

Clinical and Serological Observations of Bovine *Neospora* Abortion in Three Dutch Dairy Herds

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Abstract

Three major outbreaks of *Neospora* abortion in different dairy herds in The Netherlands during 1992-1993 are reported. All outbreaks lasted three weeks and were followed by the birth of mummified fetuses during subsequent months. In addition unexpected heat in herd mates previously diagnosed pregnant was seen. A total of 85 animals lost their fetus. The length of gestation for 50 *Neospora* fetuses was 180 ± 36 days (range 100-260). Within one year after the outbreak 50% of the animals were culled. The economic effect was \$586 loss per aborted cow ($n=67$). In total 49 animals were rebred. Fertility immediately after abortion was not impaired. The interval abortion - last insemination was 75 ± 43 days. In total 75 pregnancies were followed, including 21 during the second and 8 during the third year following the outbreak. 70 apparently healthy calves, including 5 sets of twins, were born. Three calves were stillborn. Three animals aborted a *Neospora*-infected fetus for the second time. Serial blood samples from 43 cows tested by an ELISA indicated a decrease in antibodies to *Neospora* to low or undetectable levels within two months after abortion. A rise of antibodies during the second half of gestation was recorded in 58% of the animals. Precolostral serum antibodies to *Neospora*, indicating intrauterine infection, were present in 68% of the calves tested. Seronegative cows produced seronegative calves. In all herds seroprevalence increased following the outbreak. Serotitres in parity zero animals showed a marked increase, *Neospora* continued to be the major cause for abortion.

Keywords: *Neospora caninum*, bovine abortion, fertility

Introduction

Neospora is a recently recognized protozoan parasite, closely related to *Toxoplasma gondii*. The parasite was first isolated from a dog and named *Neospora caninum* (Dubey *et al*, 1988) Thilsted (1989) and Anderson (1991) reported *Neospora* as a cause for abortion and congenital infection in cattle in the USA. The infection causes characteristic histopathological lesions in fetal tissues, especially in the brain. *Neospora caninum* has been diagnosed as a leading cause for bovine abortion in the northern Netherlands (Wouda *et al*. 1995) with more than 800 (17%) fetuses submitted during the past three years, representing a little over 400 herds. The infection causes sporadic or multiple abortions, occasionally an explosive epizootic is seen. Although *Neospora* abortions occur year round epizootic outbreaks are seen more often in summer and early fall (Moen *et al.*, 1995). Three outbreaks of *Neospora* abortion are reported here. Possible spreading mechanisms of *Neospora* in the herds were investigated in a serological follow-up study of aborting cows, their offspring and herd mates.

Materials and Methods

Dairies joined by study between June '92 and September 1993. The three herds (almost 100% Holstein) were managed as pasture-based herds. The basic ration was the same for all three herds; pasture grass, corn/grass silage and various other commodity feeds. Milk production of all farms was above average (± 8000 kg compared to 7200 kg of 0.8 milijoules, animals recorded). The calving interval before the outbreak was ± 395 compared to 390 days. Herd A consisted of 204 animals, including 99 milking cows, had an autumn calving pattern and was served by artificial insemination exclusively. No replacement cattle had been bought for over

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a year. Undiagnosed abortions in previous years had not alarmed the farmer. Herd B consisted of 168 animals, including 73 milking cows, had an autumn calving pattern. AI was done by the farmer. Three lactating replacement animals had been added a few months before. There was no history of abortion. Herd C consisted of 198 animals, including 103 milking cows and had an autumn calving pattern. AI was performed by the farmer. In the spring of 1993 ten lactating heifers from a neighboring farm were added. There was no history of abortion. Blood sampling of cows that previously aborted during the outbreaks or later on were sampled with intervals of one to two months. Immediately after calving the dam and the calf (before receiving colostrum) were sampled. Animals that aborted were sampled within a week after the abortion. Twice a year, in spring and fall, blood samples of the whole herd were taken. An ELISA to detect antibodies to *Neospora* in bovine sera was developed (De Gee *et al*, 1995) following the method described by Ruitenbergh and Van Knapen (1977). Antigens for the coating of microtitre plates were prepared using sonicated *Neospora caninum* tachyzoites (NC-1 isolate). Sera were tested in twofold dilutions beginning at 1:50, following a standard ELISA procedure. Titres of 50 or more were defined as seropositive.

