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Association between seropositivity for *Neospora caninum* and reproductive performance of beef heifers in the Pantanal of Mato Grosso do Sul, Brazil

Associação entre soropositividade para *Neospora caninum* e o desempenho reprodutivo de novilhas de corte no Pantanal Sul-Mato-Grossense, Brasil

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Abstract

Neospora caninum is an obligate intracellular parasite that can infect domestic and wild canids, ruminants, and horses. The purpose of this study was to evaluate the association between the presence of antibodies to *N. caninum* and reproductive loss in beef heifers in the South Pantanal region of Mato Grosso, Brazil. A total of 1098 heifers were evaluated from breeding to calving with regard to reproductive performance, and serology for neosporosis was assayed by indirect immunofluorescence reaction. The prevalence of *N. caninum* in cows that had failed to conceive was 22.13% higher than in those that had conceived ($p < 0.0001$), indicating a significant correlation between non-conception and presence of anti-*N. caninum* antibodies. The extraction rates for seropositive and seronegative heifers were 28.24 and 50.12%, respectively, with a statistically significant value ($p < 0.0001$) that showed a decrease of 44% in the extraction rate of seropositive animals. The results emphasize the importance of diagnosis for monitoring of neosporosis in primiparous cows, and indicators of seropositive females as a factor in strategies for culling the beef cattle herd.

Keywords: Reproduction, bovine, diagnostic, abortion, *Neospora caninum*.

Resumo

Neospora caninum é um parasita intracelular obrigatório que pode infectar canídeos domésticos e selvagens, ruminantes e eqüídeos. Neste trabalho estudou-se a associação entre soropositividade para *Neospora caninum* e perda reprodutiva em novilhas de corte na região do Pantanal Sul-Mato-Grossense. Foram avaliadas 1098 novilhas da estação de reprodução ao nascimento dos bezerros com relação ao desempenho reprodutivo e realizada a sorologia para *N. caninum* através da reação de imunofluorescência indireta. A soroprevalência de *N. caninum* nas vacas que falhara na concepção foi 22,13% maior que nas que conceberam ($p < 0,0001$), indicando que há uma correlação significativa entre a não concepção e a presença de anticorpos anti-*N. caninum*. As taxas de desfrute para as novilhas soropositivas e soronegativas para neosporose foram de 28,24 e 50,12%, respectivamente, mostrando uma redução de 44% na taxa de desfrute de novilhas soropositivas para *N. caninum*. Ressalta-se a importância do monitoramento do diagnóstico da neosporose em primíparas, e o indicador de fêmeas soropositivas como um fator nas estratégias de descarte no rebanho bovino de corte.

Palavras-chave: Reprodução, bovinos, diagnóstico, aborto, *Neospora caninum*.

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Introduction

Neospora caninum is an obligate intracellular parasite that until 1988 was confused with *Toxoplasma gondii* (LINDSAY; DUBEY; McALLISTER, 1988). Dogs and coyotes are definitive hosts, which eliminate parasite oocytes in their feces. After sporulation, these oocytes are ingested by an intermediary host and form tissular cysts (LINDSAY et al., 1999; McALLISTER et al., 1998; GONDIM et al., 2004).

Neosporosis has emerged as an important disease in bovines worldwide and is related to reproductive loss in these animals, causing abortions and neonatal mortality (ANDERSON et al., 1991; BARR et al., 1991; THORNTON; THOMPSON; DUBEY, 1991; WOUDE et al., 1999). Establishing the diagnosis of neosporosis is therefore important, as a step in re-breeding and possibly switching dams according to their history of abortions (DUBEY; SCHARES; ORTEGA-MORA, 2007).

Besides being associated with bovines, this protozoon has been associated with the occurrence of sporadic disease in other animals species, including sheep (DUBEY; SCHARES; ORTEGA-MORA, 2007), goats (BARR et al., 1992), and horses (MARSH et al., 1996).

