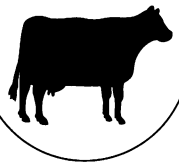

Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Québec

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Abstract — *Neospora caninum* is an important cause of abortion in dairy cattle. The objective of this observational study was to estimate the rate of vertical transmission of *N. caninum* in dairy herds in Québec and to investigate horizontal transmission in the same herds. The genealogy of cows from 23 dairy herds were examined. Prevalence of seropositive animals in herds studied varied from 4.3% to 61.8% (average, 21.9%). The overall rate of vertical transmission was estimated to be 44.4%, varying from 0% to 85.7%. Seven cases of horizontal transmission were identified in 6 of the 23 herds studied. Estimated vertical transmission rate varied from herd to herd, but appeared to be higher in herds with a high prevalence of seropositive animals. Although horizontal transmission was identified in 6 herds, it does not appear to be the major route of infection for *N. caninum*.

Résumé — *Neospora caninum* est une cause d'avortement importante chez les vaches laitières. Les objectifs de cette étude sont d'estimer la prévalence de transmission verticale de *N. caninum* dans les troupeaux laitiers du Québec et d'examiner la possibilité de transmission horizontale dans les mêmes troupeaux. La généalogie des vaches de 23 troupeaux a été étudiée. La prévalence des animaux séropositifs varie de 4,3 % à 61,8 % (moyenne, 21,9 %). La transmission verticale a été estimée à 44,4 % pour l'ensemble des 23 troupeaux, variant de 0 % à 85,7 %. L'évidence de transmission horizontale a été identifiée 7 fois dans 6 troupeaux différents. La transmission verticale estimée varie d'un troupeau à un autre, mais elle semble plus élevée chez les troupeaux où la prévalence des animaux positifs est plus élevée. La transmission horizontale, étant identifiée que dans 6 troupeaux, ne semble pas être la voie d'infection la plus importante pour *N. caninum*.

(Traduit par les auteurs)

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Introduction

Since the early 1990s, *Neospora caninum* has been considered, worldwide, as an important cause of abortion in cattle (1,2). Abortions are costly to the dairy industry with an estimated annual lost of 35 billion dollars in the United States (3,4).

Currently, vertical transmission of *N. caninum* is the only proven route of transmission and appears to be the main means of transmission in dairy herds. In previous studies of a limited number of herds, rate of vertical transmission varied from 72% to 93% (5–7). The similarities between *N. caninum* and *Toxoplasma gondii* suggest the existence of horizontal transmission via a definitive host. Dogs are identified as a definitive host for *N. caninum* (8), and a significant association between the presence and number of dogs on a farm and the prevalences of seropositive animals in a herd has been reported (9).

The protozoan was reported for the first time in Québec in 1993 (Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec, (MAPAQ)). In 1996, 11.4% of aborted fetuses submitted to the provincial Québec veterinary diagnostic laboratory were infected with *N. caninum* (MAPAQ). Results of a case-control study indicates that *N. caninum* is present in the majority of dairy herds in Québec (9).

The primary objective of this observational study was to estimate the rate of vertical transmission of *N. caninum* in dairy herds in Québec and to compare these results with those of previous studies (5–7). A secondary objective was to investigate horizontal transmission of this protozoan in dairy herds in Québec.

Materials and methods

Herd selection and data collection

A letter was sent to all veterinarians who are members of the Québec Association of Large Animal Practitioners requesting their collaboration in finding herds that

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Table 1. Herd size, % of cows tested, % prevalence, % vertical transmission rate, presence of horizontal transmission, and the number of dogs during the 3 y prior to test date for each herd studied

