Epidemiology of *Neospora caninum* in Canadian dairy farms

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Abstract

Neospora caninum was first recognized in dogs in Norway in 1984 and in 1988, a new protozoan species, *N. caninum*, was proposed under a new genus, *Neospora.* It is an apicomplexan protozoan, with dogs and coyotes as the definitive host.

The overall objective of this thesis was to acquire a more complete appreciation of the epidemiology of *N. caninum* infection in Canadian dairy farms, by answering important questions about the epidemiology of this disease. The epidemiology of neosporosis was investigated through four different studies and one extensive literature review.

The review covers the life cycle of the agent, its mechanisms of transmission, clinical signs, and tests for diagnosing the infection. Data on the prevalence of the infection in Canadian dairy and beef cattle are reviewed and briefly compared with estimates from other parts of the world. Most importantly for Canadian bovine practitioners, the impacts of the infection, risk factors for its occurrence and methods of control are also discussed.

A total of 6,662 blood samples were collected from cows on 240 dairy farms selected randomly from 6 provinces in Canada. Enrollment in a monthly, individual milk-testing regimen through a dairy herd improvement (DHI) program was a prerequisite for participation. Serum samples were stored at –20°C, and then tested for antibodies to *N. caninum* using an indirect ELISA.

The overall Canadian cow level prevalence was 11.9%, while for the maritime provinces it was 19.5% and for the western provinces it was 10.4%. The herd level, prevalence was 81.9% at the national level, and 86.7% and 80.0% for maritimes and western provinces, respectively. *N. caninum* infection was spatially clustered in both western and maritime Canadian provinces, occurring more commonly in Alberta in the western provinces and New Brunswick in the Maritimes.

A study was carried out to investigate the impact of serostatus for *Neospora caninum* (NC), bovine viral diarrhea virus (BVDV), bovine leukosis virus (BLV) and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and their possible interactions on reproductive efficiency (specifically, the ability to conceive and assumed fetal loss) in dairy cows. A Cox proportional hazards model with shared (herd-level) frailty was used to analyze the calving to conception interval data while controlling for herd-clustering effects. In this model, only BLV serostatus was associated with calving to conception intervals (CCI). Peak milk production before 73 days, SCC (linear score) and peak milk production after 73 days also had significant effects on the interval between calving and conception.

Logistic regression models of CCI greater than 200, 250, and 300 days were built, controlling for herd as a random effect. *N caninum*-seropositive cows had a 1.27 times higher risk of exhibiting a CCI exceeding 200 days, a 1.37 times higher risk of a CCI exceeding 250 days, and a 1.54 times higher risk of a CCI exceeding 300 days. BLV status also interacted with lactation numbers in the CCI200 model only, with older seropositive cows being less likely to have a CCI over 200 than 1st lactation seropositive cows. Neither BVDV nor MAP showed any significant effect in the models.

A logistic regression model of first service conception (FSC) revealed a significant interaction between *N. caninum* and BVDV infections. Increased peak milk production also reduced FSC. BLV and MAP had no significant impact on FSC.

Another study had the objective of determining important cow and herd level risk factors for seroprevalence for *N. caninum* in a population of randomly selected Canadian dairy cattle. A mixed logistic regression model was built using *N. caninum* serostatus at the cow level as the outcome variable, with herd as a random effect and province as a fixed effect. BLV serostatus was the only cow-level variable that remained in the model. Dogs being present on the farm, but not eating placentas and/or fetuses increased the odds of a farm having *N. caninum* by a factor of 1.54. If the dogs also ate placentas and/or fetuses, the odds ratio was 2.22. Other variables that remained (and appeared to be protective) in the final model included: "farmer asks for BVDV-negative exam before introduction of animal"; "number of milk cows on farm"; "dry cows receive Rumensin (monensin) in diet"; "embryo transfer used on farm"; "area of farm used for forage production per cow"; and "heifers have nose-to-nose contact with calves". These findings indicate management factors that could be considered to help control *N. caninum* seroprevalence and its related impacts.

An economic analysis that estimated the production losses (and their ranges) due to *N. caninum* in the Canadian dairy industry and the possible range of losses for individual Canadian dairy herds was conducted. For the Canadian dairy industry, annual losses attributable to *N. caninum* were estimated to be $1,838.76 per 100 cows ($18 per cow) (95% CI = $7 to $32 per cow). For herds (assumed to be 61 cow herds), losses were estimated to be $1,494.38 but there was a wide range of losses (95% CI = $37 to $5,309), primarily due to the variation in the within-herd prevalence.

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1 Introduction

1.1 Canadian Dairy Industry

The dairy industry, after grains, red meats and horticulture, is the fourth largest sector of the Canadian agri-food economy. In 2004, dairy farming generated 4.6 and 11.5 billion Canadian dollars in total farm cash receipts and sales from Canadian dairy processors, respectively. This represents 14.9% of all processing sales in the Canadian food and beverage sector. For the period of 2003/2004, there were approximately 64,000 people working in the Canadian dairy industry (http://www.dairyinfo.gc.ca/cdiccdi.htm).

1.2 Production Limiting Disease Committee

Infectious diseases negatively affect the net return to dairy farmers through:

- lower milk production and reproductive efficiency levels,
- reduction in the efficiency of cows in early lactation and additional culling,
- loss of opportunity in international and local sales, and
- cost of prevention and treatment of the direct effects of those diseases.

The Production Limiting Disease Committee (PLDC) was created in 1997 in Canada with the objective to reduce all these impacts of infectious diseases. Four diseases in Canadian dairy cattle: (enzootic bovine leukosis, bovine viral diarrhea, Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*), and neosporosis (*Neospora caninum*)), were selected on the basis of their assumed impact on the Canadian industry. The PLDC then initiated research to determine the prevalence, spatial distribution, impacts, risk factors, and economics losses of each of the infectious agents causing these diseases. In

addition, it was recognized that there had been very little research on how the agents that cause these diseases might interact if co-infecting the same farms and the same animals, and this needed to be addressed. A long-term goal of the PLDC is to develop cost-effective control programs for these diseases, for use by Canadian dairy producers.

1.3 Neosporosis

This thesis deals with the prevalence, distribution, production impacts, risk factors, and economic effects of neosporosis in the Canadian dairy industry. While the focus is on neosporosis, the effects of co-infections with the other three organisms will also be addressed. A review of neosporosis, as it pertains to the Canadian cattle industry, is presented in Chapter 2. Nevertheless, a few key aspects are presented below.

1.4 Epidemiology of *Neospora caninum*

Neospora caninum was first recognized in dogs in Norway in 1984, and in 1988, a new protozoan species, *N. caninum*, was proposed under a new genus, *Neospora*. It is an apicomplexan protozoan, with dogs and coyotes as the definitive host[1]. Many domestic mammals, including beef and dairy cattle, can act as intermediate hosts.

Both horizontal (via ingestion of canine feces containing oocysts) and vertical (*in utero*) transmission (via tachyzoites) occur, with vertical transmission being the primary route of transmission on most dairy farms. Infected animals remain infected for life, and therefore seropositive animals are typically subclinically infected, except for the brief time during which they may abort. The epidemiology of *N. caninum* is covered in more detail in section 2.4 of Chapter 2.

1.5 Clinical signs / subclinical effects

Possible effects of *N. caninum* in dairy cattle include: abortions; stillbirths; newborn calves with central nervous system malformations and low birthweights; reduced milk production; premature culling; and decreased value of breeding stock with known *N. caninum*-positive status. Cows of any age can abort at any point during the gestation period, but primarily from 3 months to term. This parasite is now recognized as an important cause of reproductive problems and abortion in cows. It is found worldwide, with widespread occurrence. Diagnosis of *N. caninum*-associated abortions in dairy cattle in Ontario increased from 1.6% of abortion submissions in 1993-94 to 5.7, 11.4, 12.5, and 14-15% in 1994-95, 1995-96, 1996-97, and 1997-00, respectively. In Quebec, 11.4% of all aborted bovine fetuses submitted to diagnostic laboratories in 1996 were infected with *N. caninum* . Similar estimates of 15% to 20% have been found in California and The Netherlands, demonstrating the large impact of *N. caninum* in dairy producing areas of the world .

There are three main subclinical effects of infection with *N. caninum.* Two effects which affect dairy cattle productivity, reduction in milk production and premature culling, are described in more detail in sections 2.7.2 and 2.7.3 respectively. The third effect is reduction of weight gain and feed efficiency, which has greater impact in beef cattle and is discussed in more detail in section 2.7.4 of the thesis.

1.6 Prevalence / Spatial Distribution

There have been three Canadian studies of the prevalence of *N. caninum* in dairy cattle and two for beef cattle. Two studies, in the United States and Italy, have described

the spatial distribution of *N. caninum*[17].The literature relating to the prevalence and spatial distribution of *N. caninum* is presented in Section 2.6 of Chapter 2.

1.7 Risk Factors

There is little information about risk factors for neosporosis, and some important unanswered questions remain about the epidemiology of neosporosis (e.g. other possible definitive hosts). There are two Canadian risk factor studies[18]. There are many remaining questions about risk factors and their role in the epidemiology of the disease and their potential modification to prevent and control the incidence of new infections and of abortions. The literature relating to risk factors for *N. caninum* is presented in Section 2.9 of Chapter 2.

1.8 Economic Impact

A previous Canadian study estimated direct losses attributable to *N. caninum* were $2,304.98 per infected herd of 50 cows per year . However, this estimate was based on prevalence data from a single region of the country (Atlantic Canada) and involved some untested assumptions. The global literature relating to the economic impacts of *N. caninum* is presented in Section 2.8 of Chapter 2.

1.9 Objectives of Thesis / Thesis Outline

The overall objective of the research program on which this thesis was based was to acquire a more complete appreciation of the epidemiology of *N. caninum* infection in Canadian dairy farms. By answering important questions about the epidemiology of this

disease, constructive recommendations could be made to farmers to minimize the occurrence and the impact of the disease.

The specific objectives which have been addressed in this thesis are related to:

- the prevalence and spatial distribution of *N. caninum* infection in dairy cattle in Canada;
- the impact of *N. caninum* on reproductive efficiency in dairy cattle in Canada;
- the identification of risk factors for *N. caninum* in dairy cattle in Canada;
- an analysis of the economic impact of *N. caninum* in dairy cattle in Canada.

There are 5 main chapters in this thesis.

- Chapter 2 is a review of the current state of knowledge of neosporosis in dairy and beef cattle, with a particular focus on its relevance to the Canadian cattle industry.
- Chapter 3 presents the results from a study designed to estimate the current seroprevalence of *N. caninum* in Canadian dairy cattle and to better understand the spatial distribution of the disease in Canada.
- Chapter 4 presents a study which aimed to investigate the impact of seropositivity for the four diseases studied in the PLDC, and their possible interactions on reproductive efficiency (specifically, the ability to conceive and assumed fetal loss) in dairy cows. This four-disease study complements two other studies (present in Tiwari, 2005, PhD Thesis) which investigated the

impacts of seropositivity for these infectious agents on levels of milk production and on the risk of culling.

- Chapter 5 presents an evaluation of a wide range of risk factors related to co-infections with other pathogens, and herd management practices on dairy farms on *N. caninum* serostatus in randomly selected dairy cattle and herds.
- Chapter 6 gives an economic analysis that estimates the production losses (and their ranges) due to *N. caninum* in the whole Canadian dairy industry and the possible range of losses for individual Canadian dairy herds.
- Chapter 7 provides an overall discussion of the results.

2. A review of *Neospora caninum* in dairy and beef cattle – a Canadian perspective[1]

2.1 Introduction

Neospora caninum is an apicomplexan protozoan that was first recognized in dogs in Norway in 1984. In 1988, a new protozoan species, *N. caninum*, was proposed under a new genus, *Neospora*. This parasite is now recognized as an important cause of reproductive problems and abortion in cows. It is found world-wide, with widespread occurrence of neosporosis in beef cattle, dairy cattle, or both in most provinces in Canada, including the maritime provinces, Ontario, Quebec, Manitoba, Saskatchewan, Alberta, and British Columbia. Since its discovery, there has been much research about this parasite, and several general reviews have been written. The aim of this paper is to summarize the current state of knowledge of neosporosis in dairy and beef cattle, with a particular focus on its relevance to the Canadian cattle industry.

2.2 Methods

1Medline (accessed via PubMed from 1966 to 2004), The Commonwealth Animal Bureaux (CAB) (accessed via VetCD and ParasiteCD from 1973 to 2004), and Agricola, produced by the National Agricultural Library of the U.S. Department of Agriculture (accessed via National Agricultural Library from 1970 to 2004) were used to collect the majority of the references that were used in this paper. The keywords used in the search of the databases were *Neospora*, neosporosis, Canada, Canadian, and cattle. In

[1] Haddad JPA, Dohoo IR, VanLeeuwen JA. A review of *Neospora caninum* in dairy and beef cattle – a Canadian perspective. Can Vet J 2005 Mar;46(3):230-43.

addition, a small number of papers were identified from the reference lists of other papers, or through personal knowledge of reports or conference proceedings.

2.3 History

Before Dubey *et al.* described *N. caninum* in 1988, many researchers already suspected that a new, different genus of protozoa was causing abortion in cows. In 1987, O'Toole and Jeffrey described a sporozoan associated disease in a weak newborn calf in England that was tested for toxoplasmosis and sarcocystis by an immunoperoxidase test with negative results. It would later be confirmed as *N. caninum*.

The characteristics of the oocyst of *N. caninum* are quite similar to those of oocysts of *Hammondia heydorni* in dogs and of *Toxoplasma gondii* and *Hammondia hammondi* in cats. Furthermore, their tachyzoites and bradyzoites appear similar under light microscopy, but they can be distinguished by electron microscop by the number, appearance, and location of their rhoptries, leading to the conclusion that they are different protozoa.

In Canada, the first report of *N. caninum* associated with clinical disease was in 1994 when a 3-day-old calf in Alberta presented with clinical neurological signs. Histopathologic examination revealed tissue cysts and lesions in the central nervous system (CNS). In the same journal issue, it was reported that a Santa Gertrudis cow in British Columbia had aborted in her 8th month of pregnancy. The calf and the fetus were both confirmed as *N. caninum* positive by immunohistochemical (IHC) staining, while tests for *Toxoplasma gondii* and *Sarcocystis* spp. were negative.

The earliest known outbreak of abortions due to *N. caninum* in Canada was also reported in 1994. On a dairy farm in eastern Ontario, 15 of 80 cows aborted in an 18-day

period in January and February 1994. The cows were 3 to 7 y of age and aborted at 4 to 8 mo gestation. From the 15 abortions, 4 fetuses were submitted to a provincial veterinary diagnostic laboratory, where typical lesions of *N. caninum* were found and the diagnosis was confirmed by IHC. Comparative testing was carried out for infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), and leptospirosis with negative results. From the same laboratory, lesions of neosporosis were subsequently identified in cattle from stored samples received from 24 other farms between February 1993 and July 1995, using histologic examination and IHC tests. Since that time, *N. caninum* has been a commonly diagnosed cause of abortion in cattle in many parts of Canada.

2.4 Biology and life cycle of *Neospora caninum*

Figure 2.1 depicts the life cycle of *N. caninum* as it is understood today. The dog is a definitive host of *N. caninum*, although it is suspected that it may also serve as an intermediate host. As a definitive host, the dog sheds unsporulated oocysts in the environment for 5 to 17 d after the ingestion of tissue cysts. After 3 d in the environment, the oocysts (the sexual stage) sporulate to form 2 sporocysts, each containing 4 sporozoites. It is unclear how long oocysts will survive in the environment. Intermediate hosts (cattle) ingest oocysts that are found in contaminated food and water. Sporozoites are released in the intestinal tract where they penetrate cells and become tachyzoites (a rapidly dividing asexual phase). Tachyzoites divide and quickly spread to other host cells, which they invade and often destroy. Tachyzoites have been found in neural cells, macrophages, fibroblasts, vascular endothelial cells, hepatocytes, and muscular cells including those of myocardium, and the placenta in pregnant cows. The tachyzoites can be transmitted vertically from a dam through the placenta to the fetus. In neural cells,

tachyzoites can transform into bradyzoites (a slowly dividing asexual phase) when a strong immune response is mounted against the protozoa elsewhere in the body. The bradyzoites form tissue cysts around themselves for protection; they remain latent until the immune system of the intermediate host is suppressed, allowing them to recrudesce. Cysts have been found in the brain, spinal cord, and retina. Tachyzoites in placental tissue, (and likely bradyzoites in tissue cysts), when consumed by a dog, implant in the gastrointestinal tract where they mature, begin to shed oocysts, and complete the horizontal transmission cycle.

When calves were infected experimentally by oral inoculation with *N. caninum* oocysts collected from dog feces, the calves seroconverted within 2 to 4 wk. In another experiment, uninfected calves that were bottle-fed colostrum with added tachyzoites also seroconverted, introducing another possible mechanism of transmission, which needs confirmation under commercial conditions. It is unknown whether colostrum with tachyzoites from naturally infected cows would infect calves.

Serological evidence of *N. caninum* infection or confirmation of its presence by using IHC or polymerase chain reaction (PCR) has been found in many mammals other than cattle and dogs; these include goats, sheep, horses, deer, foxes, dingoes, raccoons, and coyotes. A recent article has confirmed that coyotes are a definitive host as well. The other canids listed above may also be definitive hosts, while the herbivores listed above may also be intermediate hosts. It has been suggested that birds may also act as reservoir hosts for *N. caninum*, but none of these suppositions has yet been proven, and further research on these potential hosts is required.

The principal route of infection in cattle is transplacental (vertical) transmission and the same cow can pass the infection to multiple offspring. The probability of a seropositive dam producing a calf that is seropositive, prior to consumption of colostrum, has been widely reported as ranging between 81 and 100%. However, these reports are based on a small number of herds with a high prevalence of seropositive animals, including a large cow-calf herd in northern Alberta following a *N. caninum* associated abortion epidemic. This high probability of vertical transmission may be appropriate for high prevalence herds, particularly if they are having abortion problems. However, lower probabilities of vertical transmission were found in a study of 23 dairy herds in Quebec with a range of seroprevalences (from 4.3 to 61.8%); these farms may be more representative of the majority of infected dairy and beef herds in Canada. In this Quebec study, a range of vertical transmission probabilities, from 0% to 86% were found, with the high probabilities occurring in the herds with high seroprevalences.

A number of factors may have contributed to the lower vertical transmission probabilities in the Quebec herds compared with those reported elsewhere. First, there is an increased likelihood of false-positive test results among dams in low prevalence herds due to low predictive values of positive tests in these herds. False-positive dams will not deliver a congenitally infected calf, so when they are mixed in with true positive dams a lower perceived probability of vertical transmission compared with what would be observed if only truly positive dams were included will be obtained. Second, vertical transmission estimates could differ, depending on the tests used to determine seropositivity, with their associated sensitivities and specificities. Third, selective culling of infected cattle without identification of their serological status prior to culling could

also bias vertical transmission probabilities downward. Congenitally infected cows that abort are more likely to be culled for abortion-related reasons (see details in impacts section below); consequently, they are lost to follow-up for vertical transmission assessments, leading to a possible downward bias. Fourth, the use of embryo transfer will prevent *N. caninum* infection in daughters of seropositive cows, provided that the embryos are implanted in seronegative recipients and will also reduce the apparent probability of vertical transmission in these herds. Finally, in small herds with moderate seroprevalences or large herds with low seroprevalences, the estimates of vertical transmission will be dependent on only a few seropositive dams and their progeny, making these estimates highly unstable and susceptible to the biases mentioned above. Therefore, the low probabilities of vertical transmission are likely underestimated.

While the above reasons may partially explain the differences found in the results between the herds in Quebec and in high prevalence herds, there are also possible biological reasons for the lower probabilities of vertical transmission in the herds in Quebec. First, in low and moderate prevalence herds where there is no horizontal transmission; infections occur *in utero* and turn into latent infections by the time female calves reach a reproductive age. Without circulating tachyzoites, daughters of latently infected dams are unlikely to become infected congenitally unless there is sufficient down-regulation of the immune system in mid-gestation to allow recrudescence. Evidence for this effect was recently demonstrated in a study of dairy herds in The Netherlands with a history of abortion problems. Even with abortion problems evident in the herd, vertical transmission decreased with age, with 66% of seropositive cows in their 4th or higher parity having seropositive calves, compared with >80% in their 1st lactation.

Second, differences in herd factors (such as herd size, average age of cows, and production level) between the herds in Quebec and those large herds citing high vertical transmission risks may result in lower stress levels in the herds in Quebec, thereby reducing the down-regulation of the immune system in mid-gestation and recrudescence of predominantly latently infected cattle.

True vertical transmission probabilities for most Canadian dairy (and perhaps beef) herds not having abortion problems are unlikely to be 0%. A study in a population of randomly selected Canadian dairy herds would be useful to confirm whether the average vertical transmission level of 44% found in the case control study in Quebec is representative of the Canadian dairy industry as a whole. Because *N. caninum* infection is thought to be maintained by vertical infection on most farms, the true level of vertical transmission would be important to determine in order to assess the need for culling seropositive cattle.

Based on available reports in California and England, estimated incidence rates of horizontal transmission of infection appear to be generally quite low, 1 infection per 100 cow-years at risk in one study and 1.9 infections per 100 heifer-years at risk in another study, respectively. Specific farms undergoing a horizontally transmitted abortion outbreak due to *N. caninum* could have a much higher incidence rate, but because outbreaks are not common, the overall rate is likely to be low.

Dogs shed oocysts after ingesting tissue from a number of different infected species: cattle, goat, sheep, guinea pigs, rats and mice. One study showed that 2 dogs already infected with *N. caninum* did not resume shedding oocysts again upon re-exposure. However, confirmation of this finding is required in a larger number of dogs

(and possibly wild canids) with different types of tissues having varying levels of *N. caninum*. Recrudescence of shedding in dogs (and possibly wild canids) also needs to be investigated further to determine the possibility of it occurring under varying circumstances and stress levels (pregnancy).

The relative proportion of vertical versus horizontal transmission in a farm or region is likely dependent on the current seroprevalence of infection in the cattle population, and the distribution of infected dogs and, maybe, other canids in the region and their access to cattle and their feeds.

2.5 Diagnosis

2.5.1 Clinical signs and lesions

When a naïve cow is infected with *N. caninum*, there are 2 main factors that determine which of 4 manifestations (early embryonic death (EED), abortion, stillbirth or birth of a feeble abnormal calf, and birth of a normal calf with no obvious effect of *N. caninum* infection) occurs; whether the animal is pregnant or not at the time of infection, and the phase of gestation - early, mid, or late.

If a naïve cow is not pregnant when infected, the infection usually produces no clinical signs but seroconversion occurs, along with the development of cell-mediated immunity (CMI) (involving cell proliferation and interferon-gamma (IFN-J) production). Infection leads to limited multiplication of intracellular parasites due to IFN-gamma produced by CD4-T cells, with persistent infection (as bradyzoites) within tissue cysts in the CNS.

If a naïve, cow is pregnant and in early gestation (< 2-3 mo) when infected, the infection leads to EED. It is likely that the EED is caused by the presence of pro-inflammatory T helper (Th) type-1 cytokines at the maternal-fetal interface (placenta) damaging the placental connection, because the maternal immune system develops a strong cell proliferation response with production of IFN-gamma in response to parasite antigen.

If a naïve, cow is pregnant and in mid-gestation (3-7 mo) when infected, the infection leads to either abortion or birth of a weak, abnormal calf, depending on the month of gestation. At this stage of gestation, the fetus has an immature immune system and is unable to fully fight off the infection. With the down-regulation of the maternal TH1-cell response by the placental TH2-cell production of cytokine, the immunological defense of the cow is reduced at this stage, allowing for an increase in *N. caninum* population, with subsequent invasion of the placenta and calf by the *N. caninum* tachyzoites. If the *N. caninum* exposure completely overcomes the immune system of the calf, tachyzoites dramatically increase in number, leading to extensive tissue damage and the abortion of an autolysed fetus. If the immune system of the calf is nearly completely developed, a weak abnormal calf is born with poor formation of the CNS or encephalomyelitis due to the mild or moderate tachyzoite-induced tissue damage, leading to neurological symptoms and low weight at birth[1].

If the naïve cow is pregnant and in late gestation when infected, the infection leads to the birth of a weak or normal calf that is seropositive for *N. caninum*. During this stage of gestation, the immune system of the fetus is more mature than that of a younger

fetus; therefore, it is more able to control the infection, leading to limited or no clinical signs in the newborn calf.

2.5.2 Diagnostic tests

Since the discovery of *N. caninum,* many diagnostic tests have been developed to help in diagnosing this parasitic infection. They include immunohistochemical (IHC) staining, indirect fluorescent antibody tests (IFAT), enzyme-linked immunosorbent assays (ELISA), direct agglutination tests (DAT), western blot analysis (WB), and polymerase chain reaction (PCR). This paper will focus on the tests used most frequently and emphasize those that are commercially available in Canada.

Immunohistochemical (IHC) staining in which an avidin-biotin-peroxidase complex was used was the first test produced to identify the parasite and demonstrate that there was no cross-reaction with the closely related *Toxoplasma gondii* or other extra-intestinal coccidia. This test is still used to confirm *N. caninum* parasites in tissue where characteristic inflammatory lesions are observed on histologic examination; however, IHC staining can underestimate the true prevalence of infection due to low sensitivity in severely autolysed fetuses. Brain is the tissue of choice from the fetuses for the diagnosis of *N. caninum* when IHC staining or IFAT are used, although frequently tissue cysts or tachyzoites can also be found in lung, kidney, and skeletal muscle. From cows, IHC staining can be carried out in samples of brain, liver, or heart.

The main tests used for serologic detection of *N. caninum* infection are IFAT and ELISA. The IFAT is very specific and there are no cross-reactions between *N. caninum* and *T. gondii*, although they share several antigens. Comparisons of IFAT results from different laboratories is extremely difficult, given the different antigen preparations,

reagents, and serum dilutions used and a variety of cutpoints are used. Cutpoint dilutions of 1:200 to 1:640 have been suggested for infections in adult cattle. For bovine fetuses and pre-colostrum newborn calves, lower values, such as 1:80 have been suggested as being indicative of infection. For 1-week-old heifers that have been fed colostrum, a cutpoint of $\geq$ 1:5120 has been reported to be a good test for the early diagnosis of seropositive heifers.

However, the IFAT is time-consuming and expensive compared with ELISA, therefore, it is not used routinely for screening cattle populations for *N. caninum* infection. An indirect ELISA (i-ELISA), using a crude tachyzoite antigen derived from an aborted fetus, was first reported in 1994 and was reported to have a sensitivity and specificity of 88.6 and 96.5%, respectively. This test is the basis of both the IDEXX (IDEXX Laboratories, Westbrook, Maine, USA) and Biovet (BIOVET Laboratories, St. Hyacinth, Quebec, Canada) ELISA test kits, 2 indirect *N. caninum* ELISAs available in Canada. Sample-to-positive control (S/P) ratios of 0.45 and 0.60 for the IDEXX and Biovet ELISAs, respectively, were determined as the optimal cutpoints in order to differentiate between infected and noninfected cattle. A recent independent validation study of the IDEXX and Biovet ELISAs compared them to immunoblotting (the gold standard) by using 150 field sera from an infected beef herd. The results showed that the 2 ELISAs worked equally well and there was no statistically significant difference between the performances of the 2 tests. Both tests showed high reproducibility, repeatability, and substantial agreement with results from 2 other laboratories. Sensitivities for the Biovet and IDEXX ELISAs on the field samples were 95.1% and 97.6, respectively, while specificities were 100% and 98.5%, respectively.

However, there has been concern regarding cross-reaction of the indirect ELISA with antibodies to infection with *Sarcocystis* spp., leading to false-positive test results. Use of higher, more specific cut-off values would reduce the number of false-positive test results, but would lower the sensitivity of the test for identifying *N. caninum* infected cattle.

A competitive inhibition ELISA a stands for assay (c-ELISA) (VMRD Laboratories, Pullman, Washington, USA) has been shown to be unreactive to antigens of 2 closely related apicomplexan protozoa, *Toxoplasma gondii* and *Sarcocystis cruzi*. An independent assay to validate the test used a "gold standard" set of 184 cow sera (42 positives and 142 negatives) defined by fetal histopathologic examination and *N. caninum* immunohistochemical staining and by maternal *N. caninum* IFAT at a 1:200 serum dilution. The sensitivity was 97.6%, and specificity 98.6%. This ELISA has recently been adopted by many laboratories in Canada as the test of choice for detecting antibodies against *N. caninum*.

All serological results for *N. caninum* should, however, be interpreted with caution, because the immune system is not static and antibody levels fluctuate, particularly for parasites that form cysts that wall themselves off from the host's immune system and can recrudesce with immunosuppression. A single serum sample from an individual cow may not accurately reflect her infection status, particularly on farms without a history of *N. caninum* abortions. On these farms, only consistent results on multiple tests during different years or seasons of the year should be used to make culling decisions, particularly when S/P ratios are close to the cut-off values of the test. For example, congenitally infected heifers that have had a history of positive *N. caninum*

titres have had negative titres at calving, while giving birth to a *N. caninum*-infected calf. Similarly, cows that abort a *N. caninum*-infected fetus may no longer have a significantly elevated titre at the time of abortion.

In a Dutch study of 21 dairy farms with a history of *N. caninum* abortion, blood was sampled multiple times to evaluate a single serological screening. Based on subsequent test results, the first test provided a relative sensitivity and specificity of 94.7 and 95.6%, respectively, and positive predictive value (PPV) and negative predictive value (NPV) of 92.4 and 97%, respectively. However, there are two concerns related to applying the results of this study to Canadian dairy herds. First, no gold standard tests were used in this study to determine the true *N. caninum* status of the tested animals, and therefore, cross-reactions or other misclassification problems may have occurred. In addition, 36.8% of animals tested positive in this study, a prevalence that is considerably higher than most Canadian dairy and beef farms (as discussed in detail below). This difference is a concern because PPV and NPV of test results are affected by the estimated true prevalence of infection in the population being tested. With lower seroprevalences, such as those found in Canada, PPV decreases, making confirmation of an initial positive test necessary, particularly if the positive titre is close to the cut-off value. Conversely, on the small number of Canadian farms with seroprevalences approaching 80-100%, NPV may decrease, making confirmation of an initial negative test necessary.

Accurate comparison of results between samplings or studies that use different tests is also challenging unless duplicate samples, tested by the different tests, have produced similar results, something that is rarely done. Using estimated true prevalences, adjusted for test sensitivity and specificity, can reduce the bias in prevalence estimates.

However, populations of cattle with a high average age could demonstrate low *N. caninum* prevalence simply because most infected animals are in latent stages that are less likely to test positive.

One potentially useful test for *N. caninum* diagnosis in outbreak investigations is an immunoglobulin (Ig) G avidity ELISA, which can be used to differentiate acute and chronic infections. With this test, the binding strength (avidity) of the IgG antibodies to a *N. caninum* antigen is measured. The IgG avidity increases with time after infection; consequently, low (<50) and high (>50) avidities indicate recent and chronic infection, respectively. This test is not yet commercially available in Canada as further research is needed to validate it.

At the time of writing (2004), the following Canadian laboratories offer the c-ELISA (VMRD Inc.) test: Diagnostic Services of the Atlantic Veterinary College (AVC/UPEI) in Charlottetown, Prince Edward Island; the Animal Health Centre in Abbotsford, British Columbia; Prairie Diagnostic Services in Regina, Saskatchewan; and Veterinary Laboratory Services in Guelph, Ontario. The Biovet ELISA is available at the source laboratory. The IDEXX ELISA is available at the International Bio Institute in Fergus, Ontario, and the Veterinary Services Laboratory in Winnipeg, Manitoba. The IFAT is available for diagnostic confirmation at the Animal Health Centre in Abbotsford. The PCR for aborted fetuses is available at the Veterinary Services Laboratory in Winnipeg. The IHC test is available at Prairie Diagnostic Services in Saskatoon, Saskatchewan.

