PEER REVIEWED

Seroprevalence of *Neospora caninum* Infection in 25 Ontario Dairy Herds and its Association with Periparturient Health and Production

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Abstract

Serum samples were collected in 1995/1996 from 758 animals on 25 Ontario Holstein dairy farms at three weeks prior to expected calving. The frozen sera were subsequently analyzed for anti-*Neospora caninum* antibodies. Herd seroprevalence was 60% and 73% using two different cut-off values for defining infection. Using the lower cut-off, within-herd seroprevalence ranged from 0 to 38% with a mean of 9.0% in seropositive herds. Seropositivity was associated with a 3.8-fold increased risk of retained placenta. No significant impact of infection on milk production was detected for the first three Dairy Herd Improvement test-day evaluations of each cow's lactation.

Résumé

Des échantillons de sérum ont été pris en 1995/96 chez 758 animaux, provenant de 25 fermes laitières Holstein de l'Ontario, trois semaines avant la date de vêlage. Le sérum congelé a été par la suite analysé pour des anticorps anti-*Neospora caninum*. La prévalence sérologique était de 60% et 73% en utilisant deux valeurs seuils différentes pour définir l'infection. En utilisant la valeur seuil la plus basse, la prévalence sérologique à l'intérieur des troupeaux variait entre 0 et 38% avec une moyenne de 9.0% dans les troupeaux affectés. La prévalence sérologique était associée à une augmentation de plus de 3.8 fois du risque de rétention du placenta. Aucune différence n'a été détectée au niveau de la production de lait pour la lactation de chacune des vaches suite aux trois premiers tests journaliers du programme d'amélioration des troupeaux laitiers.

Introduction

Neospora caninum is a protozoan parasite that was first identified as a possible cause of abortion in cattle in New Mexico in 1987.[9] Subsequently, the parasite has been shown to be an important cause of endemic fetal loss, and occasional abortion epidemics, in dairy cattle in Canada, USA and many countries around the world.[5] In Ontario *N. caninum* is currently the most commonly diagnosed infectious cause of abortion.

Preliminary studies also indicate that neosporosis may be associated with premature culling and decreased milk production in dairy cows.[10,11] As a result, *N. caninum* may have a significant impact on the productivity of the dairy industry in addition to abortion losses. Within Canada the prevalence of infection has not been extensively investigated. Research on 46 Québec dairy herds selected for a case-control study showed that at least 70% of the herds had seropositive cattle.[7] The *N. caninum* seroprevalence in this case-control study was 22.5% within case herds, while the control herds had an average seroprevalence of 7.5%. In other work, eight beef herds in central Alberta had a reported seroprevalence of 16 to 27% over a four year sampling period. *Neospora caninum*-seropositive cows were 5.7 times more likely to abort compared to seronegative cows, and *N. caninum* was associated with an increase in stillbirths.[13] Finally, recent work from the Atlantic provinces of Canada has indicated that 19.2% of dairy cattle in this region were seropositive for *N. caninum*.[6]

Currently there is limited information on the *N. caninum* seroprevalence in southern Ontario dairy cattle and the impact of this parasite on health and production. The objectives of this study were, therefore, to determine the prevalence of *N. caninum* antibody in an existing serum bank from a study conducted in 1995-1996 involving 758 Holstein dairy cows from 25 dairy herds. In addition, the impact of *N. caninum* infection on periparturient cow health and milk production was investigated since these data were available for all cows on the study.