Herd	Herd size	% cows tested	% prevalence[a]	% VT	Presence of HT	No. of dogs[b]
1	205	100	4.3	20.0	No	2
2	100	100	4.8	0.0	Yes	1
3	48	81	5.1	0.0	No	1
4	156	100	9.6	25.0	No	2
5	54	100	10.0	0.0	No	1
6	120	86	14.6	12.5	No	2
7	122	85	16.3	57.1	No	1
8	100	96	17.7	20.0	No	NA
9	61	100	17.9	50.0	No	2
10	63	100	19.4	20.0	No	0
11	117	71	20.5	60.0	No	3
12	167	100	22.4	35.7	Yes	1
13	90	91	25.6	60.0	Yes	1
14	78	100	28.3	33.3	No	2
15	64	92	30.5	28.6	No	1
16	36	100	35.6	0.0	No	1
17	93	99	40.2	53.3	No	1
18	78	65	41.2	71.4	Yes	2
19	51	100	41.2	85.7	Yes	2
20	69	100	42.5	53.8	Yes	1
21	53	92	44.9	80.0	No	2
22	50	64	46.9	66.7	No	1
23	105	52	61.8	83.3	No	2

HT — horizontal transmission; NA — not applicable; VT — vertical transmission
[a]% prevalence of seropositive animals
[b]Number of dogs during the 3 y prior to the test date

met the following criteria: 1) The serological status to *N. caninum* of a majority (more than 50%) of animals over 6 mo of age was available, and 2) the herd records allowed the study of genealogy (dam identification, birthdates).

Each herd provided, for each subject tested, the *N. caninum* ELISA ratios, the birthdate, and the dam's identification and birthdate. Based on this information, the genealogy was built for each family in each herd. Information on the presence of dogs on the farms was recorded for the 3 y prior to the test date.

Serology

Determination of the status of the animal was based on the ELISA ratio results. Two different laboratories were used to obtain the ELISA results. Reported sensitivities and specificities varied from 88.4% to 99%, and 92.9% to 98.4%, respectively (Biovet, St-Hyacinthe, Québec, and MAPAQ, St-Hyacinthe, Québec) (9).

Genealogy study

The pedigree of each cow tested was traced on the dam's side by using diagrams. Similar to the definition proposed by Shares et al (7), horizontal transmission was considered to have occurred if a seropositive cow was born to a seronegative dam and was known to have a minimum of 2 seronegative sisters.

Statistical analysis

The prevalence of seropositive animals in each herd was computed as the number of seropositive cows divided by the total number of animals tested within that herd. The rate of vertical transmission was calculated within each herd and for all herds combined. The rate of vertical transmission was computed as the proportion of seropositive heifers born to seropositive dams. To ensure independence, when a dam had more than one heifer tested, one dam/heifer pair was selected randomly. The overall rate of vertical transmission was compared with that of 3 previous studies (5–7) by use of a chi-squared test (10). A correlation coefficient between vertical transmission and prevalence of seropositive animals was also computed (10). A Mann-Whitney test was used to test the association between the number of dogs on the farms and presence of horizontal transmission (10).

Results

Eleven veterinarians responded to the letter and provided a total of 23 dairy herds. Study herds were located in 5 different geographical regions of Québec. Herd size, as evaluated by the number of animals over 6 mo of age, varied from 36 to 205 (average, 90) (Table 1). In each herd, 52% to 100% of animals over 6 mo of age had been sampled and, in 16 of the 23 herds studied, the proportion of animals sampled was above 90% (Table 1). For computation of vertical transmission, the number of dam/heifer pairs studied per herd varied from 1 to 15, for a total number of 144 dam/heifer pairs. The number of families studied per herd varied from 13 to 110, for a total number of 832 families. The number of generations per family varied from 2 to 5.

