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A Long-Term Study of *Neospora caninum* Infection in a Swedish Dairy Herd

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Stenlund S, Kindahl H, Uggla A, Björkman C: A long-term study of *Neospora caninum* infection in a Swedish dairy her. Acta vet. scand. 2003, 44, 63-71. – A longitudinal study was performed in a Swedish dairy herd where *Neospora caninum* had been isolated from a stillborn calf. Starting in autumn 1994, blood samples from all female animals in the herd were collected once yearly until 1999. The sera were analysed for presence of IgG_1 antibodies to *N. caninum* by the iscom ELISA, and by an avidity ELISA to establish the timing of infection. In addition, data on reproductive performance were compiled. During the study the percentage of seropositive female animals increased from 63% to 87%. In 1994 a large number of young animals tested seropositive although their dams were seronegative, indicating that a transmission of the parasite other than the vertical had recently occurred. Low avidity values supported this assumption. The annual abortion rate increased from a mean of 2% before the initiation of the study to 9% in 1994-1998. During the same time, as judged by the avidity data, a large proportion of the animals shifted from being recently to being chronically infected. The source of the external infection in the herd could not be identified.

Neospora caninum; parasite; cattle; transmission; abortion; antibodies; IgG avidity.

Introduction

Neospora caninum is a cyst-forming coccidian parasite which may infect several mammalian species although it is clinically most important in cattle where it can cause abortion, stillbirth and the birth of feeble calves (*Dubey* 1999). In the bovine, transplacental transmission of *N. caninum* from dam to foetus is considered the most important mode of infection and can occur in consecutive pregnancies (*Anderson et al.* 1995, *Björkman et al.* 1996). However, post natal *N. caninum* infection has also been observed in cattle herds (*Yaeger et al.* 1994, *Davison et al.* 1999a, *Hietala & Thurmond* 1999, *Dijkstra et al.* 2001a). The dog, a definitive host of the parasite (*McAllister et al.* 1998), has been suggested as a source of such a transmission (*Dijkstra et al.* 2001b). The risk of abortion is generally higher in cows congenitally infected with *N. caninum* than in non-infected animals (*Björkman et al.* 1996, *Wouda et al.* 1998). However, abortion outbreaks have also been associated with recently acquired *N. caninum* infection (*McAllister et al.* 1996, *Moen et al.* 1998).

The presence of antibodies to *N. caninum* in the serum of an individual indicates that it is, or has previously been, infected with the parasite. Antibodies can be demonstrated by different serological tests (*Björkman & Uggla* 1999), including the IgG avidity ELISA that can be used to

discriminate between recent and chronic *N. caninum* infections (*Björkman et al.* 1999).
In this paper, we describe a long-term serological investigation of the dynamics of *N. caninum* infection in a Swedish dairy herd, and its effects on reproduction.

Materials and methods

Animals

A herd of Swedish red and white breed dairy cattle, in which *N. caninum* was isolated from a stillborn calf (*Stenlund et al.* 1997), was investigated in a longitudinal study from 1994 to 1999. The farm, situated in central Sweden, included around 40 milking cows. The heifer calves were kept for dairy replacement and the bull calves were reared until slaughter. The herd was free from bovine virus diarrhoea virus (BVDV) infection when it joined the Swedish control program for BVDV in 1993 (*Lindberg* 2002), and has remained free since then. The milking cows were housed indoors in tie stalls during winter and were pastured during summer. Heifers and bull calves were kept outdoors in a loose housing system in winter and were pastured during the summer. Calves younger than 3 months were kept indoors in pens. The animals were fed hay and concentrate according to Swedish standards.
The heifers and cows were artificially inseminated. Heifers were covered by a bull if inseminations were repeatedly unsuccessful. Pregnancies were diagnosed by transrectal palpation at 6-8 weeks. The majority of the calvings took place during summer or autumn. Calvings that occurred during summer or early autumn took place on pasture, while during the cold period the cows calved indoors in a separate calving box. The cow and calf spent at least 4 days together. Placentas, if recovered, were usually disposed of outdoors with no special care taken to prevent access to predators and scavengers.
Eleven of the 1-year-old heifers present in the herd in 1994 had suckled foster dams for a period during the previous year. As the cows and calves moved freely the calves could also have suckled cows other than the foster dam. All foster cows had been culled at our first visit to the farm, and were not sampled.
All animals in the herd were descendants of 2 cows, one of which had been purchased in 1933, and the other in the 1940s. A few cows had been introduced in the 1970s but by 1994 no descendants of these remained in the herd. In January 1994 a dog was purchased. Before that there had been no dogs on the farm at least since 1952. In March 1998 a second dog was bought. The dogs were working dogs and had free access to the stable and to the pastures.

