Experimental Parasitology 168 (2016) 62–69

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Foetal death in naive heifers inoculated with *Neospora caninum* isolate Nc-Spain7 at 110 days of pregnancy

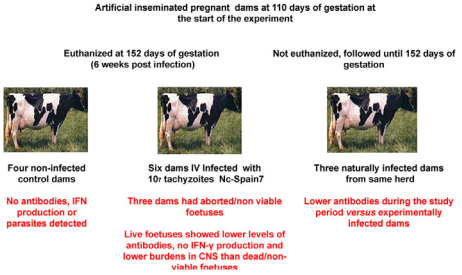
Sonia Almería [a, b, *], Beatriz Serrano-Perez [c], Laila Darwich [a, b], Mariano Domingo [a, b], Ramon Mur-Novales [c], Javier Regidor-Cerrillo [d], Oscar Cabezón [e], Mónica Pérez-Maillo [a], Irene Lopez-Helguera [c], Xavier Fernández-Aguilar [e], Maria Puig-Ribas [e], Luis Miguel Ortega-Mora [d], Irina García-Ispierto [c], Jitender P. Dubey [f], Fernando López-Gatius [c]

[a] Centre de Recerca en Sanitat Animal (CReSA) – Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus UAB, 08193, Bellaterra, Barcelona, Spain
[b] Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona (UAB), 08193, Bellaterra, Barcelona, Spain
[c] Departament de Producció Animal, Universitat de Lleida, Agrotecnio Center, Lleida, Spain
[d] SALUVET Group, Animal Health Department, Veterinary Sciences Faculty, University Complutense of Madrid, Spain
[e] SEFaS, Dept. Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona (UAB), Spain
[f] Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, Agriculture Research Service, United States Department of Agriculture, Beltsville, MD, USA

HIGHLIGHTS

- The study analysed the effect of virulent isolates of bovine neosporosis at mid-gestation.
- Three dams experimentally infected in the second term of gestation suffered fetopathy.
- Changes in temperature, antibody, parasite detection and IFN-γ levels were observed in infected dams and their fetuses.
- Experimentally infected dams had significantly higher antibody levels than naturally infected dams.
- Live foetuses showed lower levels of antibodies, no IFN-γ production and lower burdens in CNS than dead/non-viable foetuses.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form
23 May 2016
Accepted 22 June 2016
Available online 5 July 2016

ABSTRACT

Neospora caninum infection is a leading cause of abortion in cattle worldwide. The pathogenesis of bovine neosporosis, particularly during the second term of gestation when most abortions occur in naturally infected dams, is poorly understood. In the present study foetal death was observed in 3 of 6 experimentally infected dams at 110 days of gestation after 6 weeks of experimental period. All experimental heifers were febrile between 3 and 5 days post infection (dpi). Inoculated dams seroconverted by 3–4 weeks post-infection with higher mean antibody titres in aborting dams compared to non-aborting heifers, although not significantly ($p > 0.05$). *Neospora caninum* DNA was detected in all infected

* Corresponding author. Centre de Recerca en Sanitat Animal (CReSA) – Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Campus UAB, 08193, Bellaterra, Barcelona, Spain.
E-mail address: Sonia.Almeria@uab.cat (S. Almería).

http://dx.doi.org/10.1016/j.exppara.2016.06.009

Keywords:
Neospora caninum
Pathogenesis of abortion
Second term of gestation
Foetal IFN-γ
Antibodies

foetuses and placentas, and three infected foetuses also had *N. caninum* antibodies. The parasite burden was higher in the brain of dead/aborted foetuses than in live foetuses. Interestingly, high IFN-γ production was detected in foetal fluids of a dead foetus found upon euthanasia of its dam, while no IFN-γ was observed in amniotic, allantoic and/or foetal fluids in the three infected foetuses that were alive upon maternal euthanasia. The present study confirms that the infection of dams on gestation day 110 with 10^7 tachyzoites of the Nc-Spain7 isolate causes abortion. The fact that some infected dams aborted and some did not is relevant to the understanding of *N. caninum* pathogenesis of abortion in naturally infected cows.

1. Introduction

Neospora caninum is considered as one of the main causes of bovine abortion worldwide (Almeria and López-Gatius, 2013; Dubey et al., 2007; Dubey and Schares, 2011).

