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INCREASED RISK OF ABORTION FOLLOWING Neospora caninum ABORTION OUTBREAKS: A RETROSPECTIVE AND PROSPECTIVE COHORT STUDY IN FOUR DAIRY HERDS

A.R. Moen,[1] W. Wouda,[1a] M.F. Mul,[2]
E.A.M. Graat[2] and T. van Werven[3]

[1] Animal Health Service
P.O. Box 361, 9200 AJ Drachten
The Netherlands
[2] Group of Animal Health and Reproduction
Institute of Animal Science
Wageningen Agricultural University
Wageningen, The Netherlands
[3] Department of Herd Health and Reproduction
Faculty of Veterinary Medicine
Utrecht, The Netherlands

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ABSTRACT

Explosive abortion outbreaks in 4 Dutch dairy herds during 1992 to1994 are reported. In 50 of 51 fetuses submitted during the first 3 wk of the outbreaks characteristic histological lesions compatible for infection with Neospora caninum were seen. Diagnosis of infection was confirmed by immunohistochemistry in 40 fetuses (78%). No evidence for other abortifacients was found.

The abortion risk of the herds was investigated in a prospective and retrospective cohort study. The prospective study showed that cows aborting during the outbreaks and N. caninum seropositive nonaborting cows had a two- to three-fold increased risk of abortion compared with N. caninum seronegative cows. Retrospective examination showed that seropositive nonaborting cows had an increased risk of abortion before the outbreaks, which may indicate that these animals were infected with N. caninum prior to the outbreaks.

It is concluded that serostatus can be used for selective culling of cows to decrease future risk of abortion in dairy herds.

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Key words: Neospora caninum, bovine, abortion risk, serology, cohort

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[a] Corresponding author.

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INTRODUCTION

Neospora caninum is a newly discovered protozoan parasite, that can cause fetal death and neonatal disease in companion animals and livestock (for review, see 7). At present neosporosis is recognized as a major cause of bovine abortion throughout the world (1,4,6,7,11,16,20). The infection has been associated with abortion epidemics in dairy herds which have had a pattern of occurrence, suggesting a point source exposure (8,17,22), although an environmental source of infection is not known. Contamination of feed by fecal oocysts shed by an unknown definitive host has been postulated (8,22). At present congenital transmission is the only reported mode of infection (2,5,6,13,19).

Repeated abortions due to neosporosis have been reported (1,10), and a recent study found a two-fold increased risk of abortion in N. caninum seropositive cows compared with seronegative cows (14). However, it is still debated whether cows that have aborted a N. caninum-infected fetus or have serum antibodies to N. caninum should be culled (18), or whether their presence in the herds might act as a means of natural vaccination (4).

In this paper we describe the results of a cohort study of 4 dairy herds that had had an outbreak of N. caninum associated abortions. More than 50% of the aborting animals were rebred and were followed for a period of 1 to 4 years. The main objective of this study was to assess the risk of subsequent abortion in cows that aborted during the epidemic outbreaks compared with that of nonaborting N. caninum seropositive and nonaborting seronegative cohorts.

MATERIALS AND METHODS

Dairy Herds

Data were collected from 4 dairy herds (A,B,C,D) that had had N. caninum associated abortion outbreaks in July 1992, June 1993, September 1993 and October 1994, respectively. The herds were located in The Netherlands in the provinces Friesland (Herds A,C), Drenthe (Herd B) and Utrecht (Herd D). The herds consisted mainly of Holstein cows. All herds were pasture-based in the summer, and most of the cows calved in the autumn. The basic winter ration was the same for the 4 herds: corn silage, grass silage and a number of other commodity feeds. Breeding was done by artificial insemination, and pregnancy was diagnosed by transrectal palpation at 6 to 8 weeks post insemination. Herds A, B, and C had no serious abortion problems before the outbreaks. Herd D had an increased abortion rate of unknown cause during a 3-yr period prior to the outbreak in 1994, and 6 cows had been culled because of abortion, 3 of which had aborted twice.

Data were collected for all cows that were present in the 4 herds at the time of the abortion outbreaks, and included the dates of all inseminations and calvings or abortions untill March 1, 1997. Therefore, the follow-up period after the abortion outbreaks of 1992 to 1994 ranged from 2 yr 4 mo (Herd D) up to 4 yr 8 mo (Herd A). Pregnancies completed before the abortion outbreak were considered as pre-outbreak pregnancies, whereas those completed during and after the epidemic counted as post-outbreak pregnancies. The dairy farmers were encouraged to submit fetuses aborted during and after the outbreaks for diagnostic evaluation.