Results

Outbreaks occurred in summer or early fall and lasted approximately three weeks. The percentage of interrupted pregnancies ranged from 22 to 35%. Within two months mummified fetuses started to be born. Previously confirmed pregnant animals appeared to be empty at pregnancy check (Table 1). No other clinical signs were recorded, except for a retained afterbirth in twelve animals. Animals tend to get culled for production reasons mainly. (Table 1) Cows aborted at a various age and the gestational stage of the aborted fetuses was 180 ± 36 days. The incidence of abortion was highest in cows. No abortions were seen in heifers of herd C (Table 2).

Table 1. Incidence of abortions, mummified fetuses¹ and unexpected heat¹ in three dairy herds.

Herd	Period of outbreak	Animals pregnant (at risk)	Abortions during outbreak	Mummies/ (unexpected heat)	Total interrupted pregnancies	%
A	26/06-15/07/92	105	22	4 (11)	37	35
B	19/06-05/07/93	85	18	6 (2)	26	31
C	01/09-20/09/93	99	18	1 (3)	22	22

¹up to three months after the outbreak

Table 2. Incidence of interrupted pregnancies per category (cow/heifer) in three dairy herds.

Herd	Cows			Heifers		
	# at risk	abortions	%	# at risk	abortions	%
A	70	32	45	35	5	14
B	61	18	29	24	8	33
C	72	22	31	27	0	0

Not all fetuses were submitted for diagnostic investigation because not all of them were found. Lesions suggestive for neosporosis were confirmed after immunohistochemical staining in 86% of the fetuses submitted (Table 3).

Table 3. Number of fetuses submitted per farm found positive for *Neospora*

Herd	Fetuses submitted	Histological lesion suggesting Neosporosis	Diagnosis confirmed by immuno histochemistry
A	19	18	17
B	16	16	14
C	11	11	8

Cows that aborted in an early stage of the gestation were successfully rebred. Fertility did not seem to be impaired immediately after the abortion. In forty cows the first heat was recorded 14 ± 12 days after abortion. Forty-nine animals were first inseminated at 42 ± 18 days after abortion. The interval Abortion - Last insemination, indicative for conception of the new calf after the abortion, did not differ greatly from the interval Calving - Last insemination, this being the insemination of the aborted calf (Table 4). No calving problems were seen in cows with an extended calving interval. Culling of low producers or dried-off animals (15%) started immediately after abortion (Table 4).

Table 4. Fertility results of aborted animals.

Herd	Interval (days)			Calving Interval
	Calving-Last insem.	Abortion-Last insem.	Abortion-Culling*	
A	83±23	81±38	79±75	594±65
B	93±28	65±43	153±133	610±61
C	96±35	82±49	188±100	629±64

*Range for the interval Abortion-Culling: A 10-225 d., B 9-387 d., C 91-351 d.

Forty two animals were rebred after the abortion or delivery of a mummified fetus. The outcome of 67 completely observed pregnancies during subsequent years is summarized in table 5. 61 pregnancies ended in the birth of live apparently healthy calves, including

5 sets of twins. Three (6.1%) calves were stillborn. One of these was examined postmortem and showed residual lesions, suggesting a *Neospora* infection. No parasites were detectable after immunohistochemical staining. Three (6.1%) cows aborted a second time, one in the first, one in the second and one in the third pregnancy after the abortion storm. All three fetuses were examined and shown to be *Neospora*-infected by immunohistochemistry. Four cows in herd A appeared to be empty during the second year after the outbreak and one came unexpectedly in heat during the third gestation. No abortion was noticed in these animals (Table 5).

Table 5. Reproductive performance of aborted cows in subsequent pregnancies.

Herd year	Cows	Calves	Abortion	Stillborn	Open	Still pregnant
A 93	16	15	0	1	0	0
94	12	8	0	0	4	0
95	9	6	1	0	1	1
B 94	13	13	0	0	0	0
95	8	3	1	0	0	4
C 94	13	10	1	2	0	0
95	12	6	0	0	0	6

A general increase in seroprevalence to *Neospora* was observed in all herds. The results of whole herd serum in the three herds are shown in table 6.

Table 6. Seroprevalence of *Neospora* antibodies on whole herd serum sampling in four herds, during the last three years.

Herd	Seroprevalence (%) per sample year		
	1993	1994	1995
A	ND	40	54
B	ND	39	40
C	7	11	23

The increase in seroprevalence was particularly attributable to the young stock (Table 7).

Table 7. Seroprevalence per parity category over the last three years.