The herd prevalence of seropositive animals varied from 4.3% to 61.8% with an overall prevalence of seropositive animals of 21.9% (447/2037) (Table 1, Figure 1). The vertical transmission rate varied from 0% to 85.7% with an overall rate of 44.4% (64/144) (Table 1,

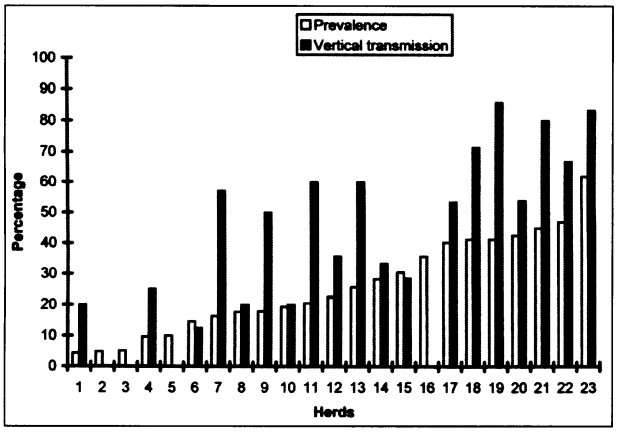

Figure 1. Prevalence and vertical transmission for each herd studied.

Figure 1). The correlation coefficient between vertical transmission rate and prevalence of seropositive animals was 0.74 ($P < 0.0001$). No association was identified between the number of dogs on farm and identification of horizontal transmission ($P = 0.63$).

Overall, 33 families were eligible for study of horizontal transmission by presence of 3 tested siblings. Seven horizontal transmissions were identified in 6 different herds (Figure 2). Two horizontal transmissions were observed within the same family.

Discussion

A high degree of variability in the rate of vertical transmission of *N. caninum,* from 0% to 85.7%, was noticed among the 23 herds studied. Compared with other studies (5–7), where 1 to 4 high prevalence herds (average, 42.4%) were examined, the overall estimated rate of vertical transmission in this study was significantly lower (average, 44.4%) than estimates reported previously (81% (93/115), $P < 0.0001$; 92% (46/50), $P < 0.0001$; 72% (76/105), $P < 0.0001$; and 93% (14/15), $P = 0.0003$) (5–7). In the present study, multiple herds with various proportions of seropositive animals were examined cross-sectionally. Results from this study may, therefore, be representative of what one might encounter when examining the genealogy of a herd tested for *N. caninum* in the field.

Disparities between rates of vertical transmission reported previously (5–7) and that estimated in this study may be explained by the variability in prevalence of seropositive animals. In fact, the high degree of correlation between rate of vertical transmission and prevalence of seropositive animals suggests that only in high prevalence herds are high levels of vertical transmission observed. Two explanations for this correlation are worth examining. First, high herd prevalence of seropositive animals may reflect a high proportion of active versus latent infections. One would expect higher rates of vertical transmission due to reactivation of infection in pregnant cows in these herds. Second, positive predictive value of the ELISA result must be considered in interpreting herd results. In low prevalence herds, the predictive value of a positive test is low, because of the high proportion of noninfected animals. Therefore, low prevalence herds may expect a higher proportion of false positive results, relative to high prevalence herds. When examining the daughters of false positive dams, the vertical transmission rate would be decreased. It appears that estimated vertical transmission rates are more reliable in high prevalence herds than in low prevalence herds.