Diagnostic Procedures

Autopsies were performed using a standard protocol, including bacteriological culture of organs and abomasal content, and histology of the brain, heart and liver (21). Diagnosis of N. caninum infection was based on the presence of typical histological lesions and immunohistochemical identification of the organisms (3,21). In addition, immunohistochemistry was done for bovine herpesvirus 1 (BHV1) on fetal liver (15). Sera from aborting cows were screened for antibodies to Brucella abortus, Leptospira hardjo, bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BHV1).

ELISA for Detection of Antibodies to N. caninum

An enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies to N. caninum in bovine sera. Antigens for coating of microtitre plates were prepared by sonication of culture-derived N. caninum (NC-1) tachyzoites (12). A cut-off value of optical density (OD) 0.400 was determined at serum dilution 1:50, using a collection of negative sera obtained from herds with no history of abortion. Sera were screened at 1:50 and positive samples were titrated in two-fold dilutions. Repeatability of the assay was within a 10% range of average measured optical densities. No cross reactions were found with reference sera to Toxoplasma gondii and Sarcocystis cruzi. The assay was shown to have a sensitivity of 92% and a specificity of 96%, using a panel of 90 post abortion sera from cows that aborted due to infection with N. caninum (n= 60, confirmed by IHC of fetus) and other identified or unidentified causes (n=30). A high level of agreement was found with 3 commercially available ELISA's[abc] using this panel of post abortion sera (unpublished data).

Blood Samples for Analysis by ELISA

Serial blood samples with 2 to 3-mo interval were taken from 50 aborting cows and from an equal number of nonaborting controls in Herds A, B and C. Sampling was started within 4 wk after the epidemic in Herds B and C, and 10 mo after the epidemic in Herd A. Samples taken within 1 mo post abortion were available from 8 cows in Herd B and from 12 cows in Herd C. Complete blood sample collections from the herds were done in April 1994 for Herds B and C, for all 4 herds in November 1994 and in May 1995 and in October 1995 for Herd D. Sera from 2 pre-outbreak collections of Herd D in April 1992 and May 1993, respectively, had been stored and were available for analysis.

Definitions

Abortion was defined as 1) the termination of pregnancy by an observed expulsion of the fetus between 45 and 265 d post insemination or 2) by the assumed expulsion of a fetus occurring within a greater than 120-d interval between 2 inseminations.

Abortion outbreak was defined as a cluster of abortions within a 4-wk period involving >15% of the animals at risk (pregnant cows and heifers).

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[a] BIOVET Inc., St-Hyacinthe, Québec, Canada

[b] MAST Diagnostics, Bootel, UK

[c] IDEXX Laboratories, Westbrook, Maine, USA

Abortion risk was calculated as the percentage of pregnancies that ended in an abortion. All pregnancies recorded for a group of animals during the period of observation were included in the data.

A cow was considered to be seropositive if at least 1 blood sample was seropositive for N. caninum in the ELISA procedure.

Cohorts

Cows were assigned to 3 cohorts. Cohort 1 consisted of animals that aborted during the outbreak. Nonaborting cows were assigned to Cohort 2 if seropositive for N. caninum and to Cohort 3 if seronegative for N. caninum.

Statistical Analysis

Logistic regression was used to assess differences in abortion risk among cohorts. The regression model was: risk of abortion = intercept + herd + cohort + error, where herd represents a set of indicator variables for Herds A, B, C, D and cohort represents a set of indicator variables for Cohorts 1, 2, and 3. The unit of analysis was pregnancy. Individual cows could, therefore, contribute more than 1 record to the dataset. To account for correlation of events within a cow, the intraclass correlation was used to update the variance estimates of the regression coefficients (9). Logistic regression coefficients were exponentiated to obtain odds ratios. In a separate model using Chi-square analysis, abortion risk before the outbreaks was compared with abortion risk after the outbreaks. Significance levels of the Chi-square analyses were similarly updated using a variance inflation factor (9).

RESULTS

Description of Abortion Outbreaks

In all 4 herds explosive abortion outbreaks occurred. These lasted for approximately 3 wk, with a peak of abortions occurring during the second week. No other signs of disease were noticed in the aborting animals and their herdmates. A total of 67 fetuses was recovered during the storms. During a period of 3 mo following the epidemics another 15 mummified fetuses were aborted, and 17 cows that were not seen to have lost their fetus were found to be open either by showing estrous behavior or at drying off after pregnancy check. Table 1 presents the number of abortions and mummies found in each herd. The age of aborting cows varied from 2 to 11 yr (mean 4.3 yr). Of 99 aborting cows 42 were culled within 6 mo of the abortion. Culling of low producers and dried-off animals was started immediately after the outbreaks of abortion. The remaining 57 aborting cows were rebred.

