Antibody dynamics during gestation in cows naturally infected with *Neospora caninum* from four dairy herds in Brazil

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Abstract

Neospora caninum has been described as an important cause of abortion in bovine worldwide. The objective of the present study was to characterize patterns of antibody dynamics during gestation in dairy cows naturally infected with *N. caninum*. Twelve *N. caninum* naturally infected cows were selected from four dairy herds from Brazil and blood samples were monthly collected during pregnancy. Serum were tested for antibodies against *N. caninum* by Indirect Fluorescent Antibody Test (IFAT). During this period, all cows remained clinically normal and gave birth to healthy calves. The cows remained seropositives during the study and *N. caninum* IFAT titers ranged from 100 to 12,800; only animal 234 presented one negative result in the first month of pregnancy. Significant differences of *N. caninum* IFAT titers were found between months from 1 to 9 of pregnancy by the Friedman Test ($P<0.001$). The statistical analysis showed an increase of *N. caninum* antibody titers from second and third trimester of pregnancy in relation to first trimester. High titers were observed in few cows after month fifth of pregnancy. This study showed a variation of specific antibody levels in seropositive cows during different gestational periods. The highest values were observed during the second and third trimester. The antibody increase after the fifth month of gestation was not associated to abortion.

Key words:
Neospora caninum.
Antibody dynamics.
Antibody titer.
Pregnancy.
Bovine.

Introduction

Neospora caninum is an apicomplexan parasite that was first recognized in dogs by Norwegian scientists[1] and isolated, characterized and named in 1988 in the United States[2]. *N. caninum* has been described as an important cause of abortion in bovine worldwide[3,4,5].

Despite the discovery that dogs and coyotes can serve as a definitive host for *N. caninum*[6,7], congenital infection is generally accepted as the main way of transmission and maintenance of *N. caninum* in cattle and can occur in subsequent pregnancies and over several generations[8,9,10,11,12,13,14,15]. Whereas some fetal infections lead to abortion, birth of congenitally infected calves is the most frequent observation.

Bovine neosporosis develops either as a result of maternal infection during gestation or as consequence of a recrudescence of a persistent infection of the dam during gestation, denoted exogenous or endogenous transplacental infection, respectively.[16] The study of the dynamics of antibodies allows better interpretation of the serological analyses. It is generally accepted that this enhanced humoral response shown by *N. caninum*-infected pregnant dams reflects reactivation of the parasite and its transplacental transmission to the fetus.[17]

The objective of the present study was to characterize patterns of antibody dynamics during gestation in dairy cows naturally infected with *N. caninum*.

Material and Method

This study was conducted in four dairy herds, three in São Paulo and one in Paraná State, Brazil. The cattle were of Holstein Friesian crossbreed and were reared in the semi-extensive system. The herds had previously confirmed cases of *N. caninum* infection in prospective studies using serological assays. Twelve pregnant cows naturally infected with *N. caninum* were selected. All the cows were artificially inseminated and pregnancy was diagnosed by rectal palpation on day 40 after insemination. The pregnancies were not coordinated in time, but spread over the year. All animals were tuberculosis and brucellosis free and vaccination programs were adopted for the prevention of main bovine diseases

From October 2005 to May 2008, blood samples of the cows were monthly collected by coccygeal venipuncture from breeding to calving. Only one pregnancy of each cow was evaluated. After centrifugation at 1,000 x g for 15 min, serum was removed and stored at –20°C until analysis.

Serum were tested for antibodies against *N. caninum* by Indirect Fluorescent Antibody Test (IFAT) using whole culture-drived tachyzoites (NC-1 strain) as antigen.[18] Serum samples were titrated in doubling dilution from 1:100. Fluorescein isothiocyanate conjugated rabbit antibovine IgG (Sigma, St Louis, MO, USA) was used at 1:3,000 dilution.

IFAT data from the individual sera did not follow a normal distribution, requiring the use of a nonparametric statistical comparison, the Friedman Test, using SPSS 9.0 program (SPSS Inc., 2004). Multiple comparisons between titers of anti-*N. caninum* antibody during pregnancy months were carried out by Wilcoxon Signed Ranks Test. Difference was considered statistically significant for $P<0.05$.

Results and Discussion

During the pregnancy, all cows remained clinically normal and gave birth to healthy calves. Blood samples of calves were not collected because it was not possible collect pre-colostral samples. Individual serological results are shown in table 1.

