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Dynamics of lymphocyte subpopulations in *Neospora* chronically infected cows throughout pregnancy

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Abstract

Vertical transmission of *Neospora caninum* is very efficient (80-90%) and can occur in chronically infected cows through successive pregnancies probably due to reactivation of the parasite during gestation. The factors involved in the reactivation are unknown. The purpose of the present study was to investigate the immune responses related to reactivation of *N. caninum* chronic infections in pregnant cattle.

Phenotypic analysis of peripheral blood lymphocyte subpopulations (PBLs) by flow cytometry, presence of *N. caninum* DNA in blood samples by the polymerase chain reaction (PCR) and quantification of *Neospora* specific serum antibodies by ELISA were performed throughout pregnancy in *Neospora* seropositive dams and compared to seronegative control pregnant dams. Blood samples were collected by caudal vein puncture at 60 d , 120 d, 160 d, 180 d, 215 d 240 d of pregnancy and at parturition and isolation of PBLs and DNA extraction were performed. Sera samples were also collected at same time and antibodies titres analysed by ELISA.

Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Ficoll density gradients followed by repeated washing with RPMI-1640. Immunofluorescence staining was performed using monoclonal antibodies MM1A (IgG1, Anti-CD3), CACT138A (IgG1, anti-CD4), CACT80C (IgG1, anti-CD8), BAQ155A(IgG1, anti-B-B4) and CACT116A (IgG1, anti-A5/IL-2R). Flow cytometry analyses were performed with an Epics Profile II flow cytometer (Coulter, Hialeah, FL, USA).

No statistically significant differences were observed at any sampling date in PBLs subpopulations between seropositive and seronegative pregnant dams. Similar dynamics were observed in both groups with a rise of LT (CD3) and LB from day 215 to parturition. High individual variation was observed among animals in both groups.

Neospora antibody titres remained well above the cut-off limit for the test in all seropositve animals during the study while seronegative animals remained negative. In seropositive animals, mean antibody titres rose during the study to reach a maximum around 210-240 days of pregnancy and slightly decreased at parturition.

The presence of *N. caninum* DNA in blood was observed intermittently in several seropositive animals.

As conclusion, the reactivation of chronic infection in *N. caninum* pregnant dams was followed by parasitaemia, but no significant modulation of PBL cell populations was observed during the course of pregnancy in infected animals compared to non-infected animals. Peripheral blood subpopulations might not reflect the modifications around the cysts at tissue level or vertical transmission could be the result of an occasional rupture of cysts in too low numbers to activate a specific peripheral immune response.