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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.

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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).

Table 1
Number of animals with the same (negative or positive) or converted serostatus (from negative to positive or reverse) at six pairs of consecutive blood samplings within a period of 3.5 years

Serostatus	June 1999/ January 2000	January 2000/ August 2000	August 2000/ February 2001	February 2001/ December 2001	December 2001/ April 2002	April 2002/ January 2003
N to N	50	50	57	48	71	67
N to P	45	6	2	0	4	2
P to P	4	57	60	47	43	30
P to N	0	2	0	8	4	5

N = seronegative; P = seropositive.

Animals younger than 6 months were not included in the analysis because of the possible misinterpretation of their infection status due to maternal antibodies (Alvarez-Garcia et al., 2007).

3. Results

At the seven consecutive sampling dates, 3% (4/134), 52% (64/124), 51% (65/128), 53% (64/121), 41% (62/151), 39% (53/135) and 30% (36/120) of the animals were found to be seropositive.

Table 1 presents an overview of the seroconversions during the period of investigation. It can be seen that after the mass seroconversion (45 animals) between the first and the second bleeding another six animals seroconverted between the second and the third bleeding. These six animals were born before January 2000 and were, at the time of the point source exposure, housed together with the group of 45 animals that seroconverted earlier. The eight later seroconversions were of three cattle (animals 38, 43 and 49, Table 2) with fluctuating test results and of five cattle with a single positive test result. Seroconversions from positive to negative were seen in animals with fluctuating or single positive test records.

Table 2 summarizes the available serological records of the 45 and 6 cattle with seroconversions in January and August 2000, respectively. Twenty-one of the 45 postnatally infected cattle (animals 1–14 and 38–44) could be followed for 3.5 years. The other animals were culled at various time points during the investigation period. Fourteen cattle (animals 1–14) remained seropositive during the whole period of 3 years. Thirty-six of 45 animals (animals 1–45) were high seropositive in January 2000. Eight of 14 seropositive animals (animals 1–14) were still high seropositive in January 2003. Six cattle (animals 39–44) had at least three consecutive seropositive test results and became

Table 2
Animals with seroconversion in January (1–45) and August 2000 (46–51)

Animal	June 1999	January 2000	August 2000	February 2001	December 2001	April 2002	January 2003
1–14	N	P	P	P	P	P	P
15–20	N	P	P	P	P	P	
21	N	P	P	P	X	P	
22–25	N	P	P	P	P		
26	N	P	P	X	P		
27–32	N	P	P	P			
33	N	P	P				
34–37	N	P					
38	N	P	P	P	P	N	P
39–40	N	P	P	P	P	P	N
41–42	N	P	P	P	P	N	N
43	N	P	P	P	N	P	N
44	N	P	P	P	N	N	N
45	N	P	N				
46–47	N	N	P	P	N	N	N
48	N	N	P	P			
49		N	P	P	N	P	N
50		N	P	P	N	N	N
51		N	P	P	P	N	

N = seronegative; P = seropositive; X = missing value.

Table 3
Seropositive and seronegative offspring of postnatally infected dams that were infected after and before insemination

Moment of postnatal infection of the dam	After insemination	After or before insemination	Before insemination	Total
Seropositive offspring	3	2	9	14
Seronegative offspring	0	3	7	10
Total	3	5	16	24

seronegative after 12–27 months. Four of the nine animals, which were low seropositive in January 2000, were part of the group animals 39–44. In two cases a positive test result was found in a later blood sample (fluctuating test results, animals 38 and 43). All further test records of the other cattle were positive except for one cow (animal 45) which tested low seropositive in January 2000 and seronegative in August 2000, but was culled after that.

All six cattle (animals 46–51) that seroconverted between the second and the third blood sampling had at least two consecutive seropositive test results. Five of these six animals were low positive in August 2000. Five animals (animals 46–47, 49, 50, 51) remained seropositive for 6–14 months, before they became seronegative. One animal (animal 48) was culled after two seropositive test results. Another animal (animal 49) had a positive test record later on.

Table 3 summarizes data on the serostatus of offspring of the postnatally infected dams. In total 58% (14/24) of the offspring was seropositive. Three seropositive daughters were from dams that had been inseminated before June 1999 and thus before the period of postnatal infection. These dams had seroconverted during their pregnancy, causing exogenous TPI of their calves. Two seropositive daughters were born from dams that were inseminated during the period of postnatal infection between June 1999 and January 2000. In these cases no conclusion on the origin of the transplacental infection – endogenous or exogenous – could be made. Nine seropositive daughters were born from dams that were inseminated after January 2000 when they were already seropositive. Five of these daughters were from a first pregnancy and four from a second pregnancy after the postnatal infection of their dams.

