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Association of abortion history with the presence of anti-*Neospora caninum* antibodies in dairy cows

Associação do histórico de abortamento com a presença de anticorpos anti-*Neospora caninum* em vacas leiteiras

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Abstract

Neospora caninum is an important cause of abortions in dairy cattle, leading to severe economic losses. The objective of this study was to determine the association of serum antibodies against *N. caninum* with a history of abortion in dairy cows. A total of 46 dairy cows from nine dairy farms were evaluated; 28 with a history of one or more abortions and 18 without a history of abortions. Antibodies against *N. caninum* were detected by indirect immunofluorescence, and the reaction was considered positive when the titer was ≥1:50. Serum antibodies against *N. caninum* were detected in 39.1% (18/46) of all cows, 57.1% (16/28) of cows with a history of abortion, and 11.1% (2/18) of cows without a history of abortion. Among the seropositive cows, 88.9% had a history of abortion. All farms showed at least one animal positive for *N. caninum*. These results indicate an association between seropositivity for *N. caninum* and a history of abortion in cows. Cows with a history of abortion were 10.6 times more likely to be seropositive for *N. caninum* than cows without a history of abortion. Therefore, we concluded that there is an association between a history of abortion and the presence of antibodies against *N. caninum* in dairy cows.

Key words: Neosporosis. Bovine. Pregnancy. Abortion.

Resumo

O *Neospora caninum* é um importante agente causador de abortos em bovinos leiteiros, promovendo severos prejuízos econômicos. Objetivou-se neste trabalho associar a presença de anticorpos contra *N. caninum* com histórico de abortamento em vacas leiteiras. Um total de 46 vacas leiteiras foram avaliadas, sendo 28 com histórico de um ou mais abortamentos e 18 sem histórico de abortamentos, provenientes de nove propriedades leiteiras. Para a detecção de anticorpos contra *N. caninum* foi realizada a reação de imunofluorescência indireta, sendo consideradas positivas quando os títulos foram maiores ou iguais a 1:50. A soro ocorrência de anticorpos contra *N. caninum* entre todas as vacas avaliadas foi de 39,1% (18/46), entre as vacas com histórico de abortamento foi de 57,1% (16/28) e de 11,1% (2/18) nas vacas que não haviam abortado. Dentre as vacas soropositivas, 88,9% possuíam histórico de aborto. Todas as propriedades avaliadas apresentaram animais com anticorpos contra o *N. caninum.* Há uma associação entre vacas soropositivas para *N. caninum* e histórico de abortamento. Vacas soropositivas apresentaram 10,6 vezes mais chances de serem sororreagentes ao *Neospora caninum* do que vacas sem histórico de

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abortamento. Conclui-se que existe associação entre histórico de abortamento e presença de anticorpos contra *Neospora caninum* em vacas leiteiras.
Palavras-chave: Neosporose. Bovinos. Prenhez. Aborto.

Introduction

Neospora caninum is considered to be the main cause of abortion in dairy and beef cattle in several countries, including Brazil (ALMERÍA; LÓPEZ-GATIUS, 2015). This protozoan belongs to the class Apicomplexa and was first described in dogs and subsequently found to cause abortions and neonatal infections in cattle, horses, sheep, and goats (DUBEY, 2003). The definitive hosts of this protozoan are canids, which can be infected by ingesting cysts (McALLISTER et al., 1998).

N. caninum-infected cattle present reproductive problems, including abortion, stillbirth, and neonatal death (HADDAD et al., 2005). Melo et al. (2001) reported that this protozoan causes significant economic losses in the dairy industry because infected cows are three times more likely to have abortions than healthy cows. In addition, these authors also reported that these infections increase the costs related to abortions, including new inseminations or coverings, reduction of useful cow life-span, and decreased milk production due to the longer intervals between deliveries.

Since the identification of this protozoan by Dubey et al. in 1988, infection of cows has been reported worldwide. In some countries, *N. caninum* infection (neosporosis) is considered to be the leading cause of reproductive failure, and it is associated with considerable economic losses (GUIMARÃES JÚNIOR; ROMANELLI, 2006; WOUDA, 2005).

The estimated worldwide total losses caused by *N. caninum* exceeds USD 1.298 billion per year, and may reach USD 2.38 billion. Approximately two-thirds of this total (USD 1.58 billion) is related to production losses in dairy cattle (REICHEL et al., 2013). Reichel et al. (2013) and Nicolino et al. (2015) estimated the costs related to *N. caninum* infections in Brazil at R$ 291 million and USD 51.3 million, respectively. Therefore, losses due to neosporosis account for approximately 1% of the gross value of dairy production in Brazil, which has high socioeconomic importance.

