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Natural postnatal *Neospora caninum* infection in cattle can persist and lead to endogenous transplacental infection

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Abstract

A serological follow-up study of 3.5 years duration was done of a dairy herd that had experienced a mass seroconversion to *Neospora caninum* following a point source exposure shortly before the 17th of January 2000. A total of 913 blood samples of 244 animals at seven sampling dates were used to investigate the seroprevalence dynamics in the herd.

Most postnatally infected cattle remained seropositive during the period of investigation but 11 animals became seronegative after 6–27 months indicating transient infection. Six animals seroconverted later than the main group of 45 animals and 5 animals became seronegative after at least two seropositive records possibly due to a low infection dose or difference in the haplotypes of the infected animals. In total 58% (14/24) of the offspring of postnatally infected dams was seropositive. Nine of 16 (56%) daughters originating from inseminations after the postnatal infection of their dams were seropositive indicating endogenous transplacental infection.

Keywords: Cattle; *Neospora caninum*; Persistent infection; Postnatal infection; Transplacental transmission

1. Introduction

Neospora caninum has been recognised as the most important cause of abortion in cattle throughout the world (Dubey et al., 2006). Prenatal (vertical, congenital) and postnatal (horizontal, lateral) infection are the two modes of transmission in cattle. The prenatal infection, from an infected dam to her foetus during pregnancy, is the major route of infection. Prenatal infection occurs in less than 100% of the cases, so without postnatal infection the infection would extinct (Dubey et al., 2007). Previous studies in the Netherlands have shown that postnatal infections with *N. caninum* occur regularly in association with abortion outbreaks, (Dijkstra et al., 2001) but may also occur without an increased incidence of abortions (Dijkstra et al., 2002). Bartels et al. (2007) calculated an incidence rate for horizontal transmission of 1.4 infections per 100 cows-years at risk, based on a random sample of 108 infected Dutch dairy herds.

Trees and Williams (2005) advocated the use of the more precise terminology 'endogenous transplacental infection (TPI)' and 'exogenous TPI' to describe respectively a foetal infection after reactivation (recrudescence) of a pre-existing chronic infection of

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the dam and a foetal infection that results from a primary infection of a susceptible dam during pregnancy. Exogenous TPI and abortion was demonstrated in cows experimentally infected with tachyzoites or oocysts (Trees and Williams, 2005; Dubey et al., 2007). However, endogenous TPI could not be demonstrated in experimentally infected cattle. Cows experimentally infected before insemination gave birth to uninfected calves (Williams et al., 2000; Innes et al., 2001). Also, seven cows experimentally infected with oocysts during their first pregnancy gave birth to uninfected calves in a subsequent pregnancy (McCann et al., 2007). These experimental studies suggest that postnatally infected adult cows fail to establish a persistent infection.

The objective of the present study was to present evidence that cattle with a naturally acquired postnatal infection with *N. caninum* can become persistently infected and can transmit the infection to their offspring during a subsequent pregnancy (endogenous TPI).

2. Materials and methods

2.1. Herd and animals

The herd of this study was used in an earlier study, based on repeated herd serology. Hundred thirty-four of 144 animals which were older than 3 months were blood sampled on June 1999, and the whole herd was blood sampled on January, and August 2000, which showed a mass seroconversion between June 1999 and January 2000, without an increased incidence of abortions (Dijkstra et al., 2002). There was a lack of association between the serological status of daughters and mothers and an extreme overrepresentation of seropositive animals in the age group of 8–30 months, which were housed together during a period of 4 months, suggesting a point source infection of this age group. A recent postnatal infection shortly before the 17th of January 2000 was substantiated by an IgG avidity analysis of sera. The present study is a follow-up study of this herd based on further whole herd blood samplings in February 2001, December 2001, April 2002 and January 2003. Part of the data of the previous study was included in the present study to give an overview of the infection dynamics in this herd during the period of investigation.

On average 132 female animals were present on the farm during June 1999 to January 2003. All animals were of Holstein Friesian breed and were housed in the same free-stall barn. Adult cows were pastured in summer whereas young stock was kept indoors until calving. Ear tags of the Dutch Identification and Registration (I&R) System (Royal Dutch Dairy Syndicate, Arnhem, The Netherlands) identified all animals. The farmer had a closed herd policy and reared his own replacement. The calves were only fed colostrum of their own dams. Thus, false-positive results due to the feeding of pooled colostrum could be excluded.

At the seven consecutive sampling dates, 134, 124, 128, 121, 151, 135 and 120 blood samples were collected, respectively (in total 913). Serological data of 244 animals were evaluated.

2.2. Blood sample collection

Blood samples were taken using disposable needles and 8.5 ml SST™ Gel and Clot Activator Vacutainer® Plus serum-tubes (Becton Dickinson Vacutainer Systems Europe). All samples were immediately transported to the laboratory of the GD-Animal Health Service (GD-AHS), Deventer, The Netherlands. Serum was removed after centrifugation at $2000 \times g$ for 10 min and analysed in the ELISA of the GD-AHS within 24 h.

2.3. Serology

All sera were tested for antibodies to *N. caninum* using the GD-AHS ELISA (Deventer, The Netherlands). This ELISA is based on a detergent lysate of whole sonicated tachyzoite antigens and detects all Ig classes. This test has a sensitivity of 98% (95% CI 93–100%) using post-abortion sera and a specificity of 92% (95% CI 85–98%) using non-suspect sera (Wouda et al., 1998a). The results of the ELISA kit were calculated as S/P ratio = {(OD test sample − OD negative control)/(OD positive control − OD negative control)}. A cut-off S/P ratio of <0.5 was defined as negative, and a S/P ratio ≥ 0.5 as positive. A positive S/P ratio of 0.5–1.5 was defined as low positive and a positive S/P ratio ≥ 1.5 as high positive (Dijkstra et al., 2003).

2.4. Analyses

Data on insemination, birth, culling, and pedigree were obtained from the Dutch I&R System. A software program Neospora© (Beiboer, Veterinary Software design, Ureterp, The Netherlands) was used to link all serologic test results to the data of the Dutch I&R system. By this software animals can be sorted by date of birth so that clusters of seropositives can be easily recognised. In addition daughter–mother relationships can be easily matched (Dijkstra et al., 2001).