4. Discussion

This longitudinal study of a dairy herd that had experienced a mass seroconversion to *N. caninum* following a point source exposure, shows that most high seropositive and postnatally infected cattle remained high seropositive during the period of investigation (3 years) but 11 became seronegative after 6–27 months. Another field study also indicates that some infected cattle can get rid of the infection (Chanlun et al., 2007). There is a decrease in S/P ratio during the study. Most low seropositive animals became seronegative and few high seropositive animals became low seropositive after 3 years.

Five of the six animals, seroconverted later than the group of 45 animals, became seronegative within the investigation period after at least two seropositive records. These six animals were mainly low seropositive in August 2000. The six animals had been housed together with the group that had seroconverted earlier. We speculate that these six animals may have been infected during the point source exposure by a low infection dose and that the duration of the infection may be dose dependant. Björkman et al. (2006) found lower avidities and less consistent results in experimentally oocysts-infected animals with a low infection dose than in the tachyzoite-infected animals with a high infection dose. In their view avidity maturation of specific antibodies for particular antigens might occur only late after infection and might be influenced by the infection dose. Alternative explanation of dose dependant infection is difference in the haplotypes. So, some animals are more resistant than the others.

Five animals with seroconversions later than August 2000 were single test-positive results, which we consider as false positive test results (Dijkstra et al., 2003).

Nine out of 16 (56%) daughters, originating from inseminations of postnatally infected dams after the infection period, were seropositive indicating endogenous transplacental transmission. Postnatal infection of these daughters cannot be completely excluded but appears to be very unlikely in view of the seroprevalence dynamics in the herd. These findings are in contrast with those of experimental studies (Williams et al., 2000; Innes et al., 2001; McCann et al., 2007). 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 findings suggest that adult cows fail to establish a persistent infection. On the other hand Maley et al. (2001) found indirect evidence for a persistent infection of cattle after experimental infection with a NC1 isolate of *N. caninum*, based on the serological profile, which was similar of that in sheep infected with a complete isolate of *Toxoplasma gondii* leading to tissue cyst formation and thus a persistent infection (Buxton et al., 1991).

The transplacental transmission of postnatally infected cattle was 58% (14/24) in this herd, without an obvious increased incidence of abortions. In a previous study of three herds with *N. caninum* abortion rates of 23–43%, we found a transplacental transmission of approximately 45% in postnatally infected animals (Dijkstra et al., 2006). These figures are lower than the rates varying from 81 to 95% reported in some studies (Paré et al., 1996, 1997; Schares et al., 1998; Wouda et al., 1998b; Davison et al., 1999), but in the same range as in other studies in herds with postnatally infected cattle: 56% (Mainar-Jaime et al., 1999), 44% (Bergeron et al., 2000), 39.3% (Dyer et al., 2000) and 63.7% (Romero and Frankena, 2003). We suggest that TPI may be more likely to occur in dams that were themselves prenatally infected compared with postnatally infected dams.

It appears from this study that postnatally and prenatally infected dams have different rates of transplacental transmission and differ in persistence of infection. This should be considered in culling strategies. Selection of postnatally infected dams and their offspring for breeding is preferable compared with prenatally infected animals because of a higher chance of a transient infection and a lower TPI in postnatally infected dams.

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References

Alvarez-Garcia, G., Ortega-Mora, L.M., Dijkstra, Th., Wouda, W., 2007. Diagnostic applications and recommended diagnostic schemes (cattle). In: Ortega-Mora, L.M., Gottstein, B., Conraths, F.J., Buxton, D. (Eds.), Protozoal Abortion in Farm Ruminants. Guidelines for Diagnosis and Control. Athenaeum Press, Gateshead, pp. 89–98.

Bartels, C.J., Huinink, I., Beiboer, M.L., van Schaik, G., Wouda, W., Dijkstra, T., Stegeman, A., 2007. Quantification of vertical and horizontal transmission of *Neospora caninum* infection in Dutch dairy herds. Vet. Parasitol. 148, 83–92.

Bergeron, N., Fecteau, G., Pare, J., Martineau, R., Villeneuve, A., 2000. Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Quebec. Can. Vet. J. 41, 464–467.

Björkman, C., Alvarez-Garcia, G., Conraths, F.J., Mattsson, J.G., Ortega-Mora, L.M., Sager, H., Schares, G., 2006. *Neospora caninum* IgG avidity tests: an interlaboratory comparison. Vet. Parasitol. 140, 273–280.

Buxton, D., Thomson, K., Maley, S., Wright, S., Bos, H.J., 1991. Vaccination of sheep with a live incomplete strain (S48) of *Toxoplasma gondii* and their immunity to challenge when pregnant. Vet. Rec. 129, 89–93.

Chanlun, A., Emanuelson, U., Frossling, J., Aiumlamai, S., Bjorkman, C., 2007. A longitudinal study of seroprevalence and seroconversion of *Neospora caninum* infection in dairy cattle in northeast Thailand. Vet. Parasitol. 146, 242–248.