Materials and Methods

Existing frozen serum samples were sorted by herd and cross referenced with an existing database from a purposive field study conducted in 1995-1996.[4] Herds were selected based on the owners' willingness to participate in the study and the requirement that they be enrolled in the Ontario Dairy Herd Improvement (DHI) milk recording service. The study was designed to assess the impact of monensin on metabolic disease in transition dairy cows. Further details of the results of this study can be found elsewhere.[4] Sera from 758 Holstein dairy cows from 25 farms were obtained at 3 weeks prior to expected calving, at the same time that treatment with monensin was administered. Serum samples were submitted to the California Animal Health & Food Safety Laboratory System at the University of California, Davis for assessment of anti-*N. caninum* antibody using a kinetic ELISA.[8] The laboratory has determined two cut-off sample-to-positive ratios (s/p) for classification of samples as either positive or negative. The lower threshold of 0.45 yields a sensitivity of 88.6% and a specificity of 96.5%, while the higher threshold of 0.70 yields a sensitivity of 79% and a specificity of 100%.[8] When determining seroprevalence both thresholds were utilized. However, all risk factor analyses and health and production effects were measured using the higher cut-off value to minimize the number of false-positive animals. In addition, only the 15 herds having at least one seropositive cow were evaluated.

Results from the evaluation of sera for *N. caninum* antibodies were merged with the existing production and health databases from the 1995-1996 study. Simple prevalence values were calculated by herd and overall using both cut-off s/p values. Milk production effects associated with seropositivity were assessed within seropositive herds using repeated measures analysis of variance using the mixed procedure in SAS. Infection was coded as a dichotomous independent variable and included in the model with other possible confounders including parity, test day number, the interaction between test day number and infection, days in milk, season of calving and linear score. Test day milk production was the dependent variable and both herd and cow were random variables. Only data from each cow's first three DHI milk production tests were available for analysis. On average these tests occurred at 23, 55, and 92 days-in-milk.

Data on individual cow health included the following: milk fever, calving difficulty, twins, retained placenta, metritis, mastitis, clinical ketosis, displaced abomasum and removal (died or sold). All health variables were screened for associations with seropositivity using two by two tables and the Chi Square statistic. Any significant effects ($p<0.05$) were then submitted to logistic regression modelling using the generalized estimating equation in SAS to account for herd clustering. Possible confounding variables such as parity, twins, calving difficulty, and other preceding diseases were offered to the logistic model and included if they were significant or altered either the statistical association or the estimate of the impact of *N. caninum*. Potential herd-level risk factors included: stall design (freestall or tiestall), feeding method (total mixed ration or component), production level (≥ 18 700 lb rolling herd average [high], < 18 700 lb rolling herd average [low]), summer turnout (yes or no), ionophores fed to heifers (yes or no), and major ration components (ie dry corn, dry hay, corn silage). These herd-level risk factors were compared between seropositive and seronegative herds using the simple Chi square statistic. Fisher's exact test was used for confirming statistical associations ($p<0.05$) when there were less than 5 observations in a cell of any two by two table.

Results and Discussion

Seroprevalence

Neospora caninum seroprevalence data are illustrated in Tables 1 and 2 for s/p cut-off values of 0.45 and 0.7, respectively. Depending on the cut-off used, 72% and 60% of herds had at least one seropositive cow. Within-herd seroprevalence ranged from 0 to 38% and from 0 to 34% for the lower and higher cut-off values, respectively. Within seropositive herds, the mean seroprevalence was 9.0% or 8.6%, respectively. The herd seroprevalence is similar to that found in Québec, where 73% of control herds in a case control study had at least one seropositive cow.[7] Similarly, a recent study conducted in the Atlantic region of Canada found a herd

Table 1. Seroprevalence data for *N. caninum* from 758 Holstein dairy cows in Ontario using an ELISA s/p ratio cut-off of 0.45.

	Herds	Cows	Number seropositive
Overall	25	758	51
Zero prevalence	7	190	0
Low prevalence <10%	10	411	15
High prevalence ≥ 10 %	8	157	36

Herd prevalence (at least one positive cow): 18/25 = 72%
Within-herd prevalence (all herds): 51/758 = 6.7%
Within-herd prevalence (positive herds): (15+36)/(411+157) = 9.0%

Table 2. Seroprevalence data for *N. caninum* from 758 Holstein dairy cows in Ontario using an ELISA s/p ratio cut-off of 0.70.