The serologic prevalence of *N. caninum* in beef cattle in Canada, as obtained from random samples of confined animals, was 6.5% positive animals. Prevalence in blood samples from auction animals was 9.0%. As opposed to this low seropositivity, a study on the association between *N. caninum* infection and reproductive performance found that 30% of the cattle selected for the study were seropositive (HADDAD; DOHOO; VANLEEWEN, 2005). As for groups in which abortion had been documented and no antibodies for other pathogens were found, between 17 and 45% of aborted fetuses were seropositive for *N. caninum* (PITEL et al., 2001).

The presence of positive serology for *N. caninum* in beef cattle in the State of Mato Grosso do Sul (MS), Brazil has been established as 30% in heifers (ANDREOTTI et al., 2004) and 14.9% in cows (OSHIRO et al., 2007). An association between seropositivity and abortion was found in 69.8% of bovine herds (OSHIRO et al., 2007). Seropositivity was 29.5% for anti-*N. caninum* antibodies in embryo-receptor heifers of beef cattle raised extensively (PAZ; LEITE; ROCHA, 2007).

Most abortions due to infection by *Neospora* are diagnosed between 3 and 8 months of gestation, although abortions that occur early in the pregnancy are more difficult to diagnose due to a lack of fetal material necessary for analysis (INNES, 2007).

As for vertical transmission of *N. caninum*, infected heifers that become healthy adults may transmit the agent to the fetus, which is especially vulnerable, thereby maintaining the parasite in the herd (ANDERSON et al., 1997; INNES et al., 2005). Transplacental transmission has been reported in at least 36.8% of neosporosis-positive heifers and may reach 100% in experimental infections (THURMOND; HIETALA, 1996; WILLIAMS, et al., 2000; INNES, et al., 2005; VIANA et al., 2008).

Here we studied the association between *N. caninum* antibodies and reproductive loss in heifers of beef cattle of the south Pantanal region of Mato Grosso State, Brazil. Our results allow us to measure the bottleneck of the reproductive efficiency in these animals.

Material and Methods

1. Study area

The study was done in a farm located in the South Pantanal region of Mato Grosso State, Brazil (20° 06' 05" S and 56° 47' 43" W). It is an area of plains subjected to dry and flood seasons and suffers periodic inundations. The climate is hot and rainy in the summer and dry and pleasant in the winter, with average temperatures of 32 and 21 °C, respectively. The ground has low natural fertility (CADAVID GARCIA, 1986).

The reproductive system on the farm is comprised of breeding, rebreeding, and fattening. The areas dedicated to cattle have cultivated pastures of marandu-grass (*Brachiaria brizantha* cv. Marandu) and guinea-grass (*Panicum maximum*). The field system is characterized by a low number of pastures and a defined season for mating in which andrologic examination of the bulls is done and separate handling is adopted for heifers and reproductive cows.

2. Sample collection

A total of 1098 Nelore heifers aging around 24 months and with average weight of 259.14 kg were evaluated. The mating season regime was artificial insemination with repeated bulls (1:35) during the period of November 2007 and March 2008. Diagnosis of gestation was based on a rectal palpation done around 60 days after mating. Animals were followed during the reproductive regime until the calves were one month of age.

Information about the herds was collected based on the handling routine at the farm. We collected blood samples from cows twice, once at the diagnosis of gestation, around 60 days after mating, and again a month after delivery. Blood samples were also collected from heifers when they were around one month old. After identification, sera were kept in -20 °C until serologic tests were done.

3. Indirect immunofluorescence reaction (RIFI)

Samples were examined by RIFI to detect the presence of antibodies against *N. caninum*. The antigen was produced in cultures of tachyzoites of *N. caninum*, strain NC-1 (DUBEY et al., 1988) in Vero cells (OLIVEIRA et al., 2004). A commercial conjugate anti-IgG bovine (Sigma) was used at a dilution of 1:10,000 and the samples were tested at a dilution of 1:50 (PARÉ; THURMOND; HIETALA, 1996). A positive and negative serum sample was included in each slide.

4. Statistical analysis

To determine sample size we used the population proportion estimate ($n = (Z\alpha/2)^2 \cdot 0.25/E$) according to Levine, Berenson e Stephan (2000), with 95% of confidence and 3% error. A total of 1098 primiparous Nelore heifers were analyzed.

