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Protection against abortion linked to gamma interferon production in pregnant dairy cows naturally infected with *Neospora caninum*

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Abstract

Many immunological aspects of pregnancy, such as the role played by gamma interferon (IFN-γ) in abortion, are not well understood. *Neospora caninum* is an intracellular protozoan considered to be among the main causes of abortion in cattle worldwide. The present study analyzes the interaction between IFN-γ production and *N. caninum* infection in naturally infected pregnant cows. Data were obtained from 126 pregnant cows: 86 seropositive and 40 seronegative for the parasite. Pregnancy diagnosis and blood sample collection were performed on days 40, 90, 120, 150, 180 and 210 post-insemination or until the time of abortion detection. Plasma was tested for antibodies against *N. caninum* and IFN-γ. Interferon-gamma was detected at some point along the pregnancy in 16 (19%) of the 86 *Neospora*-seropositive cows yet was undetectable in the 40 seronegative animals. Of the 126 pregnancies examined, 22 (17.5%) ended in abortion. Abortion occurred in 24.4% of seropositive cows (21/86) and in 2.5% of seronegative animals (1/40). Significant ($P < 0.0001$) interaction was observed between *Neospora*-seropositivity and IFN-γ production. Based on the odds ratio, the risk of abortion was 15.6 times higher in seropositive cows not producing IFN-γ than in seronegative animals, whereas neosporosis had no effect in seropositive cows with IFN-γ production. A significant ($P = 0.001$) negative effect of IFN-γ production on the *Neospora* titer was furthermore observed in the 65 non-aborting seropositive animals. These results indicate that IFN-γ production affords protection against abortion in *Neospora*-infected cows and also point to a reduced humoral immune response to *N. caninum* during gestation in cows producing IFN-γ.

Keywords: *Neospora caninum*; Abortion; Gamma interferon; Dairy cows

1. Introduction

Neospora caninum, an obligate intracellular protozoan closely related to *Toxoplasma gondii*, was first described in the 1980s [1] and has been identified in a wide range of warm-blooded animals [2]. Since then, bovine neosporosis has emerged as a disease of

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international concern, being recognized as a major cause of abortion and congenital infection in cattle worldwide [3–5]. Parasites cross the placenta and infect the fetus causing abortion or congenital infection. The incidence of *N. caninum*-associated abortions peaks in the 5th–7th month of gestation [6–8]. Bovine neosporosis appears either as the result of maternal infection during gestation or following recrudescence of a persistent infection of the dam during gestation, denoted exogenous and endogenous transplacental infection, respectively [9]. Infection seems to persist for the life of a congenitally infected cow, which may abort or infect the fetus in successive gestations [2], and can cause repeated abortion [10]. In effect, *Neospora*-seropositive cows are more likely to abort than seronegative cows [11,12]. For example, we observed that cows with *N. caninum* antibodies in infected herds in northeast Spain had a 12- to 19-fold higher risk of abortion than their seronegative partners, and abortion rates ranged from 30 to 44% in seropositive animals [13,14]. In addition, endogenous transplacental infection is associated with an acute increase in maternal antibodies [15].

There is evidence that *N. caninum* is a primary cause of abortion, but it is difficult to explain why infected cows abort or not [2]. As for infections caused by *Toxoplasma* or other intracellular parasites, cell-mediated immune mechanisms are attributed the most important role in reducing the multiplication of *N. caninum* within the host, hence reducing parasitemia. Th1-type immunity involving proinflammatory cytokines such as gamma interferon (IFN-γ) is critical for the development of host protective immunity [16], and IFN-γ production has been shown to protect against *N. caninum* both in murine models [17,18] and in experimentally infected cattle [19–22]. However, the production of IFN-γ, although very effective in non-pregnant animals, may be involved in the pathogenesis of fetal rejection during gestation [21]. In pregnant mice infected with the protozoan *Leishmania major*, a bias toward Th1 cytokines has been associated with smaller lesions and resolving infection, but also with increased fetal mortality [23]. Similarly, natural immune-modulation of gestation can be related to abortion control, but not to the control of neosporosis infection [24–26]. Transitory immune-suppression of T lymphocytes, starting at around 18 weeks of gestation, has been observed in cattle experimentally infected with *N. caninum* [27] and could be the cause of the increased susceptibility of these animals to parasitemia at that time [18,28]. Infection by environmental *N. caninum* oocysts or reactivation of a chronic infection leading to the release of bradyzoites from the tissue cyst in infected animals, will elicit high levels of IFN-γ, and may notably contribute to bovine abortion [29,30].

Presently, it remains unknown if host IFN-γ production in pregnant cattle naturally infected with *N. caninum* promotes abortion or, conversely, protects against abortion. No drugs exist to treat bovine neosporosis nor do we have effective vaccines for its control [21]. Understanding the immune response in cattle naturally infected with *N. caninum* may help design vaccination strategies. The purpose of the present study was to investigate the possible relationship between IFN-γ production and pregnancy outcome in chronically *Neospora*-infected dairy cows.

2. Materials and methods

2.1. Cattle and herd management

This 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 fetuses and were free of dogs. Cows that became pregnant from March 2003 to December 2005 were included in the study, comprising a mean of 730 mature cows in the two herds (160 and 570 animals per herd). The cows, reared within the herds, calved all the 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, annual control for neosporosis was also performed 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 receiving their last vaccine four months or longer before the first blood collection. For every two *Neospora*-seropositive cows used in the study, one seronegative cow was added as a control. The final data analyzed were obtained from 126 pregnant parous cows: 86 seropositive and 40 seronegative. The seropositive cows were known to be *Neospora*-seropositive at least one year before they became pregnant.