2.6 Prevalence of *N. caninum* Infection

2.6.1 Prevalence in dairy cattle

Based on i-ELISA testing of 30 randomly selected dairy cattle in each of 181 randomly selected herds in Manitoba, Saskatchewan and the 3 maritime provinces, and from a representative population of 51 herds in Ontario, *N. caninum* infection can be found in a large number of herds in Canada. Cow-level prevalences were 7.0, 5.6, and 8.2% for Manitoba, Saskatchewan, and Ontario, respectively, and 10.4, 21.3, and 25.5% for Prince Edward Island (PEI), Nova Scotia, and New Brunswick, respectively. These *N. caninum* seropositive cows were widely distributed throughout the herds, with 38, 44, 71, 63, 83, and 90% of herds having at least 2 test-positive cows in Manitoba, Saskatchewan, Ontario, PEI, Nova Scotia, and New Brunswick, respectively. Analysis of samples from PEI in 1979, 1989, and 1998 showed the same prevalence in 1998 and 1989, but a lower level in 1979, suggesting a possible expansion of the disease in the 1980's but a stable prevalence throughout the 1990s.

In a case-control study in Quebec, 3059 dairy cows were i-ELISA tested from 24 case herds (presence of a *N. caninum* aborted fetus confirmed histologically and immunohistochemically) and 22 control herds (no presence of *N. caninum* suspected). All case herds and 73% of the control herds had at least 1 seropositive cow. Based on the within-herd prevalence in the control group, it was estimated that the provincial cow-level prevalence was at least 7.5% and that *N. caninum* exists in the majority of farms in the province. However, this estimate is likely to be an underestimate of the true prevalence in Quebec, because it is based only on herds that have never had *N. caninum*

infections reported, rather than a random sample of herds that would likely include some herds with reported *N. caninum* infections. Therefore, the true seroprevalence in Quebec is likely to be closer to that of Ontario or the maritime provinces, rather than that of western Canada.

Based on the data from dairy cattle, there appears to be some ecological (proximity of farms to domestic or wild canid populations) or management (pasture use or cattle density on pasture) differences that have lead to substantially higher *N. caninum* infection levels in eastern Canada compared with western Canada. The *N. caninum* prevalences found in eastern Canada are similar to those reported among dairy cattle in the United States and elsewhere.

2.6.2 Prevalence in beef cattle

In a 2004 report on Canadian beef cattle, a random sample of 1976 steers and bull calves from 4 feedlots in northern Alberta were tested by i-ELISA, with 128 (6.5%) testing positive. The cattle came from British Columbia, Alberta, Saskatchewan, and Manitoba. Another study involved the random collection of blood samples from 1806 cows from 174 herds at auction in northern Alberta in 1998; 162 (9.0%) cows were positive and 62 (36%) herds had at least 1 positive cow by an i-ELISA. A total of 260 samples had been collected in the same region in 1980, when 35 (11.5%) were positive.

In contrast to these low seroprevalences in randomly selected cattle, a study to determine associations between *N. caninum* infection and reproductive performance estimated the *N. caninum* seroprevalence in 419 cows from 8 progressive cow-calf herds in central Alberta to be 30% in beef cattle, based on IFAT results from blood samples taken between 1992 and 1995. However, this study likely overestimated the true

prevalence in the beef industry in Alberta, because the authors selected herds in order to achieve their primary objective, which was to determine the association between serologic status and rate of abortion, stillbirth, calf mortality, and reproductive failure, not to estimate the true prevalence of *N. caninum* infection in Alberta. Eight herds would be an inappropriate number of herds to give a valid representation of the estimated true prevalence in the whole province. Another reason for the likely overestimation is that horizontal transmission of *N. caninum* had occurred in at least some of this select group of herds. Indeed, in an outbreak investigation of a herd that suffered an abortion storm in northern Alberta, over 80% of cows, heifers, and calves were seropositive shortly after the outbreak, demonstrating how widespread the infection can become in some herds.

Based on the studies specifically designed to determine *N. caninum* seroprevalence in representative cattle, dairy and beef cattle in Saskatchewan and Manitoba appear to have similar *N. caninum* seroprevalences, albeit based on studies with some differences in methods and results. Currently, there are no data on the seroprevalence of *N. caninum* in beef cattle in eastern Canada to confirm that dairy and beef cattle have similar prevalences.

2.6.3 Prevalence in dogs

There is little reported information about the prevalence of *N. caninum* infection in dogs in Canada. One study, which involved 1077 serum samples collected from dogs in 35 US states and 3 Canadian provinces, determined that 75 dogs (7.0%) were IFAT positive, with no difference in prevalence between males and females. In PEI, all 3 samples were negative. In Alberta, 6 of 8 (75%) samples were positive, while in Ontario, 5 of 77 (6.5%) samples were positive. In one other North American study, a 2%

prevalence was reported in dogs that were tested in Kansas. However, these estimates of seroprevalence should be interpreted with caution, because diagnostic tests for *N. caninum* infection in cattle have not been evaluated to determine their sensitivity and specificity in dogs. Furthermore, the small sample sizes in 2 of the 3 provinces certainly cannot be considered representative of the dog population in these provinces. Also, while dogs have been shown to develop a measurable antibody response to *N. caninum*, some dogs remain seronegative even after producing *N. caninum* oocysts. In studies from Japan, Korea, and Mexico, a higher level of infection was reported in rural dogs than in urban dogs, which is what would be expected where farm dogs are likely to have greater access to cow placenta and dead stock tissue.

2.7 Effects on productivity

The possible effects of neosporosis on productivity in cattle include reproductive losses, reduction in milk production, premature culling, and reduced weight gain.

2.7.1 Reproductive losses

Neosporosis can cause abortions at sporadic, endemic, and epidemic levels in herds. In herds with low seroprevalence of *N. caninum* (< 5%), abortions due to *N. caninum* infection may occur at a rate of 1 per 100 cow-years, or less, because of the low seroprevalence and unpredictability with which seropositive cattle recrudesce and abort the fetus. In herds with moderate (10% – 20%) or high (> 20%) seroprevalence of *N. caninum* infection and no evidence of horizontal transmission, abortion due to *N. caninum* infection may be frequent and distributed throughout the year. Abortion storms, involving 10 to 60% of cows, can occur either in herds with recently infected cows

(horizontal transmission) or in herds with moderate or high seroprevalence due to previous *N. caninum* infection that have been exposed to factors that have lead to recrudescence and abortion.

The risk of abortion is 2-3 times higher in seropositive than in seronegative dairy cows . However, this risk is age-dependent and can be 7 times higher, as was observed in congenitally infected heifers in their 1st pregnancy in a large dairy herd in California. On this same farm, seropositive cows were only 1.7 times more likely to abort in their 2nd pregnancy (during their first lactation).

A study of 8 beef herds in central Alberta with moderate to high levels of *N. caninum* infection demonstrated a similar increased risk of abortion (OR = 5.7) and even stillbirth (OR = 2.8) among seropositive cows compared with seronegative cows. In at least 2 of these herds, there was evidence for horizontal transmission, which perhaps explains the higher risk of abortion compared with that in many reports in dairy cattle. From an investigation of an abortion storm on a beef farm in Alberta with evidence of *N. caninum* horizontal transmission, seropositive cows were also 6 times more likely to abort or be open at pregnancy check compared with seronegative cows. Therefore, the risk of abortion appears to be highest soon after the time of initial *N. caninum* infection. A possible explanation for this time dependency could be that as time passes, the encysted bradyzoites are less likely to recrudesce. Support for this theory can be seen with the negative dose: response relationship between cow age and level of vertical transmission, as discussed earlier.

While it is clear that seropositive cows are more likely to abort than are seronegative cows, it is also clear that many seropositive cows do not abort. The factors

that enhance the likelihood of any given seropositive cow aborting remain largely unknown, as are the factors that enhance the likelihood of any given seropositive cow to abort a second time. Dairy cows that are seropositive for *N. caninum* and have aborted are 2 to 3 times more likely to have a subsequent abortion than are seronegative cows. However, in the investigation of an outbreak of abortions on a beef farm in Alberta, seropositive cows that aborted during the initial abortion storm were not at increased risk of abortion, compared with seronegative herd mates during the subsequent 2 years of observation. Perhaps the stress of high milk production in dairy cows, accompanied by the normal down-regulation of the immune system during mid-gestation, may explain the increased risk of multiple abortions in dairy cows but not in beef cows.

Since the discovery of *N. caninum* as a cause of cattle abortions in the late 1980s, *N. caninum* has become the most commonly diagnosed cause of abortions in many parts of Canada and elsewhere. 1Diagnosis of *N. caninum*-associated abortions in dairy cattle in Ontario increased from 1.6% of abortion submissions in 1993-94 to 5.7, 11.4, 12.5, and 14-15% in 1994-95, 1995-96, 1996-97, and 1997-00, respectively. Since 1994, *N. caninum* has been the most commonly diagnosed cause of abortion in dairy herds in Ontario. In Quebec, 11.4% of all aborted bovine fetuses submitted to diagnostic laboratories in 1996 were infected with *N. caninum*. Similar estimates of 15 to 20% have been found in California and The Netherlands, demonstrating the large impact of *N. caninum* in other dairy producing areas of the world.

2.7.2 Reduced milk production

The association between seropositive dairy cows and milk production depends on whether *N. caninum* is causing abortions. In 90 randomly selected herds in maritime Canada, with seroprevalence levels ranging from 0 to 73%, *N. caninum*-seropositive cows did not have a significantly different 305-day milk production compared with *N. caninum*-seronegative cows. However, in a case control study of 83 herds in Ontario, 305-day milk production for seropositive cows was 250 to 300 kg less than for seronegative cows in herds with a history of *N. caninum* abortion problems, but was not in herds without a history of abortion problems. These results would explain previous studies in large herds with abortion problems in the United States that demonstrated similar reductions in milk production associated with seropositive cows compared with seronegative herdmates.

2.7.3 Premature culling

The association in dairy cows between seropositivity and premature culling also depends on whether *N. caninum* is causing abortions. In 56 dairy herds in Ontario, *N. caninum* serostatus was not significantly associated with either time to culling or risk of culling. In 90 randomly selected dairy herds in maritime Canada, *N. caninum*-seropositive cows also did not have an increased risk of culling compared with *N. caninum*-negative cows. There was no information on abortions in either of these Canadian studies. Conversely, in 1 large dairy herd with *N. caninum* abortion problems in California, seropositive cows were 1.7 times more likely to have been culled during the study period than were seronegative cows and were 6.3 mo younger at culling. An

interaction was found between the number of abortions and the serological status of the animal, indicating that cows were more likely to be culled if they were seropositive and had aborted, than if they were seropositive but didn't abort, or if they aborted but were not seropositive. For example, a seropositive cow that had aborted was 3 times more likely to be culled than a seronegative cow that had aborted. Seropositive cows were also twice as likely to have been culled for low milk production or mastitis compared with seronegative cows.

There is limited information on the risk of culling among seropositive beef cattle. In a study of 8 beef herds in central Alberta with moderate to high levels of *N. caninum* infection, seropositive cows had a 1.9 times higher risk of being culled for any reason, and 2.5 times higher risk for reproductive failure compared with seronegative herdmates. It is unlikely that these herds are representative of the majority of beef herds in Canada, because their seroprevalences, ranging from 20% to 50%, were considerably higher than those found on most beef farms. Additional research is required to determine if seropositive beef cows are at higher risk of premature culling in herds that do not have abortion problems.

2.7.4 Reduced weight gain/feed efficiency

Following the outbreak investigation of a *N. caninum* abortion storm in a beef farm in Alberta, fall calf weights for 75 calves with antibodies to *N. caninum* at birth were slightly lower (4.2 kg) but not significantly different from those of 37 calves with no antibodies, after adjusting for calf sex, dam age, calf birth weight, calf age at weighing, and sire group.

In another recent, larger study of 1976 male calves from all 4 of the western Canadian provinces, 128 seropositive calves also had a slightly lower (0.04 kg/d) but not significantly different postweaning average daily gain (ADG) compared with the seronegative calves. These findings can be compared with results from a study of 1009 weaned steers from 92 herds in Texas. This latter study found a significantly lower postweaning ADG (0.05 kg/d) and slaughter weight (7.5 kg) in seropositive steers compared with seronegative steers. No significant difference was found in feed conversion. However, in a small, detailed longitudinal study of 34 feedlot steers in Texas, seropositive steers required 2.2 kg more feed (on a dry matter basis) for each 1.0 kg of live body weight gain than did seronegative steers. This extra feed requirement demonstrated a significant impairment in feed efficiency.

The 2 Canadian studies, neither of which examined feed efficiency, showed a trend toward lower ADG in seropositive cattle compared with seronegative cattle, a trend that may have developed into a statistically significant difference with a larger sample size. With the ADG and total weight gain findings being numerically similar in the studies from both Canada and the United States, it would appear that *N. caninum* infection has a negative impact on growth in beef cattle under North American production systems.

2.8 Economic impact

There are few firm data on the economic losses due to *N. caninum* in the dairy or beef cattle industries in Canada or elsewhere. As indicated in the previous section, direct productivity losses due to *N. caninum* include reproductive problems, such as stillbirths; abortions; early fetal death and resorption, manifested as return to service; increased time

to conception; or infertility. Other direct costs include loss of milk yield in cows aborting due to *N. caninum*, increased culling of cows aborting due to *N. caninum*, and reduced growth and feed efficiency. A reduction in the value of breeding stock is also likely, although there is no documented evidence of this impact. Potential indirect costs include professional costs and costs associated with diagnoses, rebreeding, increased lactation time, and replacement of a positive cow that has been culled.

In a Canadian study, based on data from the maritime provinces, the cost of neosporosis was estimated at the herd and regional level. At the herd level, direct costs (premature culling, reduced cull value, abortions, and reproductive losses) and treatment costs (the cost of veterinary services, medication, and extra labor) were estimated at $2,305 per year per infected herd of 50 cows, based on a 20% within-herd prevalence of infection. At the regional level, annual losses were estimated to be $1,909,794 for the 3 provinces.

A California study, based only on the number of possible abortions, estimated the loss at US$35 million per year in California alone. By applying the assumptions used in the California study that 2 to 5% of all pregnancies end in abortion due to *N. caninum* infection, annual losses in Canada could be between 24,000 and 60,000 pregnancies/year.

There is one estimate of the economic losses associated with *N. caninum* infection in beef cattle, based on effects estimated in beef herds in Texas. The predicted loss, using a stochastic model that allows for ranges of estimated costs with likely distributions of these ranges, was between US$ 23 to 35 per head or US$ 978 to 1,479 per 42-head infected herd with a 20% prevalence of infection. Estimated financial losses to the Texan beef industry ranged from 15 to 24 million US dollars. However, this estimate may not be

applicable to Canadian herds due to differences in prices and market conditions between the 2 countries.

2.9 Risk factors

Identified risk factors for *N. caninum* transmission can be subdivided, based on the type of outcome that was investigated, into those associated with seroprevalence, confirmed postnatal infection, and abortion storms. However, it is unlikely that the risk factors associated with each of the types of investigated outcomes are independent. Documented abortion storms have frequently resulted from infection of previously uninfected pregnant animals, although they could result from group recrudescence among congenitally infected cattle. High seroprevalence herds could have reached that status through long-term accumulation of congenitally infected cattle, widespread postnatal infection or both. It is likely that there are factors that contribute to horizontal transmission, and factors that contribute to vertical transmission on farms with and without existing *N. caninum* infection on the farm. However, many exploratory risk factor studies are unable to specifically identify whether the factors are contributing to horizontal or vertical transmission, unless the factors are biologically associated with one or the other, as demonstrated below.

There has been only one published Canadian study to determine risk factors of *N. caninum* seroprevalence and *N. caninum* abortion on dairy farms. The presence and number of farm dogs on the dairy farm premises at the time of the study visit, as well as during the previous 3 years, were the only 2 factors significantly associated with a herd being a case herd (*N. caninum* confirmed abortion) and a herd having a high ($\geq$ 10%) seroprevalence. These findings have been corroborated elsewhere and with additional

detail from a study on 8 dairy farms in The Netherlands with abortion storms and evidence of horizontal transmission. All 8 farms reported the introduction of a new farm dog within a period of 1.5 y before the first indication of *N. caninum* infection (either a *N. caninum* abortion or *N. caninum* infected calf). As there was evidence in all herds of vertical transmission of *N. caninum* for years, it was hypothesized that the newly introduced (probably naïve) dog was infected with *N. caninum* through ingestion of placenta (or other material) from already infected cattle and that it subsequently transmitted the infection to other cattle by shedding oocysts. However, there were no uninfected farms in this small study; therefore, it is unknown whether a randomly selected group of uninfected dairy farms would also have had new farm dogs introduced within the past 1.5 years, or whether the 8 farms are representative of typical management seen in the dairy industry in The Netherlands or Canada.

Another Dutch study found that female dogs were twice as likely to be seropositive as male dogs. Recrudescence of infection during pregnancy and a subsequent rise in antibodies may explain this finding, a phenomenon that is well known to exist in cows and has been demonstrated to occur in a single dog. Confirmation of this theory is required in a larger population of dogs.

Various other risk factors have been reported to be related to *N. caninum* seroprevalence in cattle from studies done outside Canada, factors that may, in the future, prove to be relevant to the control of *N. caninum* in dairy and beef farms in Canada. In one study of 42 dairy farms in France, 27 seropositive farms were associated with having contact with rabbits, ducks, and poultry, as well as with tethered housing and pond water supply. A Dutch study of risk factors among 50 dairy farms with *N. caninum* abortion

storms also found a significant association with the number of poultry present on the farm. With a number of avian species having been shown to have antibodies against *N. caninum*, it is possible that poultry and other avian species will be another intermediate host, although this still requires confirmation. The Dutch study also found an association between the farms with abortion storms and the feeding of moldy corn-silage during the summer. This finding may be related to the possible immunosuppression that moldy feed can impart on cattle, leading to recrudescence of already infected cattle.

In the above mentioned French study, long calving intervals, high somatic cell counts, and the presence of cats and rodents were found to be negatively associated with the risk of *N. caninum* infection, although it is unclear by what mechanism these factors could protect against a positive antibody test for *N. caninum*.

In a Dutch study, 12 dairy farms with demonstrated horizontal *N. caninum* infection were compared with 21 control farms with no evidence of post-natal infection. The 12 farms were significantly more likely to have farm dogs which ingested colostrum, milk, uterine discharge, and placental material, and defecate in feed alleys and storage areas for grass and corn silage compared to control farms. However, multivariable regression analyses were not conducted on these data to determine which of these variables were most significantly associated with horizontal infection. For example, it is conceivable that most farm dogs that ingested placental material also ingested uterine discharge, and without this additional analysis, one could prematurely conclude that uterine discharge consumption can lead to horizontal transmission of *N. caninum*. Two dogs fed colostrum spiked with culture-derived tachyzoites did not develop a titre or shed oocysts; so, based on this small Dutch study, consumption of tachyzoites contaminated

colostrum appears not to be a means of transmission to dogs. Confirmation of this finding from naturally contaminated colostrum from *N. caninum* infected cows under a commercial setting is required.

A number of authors have suspected that concurrent infections with other agents may lead to immunosuppression, allowing recrudescence in latently infected cattle. However, herd-level prevalence of antibodies to bovine herpesvirus 1, *Leptospira hardjo* or *Salmonella dublin* were not associated with the risk of abortion storms on Dutch dairy farms. Similarly, an increased risk of abortion was not observed when cows were seropositive to both *N. caninum* and BVDV infections.

Interesting possible risk factors for transmission of infection were identified among 760 calves from 76 herds in Texas, where 99 (13%) calves were positive and 59% of the herds had at least 1 seropositive calf. In the final multivariable regression model, the following factors (possibly related to horizontal transmission) were associated with an increased risk among seropositive calves: calving in the spring, stocking density > 1 cow/calf unit/2.2 ha, use of a round-bale feeder, and wildlife access to the weaning supplement. The use of a self-contained feeder for cow supplements was associated with a reduced risk for seropositivity, potentially reducing the risk for horizontal transmission. Ranches that self-reared replacement heifers also had an increased risk for seropositivity, supporting the hypothesis that some of the calves were exposed to *N. caninum* through vertical transmission. A decrease in the risk for seropositivity was associated with the use of a cattle-working dog, which, the authors speculated, may have prevented contamination of feed and water sources by other canids.

A spatial analysis of seropositivity among 131 steers on 54 of 94 ranches tested in Texas showed associations between seroprevalence of *N. caninum* and cattle density and abundances of gray foxes, coyotes or both, which seems to corroborate the association between cattle density and seroprevalence found elsewhere, and also is indicative of how coyotes and perhaps foxes may be responsible for sylvatic transmission of *N. caninum*. Additional studies are needed to confirm whether these possible risk and protective factors are representative of other cattle-rearing locations, including Canada.

In 5 north-western states in the United States, which have an ecology more representative of western Canada than Texas, a significant association between seroprevalence and higher winter stocking density was found.

2.10 Prevention and control

2.10.1 Uninfected herds

On uninfected farms, preventing the introduction of the parasite through normal biosecurity measures is the primary focus. With the waxing and waning of titres in infected animals, the best method to ensure that the parasite is not introduced by carrier cattle is to maintain a closed herd. If animals are purchased, they should be obtained only from herds that have been tested and are known to be test-negative. Alternatively, purchased animals should be test-negative, but false-negative identifications on single tests at the individual animal level are more likely to occur than false-negative herd tests, especially with repeated testing within the herd.

Even with careful purchasing of animals, *N. caninum* could be introduced into a herd; therefore, risk factors for horizontal transmission should be minimized. Access of

dogs (both farm and feral) to ruminants and their feed and calving areas should be restricted as much as possible. On farms where cows are pastured, it is impossible to keep dogs out of pastures, so mangers and feed storage areas should be the focus of protection from contamination by dog feces. Any feeding equipment used on neighbouring farms should be cleaned well before use on an uninfected farm.

An effective monitoring program to confirm that *N. caninum* is not in the herd is also recommended. This program should include the serological testing of all cows that aborted their fetuses, and the examination of their placentas for *N. caninum* using IHC. A cow with a negative test in a herd with no history of *N. caninum* is likely to be a true negative (have a very high negative predictive value of the test, due to a low prevalence and high test sensitivity). A positive antibody test from a cow that aborts should only implicate *N. caninum* as the cause of the abortion in the absence of negative tests for other abortifacients. A positive IHC test for *N. caninum* on fetal or placental tissue would confirm the cause of the abortion.

If herd status is important for genetic sales, periodic testing of the herd (every 1 or 2 y) to confirm that *N. caninum* has not been introduced into the herd may be cost-beneficial. The ability to detect *N. caninum* infected herds through bulk tank milk ELISA for antibodies to *N. caninum* is available in other countries and is currently being evaluated in Canada, and may become a useful monitoring tool for early detection of *N. caninum* in uninfected herds before *N. caninum* associated abortions occur. The testing of a farm dog for *N. caninum* infection status is unlikely to yield useful results, due to the limited information on the interpretation of test results in dogs for tests intended for cattle

use only. There are no tests commercially available specifically for *N. caninum* infection in dogs.

2.10.2 Infected herds

The primary management goals for infected herds include preventing abortions and reducing the risk of both vertical and horizontal transmission of *N. caninum,* so that the prevalence of infection in the herd is reduced in the long-term. Reducing the risk of introduction of the parasite into the herd, as discussed above, is also important, so that on-farm transmission control efforts are not offset by the reintroduction of *N. caninum* from outside the farm.

In order to determine the extent of *N. caninum* infection in the herd, systematic serological testing of old and young stock (precolostrally or after 6 mo of age to avoid maternal antibody false-positives) should be instituted to identify infected and uninfected animals. Based on serological test results, selective rearing of seronegative youngstock should be the backbone for long-term prevalence reduction. In herds with a seroprevalence above 30%, a single positive ELISA is sufficient to consider an animal infected and a potential cull. In herds with a lower seroprevalence, all cattle with more than 1 positive antibody titre, or a strong positive antibody titre on a single test, should be classified as *N. caninum* positive and considered for culling. However, due to the limited impacts on production parameters that have been confirmed to date, other than when *N. caninum* abortions occur, *N. caninum* infected cattle should not be culled automatically. Even though a *N. caninum* infected cow is more likely to abort than an uninfected cow, and cows that abort due to *N. caninum* in the past are more likely to abort due to *N. caninum* again in the future, it is impossible to predict whether a specific *N. caninum*

infected cow will abort in the future. Furthermore, while many calves born to seropositive cows become congenitally infected, the probability of vertical transmission is not 100% and can vary considerably from farm to farm. Also, embryo transfer can be used on seropositive donor cows to harvest uninfected embryos for implantation into seronegative recipient cattle, a practice that has been shown to produce seronegative calves. Although various antiprotozoal agents have been tested against *N. caninum in vitro*, there is currently no known effective treatment to clear a cow of *N. caninum* infection.

Farmers do, however, need to consider a *N. caninum* infected cow as a reservoir for *N. caninum* that could be spread to the rest of the herd, either slowly and insidiously through vertical transmission or rapidly and explosively through horizontal transmission, if risk factors for this are in place on the farm. A *N. caninum* infected cow in a herd could lead to a *N. caninum* abortion outbreak if she has a *N. caninum* abortion and an uninfected dog has access to and consumes some of the *N. caninum* infected placenta or fetus. A dog could also consume the parasite through *N. caninum* infected deadstock, if it is not properly disposed of. If this same dog becomes *N. caninum* infected and defecates in areas where cattle feed or water is stored, prepared or consumed when it is shedding oocysts, and if uninfected cattle subsequently consume the contaminated feed or water before the oocysts have been destroyed by environmental conditions, the horizontal transmission cycle will be completed. An extraneous source of the parasite may also be other domestic or wildlife reservoirs on or off the farm. These factors should be carefully assessed prior to the culling decision, particularly if the infected animal is valuable for breeding. For some farms, particularly those with confined herds, it may be more cost-

effective to mitigate these risk factors (control access of dogs to the barn and feed storage areas, and properly dispose of placenta, fetuses, and all deadstock) than to lose genetically valuable animals.

There is a killed vaccine on the market in the USA, conditionally approved in December of 1998 by the U. S. Department of Agriculture (USDA). It is recommended that the vaccine be used twice in early gestation. Preliminary evaluations of this vaccine suggest that it might be able to induce protection against abortion, although this protection may only be measurable on farms with on-going risks of abortion in the 10% or higher range, as demonstrated in a field trial in New Zealand.

However, there are still concerns about the use of this vaccine. Test-and-cull control strategies using immunological (ELISA) tests can no longer be used in vaccinated herds. Additionally, in the vaccine efficacy trial in New Zealand, more vaccinates were found to be open at their expected calving date compared with nonvaccinates, possibly due to early embryonic death (EED) from the immune response to the vaccine and its subsequent effects on placental attachment in early gestation. Vaccination prior to gestation may be preferred to avoid this EED. While exposure to tachyzoites prior to gestation has been shown to prevent congenital infection with *N. caninum* in a majority of the experimental animals, there is still no scientific evidence to indicate that the vaccine can prevent fetal infection in commercial herds. Therefore, the use of this vaccine as it is currently recommended is controversial and requires further evaluation.

2.11 Conclusions

N. caninum is a major cause of abortion on dairy farms worldwide and is widespread in Canada. In the past 15 years, great progress has been made in understanding the pathogenesis of neosporosis. However, there are still risk factors of transmission that must be better understood, and gaps remain in the knowledge about the impact and control of the disease. Vertical transmission is the major route of transmission, but elimination of vertical transmission may not be enough to eliminate the infection from a herd, because horizontal transmission may occur. Control of *N. caninum* involves the prevention of both vertical and horizontal transmission, including testing and culling seropositive cows, where appropriate; use of embryo transfer; and limiting access of dogs to cattle and their feed. The ELISA has sensitivity $\geq$ 95% and specificity $\geq$ 97%, making it a good test for the determination of infected animals and herds, particularly with multiple or strong positive results or both. The economic impact of *N. caninum* on dairy production is substantial and losses from abortion alone are enough to warrant control of this disease.

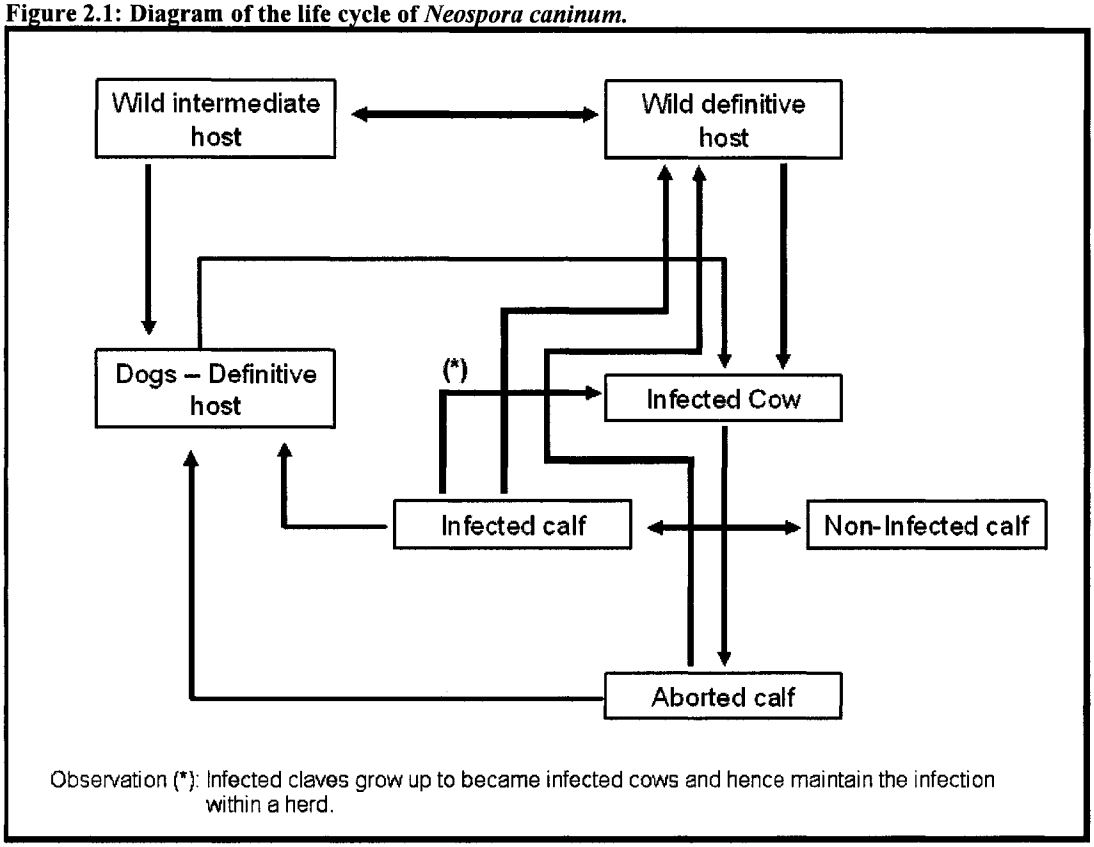
2.12 Table 2.1: Summary of seroprevalence for *Neospora caninum* in dairy and beef cattle in Canada

	Number of Herds	Number of cows	Herd level prevalence	Cow level prevalence	Reference
Dairy prevalence					
New Brunswick	30	900	90.0	25.5	3 and 80
Nova Scotia	30	900	83.0	21.3	3 and 80
Prince Edward Island	30	900	63.0	10.4	3 and 80
Quebec[a]	22	1,463[b]	73.0	7.5	4
Ontario	51	1,530	70.8	8.2	3 and 80
Manitoba	40	1,200	38.0	7.0	3 and 80
Saskatchewan (Sask)	51	2,040	44.0	5.6	3 and 80
Beef prevalence					
Alberta (Alta)	174	1,806	36.0	9.0	5

Manitoba (Man)	49	1,417	78.0	8.8	unpublished data
Man, Sask, Alta, British Columbia	-	1,976	-	6.5	84

[a] Only control data were used from the case-control study to estimate prevalence
[b] Estimated because the exact number of control group cows was unavailable from the paper

Figure 2.1: Diagram of the life cycle of *Neospora caninum*.