For statistical analysis of prevalence results of the neosporosis diagnosis in pregnant heifers, as well as of the abortion or conception (birth) prevalence, we used 2 × 2 contingency tables as determined by chi-square (χ^2) test (SAMPAIO, 2007), using the statistical program MedCalc® 8.0.0.0.

Results and Discussion

There is no consensus for a cut-off value to be used as reference for the diagnosis of neosporosis. Additionally, this value could be affected by the animal age or disease stage, the strategy used for diagnosis, and laboratory conditions (ALVAREZ-GARCIA et al., 2003; Von BLUMRÖDER et al., 2004; DUBEY; SCHARES, 2006). Serological tests for the identification of infected animals may require higher sensitivity and lower cut-off than suggested for the diagnosis of bovine abortion. For these reasons we defined a cut-off value that would provide greater sensitivity to the test (1:50).

Cow blood serum samples of the first and second collection by RIFI showed similar results, with only three sera being converted into positive in the second collection. Therefore, we considered the information from animals with two positive results for the serological analysis in order to co-relate the results with births.

The diagnosis of gestation by palpation verified a total of 546 pregnant cows, which corresponded to 49.73% of the whole herd. The diagnosis of gestation in the positive and negative categories for *N. caninum* serology showed significant difference ($p < 0.0001$) between groups (Table 1).

Loss of pregnancy was 15% higher in seropositive cows (55.89%) as compared to loss in *N. caninum* seronegative cows (40.88%), showing that in this bovine herd the presence of positive *N. caninum* serology is a relevant factor for female reproduction. It has been shown that a seropositive cow that suffers an abortion has a 5.7 times greater risk of having an abortion in subsequent gestations (THURMOND; HIETALA, 1997).

During the period between the beginning of the mating season and the gestation diagnosis in cows negative for *N. caninum*, we verified a loss of 40.88%, with an additional percentage of 9% of loss until birth. In cows positive for *N. caninum*, loss in the first period was 55.89%, and in the second it was 15.87%. The results revealed a higher impact of reproductive loss in both categories between the beginning of the mating season and the diagnosis of gestation.

Seroprevalence of *N. caninum* in cows that did not conceive was 22.13% higher than in those that conceived ($p < 0.0001$), which indicates a significant correlation between not conceiving and the presence of anti-*N. caninum* antibodies. These results reveal that the loss between the gestation diagnosis period and conception in seropositive cows was 36%, while in seronegative cows it was 15.23%, indicating that positive serology for *N. caninum* affects the conception ratio.

One of the main symptoms of seropositivity in bovines is abortion, which may occur due to activation of tachyzoites and their migration to the uterus (DUBEY; SCHARES; ORTEGA-MORA, 2007). Thus, the association between positive serology for *N. caninum* and abortion during the reproductive season, when compared to negative cows, can confirm the presence of this disease in this herd. The positive serology suggests the existence of antibody production from tachyzoites and the presence of at least the latent form, bradyzoites, which remains in the host for the rest of its life.

The prevalence of *N. caninum* in calves in relation to heifers is shown in Table 2. The highest prevalence of seropositive calves

among those born from *N. caninum* seropositive heifers was statistically significant ($p < 0.0001$), suggesting the occurrence of vertical transmission of neospora between the mother and calves, although it was not possible to determine the ratio of vertical transmission in this herd.

There were 38.4% more *N. caninum* seropositive calves born from seropositive cows as compared to seropositive calves born from seronegative cows. However, in this study, blood collection from calves was performed during the first month after birth, which affected the results due to the transmission of immunoglobulin through the colostrum, so it was not possible to verify the immune response in these calves. It is noteworthy that in the conditions found in the field we assume that calves usually feed from their own mothers. Vianna et al. (2008) found positive serology of 36.8% in fetuses from *N. caninum* seropositive cows.

A total of 29.12% of calves positive for *N. caninum* serology from negative cows show an infection ratio from the environment that suggests a careful analysis. In endemic conditions, the oocytes may be found in the pastures and in the water. The definitive host and other native species of the region favor the maintenance of the agent in the environment. This combined with a lack of control of the disease on the farm and the discarding of the carcasses of dead animals in the field favor the persistence of the risk factors associated with contamination.