Sampling

A first set of blood samples from all female animals older than 4 months was collected in autumn 1994. Until 1999, inclusive, samples were then collected once yearly, in spring, from all female animals older than 4 months. Blood samples from the farm dogs were collected at the start and end of the study. The bovine blood samples were collected from the coccygeal vein into plain evacuated tubes (Becton-Dickinson). After centrifugation at 1000 × *g* for 20 min, the sera were removed and stored at -20 °C until analysis.

Antibodies

The serum samples were diluted 1:100 in phosphate-buffered saline, pH 7.4, with 0.05% Tween-20 and analysed for the presence of IgG_1 antibodies to *N. caninum* by the iscom ELISA, as described by *Björkman et al.* (1994, 1997). Bovine and canine sera with absorbances ≥0.20 were judged positive. The bovine sera with absorbances ≥0.40 in the samplings of 1994 and 1998 were analysed by avidity ELISA according to *Björkman et al.* (1999). An IgG avidity of ≤35 was considered indicative of an acute in-

Table 1. Number of *Neospora caninum* seropositive female animals above 4 months of age in a Swedish dairy herd. The animals were classified into age groups according to year of birth.

Age	Year					
years	1994*	1995*	1996*	1997*	1998*	1999*
≥4	5/15 (33%)	4/10 (40%)	15/23 (65%)	19/23 (83%)	18/25 (72%)	18/20 (90%)
3	6/12 (50%)	4/8 (50%)	13/13 (100%)	9/16 (56%)	10/14 (71%)	20/23 (87%)
2	11/17 (65%)	18/18 (100%)	14/19 (74%)	13/16 (81%)	20/26 (77%)	14/16 (88%)
1	18/18 (100%)	11/16 (69%)	12/18 (67%)	20/26 (77%)	15/16 (94%)	16/19 (84%)
<1	4/8 (50%)	0/0	0/0	1/2 (50%)	1/2 (50%)	5/6 (83%)
Total	44/70 (63%)	37/52 (71%)	54/73 (74%)	62/83 (75%)	64/83 (77%)	73/84 (87%)

* number of seropositive animals/total number of animals (percentage).

fection while an avidity of >50 indicated a longer infection period. Student's t-test was performed to compare avidity results from 1994 with those from 1998.

Data collection

Data regarding age, identity of mother, and reproductive performance for each individual animal were collected from the farm records and from the Milk Recording Service of the Swedish Dairy Association. Data were retrieved for the 5 years preceding the study (1989-1993) and for the years 1994-1998.

"Abortion" was defined as premature parturition occurring between 42 and 260 days of gestation (*Anonymous* 1972), and "stillborn" was used if the calf was dead at birth or died within 24 h. An abortion was recorded when a foetus or foetal membranes were found, or when a previously confirmed pregnant cow was found non-pregnant at a second pregnancy control initiated by a new oestrus. The gestational age could only be estimated from those abortions where a dead foetus or foetal membranes were observed.

Results

Serology

Forty-four (63%) of the 70 animals blood sampled in 1994 were seropositive to *N. caninum* (Table 1). At this first sampling 11 (41%) out of 27 cows ≥3 years of age and all 18 1-year-old heifers had antibodies to the parasite. During the study the overall percentage of seropositive animals increased progressively, and was 87% at the last sampling in 1999 (Table 1). This increase was seen in all age groups except in the 1-year-olds. The mean antibody levels of the seropositive animals was relatively constant during the study period with absorbances varying between 0.55 and 0.85. The 8 animals present in the herd during the entire study period exhibited constantly high antibody levels, with a mean absorbance of 0.74. The minimum absorbance recorded in any of these animals was 0.35.