	Herds	Cows	Number seropositive
Overall	25	758	42
Zero prevalence	10	268	0
Low prevalence <10%	9	354	12
High prevalence ≥ 10%	6	136	30

Herd prevalence (at least one positive cow): 15/25 = 60%
Within-herd prevalence (all herds): 42/758 = 5.5%
Within-herd prevalence (positive herds): (12+30)/(354+136) = 8.6%

seroprevalence of 79%.[6] At the lower threshold, the within-herd seroprevalence in the current Ontario study (6.7%) appears to be similar to that in Québec (7.5%)[7] and Prince Edward Island (11.1%)[12] but is considerably lower than that found in New Brunswick (24.9%) and Nova Scotia (22.2%).[12] Reasons for these regional differences in sero-prevalence are presently unknown. They may however, be a reflection of experimental design; the current study and that of Québec were purposive studies while the Maritime study involved a random selection of farms.

Periparturient disease

Simple associations between *N. caninum* seropositivity and periparturient disease are shown in Table 3. There were no significant associations found for *N. caninum* serological status and calving difficulty, milk fever, metritis, ketosis, mastitis and removal. However, both retained placenta and abomasal displacement were more likely in seropositive cows (Table 3). When analyzed using logistic regression, the association between *N. caninum* and abomasal displacement was confounded by retained placenta and thus was not statistically significant. The retained placenta association was strong (Odds Ratio 3.8 [95% confidence interval: 1.7, 8.3]) even after accounting for herd clustering and the occurrence of twins (Table 4). Thus, it appears that *N. caninum* infection may be a risk for the subsequent development of retained placenta. To our knowledge this is the first report linking seropositivity with an increased risk of retained placenta at the cow level. However, this result supports that found in a study conducted in the Netherlands in which herds with a high prevalence of *N. caninum* antibody were significantly associated with a high herd prevalence of retained placenta.[3] The current finding suggests that an observed increase in a herd's incidence of retained placenta could, in some situations, be attributable to an increase in the herd seroprevalence

Table 3. Disease incidence by *N. caninum* serological status (positive/negative) within seropositive herds (n=15) based on a serum sample obtained 3 weeks prior to calving and an ELISA cut-off s/p ratio of >0.7.

Disease	Seropositive Incidence	Seropositive n=42	Seronegative Incidence	Seronegative n=448	Chi square *p*-value
Calving difficulty	2.4%	1	2.2%	10	0.95
Retained placenta	21.4%	9	7.6%	34	0.002
Milk fever	9.5%	4	6.3%	28	0.41
Metritis	2.4%	1	4.0%	18	0.60
Ketosis	2.4%	1	1.8%	8	0.78
Abomasal displaced	7.1%	3	2.0%	9	0.04
Removed (died or sold)	4.8%	2	1.6%	7	0.14
Mastitis	11.9%	5	14.1%	63	0.67

Table 4. Logistic regression model assessing the impact of *N. caninum* seropositivity on the occurrence of retained placenta in 15 seropositive herds controlling for the fixed effect of twins and disease clustering by herd.

Variable	Standard error	Odds ratio	95% Confidence interval	*p*-value
N. caninum positive	0.3998	3.80	1.73, 8.32	0.001
Twins	0.3373	9.0	4.63, 17.38	<0.001

for *N. caninum*. Since lesions associated with *N. caninum* have been identified in the placenta of cows,[2] the placenta should be presumed to be a potential source of infection for other cows either directly or indirectly through an intermediate or definitive host. Thus placentas, and particularly cows with retained placentas, should be managed with a view to limiting contact with other animals. It should, however, be recognized that on most farms, horizontal transmission appears to play a minor role in transmission of *N. caninum* to cows.[1]

It should be noted that if a more conservative *p*-value cut-off (ie < 0.2) had been used for entry to logistic regression then the variable 'removed' would have been included in further analysis. The crude numbers (Table 3) show a numerically higher incidence of cows being removed in the seropositive group. However, a simple regression model adjusting for herd clustering showed that *N. caninum* seropositivity was not significantly (p=0.70) associated with risk of being removed from the herd. This should be assessed in subsequent research with a larger number of seropositive cows.