3 Prevalence and spatial distribution of *Neospora caninum* infection in Canadian dairy herds

3.1. Introduction

Neosporosis is caused by *Neospora caninum*, a protozoan parasite that was first reported in the late 1980s and is now considered a major cause of abortion in cattle in many countries, including Canada. In Canada, *N. caninum* abortion was first reported in 1994, in both British Columbia and Prince Edward Island. 1Diagnosis of abortions due to *N. caninum* in dairy cattle in Ontario increased from 1.6% of abortion submissions in 1993-94 to 5.7%, 11.4%, 12.5%, and 14%-15% in 1994-95, 1995-96, 1996-97, and 1997-2000, respectively. In Quebec, 11.4% of all aborted bovine fetuses submitted to diagnostic laboratories in 1996 were infected with *N. caninum*. Similar estimates of 15 to 20% have been found in California and The Netherlands, demonstrating the large impact of *N. caninum* in other dairy producing areas of the world. In a case-control study from Quebec, 22.5% of cows within herds with a previously diagnosed *N. caninum* abortion were seropositive, while the seroprevalence in herds without diagnosed *N. caninum* abortion was 7.5%.

Dogs are the definitive host, and it has been verified that coyotes can also act as a definitive host. A spatial analysis of seropositivity among 131 steers on 54 of 94 ranches tested in Texas showed associations between seroprevalence of *N. caninum* and cattle density and abundances of gray foxes, coyotes, or both, which corresponds with how coyotes and perhaps foxes may be responsible for sylvatic transmission of *N. caninum*.

The objective of this study was to determine the current seroprevalence of *N. caninum* in Canadian dairy cattle and to better understand the spatial distribution of the disease in Canada.

3.2 Material and methods

3.2.1 Data collection

We collected blood samples for *N. caninum* testing from up to 30 (if available) randomly selected milking cows from 240 farms in 6 provinces in Canada. Blood sampling was undertaken in 1998 on 90 randomly selected herds in the maritime provinces (30 each from Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS)). In Saskatchewan (SASK), Manitoba (MAN) and Alberta (ALTA), samples were collected in 2001, 2002 and 2002-2003 from 44, 40 and 66 randomly selected herds, respectively. Herd selection in all provinces was conducted through random sampling from all herds on a monthly, individual milk-testing regimen through the dairy herd improvement (DHI) program. In ALTA, veterinary clinics in areas with dairy herds were identified, and then dairy herds were randomly selected from within clinics. Participation of herds was sought through either direct phone calls (PEI, NS, NB, MAN, ALTA), or initial letters of request followed by a phone call (SASK). Funding was not available to obtain samples from randomly selected herds from the other 4 provinces in Canada.

During the blood sampling a questionnaire was used to collect information on whether dogs were present on the farm and whether foxes, coyotes, raccoons, or stray cats and dogs not belonging to the farm had been seen on the farm. This information was used for covariate analysis in the Poisson model in the spatial scan statistics (see below).

3.2.2 Serological testing

Serum samples were stored at –20°C until all samples for that province were ready for testing. To determine serologic status for *N. caninum*, commercially available ELISA tests were used. For all provinces except Alberta, the samples were tested with the indirect BIOVET ELISA (BIOVET Inc., Quebec, Canada) at the BIOVET Laboratories in St. Hyacinthe, Quebec. This test has been reported to have a sensitivity of 99.0%, and a specificity of 98.4%, at a serum-to-positive (S/P) ratio cutpoint of 0.60. For our study, a cow was considered to be infected with *N. caninum* if the serum-to-positive (S/P) ratio was 0.60, as recommended by the manufacturer of the test kits. Serum samples were tested in duplicate for *N. caninum*.

In Alberta, the indirect IDEXX ELISA (IDEXX Laboratories, Westbrook, Maine, USA) was utilized for serologic testing at the Alberta Agricultural Laboratory in Edmonton, Alberta. This test has been reported to have a sensitivity of 88.6%, and specificity of 96.5%, at a serum-to-positive (S/P) ratio cutpoint of 0.50. For our study, a cow was considered to be infected with *N. caninum* if the serum-to-positive (S/P) ratio was 0.50, as recommended by the manufacturer of the test kits.

3.2.3 Statistical analyses

3.2.3.1 Descriptive statistical analyses

Seroprevalence estimates and 95% confidence intervals (CIs) were determined for the proportion of cattle and herds that were seropositive for *N. caninum* by utilizing survey data analysis procedures which adjusted for within herd clustering and sampling weights (STATA, v.8; Stata Press, College Station, Texas, USA). Due to the large number of animals tested per herd and the less-than-perfect specificity of the test for *N. caninum*, false-positive test results were likely. Therefore, some herds with only 1 seropositive animal may erroneously be considered a positive herd. As a result, herd level seroprevalence was calculated by using 2 definitions for a positive herd: 1) a lenient definition - having at least 1 test-positive animal, and 2) a more restrictive definition - having at least 10% of the sample (e.g. 3 in 30) positive. Furthermore, due to the inaccuracies of the indirect ELISAs for identifying *N. caninum* infected animals, the estimated true animal and herd prevalence and 95% CIs, correcting for test sensitivity and specificity, were calculated[14].

3.2.3.2 Spatial analyses

As information regarding the exact location (latitude, longitude) of each herd included in the study was unavailable, latitude and longitude coordinates were determined for each participating farm using full postal code conversion.

Visualization of geographic differences in seroprevalence was enabled through the creation of regions within provinces. In order to produce reasonably stable and

representative regional estimates, the regions were assembled from aggregations of contiguous census divisions within each province until there were at least 4 study herds per region. Other factors which were considered in the assembly of regions included: ecological divisions (e.g. bays, lakes, large rivers, and straits), current administrative regions (e.g. health units), and ability to identify individual farms if there were very few in an area (ie. to maintain confidentiality of participating farms)

Three statistical tests were used to identify spatial clustering of seropositivity for *N. caninum*: the spatial scan statistic, the Cuzick and Edwards' test for heterogeneous populations, and the Moran's I autocorrelation statistic. The analyses were carried out separately in the two parts of the country, 90 herds in the three maritime provinces, and 150 herds in three western provinces.

For the spatial scan statistic, two approaches were used in order to determine clusters in the two parts of the country studied. First, a Bernoulli model was used, in which herds were classified as a case (positive) or as a control (negative). A case was defined using the same definitions in the herd-level seroprevalence study (i.e. at least one animal positive, or having at least 10% of the sample positive). The second approach used a Poisson model, and was based on the number of positives in each herd compared with the number of samples in each herd. The Poisson models were first built without covariates, and six other models included the presence of dogs (yes/no), foxes, raccoons, coyotes, stray cats, and other dogs (dogs not belonging to the farm) as covariates. The data set was only scanned for clusters with more cases of *N. caninum* than expected, equivalent to a one-sided statistical test. Scanning windows of up to 50% of the study area were used to identify clusters of *N. caninum*, and a likelihood-ratio test statistic was

calculated for each window. The distribution of the likelihood ratio and its corresponding P-value was obtained by Monte-Carlo simulation so that the overall type I error was set at 0.001 (i.e., 999 simulations). All calculations were performed using SaTScan software version 4.0.3, released February 3rd 2003 (http://www.satsacan.org; Kulldorff *et al.*, 1998).

The Cuzick and Edwards' test using nearest-neighbour levels from 1 to 10 were examined. Overall significance of clustering (k = 1 to 10) was assessed by combining P-values using either a Bonferroni correction or Simes' correction method, in which the overall P-value is estimated by ranking the array of P-values. The latter method appears particularly advantageous over the classical Bonferroni procedure when several highly-correlated test statistics are involved. All calculations were performed using ClusterSeer2 Software (TerraSeer, 2001-2003).

The significance of Moran's I can be assessed employing a Z-statistic. All calculations for this method were performed using GeoDa version 0.95. The analysis in this case was done using the number of positive cows divided by the total cows sampled in each herd. The points referring to herds were converted into polygons in order to use a contiguity matrix. Two kinds of weight matrices were used in the analysis: a queen contiguity spatial weight matrix, and a 5 nearest-neighbours spatial weight matrix. The first spatial weight matrix was based on having a denser connectedness structure (more neighbours), while the second matrix used a simple distance threshold criterion with very few neighbours (or none, yielding "islands"). Maps were produced for the visualization and exploration processes, using MapInfo Version 5.0.

3.3 Results

3.3.1 Prevalence

Overall, 6,662 cows from 90 herds in the Maritimes and 150 herds from the western provinces were in the final database of *N. caninum* test results. The overall Canadian cow level seroprevalence was 11.9%. For the Maritimes, it was 19.5%, and for the western provinces, it was 10.4%. In the Maritimes, Prince Edward Island had the lowest seroprevalence, with 11.0% (near the national average). In the western provinces, Alberta had the highest seroprevalence with 14.6% (Table 3.1).

Using a herd classification based on one or more cows testing positive, all provinces had herd seroprevalences equal or greater than 60.0%, ranging from 60.0% to 96.7%, with New Brunswick having the highest value among the 6 provinces in the study. The herd seroprevalence at the national level was 81.9% (Table 3.2).

Using a herd classification of >10% of cows positive, New Brunswick again had the highest seroprevalence (73.3%) while Saskatchewan had the lowest value of only 9.1%. Nationally 47.1% of the herds had more than 10% of the sample being test positive, with 61.8% for the maritime provinces, and 41.3% for the western provinces (Table 3.3).

The maps in Figure 3.1 and 3.2 show the average seroprevalence in different regions of the 6 provinces in the study. In the Maritimes (Figure 3.1), Prince Edward Island and northern Nova Scotia had lower seroprevalence than the other regions of the

three provinces. In the western provinces (Figure 3.2), Alberta's regions had average seroprevalences higher than Manitoba and Saskatchewan.

3.3.2 Spatial analysis for maritime provinces

The spatial scan statistic based on the Bernoulli model with at least one case positive as a definition of a case herd, identified one cluster of positive herds, with a 262 km radius (P=0.04) located in Nova Scotia and New Brunswick. Using the second case herd definition (at least 10% of the herd sample testing positive), a cluster with a 113 km radius (P=0.017) was identified with its centre in New Brunswick (Figure 3.3). The overlap of the two clusters was located in southern New Brunswick.

The spatial scan statistic using the Poisson model without covariates identified one cluster with a 167 km radius (P=0.001). In the model with coyotes as a covariate, the cluster identified in the other models "shrank" to a cluster with a 36 km of radius (P=0.001)(Figure 3.4). Including the covariates foxes, raccoons, dogs, stray cats, and dogs that did not belong to the farm, (other dogs) did not substantially change the original model.

Overall, P-values of 0.148 and 0.022 for Bonferroni and Simes corrected versions of the Cuzick and Edwards' test were obtained for detecting clustering. Significant clusters were detected at k = 1, and where k was 7, 8, 9 and 10 (Table 3.4) when the case definition was one or more positive cow in a herd. Using a case definition of at least 10% of the sample being positive, the clustering was significant in almost all levels (Table 3.4).

The autocorrelation statistic (Moran's I), using a queen contiguity spatial weight matrix(considering the eight neighbours, like the game of chese the queen movements), had a value of 0.1024 and a P-value of 0.048, and for a 5 nearest-neighbours spatial weight matrix a value of 0.1192 and P-value of 0.018.

3.3.3 Spatial analysis for western provinces

The spatial scan statistic based on the Bernoulli model with at least one case positive as a definition of case, identified one cluster with a 388 km radius (P=0.001) located in central and southern Alberta. Using the second case definition (at least 10% of the sample positive), a cluster with a 244 km radius (P=0.001) was identified with its centre in central Alberta (Figure 3.5). The overlap of the two clusters covered much of central Alberta.

The spatial scan statistic using Poisson model without covariates identified a cluster with 222 km of radius (P=0.001) (Figure 3.6). Including the covariates foxes, dogs, stray cats, and coyotes did not substantially change the model, showing the same region clusters with radii of 168 km, 173 km, 206 km, and 222 km, respectively (all with a P-value of 0.001). In the model using other dogs or raccoons as covariates, there were bigger clusters with radii of 419 km and 426 km, respectively (P=0.001). The cluster derived from the model without covariates was almost entirely within these larger clusters (Figure 3.6).

Overall, P-values of 0.092 and 0.009 for Bonferroni and Simes corrected versions of Cuzick and Edwards' test were obtained for detecting clustering. Significant clusters were detected with k = 3, 5, 6, 8, 9, and 10 (Table 3.5), based on the case definition of

one or more positive cows in a herd. Results were highly significant with the case definition of at least 10% of the sample positive per herd (Table 3.5). Based on this second case definition, clustering was highly significant with P-values < 0.001 at all k levels.

The autocorrelation statistic (Moran's I) using a queen contiguity spatial weight matrix had a value of 0.1637 and a P-value of 0.001, and for a 5 nearest-neighbour spatial weight matrix, a Moran's I of 0.2260 and P-value of 0.001 were obtained.

3.4 Discussion

The overall prevalence estimates for Canada were 11.9% and 81.9% for cow-level and herd-level seroprevalence, respectively. In the United States, a study of 4,907 cows from 93 dairy and five beef herds recruited from 20 states and the territory of Puerto Rico produced slightly higher estimates: a cow level prevalence of 16% and a herd level prevalence of 90%[20].

The spatial scan statistic is defined by a circular geographic window that is moved over the study area of interest. It is centreed, in turn, on each of several possible clusters in the study region, with the window radius being allowed to vary between zero and some upper limit. Each distinct geographical circle created includes a unique set of neighbouring locations or administrative areas, and is assessed as a cluster by comparing the number of cases within the window with the number expected if cases were randomly distributed across the population of herds and/or cows. The spatial scan statistic is a cluster-detection test, able both to identify and to test the significance of specific clusters. An advantage provided by both the spatial scan statistic and Cuzick and Edwards' test is

the ability to account for uneven (heterogeneous) distributions of the population-of-interest. This is particularly important when investigating the clustering of disease in livestock populations.

In the Maritimes Canadian provinces, using spatial scan statistics, the effects of all covariates except coyotes were not significant, because the model with those 5 covariates (foxes, raccoons, dogs, stray cats and other dogs) produced the same result as the model with no covariates. The reduction of the cluster size when coyotes were included in the model suggests that frequent coyote sightings played an important role in the bigger cluster (light grey in Figure 3.4). Once the effect of coyotes was accounted for, only a smaller cluster (medium grey in Figure 3.4) remained. The model including coyotes produced the tightest cluster in the maritimes provinces, which supports the hypothesis that these animals play a role in the transmission of *N. caninum*. Barling *et al.*, in a Texas, US study, found an association between coyotes and foxes and the presence of *N. caninum*. This finding agrees with a study that showed that coyotes can act as a definitive host for *N. caninum*.

In the western provinces when covariates were added to spatial scan models, there was no effect for the covariates foxes, dogs, stray cats, and coyotes because the cluster identified for each was nearly the same size as the one identified without covariates – a small cluster centreed around Edmonton. For the models with other dogs and raccoons sighted frequently, there was an expansion in the cluster identified. These results suggest that the clustering observed in the model without covariates (small cluster around Edmonton) was driven, in part, by the effects of peri-urban animals (other dogs and raccoons). Once this effect was removed, a much more diffuse cluster covering much of

southern Alberta was observed. The lack of an effect of coyotes as a covariate does not necessarily indicate that coyotes are not important in the transmission of *N. caninum* in Alberta. If coyotes are relatively evenly distributed throughout the region, they would not have an effect on the clusters detected.

The autocorrelation statistic (Moran's I) relates the occurrence of a variable of interest, such as disease prevalence, to its spatial location with regard to other cattle herds. Thus, it is similar to the Pearson's correlation coefficient (r) except that correlation between different values of the same variable is examined, rather than correlation between values of different variables. A weight matrix is included to define the spatial relationships, for example, it may be based on distance between points so that points that are close in space are given greater weight in the statistic than those that are further apart. A value of Moran's I close to 0 implies that points are randomly distributed in space. A positive value of Moran's I indicate clustering. In, the Atlantic provinces and western Canada, the Moran's I confirmed the presence of clustering of cases.

Cuzick and Edwards' test compares the spatial coordinates (longitude, latitude) of case and control locations. It is appropriate for assessing clustering in populations in which the distribution of members is uneven. There is flexibility for defining case and control locations. Case locations may be where disease has been detected or locations at which disease is above a threshold value such as mean or median disease prevalence. This flexibility is advantageous when analyzing clustering of disease in livestock populations. The nearest-neighbour order (k # of neighbours evaluated) can be selected by the researcher. As k increases, more subtle and complex forms of clustering can be detected. The test statistic T_k is the number of cases that are nearest neighbours to each

individual case. A Z-statistic can be calculated to test the overall significance of T_k across all k levels examined, as well as the significance of each T_k individually. As with Moran's I, these test statistics confirmed the presence of clustering. Clustering was most evident at higher values of k, suggesting a pattern of general clustering in a region as opposed to direct spread of *N. caninum* to the nearest one or two neighbouring farms.

The second case definition (10% or more cows positive in the sample) was used to reduce the probability of false-positive classifications at the herd level. Indirect ELISAs may produce false-positives primarily from cross reaction with *Sarcocystis* sp.. Requiring 10% of the herd to be positive reduces the probability of false-positive results at the herd level.

Provinces with higher regional prevalence, such as Alberta and New Brunswick, showed the presence of clusters regardless of whether a case herd was defined as at least one positive cow, or at least 10% of the samples positive. In western Canada, these clusters were more consistent across the Bernoulli and Poisson models used in the spatial scan statistics, and had a higher level of statistical significance. They also had high levels of statistical significance based on the Cuzick and Edwards' test and autocorrelation tests.

Regardless of whether the Bernoulli model was used with of the two case definitions (Figure 3.5), or the Poisson model was used with various sets of covariates, there was strong evidence of a cluster of *N. caninum* in central Alberta.

In the Maritimes provinces, the seroprevalence was higher than in the western provinces and the clusters defined were more "diffuse" with more marginally significant P-values. In addition, clusters defined by the spatial scan statistics were not the same in

the four models (two Bernoulli models based on different herd classification and two Poisson models with/without coyotes as a covariate). It was observed that the models could identify a higher seroprevalence in much of southern New Brunswick. However, frequency of coyotes sighting was the covariate that had the greatest effect in identifying the cluster. This potentially reflects the importance of coyotes in that ecosystem and their effects on the prevalence of *N. caninum* infection in dairy cattle.

Significantly fewer cows and herds in western provinces were seropositive for *N. caninum* as compared with maritime Canada. These differences may be due to different vertical or horizontal transmission rates, related to their respective housing, nutrition, biosecurity, demographics, or frequency of dogs or wild canine populations. Future analyses will investigate which of these risk factors were associated with seroprevalence for *N. caninum*.

The differences in seroprevalence levels for *N. caninum* between herds in the maritime provinces and in western Canada were very unlikely to be due to differences in test accuracies, because exactly the same tests at the same laboratories were utilized for all herds except those in Alberta. It is unlikely that the higher prevalence observed in Alberta was due to differences in test sensitivity. However, because the maritime samples were tested in 1998-99, Saskatchewan samples were tested in 2001, and samples from Manitoba and Alberta were tested in 2003, temporal differences in test lots or laboratory conditions may have created a systematic bias in the results, leading to some of the differences seen. However, the manufacturers of the tests and the laboratories that used them employ careful quality control efforts to minimize this bias.

Furthermore, due to variable response rates among provinces, a selection bias in the farms surveyed in Saskatchewan may also have been responsible for some of the differences seen. For example, the low response rate of 39% in Saskatchewan may have lead to results for the sample population that would not be representative of the target population, all dairy farms in Saskatchewan. The reasons for not participating were varied (uninterested, planning to sell, too busy, no handling facilities for taking blood), making it unclear whether a selection bias would be likely. However, the average herd size and milk production level of the sample population farms were similar to those of all dairy farms in Saskatchewan, leading one to believe that the bias, if present, was likely small, making comparisons between provinces possible.

3.5 Conclusions

Based on data from 6 Canadian provinces (3 in the Maritimes and 3 in western Canada), the overall cow and herd level prevalences were 11.9% and 81.9% respectively. The prevalence in the Maritimes (19.5%) was much higher then in western Canada (10.4%). In western Canada, *N. caninum* infection was spatially clustered in central Alberta. In the maritimes provinces, the infection was clustered in southern New Brunswick.

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Table 3.1: Cow level seroprevalence for *Neospora caninum* in 6 Canadian provinces.

	N cows	N positive	Prevalence[a]	S. E.[a]
Prince Edward Island	764	75	11.0%	1.81%
Nova Scotia	777	165	21.7%	3.72%
New Brunswick	752	191	24.0%	3.50%
Manitoba	1,147	86	6.9%	1.55%
Saskatchewan	1,278	55	4.2%	0.58%
Alberta	1,944	299	14.6%	1.86%
maritime provinces	2,293	431	19.5%	1.92%
western provinces	4,369	440	10.4%	1.00%
All 6 provinces	6,662	871	11.9%	0.92%

a – Prevalence estimates (and their standard errors) are adjusted for sampling weights and clustering of cows with herds.

N – Number

S. E. – Standard Error

Table 3.2: Herd level seroprevalence for *Neospora caninum* in 6 Canadian provinces.

	N herds	N positive	Prevalence[a]	S. E.[a]
Prince Edward Island	30	23	76.7%	7.85%
Nova Scotia	30	26	86.7%	6.31%
New Brunswick	30	29	96.7%	3.33%
Manitoba	40	24	60.0%	7.84%
Saskatchewan	44	32	72.7%	6.79%
Alberta	66	63	95.4%	2.58%
maritime provinces	90	78	86.7%	3.55%
western provinces	150	119	80.0%	3.02%
All 6 provinces	240	197	81.9%	2.39%

a – Prevalence estimates (and their standard errors) are adjusted for sampling weights and clustering of cows with herds.
N – Number
S. E. – Standard Error

Table 3.3: Herd with 10% of cows positive for *Neospora caninum* in 6 Canadian provinces.

	N herds	N positive	Prevalence[a]	S. E.[a]
Prince Edward Island	30	12	40.0%	9.10%
Nova Scotia	30	21	70.0%	8.51%
New Brunswick	30	22	73.3%	8.21%
Manitoba	40	10	25.0%	6.93%
Saskatchewan	44	4	9.1%	4.38%
Alberta	66	44	66.7%	5.85%
maritime provinces	90	55	61.8%	4.98%
western provinces	150	58	41.3%	3.59%
All 6 provinces	240	113	47.1%	2.94%

a – Prevalence estimates (and their standard errors) are adjusted for sampling weights and clustering of cows with herds.

N – Number

S. E. – Standard Error

Figure 3.1: Descriptive map of maritime provinces with average cow level seroprevalences of *Neospora caninum*, by region.

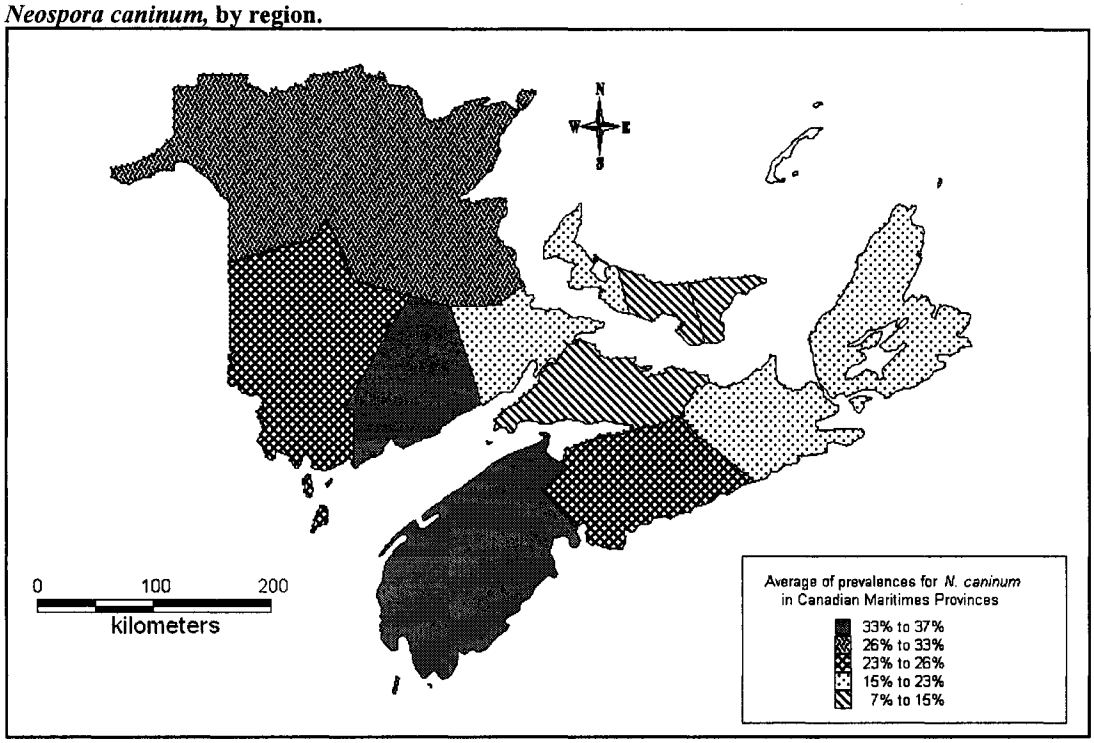


Figure 3.2: Descriptive map of western provinces with average cow level seroprevalences of *Neospora caninum*, by region.

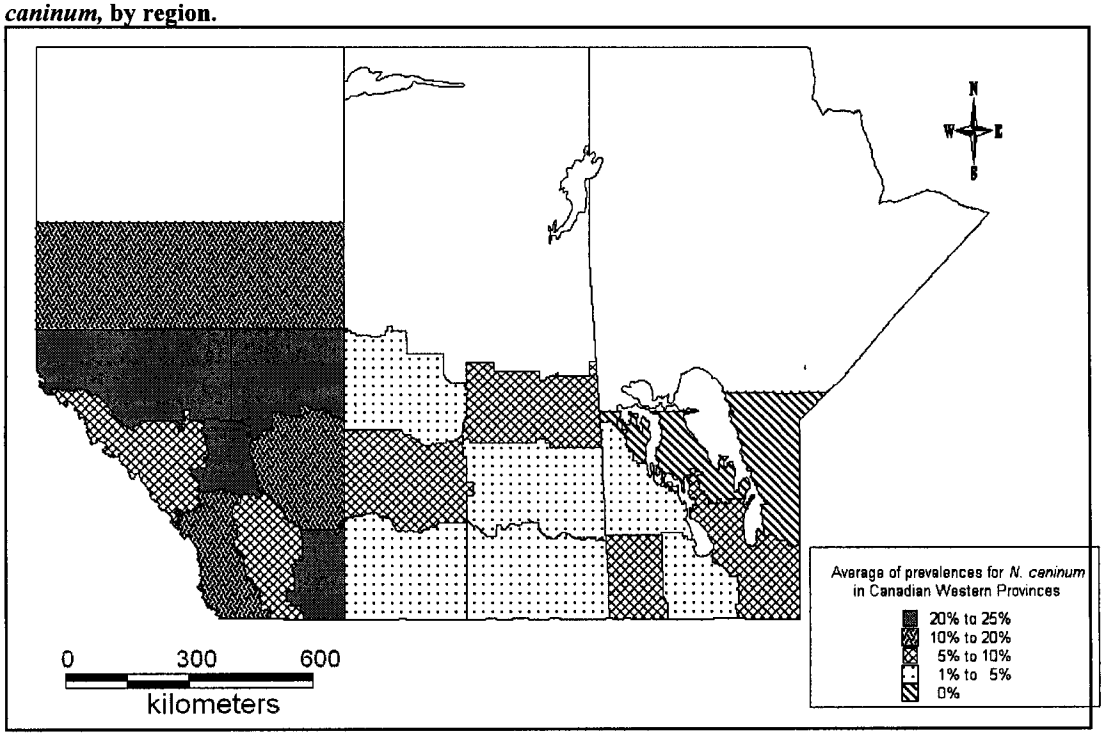


Figure 3.3: Map of maritime provinces with *N. caninum* seropositivity clusters in 90 sampled dairy herds identified by the spatial scan statistics using the Bernoulli model. Light grey ( ) cluster identified using case definition of at least 10% of positive sample; medium grey ( ) cluster identified using case definition of at least one cow positive in a herd; dark grey ( ) area is the overlap of the two clusters.

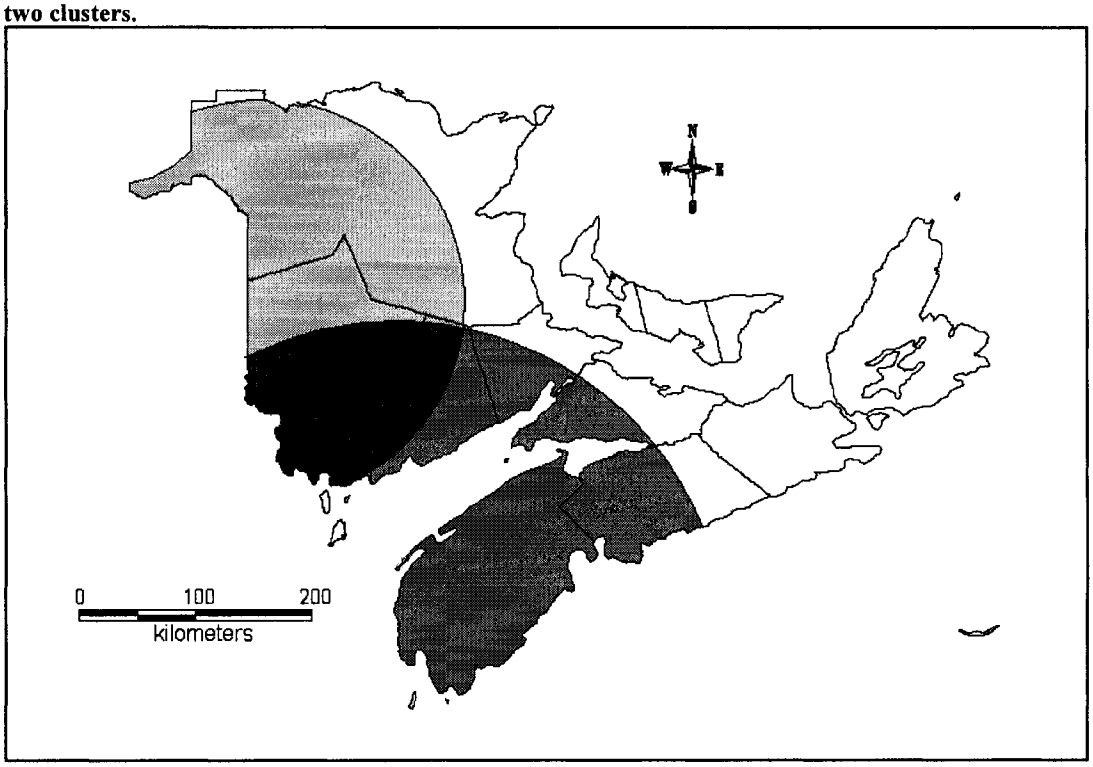


Figure 3.4: Map of maritime provinces with *N. caninum* seropositivity clusters in 90 sampled dairy herds identified by the spatial scan statistics using the Poisson model. Light grey ( ) cluster identified without covariates; medium grey ( ) cluster identified using coyotes as covariate.