Serological techniques capable of detecting specific anti-*N. caninum* serum antibodies are used to confirm infection in bovine herds and assess whether neosporosis is related to the occurrence of abortion (THURMOND; HIETALA, 1995). The objective of this study was to evaluate the possible association between a history of abortion and the presence of anti-*N. caninum* antibodies in dairy cows.

Materials and Methods

This study was approved by the Animal Research Ethics Committee of the Pontifical Catholic University of Paraná (Pontifícia Universidade Católica do Paraná-PUCPR) (Protocol No. 0971).

Study sites and study subject characteristics

The city of Marechal Cândido Rondon (24°33'24'' S and 54°3'24'' W) is located in the western region of Paraná state and has an altitude of 420 meters. The climate is subtropical, with a mean annual temperature above 20.1°C. Rainfall occurs throughout the year, but is more common in the summer. According to the last livestock census, this municipality is the eighth largest producer of milk in Brazil (IBGE, 2015).

This case-controlled study was conducted on nine properties selected by convenience sampling with the assistance of a single veterinarian. Each farm had an average of 42 lactating cows with a history of abortion. Health management included

annual examinations, diagnosis of brucellosis, and vaccination against leptospirosis, infectious bovine rhinotracheitis, and bovine viral diarrhea every 6 months.

Biological samples

From March to December 2014, blood samples were collected from 46 dairy cows, including 28 animals with a history of abortion and 18 animals without a history of abortion. These cows were lactating, *i.e.*, they had given birth at least once. The collected serum was stored at -20°C until indirect immunofluorescence (IIF). Abortions occurred in the third trimester of gestation and were diagnosed by the owners or caretakers.

Detection of anti-N. caninum antibodies

Anti-*N. caninum* IgG antibody titers were quantified using the IIF technique reported by Conrad et al. (1993). For IIF, histological slides were prepared for antibody screening using crude tachyzoite forms of *N. caninum* (strain Nc-1) and were considered positive when the titer was ≥50 (VENTURINI et al., 1999). FITC-conjugated anti-bovine IgG (Sigma-Aldrich®) was used. Positive samples were diluted sequentially at a ratio of 1:1 until they became negative. Positive and negative controls were included in all assays.

Statistical analysis

The correlation between a history of abortion and *N. caninum* seropositivity was analyzed by the chi-square (X^2) or Fisher's exact test using EpiInfo software (version 6), and p-values less than 0.05 were considered statistically significant. The degree of correlation was assessed by calculating the odds ratio (OR) with the 95% confidence interval.

Results and Discussion

Animals with a history of abortion were 10.6 times (95% CI: 1.17-82.8, p = 0.004) more likely to be seropositive for anti-*N. caninum* antibodies compared to animals with no history of abortion. The rates of seropositivity in the groups with and without a history of abortion were 57% (16/28) and 11.1% (2/18), respectively. Of the 18 seropositive animals evaluated, 16 (88.9%) had a history of abortion (Table 1). All seropositive animals had antibody titers <400, indicating the absence of acute or active infections, as antibody titers in acute or active infections are ≥800 (RAGOZZO et al., 2003).

Table 1. Rate of seropositivity and seronegativity to *Neospora caninum* in cows with and without a history of abortion.

Abortion	Seropositive (%)	Seronegative (%)	Total (%)
YES	16 (57.1)	12 (42.8)	28 (60.9)
NO	2 (11.1)	16 (88.9)	18 (39.1)
Total	18 (39.1)	28 (60.9)	46 (100)

* OR = 10.6 (1.77 < OR < 82.8), p = 0.004.

The rate of abortion due to *N. caninum* infection in dairy cows in Brazil is estimated at 10.04%, corresponding to more than 474,000 abortions per year and resulting in severe economic losses to the dairy industry (NICOLINO et al., 2015).

Dubey et al. (1997) reported that the detection of anti-*N. caninum* antibodies in the sera of cows with a history of abortion was evidence of maternal exposure to the protozoan. However, these infections may not be the primary cause of abortion.

Nevertheless, the presence of high antibody titers in aborting cows suggests the presence of *N. caninum* infection.