Diagnostic Examinations

Fifty-one fetuses that were aborted during the outbreaks were submitted for diagnostic evaluation. The gestational age of these fetuses varied from 4 to 7 mo (mean 6 mo). Histological lesions compatible with neosporosis were found in 50 of 51 fetuses, and N. caninum was identified by immunohistochemistry in 1 or more tissues in 40 fetuses (78%). In 9 fetuses originating from Herds A and B large numbers of tachyzoites were found in the liver, with associated extensive hepatic necrosis as reported elsewhere (21). No other abortifacients were identified by bacteriological examination. In addition, brucellosis, leptospirosis (L. hardjo), and BVDV infection were excluded by maternal serology. In Herd A a high prevalence of BHV1 antibodies was found both in aborting and nonaborting cows. A few animals in Herd B including

1 aborting cow, had serum antibodies to BHV1. However, no BHV1 antigen was detected in fetal tissues by immunohistochemistry.

Table 1. Characteristics of Neospora caninum abortion outbreaks in 4 dairy herds in The Netherlands between July 1992 and October 1994

	Pregnant cows and heifers	Fetuses recovered during outbreak	Mummies recovered within 3 months after outbreak	Additional open cows	Total abortions
	n	n	n	n	n (%)
Herd A	105	22	4	11	37 (35)
Herd B	85	18	6	2	26 (31)
Herd C	99	18	6	2	26 (22)
Herd D	38	9	4	1	14 (37)
All herds	327	67	15	17	99 (30)

Serostatus to N. caninum of aborting and nonaborting Cows

Antibodies to N. caninum were detected in 17/20 (85%) samples from aborting cows taken within 4 wk post abortion. Table 2 presents the proportions of aborting and nonaborting cows seropositive for N. caninum, derived from blood collections for the 4 herds. The proportions seropositive were significantly higher in cows that aborted during the outbreak as well as before and after the outbreak compared with nonaborting cows (P=0.0000). The pre-outbreak seroprevalence of N. caninum in Herd D was 16.8% (based on herd blood samples in 1992 and 1993). The proportion of seropositive animals was 5/6 (83%) for aborting cows and 14/107 (13%) for nonaborting cows. None of the cows that aborted during the outbreak in 1994 were seropositive in their earlier blood samples.

Table 2. Proportions of aborting and nonaborting cows seropositive for Neospora caninum in 4 herds that had had abortion outbreaks

	Aborting cows		Nonaborting cows
	during outbreak	pre/post outbreak	
Herd A	11/12	17/24	35/67
Herd B	14/15	19/27	41/81
Herd C	7/17	9/19	25/111
Herd D	8/11	15/27	15/58
All herds	40/55 (72.7%)	60/97 (61.8%)	116/317 (36.6%)

Abortion Risk in Cohorts 1, 2 and 3 before the Abortion Outbreaks

In the period before the outbreaks, 21 (4.1%) abortions originating from 507 pregnancies were recorded (Table 3). These abortions were of unknown cause in Herds A, B and C as no fetuses were submitted. In 1 fetus from Herd D that was aborted in 1992, N. caninum was identified retrospectively in paraffin embedded brain tissue.

The pre-outbreak abortion risk was at a low level in Cohorts 1 and 3 (1.8 and 2.3%, respectively). In Cohort 2 the risk of abortion was 9.8%, significantly higher than in Cohorts 1 and 3 (P=0.02). Abortion risk in Herds A, B, C and D before the outbreak was 5.0, 4.3, 0.9 and 6.1%, respectively. There were no significant differences between the herds (P>0.05).

Table 3. Abortion risk prior to and following Neospora caninum abortion outbreaks in 3 cohorts of cows from 4 dairy herds

Cohort	Herd	No. of cows	Abortion risk pre-outbreak	Abortion risk post-outbreak
1. Cows aborting during outbreak	A	35	1/85[a]	11/52
	B	20	1/30	2/33
	C	20	0/23	6/42
	D	14	1/25	4/10
	A-D	89	3/163 (1,8%)	23/137 (16.8%)
2. Nonaborting seropositive cows	A	52	5/39	14/158
	B	60	3/35	22/139
	C	34	1/24	9/84
	D	30	4/34	13/46
	A-D	176	13/132 (9,8%)	58/427 (13.6%)
3. Nonaborting seronegative cows	A	39	2/37	5/121
	B	48	1/52	8/103
	C	96	0/69	10/219
	D	55	2/54	12/85
	A-D	238	5/212 (2,3%)	35/528 (6.6%)
Total	A-D	503	21/507 (4.1%)	116/1092 (10.6%)

[a] Number of abortions/number of pregnancies.