Herd	Seroprevalence (%) per parity (0,1,>1) category								
	1993			1994			1995		
	0	1	>1	0	1	>1	0	1	>1
A	ND			27	63	38	59	47	53
B	ND			33	40	48	45	36	32
C	ND	14	1	28	12	7	32	18	12

The reproductive performance after the abortion outbreak of herd mates is combined with their serological status before the abortion in Table 8. 30%(10/33) of

the aborted animals had developed a titre against *Neospora* before abortion. In addition to the abortion problems 6 animals in herd A, previously diagnosed to be pregnant were found empty, and 2 were carrying a mummy. In addition to the abortion problems in herd B, 12 mummified fetuses were delivered in 1994 and 3 in 1995. 14 animals of herd C previously diagnosed pregnant during the end of 1994 and the beginning of 1995 but found open (Table 8).

Table 8. Reproductive performance and serology of herd mates.

Herd	Year	# Abortions	Serological status before abortion	
			+	-
A	93	2	ND	
	94	1	0	1
	95	10	6	2
B	93	1	0	1
	94	2	1	2
	95	1	0	1
C	94	3	0	3
	95	6	2	4

A total of 43 animals were studied longitudinally after abortion. Antibody titres decreased after abortion in 58% of the animals, but rose again during the second half of gestation and were high during the following calving. A rise of antibody during the second half of gestation was recorded in 58% of the animals. Precolostral serum antibodies to *Neospora*, indicating intra-uterine infection, were present in 68% (32/46) of the calves tested. Seronegative cows produced seronegative calves (Table 9).

Table 9. Distribution of *Neospora* antibody titres in precolostral sera of calves born to cows with a previous abortion attributable to a *Neospora* infection.

Herd	Titre category			
	0	50-200	400-800	>1600
A (n=18)	6	2	3	7
B (n=15)	2	2	11	0
C (n=13)	7	5	1	0

Discussion

Histopathology indicative for neosporosis in 45 fetuses of which 39 (86%) were confirmed by immunohistochemistry, provides powerful evidence that *Neospora* was the causative agent in these three outbreaks. The major differential diagnosis of brucellosis, salmonellosis, BHV1, BVDV infection were excluded by the laboratory findings. There was no clinical or circumstantial or serological evidence of other causes for

abortion. The clinical picture in all three herds was similar, sudden onset of the abortion storm, without preceding symptoms of illness, lasting three weeks followed by a period of three months where mummified fetuses were delivered. Aborting animals were of various age and gestational stage. This would suggest a point source exposure of the herd (Yeager *et al.* 1994). Mummified fetuses born within a few months after the outbreak probably were exposed to *Neospora* during the same period. The age of fetuses submitted ranged between 100 to 260 days. This is probably due to the diagnostic ability of the dairy farmer. A fetus lost before 100 days of age is probably too small to be noticed. Unexpected open cows were observed in three herds, this may be indicative of mortality in fetuses less than 100 days of age. Apparently cows that became infected and aborted are at a relative low risk to abort again (Barr *et al.* 1993, Wouda *et al.* 1994, Obendorf *et al.* 1995) In our study 6% aborted a second time. When interpreting serotiters of cross-sectional samples the true number of infected animals can be underestimated because initially sero-positive animals may show substantial decrease in serotiter to an undetectable level after abortion or calving. Longitudinal sero-follow-up of animals may appear to be the better way to estimate incidence and prevalence in herds. The rise in serum antibodies during the second half of gestation and the high titers during the following calving was possibly associated with a recrudescence of the original *Neospora* infection of the dam, because 32 out of 47 calves born to cows that previously aborted showed serotiters before intake of colostrum. Hence a cross placental infection is suggested. The reproductive performance of these calves is of great interest with regard to the epidemiology of the disease. Seven of these seropositive animals have now calved themselves. Three of them have aborted. In one evidence for neosporosis was found which meant seropositivity did not protect against abortion attributable to *Neospora*. All three outbreaks took place between early summer and mid fall. In our area major *Neospora* abortion outbreaks show this seasonal trend (Moen *et al.* 1995). In

our follow-up study we noticed that neosporosis remained the major cause of sporadic abortion in these herds during the following year(s). To date, only herd C has experienced a second, small, outbreak of neosporosis during May 1995 with six animals aborting, of which 4 of the fetuses showed lesions attributable to *Neospora*. This outbreak may have been initiated by a BVDV infection going through the herd causing all animals to seroconvert between December 1994 to October 1995. Further studies are required to understand the dynamics of the *Neospora* infection within herds and animals.

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