The extraction rates of heifers that were either seropositive or seronegative for *N. caninum* were 28.24 and 50.12%, respectively, which shows a reduction of 21.88% in the extraction rate of *N. caninum* seropositive heifers ($p < 0.0001$). When compared with weaning and natality indexes of beef cattle herds that are raised in native pastures in the Pantanal, which are considered low in relation to dense and covered tropical regions of the country, the values for *N. caninum* seropositive heifers are close to 50 and 58%, as found by Brasil (1978) and Cadavid Garcia (1981, 1986).

When the extraction rate of *N. caninum* seropositive heifers is considered, we observe a strong reduction capable of causing impact in the reproductive system, to a degree that varies according to the age of the female.

In Brazilian bovine herds a discard system that considers the *N. caninum* serology of the animal is not in place. In this study we found that cows seropositive for *N. caninum* are 1.44 times more prone to lose calves than are seronegative cows. The risk of abortion for cows infected with *N. caninum* was 1.7, and 3 to 7 times higher in seropositive cows as compared to seronegative cows (THURMOND; HIETALA, 1997).

Due to differences in the effect of risk factors associated with abortion in beef and dairy cattle observed in different regions and handling conditions, control strategies should be diversified and adopted based on the cost/benefit analysis for the reproductive system. In this case, the parameters to be considered are the production system, the handling conditions, the prevalence of the disease in the herd, the predominant transmission route, the existence of biosecurity measures in the propriety, and the effects of infection calculated in the productive and reproductive performance (DUBEY; SCHARES; ORTEGA-MORA, 2007).

Our results show that the reproduction of *N. caninum* seropositive primiparous cows has an impact of 44% in relation to seronegative heifers, which suggests the need for controlling

Table 1. Association between *N. caninum* seroprevalence in heifers, reproductive status, and conception.

Heifers	Reproductive status		Conception		Extraction rate
	Pregnant (%)	Not pregnant (%)	Conceived	Did not conceive	
Seropositive	303 (44.11)	384 (55.89)	194	493	28.24%
Seronegative	243 (59.12)	168 (40.88)	206	205	50.12%
Total	546 (49.73)	552 (50.27)	400	698	36.43%

$\chi^2_g = 22.607$ ($p < 0.0001$) e $\chi^2_c = 52.233$ ($p < 0.0001$), in which χ^2_g is the relation between reproductive status and *N. caninum* seroprevalence and χ^2_c is the relation between conception and *N. caninum* seroprevalence.

Table 2. Seroprevalence of anti-*Neospora caninum* antibodies as determined by RIFI in calves born from *Neospora caninum* seropositive or seronegative heifers.

Heifers	Calves		Prevalence
	Seropositive	Seronegative	
Seropositive	131	63	67.53%
Seronegative	60	146	29.12%
Total	191	209	47.75%

$\chi^2_b = 57.518$, where χ^2_b is the relation between serologic diagnosis of *N. caninum* among calves and their mothers, with degree of significance ($p < 0.0001$). Serologic exam: RIFI (1:50).

the herds in the Pantanal region. The existence of flooding areas and wild animals, especially canids, should be further investigated to evaluate whether they are risk factors for this disease.

Losses during gestation may be the consequence of misdiagnosis of gestation, abortion, absorption of the embryo, or occurrence of infectious diseases that affect reproduction. Presence of neosporosis in this animal category in the system of beef cattle in the Pantanal contributes for a lower extraction rate of the herd. Thus, developing measures for the control of neosporosis in this animal category is very important.

In beef cattle herds of the Pantanal of South Mato Grosso, presence of *N. caninum* seropositive heifers causes a significant impact on reproduction, with a reduction of 21.88% in the extraction rate of the herd. Thus, we would like to highlight the importance of performing evaluations and procedures to control this disease in the herd, of establishing a control mechanism for buying and introducing new animals, and of the use of serology for *N. caninum* as an indication for the discarding of animals in bovine herds.

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