Although no seropositive cows had antibody titers ≥800, the high percentage (88.9%) of seropositive cows that had already aborted at least once suggests that the abortions may have been due to neosporosis since serum titers are higher during gestation when the immune system is activated to control infections, because the parasites are active and may damage the fetus or placenta, potentially resulting in abortion (ALMERÍA; LÓPEZ-GATIUS, 2015).

All evaluated properties contained animals that were seropositive for *N. caninum*. Previous epidemiological studies conducted in different states of Brazil showed that this protozoan was present in 91.7-100% of the properties (GALVÃO et al., 2011; CAMILLO et al., 2011; MARTINS et al., 2011).

Yániz et al. (2010) observed that the abortion rate in dairy cows was significantly different ($p < 0.05$) between animals that were seropositive (23.6%) and seronegative (2.3%) for *N. caninum*. Hein et al. (2012) evaluated the association between abortion history and the presence of *N. caninum* antibodies in cattle in Rio Grande do Sul, Brazil, and found a significant correlation ($p < 0.001$) between abortion and seropositivity to *N. caninum*, since the percentages of seropositive cows with and without a history of abortion were 58.5% and 16.4%, respectively. Corbellini et al. (2002) reported that the seroprevalence was higher ($p = 0.0053$) in cows that had aborted (23.3%) than in cows that had not aborted (8.3%), and found a positive correlation between the rate of abortion and *N. caninum* infection.

Comparison of the percentages of seropositive cows in this study with at least one abortion to the rates in other studies suggests that neosporosis in Marechal Cândido Rondon, Paraná, may be the primary cause of abortion in dairy cattle because 57.1% of cows with a history of abortion were seropositive for *N. caninum* antibodies.

Andreotti et al. (2010) observed that the rate of pregnancy loss (reabsorption and/or abortion) was 15% higher in seropositive cows than in seronegative cows ($p < 0.0001$), demonstrating that the presence of *N. caninum* affected the reproductive efficiency of cows. Galvão et al. (2011) reported a significant association between infection and the occurrence of abortions and stillbirths, since the rate of abortion was 3.76-fold higher ($p = 0.005$) in seropositive cows than in seronegative cows, and the rate of stillbirths was 7.44-fold higher ($p = 0.012$) in seropositive cows than in seronegative cows.

Paré et al. (1997) indicated that the risk of abortion in *N. caninum* seropositive cows at the time of diagnosis or pregnancy was two-fold higher ($p = 0.006$) than that of seronegative cows. Wouda et al. (1998), Hernandez et al. (2002), Hein et al. (2012), Corbellini et al. (2002), and Yániz et al. (2010) ($p = 0.0001$) observed that the risk of abortion in seropositive cows was 3.1- ($p = 0.0001$), 2.8- ($p = 0.02$), 7.21- ($p < 0.001$), 3.3- ($p = 0.0053$), and 3.2-fold ($p < 0.001$) higher, respectively, than that in seronegative cows. In this study, the risk of abortion in seropositive cows was 10.6-fold higher ($p = 0.004$) than that in seronegative cows.

Thurmond and Hietala (1996) reported that the seroprevalence of *N. caninum* in cow herds was 10-20% or >20%, and abortions were common and might occur throughout the year. Antoniassi et al. (2013) evaluated the cause of bovine fetus abortions in Rio Grande do Sul and found that 33% of the fetuses were infected with *N. caninum*.

Wouda et al. (1998) reported that out of every 10 abortions in seronegative cows, two (20%) might be due to infection with *N. caninum*, suggesting that the rate of postnatal infection was low, i.e., the main route of transmission was transplacental. These authors also suggested that to reduce the risk of abortion in the herd, seropositive young cows should not be used for cow replacement.

Infectious agents other than *N. caninum* may cause abortions in cattle. Santos et al. (2005) reported

that animals can be infected with more than one agent, limiting definitive diagnosis using laboratory tests. These authors performed serological tests in dairy herds with a history of abortion in the northern state of Paraná to assess the presence of antibodies against *N. caninum*, *Toxoplasma gondii*, *Leptospira* spp., *Brucella abortus*, BHV-1, and BVDV and observed that 60% of the animals presented serology compatible with infection by one or more agents and, of these animals, all were seropositive for *N. caninum*, indicating that other infectious diseases might cause abortion, although the most common disease was neosporosis.

Conclusion

In the analyzed animals, cows with a history of abortion were more likely to be seropositive for *N. caninum* than cows without a history of abortion.

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