



Abortions in bovines and *Neospora caninum* transmission in an embryo transfer center

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ABSTRACT

Neosporosis is considered to be one of the main causes of abortions in bovines. In this study we evaluated the congenital transmission and occurrence of abortions by *Neospora caninum* in an embryo transfer center in Nerópolis, Goiás. Serological samples from 101 recipients, 61 donors, and 90 calves were analyzed. Among these animals, 32.67% of the recipients, 22.22% of the donors, and 6.66% of the calves were positive for *N. caninum*. The rate of vertical transmission was 24%. There was a statistically significant difference between antibody titer of the recipient cows and the serological status of the calves. There was no statistically significant relationship between *N. caninum* serological status and reproductive rates such as estrum repetition and embryo production. Eight abortions occurred, six (75%) in positive females and two (25%) in negative females. These results indicate that neosporosis may be an important cause of failing reproduction and that in embryo transfer centers recipients should be previously examined, and those that are seronegative for *Neospora* should be chosen to reduce abortion and the birth of seropositive calves.

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1. Introduction

The protozoan *Neospora caninum* was identified in 1989 as an etiologic agent of abortions in bovines (Thilsted and Dubey, 1989). Later, dogs (McAllister et al., 1998) and coyotes (Gondim et al., 2004) were confirmed to be the definitive hosts. More recently, the DNA of *N. caninum* has been found in the feces of red foxes (*Vulpes vulpes*) in Canada (Wapenaar et al., 2006).

Neosporosis is considered to be one of the main causes of abortions in bovines in various regions of the world. The main transmission route in bovines is vertical or endogenous, being transmitted from mother to fetus. This route

may cause abortions or lead to the birth of calves chronically infected or with neurological symptoms. Cows may also have reproductive defects, such as estrum repetition and infertility (Dubey, 2003).

In this study we evaluated the congenital transmission and occurrence of abortions by *N. caninum* in an embryo transfer center in Nerópolis, Goiás. We aimed to demonstrate an association between serological status of the cows (recipient and donor) and the subsequent calves.

2. Materials and methods

2.1. Animals

This study was done in an embryo transfer center located in the county of Nerópolis, Goiás, Brazil. Data on reproduction, such as estrum repetitions, abortions, and the number of embryos produced were obtained from indi-

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vidual charts belonging to the databank of the institution itself.

Female bovine donors were of the Nelore breed and the recipients were mixed breed (Zebu/Holstein). All animals used for embryo transfer were serologically negative for brucellosis and tuberculosis and had been vaccinated against leptospirosis, infectious bovine rhinotracheitis (IBR), and bovine viral diarrhea (BVD). Additionally, they were all treated for leptospirosis with antibiotics before each program.

Embryo transfer (ET) was always done by the same technician according to the International Embryo Transfer Society (Stringfellow and Seidel, 1998). The donors were super-ovulated and the insemination done at a specific time, according to estrum synchronization between the recipients and donors.

2.2. Sample collection

Blood samples were taken from 61 donors, 101 recipients, and 90 calves. Blood samples were drawn after involution of the recipients. To disturb animals as little as possible, blood samples were not collected before the ET program, as donors were being inseminated and would pass through the process of embryo collection, and the recipients presented recent gestation. The first blood sample was collected from recipients 60 days following gestation and from donors after the ET program was over. In adult animals, each venous blood sample was collected from the coccygeal vein while in calves it was collected from the jugular vein into Vacutainer® tubes, without anticoagulant so the serum could be collected. Blood collection from the recipients was done throughout the gestation, at intervals of about 60 days, with an average of 4 or 5 collections for each animal. In calves, the samples were taken soon after birth, before the ingestion of colostrum, and until seven months of age, with an average of three to five collections for each calf. Samples were taken to the laboratory of the Center of Parasitology of the Veterinary School of the Federal University of Goiás and were centrifuged to obtain the serum, which was then identified and maintained at -20°C until analysis.

2.3. Detection of anti-*N. caninum* antibodies

The indirect fluorescent antibody test (IFAT) was used to detect specific anti-*N. caninum* antibodies. The antigen was prepared by multiplying the tachyzoites of *N. caninum* (sample Nc-1) in Marc-145 cell cultures. The reaction was done according to Álvarez-garcía et al. (2002). Positive and negative controls were added to every slide.

An initial dilution of the serum at 1:50 was performed, with subsequent double dilutions. The result was considered positive when fluorescence was observed in all the tachyzoites, at a dilution of 1:200. In positive samples, the titration of the antibodies was done in doubling dilutions, starting from 1:50. The conjugated anti-IgG bovine (Sigma®) was diluted at 1:300 in a 0.2% Evans blue/PBS solution.

Table 1

Occurrence of anti-*N. caninum* antibodies in bovines from the embryo transfer center, by IFAT (cut off $\geq 1:200$) in the period between April 2005 and October 2006, Nerópolis, Goiás.

Animals	Positive	Negative	Total
Recipients	33 (32.67%)	68 (67.33%)	101
Donors	16 (26.22%)	45 (73.78%)	61
Calves	6 (6.66%)	84 (93.34%)	90
Total	55 (21.82%)	197 (78.18%)	252

2.4. Evaluation of vertical transmission and titration of maternal antibodies

This evaluation was carried out by examining the sera collected from the calves, before the ingestion of colostrum, and by titration of the anti-*N. caninum* antibodies from the sera of positive recipients.

The probability that a vertical transmission had occurred was calculated by the formula $p = x/(x+y)$, in which x is the frequency of positive calves born from positive cows and y is the frequency of negative calves born from positive cows. The confidence interval was measured by the formula $p \pm 1.96 \sqrt{p(1-p)/(x+y)}$ (Sampaio, 1998).

Possible associations between the titre of the mother and a positive serological status of the calf, as well as that between the production of embryos from positive and negative cows, were evaluated by the chi-square test.

3. Results and discussion

3.1. Occurrence of anti-*N. caninum* antibodies

Serum samples from 101 recipients, 61 donors, and 90 calves were analyzed. Of this total, 8 of the 101 recipients aborted and 3 calves died from undetermined causes soon after birth.

Of the 101 recipients, 33 were positive for *N. caninum* by IFAT. Among the donors analyzed in the experiment, 16 were positive, and among the calves, only 6 were positive (Table 1).

A seroprevalence of 32.67% for *N. caninum* was found in this study. This result agrees with those from Melo et al. (2006), who did a study of seroprevalence in the microregions of Goiânia and Anápolis, in Brazil. In that study serum samples were collected from beef and dairy cattle, showing that an average of 30.43% of the animals were seropositive. This result and ours are higher than those found by Brautigam et al. (1996) and Gondim et al. (1999) in dairy herds; those authors found a seroprevalence of 15% in the state of São Paulo and 14% in Bahia, respectively. In Rio Grande do Sul, it was found that 23.3% of the cows with a history of abortions were seropositive for *N. caninum*, while in animals with no history of abortions this prevalence was 8.3% (Corbellini et al., 2002).

In our study, a seroprevalence of 26.2% was found in donors. This rate is lower than that of recipients. This may be explained by the special care that donors received, being kept in exclusive pastures or in individual stables. The breed and type (that is, beef or dairy cows) also may have affected this result, as it has been shown that Zebu cattle

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