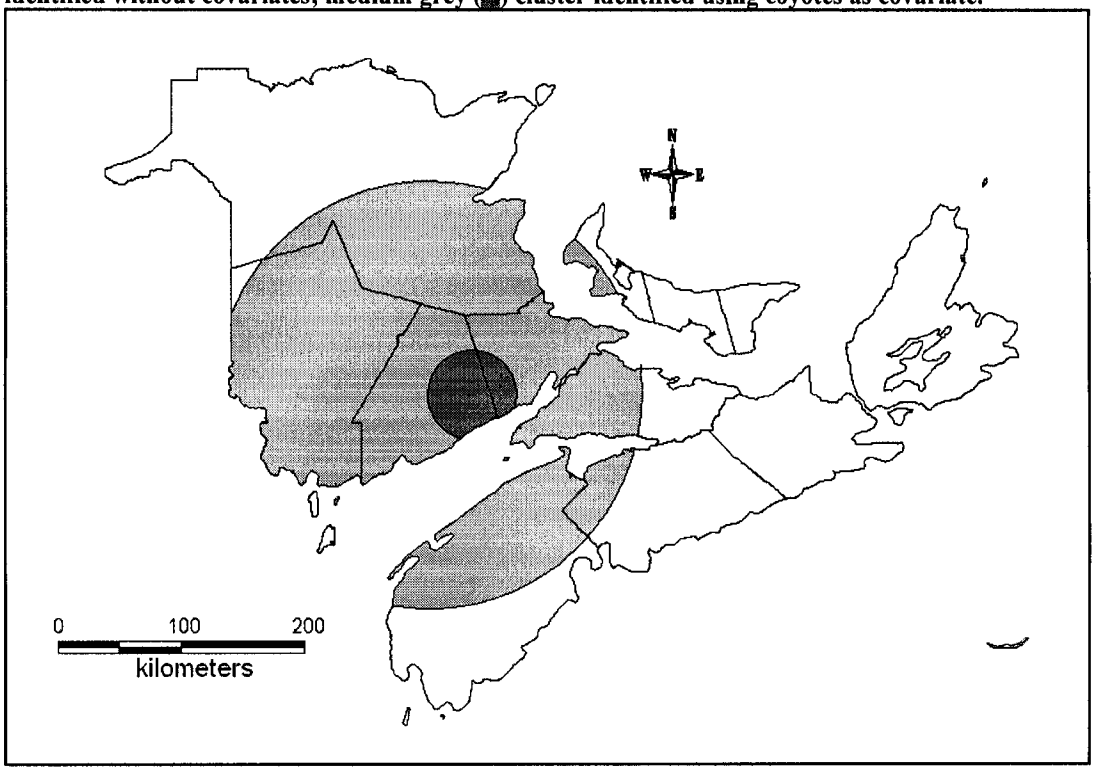


Figure 3.5: Map of western provinces with *N. caninum* seropositivity clusters in 150 sampled dairy herds identified by the spatial scan statistics using the Bernoulli model. Light grey ( ) cluster identified using a case definition of at least one cow positive in a herd; medium grey ( ) cluster identified using a case definition of at least 10% of positive sample; dark grey ( ) area is the overlap of the two clusters.

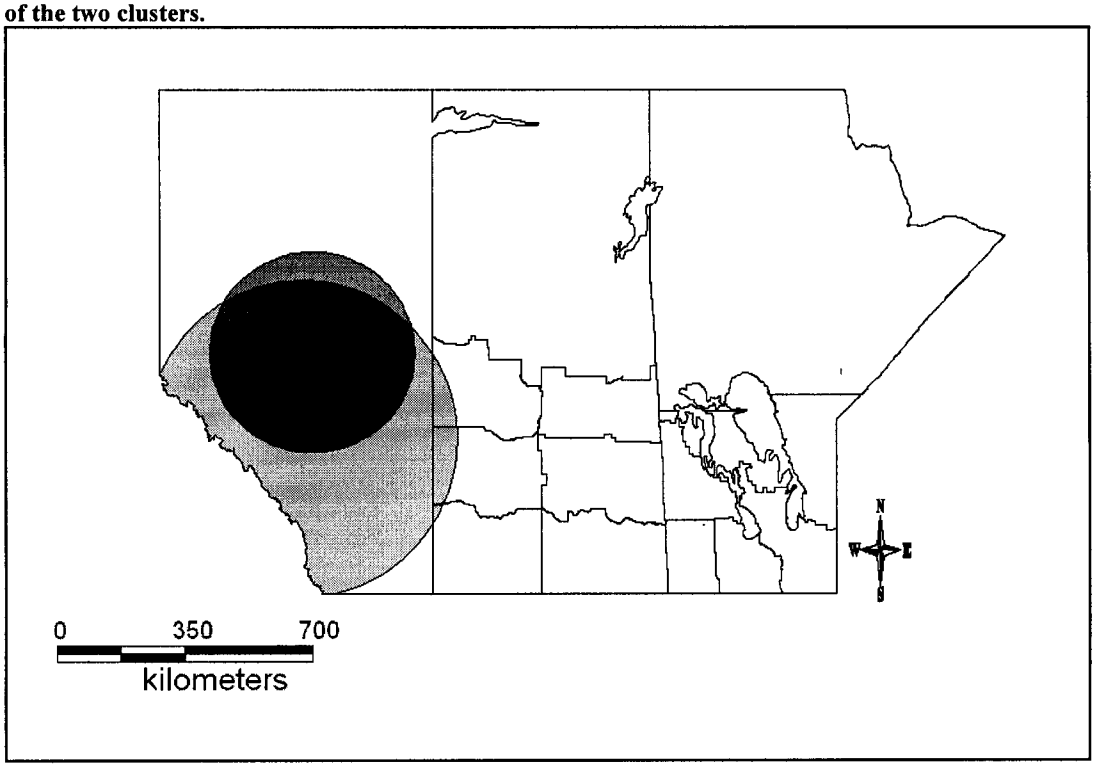


Figure 3.6: Map of western provinces with *N. caninum* seropositivity clusters in 150 sampled dairy herds identified by the spatial scan statistics using the Poisson model. Light grey ( ) cluster identified using other dogs and raccoons as covariates; medium grey ( ) cluster identified using dogs, coyotes, foxes, stray cats, and without covariates; dark grey ( ) overlap of the two clusters.

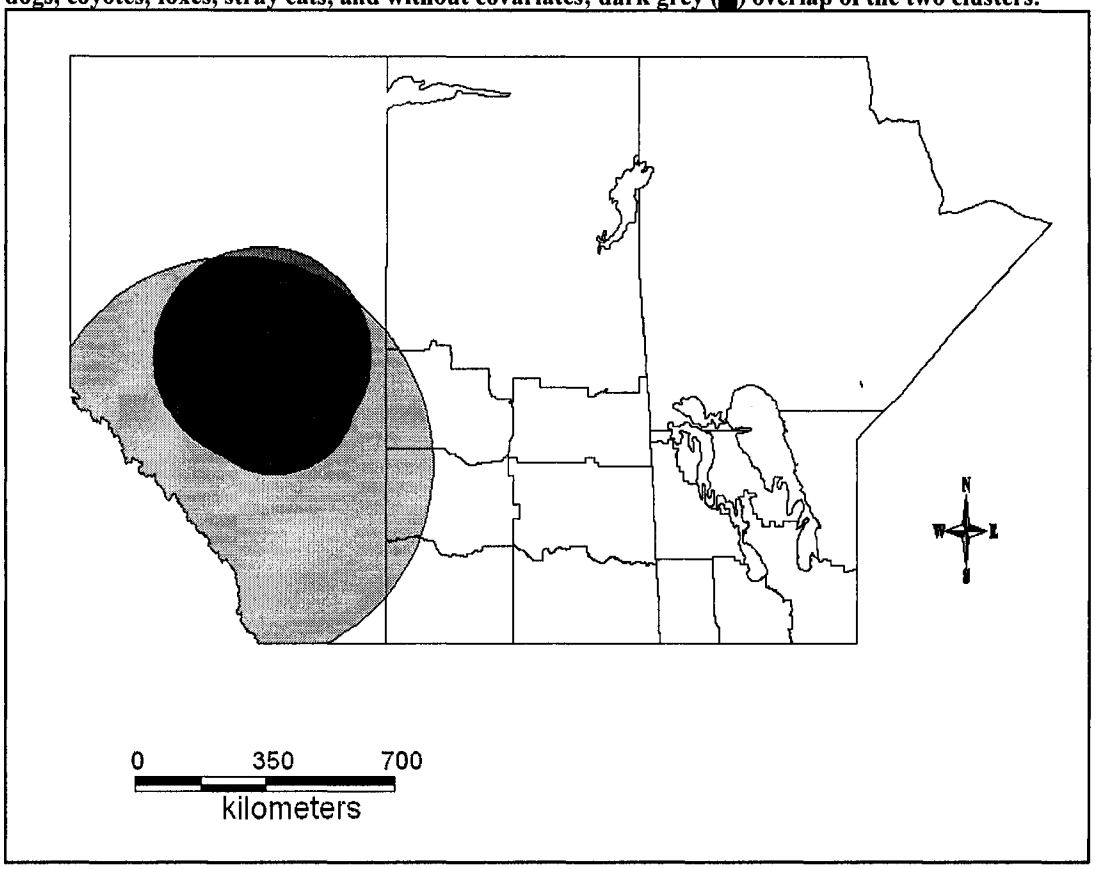


Table 3.4: Cuzick and Edwards' test results with Bonferroni and Simes corrections for *Neospora caninum* cluster detection in 90 dairy herds of the Canadian maritime provinces.

k	T[k]	E[T]	P-value	T[k]	E[T]	P-value
	Case = 1 cow positive			Case = 10% cows positive		
1	73	67.48	0.015	40	32.16	0.014
2	138	134.97	0.228	73	64.31	0.056
3	207	202.45	0.181	111	96.47	0.015
4	276	269.93	0.148	140	128.63	0.071
5	346	337.42	0.104	186	160.79	0.002
6	416	404.90	0.087	223	192.94	0.002
7	489	472.38	0.036	259	225.10	0.001
8	561	539.87	0.019	297	257.26	0.001
9	630	607.35	0.026	330	289.42	0.002
10	701	674.83	0.022	356	321.57	0.011
Bonferroni			0.148			0.007
Simes			0.022			0.002

k – Nearest-neighbourr level
T[k] - Cuzick and Edwards' test statistic
E[T] – Expected Cuzick and Edwards' test statistic

Table 3.5: Cuzick and Edwards' test results with Bonferroni and Simes corrections for *Neospora caninum* cluster detection in 150 dairy herds of the Canadian western provinces.

k	T[k]	E[T]	P-value	T[k]	E[T]	P-value
	Case = 1 cow positive			Case = 10% cows positive		
1	102	95.52	0.051	37	21.71	0.000
2	198	191.05	0.118	68	43.43	0.000
3	302	286.57	0.017	110	65.14	0.000
4	397	382.10	0.055	148	86.86	0.000
5	497	477.62	0.046	183	108.57	0.000
6	596	573.14	0.042	215	130.29	0.000
7	693	668.67	0.054	251	152.00	0.000
8	803	764.19	0.012	291	173.71	0.000
9	905	859.71	0.009	325	195.43	0.000
10	1006	955.24	0.009	366	217.14	0.000
Bonferroni			0.092			0.000
Simes			0.009			0.000

k – Nearest-neighbourr level
T[k] - Cuzick and Edwards' test statistic
E[T] – Expected Cuzick and Edwards' test statistic

4 Effects of the serostatus for bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* on reproductive performance in Canadian dairy cows.

4.1 Introduction

Neosporosis (caused by *Neospora caninum* - *N. caninum*), bovine viral diarrhea (caused by bovine viral diarrhea virus - BVDV), enzootic bovine leukosis (caused by the bovine leukosis virus - BLV), and Johne's disease (caused by *Mycobacterium avium* subspecies *paratuberculosis* - MAP) are all transmissible diseases that are considered to be of economic importance and that are significant in the international trade of animals and animal products. Recent economic studies undertaken in the maritime provinces of Canada estimated total annual costs for a 50-cow herd infected with *N. caninum* at $2,304[1]. The costs associated with BVDV, BLV and MAP infection in the same sized herd were estimated at $2,421, $806, and $2,472, respectively. These figures largely reflect decreased milk yields[1] associated with subclinical infections of each pathogen.

4.1.1 *Neospora caninum*

Clinical signs in *N. caninum*-infected cows include abortion, stillbirth and the birth of calves with central nervous system malformations and low birthweights[2,3]; *N. caninum* also produces a variety of other effects including reduced milk production (coincident with abortion), premature culling, and decreased value of breeding stock with known *N. caninum*-positive status. Cows can abort at any point in the gestation period from 3 months to term[3]. Both horizontal (via ingestion of canine feces containing

oocysts- definitive hosts) and vertical (*in utero*) transmission occur, with vertical transmission being the primary route of transmission on most dairy farms. Infected animals remain infected for life, and therefore seropositive animals are typically subclinically infected, except for the brief time during which they may abort.

The risk of abortion in seropositive cows are approximately 3 times that of seronegative cows. However, this increase in risk is age-dependent and can be as high as 7 times more likely in younger animals, as was seen in congenitally infected heifers in their first pregnancy in a large dairy herd in California. On this same farm, seropositive cows were only 1.7 times more likely to abort in their second pregnancy (i.e. during their first lactation). Aborting dairy cows that were seropositive for *N. caninum* were 2 to 3 times more likely to have a subsequent abortion than seronegative cows. However, little research has been done to determine whether seropositive cows have longer calving intervals or impaired first service conception. In a study from Costa Rica, in 94 dairy herds and 2743 cows, serostatus did not have a significant effect on the length of the calving interval, the number of abortions, and the number of services per conception[8]. However, in a Canadian study of 66 beef herds and 2516 cows and heifers, *N. caninum* seropositive cow and heifers had an increased individual animal risk of non-pregnancy and abortion[9].

4.1.2 Bovine Viral Diarrhea Virus (BVDV)

Clinical signs of BVDV infection range widely and can include diarrhea, respiratory disease, and reproductive problems[10]. BVDV-infected herds can also have a higher risk of mastitis, and a negative association between milk yield and BVDV

infection has been reported[10]. Non-pregnant cattle that become acutely infected with BVDV shed virus for a number of weeks and develop an antibody response if they survive the infection. Transmission of the virus between cattle is through direct or indirect exposure to either body secretions containing the virus from acutely or persistently infected cattle. Persistent infection develops in a fetus when the dam becomes infected with a non-cytopathic BVDV strain during the 45-125 day period of gestation before the immune response of the fetus is developed. Cytopathic strains can cause early embryonic death, abortion, or weak calves with or without birth defects such as cerebellar hypoplasia, depending on the gestational period of exposure and pathogenicity of the virus[11]. Herds with abortion storms caused by BVDV obviously have impaired reproductive performance. However, it is unclear whether herds with BVDV infected animals (either from acute or persistent infection) but not suffering abortion storms (e.g. subclinical infections) have longer calving intervals or impaired first service conception. Infection with BVD virus during the first 45 days of gestation and in later gestation (more than 210 days of gestation) did not influence the rate of return to estrus, while a statistically significant increase in the abortion rate has been reported following mid-term infections (days 46 to 210). It has been reported that 7% of fetal deaths may be attributable to infection with BVD virus. In the year following BVDV seroconversion, there was an increase in the herd average time to first calving by 14 to16 days overall in the herd and 18 days in young stock. No effect on the number of breeding services for heifers or cows was observed, indicating a need to search for determinants other than reduced conception risk for the increased calving interval. Beef herds with high

pregnancy rate (>90%) showed a significant association between antibodies to BVDV type 2 (titre level above 1:3000) and increased risk of abortion[9].

4.1.3 Bovine Leukosis Virus (BLV)

Transmission of BLV occurs horizontally, through fluids containing white cells (e.g. blood, milk, semen) and vertically (*in utero*), leading to lifelong infection. Lymphocystosis can develop in approximately 30% of BLV-seropositive cows, but less than 5% of BLV-seropositive cows go on to develop clinical signs of lymphosarcoma. Subclinical BLV infections are a concern, as they can have a large negative impact on the international trade of animals and animal products. Decreased milk production due to BLV infection has also been reported.

In Canadian cattle, after controlling for age and milk production, a significant positive association (increasing calving interval) between BLV-positive cows and the length of the calving interval was found. In Sweden, losses to reproduction were evident by a slightly increased calving to last service interval and a somewhat greater risk of cystic ovaries. However, in other studies, variables such as age at first calving, duration of most recent calving interval, number of days open, and number of times bred have not been found to be associated with BLV status[20;21;22]. Therefore, additional evidence is required to determine if BLV-seropositive cows have longer calving intervals or impaired first service conception.

4.1.4 *Mycobacterium avium* subspecies *paratuberculosis* (MAP)

Clinical signs of Johne's disease in cattle include intermittent or chronic watery diarrhea, and chronic weight loss despite normal appetite. Subclinical infection has been associated with decreased milk production, premature culling and reduced slaughter value[24]. Transmission is typically horizontal, occurring primarily in calves less than 6 months of age through the ingestion of contaminated fecal matter. However, clinical signs usually do not develop until much later in life, and therefore most MAP-infected animals are subclinically infected. There is growing evidence that the milk production and culling effects of MAP occur in subclinically infected animals as well, however the reproductive effects of subclinical MAP infection are not clear. Cows that were MAP-positive by enzyme-linked immuno-sorbent assay (ELISA) testing exhibited a 28-day increase in days open compared with MAP-negative cows, but the same relationship was not observed when the diagnosis was based on a radiometric fecal culture. Due to the lower sensitivity and specificity of the ELISA tests compared to fecal culture, this association may be spurious because of misclassification bias. Again, additional evidence is required to determine if MAP-seropositive cows have longer calving intervals or impaired first service conception compared to MAP-seronegative cows.

Possible interactions between these infectious agents(*N. caninum*, BLV, BVDV, MAP) have been theorized, but there has been limited published research on this hypothesis. Concurrent infection of *N. caninum* and other agents may lead to immunosuppression, allowing recrudescence of latently infected cattle. However, herd-level prevalence of antibodies to bovine herpesvirus 1, *Leptospira hardjo* or *Salmonella dublin* were not associated with the risk of abortion storms on Dutch dairy farms.

Similarly, an increased risk of abortion was not observed when cows were seropositive to both *N. caninum* and BVDV infections. A Canadian study tested serum for BVDV, IBR and *N. caninum* antibody and there were no associations between *N. caninum* and the other two agents[9]. No previous study has ever looked at all four of these agents in the same sample population of cattle.

The present study aimed to investigate the impact of seropositivity for the aforementioned diseases, and their possible interactions, on reproductive efficiency (specifically, the ability to conceive and assumed fetal loss) in dairy cows.

4.2 Materials and methods

In order to assess overall reproduction performance, and fetal loss and conception ability specifically, three different approaches were used, based on the restriction that the only source of reproductive data on the sampled cattle was from monthly milk testing data from dairy herd improvement (DHI) records. Initially the calving to conception interval (CCI), with observations restricted to 30 to 200 days, was used to study the impact of *N. caninum*, BVDV, BLV, and MAP exposure on overall reproductive performance. Secondly, three dichotomous variables, calving to conception interval greater than 200, 250 and 300 days (CCI200, CCI250, and CCI300), were created under the assumption that prolonged CCI was a surrogate for fetal loss. Finally, first service conception (FSC) was used to assess the conception ability specifically.

4.2.1 Data collection

Blood samples were collected for BLV, *N. caninum* and MAP testing from 30 randomly selected cows from 165 farms in 5 provinces in Canada. Blood sampling was undertaken in the summer of 1998 on 90 randomly selected herds in the maritime provinces (30 each from Prince Edward Island, New Brunswick, and Nova Scotia) and on a further 31 herds in Ontario. In Saskatchewan, samples were collected in spring 2001 from 44 randomly selected herds. Enrollment in a monthly, individual milk-testing regimen through the dairy herd improvement (DHI) program was a prerequisite for participation.

Testing these herds for BVDV infection required a different sampling approach as vaccination of both heifers and adults against BVD is common in Canada and limits the number of animals on a farm that can provide a meaningful blood sample for BVDV antibody analysis. Serology data for vaccinated animals and young (<6 months) offspring of vaccinated animals are difficult to interpret given the potential for interference from vaccine-induced or maternal antibodies. We therefore selected 5 cows in those herds that had not been vaccinated for BVDV, or 5 heifers that had not yet been vaccinated but were over 6 months of age (if available) in the vaccinated herds. Details of overall prevalence estimates for the maritime provinces and Saskatchewan can be found elsewhere[31;32].

DHI data of sampled cows were obtained for all lactations (lactation in which the blood sampling was done, plus lactations before and after that) between July 1996 and October 2002, based on the assumption that infection with BLV, MAP and/or *N.*

caninum likely occurred prior to the cows' first lactations for most seropositive cattle. The production and reproductive data for these lactations, such as somatic cell count, peak milk production, days in milk, dates of breeding and number of breedings, were obtained electronically from Canadian Dairy Herd Management Services (CDHMS), which processes DHI records for all of Canada. There were no DHI data on abortions in this database, and therefore none were available for analyses at the cow level. The data thus derived were structured on 4-levels: lactation, cow, herd, and province.

4.2.2 Serological testing

All serum samples were stored at –20°C until all samples for that province were ready for testing. To determine serologic status for BLV, *N. caninum*, and MAP, a commercially available ELISA tests were used. For BLV, the samples were tested using the indirect IDEXX ELISA at the Canadian Food Inspection Agency (CFIA) laboratory in Charlottetown, which was certified as the national laboratory for BLV testing for international trade purposes. The test has been reported to have a sensitivity of 98.5%, a specificity of 99.9%, at a sample-to-positive ratio (S/P ratio) cutpoint of 0.50. This test requires a confirmation of positive tests, using a sample-to-negative host-cell ratio of 1.8. For *N. caninum*, the samples were tested with the indirect BIOVET ELISA at BIOVET Inc., St. Hyacinthe, Canada. The test has been reported to have a sensitivity of 99.0%, and specificity of 98.4%, at a S/P ratio cutpoint of 0.60. For MAP, the samples were tested using the indirect IDEXX ELISA at Prairie Diagnostic Services, Saskatchewan, Canada. The test has been reported to have a sensitivity of 43.0% and specificity of 99% compared to fecal culture results, at the recommended S/P ratio of 0.25[7]. However, more

recent test evaluations suggest lower test sensitivity, particularly when compared to low fecal shedders and tissue culture positive cows[35].

The serum samples from the 5 unvaccinated animals per herd were tested for the presence of BVDV antibodies using virus neutralization at Animal Diseases Research Institute, Alberta, and Canada. Herd-level sensitivity to identify the presence of a persistently infected animal on a farm has been reported at ≥95% while herd-level specificity to correctly identify herds without persistently infected animals has been reported at ≥98%, using a sample of five unvaccinated animals. However, for the current study, a herd was considered positive for BVDV infection when at least one animal with a titre greater than or equal to 1:64 was present due to the distribution of titres found. Herds with animal titres ≥1:64 usually also had animal titres ≥1:256, the maximum titre tested.

4.2.3 Variables

CCI was estimated from the calving interval in the DHI data by subtracting an average gestation length of 284 days from the CI. Inherently, this lead to the exclusion of lactations that did not have a subsequent calving, thereby not producing a calving interval. All lactations starting after 1st January 2001 were also excluded to allow a minimum of 22 months of follow up for each calving in order to overcome selection bias (otherwise, later lactations would only include those that successfully conceived quickly). Lactations with CCIs fewer than 30 days were also excluded, assuming these to be recording errors or outliers.

CCIs ranging from 30 days to 200 days were used as an overall assessment of reproductive performance for the following reasons. CCIs combine the potential impact of the pathogens and other possible factors on multiple breedings. However, we wanted to separate these effects from the impacts related to fetal loss, and a lactation with a CCI of less than 200 days was considered unlikely to have had an abortion event. Subsequently, we used various cutoff values of CCI to determine which cows had unusually long CCI. This variable initially used 200 days as a cutpoint (CCI200), and this was considered an indirect indicator of fetal loss or abortion. Cutpoints of 250 days (CCI250) and 300 days (CCI300) were also investigated.

FSC was used as a direct measure of the ability of each cow to conceive. Each first breeding was classified as successful (pregnancy = 1) if it was the cow's only service and produced a subsequent calving 270-290 days later. Breeding was classified as unsuccessful (pregnancy = 0) if: it was not the last service; the interval between the service to next calving fell outside the 270 to 290 day range; or the interval between service and dry-off exceeded 270 days. Multiple inseminations of the same cow within a 72-hour period were considered a single breeding, with the date of the last insemination considered the date of breeding. Services not meeting the criteria for successful or unsuccessful breedings remained unclassified and were not used in our analysis. Unconditional associations between demographic variables and classification status were carried out to confirm that there were no significant ($P < 0.05$) differences between the classified and unclassified records.

For FSC, an observation was excluded if DIM at service date was < 30 or > 300 days, as these were likely outliers for first breeding or recording errors. Herds with services per conception equal to 1 were also excluded from the analyses because only successful breedings were being entered into the DHI database for these herds, making all breedings effectively first service, potentially biasing the analyses.

N. caninum, BLV, BVDV, and MAP were all coded as positive or negative for each cow sampled. BVDV was a herd-level variable while; *N. caninum*, BLV, and MAP were cow-level variables. Lactation number was coded as a 3-level (1^{st}, 2^{nd}, and 3^{rd} +) categorical variable. The 1^{st} lactation was the baseline level for all models. We also coded provinces as a categorical variable, with Prince Edward Island as the baseline.

The potential effect of somatic cells count (SCC) on the reproductive parameters was determined by calculating the median value of the linear score from the observations measured between 30 and 200 days of lactation. Peak milk production was recorded as the highest level of milk production at any of the DHI tests taken between 30 and 200 days in milk.

4.2.4 Statistical analysis

For determining the significant predictors of CCI, a survival analysis using Cox's proportional hazards models and Aalen's linear hazards models was conducted. The latter was used to investigate the time-varying influence of the predictors used in the Cox model. This model produced graphs with time on the X-axis and the cumulative coefficient of each predictor on the Y-axis. If the effect of the predictor was constant over

time, the graph would form a straight line. Any change in the slope of the line was indicative of a time varying effect.

Two types of Cox proportional models were fit: unconditional models to determine the effect of each predictor on the outcome variable without controlling for other predictors or herd effects; and shared frailty survival models, controlling for other significant ($P \leq 0.05$) predictors and shared herd effects, assuming a gamma frailty distribution.

To analyze CCI200, CCI250, CCI300, and FSC, mixed logistic regression models were used, with provinces entered as a fixed effect and herds as a random effect. Clustering of herds within provinces and lactations within cows were also investigated as random effects. Backward elimination was utilized to determine all final models. All main effects and first-order interactions were evaluated and retained if significant ($P \leq 0.05$). Model diagnostics were performed, including a normal probability plot of the higher level residuals from each of the logistic models. All analyses were conducted using the statistical software package STATA (version 8)

The effects of peak milk on each of the outcomes evaluated were handled in three different ways. In the survival analysis of CCI, the effects of peak milk was different early in the breeding period (<73 days) compared to later. This can be seen in Figure 4.1 (Aalen's linear hazards model – discussed in Results) in which peak milk can be seen to be having a suppressive effect on CCI up to 73 days but not thereafter. Consequently, two variables (PMEarly and PMLate) were created in order to separately estimate these two effects. In our logistic regressions with CCI200, CCI250, and CCI300 as the response

variables, we used a single estimate of peak milk as a continuous variable on its own, the test with the highest milk production for that lactation. However in logistic regression of FSC, we included PM as a 3-level categorical variable (<32Kgs, 32-41Kgs, >41Kgs) because the relationship between PM and logit FSC was not linear. The cutpoints were defined based in the change of the behavior of the risk relation and the peak milk production based in the figure 4.2.

4.3 Results

4.3.1 Descriptive Analysis

Descriptive statistics for the main variables in the three analyses are found in Table 4.1, which highlight three points. The overall average FSC and CCI were 51% and 109 days, respectively, with 18%, 9% and 5% of lactations having CCI greater than 200, 250 and 300 days, respectively. The apparent prevalence of MAP infection was very low, probably due, in part, to the low sensitivity of the diagnostic test. The sample size changed for each analysis, in part due to the distribution of the exclusions mentioned for each of the outcome variables. In order to avoid the reduction of sample size (and associated power) even more, herds that could not be classified as BVDV infected (BVDV=1) or not infected (BVDV=0), due to the lack of unvaccinated animals older than 6 months of age, were classified as unknown BVDV status (BVDV = 2) instead of missing. There were 15, 2 and 3 herds in Saskatchewan, New Brunswick and Ontario, respectively, requiring this additional classification.

4.3.2 Analysis of CCI

In the final CCI model (Table 4.2), only one of the pathogens, BLV serostatus, was marginally significantly associated with CCI; BLV seropositive cows had a 7% lower rate of conception compared to seronegative cows due to a borderline significant hazard ratio (table 4.2) of 0.93 (P=0.06). *N. caninum*, BVDV, and MAP exposure and their possible confounders investigated had no significant effects in the final model in which clustering within herds was controlled. Expected confounders of reproductive performance (parity, province) that were found to be non-significant were retained in the final model to account for their possible confounding effects First level interactions between main effects revealed no significant effects.

The peak milk production (PM) had a time-varying effect (Figure 4.1), violating an assumption of the Cox proportional hazards model for which the ratios of the hazards should be constant overtime. The global test for proportional hazards had a P-value of 0.507 and 0.191 on the time and log (time) scales, respectively, while the specific test for PM had P-values of 0.033 and 0.004, respectively. PM was therefore divided, using 73 days as the cutpoint (based on the data), into two effects (early and late). After the division of PM into early and late segments, the P-values for the tests of proportional hazards were no longer significant. Therefore, in this final model (Table 4.2), SCC and PM yields before and after 73 days had significant effects on the CCI, with hazard ratios of 0.98, 0.98 and 0.996, respectively, while BLV was borderline significant (hazard ratio of 0.93).

The variance associated with the random herd effect of cows clustering within herds was 0.028 (P<0.001). Province was included as a fixed effect in the model because the small number of provinces in the dataset (attempts to include it as a random effect

produced very low variance estimates). Clustering of lactations within cows was not controlled for in the final model because there were only 1.59 lactations per cow, on average, so any clustering effect would have been small. Nine herds were excluded from the analysis due to the presence of fewer than 5 observations (lactations) per herd. In these herds, cow barn names rarely matched with DHI database names, thereby reducing the number of cows with applicable reproductive data. The cows from these herds had undue influence on the final model and high standardized residuals and therefore were excluded.

4.3.3 Logistic Regression using CCI200, CCI250 and CCI300

For the CCI200 model, *N. caninum*-seropositive cows had a 1.27 (p=0.027) times higher risk of having a CCI greater than 200 days compared to *N. caninum*-seronegative cows, and this risk increased to 1.37 (p=0.025) times in the CCI250 model and to 1.54 (p=0.020) times in the CCI300 model (Table 4.3). BLV seropositive cows also had a higher risk of having a CCI above 200 days compared to seronegative cows. BLV status also interacted with lactation number in the CCI200 model only, with older seropositive cows being less likely to have a CCI over 200 days than younger seropositive cows. Neither BVDV nor MAP exhibited a significant impact on our models.

Control of the effects of cows clustering in herds was achieved using a mixed logistic model, with herd as a random effect, with variances (± standard error) of 0.35± 0.07, 0.51±0.11, and 0.58±0.16 for CCI200, CCI250, and CCI300 outcomes, respectively. As with the Cox proportional hazard model, province was included as a fixed effect. Clustering of lactations within cows was not controlled for in the final model

because there were only 1.72 lactations per cow, on average, and hence not much potential for a clustering effect. Five herds were excluded from the analysis due to the presence of fewer than 5 observations (lactations) per herd. These herds also might otherwise have had an undue influence on the outcome of our study owing to the high values of their standardized residuals.