Abortion Risk in Cohorts 1, 2 and 3 following Abortion Outbreaks

After the outbreaks 116 (10.6%) abortions out of 1092 pregnancies were recorded, 68 (59%) of which were diagnosed based on a between-insemination interval longer than 120 d. Neospora caninum was identified in 25 of 48 submitted fetuses. In 9 fetuses there were no lesions to suggest neosporosis. In 3 of these fetuses another abortifacient agent was identified (Actinomyces pyogenes in 2 cases and Campylobacter sp. in 1 case). Fourteen submitted fetuses were unsuitable for diagnostic evaluation because of mummification or severe autolysis. The proportion of confirmed N. caninum abortions was 5/23 (21.7%) in Cohort 1; 18/58 (31.0%) in Cohort 2; and 2/35 (5.7%) in Cohort 3. The proportion in Cohorts 1 and 2 differed significantly from that in Cohort 3 (P=0.0001 and P=0.0000 respectively).

The risk of abortion after the outbreaks was 16.8 % in Cohort 1, 13.6% in Cohort 2 and 6.6% in Cohort 3 (Table 3). Adjusted odds ratio's were 3.01 (95% CI 1.5, 6.0) for Cohort 1 and 2.4 (95% CI 1.4, 4.0) for Cohort 2 when compared with Cohort 3. Risk of abortion was significantly higher post-outbreak compared with pre-outbreak in Cohort 1 (P=0.000) and Cohort 3 (P=0.03) but was not different in Cohort 2 (p=0.31). The observed within-cow correlation of abortion events was intermediate (rho=0.12). However, due to the small cluster size (mean parities per cow = 3.19) the effect of intraclass correlation was small.

DISCUSSION

The described outbreaks of abortions in 4 dairy herds were attributable to neosporosis. Fifty percent of the fetuses aborted during the outbreaks were submitted for diagnostic evaluation. In all but 1 fetus histological lesions compatible with N. caninum infection were found, and in 78% of fetuses N. caninum was identified by immunohistochemistry. In addition, bacteriological examination of fetuses and immunohistochemical examination for BHV1 were negative, and no serological evidence was found for brucellosis, leptospirosis, and BVDV infection. The ELISA results also indicate that infection with N. caninum was associated with the abortions, since the proportions of seropositive cows were significantly higher in the group of aborting cows than in nonaborting cows. The explosive character of the outbreaks involving cows of all age classes and in various stages of gestation suggests a point source exposure as reported previously (8,17,22).

The overall abortion risk in all 4 herds increased from 4.1% prior to the outbreaks to 10.6% after the outbreaks. Neosporosis was the major diagnosed cause of abortion during the years after the outbreaks, indicating that the infection remained in the herds at an endemic level.

There are limitations with regard to cohort classification described in this paper. Only post-outbreak serology was known for Herds A, B and C. In addition, the time span between the start of the outbreak and the date of sampling of the entire herd was rather long for Herds A and B. A proportion of the Cohort 2 cows in these herds may have seroconverted after the outbreaks. Further, it is possible that insufficient sensitivity of the ELISA to detect chronically infected animals resulted in the assignment of infected cows to the seronegative group.

However, the results of this study show clearly that aborting cows and seropositive nonaborting cows had a two- to three-fold higher risk of abortion than seronegative cows. These results corroborate previously reported findings (14) and justify selective culling of N. caninum seropositive cows as a means for decreasing the future risk of abortion in dairy herds.

Before the abortion outbreaks Cohort 2 had a significantly increased risk of abortion compared with the 2 other cohorts in all 4 herds. This may indicate that neosporosis was present before the outbreaks in this cohort. Retrospective serological examination and immunohistochemical identification of N. caninum in stored fetal tissue blocks proved the presence of the infection in Herd D 2 years prior to the abortion outbreak. The pre-outbreak serological status of the other 3 herds is not known, and no fetuses were examined.

In contrast with Cohort 2, Cohort 1 had a low pre-outbreak abortion risk. In addition, none of the Cohort 1 cows in herd D were found to be seropositive in pre-outbreak blood samples. These findings suggest that the abortion outbreaks were the result of a primary infection in these cows. Evidence for postnatal infection of cows during an epidemic has been reported previously (19). Whether cows in Cohort 2 may have served as a reservoir for infection or whether the infection was newly introduced into the herds is not known. There is no reported evidence of lateral spread of neosporosis among cows. It could be speculated that a definitive host may have been infected by ingesting abortion material from Cohort 2 cows and subsequently may have spread the infection by shedding oocysts. However, as long as the life cycle of N. caninum has not been elucidated such questions cannot be answered.

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