 4-6, 2003. Depto de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Orlando Marques de Paiva 87, Cidade Universitária, 05508-000, Brazil. E-mail: sgennari@u_W.br

The indirect fluorescent antibody test (IFAT) and *Neospora agglutination* test (NAT) are two serologic test that detect antibodies to whole tachyzoites. In this study IFAT and NAT were, compared for the detection of *N. caninum* antibodies in naturally exposed dogs. Sera from 157 dogs from the county of Monte Negro, Rondônia, with 8.3% seropositivity for *N. caninum* using IFAT (≥ 50) were analyzed by NAT (≥ 25), presenting sensitivity of 61.5% (Confidence Interval 95%: 53.9 to 69.2%), specificity of 89.6% (C.I. 95%: 84.8 to 94.4%) and positiveness of 14.7% (23/157).

KEY WORDS: Indirect fluorescent-antibody test, *Neospora agglutination* test, specificity, sensitivity *Neospora caninum*, dogs.

RESUMO

A reação de imunofluorescência indireta (RIFI) e o teste de aglutinação modificada para *Neospora caninum* (NAT) são duas provas utilizadas na detecção de anticorpos anti-*N. caninum*. Neste estudo RIFI e NAT foram comparadas na detecção de anticorpos anti-*N. caninum* em cães naturalmente infectados. Soro de 157 cães do município de Monte Negro, Rondônia, com 8,3% de soropositividade para *N. caninum* pela RIFI (≥ 50) foram analisados pelo NAT (≥ 25), que apresentou uma sensibilidade de 61,5% (Intervalo de Confiança 95%: 53,9 a 69,2%) e uma especificidade de 89,6% (I.C. 95%: 84,8% a 94,4%) e positividade de 14,7% (23/157).

¹Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia - Universidade de São Paulo (USP). Av. Prof. Orlando Marques de Paiva, 87, CEP 05508-000 - Cidade Universitária, São Paulo, SP, Brasil. E-mail: sgennari@u_W.br

²Departamento de Epidemiologia, Faculdade de Saúde Pública, USP.

³Departamento de Parasitologia, Instituto de Ciências Biomédicas, USP.

* Author for correspondence: S. M. Gennari- E-mail: sgennari@u_W.br
phone: 11-309176-54, fax: 11 3091-7928.

PALAVRAS-CHAVE: Reação de Imunofluorescência Indireta, Teste de Aglutinação para *Neospora caninum* especificidade, sensibilidade, *Neospora caninum*, cães.

INTRODUCTION

Antibodies to *Neospora caninum* have been reported in dogs worldwide (DUBEY; LINDSAY, 1996; LINDSAY; DUBEY, 2000); including from Brazil, both in rural and urban areas (SOUZA et al., 2002; GENNARI et al., 2002). A titer of 1:50 is considered indicative of *N. caninum* infection and indirect fluorescent antibody test, (IFAT) has been used as goldstandard to detect *N. caninum* antibodies (DUBEY et al., 1988; BJÖRKMAN; UGGLA, 1999) however, IFAT requires a species specific conjugate and special equipment. *Neospora agglutination* test (NAT) is simple to perform and does not require species specific conjugate (ROMAND et al., 1998). However, the sensitivity and specificity of this test, in dogs, have not been clearly described. The objective of the present study was to compare the performance of the NAT and IFAT for detecting *N. caninum* antibodies in dogs.

MATERIAL AND METHODS

Sera from 157 naturally exposed dogs from the county of Monte Negro, state of Rondônia - Brazil, were first tested by the IFAT using culture-derived tachyzoites, of NC-1 isolate (DUBEY et al., 1988b) and the rabbit anti-canine IgG conjugate (Sigma, St. Louis, MO). Sera were tested at 2-fold dilutions starting at 1:50, using the procedure described by Dubey et al. (1988b). The same sera were later evaluated by NAT, performed as described by ROMAND et al. (1998) using formalin fixed whole tachyzoites and tested at 2-fold dilution starting at 1:25 as recommended by Bjorkman and Ugglä (1999).

Performance of the NAT was assessed by measures of sensitivity, specificity and global concordance and their respective confidence intervals (C.I.) of 95% (GARDNER; GREINER, 2000) using IFAT as gold test.

RESULTS

By IFAT antibodies to *N. caninum* were found in 13 (8.3%) of dogs and prevalence study was published elsewhere (CAÑON-FRANCO et al., 2003). Using NAT the seropositivity for antibodies anti-*N. caninum* was 14.7%, with 23 positive dogs. Among the positives ones, 18 (78.30%) dogs presented titer of 25 and 5 (21.7%) titer of 50.

When compared with IFAT (≥ 50), the performance study for the NAT (≥ 25), to detect *N. caninum* antibodies, showed 61.5% sensitivity (CI 95%: 53.9 to 69.2%) and 89.6% specificity (CI 95%: 84.8 to 94.40%). Global concordance was 87.3%. Fifteen samples that were negative by IFAT were positive by NAT and five samples negative by NAT were positive by IFAT (Table 1). A fall in sensitivity and a rise in specificity of the test were observed with the increase of the cut-off point as illustrated in Table 2.

Table 1. Detection of *Neospora caninum* antibodies by *Neospora* agglutination test (NAT ≥ 225) and by indirect fluorescent antibody test (IFAT ≥ 50), in dogs naturally infected, from the county of Monte Negro, Rondônia.

Tests		IFAT		Total
		+	-	
NAT	+	8	15	23
	-	5	129	134
Total		13	144	157

Table 2. Values for sensitivity and specificity for *Neospora* agglutination test (NAT) compared to indirect fluorescent antibody test (IFAT ≥ 50) by different NAT cut-off points to detect *Neospora caninum* antibodies.

NAT cut-off point	Sensitivity (%)	Specificity (%)
≥ 25	61.5	89.6
≥ 50	15.4	97.9

DISCUSSION

In the present study the NAT performed poorly, owing to low sensitivity (61.5%) for cut-off point ≥ 25 . Several cut-off points have been evaluated for the NAT; the most widely used in dogs is ≥ 25 (BJÖRKMAN; UGGLÄ, 1999).

The differences between the performances of NAT versus IFAT may be due to types of antibodies measured, and different epitopes detected. The NAT detects only IgG because the 2-mercaptoethanol used in the test destroys specific and non-specific IgM, whereas IFAT detects both types of antibodies IgG and IgM.

Further studies are needed to compare these tests in experimentally infected dogs.

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