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Dynamics of anti-*Neospora caninum* antibodies during gestation in chronically infected dairy cows

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Abstract

The dynamics of antibody production against *Neospora caninum* during the gestation period was examined in chronically infected dairy cows. Data were obtained from 86 pregnant parous dairy cows, 21 of which had suffered abortion. The cows belonged to two herds in which a diagnosis of *N. caninum* infection had been previously confirmed in aborted foetuses. Pregnancy diagnosis and blood collection were performed on post-insemination Days 40, 90, 120, 150, 180, 210, and at parturition or until the time of abortion detection. Blood plasma was tested for antibodies against *N. caninum* using ELISA. The non-aborting cows were divided into two groups according to whether their antibody values in the second half of gestation had increased or not, while aborting cows were classified as those showing an antibody peak before abortion or those not showing a pre-abortion peak. Differences in antibody values throughout pregnancy in each group of non-aborting and aborting cows were analysed by GLM repeated measures of analysis of variance. While 32 non-aborting cows (49%) showed a significant and consistent increase in anti-*Neospora* antibody values during the second half of gestation, antibody values in the remaining 33 non-aborting cows were practically constant throughout gestation. An antibody peak around abortion was observed in 11 aborting cows (52%), while antibody values in the remaining 10 aborting cows were similar before and at abortion. Seroprevalence fluctuations, defined as seronegative blood samples at some point during the gestation period, were, furthermore, observed in 2 aborting and 11 non-aborting cows. Our results indicate two clearly distinguishable types of humoral immune dynamics throughout gestation: an increased or flat production of antibodies during the second half of gestation in non-aborting animals and before abortion in aborting cows. The observation that some *Neospora*-infected dams can exhibit negative antibody values at any time during gestation, particularly at parturition or abortion, prompts future studies designed to explore the use of new ELISA strategies at the farm level.

Keywords: *Neospora caninum*; Antibody response; Gestation; Dairy cows

1. Introduction

Neospora caninum is an obligate intracellular parasite recognized, since its link with abortion in the late 1980s, as a major cause of abortion and reproductive failure in

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cattle throughout the world (Dubey and Lindsay, 1996; Dubey, 2003). Cows infected with the parasite are three to seven times more likely to abort compared to uninfected cows (Innes et al., 2005) although there have been reports of seropositive animals showing a 23-fold risk of abortion relative to healthy cows (Weston et al., 2005). In our geographical area of study, the risk of abortion is 12–19 times greater in *Neospora*-seropositive dairy cows than in their seronegative counterparts (López-Gatius et al., 2004a,b).

Bovine neosporosis develops either as the result of maternal infection during gestation or following recrudescence of a persistent infection of the dam during gestation, denoted exogenous or endogenous transplacental infection, respectively (Trees and Williams, 2005). Infection seems to persist during the life of a congenitally infected cow, which may abort or infect the foetus in successive gestations (Hietala and Thurmond, 1999; Davison et al., 1999; Dubey et al., 2006). Such endogenous transplacental infection is associated with an acute increase in maternal antibodies (Guy et al., 2001; Weston et al., 2005). Using ELISA tests in naturally infected animals, a peak in *N. caninum* antibody values at 5–6 months of gestation in both aborting and non-aborting animals was described in 18 heifers during two consecutive pregnancies (Stenlund et al., 1999) and in another group of 18 heifers (Weston et al., 2005). The serological profile of three cows during three consecutive gestations showed a clear increase during the third trimester of gestation (Piergili Fioretti et al., 2003). Moreover, in 16 non-aborting cows, there was a significant increase in antibody values during the second half of gestation compared to the first half (López-Gatius et al., 2007). It has been suggested (Stenlund et al., 1999; Guy et al., 2001; Weston et al., 2005) that different antibody responses are produced throughout gestation in cattle depending on the time when parasitaemia can occur (early-, mid- or late- gestation). Based on monthly serological screening, the objective of the present study was to characterize possible patterns of antibody dynamics during gestation in dairy cows naturally infected with *N. caninum*. Seroprevalence fluctuations throughout the gestation period were also evaluated.

2. Materials and methods

2.1. Cattle and herd management

The study was performed on two commercial Holstein–Friesian dairy herds in northeast Spain. The herds had previously confirmed cases of *N. caninum* infection in aborted foetuses and were free of dogs. Cows that became pregnant from March 2003 to December 2005 were included in the study (160 in one herd and 570 animals in the other), to give 730 mature cows in the two herds. The cows, reared within the herds, calved all year round and were milked three times per day. Mean annual milk production was 10,900 kg per cow. All animals were tuberculosis and brucellosis free, as shown by yearly tests from 1985 to 2006. Coinciding with these tests, yearly checks for neosporosis had also been undertaken from 2002 to 2006. The mean *Neospora* seroprevalence for both herds was 25% during the study period. Vaccination programs were undertaken for the prevention of bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR). Modified live vaccines were used for animals 6–8 months old. Pregnant animals were given killed vaccines during the 7th month of each gestation period. Parous cows that were not pregnant on Day 150 post partum received a further killed vaccine. All animals were bred by artificial insemination using semen from 22 independent bulls of proven fertility. The study population only included animals testing positive for *Neospora* at least two times before gestation. The final data analysed corresponded to 86 pregnant parous cows.

2.2. Pregnancy diagnosis and blood sample collection

Pregnancy was diagnosed by trans-rectal ultrasonography on Day 40 post-insemination and by palpation per rectum on Days 90, 120, 150, 180 and 210. Abortion was recorded when signs of abortion were observed after Day 90. The outcome of pregnancy was recorded for all non-aborting animals. Animals were observed daily for signs of abortion from 120 days of gestation until parturition. Blood samples were collected from each animal into heparinized vacuum tubes (BD Vacutainer™, Becton-Dickenson and Company, Plymouth, UK) immediately before each pregnancy diagnosis, during gestation and at parturition in non-aborting cows or during gestation until the time of abortion detection and at abortion in aborting cows. These blood samples were centrifuged (10 min, 1600 × *g*) and the plasma stored at −20 °C until analysis.

2.3. Serological diagnosis

Samples were tested for antibodies against *N. caninum* using a commercial enzyme-linked immunosorbent assay (ELISA) kit (CIVTEST® anti-*Neospora*; Hipra, Girona, Spain), based on the whole tachyzoite lysate of *N. caninum* NC-1. This test has been previously validated (López-Gatius et al., 2004b) and is performed