4.3.4 Logistic Regression using FSC

An interaction between *N. caninum* and BVDV was observed with odds ratio of 0.65, 1.04 and 0.84 for *N. caninum* positive cows (compared to *N. caninum* negative) in BVD-negative, BVD-positive and BVD-missing herds, respectively (Table 4.4).

The peak milk yield (PM) was divided into three categories, using 2 cutpoints, 32Kg/day and 41Kg/day. These categories were based on Figure 4.2, which shows the non-linear effect of PM on log odds of FSC. PM had odds ratio for FSC of 0.85 in PM between 32 and 42 kg and 0.74 in PM greater than 42 kg compared with PM lower than 32 kg.

Control of herd effects was achieved using the mixed logistic model, with herd included as a random effect, with variance of 0.20 ± 0.04. Again, province was included as a fixed effect in the model due to the small amount of clustering of herds within provinces when it was included as a random effect. FSC differed among the 5 provinces ($P < 0.001$). In addition, clustering of lactations within cows was again not controlled for in the final model. Four herds were excluded from the analysis due to the presence of fewer than 5 observations (lactations) per herd.

4.4 Discussion

This is the first study that has investigated the reproductive effects of these four pathogens in the same sample population of dairy cattle. A number of outcome variables were used to assess reproductive performance, based on available DHI data: overall reproductive performance (CCI); fetal loss (CCI200/CCI250/CCI300); and the ability to conceive (FSC). Although on some farms, complete breeding information is usually recorded by DHI technicians, this practice is not consistent across all farms and all technicians. We considered CCI to offer a more unbiased estimate of reproductive performance for lactation than other reproductive parameters based on available DHI data. CCI only requires 2 calving dates, which are consistently reported, whereas days to first service, overall conception rate or services per conception require accurate entry of breeding data beyond the successful breeding. Three outcome variables (CCI200, CCI250, and CCI300) were used as surrogate measures of fetal loss, because abortion records were not available. We used FSC as a measure of conception ability, although early fetal loss may also show up as failure to conceive.

Based on these different outcome variables different statistical analyses were used. The Cox proportional hazards model with herd level frailty was used because allows for the evaluation of time until conception while controlling for clustering of the outcome within herds. The mixed logistic model was selected for analyzing FSC, CCI200, CCI250, and CCI300, based on the dichotomous outcomes and on the hierarchical structure of the data: lactations clustered within cows, cows clustered within herds, and herds clustered within provinces. The provincial and cow-level clustering were investigated as random effects, but only the clustering of cows within herds showed

enough variance at that level to warrant its inclusion as a random effect. Province was included as a fixed effect in all models and cow was not included as a random or fixed effect in any model because, on average, these were fewer than 2 lactations per animal.

Effects of seropositivity for *N. caninum* on CCIs less than 200 days in our survival model were not significant, and this is consistent with the findings of Bjorkman, 1996 and Jensen, 1999. We also discovered no effect of *N. caninum* on FSC, the probability of conceiving at the first service. (also It was also evaluated the effects of *N. caninum* on the number of inseminations per confirmed pregnancy, or on the number of cows requiring more than one insemination per pregnancy and found no effects). Seropositive cows performed as well as seronegative cows, suggesting that, in general, overall reproductive performance were not impaired by *N. caninum* seropositivity for the sampled population.

Conversely, using outcomes of CCI200, CCI250 and CCI300 we consistently found an association between *N. caninum* seropositivity and logit of CCI for each cut-point. Although the number of positive outcomes decreased as the cutpoint was raised from 200 to 300 days and hence the power of the model was expected to fall, there was no decrease in the significance of the observed odds ratio, due to the increased strength (odds ratio) of the observed association as the cutpoint was raised. We speculate that this increased strength of association was due in part to a reduction of the number of cows that had not experienced a fetal loss or abortion in the positive group as the cut-point was raised (i.e. fewer "problem breeder" cows in this group that was assumed to be suffering fetal loss). Although we have no data to confirm this speculation, we expect that proportionately more problem breeder cows were among the cows with CCI $\geq$200 days

than among cows with CCI ≥300 days (eventually many problem breeders would become pregnant or culled) effectively weakening the association between *N. caninum* seropositivity and having a long CCI.

BLV-positive cows exhibited a borderline significant lower hazard of conception in lactations with CCIs less than 200 days, Furthermore; there was an interaction between BLV and lactation number in the CCI200 model, but not in the CCI250 and CCI300 models, with older seropositive cows being less likely to have a CCI over 200 than 1st lactation seropositive cows. It is unclear how to interpret these findings. The loss of power in the models with higher cut-points may have lead to an inability to detect the interaction effect in these models. Management of first lactation animals is considerably different from heifers. Heifers often undergo minimal handling and health management interventions, and have much less disease incidence compared to cows. Conversely, cows have more opportunities for virus transfer in the form of shared needles or syringes for treating or preventing disease, and shared rectal sleeves during multiple heat checks and pregnancy checks. Therefore, it may be possible that first lactation cows are becoming infected during this first lactation, with the effects of this new infection reducing reproductive performance. Alternatively, we may have detected a spurious interaction in CCI200 that was not in the CCI250 and CCI300 models.

The BVDV results were surprising. While controlling for *N. caninum* status, they revealed a higher likelihood of conception at first service in BVDV seropositive herds than in BVDV seronegative herds, suggesting that the presence of BVDV can improve FSC rates. This result is contrary to some previous research showing adverse reproductive effects of BVDV infection. Infection with BVDV during the first 45 days

and after 210 days of gestation has been shown to not influence the rate of return to estrus, but mid-term infections (Days 46 to 210) resulted in a statistically significant increase in the abortion rate. It has also been reported that 7% of fetal deaths were associated with infection with BVD virus. Seroconversion for BVD after two years of age had an effect on the herd average time to first calving by 14-16 days and 18 days for young stock. No effect on the number of breeding services for heifers or cows was observed, indicating that other factors are reducing conception risk. BVDV has also been reported to have no effect on the herd average calving interval. BVDV-infection at the herd level was not significantly associated with the risk of cow return to service before 25 days of insemination (early return), but was significant for return to service after 25 days of insemination (late return). BVDV-infection was found to increase the risk of embryonic and fetal death. One possible reason for the unexpected finding of a beneficial effect of BVDV in this study was a possible selection bias. Herds that did not have 5 or more unvaccinated cattle over 6 months of age were not included in the study. Herds with aggressive vaccination schedules due to on-going BVD problems, leading to vaccination of calves and heifers would be ineligible for inclusion in the study and these are the herds in which negative effects of BVDV might be expected.

We found no impact of MAP on outcome variables in any of our models, similar to the findings of Johnson-Ifearulundu *et al.*. This might reflect the low sensitivity of the diagnostic test (ELISA). The power of the analysis was also low due to the small number of seropositive animals in the sample.

4.5 Conclusion

Seropositivity for *N. caninum* was associated with two reproductive performance measures. *N. caninum* seropositive cows were at higher risk for prolonged CCI, presumably due to fetal loss or abortion. In herds negative for BVDV exposure, *N. caninum* seropositive cows were also less likely than seronegative cows to conceive on their first service but not in BVDV-positive herds. It is unclear why herds with evidence of exposure to BVDV had a higher likelihood of success on first breeding than herds with no evidence of BVDV. BLV-positive cows exhibited a borderline significantly increased CCI, and a higher risk of prolonged CCI, in first lactation cows, in particular. MAP seropositive cows were not associated with any of the reproductive parameters examined.

4.6

Table 4.1: Descriptive statistics of reproductive efficiency and apparent prevalence of *N. caninum*, BLV, BVD, and MAP by outcome variable and province.

Variable	Level	provinces PEI	NS	NB	ONT	SASK	TOTAL
Data used in survival analysis using CCI as an explanatory variable							
Herds		29	28	28	26	40	151
Cows		483	498	482	529	884	2876
Lactations		918	866	883	928	980	4569
CCI average (days)		110	110	108	108	107	109
Diseases prevalence							
N. caninum	cow	8.3%	21.3%	24.5%	8.1%	4.0%	11.9%
BLV	cow	15.9%	14.1%	29.7%	29.5%	35.8%	26.5%
MAP	cow	1.4%	2.0%	2.1%	2.6%	2.5%	2.2%
BVDV	Herd	51.7%	42.3%	46.4%	39.1%	32.0%	42.7%
Data used in logistic regression using CCI200, CCI250 and CCI 300 as an explanatory variable							
Herds		29	29	29	26	43	156
Cows		555	563	567	584	1262	3531
Lactations		1201	1077	1124	1148	1528	6078
CCI200	Lactation	19.4%	16.2%	17.7%	16.8%	17.7%	17.6%
CCI250	Lactation	10.9%	7.8%	8.5%	8.2%	8.9%	8.9%
CCI300	Lactation	5.8%	3.6%	4.4%	4.2%	4.7%	4.6%
Diseases prevalence							
N. caninum	cow	8.5%	21.9%	25.2%	8.1%	4.2%	11.7%
BLV	cow	16.0%	13.1%	30.5%	30.8%	34.7%	27.0%
MAP	cow	1.3%	2.7%	2.3%	2.6%	3.0%	2.5%
BVDV	Herd	51.7%	40.7%	44.8%	39.1%	35.7%	42.7%
Data used in logistic regression using FSC as an explanatory variable							
Herds		28	29	29	24	37	147
Cows		472	494	502	430	970	2868
Lactations		1042	948	1010	855	1183	5038
FSC	Lactation	52.8%	59.7%	47.4%	43.0%	50.3%	50.8%
Diseases prevalence							
N. caninum	cow	8.9%	22.5%	25.1%	8.6%	4.6%	12.6%
BLV	cow	17.4%	13.6%	29.7%	31.4%	34.2%	26.7%
MAP	cow	0.9%	2.6%	1.8%	2.3%	3.2%	2.3%
BVDV	Herd	50.0%	40.7%	44.8%	36.4%	34.6%	41.7%

Table 4.2: The Cox proportional hazards model for calving to conception interval in 2876 cows in 151 Canadian dairy herds.

VARIABLE	HR	SE	P VALUE
N. caninum	0.95	0.05	0.294
BVDV – Neg	BASELINE - Overall P value: 0.177		
BVDV – Pos	1.07	0.05	
BVDV – Missing	1.10	0.08	
BLV	0.93	0.04	0.062
MAP	0.97	0.10	0.764
LACT 1	BASELINE - Overall P value: 0.143		
LACT 2	1.02	0.05	
LACT 3+	1.08	0.05	
PEI	BASELINE - Overall P value: 0.231		
NB	1.02	0.07	
NS	1.09	0.07	
ONT	1.12	0.08	
SASK	1.14	0.08	
SCC	0.98	0.009	0.047
PM EARLY	0.98	0.003	0.000
PM LATE	0.996	0.002	0.049
VARIANCE	0.028	0.007	

Table 4.3: Three logistic mixed models for length of calving to conception interval in 3531 cows in 156 Canadian dairy herds.

VARIABLE	CCI 200			CCI 250			CCI 300		
	ODDS RATIOS	S. E.	P VALUE	ODDS RATIOS	S. E.	P VALUE	ODDS RATIOS	S. E.	P VALUE
N. caninum	1.27	0.14	0.027	1.37	0.19	0.025	1.54	0.28	0.020
BVDV – Neg	BL – Overall P value: 0.569			BL – Overall P value: 0.372			BL – Overall P value: 0.653		
BVDV – Pos	0.92	0.12		1.06	0.18		1.11	0.22	
BVDV – Missing	0.81	0.17		0.71	0.19		0.82	0.27	
BLV	1.66	0.28	0.003	0.97	0.12	0.826	1.12	0.18	0.477
MAP	1.05	0.24	0.839	0.99	0.32	0.982	0.98	0.43	0.961
1st Lactation	BL – Overall P value: 0.888			BL – Overall P value: 0.269			BL – Overall P value: 0.065		
2nd Lactation	1.01	0.13		0.99	0.15		1.08	0.21	
3rd + Lactation	0.96	0.12		0.84	0.12		0.76	0.15	
BLV*1st Lactation	BL – Overall P value: 0.008								
BLV*2nd Lactation	0.50	0.12							
BLV*3rd + Lactation	0.61	0.12							
PEI	BL – Overall P value: 0.668			BL – Overall P value: 0.432			BL – Overall P value: 0.362		
NB	0.77	0.15		0.68	0.17		0.57	0.18	
NS	0.82	0.16		0.71	0.18		0.66	0.20	
ONT	0.78	0.16		0.69	0.18		0.65	0.20	
SASK	0.87	0.17		0.88	0.22		0.83	0.24	
SCC	1.00	0.02	0.886	0.98	0.03	0.524	0.93	0.04	0.109
PM	1.02	0.00	0.002	1.02	0.00	0.015	1.02	0.00	0.079
		5			7			9	
VARIANCE (Herd)	0.35	0.07		0.51	0.11		0.58	0.16	

BL = Baseline

Table 4.4: Logistic mixed model results for first service conception in 2868 cows in 147 Canadian dairy herds.

VARIABLE	ODDS RATIOS	S. E.	P VALUE
N. caninum	0.65	0.08	0.001
BVDV – Neg	BASELINE – Overall P value: 0.112		
BVDV – Pos	1.24	0.13	
BVDV – Missing	1.11	0.20	
N. caninum * BVDV Neg	BASELINE – Overall P value: 0.048		
N. caninum * BVDV Pos	1.60	0.29	
N. caninum * BVDV Missing	1.31	0.47	
BLV	1.14	0.09	0.082
MAP	0.98	0.21	0.907
LACT 1	BASELINE – Overall P value: 0.106		
LACT 2	1.10	0.10	
LACT 3+	1.21	0.11	
PEI	BASELINE – Overall P value: 0.0001		
NB	1.44	0.22	
NS	0.82	0.12	
ONT	0.69	0.11	
SASK	0.95	0.15	
SCC	1.04	0.02	0.049
PM 1	BASELINE – Overall P value: 0.001		
PM 2	0.85	0.07	
PM 3	0.74	0.07	
VARIANCE (Herd)	0.20	0.04	

Figure 4.1: Aalen's linear hazards models for the peak milk production using calving to conception interval as an explanatory variable for 3531 cows in 156 Canadian dairy herds.

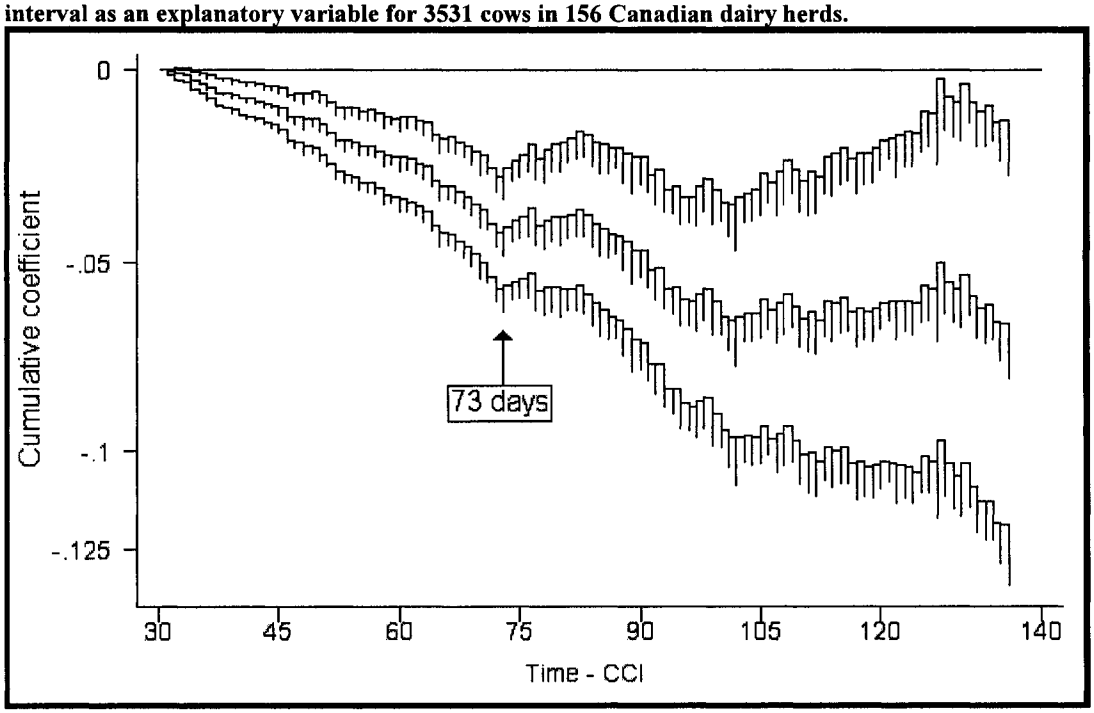
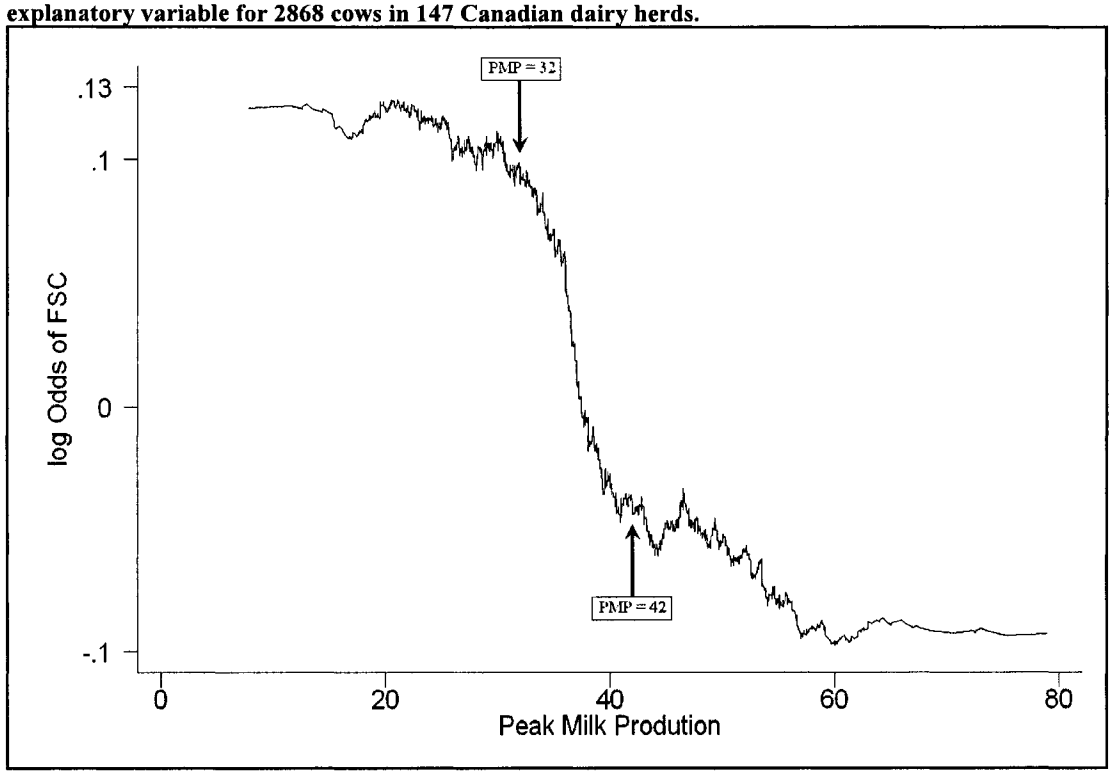


Figure 4.2: Smoothed curve of log odds of first service conception using peak milk production as explanatory variable for 2868 cows in 147 Canadian dairy herds.



5 Risk factors for seropositivity for *Neospora caninum* in Canadian dairy cows

5.1 Introduction

Neospora caninum (*N. caninum*) is an apicomplexan protozoan that was first recognized in dogs in Norway in 1984. In 1988, a new protozoan species, *N. caninum*, was proposed under a new genus, *Neospora*. This parasite is now recognized as an important cause of abortion in cows. It is found worldwide, with widespread occurrence of *N. caninum* infection in dairy cattle.

Direct productivity losses due to *N. caninum* include abortions and potentially other reproductive problems, such as stillbirths, early fetal death and re-absorption manifested as return to service, increased time to conception, or infertility although these losses have not been clearly established. Other direct costs associated with *N. caninum* include loss of milk yield in cows aborting due to *N. caninum*, increased culling of cows aborting due to *N. caninum*, and reduced growth and feed efficiency. A reduction in the value of breeding stock is also likely, although there is no documented evidence of this impact. Potential indirect costs associated with *N. caninum* include professional costs and costs associated with diagnoses, rebreeding, and replacement of a positive cow that has been culled.

In a Canadian study, based on data from the maritimes provinces, the cost of neosporosis was estimated at the herd and regional level. At the herd level, direct costs (premature culling, reduced cull value, abortions and reproductive losses) and treatment costs (which included the cost of veterinary services, medication and extra labor costs) were estimated at CAD$2,305 per year per infected herd of 50 cows, based on a 20% within-herd prevalence of infection. At the regional level, annual losses were estimated to

be CAD$1,909,794 for the three provinces. Another study from California, based only on the number of possible abortions, estimated the loss at $35 million US per year in this state, using the assumption of 2% of all pregnancies ended in abortion due to *N. caninum* infection.

Reduction of these costs depends on successful control of the transmission of *N. caninum* to a herd and between cattle within that herd, and a reduction in the likelihood of abortions occurring in infected animals in the herd. Epidemiologic studies have an important role in identifying the risk factors for *N. caninum* transmission between farms and between animals within a herd. Risk factor studies on *N. caninum* can be subdivided, based on the type of outcome that was investigated, into those associated with seroprevalence, confirmed post-natal infection, and abortion storms. However, it is unlikely that the risk factors associated with each of the types of outcome are independent. Documented abortion storms have frequently resulted from infection of previously uninfected pregnant animals, although they may also result from recrudescence among congenitally infected cattle. Furthermore, high-seroprevalence herds could have reached that status through long-term accumulation of congenitally infected cattle, and/or widespread post-natal infection. Therefore, exploratory risk factor studies cannot specifically identify whether the factors are contributing to horizontal or vertical transmission, unless the factors are biologically associated with one or the other.

Because the obvious clinical manifestation of *N. caninum* is abortion, the majority of published studies on risk factors of *N. caninum* are investigations of non-random samples of farms with recorded abortions due to *N. caninum*[14]. These studies have identified numerous potential risk factors for abortion storms due to *N. caninum*,

including the presence and number of dogs on the dairy farm at the time of the study visit, as well as during the past three years. In a study by Pare *et al.*, these were the only factors significantly associated with a herd being identified as a case herd (confirmed abortion) by *N. caninum* and a herd having a high (≥10%) seroprevalence. These findings have been corroborated by other studies with additional detail from a study of eight dairy farms with abortion storms in The Netherlands with evidence of horizontal transmission. All eight farms reported the introduction of a new dog within a period of 1.5 years before the first indication of *N. caninum* infection (either a *N. caninum* abortion or *N. caninum*-infected calf). Another study also found that female dogs were twice as likely as male dogs to be seropositive.

A study of 12 dairy farms with demonstrated horizontal *N. caninum* infection found farm dogs to be significantly more likely to ingest colostrum, milk, uterine discharge and placental material, and defecate in feed alleys and storage areas for grass and corn silage, compared with 21 control farms.

A Canadian study using sample from 88 herds and 5080 cows, associating *N. caninum* infection and abortion, identified the *N. caninum* herd seroprevalence, the total number of dogs on a farm, the frequency that dogs were observed defecating in mangers, the number of horses on a farm, the observed annual rate of retained fetal membranes, and the observed annual rate of cows returning to estrus after pregnancy confirmation as risk factor for abortion. Factors negatively associated with abortion due to *N. caninum* infection were the frequency of stray cats and wild canids observed on a farm, and the housing of heifers on loafing packs (a housing pen divided into feed manger, scrape alley and bedded pack areas)[14].

There have been other risk factors associated with *N. caninum* seroprevalence in cattle. A study of risk factors among 50 dairy farms with *N. caninum* abortion storms found a significant association with the number of poultry present. As a number of bird species have been shown to have antibodies against *N. caninum*, it is possible that poultry (and/or other bird species) may be an intermediate host, although this still requires confirmation. This study also found an association between the farms with abortion storms and the feeding of moldy corn-silage during the summer. This finding may be related to the possible immunosuppression caused by organisms or toxins in moldy feed, which leads to recrudescence of symptoms in already infected cattle.

However, many farms that are infected with the protozoan do not sustain abortion storms and their owners may be unaware that their cattle are infected. Therefore, it is important to investigate the risk factors that are associated with seroprevalence of *N. caninum*. In one study of 42 dairy farms, 27 seropositive farms were more likely to have contact with rabbits, ducks, and poultry, as well as have tethered housing and a pond water supply. In this same study, long calving intervals, high somatic cell counts, and the presence of cats were found to be negatively associated with *N. caninum* infection. However, it is unclear how these factors could protect against a positive antibody test for *N. caninum*.

The objective of this study was to evaluate a wide range of risk factors of *N. caninum* seropositivity, including co-infections with other pathogens and herd management practices in randomly selected dairy cattle and herds.

5.2 Material and Methods

5.2.1 Herd and cattle selection

We collected blood samples from 240 farms from 6 provinces in Canada. For all provinces except Alberta (ALTA), herds were randomly selected from a provincial list of herds enrolled in a monthly, individual milk-testing regimen through a dairy herd improvement (DHI) program. On the request of the study director in Alberta, sampling of herds in ALTA was first stratified by veterinary practices servicing dairy herds in the province, and then within these practices, herds on DHI were randomly selected.

Within each herd, blood samples from 30 cows randomly selected (where available) were taken. Collection occurred in the summer of 1998 for the 90 herds in the maritime provinces (30 each from Prince Edward Island, New Brunswick, and Nova Scotia). In Saskatchewan, blood samples were collected in winter 2001 from 44 herds. In Manitoba, blood samples were collected in October-November 2002 from 40 herds. In Alberta, blood samples were collected throughout 2002 from 66 herds. These samples were tested for exposure to *N. caninum*, the agent of interest for this paper, and for two other agents used as predictors in the risk factors analyses, bovine leukosis virus (BLV) and *Mycobacterium avium* subspecies *paratuberculosis* (MAP).

Sampling for Bovine Viral Diarrhea Virus (BVDV) exposure, another predictor of interest, required a different approach, as vaccination of both heifers and adults against BVDV was common in the study population and limited the number of animals on a farm that could provide a meaningful sample. Serology data for vaccinated animals and young (<6 months) offspring of vaccinated animals would be difficult to interpret given the potential for interference from vaccine-induced or maternal antibodies. We therefore selected five cows in those herds that had not been vaccinated for BVDV, while in the vaccinated herds; five heifers that had not yet been vaccinated but were over six months

of age were blood sampled. Farms where five unvaccinated animals over six months of age were unavailable for sampling, as indicated during herd recruitment, were excluded.

5.2.2 Serological testing

Serum samples were stored at –20°C until all samples in the respective province were obtained. Two commercial tests were utilized to detect *N. caninum* antibodies; 1) the indirect BIOVET ELISA (BIOVET Inc. - St. Hyacinthe, Quebec, Canada - sensitivity 99.0%, specificity 98.4%) was used for samples from NS, NB, PEI, MAN, and SASK; and 2) the indirect IDEXX ELISA (IDEXX Laboratories, Westbrook, Maine, USA - sensitivity 88.6% specificity 96.5%) was used in ALTA. A cow was considered to be infected with *N. caninum* if the serum-to-positive ratio on the Biovet ELISA and IDEXX ELISA were $\geq$0.60 and $\geq$0.50, respectively, as recommended by the manufacturers of the test kits. Serum samples were tested in duplicate for *N. caninum*. The *N. caninum* testing was conducted at the BIOVET Inc. laboratory in Quebec for dairy farms from NS, NB, PEI, MAN, and SASK and the Alberta Agricultural Laboratory for dairy farms in ALTA.

For BLV, the samples were tested using the IDEXX ELISA (IDEXX Laboratories, Westbrook, Maine, USA) at the National BLV Testing Laboratories in Prince Edward Island (until 2001) and subsequently in Quebec, Canada. The test is reported to have a sensitivity of 98.5%, a specificity of 99.9%, at a sample-to-positive ratio (S/P ratio) cutpoint of 0.50. This test requires a confirmation of positive tests, using a sample-to-negative host-cell ratio of 1.8.

The test utilized by all provinces except ALTA for MAP antibodies was the IDEXX ELISA (IDEXX Laboratories, Westbrook, Maine, USA - sensitivity 43.0%,

specificity 99.0%) compared to fecal culture results. The BIOCOR ELISA (Biocor Animal Health Inc., Omaha, Nebraska, USA) was utilized in ALTA, with a reported sensitivity and specificity of 47.3% and 99.0%, respectively compared to fecal culture results. An animal was considered to be infected with MAP if the serum-to-positive (S/P) ratio on the IDEXX ELISA was 0.25, and the optical density was greater than the negative control plus 0.1 on the BIOCOR test kits, as recommended by the manufacturer. Serum samples were tested in duplicate for MAP. The MAP testing for NS, NB, PEI, and SASK was conducted at Prairie Diagnostic Services in Saskatchewan, which is accredited for MAP ELISA testing by the United States Dept of Agriculture. Upon request by the directors of the studies in Manitoba and Alberta, the Manitoba Agricultural Laboratory in Manitoba was utilized for the dairy farms in MAN, and the Alberta Agricultural Laboratory in Alberta for the dairy farms in ALTA.

For BVDV, the samples were tested for the presence of antibodies using virus neutralization to the cytopathic Singer strain at Animal Diseases Research Institute, Alberta. Animal-level sensitivity has been reported at 99.6% and specificity at 100%. Herd-level sensitivity has been reported at ≥95% and herd-level specificity at ≥98% for correctly identifying or ruling out BVDV infection in herds, using as a sample of five unvaccinated animals over 6 months of age.

5.2.3 Cow-level predictor data collection

The data for the sampled cows were obtained electronically from Canadian Dairy Herd Management Services (CMHDS), which processes DHI records for all of Canada. The cow-level predictors included: lactation number (used as both a continuous and categorical variable – 1st, 2nd, 3rd, and 4th+), days in milk, 24-hour milk yield and linear

score somatic cell count from the milk test nearest the day of blood collection. Peak milk production, 305-day milk yield, 305-day fat yield, and 305-day protein yield were obtained to represent production data for the lactation during which the blood sample was taken.

Other cow-level predictors investigated were the serostatus of MAP and BLV. BLV and MAP were defined as dichotomous variables (positive and negative, based on the manufacturers' cutpoints of the diagnostic tests).

5.2.4 Herd-level predictor data collection

A herd-level predictor was determined through serological testing as outlined previously (BVD status). A herd was considered positive for BVDV infection when at least one animal with a titre greater than 1:64 was present, based on the distribution of titres found. Herds with animal titres ≥1:64 usually also had animal titres ≥1:256, the maximum titre tested.

The majority of the herd-level predictors were obtained through questionnaires administrered on each farm. We had 224 of the 240 farms participating in the survey (93.3% response rate) with a high acceptance of the structure and format of the questionnaire. The questionnaire was comprised of 10 sections with 21 pages and 423 variables and was pre-tested on 5 farm owners/employees for clarity and ease of administration. The questionnaires were completed in an average of 45 minutes each.

One section collected information about the farm and farmer, including: age of the farmer, physical size of the farm, cow breed, and number of employees. The herd population section included: the number of dairy cattle present on the farm, sold, culled,

died and purchased in the last 12-month period. These data were subdivided by age-sex categories such as: pre-weaned calves, open heifers, bred heifers, milking cows, dry cows, and bulls. Housing sections covered the method of housing for each type of animal in the winter and summer, and management practices for pasture. Biosecurity was examined under various criteria: purchase practices; biosecurity requirements for introduction of purchased animal; contact between other domestic animals present on the farm and cattle or their water and food (with particular focus on dogs and cats); possible contacts with wild animals and cattle from other farms; possible contact with other people and their vehicles or equipment; and use of coccidiostats and vaccines. The calving and calf management section covered the colostrum and milk feeding practices with calves, management of newborn calves and calving area, and management of placentas and aborted fetuses. Another section covered the origin of food and water: how food and water were stored; type of food given to the dairy cattle; and general management of manure. The final section looked at the prevalence of diseases. Farmers were questioned about previous disease events and diagnostic tests carried out on the farm. The complete questionnaire is available in Appendix A.