The cows remained seropositives during the study and *N. caninum* IFAT titers ranged from 100 to 12,800, only animal 234

Table 1 - *Neospora caninum* IgG antibody titers by Indirect Fluorescent Antibody Test (IFAT > 100) during pregnancy in 12 seropositive dairy cows

Farm	Animal	IFAT Titer during month of pregnancy								
		1	2	3	4	5	6	7	8	9*1
I	6	400	400	800	800	800	200	800	1600	800
I	215	400	200	200	100	400	800	800	800	800
II	78	200	200	400	800	800	400	800	400	800
II	234	Neg*2	200	800	400	12800	6400	12800	1600	3200
II	322	400	400	400	800	400	400	400	400	800
III	662	800	800	800	800	800	800	800	800	800
III	657	400	400	400	400	800	800	12800	800	800
IV	359	400	200	400	400	200	400	400	800	800
IV	374	100	400	200	400	200	200	200	200	1600
IV	557	800	200	200	800	800	800	1600	1600	800
IV	368	200	800	800	800	800	800	800	800	800
IV	547	400	800	800	800	800	1600	800	800	400

*1 Date of calving; *2 Negative

presented one negative result in the first month of pregnancy, probably caused by fluctuations in the levels of antibodies that occasionally fall below of detection of serological tests.[13,14,19,20,21]

Significant differences of *N. caninum* IFAT titers were found between months from 1 to 9 of pregnancy by the Friedman Test (P<0.001). In the multiple comparisons, months 1 and 2 were significantly different (p<0.05) and presenting low titers when compared to months 4 to 9 (second and third trimester of pregnancy). Month 3 was different (P<0.05) to months 7, 8 and 9 (third trimester of pregnancy) showing lower titers. No significant difference (P<0.05) in titers occurred between months 4, 5 and 6 (second trimester) and months 7, 8 and 9 (third trimester). These results show an increase of *N. caninum* antibody titers from second and third trimester of pregnancy in relation to first trimester. The increase of antibody level during mid- and late-gestation has been reported[13,14,22,23,24,25,26], however other few studies found no difference in the levels of antibodies during pregnancy[21,27].

In the present study, high titers were observed in few cows after month fifth of pregnancy (Table 1). Several authors demonstrated that cows with high *N. caninum* antibody titer had higher risk of abortion than seronegative or cows with low titer[14,23,28,29,30,31,32], however no abortion occurred, even in the cows they showed increase of *N. caninum* antibody titers after the fifth month of pregnancy.

This study showed variation of specific antibody levels in seropositive cows during different gestational periods. Highest values were observed during the second and third trimester. The level of antibodies increased after the fifth month of gestation; however this was not associated to abortion.

Dinâmica de anticorpos durante a gestação em vacas naturalmente infectadas com *Neospora caninum* de quatro rebanhos leiteiros no Brasil.

Resumo

Neospora caninum é descrito como uma importante causa de abortamento em bovinos por todo o mundo. O objetivo do presente estudo foi caracterizar o padrão da dinâmica de anticorpos durante a gestação em vacas leiteiras infectadas naturalmente por *N. caninum*. Doze vacas gestantes infectadas naturalmente com *N. caninum* foram selecionadas de quatro rebanhos leiteiros do Brasil e amostras de sangue foram mensalmente colhidas da concepção até o parto das vacas. Soros foram testados para anticorpos contra *N. caninum* pela reação de imunofluorescência indireta (RIFI). Durante a gestação, todas as vacas permaneceram clinicamente normais e geraram bezerros saudáveis. As vacas permaneceram soropositivas durante o estudo e títulos de anticorpos anti-*N. caninum* variaram de 100 a 12.800; somente o animal 234 apresentou um resultado negativo no primeiro mês de gestação. Diferenças significativas dos títulos da RIFI para *N. caninum* foram encontradas entre os meses de 1 a 9 de gestação pelo Teste de Friedman (P<0,001). As análises estatísticas mostraram um aumento dos títulos de anticorpos anti-*N. caninum* no segundo e terceiro trimestre de gestação em relação ao primeiro trimestre. Altos títulos de anticorpos foram observados em algumas vacas após o mês cinco de gestação. Este estudo mostrou variação dos níveis de anticorpos em vacas soropositivas durante diferentes períodos gestacionais. Altos títulos foram observados durante o

Palavras-chave:
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Título anticorpos.
Gestação.
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segundo e terceiro trimestre e o aumento dos títulos de anticorpos após o quinto mês de gestação não foi associado a abortamentos.

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