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# *Neospora caninum* in cattle: Experimental infection with oocysts can result in exogenous transplacental infection, but not endogenous transplacental infection in the subsequent pregnancy

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**Abstract**

Whilst it is presumed that infection of pregnant cattle with *Neospora caninum* oocysts can provoke abortion and is the likely cause of epidemic abortion outbreaks, only two previous experiments have involved inoculation of pregnant cows with oocysts (and only one abortion was provoked in 22 pregnancies). Here, we describe the oral oocyst challenge of 18 cows synchronously bred and inoculated precisely at 70 ($n = 6$), 120 ($n = 6$) and 210 ($n = 6$) days in pregnancy with a nominal dose of 40,000 oocysts. Only one abortion occurred (at the 120 days challenge) which could be definitively ascribed to *N. caninum* and no transplacental infection (TPI) was detected in any of the other 11 calves born in the 70 and 120 day challenge groups. In contrast, 4/5 live calves born to cattle challenged at 210 days were transplacentally infected. When cows which had transplacentally infected their calves in the first pregnancy were rebred, no TPI occurred. The results show that the timing of challenge influences clinical and parasitological outcomes and that cattle in late pregnancy are exquisitely sensitive to oocyst challenge leading to exogenous TPI and congenitally infected calves. However, cattle which were indisputably systemically infected in their first pregnancy did not induce endogenous TPI in their subsequent pregnancy. This confirms previous results with experimental tachyzoite challenge and suggests that post-natal infection does not lead to persisting infections which can recrudesce in pregnancy.


*Keywords:* *Neospora caninum*; Transplacental infection; Oocysts; Cattle

## 1. Introduction

The intracellular protozoan parasite *Neospora caninum* was first recognised in 1984 in dogs and in 1989 in cattle and is an important cause of bovine abortion worldwide (Dubey, 2003). Dogs and coyotes are definitive hosts and have been shown to shed unsporulated *N. caninum* oocysts following the ingestion of infective tissue from intermediate hosts (McAllister et al., 1998; Lindsay et al., 1999; Schares et al., 2001; Dijkstra et al., 2001a; Gondim et al., 2004b). Transplacental infection (TPI) is a major route of transmission of *N. caninum* in cattle and may result in foetopathy or the birth of congenitally infected calves. Recently, two types of TPI have been defined, namely endogenous and exogenous TPI (Trees and Williams, 2005). Endogenous TPI refers to foetal infection occurring as a result of the recrudescence of a pre-existing persistent maternal infection during pregnancy; exogenous TPI occurs as a result

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of a de novo infection of a pregnant dam and has been shown experimentally following the administration of *N. caninum* tachyzoites (Barr et al., 1994; Williams et al., 2000; Innes et al., 2001) and *N. caninum* oocysts (Gondim et al., 2004a). Natural shedding of *N. caninum* oocysts by dogs has been described but the prevalence and intensity is low (Basso et al., 2001; McGarry et al., 2003; Schares et al., 2005). Nevertheless, oocysts are the most likely parasite stage to provide the point source of infection which has been associated with epidemic abortions (McAllister et al., 1996, 2000; Dijkstra et al., 2001b, 2002; Crawshaw and Brocklehurst, 2003). However, whilst there is epidemiological evidence that infection with oocysts during pregnancy may result in abortion, the experimental data are more circumspect – to date, in 22 pregnant cows inoculated with oocysts, there has only been a single *N. caninum* confirmed abortion (Trees et al., 2002; Gondim et al., 2004a).

Whilst the ability of *N. caninum* oocysts to provoke abortion by exogenous TPI requires further investigation, another significant question is whether post-natal infections by oocysts can establish persistency and result in endogenous TPI in subsequent pregnancies. Cattle infected with tachyzoites before pregnancy do not infect their foetuses in a subsequent pregnancy (Williams et al., 2000; Innes et al., 2001), but none of the cattle that have been experimentally inoculated with oocysts during pregnancy have been rebred to determine if a persistent infection was established that could recrudesce during a subsequent pregnancy.