5.2.5 Statistic analysis

Analyzing the relationships between *N. caninum* serostatus (the outcome variable) and possible risk factors was a three-stage process. First, an unconditional analysis using logistic regression for all cow-level and herd-level variables was conducted, selecting those for which the P-value was ≤ 0.15. A mixed logistic regression model with herd as a random effect and province as a fixed effect was used for this process.

The second stage of the analysis included the 44 herd-level and 3 cow-level variables selected in the unconditional analyses outlined above. Section specific models were built for variables with $p<0.05$ within each section of the questionnaire in order to understand their relationships in the biological context, as well as the relationship with herds, provinces and the three cow-level variables previously selected. A "manual forward" stepwise process was conducted using mixed logistic regression models with herd as a random effect and province as a fixed effect.

The final stage included model building with the variables found significant in the section specific models. With this final model of significant ($p\leq0.05$) main effects, all first-order interactions were evaluated and retained if significant. Maximum likelihood estimates of all parameters were obtained from an algorithm that used numerical integration with adaptive quadrature for estimation.

We performed model diagnostics, including normal probability plots of the herd level residuals for the logistic models, and an evaluation of outlying observations. All analyses were conducted using the statistical software package STATA (version 8).

5.3 Results

5.3.1 Descriptive analysis and unconditional associations

The final dataset comprised 6,267 cows from 224 herds and 6 Canadian provinces. A total of 801 (12.8%) cows were seropositive for *N. caninum*. There was at least one positive cow in 183 (81.7 %) herds, and 106 (47.3 %) herds had at least 10% of sampled cows testing positive. In the case of BLV infection, a total of 2,095 of 6,257

(33.4%) cows were seropositive, while 272 of 6,249 (4.4%) cows tested positive for MAP.

In the first stage of the cow-level variable analyses, when controlling for clustering (mixed model) using herd as random effect and province as a fixed effect, BLV serostatus, days in milk, and somatic cell count had P-value ≤ 0.15 (Table 5.1).

With herd as a random effect and province as a fixed effect, 44 herd-level variables had a P-value ≤ 0.15 (Table 5.2). The sections in which each variable was located in the questionnaire are identified in the table, where variables were highly correlated; those variables that were retained in the models for each section of the questionnaire are identified by an asterisk beside the section identifier.

5.3.2 Final model

During the diagnostic examination of the final model, we observed two herds from NS, one herd from PEI and two herds from MAN with very high standardized herd level residuals. We built another model excluding these five herds and obtained slightly modified results, but none of the modifications was large enough to alter the variables included in the final model. The most notable change was that the variance between herds dropped from 0.682 to 0.538, showing a lower clustering effect after excluding the five aforementioned outlier herds. In addition, herds with heifers having nose-to-nose contact with calves had an decreased odds of being seropositive from 0.662 to 0.724, with this variable becoming borderline significant with a P = 0.08. Table 5.3 compares the models with and without the outlier herds.

In the final model (Table 5.3), one categorical variable was identified. The baseline was the absence of dogs on the farm, and the other two levels were "dog was

present but do not eat placenta and/or fetuses" and "dog was present and eat placenta and/or fetuses"; with odds ratio of 1.54 and 2.22, respectively. For the cow level variables, BLV serostatus was the only variable that was kept in the model, with an odds ratio of 1.4, meaning that a BLV-seropositive cow was more likely to be positive for *N. caninum*.

Six other variables, all of them herd-level variables indicating practices that decreased the risk of a herd being seropositive for *N. caninum*, remained in the final model: "If the farmer asked for a BVDV-negative test before introduction of an animal" (n=82/170); "Number of milk cows on the farm" (mean = 67.6, standard deviation = 47.5); "If heifers had nose-to-nose contact with calves" (n = 48/224); "If dry cows received Rumesin (monesin) in their diet" (n = 59/157), "If embryo transfer was used on the farm" (n = 48/223), and "Area of the farm used for forage production per cow" (mean = 405 acres, standard deviation = 988) (Table 5.3).

5.4 Discussion

This is the first study that has investigated the relationship between *N. caninum* seropositivity, a number of risk factors at both the herd level and the cow level and two other infectious agents at the cow level (BLV and MAP infection) and one other agent at the herd level (BVDV infection). This study was conducted in 224 randomly selected herds across 6 Canadian provinces under various conditions of management and dairy production. Therefore, it has made a substantial contribution towards understanding the relationship between *N. caninum* seropositivity (regardless of abortion status) and many investigated factors, confirming previously determined factors, refuting other unlikely

factors found elsewhere and identifying previously undiscovered protective factors that require confirmation.

Dogs are a definitive host of *N. caninum*. The categorical variable present in our final model yields similar results to those previously reported, in that the presence of farm dogs on the dairy farm and whether or not the dogs ate placenta and fetuses were associated with the seroprevalence of *N. caninum*. In a previous study, a herd becoming a case herd (*N. caninum* confirmed abortion) and a herd having a high (>10%) seroprevalence were associated with the number of dogs on a farm. In a study of eight dairy farms that had seen abortion storms in The Netherlands, there was evidence of horizontal transmission following the introduction of a new farm dog within a period of 1.5 years prior to the first indication of *N. caninum* infection (either abortion or an infected calf). Another study also found that female dogs were twice as likely to be seropositive as male dogs, however our results found no association between *N. caninum* seroprevalence and the gender of dogs or whether they had been neutered. A study of 12 dairy farms with demonstrated horizontal *N. caninum* infection found that farm dogs from these 12 farms were significantly more likely to have ingested uterine discharge and placental material than dogs on control farms. An Ontario study demonstrated that dogs were more likely to eat placentas and fetuses in seropositive herds, than in seronegative herds. The total number of farm dogs had an odds ratio of 2.8 for its association with *N. caninum* abortion[14].

A number of authors have suspected that concurrent infections with other agents may lead to immunosuppression, allowing recrudescence of latently infected cattle. However, herd-level prevalence of antibodies to bovine herpes virus I, *Leptospira hardjo*

or *Salmonella dublin* were not associated with the risk of abortion storms on Dutch dairy farms. Similarly, an increased risk of abortion was not observed when cows were seropositive to both *N. caninum* and BVDV infection[26]. Our study found no associations between BVDV and MAP seroprevalence and *N. caninum* serostatus. We did find an association between BLV and *N. caninum* serostatus, but more study is needed to understand this relationship. A possible reason for this association could be that latent *N. caninum* infections may be easier to detect with the persistent lymphocytosis that can occur in up to 30% of BLV seropositive cattle. It has been well established that antibody levels specific to *N. caninum* do fluctuate over time, potentially falling below the cutoff values typically used by commercial ELISA tests. Further research is needed to determine if an increase in the number of BLV-infected lymphocytes, in a *N. caninum* infected cow may lead to fewer false-negative antibody test results for *N. caninum* exposure.

Embryo transfer can be used on seropositive donor cows to harvest uninfected embryos for implantation into seronegative recipient cattle, a practice that has been shown to produce seronegative calves. Our findings confirm that, in the field, commercial herds using embryo transfer had a lower odds of infection (odds ratio = 0.652), showing a protective effect of the practice on *N. caninum* seroprevalence.

Four of the other five protective factors are possible measurements or indicators of control plans of disease, which may be directly or indirectly related to *N. caninum* control. When farmers ask for BVDV-negative testing before introducing an animal, this may be an indirect assessment of the quality of biosecurity measures used by the farmer. Among the farmers that had purchased an animal in the last 5 years (170 from the 224

herds), only 6 asked for a negative *N. caninum* test result, producing little power to detect a significant difference between positive and negative herds for *N. caninum*. Conversely, 82 farmers asked for a negative test result for BVDV, a frequency that may be overestimated due to social desirability bias. Neosporosis is a relatively new disease, first recognized in 1984 while BVD has been recognized for more than 60 years, so it is more likely that the producers would have more knowledge about BVD. Asking for *N. caninum* test results, biologically plausibly related to *N. caninum* seroprevalence, did not remain in the final model, probably due to the very infrequent use of this biosecurity measure.

"Number of milk cows on the farm" is considered a "herd size" parameter, with larger farms having a lower seroprevalence of *N. caninum*. A number of reasons may explain this association. Large farms were more likely to have free-stall than tie-stall barns for ease of labor, and producers with free-stall barns were more likely to keep cattle indoors during the summer (43% vs. 15% for tie-stall barns). Intuitively indoor cattle are less likely to become exposed to neighbours' dogs or wild canids. However, further research is needed to examine these possible explanations.

"Area of the farm used for forage production per cow" is a factor that shows an inverse relationship with cattle density on pasture. Lower density may lead to decreasing contact with possible sources of horizontal transmission of *N. caninum*, such as water or feed containing infected dog feces. Again, this speculation needs confirmation.

"Dry cows receiving Rumesin (monesin) in diet" as a protective factor for *N. caninum* seropositivity is an interesting result which must be studied more. Rumensin is used for treating other protozoal infections in ruminants, such as coccidiosis in cattle and

toxoplasmosis in sheep. Rumensin is used on some dairy farms, but further research is needed to determine if it has any effect on *N. caninum.*

We cannot explain why there was an association between *N. caninum* seropositivity and "Heifers having nose-to-nose contact with calves", and therefore we suspect that this is probably a spurious association. Future research may shed light on this putative protective factor. Research is also needed to incorporate all of the findings from this research into a control program, and determine their costs and benefits in an economic framework.

Comparing our results with a study of 42 French dairy farms of which 27 seropositive farms were found to have had contact with rabbits and poultry as well as with pond water supply, we found no such associations. In regards to water supply, our study analyzed 10 variables: the distance of the pond and of the well from the dairy barn; and the kind of water supply for 4 separate categories of dairy animal (open heifers, bred heifer, milking cows, and dry cows) in the winter and in the summer. None of these variables had an association with *N. caninum* seroprevalence in the herd.

In the 42-farm French study, high somatic cell counts and the presence of cats were found to be negatively associated with the risk of *N. caninum* infection. Our study of 224 randomly selected dairy farms looked at the number of cats overall on the farm, as well as the number of females (both spayed and intact) and males, and where cat litters were born. None of these variables had an association with *N. caninum* seroprevalence suggesting that the cat associations found in the French study were likely spourious. In our study, linear score somatic cell count was found to have a borderline negative

association with *N. caninum* seropositivity but this was only an unconditional effect (P-value= 0.149), and it did not remain significant in our final model.

5.5 Conclusion

There were several important factors that were associated with *N. caninum* seroprevalence in this study. The most important evidence was the confirmation that dogs eating placenta and fetuses were associated with higher *N. caninum* seroprevalence. BLV seroprevalence on farm was also associated with *N. caninum* seroprevalence.

From a disease management perspective, some of the factors identified here may be important as control measures because six factors were determined to be protective and may act to reduce the level of infection in the herd. The protective factors were: "If farmer asks for BVDV-negative exam before introduction of animal"; "Number of milk cows on farm"; "dry cows receive Rumesin (monesin) in diet"; "If embryo transfer used on farm"; "Area of farm used for forage production per cow"; and "If heifers have nose-to-nose contact with calves". Further research is needed to corroborate some of these findings.

5.6 Literature cited

Table 5.1: Unconditional analyses of cow level variables on *N. caninum* seropositivity in 6267 dairy cattle on 224 Canadian herds in 6 provinces.

Variables	Cows	Herds	Clustering controlled using herd as random and province as fixed effects		
			Odds Ratio	S. E.	P-Value
lactation number	6267	224	0.985	0.026	0.55
1st lactation	1859		Baseline - overall P-value: 0.55		
2nd lactation	1546		0.881	0.105	
3rd lactation	1147		1.032	0.130	
4th or more lactation	1715		0.918	0.109	
Days in milk	6257	224	1.001	0.000	**0.08**
24-hour milk yield	5659	223	0.965	0.049	0.48
Somatic cell count	5548	220	0.999	0.000	**0.15**
Linear score of somatic cell count	5545	220	0.994	0.024	0.79
305-day milk yield	5675	224	1.000	0.000	0.84
305-day fat yield	5675	224	1.000	0.001	0.61
305-day protein yield	5675	224	1.000	0.001	0.78
BLV serostatus	6257	224	1.432	0.148	**> 0.01**
MAP serostatus	6249	224	1.034	0.212	0.87

Table 5.2: Results of the unconditional analyses ($p \leq 0.15$) of herd level variables on *N. caninum* seroprevalence in 6267 dairy cattle on 224 Canadian herds in 6 provinces. Variables are listed in order of statistical significance.

Variables	Questionnaire Section	Cows	Herds	Herd random and Prov fixed		
				Odds Ratio	S. E.	P-Value
Total number of employees	Farm and farmer (*)	6237	223	0.870	0.038	> 0.01
Percent of family income from dairy production	Farm and farmer (*)	5669	202	1.007	0.003	0.02
Total area of the farm	Farm and farmer (*)	6119	219	0,999	0.000	0.02
Total number of part time employees	Farm and farmer	6197	222	0.873	0.053	0.03
Total number of full time employees	Farm and farmer	6178	220	0.868	0.051	0.03
Area of the farm not used as forage and pasture	Farm and farmer	6102	219	0,999	0.000	0.05
Area of the farm used as forage production	Farm and farmer	6239	223	1.000	0.001	0.06
Breed of dairy cows are other than Holstein	Farm and farmer	6267	224	0.382	0.199	0.06
Number of milk cows on farm	Herd population (*)	6127	219	0.995	0.002	0.01
Percentage of cows raised on farm	Herd population (*)	5901	210	0.996	0.002	0.02
Number of bred heifers on farm	Herd population	6127	219	0.990	0.005	0.02
Number of cows on farm	Herd population (*)	6127	219	0.996	0.002	0.03
Number of heifers on farm	Herd population (*)	6127	219	0.995	0.002	0.03
Number of dairy cattle on farm	Herd population (*)	6153	220	0.998	0.001	0.04
Number of dry cows culled	Herd population	6179	221	0.956	0.022	0.05
Number of open heifers on farm	Herd population	6127	219	0.994	0.003	0.08
Pasture was dragged	Housing (*)	6165	221	1.472	0.299	0.06
Farmer asks for BVDV negative before introduction of animal	Purchase (*)	6267	224	0.418	0.183	0.05
Farmer asks for Johne's negative test before introducing animal	Purchase	6267	224	0.223	0.192	0.08
Farmer asks for *Neospora*-negative test before introducing animal	Purchase (*)	6267	224	0.404	0.231	0.11
Presence of dogs on farm	Contact (*)	6267	224	1.760	0.455	0.02
Number of goats on farm	Contact	6239	223	1.226	0.115	0.03
Farms borrow equipment from other farmers	Contact (*)	6267	224	1.417	0.243	0.04
Farmer sees foxes on farm	Contact (*)	6267	224	0.714	0.131	0.07
Number of AI technicians entered dairy barn	Contact	6236	222	1.003	0.002	0.11
Other farmers use test farmer's trailer to transport dairy cows	Contact (*)	6229	223	1.455	0.362	0.13

Calves vaccinated with Bovishield	Vaccination and medication practices	6056	216	0.321	0.144	0.01
Dry cows receive Rumesin (monesin) in diet	Vaccination and medication practices (*)	6248	223	0.659	0.128	0.03
Heifers vaccinated with other vaccine (not listed in questionnaire)	Vaccination and medication practices	5099	188	2.330	0.963	0.04
Calves receive Rumesin (monesin) in diet	Vaccination and medication practices (*)	6212	222	0.611	0.151	0.05
Milk cows receive Rumesin (monesin) in diet	Vaccination and medication practices (*)	6248	223	0.735	0.135	0.09
Cows vaccinated with Cattlemaster	Vaccination and medication practices	6056	216	0.615	0.181	0.10
Dogs eat remains of aborted fetuses - never	Calving and calf management	6267	224	Baseline - overall P-value: > 0.01		
Dogs eat remains of aborted fetuses - sometimes	Calving and calf management	6267	224	2.210	0.782	> 0.01
Dogs eat remains of aborted fetuses - often	Calving and calf management	6267	224	0.887	0.416	> 0.01
Dogs eat remains of placenta and fetuses	Calving and calf management (*)	6267	224	2.788	1.070	> 0.01
No dogs on farm	Calving and calf management (*)	6267	224	Baseline - overall P-value: > 0.01		
Presence of dogs but do not eat placenta and fetuses	Calving and calf management (*)	6267	224	1.69	0.42	> 0.01
Presence of dogs which eat placenta and fetuses	Calving and calf management (*)	6267	224	2.38	0.72	> 0.01
Dogs eat remains of placenta - never	Calving and calf management	6267	224	Baseline - overall P-value: 0.04		
Dogs eat remains of placenta - sometimes	Calving and calf management	6267	224	1.663	0.356	
Dogs eat remains of placenta - often	Calving and calf management	6267	224	1.214	0.370	
Remove all manure from calving areas - each calving	Calving and calf management	6151	220	Baseline - overall P-value: 0.05		
Remove all manure from calving areas - every 2 to 4 calvings	Calving and calf management	6151	220	1.248	0.294	
Remove all manure from calving areas - every 5 calvings or more	Calving and calf management	6151	220	0.700	0.147	
Heifers have nose-to-nose contact with calves	Calving and calf management (*)	6267	224	0.719	0.123	0.05
Use embryo transfer	Calving and calf management (*)	6227	223	0.668	0.140	0.05
Heifers have nose-to-nose contact with other heifers	Calving and calf management (*)	6267	224	0.686	0.143	0.07
Number of embryo collected in the last 12 months	Calving and calf management	6107	219	0.984	0.010	0.09
Percentage of calving had multiple cows in the barn	Calving and calf management (*)	5725	203	0.995	0.003	0.12
Remove surface manure from calving areas - each calving	Calving and calf management	5526	200	Baseline - overall P-value: 0.13		
Remove surface manure from calving areas - each 2 to 4 calvings	Calving and calf management	5526	200	0.869	0.206	
Remove surface manure from calving areas - each 5 or more calvings	Calving and calf management	5526	200	0.677	0.136	
Cows are fed greenchop	Feed, water, and manure (*)	6267	224	1.396	0.318	0.14
Number of cows tested for BLV	Prevalence of disease (*)	5753	205	0.988	0.008	0.08

(*) - Variables that were retained in the models for each section of the questionnaire and used in the final stage of modeling across all sections.

Table 5.3: Results of the final modeling ($p \leq 0.05$) of cow and herd level variables on *N. caninum* seroprevalence in 6061 dairy cattle on 217 Canadian herds in 6 provinces, with and without 5 outlier farms

Variables	Final model with 217 herds and 6061 cows included			Final model with 212 herds and 5922 cows included[a]		
	Odds Ratio	S. E.	P-Value	Odds Ratio	S. E.	P-Value
Prince Edward Island	Baseline - overall P-value: < 0.01			Baseline - overall P-value < 0.001		
Nova Scotia	2.621	0.762		2.266	0.638	
New Brunswick	3.058	0.892		3.330	0.917	
Manitoba	0.745	0.240		0.690	0.219	
Saskatchewan	0.470	0.141		0.511	0.145	
Alberta	2.097	0.766		1.930	0.661	
No dogs on farm	Baseline - overall P-value < 0.01			Baseline - overall P-value < 0.01		
Presence of dogs but do not eat placenta and fetuses	1.777	0.434		1.549	0.354	
Presence of dogs which eat placenta and fetuses	2.172	0.624		2.221	0.590	
BLV serostatus	1.433	0.147	< 0.01	1.500	0.155	< 0.01
Use embryo transfer	0.652	0.130	0.04	0.682	0.125	0.03
Farmer asks for BVDV-negative test before introducing animal	0.285	0.133	< 0.01	0.317	0.149	0.01
Number of milk cows on farm	0.995	0.002	0.01	0.995	0.002	0.01
Area of farm used for forage production per cow (density)	0.991	0.004	0.05	0.992	0.004	0.05
Dry cows receive Rumesin (monesin) in diet	0.652	0.116	0.02	0.690	0.115	0.03
Heifers have nose-to-nose contact with calves	0.662	0.129	0.03	0.724	0.131	0.08
Random effects						
	σ^2	S. E. (σ^2)		σ^2	S. E. (σ^2)	
Herd level variance	0.682	0.117		0.538	0.102	

[a] During the diagnostic examination of the final model was observed two herds from NS, one herd from PEI and two herds from MAN with very high standardized herd level residuals. This model was built excluding these five herds.

6 Production losses and treatment costs from *Neospora caninum* infection in Canadian dairy herds

6.1 Introduction

Neosporosis is an infectious disease caused by *Neospora caninum* (*N. caninum*) that is found on many dairy farms in Canada and around the world. It has been associated with substantial productivity losses on affected farms and has become the most commonly diagnosed cause of abortions in the world. Diagnosis of *N. caninum* - associated abortions in dairy cattle in Ontario increased from 1.6% of abortion submissions in 1993-94 to 14%-15% in 1997-00. In Quebec, 11.4% of all aborted bovine fetuses submitted to diagnostic laboratories in 1996 were infected with *N. caninum*. Similar estimates of 15% to 20% of abortions attributed to *N. caninum* have been found in California and The Netherlands, demonstrating the large impact of *N. caninum* in other dairy producing areas of the world.

Direct productivity losses associated with *N. caninum* include reproductive losses, reduced milk production in aborting cows or subclinically infected cows, and increased risk of culling. In addition to abortion, other potential reproductive losses include stillbirths, early fetal deaths and reabsorption, increased time to conception and infertility, although these losses have not been clearly established. Reduced milk production may result from subclinical *N. caninum* infections, although cows aborting due to *N. caninum* may have more dramatic reductions in milk production. Similarly, *N. caninum* may be associated with an increased risk of culling in aborting cows or, potentially in cows subclinically affected.

A Canadian study suggests that the association between *N. caninum* serostatus and milk production may depend on abortion status of the herd. In herds with abortion problems, *N. caninum*-

seropositive cattle produced less milk, whereas in herds without abortion problems, *N. caninum*-seropositive cattle produced the same amount of milk as seronegative cattle[8].

In a study from California, USA[9], based on the assumptions that 2 – 5% of all pregnancies end in abortion due to *N. caninum*, losses from *N. caninum* abortions were estimated to be US$35 million per year. By applying the assumptions used in the California study to the Canadian context, annual losses in Canada would be between 24,000 and 60,000 pregnancies/year.

In a Canadian study, based on data from the maritime provinces, the cost of neosporosis was estimated at the herd and regional level. At the herd level, direct production losses and treatment costs (the cost of veterinary services, medication, and extra labor) together were estimated at $2,304.98 per year per infected herd of 50 cows, based on a 24% within-herd seroprevalence of infection. At the regional level, annual losses were estimated to be $1,909,794.00 for the 3 provinces[10].

The objective of this study was to update these Canadian cost estimates, to estimate production losses (and their ranges) associated with *N. caninum* seropositivity in the Canadian dairy industry, and to determine the average and possible range of losses for individual Canadian infected dairy herds. To accomplish this objective, data from 6 of 10 Canadian provinces were included, as well as new information about the epidemiology of the disease and its impacts on productivity. A stochastic simulation partial budget model with three components of production losses (impact on milk yield, impact on culling, and losses and treatment costs associated with abortions) was developed. The model took into account the variability and uncertainty in the required input values and consequently produced probability distributions of the estimated losses.

6.2 Materials and Methods

6.2.1 Partial-budget model

A partial budget model is one which deals only with those aspects of an enterprise, which are affected by a factor being investigated. The model used in this study was adapted from one originally published by Bennett *et al.*. (1999)[11] and subsequently used by Chi *et al.*.[10] and included the 3 components such as impact on milk yield, on culling, and tretment costs associated with abortion. Considerations such as possible effects of *N. caninum* on the ability of the farm to market livestock or other products, and other potential indirect costs were not included in the model.

6.2.2 Input Parameters

Table 6.1 lists all of the input parameters used in the partial budget model, the distribution that was assumed to represent the range of possible values that each parameter might have, the characteristics that defined that distribution, and the source of the information about the parameter and its distribution. Each is discussed in more detail below.

6.2.2.1 Farm characteristics and prices

Two herd sizes were used in the analyses. To estimate the average losses for the Canadian dairy industry, a herd size of 100 cows was used and losses were expressed as being "per 100 cows". For estimating the range of possible losses at the individual infected herd level, the average size of a Canadian dairy herd, as reported by Dairy Farmers of Canada in 2002 (n=61 cows) was used. The average milk production per cow per 305-day lactation (9,519 kg) was obtained from Canadian Dairy Herd Improvement (DHI) data (http://www.dairyinfo.gc.ca/pdf_files/tab26.pdf). The average milk price ($0.59/kg) was obtained from The Canadian Dairy Information Centre web site

(http://www.dairyinfo.gc.ca/pdf_files/pcan0304.pdf). Herd sizes, production levels and milk price were all treated as fixed values so that all estimates of *N. caninum* associated losses were independent of differences in those parameters across herds.

Replacement cost of a cow (triangular distribution, min. = $1,500, max. = $2,500, most likely = $2,000 per head), average slaughter value (triangular distribution, min. = $300. max. = $700, most likely = $500 per head), and newborn calf value (triangular distribution, min. = $200, max. = $600, most likely = $400 per head) were representative values assigned following consultation with dairy clinicians familiar with current costs.

6.2.2.2 Seroprevalence of *Neospora caninum*

Data about the seroprevalence of *N. caninum* were obtained from a stratified two-stage random sample of 240 herds in 6 Canadian provinces. In that study, herds were chosen randomly from a list of all herds enrolled in a monthly milk recording program provided by the local branch of the Canadian DHI. Herd testing was undertaken in 1998 on 90 randomly selected herds in the maritime provinces (30 each from Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS)). In Saskatchewan (SASK), Manitoba (MAN) and Alberta (ALTA), samples were collected in 2001, 2002 and 2002-2003 from 44, 40 and 66 randomly selected herds, respectively. Herd selection in ALTA involved an additional step in which veterinary clinics in dairy producing regions were selected and then random sampling of dairy herds within clinics was conducted. Participation of herds was sought through either direct phone call (PEI, NS, NB, MAN, ALTA), or an initial letter of request followed by a phone call (SASK). Within each herd, 30 cows (if available) were randomly selected from the herd list and sampled. Details of the sampling and testing protocols are reported elsewhere. Overall 11.9% (SD=0.92%) of dairy cattle had positive tests for antibodies against *N. caninum*. For the model

estimating overall losses for the Canadian dairy industry, the overall prevalence of *N. caninum* was assumed to fall within a normal distribution with a mean of 11.9% and SD of 0.92%.

A much wider distribution for the within-herd seroprevalence was utilized for the estimate of the range of possible losses that individual Canadian herds might encounter. The within herd prevalence ranged from 0 to 74% with a mode of 0, and 95% of values less than 43%. Consequently, for the within herd prevalence used when estimating the range of individual infected herd losses, a beta distribution (α=1.0004, β=5.33) was used to get a distribution with 95% of values <43% and a mode of 0.01% (a mode of 0% is not feasible). This produced a mean within-herd prevalence of 15.8%, which was slightly higher than the observed overall prevalence (11.9%).

6.2.2.3 Impact of *Neospora caninum* on milk yield

A number of studies have investigated the effect of subclinical infection with *N. caninum* on milk production with varying results. An observational study with 565 Holstein cows classified as seropositive or seronegative to *N. caninum* within 7 days after calving by use of a kinetic ELISA, compared milk production between those animals. On the basis of 305-day lactation data, seropositive cows produced less milk (2.8 lb/cow per day) than did seronegative cows. Showing that exposure to *N. caninum* was associated with a 3 to 4% decrease in milk production[13] One California study, that was limited to first lactation animals in a herd with a history of abortions due to *N. caninum*, reported that *N. caninum* seropositive cows produced approximately 1 kg per cow per day (305 kg per lactation) less than seronegative cows However, in an Ontario study, milk production was reduced in seropositive herds with high abortion rates, but unaffected in seropositive herds with normal abortion rate histories. This suggests that variation in the effects of *N. caninum* on milk production may in part be explained by the abortion history of the herd. In the largest evaluation of the effect of *N. caninum* on milk production (26,059 lactations from 11,177 cows in 364 herds), an interaction was found between *N.*

caninum infection and lactation number with a statistically significant reduction in milk yield being observed only in 1st lactation animals. This effect (loss of 159.5 kg in a 305-day lactation -equivalent to a yearly loss) would represent 1.68% of the average yield observed in that study. Assuming that 30% of animals are in first lactation, this loss would equate to a 0.5% loss over the whole herd (with a SD of 0.2%). Consequently, this parameter was entered into the model as a normal distribution with $\mu = 0.005$ and $\sigma = 0.002$.

6.2.2.4 Premature culling and reduced slaughter value

Two studies have evaluated the impact of infection with *N. caninum* on culling. In a single California dairy herd with abortion problems associated with *N. caninum*, Thurmond and Hietela (1996) estimated the risk of culling during the first 3 lactations to be 35.8% for seropositive animals and 30.6% for seronegative animals – a risk difference of only 5.2% over 3 years[1]. In contrast, Tiwari *et al.* (2005) studied culling in 134 randomly selected Canadian herds with a follow-up period that ranged from 1.3 to 4 years. A total of 1981 of the 3531 cows were culled but the small increased risk in overall culling that was associated with *N. caninum* seropositivity was not statistically significant. However, there was a significant increased risk of culling for reproductive reasons in *N. caninum* seropositive cows (16.2% of cows were culled for reproductive reasons) with a hazard ratio of 1.44 (95% CI, 1.15 – 1.79). Interpreting the hazard ratio as a risk ratio, assuming an average follow-up period of 2 years and using the prevalence of *N. caninum* (11.9%) and the risk of culling for reproductive reasons (16.2%) from this study, the annual risk difference associated with *N. caninum* seropositivity was estimated to be 3.5% (SD = 1.2%). Consequently, this parameter was added to the model as a normal distribution with $\mu = 0.035$ and $\sigma = 0.012$.

6.2.2.5 Abortion losses

For *N. caninum*, the reproductive losses are mainly attributable to abortions. One study in California, found between 5 and 15% of pregnancies ended in abortions each year and about one-third of the abortions were caused by *N. caninum*[9]. A number of studies have reported abortion risks in *N. caninum* infected and non-infected animals. These are summarized in Table 6.2. Random effects meta-analysis methods were used to obtained weighted average estimates of the risk difference for cows in each of three age categorizations: nulliparous, parous and all parities combined. Estimates of between study variations were also determined. The effects (risk ratio and risk difference) of *N. caninum* on the risk of abortion were larger in nulliparous animals than parous. However, because the partial budget used in this study was at the herd level; values of all parities combined were used as input parameters. The risk difference values (point estimate = 0.18, and standard deviation = 0.1) were used to define a normal distribution of the increased risk of abortion in *N. caninum* seropositive animals.

Bennett *et al.*. (1999)[11] estimated the cost of dairy cow abortions as the reduction in milk yield associated with the abortion event and the value of the calf lost. The impact of *N. caninum* on milk production described in section 2.2.3 included losses attributable to abortions and to any losses that occurred in the absence of an abortion. Consequently, the cost associated with abortions in this section was restricted to the value of the calf and costs associated with treating the abortion. Abortion treatment costs (triangular distribution, min. = $0, max. = $100, most likely = $50 per head) were estimates derived from dairy clinicians familiar with current treatment costs. The value of the calf lost (triangular distribution, min. = $200, max. = $600, most likely = $400 per head) was estimated from current market values. Calculations of abortion losses in the partial budget were carried out on a per lactation basis. To convert this to an annual loss, an average calving interval of 13 months was assumed and the abortion losses per lactation were multiplied by 0.923 (12/13) to get annual abortion losses.

