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Communication

(*Comunicação*)

Dairy cow abortion associated with *Neospora caninum* and other infectious agents

[*Aborto em vacas leiteiras associado a* Neospora caninum *e a outros agentes infecciosos*]

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The protozoa *Neospora caninum* has been described in several countries as an important cause of abortion in dairy cattle, mainly from the third month of pregnancy. The infection can occur by ingestion of animal tissues infected with bradyzoites or tachyzoites, through water or food contaminated with infectious oocysts, besides by vertical transmission. Abortion may be the only sign observed in adult cows (Björkman et al., 1996; Dubey, 1999a). Vaccines are not available to prevent oocysts elimination by dogs, the definitive host of *N. caninum* (McAllister et al., 1998).

This study aimed to verify antibodies to *N. caninum*, *T. gondii, B. abortus, Leptospira* spp., Bovine Herpesvirus-1 (BHV-1) and Bovine Viral Diarrhea Virus (BVDV) in serum of dairy cattle herds that miscarried, one or more times, between the third and the seventh month of pregnancy, in Northern Paraná State, Brazil.

Thirty-five cow blood samples were collected on the day of abortion, and 20 and 65 days later. The separated sera were processed by indirect immunofluorescent assay (IFA) for the detection of anti-*N. caninum* (Conrad et al.,1993) and anti-*T.gondii* (Camargo, 1973) antibodies, using slides adsorbed with anti-*N. caninum* sera (VMRD-Pullman-EUA) and anti-*T. gondii* sera (DMVP-UEL), respectively. Reactions with titles $\geq$ 200 to *N. caninum* and $\geq$ 64 to *T. gondii* were considered positive. Microscopic serum-agglutination test (SAM) was carried out for the detection of *Leptospira* sp. antibodies. Live cultures of 22 *Leptospira* spp. serovars were used. The sera were initially diluted at 1:100 and those that presented 2+ or higher agglutination at this dilution were considered positive. Acid buffered antigen test was used to detect antibodies to *B. abortus* and seroneutralization technique, using MDBK cells, was performed to detect BHV-1 and BVDV antibodies. Reactions with titles $\geq$ 8 were considered positive. The sera were serially diluted until the maximum positive dilution was determined.

The interpretation of seroposotivity, as indicative of infection or vaccinal response, was based on consecutive serology and epidemiologic survey of aborted cows. Overall 35 aborted cows, 21 (60%) were seropositive to some of the investigated agents. The serological titles observed in 12 (34.3%) aborted cows were in line with vaccinal response to *Leptospira* sp., BHV-1 and BVDV. Two (2/35) animals (5.7%) were seronegative to all the evaluated agents.

From 21 (21/35) seropositive cows, five (5/21) were seroreactive to *N. caninum*, two (2/21) to *T. gondii*, nine (9/21) to *Leptospira* sp., 12 (12/21) to BVDV and 15 (15/21) to BHV-1. Seven cows

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(7/21) were seropositive to a single agent (four to BHV-1 and three to BVDV). Nine different associations among two or more investigated agents were observed in 14 cows (Table 1). None of the tested sera showed reactivity to *B. abortus.* Aborted fetuses from five (5/21) *N. caninum* seropositive cows were analyzed and in all of them the presence of the protozoa were confirmed by immunohistochemistry, using anti-*N. caninum* sera (Santos, 2000).

Table 1. Associations among *Neospora caninum*, *Toxocara gondii*, *Leptospira* sp., BHV-1 and BVDV observed in dairy cows with a history of abortion, with titles indicative of infection in herds from Northern Paraná State

Infectious agent	Number of seropositive cows
N. caninum, Leptospira sp., BHV-1, BVDV	2
N. caninum, T. gondii, BHV-1, BVDV	1
N. caninum, BHV-1, BVDV	1
N. caninum, BHV-1	1
Leptospira sp., BHV-1, BVDV	1
Leptospira sp., BHV-1	3
Leptospira sp., BVDV	2
BHV-1, BVDV	2
Leptospira sp., *T. gondii*	1
BHV-1	4
BVDV	3
TOTAL	21

Through the serology, 14.3% of the abortion reports cows were seroreactants to *N. canimum*. Similar results were also observed by Ogawa et al. (1999), who found 11.7% of dairy cattle seroreactants to *N. caninum*, in Northern Paraná State. All the *N. canimum* seropositive cows were also, simultaneously, seroreactants to one or more potentially abortive agent (Table 1). Alves et al. (1996) stated that out of 32 herds diagnosed with *N. caninum,* five were, simultaneously, infected by BVDV. Thurmond et al. (1997) studying 20 bovine herds, with fetal losses cases, observed that *N. caninum* contributed to the abortions in one of them, but not in the others, where BVDV and *Leptospira* sp. were also detected. Barr et al. (1991) reported that maternal viral infection (BHV-1/BVDV) increases the susceptibility to *N. caninum* infection. The serological titles observed for *T. gondii* in two cows were not considered an infection, because this parasite is not related to cattle abortion (Björkman et al., 1996).

Infection by *N. caninum* can not be the only cause of reproductive disorders in bovine (Alves et al., 1996; Otter and Wilson, 1997; Caldow, 1998). It is important to look at the synergism among *N. caninum* and other factors like virus infections, immunossupressive agents or hereditary factors (Björkman et al., 1996). Histological and immunohistochemical procedures from aborted fetuses must be performed to confirm the etiology of *N. caninum* in abortion. Serological tests have not been conclusive to discard abortions by neosporosis in *N. caninum* infections (Dubey, 1999b).

This study showed, for the first time, the presence of *N. caninum* antibodies in cows with a history of abortion in Paraná State, Brazil. Evidences of participation of other potentially abortive agents (*Leptospira* spp., BHV-1 and BVDV) associated with *N. caninum* in evaluated cows were also observed.

Keywords: cattle, abortion, *Neospora caninum,* reproductive disorder

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RESUMO

Trinta e cinco vacas de rebanhos leiteiros da região Norte do estado do Paraná, com histórico de abortamento, foram pesquisadas sorologicamente para verificar a presença de anticorpos contra Neospora caninum, Toxoplasma gondii, Leptospira spp., Brucella abortus, *BHV-1 e BVDV. Vinte e uma vacas apresentaram títulos sorológicos compatíveis com infecção. Todas elas, soropositivas para* N. caninum, *foram também soropositivas para outros agentes infecciosos, sugerindo a possibilidade de associação desses agentes nos problemas reprodutivos de bovinos, no estado do Paraná.*

Palavras-chave: bovino, aborto, Neospora caninum, *problema reprodutivo*

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