The pathogenesis of bovine neosporosis is complex and only partially understood. Abortion occurs in both naive and chronically infected cows, and abortion is not consistently induced in experimentally infected cows. Many factors including, breed, gestational age, immune status of the cow, route of inoculation, dose, stage and the strain/isolate of the parasite inoculated affect the outcome of pregnancy, and these were very recently reviewed in detail by Benavides et al. (2014). It is generally agreed that gestational age is one of the important factors; disease is most severe in cows inoculated early in gestation versus late gestation. In most experimental infections established using different isolates in early stages of pregnancy (90 days of gestation or earlier) in naïve cattle, foetal death is the most common finding (e.g. Bacigalupe et al., 2013; Bartley et al., 2012; Caspe et al., 2012; Gibney et al., 2008; Macaldowie et al., 2004; Regidor-Cerrillo et al., 2014; Rosbottom et al., 2008; Williams et al., 2000) and such deaths have been widely attributed to a lack of foetal immunocompetence. Later in gestation, after 120 days of pregnancy or later (at 210 days), infections mostly result in the birth of full-term congenitally infected foetuses (Almería et al., 2003; Andrianarivo et al., 2001; Benavides et al., 2012; Gibney et al., 2008; Maley et al., 2003; Rosbottom et al., 2008; Williams et al., 2000).

The pathogenesis of infection during the second term of gestation, when most abortions occur in naturally infected dams, is poorly understood. Transitory immune-suppression of T lymphocytes, starting at around 18 weeks of gestation, has been observed in cattle experimentally infected with *N. caninum* (Innes et al., 2001) and could be the cause of the increased susceptibility of these animals to parasitaemia at that time. A previous study in heifers experimentally infected at 110 days of gestation with an experimental period of 6 weeks after infection was the first report of foetopathy in dams experimentally infected in the second trimester of gestation (Almería et al., 2010). The objectives of the present study were to further evaluate the outcome of pregnancy in cows inoculated following the same experimental design in the second trimester of gestation, and to examine the immune response in the foetus. A proven virulent isolate of *N. caninum*, Nc-Spain7 was used. This isolate has been recently shown to induce severe neonatal neosporosis in cows inoculated in early pregnancy (65–70 days of gestation) (Caspe et al., 2012; Regidor-Cerrillo et al., 2014).

2. Material and methods

2.1. Animals and infection

Ten Friesian heifers that were seronegative for *N. caninum* (CIVTEST, Spain) and free or vaccinated against the main abortifacient agents (*Brucella abortus*, bovine viral diarrhoea virus [BVDV] and infectious bovine rhinotracheitis [IBR] virus) were synchronized and artificially inseminated. Pregnancy was assessed by ultrasonography at 30, 45 and 90 days after insemination. At 110 days of pregnancy, 6 of the heifers were intravenously (i.v.) inoculated with 10^7 culture-derived tachyzoites of the *N. caninum* isolate Nc-Spain7 (passage 15), which was kindly donated by Dr. L. M. Ortega-Mora, SALUVET, University Complutense of Madrid, Spain. These 6 heifers were euthanized at 6 weeks post-infection (wpi) (Table 1). The four remaining heifers were kept as non-inoculated controls and were euthanized at the same time as inoculated dams. In addition, three Friesian heifers from the same herd, that had tested seropositive for *N. caninum* prior to gestation were inseminated at the same time and followed during the experimental period to compare the response in naturally infected dams versus the experimentally infected dams. These chronically infected seropositive dams had healthy calves at parturition and were not euthanized.

2.2. Sample collection

Heifers were observed daily throughout the experimental period for possible abortion. Rectal temperatures were recorded daily for the first week after infection and at weekly intervals thereafter until euthanasia. Heifers with a temperature >39.5 °C were considered febrile.

Blood samples were collected from the dams by tail vein puncture on the day before infection and regularly at weekly intervals until culling 6 wpi. In the three chronically infected dams, blood was collected at the same time points. Plasma was obtained by centrifugation within 30 min of sampling and stored at −20 °C until analysis. Body condition scores were recorded in the heifers at each sample collection date.

At 6 wpi, the six experimentally infected and the four control uninfected heifers were sedated with xylazine hydrochloride (Rompun; Bayer) and euthanized by an intravenous (i.v.) overdose of embutramide and mebezonio iodide (T61; Intervet). Immediately after death, heifers were necropsied. Amniotic and allantoic fluids were collected before the placenta was opened and foetuses separated from the placenta. Foetal blood samples were obtained by cardiac puncture or peritoneal fluids were collected. Foetuses were measured from crown to rump. Two dams aborted dead foetuses at 2–3 wpi (one could not be recovered and the second was autolytic) and one dam had a non-viable foetus upon euthanasia at 6 wpi (Table 1).

Samples of nine randomly selected placentomes (three cranial, three medial and three caudal) were removed. Foetal tissue specimens collected were: CNS (brain and spinal cord), heart, lung, liver, skeletal muscle, spleen, and thymus.