During the study, 16 out of 123 females sampled more than once converted from seronegative to seropositive, and 2 animals became seronegative. The antibody levels of 3 animals fluctuated between positive and negative. The 16 seroconverting animals were between 1 and 8 years old, with 14 being older than 3 years at seroconversion. Eight of the seroconversions took place between 1998 and 1999.

In 1994, 25 seropositive female animals had their dams (n = 20) still in the herd. Seven (35%) of these dams were themselves seropos-

Table 2. *Neospora caninum* antibody serostatus in 1-year-old heifers and their dams in a Swedish dairy herd.

1994		Heifer	
		Sero-positive	Sero-negative
Dam	Sero-positive	4	0
	Sero-negative	8	0
	Not sampled	6	0

1999		Heifer	
		Sero-positive	Sero-negative
Dam	Sero-positive	15	1
	Sero-negative	1	2
	Not sampled	0	0

itive. Five years later there were 32 seropositive female offspring to 23 dams in the herd. Of these dams, 22 (96%) were seropositive. A comparison of 1-year-old heifers between 1994 and 1999 (Table 2) shows that at the start of the study many young animals were seropositive although their respective mothers were seronegative; the exact number could not be identified since several mothers were not alive to be tested. At the last sampling, only 1 (6%) out of 16 seropositive heifers had a seronegative mother.

In 1994, 16 (47%) out of 34 samples with ELISA absorbances ≥0.40 had *N. caninum* avidity values ≤50, whereas 5 years later only 3 (5%) out of 58 samples had such low avidities (Table 3). In 1994 and 1998 the mean avidity values were 49 (SD ± 16.0) and 71 (SD ± 14.2), respectively, and the difference between the 2 years was statistically significant ($p<0.001$). Notable was that on the later sampling occasion many individual animals had very high avidity values. In the first year only 3 (9%) of the animals had an avidity above 70, whereas in 1998 the corresponding number was 29 (50%). Eight individuals that were sampled on both occasions all exhibited an increase in avidity (from an average of 50 to one of 77).

The dogs were seronegative to *N. caninum* when sampled in 1994 and in 1999.

Table 3. *Neospora caninum* IgG avidity in serum samples with a *N. caninum* ELISA absorbance of >0.40.

Year	IgG avidity*		
	≤35	36-50	>50
1994	6 (18)	10 (29)	18 (53)
1998	2 (3)	1 (2)	55 (95)

* number of serum samples (percentage).

Effects on reproduction

During the years 1994-1998, 21 (9%) of 235 confirmed pregnancies ended in abortion, whereas the corresponding figure for the preceding 5 years was 5 (2%) of 230 (Fig. 1). All cows and heifers that aborted during the study tested seropositive to *N. caninum.* The average gestational age at abortion was 6 months (range 3-8). In 1994, 2 (9%) of the 22 *N. caninum* seropositive pregnant cows aborted. The following 4 years the corresponding percentage was 23% (6/26), 9% (3/33), 17% (5/30) and 14% (5/36), respectively. The percentage of stillbirths fluctuated between 2% and 9% during the study period (Fig. 1). During the 5 years 1989-1993 the average number of inseminations per confirmed pregnancy was 1.51, and during 1994-1998 it was 1.70.