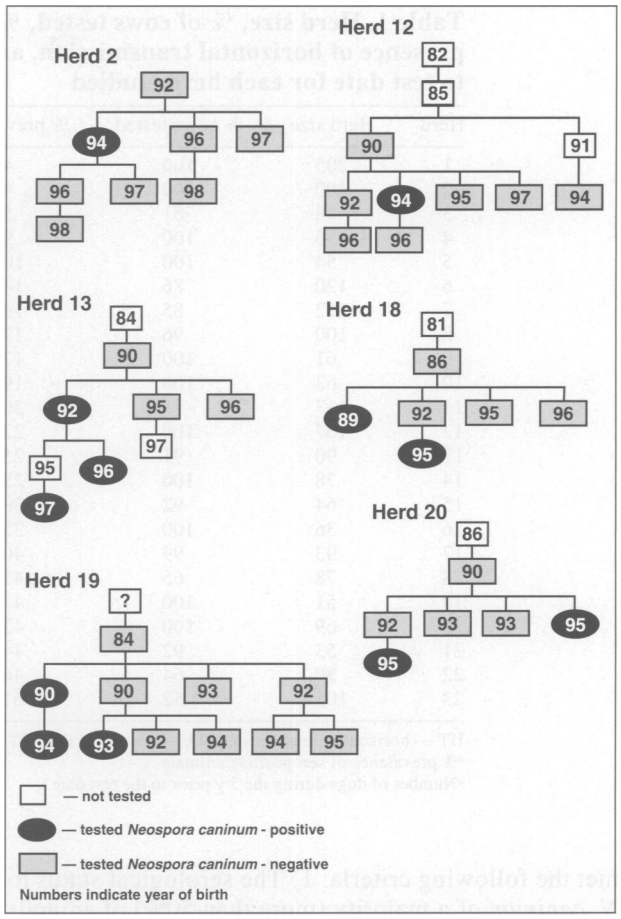

Figure 2. Genealogy of the 6 families in which horizontal transmission was identified.

Study design of the various reports of vertical transmission may also account for the differences in estimated rates of vertical transmission. A study by Paré et al (5) and one by Wouda et al (6) were prospective, whereby all calves born were sampled prior to receiving colostrum. In the present study, only cows and heifers that were currently in the herd were sampled. A higher rate of culling of seropositive animals due to poor reproductive performance and low milk production has been reported (11,12). It can be hypothesized that selective culling of infected cattle would reduce the observed rate of vertical transmission when examining only contemporary animals. In fact, in the study by Wouda et al (6), the rate of vertical transmission was lower in the retrospective part compared with the prospective part of the study. The study by Schares et al (7) reported results from cows and heifers that were currently in the herd, which might have inflated the estimated rate of vertical transmission by colostral transfer of passive immunity. The rate of vertical transmission of *N. caninum* may also be lower in dairy herds in Québec because of differences in management and environmental factors in the other countries. Herd size, climate, and production level may

alter the level of stress, thereby influencing recrudescence of latent infections and affecting the rate of vertical transmission of *N. caninum.*

The presence of horizontal transmission through a definitive host was suspected in 6 of the 23 herds studied. In 2 families studied (herds 13 and 19), the presence of seropositive daughters born to the cow suspected of having been infected through horizontal transmission provided confirmation of her infected status. In one family (herd 2), lack of transmission from a seropositive dam to her descendants suggested the possibility of a false positive result. In fact, herd 2 had a low prevalence of seropositve animals and, therefore, a low positive predictive value, which contribute to an increased likelihood of a false positive result. Use of strict criteria to define horizontal transmission, similar to those used by Schares et al (7), limited the number of families in which it was possible to identify horizontal transmission. Therefore, occurrence of horizontal transmission may be more frequent than that estimated. However, prevalence of seropositive animals should be taken into account when interpreting the presence of horizontal transmission. No association between farms with horizontal transmission and the presence of dogs was identified in this study. This could be the consequence of the small number of farms with horizontal transmission; one other explanation could be that exposure to an unidentified risk factor is necessary to have horizontal transmission.

Because the present study was an observational study, efforts were made to reduce the influence of biases on the results presented. A convenience sample was used to allow inclusion of as many herds as possible. This resulted in 2 different ELISAs being used. However, among herds, high proportions of cows were studied (16 herds had more than 90% cows sampled), reducing the possibility of biasing the estimates of prevalence of seropositive animals. Multiple generations were used to estimate the rate of vertical transmission. Also, to minimize impact of a false positive or false negative result on estimates of rate of vertical transmission, a random selection of dam/heifer pairs was used in computations, when a cow had more than one daughter tested.

Results from this study confirm the importance of vertical transmission in the epidemiology of *N. caninum,* particularly when there is a high prevalence of seropositive animals in a herd. The variability observed among herds in the rates of vertical transmission warrants testing of offspring for *N. caninum,* ideally on precolostral samples. Although horizontal transmission was identified in a 6 herds, it does not appear to be the major route of infection for *N. caninum.* The study of risk factors in these herds may contribute to better understand the epidemiology of horizontal transmission of *N. caninum* in cattle.

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