Thus this study had two aims: firstly, using precisely timed challenges in synchronously bred cattle to determine if the stage of pregnancy at oocyst challenge affected the parasitological and clinical outcomes; and secondly, to determine if cattle infected with oocysts in one pregnancy in which TPI or abortion had occurred could infect their foetus in a subsequent pregnancy.

## 2. Materials and methods

Infections with *N. caninum* were carried out in cattle and gerbils in accordance with the Animals (Scientific Procedures) Act, 1986, under licence from the UK Home Office.

### 2.1. Neospora caninum oocyst production

*Neospora caninum* oocysts were produced at the University of Illinois. A 12–16 weeks old mixed breed female hound pup was fed approximately 3 kg of mixed tissue (brain, spinal cord, tongue, skeletal muscle and kidney) from four calves that have been inoculated i.v. with tachyzoites of the Nc-Liverpool strain (Barber et al., 1995) of *N. caninum* in March 2003. Oocyst shedding in the pup was observed 13 days later. All the faeces shed during the period of maximum oocyst shedding (15–23 days after the puppy consumed the calf tissue) were collected and oocyst purification was performed as described previously (Gondim et al., 2002). Sporulation of the oocysts was achieved by suspending them in 2% sulphuric acid and shaking them at room temperature for 6 days, in the dark. After this time 792,000 (71% of detectable oocysts) were sporulated. The oocysts were suspended in 2 L of 2% sulphuric acid, stored in four 500 ml containers and kept refrigerated at 4 °C prior to being shipped to Liverpool in October 2003 under refrigerated conditions. The species identity of the oocysts was confirmed as *N. caninum* by PCR (Gondim et al., 2002).

### 2.2. Oocyst infection of cattle

Twenty-one Friesian–Holstein heifers were purchased from farms in the North-West of England and housed at the University of Liverpool's Large Animal Experimental Facility in dog- and fox-proof accommodation. Serological tests for evidence of infection with the common abortifacient agents (Bovine Viral Diarrhoea virus, Infectious Bovine Rhinotracheitis virus, *N. caninum* and *Leptospira hardjo*) were performed as described previously with negative results (Williams et al., 2000). The cattle were loose-housed on straw bedding and fed straw and concentrates with a mineral block available throughout the experiment. Oestrous was synchronised by means of a progesterone releasing device (PRID, Ceva Animal Health Ltd., Chesham, Buckinghamshire, England) and the heifers were artificially inseminated (AI). Pregnancy was confirmed by transrectal ultrasonography 35 days later.

The oocysts were stored at 4 °C upon arrival in Liverpool. The four batches of oocysts were mixed and all doses drawn from a single pool. Nominal doses of 40,000 oocysts, based on the estimated concentration of 396 oocysts/ml, were prepared shortly before they were administered to the heifers. The appropriate volume (approximately 100 ml) of oocyst suspension in 2% sulphuric acid was reduced to 20 ml by centrifugation at 1,000*g* for 20 min, and made up to 1 L with water. The heifers were dosed orally with the 1 L of oocyst suspension and immediately afterwards were given a further 1 L of water. Eighteen animals were challenged with the oocysts between January and April 2004 in three groups of six animals. Group 1 was challenged on day 70 of pregnancy, Group 2 on day 120 and Group 3 on day 210. At the time when each group of animals was challenged, one control animal was dosed with 1 L of water containing 20 ml of 2% sulphuric acid. For 48 h after dosing, the infected animals were isolated from the control animal and kept on sawdust which was later collected and incinerated. The control animal was then kept with the challenged cattle and acted as a sentinel for adventitious infection. Foetal viability was assessed three times a week by transrectal ultrasonography for the first month post-challenge and thereafter weekly. If a foetus was found to be non-viable as indicated by the absence of a heartbeat, it was checked again after 24 h to confirm foetal death. One heifer in Group 2 was found to have a non-viable foetus 33 days after the challenge and was injected with a progesterone receptor antagonist,