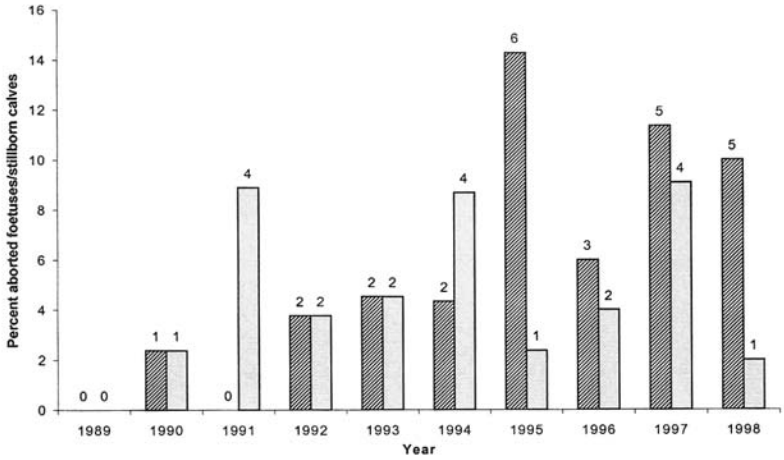

Figure 1. Abortions (striped bar) and stillbirths (dotted bar) in a Swedish dairy herd diagnosed with *Neospora caninum* infection in 1994. Results given as percentages of all pregnancies. Figures above columns denote number of cases.

Discussion

In the investigated herd the prevalence of *N. caninum* infection was high with 2 thirds of the female cattle being seropositive at the start of the study in 1994. By then, all the 1-year-old animals had antibodies to the parasite while few of the older animals were seropositive. This distribution of infection among the age groups suggests that there had been a post natal spread of *N. caninum* in the herd less than a year before the first sampling. This is supported by the fact that so many of the young animals had seronegative mothers. Such a poor correlation between the sero-status in mothers and their offspring has been suggested to reflect a horizontal transmission of the parasite (*Waldner et al.* 1998). Also, the avidity results support the assumption that the parasite had spread in the herd fairly close to the start of the investigation. *Björkman et al.* (1999) demonstrated low IgG avidity values in animals with an acute *N. caninum* infection, and other studies have shown increased avidities during the course of infection (*McAllister et al.* 2000, *Dijkstra et al.* 2002a). In our study, approximately 50% of the 34 animals tested in 1994 had IgG antibodies with a low avidity to *N. caninum,* and only 3 of them had very high avidity values (>70). Further, those individuals sampled in both 1994 and 1998 all exhibited an increase in IgG avidity.

The exact source of the post natal infection in 1994 can only be speculated upon. It could either have been a newly introduced infection, or activation of an infection already present in the herd. In both cases, a prerequisite would be the presence of a definitive host. As *N. caninum* infection in cattle is epidemiologically associated with the presence of a farm dog (*Wouda et al.* 1999, *Dijkstra et al.* 2002b), the introduction of a dog to the farm recently before the abortion problems started is notable. That the dog was seronegative to *N. caninum* does not rule out that it had excreted oocysts and thereby deposited the infection in the environment of the cattle (*McAllister et al.* 1998, *Schares et al.* 2001). In fact, later analyses by Western blot ac-

cording to *Schares et al.* (2001) of the canine sera collected in 1999 have indicated that both dogs at the farm may have been shedding oocysts (data not shown).

The fact that 11 out of the 18 1-year-old seropositive heifers were suckling calves in 1993 is also interesting. In an experimental study, *Uggla et al.* (1998) showed that *N. caninum* tachyzoites in colostrum could orally infect newborn calves. However, it is not known whether tachyzoites are present in the colostrum or milk of naturally infected cows. If this would be the case, it cannot be ruled out that any of the foster cows in our study could have transmitted the infection to the suckling calves.

The percentage of seropositive animals increased progressively during the study period, and no efforts were made to selectively cull seropositive cows or heifers. This increased seroprevalence would be expected due to the large number of seropositive heifers at the start of the study, and the efficiency of vertical transmission of *N. caninum* (*Björkman et al.* 1996, *Davison et al.* 1999a, *Thurmond et al.* 1999). Indeed, the rate of vertical transmission was considerable in this herd. In addition, it was shown that 16 out of 123 animals seroconverted during the study, reflecting continuing post natal infections. The fact that as many as 8 animals seroconverted during the last year of the study shows that the presumed external spread of the infection had not yet been controlled. At seroconversion 14 of the 16 animals were 3 years or older. A median age of 4-5 years at seroconversion was also observed in a longitudinal study of beef cattle performed by *Waldner et al.* (1998). However, these authors found that many of the cows had antibody levels fluctuating between positive and negative. It has been suggested that continuously high levels of antibody to *N. caninum* over several years indicate repeated exposure to the infection (*Hietala & Thurmond* 1999). The persistently high *N. caninum* antibody levels in the 8 animals followed throughout the study, and the constancy of the mean antibody levels of all seropositive animals, thus further supports the assumption of an infection that was active over the years.