Herd factors

Herd management factors were available for comparison between *N. caninum* seronegative and seropositive herds. These factors are illustrated in Table 5. Since the original study was not designed to investigate *N. caninum* infection, certain obvious factors such as numbers of dogs on farms were not recorded. No statistical difference was found between serological status of the herd and any of the herd variables that were available for analysis.

Milk production

A repeated measures analysis of variance model using the mixed procedure in SAS was used to evaluate the impact of *N. caninum* on milk production. This model included other important factors that may be associated with production including days-in-milk on test-day, parity, test-day linear score and the random effect of herd. The analysis revealed no significant differences in DHI test-day milk production between seropositive and seronegative animals (p=0.43). This is in contrast to a California study which reported a significant impact of 2.5 lb of milk loss per day in first lactation seropositive animals in one large dry-lot dairy.[11] The least squares means derived from the statistical model used in the present study revealed a numerical but non-significant decrease of 1.6 lb per cow per day for the first three DHI tests (76.5 lb seronegative, 74.9 lb seropositive). When the data were broken down by DHI test number (Table 6), the largest numerical difference was found at the third DHI test. If there is an impact of *N. caninum* on milk production, this might indicate it occurs later in lactation. Unfortunately data beyond the third test were not recorded in this study. In addition, the low seroprevalence combined with the large variance in milk production makes the

Table 5. Prevalence of herd management factors in *N. caninum* seropositive and seronegative herds.

Factor	Seropositive herds (15)		Seronegative herds (10)		Chi square *p*-value
	Prevalence	(n)	Prevalence	(n)	
Tiestall	80%	(12)	90%	(9)	0.50
Total mixed ration	27%	(4)	10%	(1)	0.31
Dry corn in lactating cow diet	53%	(8)	40%	(4)	0.51
Dry hay in lactating cow diet	73%	(11)	90%	(9)	0.31
Corn silage in lactating cow diet	80%	(12)	70%	(7)	0.57
Production level (>18700 lb)	47%	(7)	60%	(6)	0.74
Ionophores in heifer ration	53%	(8)	60%	(6)	0.74
Cows let outside in summer	93%	(14)	80%	(8)	0.31

Table 6. Milk production (lb/cow/day) by dairy herd improvement (DHI) test number for *N. caninum* seropositive and seronegative cows within seropositive herds (n=15) from repeated measures analysis of variance.*

DHI test number (relative to calving)	*N. caninum* serological status	Least squares means	Standard error	*p*-value
1	Positive	76.6	3.01	0.94
	Negative	76.3	1.96	
2	Positive	79.9	2.97	0.97
	Negative	79.9	2.11	
3	Positive	70.4	3.03	0.19
	Negative	73.7	2.02	

*Least squares means derived from a mixed model (using Proc Mixed in SAS) controlling for test day milk production, season of calving, linear score at test day, parity, and the random effects of herd.

power of this study too low to either refute or support the California finding. Further work in this area is needed.

Reproductive performance

There were insufficient numbers of seropositive cows to adequately assess reproductive performance.

Conclusions

This study has demonstrated that the *N. caninum* seroprevalence data at both the herd and cow level for 25 Ontario dairy herds is comparable to estimates derived from studies in Québec and Prince Edward Island, Canada. A strong association between seropositivity for *N. caninum* and retained placenta was observed. *Neospora caninum* may therefore be a potential reason for increased incidence of retained placentas on some farms. In addition, although horizontal transmission is not generally the primary route of *N. caninum* transmission, the placenta should be recognized as a potential source of infection, and therefore animal contact to placentas should be minimized. It should be recognized that the results of this study are limited by the occurrence of 9 retained placentas in seropositive cows. Thus, although attempts were made to control for potential confounding variables, these findings need to be repeated in subsequent research with a larger population of *N. caninum*-seropositive cows.

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