Davison, H.C., Otter, A., Trees, A.J., 1999. Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infection in dairy cattle. Int. J. Parasitol. 29, 1683–1689.

Dijkstra, Th., Barkema, H.W., Eysker, M., Wouda, W., 2001. Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. Int. J. Parasitol. 31, 209–215.

Dijkstra, Th., Barkema, H.W., Björkman, C., Wouda, W., 2002. A high rate of seroconversion for *Neospora caninum* in a dairy herd without an obvious increased incidence of abortions. Vet. Parasitol. 109, 203–211.

Dijkstra, Th., Barkema, H.W., Eysker, M., Beiboer, M.L., Wouda, W., 2003. Evaluation of a single serological screening of dairy herds for *Neospora caninum* antibodies. Vet. Parasitol. 110, 161–169.

Dijkstra, Th., Bartels, C.J.M., Wouda, W., 2006. High abortion rate and low congenital infection rate in cattle after a natural exogenous infection with *Neospora caninum*. In: Final Conference on Protozoal Reproduction Losses in Farm Ruminants. Liège, 6–9th September 2006, (COST 854), p. 12.

Dubey, J.P., Buxton, D., Wouda, W., 2006. Pathogenesis of bovine neosporosis. J. Comp. Pathol. 134, 267–289.

Dubey, J.P., Schares, G., Ortega-Mora, L.M., 2007. Epidemiology and control of neosporosis and *Neospora caninum*. Clin. Microbiol. Rev. 20, 323–367.

Dyer, R.M., Jenkins, M.C., Kwok, O.C., Douglas, L.W., Dubey, J.P., 2000. Serologic survey of *Neospora caninum* infection in a closed dairy cattle herd in Maryland: risk of serologic reactivity by production groups. Vet. Parasitol. 90, 171–181.

Innes, E.A., Wright, S.E., Maley, S., Rae, A., Schock, A., Kirvar, E., Bartley, P., Hamilton, C., Carey, I.M., Buxton, D., 2001. Protection against vertical transmission in bovine neosporosis. Int. J. Parasitol. 31, 1523–1534.

Mainar-Jaime, R.C., Thurmond, M.C., Berzal-Herranz, B., Hietala, S.K., 1999. Seroprevalence of *Neospora caninum* and abortion in dairy cows in northern Spain. Vet. Rec. 145, 72–75.

Maley, S.W., Buxton, D., Thomson, K.M., Schriefer, C.E., Innes, E.A., 2001. Serological analysis of calves experimentally infected with *Neospora caninum*: a 1-year study. Vet. Parasitol. 96, 1–9.

McCann, C.M., McAllister, M.M., Gondim, L.F.P., Smith, R.F., Cripps, P.J., Kipar, A., Williams, D.J.L., Trees, A.J., 2007. *Neospora caninum* in cattle: experimental infection with oocysts can result in exogenous transplacental infection, but not endogenous

transplacental infection in the subsequent pregnancy. Int. J. Parasitol. 37, 1631–1639.

Paré, J., Thurmond, M.C., Hietala, S.K., 1996. Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. Can. J. Vet. Res. 60, 133–139.

Paré, J., Thurmond, M.C., Hietala, S.K., 1997. *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. J. Parasitol. 83, 82–87.

Romero, J.J., Frankena, K., 2003. The effect of the dam-calf relationship on serostatus to *Neospora caninum* on 20 Costa Rican dairy farms. Vet. Parasitol. 114, 159–171.

Schares, G., Peters, M., Wurm, R., Barwald, A., Conraths, F.J., 1998. The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analysed by serological techniques. Vet. Parasitol. 80, 87–98.

Trees, A.J., Williams, D.J.L., 2005. Endogenous and exogenous transplacental infection in *Neospora caninum* and *Toxoplasma gondii*. Trends Parasitol. 21, 558–561.

Williams, D.J., Guy, C.S., McGarry, J.W., Guy, F., Tasker, L., Smith, R.F., MacEachern, K., Cripps, P.J., Kelly, D.F., Trees, A.J., 2000. *Neospora caninum*-associated abortion in cattle: the time of experimentally-induced parasitaemia during gestation determines foetal survival. Parasitology 121, 347–358.

Wouda, W., Brinkhof, J., van Maanen, C., de Gee, A.L., Moen, A.R., 1998a. Serodiagnosis of neosporosis in individual cows and dairy herds, a comparison of three enzyme linked immunosorbent assays. Clin. Diagn. Lab. Immunol. 5, 711–716.

Wouda, W., Moen, A.R., Schukken, Y.H., 1998b. Abortion risk in progeny of cows after a *Neospora caninum* epidemic. Theriogenology 49, 1311–1316.