One important characteristic of *N. caninum* infection in cattle is that it may lead to abortion and stillbirth (*Dubey* 1999). In the current herd the stillbirths were erratic and did not increase notably over the years. However, the number of abortions increased from 2% of the pregnancies in 1989-1993 to 9% during 1994-1998. Although the registration quality may have been improved during the study, an abortion rate of 9% is considerably higher than the average of less than 1% reported for Swedish dairy herds (*SHS Årsstatistik* 1988-1998). This last figure most probably represents an underestimation since abortions appear not always to be reported. Internationally, approximately 2%-5% sporadic abortions are expected in typical dairy herds (Roberts 1986). An increase of the abortion rate of a similar magnitude as in the present study was seen when 4 Dutch dairy herds were studied for 2-5 years after abortion outbreaks attributed to *N. caninum* (*Moen et al.* 1998).

All the abortions in the present herd were seen in *N. caninum* seropositive animals. This finding is in agreement with other studies, which have shown that seropositive cows are at a 3-7 times higher risk of aborting than are seronegative cows (*Thurmond & Hietala* 1997, *Moen et al.* 1998, *Davison et al.* 1999b). Thus, since the current herd was free from BVDV, and other abortifactive agents such as *Brucella abortus* and *Tritrichomonas foetus* are not present in the Swedish bovine population (personal communication, K. de Verdier Klingenberg, Swedish National Veterinary Institute), *N. caninum* was considered to be the primary cause of the abortions observed. The large proportion (6/26, or 23%) of pregnancies ending in abortion in the group of seropositive cows in 1995 suggests

that at least some of these cows had undergone a primary infection during pregnancy. The avidity values for these animals in 1994 ranged between 44 and 78 (individual data not shown), indicating a sub-acute infection. The later decline in the abortion rate in seropositive cows to 9%-17% may reflect a shift towards chronic infection. An increase in avidity in the individuals that were avidity tested both in 1994 and 1999 supports this idea.

The number of inseminations per confirmed pregnancy can be used to evaluate reproductive problems. However, in the current herd the number of inseminations per confirmed pregnancy never exceeded the average of 1.7 for Swedish herds (*SHS Årsstatistik* 1989-1998), indicating a normal fertility in this herd, although a slight increase from 1.5 to 1.7 was noted during the observation period. This increase in the mean number of inseminations is of interest although no final conclusions can be drawn from such a small sample.

To conclude, this longitudinal study of a dairy herd with an ongoing, active *N. caninum* infection has shown that the main detectable effect of the infection was an increased abortion rate. The frequency of abortions peaked in the year following the onset of the presumed horizontal infection after which it dropped slightly, albeit to a still high level, probably reflecting the shift towards a chronic stage of the infection in a majority of the animals.

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Svensk sammanfattning

En longitudinell studie av en svensk mjölkkobesättning infekterad med Neospora caninum.

En serologisk studie genomfördes i en mjölkkobesättning som visats vara infekterad med den encelliga parasiten *Neospora caninum.* Blodprov togs en gång per år från alla hondjur i besättningen och reproduktionsdata samlades in. Blodproverna analyserades med iscom-ELISA och IgG-aviditets-ELISA. Vid den första provtagningen 1994 var ett stort antal unga

djur i besättningen seropositiva medan deras mödrar var seronegativa. Detta, tillsammans med initialt låga aviditetsvärden som steg under de följande 5 åren, tydde på att det förekommit en horisontell spridning av parasiten i besättningen strax före den första provtagningen. Under studiens gång ökade seroprevalensen i besättningen från 63% till 87%. Abortfrekvensen ökade från i medeltal 2% under åren 1989-1993 till 9% under 1994-1998. Den mest påtagliga effekten av *Neospora*-infektionen i denna besättning var en initialt ökad och därefter fortsatt förhöjd abortfrekvens. Den horisontella spridningen av parasiten pågick under de 5 år studien genomfördes.

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