6.2.2.6 Stochastic simulation of partial budget model

A stochastic simulation model which combined the values presented in Table 6.1 into an overall estimate of the losses associated with *N. caninum* was developed using @RISK (2002) (Version 4.5.2, Palisade Corporation) with Latin hypercube sampling and 10,000 iterations for each analysis. The estimated distributions of total losses for the Canadian dairy industry and for infected dairy herds were determined and graphed. Mean and median values were determined, along with the range of values that encompassed 95% of the estimates. The losses for each of the three main components of the model were determined.

A sensitivity analysis of key parameters was carried out as follows. For each of the following parameters, analyses were repeated, assuming expected values that were either 10% lower or 10% higher than estimated: prevalence, reduction in milk yield (associated with infection), risk difference for culling, risk difference for abortion. For example, in the model evaluating overall effects in the Canadian dairy population, the mean prevalence was changed from 11.9 to 10.7 and 13.1% (the standard deviation of each of these distributions was left unchanged at 0.92%). For the model dealing with the range of losses across infected herds, the same parameters were modified. The impacts of each of these 10% changes on the estimates of loss were compared to determine which factors were most influential in the model.

6.3 Results

Tables 6.3 and 6.4 present the results from the simulation models for the Canadian dairy industry as a whole (losses per 100 cows -Table 6.3) and for individual herds of 61 cows (Table 6.4). For each value that varied in the model, regardless of whether it was an input parameter with a specified distribution or an output parameter, the mean value was presented, along with the minimum

and maximum observed values and the values that encompassed 95% of the observations. For example, the mean overall prevalence of *N. caninum* in the whole dairy population was assumed to be 11.9%, but this ranged from 8.4 to 15.9% in individual runs of the simulation.

For the Canadian dairy industry as a whole, the mean loss per 100 cows was $1,838 (95% confidence interval: $717 - $3,165) annually, or $18 per cow per year. Abortion costs were responsible for 48.3% of the total losses, with most of this loss being attributed to the loss of the value of the calf. Reduced milk production and premature culling accounted for 18.2% and 33.5% of the losses, respectively (Table 6.3 and Figure 6.1).

In the sensitivity analysis (Table 6.5), as expected, the estimates of loss were most sensitive to changes in the estimate of the overall prevalence of *N. caninum* in Canada, with a proportional reduction of 10% in the seroprevalence (e.g. 11.9 to 10.7%) reducing annual losses by 10.1%. The close connection between the seroprevalence estimate and the total annual losses was also noted in Figure 6.1, in which the distribution of total losses was very close to the shape of the distribution of prevalence estimates. Ten percent changes in the abortion risk difference, reduction in milk yield, and risk difference for culling changed the total annual losses by 5.0, 1.8, and 3.2%, respectively.

For infected dairy herds, the mean loss (per 61 cow) was $1,494, annually (95% confidence interval: $37 - $5,309) (Table 6.4 and Figure 6.2). As above, abortion losses accounted for most of the loss (48.5%), followed by premature culling costs (33.4%) and milk loss (18.2%) of the losses due to *N. caninum* infection. As expected, the estimate of the magnitude of the loss was most sensitive to the within-herd prevalence with the distribution of these losses (Figure 6.2) closely following the distribution of the within-herd prevalence.

In the sensitivity analysis for this estimation (losses for infected Canadian dairy herds with 61 cows), the total losses were once again most sensitive to the prevalence estimate, with a change of 11.0% in the annual losses following a change of 10% in this parameter. The close connection between

the prevalence estimate and the total annual losses was also noted in Figure 6.3 that was very close to the shape of the distribution of the within-herd prevalence estimates. Abortion risk difference, reduction in milk yield, and risk difference for culling changed the annual losses by 5.9, 4.3, and 3.6%, respectively, when these parameters were changed by 10% (Table 6.6).

6.4 Discussion

This paper refines the estimates of the effects of *N. caninum* seropositivity in Canadian dairy herds, from those based on a spreadsheet model[11] and applied in 3 Canadian maritime provinces for four diseases, with neosporosis being one of these four[10]. The four major updates in this study are: the prevalence estimates have been updated and are now based on data from 6 of 10 provinces; milk loss associated with subclinical infections have been added; the effects of *N. caninum* seropositivity on the risk of abortion and culling have been updated; and the model is now stochastic so that it takes into account uncertainty associated with many of the parameter estimates.

The prevalence was recalculated using data from the three original (maritime) provinces plus data from ALTA, MAN, and SASK. The overall prevalence estimate used (11.9%) was much lower than the 24% previously used. In addition, uncertainly about this estimate was included in the analysis by giving it a normal distribution instead of assuming it was a fixed value. The estimates of losses at both the national and herd levels were very sensitive to changes in seroprevalence and abortion risk difference.

The second parameter updated was the milk loss associated with *N. caninum* seropositivity. In the initial study, it was assumed that there was no reduction in milk production. While more recent evidence has shown that there is a loss of milk production in first lactation animals, this impact was relatively small and accounted for 18% of the total herd losses due to *N. caninum*.

The third parameter modified was the risk difference in culling attributable to *N. caninum* seropositivity. This parameter has now been estimated from a meta-analysis of all relevant published studies. The estimates of the risk difference varied widely across studies (from 5% to 36%), so this estimate was incorporated into the analyses as a distribution based on the estimated between-study standard deviation. The summarization of the abortion risks confirmed that *N. caninum* seropositivity had a greater impact (i.e. larger risk ratios or risk differences) on the risk of abortion in nulliparous animals compared to parous animals. However, there was wide variability in those estimates among studies. Clearly, this is an area that requires further work in large population-based studies.

The meta-analysis evaluation assumed that the between study variation had a normal distribution; therefore it was entered into the simulation model as such. However, with a large standard deviation, some of the iterations had a negative risk difference, which would suggest that *N. caninum* seropositive cows had a lower risk of aborting than *N. caninum* seronegative cows. While this is not likely, the distribution was not truncated at zero, as the possibility has not been ruled out. This negative risk difference was the main reason why some of the iterations produced negative estimates of total annual losses.

Other changes from the original study were related to items such as the value of animals and the costs of medications and veterinary visits. These items were updated to account for the effects of inflation and changed to reflect the current economic context of the dairy industry and production structure. They were included in the simulation model as triangular distributions to allow for some variability in those values.

In a California study[9], losses due to *N. caninum* abortions were estimated at US$35 million for the dairy industry in California. The California milk cow population is approximately 1.7 million (http://www.californiadairypressroom.com/pdfs/MilkFactSheet.pdf) compared with approximately 1.1 million in Canada (http://www.dairyinfo.gc.ca/pdf_files/tab1.pdf). Using the values from the California

study, and adjusting for the relative size of the dairy cow populations, we would expect Canadian losses to be approximately US$22.6 million – an estimate reasonably close to the observed estimate of Cdn$18.8 million ($17.67 per cow * 1.1 million cows).

Chi *et al.*. (2002)[10] estimated losses to be $2,305 per year per infected herd of 50 cows, based on a 24% within-herd prevalence of infection. At the herd level, our study estimated losses to be $3,220 for a 50 cow herd ($3,928 for a 61 cow herd). Although the within herd seroprevalence in infected herds was lower in the current national study, increased impacts (risk of abortion, milk loss and culling) have increased the estimate of losses.

6.5 Conclusions

For the Canadian dairy industry, annual production losses attributable to *N. caninum* seropositivity were estimated to be approximately $1,838 per 100 cows ($18 per cow with a 95% confidence interval of $7 to $32 per cow). For infected 61 cow herds, annual losses would be expected to range between $37 and $5,309, with a mean of $1,494. These update estimates of losses associated with *N. caninum* seropositivity demonstrate conservative estimate of the financial impact of *N. caninum* on the Canadian dairy industry, as they do not include considerations such as possible effects of *N. caninum* on human health, the ability of the farm to market livestock or other products, and other potential indirect costs.

6.6

Table 6.1: Variables Distributions, parameters and sources of data used in the stochastic simulation model of *N. caninum* seropositivity losses in Canadian dairy cattle.

	Variable	Distribution	Parameters	Source of data
1	Average cattle population in herd	Fixed	61 or 100	Dairy Farmers of Canada (2002)
2	Prevalence of infection in the Canadian dairy population	Normal	$\mu = 11.9\%$, $\sigma = 0.92\%$	Haddad 2005
2	Prevalence of infection in individual dairy herds	Beta	$\alpha=1.0004$, $\beta=5.33$	Haddad 2005
3	Milk yield (l per cow per year)	Fixed value	9519	CDHI
4	Milk price ($/l)	Fixed value	0.59	The Canadian Dairy Information Centre
5	Reduced milk yield (%)	Normal distribution	$\mu= 0.005$, $\sigma= 0.002$	Tiwari 2005
AM	**Milk loss ($)**	**(1)*(2)*(3)*(4)*(5)**		
6	Excess culling risk for infected cattle (%)	Normal distribution	$\mu = 0.0345$ and $\sigma = 0.0123$	Tiwari 2005
7	Replacement cost of cow ($ per head)	Triangular distribution	min=1500, most likely=2000, max=2500	Assigned by author
8	Slaughter value of healthy cattle ($ per head)	Triangular distribution	min=300, most likely=500, max=700	Assigned by author
AC	**Premature culling cost ($)**	**(1)*(2)*(6)*((7)-(8))**		
9	Value of newborn calf ($ per head)	Triangular distribution	min=200, most likely=400, max=600	Assigned by author
10	Veterinary visit and medication costs	Triangular distribution	min=0, most likely=50, max=100	Assigned by author
11	Losses per abortion (calf loss + treatment)	9 + 10		
12	Increased risk of abortion due to *N. caninum* (%)	Normal	$\mu= 0.18$, $\sigma= 0.1$	meta-analysis
LA	**Lactational Abortion losses**	**11*(1*2*12)**		
AA	**Annual Abortion losses**	**LA*(12/13)**		
TAL	**Total Annual Losses**	**AM + AC + AA**		

Table 6.2: Literature and meta-analysis summary. Estimates of the effects of *N. caninum* infection on the risk of abortion

Study	Country	Year	Number of Herds	Number of Animals	Prevalence of *N. caninum*	Risk Ratio	Risk Difference
All ages Combined							
Lopez-Gatius[20]	Spain	2004	1	164	0.232	4.78	0.35
Lopez-Gatius[21]	Spain	2003	6	2773	0.119	3.58	0.22
Vaclavek[22]	Czech Rep.	2003	5	407	0.108	4.78	0.36
Hernandez[13]	USA	2002	1	460	0.150	1.33	0.05
Thurmond[14]	USA	1997	1	1078	0.091	2.31	0.09
		meta-analysis – summary estimate				2.92	0.18
		meta-analysis – between study standard deviation				0.41 [a]	0.10
Nulliparous							
Lopez-Gatius[20]	Spain	2004	1	32	0.25	nc	0.50
Lopez-Gatius[21]	Spain	2003	6	751	0.06	10.17	0.26
Hernandez[13]	USA	2002	1	272	0.15	1.39	0.05
Thurmond[14]	USA	1997	1	468	0.05	7.05	0.11
		meta-analysis - summary estimate				4.60	0.19
		meta-analysis – between study standard deviation				1.12 [a]	0.11
Parous							
Lopez-Gatius[20]	Spain	2004	1	132	0.227	3.83	0.31
Lopez-Gatius[21]	Spain	2003	6	2022	0.141	2.87	0.20
Hernandez[13]	USA	2002	1	188	0.154	1.27	0.04
Thurmond[14]	USA	1997	1	610	0.120	1.75	0.08
		meta-analysis – summary estimate				2.33	0.15
		meta-analysis – between study standard deviation				0.09 [a]	0.01

[a] between study variance for RR is on the log scale (ln (RR))

Table 6.3: Estimates of parameters used in the simulation model and of losses attributable to *N. caninum* seropositivity for the entire Canadian industry (losses per 100 cows).

Var. #	Variable	Mean	Minimum	Maximum	95% Confidence Interval	
					Lower Limit	Upper Limit
1	Average cattle population in herd	100			Fixed effect	
2	Seroprevalence of infection in the Canadian dairy population (%)	11.9	8.4	15.9	10.1	13.7
3	Milk yield (l per cow per year)	9,519			Fixed effect	
4	Milk price ($/l)	0.59			Fixed effect	
5	Reduced milk yield (%)	0.50	-0.28	1,24	0.11	0.89
AM	**Annual Milk loss ($) (1*2*3*4*5)**	**334.28**	**-180.44**	**829.63**	**70.59**	**608.96**
6	Excess culling risk in infected cattle (%)	3.45	-1.17	8.33	1.04	5.86
7	Replacement cost of cow ($ per head)	2,000.00	1,501.31	2,495.36	1,611.62	2,388.06
8	Slaughter value of healthy cattle ($ per head)	500.00	302.59	698.18	344.68	655.23
AC	**Annual Premature culling cost ($) (1*2*6*(7-8))**	**616.24**	**-280.71**	**1,855.55**	**177.05**	**1,145.02**
9	Value of newborn calf ($ per head)	400.00	201.54	597.84	244.65	555.19
10	Veterinary visit and medication costs	50.00	0.52	99.30	11.16	88.80
11	Losses per abortion (calf loss + treatment)	450.00	211.73	683.79	291.10	609.82
12	Increased risk of abortion due to *N. caninum*(%)	18.00	-19.20	59.50	-1.60	37.58
LA	**Lactational Abortion losses**	**962.26**	**-1,358.95**	**3,579.02**	**-79.97**	**2,186.11**
AA	**Annual Abortion losses**	**888.24**	**-1,254.42**	**3,303.71**	**-73.82**	**2,017.95**
TAL	**Total Annual Losses**	**1,838.76**	**-270.08**	**4,350.16**	**717.43**	**3,165.27**

Figure 6.1: Distribution of total annual losses from Neospora Caninum seropositivity per 100 cows for the Canadian dairy industry as a whole.

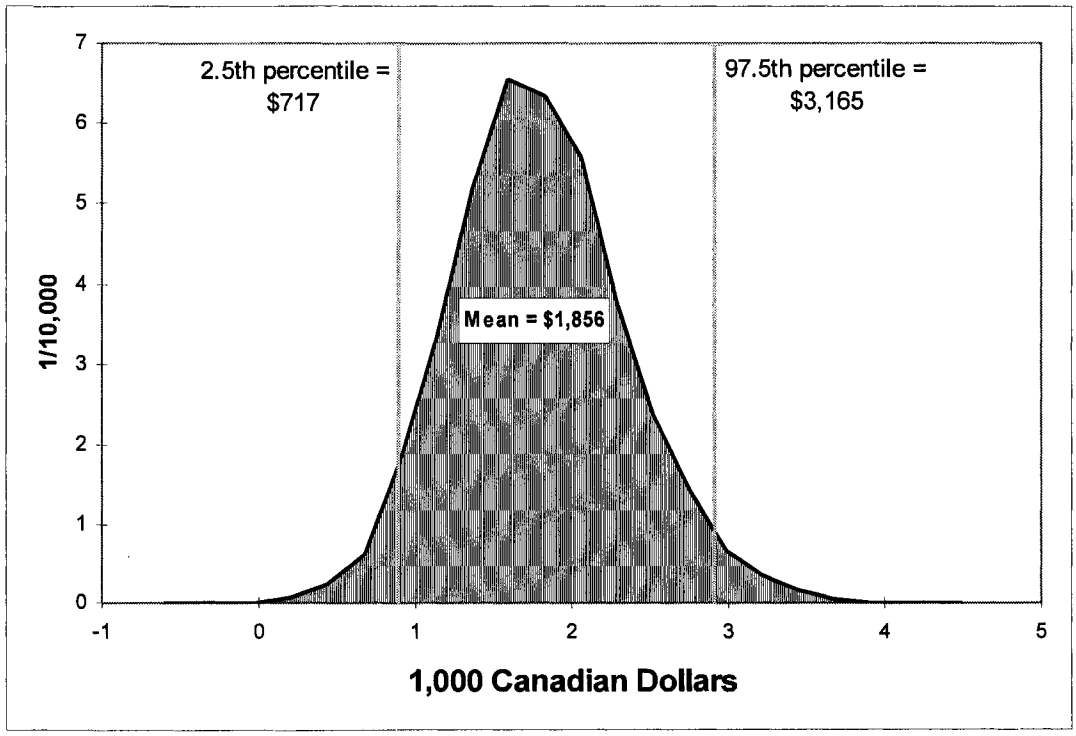


Table 6.4: Estimates of parameters used in the simulation model and of losses attributable to *N. caninum* seropositivity for infected Canadian dairy herds (losses per 61 cow herds).

Var. #	Variable	Mean	Minimum	Maximum	95% Confidence Interval	
					Lower Limit	Upper Limit
1	Average cattle population in herd	61			Fixed effect	
2	Seroprevalence of infection in infected dairy herds (%)	15.8	0.0	82.5	0.5	49.9
3	Milk yield (l per cow per year)	9,519			Fixed effect	
4	Milk price ($/l)	0.59			Fixed effect	
5	Reduced milk yield (%)	0.50	-0.27	1.34	0.11	0.89
AM	**Annual Milk loss ($) (1*2*3*4*5)**	**271.06**	**-241.51**	**2,737.32**	**4.53**	**1,004.42**
6	Excess culling risk in infected cattle (%)	3.45	-1.40	8.23	1.04	5.86
7	Replacement cost of cow ($ per head)	2,000.00	1,505.57	2,493.22	1,611.78	2,387.98
8	Slaughter value of healthy cattle ($ per head)	500.00	301.38	697.51	344.70	655.26
AC	**Annual Premature culling cost ($) (1*2*6*(7-8))**	**499.21**	**-350.51**	**4,581.67**	**10.18**	**1,799.14**
9	Value of newborn calf ($ per head)	400.00	200.83	597.28	244.65	555.27
10	Veterinary visit and medication costs	50.00	0.29	99.44	11.18	88.82
11	Losses per abortion (calf loss + treatment)	450.00	222.00	673.49	288.41	610.48
12	Increased risk of abortion due to *N. caninum*(%)	18.00	-22.67	56.65	-1.61	37.60
LA	**Lactational Abortion losses**	**784.450**	**-2,470.22**	**8,748.66**	**-30.40**	**3,378.48**
AA	**Annual Abortion losses**	**724.11**	**-2,280.20**	**8,075.69**	**-28.06**	**3,118.60**
TAL	**Total Annual Losses**	**1,494.38**	**-677.23**	**10,969.46**	**37.10**	**5,309.30**

Figure 6.2: Distribution of total annual losses from *Neospora Caninum* seropositivity per 61 cow infected dairy herd in Canada.

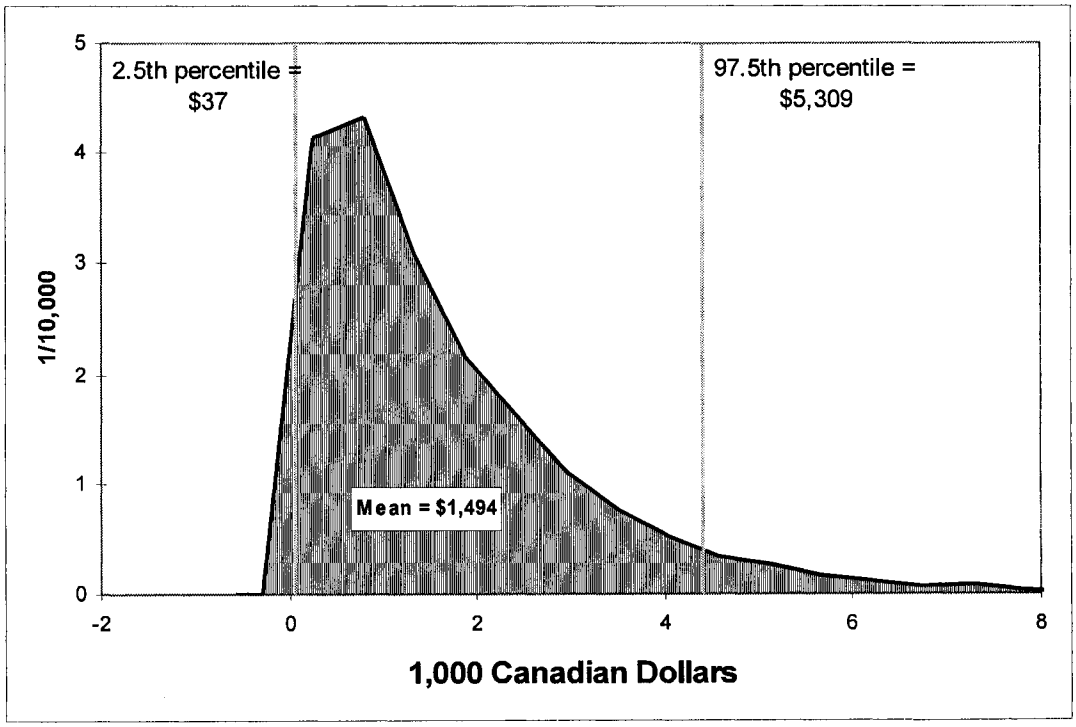


Table 6.5: Sensitivity analysis – estimates of the average loss from *N caninum* seropositivity for the entire Canadian industry (losses per 100 cows) associated with 10% changes in select input parameters.

Variable	*Original estimate*	Prevalence		Reduction milk yield		Risk difference culling		Risk difference abortion	
	s	-10%	+10%	-10%	+10%	-10%	+10%	-10%	+10%
Seroprevalence of infection in the Canadian dairy population (%)	11.9	*10.7*	*13.1*	11.9	11.9	11.9	11.9	11.9	11.9
Reduced milk yield (%)	0.5	0.5	0.5	*0.45*	*0.55*	0.5	0.5	0.5	0.5
Annual Milk loss ($)	334	300	368	301	368	334	334	334	334
Excess culling risk in infected cattle (%)	3.5	3.5	3.5	3.5	3.5	*3.1*	*3.8*	3.5	3.5
Annual Premature culling cost ($)	617	554	678	617	617	554	677	617	617
Increased risk of abortion due to *N. caninum* (%)	18.0	18.0	18.0	18.0	18.0	18.0	18.0	*16.2*	*19.8*
Lactacional Abortion losses ($)	962	867	1,061	962	962	962	962	868	1,060
Annual Abortion losses ($)	888	800	979	888	888	888	888	801	978
Total Annual Losses ($)	1,839	1,654	2,025	1,806	1,873	1,776	1,899	1,752	1,929

Table 6.6: Sensitivity analysis – estimates of the average loss from N. caninum seropositivity for Canadian dairy herd losses per 61 cow infected herd associated with 10% changes in select input parameters.

Variable	*Original estimate*	Prevalence		Reduction milk yield		Risk difference culling		Risk difference abortion	
	s	-10%	+10%	-10%	+10%	-10%	+10%	-10%	+10%
Reduced milk yield (%)	15.8	*14.2*	*17.4*	15.8	15.8	15.8	15.8	15.8	15.8
Reduced milk yield (%)	0.5	0.5	0.5	*0.45*	*0.55*	0.5	0.5	0.5	0.5
Annual Milk loss ($)	271	243	298	217	325	271	271	271	271
Excess culling risk in infected cattle (%)	3.5	3.5	3.5	3.5	3.5	*3.1*	*3.8*	3.5	3.5
Annual Premature culling cost ($)	499	455	557	506	506	448	549	506	506
Increased risk of abortion due to *N. caninum* (%)	18.0	18.0	18.0	18.0	18.0	18.0	18.0	*16.2*	*19.8*
Lactacional Abortion losses ($)	784	702	860	784	784	784	784	703	859
Annual Abortion losses ($)	724	648	794	724	724	724	724	649	793
Total Annual Losses ($)	1,494	1,346	1,649	1,447	1,555	1,443	1,544	1,426	1,570

7. Conclusions

The study was based on a stratified random sample survey of Canadian dairy cattle. A range of 30 to 66 herds (240 herds in total) in each of 6 of the 10 Canadian provinces were randomly selected from among all herds enrolled in a dairy herd improvement (DHI) program in province. Within each herd, approximately 30 cows were randomly sampled from the DHI herd list. Samples were tested for *N. caninum* using an indirect ELISA, and data on potential risk factors for *N. caninum* were collected through a questionnaire of management activities and farm characteristics. Subsequent to the testing, DHI data were downloaded for the evaluation of the effects of seropositivity for the 4 pathogens on reproductive performance. A stochastic partial budget model was used to refine estimates of *N. caninum* seropositivity in Canada dairy herds, using update data on seroprevalence, and losses due to reproductive failure, enhance culling and reduced milk production.

As with all studies, this study had its strengths and weaknesses. The fact, that the research was conducted in randomly selected herds from 6 provinces, helped ensure the representativeness of the results. Data were not available from all 10 provinces as a result of different protocols being used in some provinces, and others not taking part in the program at all. Among the 6 provinces included in this study, there were a few differences in the both the sampling and testing protocols across the provinces. In addition, not all data were obtained for the same time period as enrollment of provinces in the study took place over a period of 6 years.

One strength of the study was the ability to simultaneously investigate the effects of 4 infectious diseases and determine if there was any evidence of interaction among the diseases in their impacts on productivity. Being part of the large comprehensive research program also meant that it was possible to investigate many aspects of each disease within the framework of the single study.

A couple of specific weaknesses, which related to the *N. caninum* portion of this research program were the selection of the test used and the lack of abortion data. With regards to the test, it is now generally accepted that a competitive *N. caninum* ELISA has higher specificity than the indirect ELISA used in the project[1;2]. However, at the start of this project, the competitive ELISA was not available for routine use in laboratories in Canada. It is well known that one of the major impacts of *N. caninum* is to increase the risk of abortion in dairy cattle. This project did not have any ongoing data collection mechanisms in place within the study herds, so it was impossible to collect abortion data for individual study cows. As a consequence, prolonged calving intervals had to be used as a surrogate measure for abortions in individual cattle.

7.1 Epidemiology of *N. caninum*

The epidemiology of *N. caninum* is reviewed extensively in Chapter 2 of this thesis. The research from this thesis contributed some information to our understanding of the epidemiology of this parasite – particularly in the areas of risk factors for infection (discussed below), but the epidemiology of the disease (abortion due to neosporosis) was not the primary focus of this research.

There remain a number of unanswered questions about the epidemiology of *N. caninum*. Among these are the following.

- Most research to date has focused on cattle, as an intermediate host for *N. caninum*. It has been shown that in addition to dogs, coyotes can also serve as a definitive host for *N. caninum*[3]. The role of other wildlife species and other domestic species as reservoirs (definitive and intermediate hosts) of infection needs to be clarified. In particular, the role of birds (both wild and domestic) in the epidemiology of the disease needs to be studied. This research must include an evaluation of the prevalence of infection in a variety of species.
- This study provided the first evidence of spatial clustering of *N. caninum* and this work needs to

be expanded, with more detailed studies to investigate the role of wildlife species in this spatial clustering. The spatial distribution of the pathogen in both domestic dogs and wildlife will need to be part of this work.

- Research is needed to determine the frequency of recrudescence of shedding in infected dogs, and the circumstances under which this occurs (e.g. stress of pregnancy).
- The ability of *N. caninum* oocysts to survive in the environment, under various environmental conditions needs to be determined.
- Experimentally, it has been shown that transmission to uninfected calves through ingestion of colostrum with tachyzoites can occur, but how important this mode of transmission is under commercial production conditions is not known.

7.2 Prevalence and spatial distribution

Based on the results from this thesis, the overall Canadian cow level seroprevalence was 11.9% while the herd level seroprevalence was 81.9% (based on ≥1 positive in the herd) and 47.1% (based on ≥three positives or 10% of the sample positive test results in the herd). These overall prevalence estimates are similar to, but slightly lower than, estimates from a US study, which reported a cow level prevalence of 16% and a herd level prevalence of 90%[4]. Our study also demonstrated that there is variation in the seroprevalence across regions of the country, with the highest seroprevalence being observed in New Brunswick. Unfortunately, data were not available from Ontario and Quebec, very important dairy producing provinces.

N. caninum seroprevalence was spatially clustered in both the western and maritime Canadian provinces, occurring more commonly in Alberta in the western provinces and New Brunswick in the Maritimes. The fact that a model in which coyotes were included as a covariate produced the tightest

cluster in the maritimes provinces supports the hypothesis that these animals play a role in the transmission of *N. caninum* in this region. Barling *et al.*[5], in a Texas, US study, found a geographic association between the density of coyotes and foxes and the seroprevalence of *N. caninum* in beef cattle.

Future research will be required to document the prevalence of infection in areas not included in this study. The use of the more specific competitive ELISA (compared to the indirect ELISA) will reduce the number of false-positives in future studies. The relative lack of specificity of the indirect ELISA led us to use two different definitions for herd seropositivity. It also meant that the cow-level prevalence of infection might have been overestimated in this study. Future research related to the diagnostic tests for *N. caninum* is needed in the following areas.

- For both IFAT and ELISA, the optimum threshold for distinguishing between infected and non-infected animals in different stages of production (e.g. pregnant cow, neonate (either before or after consuming colostrum), non-pregnant cows) has yet to be established. This would be facilitated by standardization of testing protocols across laboratories.
- The potential of the avidity ELISA to distinguish between acute and chronic infections needs to be determined in a variety of populations, with a mixture of acute and chronic infections.

7.3 Impact on reproduction performance

Effects of *N. caninum* on productivity in dairy cows (e.g. milk loss, premature culling) are closely linked with the ability of the agent to induce abortions. Data on abortions were not recorded during this study, so prolonged calving to conception intervals (>200, >250 or >300 days) were used as surrogate indicators of abortion. Seropositive cows had a 1.27 times higher risk of exhibiting a CCI exceeding 200 days, a 1.37 times higher risk of a CCI exceeding 250 days, and a 1.54 times higher risk

of a CCI exceeding 300 days. Considering prolonged calving to conception intervals as a surrogate for abortion inevitably resulted in substantial misclassification bias, and under the assumption that this bias was non-differential, the estimates of the effect of *N. caninum* on abortion risk are substantially underestimated. The misclassification bias probably decreased as the cutpoint chosen increased (from 200 to 300), resulting in larger odds ratios. Other studies have shown that *N. caninum* infection increased the risk of abortion between 1.7 and 7.0 times[6; 7; 8; 9] which also supports the assumption that estimates from this study are underestimates.

It is well known that seropositive cows are more likely to abort than are seronegative cows, but infection alone is not a sufficient cause for abortion – many seropositive cows do not abort, while many seronegative do abort. Factors which result in seropositive cows aborting, or re-aborting in subsequent lactations, are not well understood.

Other reproductive outcomes which were evaluated were the calving to conception interval (CCI) and first service conception risk (FSC). No effect of *N. caninum* on CCI was observed in this study, and this was consistent with the findings of Bjorkman *et al.* (1996)[10] and Jensen *et al.* (1999)[11]. High peak milk yields were associated with longer CCI, as expected.

The model for FSC revealed a significant interaction between *N. caninum* and BVDV infections. *N. caninum* significantly reduced the probability of conception in BVDV-negative herds but not in BVDV-positive herds. The reason for this interaction is not known, nor is there an obvious explanation for why BVDV-positive herds had better FSC risks than BVDV-negative herds (no animal with a titre $\geq$1:64).

In general, with the exception of abortions (as measured by prolonged CCI), there was very little evidence of any other effects of *N. caninum* on reproductive performance, including services per conception, and number of cows requiring more than one service per pregnancy.

BLV seropositive cows had 7% lower odds of conception at first service compared to seronegative cows. BLV seropositive cows also had higher odds of having a CCI above 200 compared to seronegative cows, although an interaction with lactation number indicated that this effect was primarily seen in first lactation cows. Neither BVDV nor MAP showed any significant effect in any of the reproductive performance models.

Further research with actual abortion data will be required to investigate other factors that work in tandem with *N. caninum* to produce abortions in dairy cows. The possible interaction (antagonism) between herd BVD status and *N. caninum* seropositivity on the risk of abortion requires further investigation before any firm conclusions can be drawn about this effect.

7.4 Risk factors

Knowing the risk factors for a disease is essential for designing effective control programs. However, relatively few studies of risk factors for *N. caninum* infection have been published with more studies focusing on risk factors of abortions due to *N. caninum*. As a result, it was necessary in this study to investigate a wide range of factors, most of which were herd-level factors related to herd's environment and management, but some of which were cow-level factors. Given the large number of factors investigated, and the fact that some of them are being reported for the first time, it is important to treat these results as preliminary and requiring confirmation. Never the less, our results provide a valuable starting point for further investigations in this area. It must also be noted that any misclassification bias resulting from the use of an imperfect test for diagnosing *N. caninum* would probably have biased all of the observed risk factor associations toward the null, so estimates of measures of association presented may be underestimates of true values.

Some of the identified risk factors (e.g. the interaction between the presence of dogs on the farm and whether or not the dogs were allowed to eat aborted fetuses and/or placentas) were expected, based on our current understanding of the epidemiology of the disease. Some factors identified in other research projects, such as the apparently protective effects of farm cats and the observed association between *N. caninum* seropositivity and contact with rabbits, ducks, and poultry are interesting and support the previously mentioned need for investigations into the role of other animals and birds in the epidemiology of *N. caninum*. Some protective factors identified (e.g. "if farmer asks for BVDV-negative exam before introduction animals") were probably surrogate measures of overall biosecurity or disease control programs on the farm, while others could be useful components of *N. caninum* control programs. Some of the observed associations (e.g. apparent protective effects of nose-to-nose contact between heifers and calves") are likely spurious associations resulting from unidentified confounding effects. Future research will be required to confirm (or refute) the associations identified in this study. In particular, more detailed studies of the roles of other animals (both wild and domestic) must be undertaken to better quantify the risk that these potential reservoirs pose.

The impacts of concurrent infections with other pathogens on the risk of *N. caninum* were small or absent. BLV positivity did increase the odds of *N. caninum* positivity (odds ratio = 1.4, $p<0.05$), but the other measured agents (BVD and MAP) had no effect. Nor was there any evidence of effect of concurrent infections with other (unmeasured but evaluated in the questionnaire) agents, such as bovine herpesvirus 1, *Leptospira hardjo* or *Salmonella dublin*. This, if confirmed in future studies, is an important finding in terms of understanding the roles of those other agents when designing control programs for *N. caninum*.

7.5 Economic Impact

For the Canadian dairy industry, annual production losses attributable to *N. caninum* infection were estimated to be approximately $1,839 per 100 cows ($18 per cow with a 95% confidence interval of $7 to $32 per cow). For individual 61 cows infected herds (the mean size of Canadian dairy herds) annual losses would be expected to range between $37 and $5,309, with a mean of $1494. Approximately 50% of losses were attributed to losses associated with abortions from *N. caninum*. This economic evaluation confirms that control of *N. caninum* is a worthwhile objective for the dairy industry. Total losses from *N. caninum* would be higher than the above estimates because indirect effects, such as lost marketing opportunities, were not included in the estimates.

7.6 Overall conclusions

The research conducted for this thesis has confirmed that *N. caninum* is a substantial problem for the Canadian dairy industry. Although geographic differences were observed, the infection (as evidenced by seropositivity) was prevalent in all provinces studied. The overall cow- and herd-level prevalences were estimated to be 12 and 82%, respectively. The economic impacts of the disease were substantial, but closely linked to the occurrence of abortions. On average, it was estimated that *N. caninum* seropositivity is costing dairy producers $18 per cow per year. This study, in addition to confirming the roles of on-farm dogs as a risk factor, identified a number of other risk factors which are worthy of further investigation.

The study benefited from the fact that the data were derived from a random sample of farms on DHI within each of the 6 provinces studied. Being part of the PLDC research program meant that we were able to also investigate relationships between *N. caninum* and other pathogens (BVDV, BLV and

MAP). The quality and extent of the data enabled us to use more sophisticated statistical methods in the analysis of the data than have been used in many previous studies.

On the other hand, this study faced three major limitations. The first was that comparable data were not available from two major dairy producing regions of the country (Ontario and Quebec). Secondly, the research protocol did not provide for any ongoing on-farm data collection, so there were no data recorded about abortion events within the herd. Given the importance that abortions play in determining the economic impact of *N. caninum* within a herd, this was a substantial limitation. Finally, all of the diagnostic testing was carried out using an indirect ELISA, which has subsequently been shown to be inferior to the competitive ELISA for *N. caninum*. Despite these limitations, this study has contributed substantially to our understanding of *N. caninum* and Canadian dairy herds.

7.7 Literature cited

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Appendix A: Data Collection Farm - Risk factors for Neosporosis (and other pathogens) and their results

A. Location and identification

Name:	001
DHI number:	002
Quarter:	003
Section:	004
Township:	005
CSD:	006
County:	007
Range:	008
Meridian:	009
Postal code:	010

B. Farm and Farmer

Age (in years) of the primary person making day-to-day management decisions on the 011

farm: **Mean=41.49, Standard deviation(SD)=12.53**
province of the farm: 012

Alberta **(66)** New Brunswick **(27)** Ontario **(0)** Saskatchewan **(44)**

British Columbia **(0)** Newfoundland **(0)** Prince Edward Island **(28)**

Manitoba **(35)** Nova Scotia **(28)** Quebec **(0)**
Area of the farm (in Acres), both owned and rented, in the last summer: 013

Mean=1712.94, SD=3323.86
Area of pasture (grazing) (in Acres), both owned and rented, 014

in the last summer: **Mean=261.84, SD=722.86**
Area of forage production (in Acres), both owned and rented, 015

in the last summer: **Mean=405.04, SD=987.48**
Area of land used for other purposes (in Acres), both owned and rented, 016

in the last summer: **Mean=771.42, SD=2274.49**
Number of full-time employees, including family members, working directly in dairy 017

production: **Missing=5, Mean=2.34, SD=1.32**
Number of part-time employees, including family members, working directly in dairy 018

production: **Missing=2, Mean=1.56, SD=1.62**
Percentage of the total family income derived from dairy production: **Mean=72.98,** 019

SD=2274.49
Primary breed of your dairy cows (check one): 020

Holstein **(221)** Jersey **(3)** Ayrshire **(2)** Brown Swiss **(1)** Guernsey **(1)**

Shorthorn **(0)**

Other **(0)**

C. Herd population

Please, fill in the table below (use an estimate, if exact numbers are unavailable)

In side each cell: **Mean** **(SD)** **Missing**	Pre-weaned calves	Open heifers	Bred heifers	Milk cows	Dry cows	Bulls
Number of animals on day of blood sampling	021 **12.37** **(10.92)** **4**	022 **35.11** **(17.05)** **5**	023 **23.67** **(19.59)** **5**	024 **67.58** **(47.50)** **5**	025 **13.85** **(12.17)** **5**	026 **1.59** **(6.17)** **7**
Number of animals sold for dairy purposes in the last 12 months	027 **0.59** **(4.60)** **4**	028 **1.10** **(2.80)** **4**	029 **2.80** **(5.41)** **4**	030 **2.70** **(6.41)** **4**	031 **0.39** **(1.94)** **4**	032 **1.04** **(7.66)** **3**
Number of animals culled in the last 12 months	033 **2.08** **(9.47)** **3**	034 **1.22** **(8.70)** **3**	035 **0.14** **(0.60)**	036 **16.22** **(17.93)** **3**	037 **1.40** **(3.97)** **3**	038 **1.04** **(7.66)** **3**
Number of animals died in the last 12 months	039 **3.61** **(6.80)** **3**	040 **0.38** **(0.88)** **3**	041 **0.18** **(0.69)** **4**	042 **4.13** **(5.82)** **2**	043 **0.22** **(0.74)** **4**	044 **0.03** **(0.41)** **5**
Number of animals purchased in the last 12 months	045 **0.36** **(1.95)** **3**	046 **0.29** **(1.61)** **3**	047 **0.82** **(3.39)** **4**	048 **3.29** **(10.72)** **3**	049 **0.23** **(1.39)** **4**	050 **0.32** **(1.03)** **5**

How many of the cows (milking and dry) were raised on your farm: 051

Missing=14, Mean=66.26, SD=53.56

How many of the cows (milking and dry) are registered: 052

Missing=15, Mean=58.64, SD=55.02

D. Housing

Pre-weaned calves housing. Please, check all that apply for each season:

Barn type	Winter	Summer
Group pens	053 **No=136, Yes=92**	054 **No=137, Yes=91**
Individual pens	055 **No=106, Yes=122**	056 **No=111, Yes=117**
Hutches	057 **No=158, Yes=70**	058 **No=159, Yes=69**

Winter housing. Please, check all that apply for each group of animals on your farm:

Barn type	Open heifers	Bred heifers	Milk cows	Dry cows	Bulls
Tie-stall or Stanchion	059 **No=203, Yes=25**	060 **No=199, Yes=29**	061 **No=132, Yes=96**	062 **No=177, Yes=51**	063 **No=156, Yes=10**
Freestall	064 **No=195, Yes=33**	065 **No=189, Yes=39**	066 **No=102, Yes=126**	067 **No=176, Yes=52**	068 **No=146, Yes=20**
Loose housing	069 **No=60, Yes=168**	070 **No=73, Yes=155**	071 **No=211, Yes=17**	072 **No=101, Yes=127**	073 **No=99, Yes=67**

OBS: 063, 068, and 073 had 62 NA herds (without bulls).

Summer housing. Please, check all that apply for card group of animals on your farm:

	Open heifers	Bred heifers	Milk cows	Dry cows	Bulls
Totally confined (in barn) 24 hrs/day	0 74 **No=192, Yes=36**	075 **No=212, Yes=16**	076 **No=159, Yes=69**	077 **No=212, Yes=16**	078 **No=143, Yes=22**
Spent some time grazing and met some of their nutritional requirement from pasture	079 **No=100, Yes=128**	080 **No=67, Yes=161**	081 **No=140, Yes=88**	082 **No=80, Yes=148**	083 **No=128, Yes=37**
Given access to a concrete or dirt (non-turf) surface exercise yard (outdoor) some time each day	084 **No=167, Yes=61**	085 **No=173, Yes=55**	086 **No=175, Yes=53**	087 **No=169, Yes=59**	088 **No=132, Yes=33**
Given access to a small field for the propose of exercise (not primarily for grazing)	089 **No=166, Yes=62**	090 **No=172, Yes=56**	091 **No=166, Yes=62**	092 **No=164, Yes=64**	093 **No=142, Yes=23**

OBS: 078, 083, 088, and 093 had 62 NA herds (without bulls) and 1 missing value.

If your heifers (open/bred) have access to pasture then answer the rest of the questions in this section

How did you manage the pastures that were used by heifers in the most recent grazing season: 094

Not Applicable(NA)=31, Missing=29

continuous grazing (continuous access to the same pasture for the whole pasture season) **(118)**

controlled access grazing (rotational or strip grazing) **(50)**

Was any cattle manure mechanically spread on pastures that were used for grazing by heifers? 095

NA=22, Missing=2, No=153, Yes=51

Were these pastures dragged or harrowed this year? **NA=22, Missing=9, No=157, Yes=40** 096

Were these pastures clipped this year? **NA=22, Missing=10, No=139, Yes=57** 097

Have you used lime on heifer pastures for reducing soil acidity during the last 5 years?

NA=20, No=175, Yes=33 098

If YES, how often do the pasture fields receive lime? **NA=195** 099

every year **(0)** every 2 - 3 years **(7)** every 4 - 5 year **(17)**

every 6 –10 years **(12)** never **(2)**

OBS: This question (099) was discharged due to consistence problems.

E. Biosecurity – Purchase

Has the farm purchased any dairy animals in the last 5 years? **No=58, Yes=170** 100

If Yes,

Percentage of dairy animals purchased directly from other producers: 101

NA=58, Mean=39.82, SD=46.59

Percentage of dairy animals purchased from private dealers: 102

NA=58, Mean=12.40, SD=32.23

Percentage of dairy animals purchased through an auction: 103

NA=58, Mean=15.46, SD=34.61

When animals are transported to your farm, do you only use your own trailer? 104

NA=58, No=88, Yes=82

If YES, does other use your trailer to transport cows? 105

NA=146, Missing=1, No=55, Yes=26

Before bringing cattle (either beef or dairy) on your farm, the farm normally requires:

a negative test for BVDV from animal	**NA=58, No=88, Yes=82**	106
a negative test for Leukosis from animal	**NA=58, No=157, Yes=13**	107
a negative test for Neosporosis from the animal	**NA=58, No=164, Yes=6**	108
a negative test for Johne's disease from the animal	**NA=58, No=167, Yes=3**	109
a negative HERD test for Johne's disease	**NA=58, No=170, Yes=0**	110
a negative HERD HISTORY for Johne's disease	**NA=58, No=167, Yes=3**	111
a low somatic cell count from the cow	**NA=58, No=68, Yes=102**	112
a low bulk tank somatic cell count for the herd	**NA=58, No=117, Yes=53**	113

F. Biosecurity – Contact

Please, fill in the table below to describe contact between your dairy animals and other animal species that are **on your farm**:

	Numbers on farm	Direct animal contact with dairy cattle 0No /1Yes	Contact with feed for dairy animals 0No /1Yes	Contact with water for dairy animals 0No /1Yes
Beef cattle	114 **Mean=62.21** **SD=188.83**	115 **NA=124** **No=36** **Yes=68**	116 **NA=124** **No=51** **Yes=53**	117 **NA=124** **No=41** **Yes=63**
Sheep	118 **Mean=20.90** **SD=122.73**	119 **NA=211** **No=13** **Yes=4**	120 **NA=211** **No=15** **Yes=2**	121 **NA=211** **No=16** **Yes=1**
Goats	122 **Mean=0.13** **SD=0.77**	123 **NA=218** **No=7** **Yes=3**	124 **NA=218** **No=9** **Yes=1**	125 **NA=218** **No=8** **Yes=2**
Chicken or other poultry	126 **Mean=1134.77** **SD=4159.62**	127 **NA=155** **No=69** **Yes=4**	128 **NA=155** **No=72** **Yes=1**	129 **NA=155** **No=71** **Yes=2**
Horses and other equines **02 herds missing values**	130 **Mean=2.29** **SD=13.87**	131 **NA=154** **No=39** **Yes=33**	132 **NA=154** **No=58** **Yes=14**	133 **NA=154** **No=45** **Yes=27**
Pigs	134 **Mean=458.85** **SD=2482.48**	135 **NA=192** **No=36** **Yes=0**	136 **NA=192** **No=35** **Yes=1**	137 **NA=192** **No=36** **Yes=0**
Deer or elk	138 **Mean=0.52** **SD=7.02**	139 **NA=223** **No=5** **Yes=0**	140 **NA=223** **No=5** **Yes=0**	141 **NA=223** **No=5** **Yes=0**
Exotic ruminants (alpacas, llamas)	142 **Mean=0.02** **SD=0.21**	143 **NA=221** **No=6** **Yes=1**	144 **NA=221** **No=6** **Yes=1**	145 **NA=221** **No=7** **Yes=0**
Domestic rabbits **01 herd missing values**	146 **Mean=0.36** **SD=1.88**	147 **NA=204** **No=20** **Yes=3**	148 **NA=204** **No=20** **Yes=3**	149 **NA=204** **No=22** **Yes=1**

114 to 149

In the past 5 years have any of your dairy cattle had contact with cattle (dairy or beef) from other herds through any of the following routes:

- shared pasture (e.g.: community pasture) **No=196, Yes=32** 168
- contract raising of young stock **No=208, Yes=20** 169
- fence line contact while on pasture **No=150, Yes=78** 170
- contact at fairs/exhibitions **No=171, Yes=57** 171
- lending cows or bulls **No=198, Yes=30** 172
- borrowing cows or bulls **Missing=1, No=190, Yes=37** 173

Please, fill in the table below to describe the dogs and cats that live on your farm:

	Number of males	Number of spayed females	Number of not spayed females	Number of litters in the last 12 months	Usual Birthing locations
Dogs	151 **Missing=7 Mean=0.74 SD=1.10**	152 **Missing=5 Mean=0.40 SD=0.64**	153 **Missing=2 Mean=0.37 SD=0.67**	154 **Missing=31 Mean=0.10 SD=0.38**	155 **Missing=31 NA=186 1=1, 2=1, 3=2, 4=6**
Cats	156 **Missing=3 Mean=4.02 SD=3.29**	157 **Missing=5 Mean=0.69 SD=2.41**	158 **Missing=3 Mean=5.36 SD=4.89**	159 **Missing=18 Mean=3.86 SD=4.37**	160 **Missing=72 NA=38 1=51, 2=45, 3=1, 4=21**

(approximation)

Codes For ususal birthing location:

1-dairy barn 2-feedstorage areas 3- house 4- other

Compared with the previous years, has the number of litters of dogs in the last 12 months: 160

Missing=11, NA=62

increased **(6)** decreased **(15)** continued to be the same **(134)**

Compared with previous years, has the number of litters of cats in the last 12 months : 161

Missing=8, NA=10

increased **(20)** decreased **(37)** continued to be the same **(153)**

If there are NO dogs on the farm, how long ago (years) did one reside on the farm? 162

NA=172, Missing=22, Mean=6.82, SD=7.41

In the last 12 months how often have the following animals been seen on the farm?

	Never	1 – 3 times/year	4 – 6 times/year	More than 6 times/year
Coyotes/wolves	**36**	**61**	**21**	**110**
Foxes	**62**	**100**	**19**	**47**
Other dogs	**90**	**78**	**15**	**45**
Stray cats **Missing=1**	**47**	**99**	**31**	**48**
Raccoons **Missing=1**	**137**	**46**	**12**	**32**
Skunk **Missing=45**	**45**	**72**	**22**	**44**

OBS: Skunk variable was not included in Saskatchewan questionnaires.

Does the farm use a footbath for disinfecting visitor's boots before entering the cow and /or heifer barns? **No=211, Yes=17** 174

If YES, how many times is the disinfectant changed each month? 175

NA=211, Missing=7, 1=3, 2=4, 4=2, 8=1

During the past 12 months, give the number of times each category of people **actually entered your barn** and whether you felt their vehicles/equipment was properly cleaned.

1176
to
1199

	Number of times	Vehicles or equipment cleaned
other dairy farmers	176 **Missing=2,** **Mean=16.81,** **SD=34.87**	177 **Missing=2** **NA=16** **No=88** **Yes=122**
other beef farmers	178 **Missing=4** **Mean=12.69** **SD=43.85**	179 **Missing=1** **NA=77** **No=70** **Yes=80**
cattle dealers	180 **Missing=4** **Mean=11.45** **SD=19.57**	181 **Missing=1** **NA=75** **No=71** **Yes=81**
AI technicians + sales reps	182 **Missing=2** **Mean=30.26** **SD=45.85**	183 **Missing=24** **NA=2** **No=50** **Yes=153**
veterinarians	184 **Missing=0** **Mean=16.75** **SD=9.51**	185 **Missing=1** **NA=2** **No=30** **Yes=195**
nutrition technicians/advisors + sales reps.	186 **Missing=2** **Mean=11.12** **SD=13.35**	187 **Missing=1** **NA=26** **No=48** **Yes=153**
udder health advisers	188 **Missing=6** **Mean=0.43** **SD=1.66**	189 **Missing=0** **NA=187** **No=12** **Yes=29**
hoof trimmers	190 **Missing=2** **Mean=2.46** **SD=4.11**	191 **Missing=0** **NA=48** **No=49** **Yes=131**
dead stock collection	192 **Missing=3** **Mean=3.50** **SD=6.61**	193 **Missing=2** **NA=127** **No=61** **Yes=38**

During the past 12 months, did you borrow equipment from other farmers that could have manure contact (e.g. foot trimming chute, manure spreader, tractor, and cattle trailer)? 200

No=150, Yes=78

If YES, did you always disinfect it before using it? **NA=150, No=72, Yes=6** 201

During the past year, did you lend equipment to other farmers that could have manure contact? **No=143, Yes=85** 202

If YES, did you always disinfect it before using it again? 203

NA=143, Missing=1, No=76, Yes=8

G. Biosecurity - Transmission of disease through blood.

Do you use a new needle for every injection? **No=158, Yes=70** 204
If NOT, do you use a disinfected needle for every injection? 205

NA=70, Missing=3, No=104, Yes=518
Do you use new syringe for every injection? **No=219, Yes=9** 206
If NOT, do you use a disinfected syringe for every injection? 207

NA=9, Missing=4, No=145, Yes=70
Usual method of dehorning: **Missing=73** 208

paste **(13)** cutting (gougers, wire, etc) **(42)** burning (electric, butane, etc) **(100)**
If you use cutting equipment for dehorning, do you disinfect them between animals? 209

NA=56, No=133, Yes=39
Are the instruments used for extra teat removal disinfected between animals? 210

NA=47, No=86, Yes=95
Do people who artificially inseminate cows/heifers on your farm change rectal gloves 211

between animals? **NA=6, No=40, Yes=182**
Do people who do other rectal exams (e.g. pregnancy check) change rectal gloves between 212

animals? **NA=3, No=192, Yes=33**
Estimate the level of rodent infestation on your farm: **Missing=3** 213

low **(177)** medium **(44)** high **(4)**
What is the primary method you use for insect control? **Missing=100** 214

spray **(76)** bait **(6)** adhesive tape **(35)** other **(4)** none **(7)**
Is the equipment used for hoof trimming disinfected between animals? **Missing=6, No=206,** 215

Yes=16

H. Biosecurity - Vaccination and medication practices

Do you use coccidiostats/ionophores in calves/heifers/cows? **No=70, Yes=158** 216

If YES, please fill in the table below(check that apply): 217 to 229

	Decoquinate in feed (Deccox)	Lasalocid in feed (Bovatec)	Monensin in feed (Rumensin) premix	Monensin in bolus (Rumensin CRC)
Pre-weaned calves	217 **Missing=2** **NA=70** **No=65** **Yes=91**	218 **Missing=2** **NA=70** **No=150** **Yes=6**	219 **Missing=2** **NA=70** **No=121** **Yes=1**	
heifers	220 **Missing=1** **NA=70** **No=133** **Yes=24**	221 **Missing=1** **NA=70** **No=154** **Yes=3**	222 **Missing=1** **NA=70** **No=71** **Yes=86**	223 **Missing=1** **NA=70** **No=156** **Yes=1**
dry cows		224 **Missing=1** **NA=70** **No155** **Yes=2**	225 **Missing=1** **NA=70** **No98** **Yes=59**	226 **Missing=1** **NA=70** **No=144** **Yes=13**
milk cows		227 **Missing=1** **NA=70** **No=155** **Yes=2**	228 **Missing=1** **NA=70** **No79** **Yes=78**	229 **Missing=1** **NA=70** **No=145** **Yes=12**

Did you vaccinate any dairy animals on your farm for any disease in the last 12 months? 230

No=44, Yes=184

Did you vaccinate any dairy animals on your farm for BVD in the last 12 months? 231

NA=44, Missing=1, No=18, Yes=165

If yes, in their 1st year of vaccination, are animals boostered 2-4 weeks after their 1st shot? 232

NA=62, Missing=1, No=41, Yes=124

If YES, are these 2 injections given after the animals are 6 months of age? 233

NA=103, Missing=1, No=25, Yes=99

If you think you vaccinated your cows with BVD vaccine, indicate the vaccines you usually use in each group of animals in the table below (Check all that apply):

Vaccine Name	Cows	Heifers (+6 mo.)	Calves
Barvac 3, Barvac 3-BRSV, Barvac 3-Somnugen, Barvac 3-Somnugen-BRSV	234 **Missing=9** **NA=62** **No=153** **Yes=4**	235 **Missing=8** **NA=62** **No=153** **Yes=5**	236 **Missing=8** **NA=62** **No=153** **Yes=5**
Bovshield 3, Bovshield 4, Bovshield 4+L5	237 **Missing=8** **NA=62** **No=147** **Yes=11**	237 **Missing=8** **NA=62** **No=142** **Yes=16**	239 **Missing=8** **NA=62** **No=148** **Yes=10**
Breed back 9/Sumnagen	240 **Missing=8** **NA=62** **No=158** **Yes=0**	241 **Missing=8** **NA=62** **No=156** **Yes=2**	242 **Missing=8** **NA=62** **No=157** **Yes=1**
BRSV Vac 4, BRSV Vac 9	243 **Missing=8** **NA=62** **No=158** **Yes=0**	244 **Missing=8** **NA=62** **No=158** **Yes=0**	245 **Missing=8** **NA=62** **No=158** **Yes=0**
Cattlemaster BVD-K, Cattlemaster3, Cattlemaster 4, Cattlemaster 4+L5, Cattlemaster 4+VL5	246 **Missing=8** **NA=62** **No=136** **Yes=22**	247 **Missing=8** **NA=62** **No=136** **Yes=22**	248 **Missing=8** **NA=62** **No=142** **Yes=16**
Experess 5, Express 5 Somnugen, Express 10, Express 10 Somnugen	249 **Missing=37** **NA=62** **No=123** **Yes=6**	250 **Missing=37** **NA=62** **No=119** **Yes=10**	251 **Missing=37** **NA=62** **No=122** **Yes=7**
Herd-vac 3	252 **Missing=8** **NA=62** **No=158** **Yes=0**	253 **Missing=8** **NA=62** **No=158** **Yes=0**	254 **Missing=8** **NA=62** **No=158** **Yes=0**
Horizon 1+vac3, Horizon 4, Horizon 9	255 **Missing=8** **NA=62** **No=157** **Yes=1**	256 **Missing=8** **NA=62** **No=157** **Yes=1**	257 **Missing=8** **NA=62** **No=158** **Yes=0**

IBR Plus 4	258 **Missing=9** **NA=62** **No=153** **Yes=4**	259 **Missing=8** **NA=62** **No=154** **Yes=4**	260 **Missing=8** **NA=62** **No=155** **Yes=3**
Journey 4	261 **Missing=8** **NA=62** **No=158** **Yes=0**	262 **Missing=8** **NA=62** **No=158** **Yes=0**	263 **Missing=8** **NA=62** **No=158** **Yes=0**
Preg-guard 9	264 **Missing=8** **NA=62** **No=158** **Yes=0**	265 **Missing=8** **NA=62** **No=158** **Yes=0**	266 **Missing=8** **NA=62** **No=158** **Yes=0**
Prism 4	267 **Missing=3** **7** **NA=62** **No=129** **Yes=0**	268 **Missing=37** **NA=62** **No=129** **Yes=0**	269 **Missing=37** **NA=62** **No=129** **Yes=0**
Pyramid MVL3, Pyramid MVL4, Pyramid 4+presponse, Pyramid 9	270 **Missing=8** **NA=62** **No=153** **Yes=5**	271 **Missing=8** **NA=62** **No=147** **Yes=11**	272 **Missing=8** **NA=62** **No=150** **Yes=8**
Reliant 3, Reliant 4, Reliant 8	273 **Missing=8** **NA=62** **No=158** **Yes=0**	274 **Missing=8** **NA=62** **No=158** **Yes=0**	275 **Missing=8** **NA=62** **No=158** **Yes=0**
Respishield 4, Respishield 4L5	276 **Missing=8** **NA=62** **No=154** **Yes=4**	277 **Missing=8** **NA=62** **No=156** **Yes=2**	278 **Missing=8** **NA=62** **No=158** **Yes=0**
Resvac 3/Somnuvac, Resvac 4/Somnuvac	279 **Missing=8** **NA=62** **No=158** **Yes=0**	280 **Missing=8** **NA=62** **No=158** **Yes=0**	281 **Missing=8** **NA=62** **No=157** **Yes=1**
Sentry 4, Sentry 4/Somnugen, Sentry 9, Sentry 9/Somnugen	282 **Missing=8** **NA=62** **No=131** **Yes=27**	283 **Missing=8** **NA=62** **No=136** **Yes=22**	284 **Missing=8** **NA=62** **No=150** **Yes=8**

Starvac 3 plus, Starvac 4 plus	285 **Missing=37 NA=62 No=129 Yes=0**	286 **Missing=37 NA=62 No=127 Yes=2**	287 **Missing=37 NA=62 No=129 Yes=0**
Triangle 1, Triangle 3, Triangle 4, Triangle 4+HS, Triangle 8, Triangle 9 (OR ANY OF THESE WITH TYPE II BVD)	288 **Missing=9 NA=62 No=87 Yes=70**	289 **Missing=8 NA=62 No=101 Yes=57**	290 **Missing=8 NA=62 No=130 Yes=28**
Virabos 3, Virabos 4, Virabos 4+H. Somnus, Virabos 4 + VL5	291 **Missing=8 NA=62 No=147 Yes=11**	292 **Missing=9 NA=62 No=150 Yes=7**	293 **Missing=8 NA=62 No=155 Yes=3**
OTHER	294 **Missing=36 NA=62 No=123 Yes=7**	295 **Missing=36 NA=62 No=122 Yes=8**	296 **Missing=37 NA=62 No=119 Yes=10**

I. Calving and calf management

What is the usual amount of time after which your newborn heifer dairy calves are usually separated from their mothers (in hours)? **Missing=6, Mean=8.75, SD=9.40** 297

What percentage of heifer calves born on the farm remained with their dams for more than 24 hours? **Missing=1, Mean=4.58, SD=18.20** 298

What is the percentage of your newborn heifer dairy calves suckle their dam? **Missing=4, Mean=34.38, SD=36.88** 299

Are teats usually washed before the newborn heifer dairy calves nurse? **NA=19, Missing=48, No=153, Yes=8** 300

Are teats usually washed before colostrum is collected? **NA=1, Missing=44, No=24, Yes=159** 301

What percentage of your newborn heifer dairy calves receive colostrum :

only from their mother **Missing=1, Mean=85.97, SD=28.81** 302

pooled from all cows **Missing=1, Mean=11.96, SD=26.50** 303

pooled from BLV negative cows **Missing=1, Mean=1.24, SD=10.04** 304
pooled from Johne's disease negative cows **Missing=1, Mean=0.67, SD=7.41** 305
What percentage of your newborn heifer dairy claves receive: 306

fresh colostrums **Mean=92.30, SD=19.54**
frozen colostrum **Mean=6.47, SD=16.41** 307
fermented colostrum **Mean=0.04, SD=0.47** 308
heat treated colostrums **Mean=0.44, SD=6.62** 309
With regard to the primary source of milk given to calves, what percentage of milk fed to 310

your heifer dairy calves is:

milk replacer **Missing=1, Mean=23.00, SD=36.27**
pooled milk from all cows **Missing=1, Mean=51.34, SD=40.57** 311
pooled milk from negative for BLV cows **Missing=1, Mean=0.86, SD=7.97** 312
pooled from negative for Johne's disease cows **Missing=1, Mean=0.42, SD=4.45** 313
milk from mastitic (clinic or high SCC)cows or with antibiotic residue 314

Missing=1, Mean=23.55, SD=30.62
Was the calving area used as a hospital area for sick cows in the last 12 months? 315

Missing=2, No=108, Yes=118
Type of bedding used in calving areas. **Missing=84** 316

straw **(128)** shavings/sawdust **(13)** other **(2)** none **(1)**
For calving indoor, if it is outdoor use code -999: 317

Frequency of adding bedding to calving areas: **Missing=5**

each calving **(175)** every 2-4 calvings **(38)** every 5 or more calvings **(10)**
Frequency of removing surface manure from calving areas: **Missing=25** 318

each calving **(95)** every 2-4 calvings **(39)** every 5 or more calvings **(69)**
Frequency of removing ALL manure from calving areas: **Missing=5** 319

each calving **(56)** every 2-4 calving **(47)** every 5 or more **(120)**
After separation from the mother, but before weaning, do dairy heifer calves have physical 320

contact (nose to nose) with other pre-weaned calves? **No=80, Yes=148**
After separation from the mother, but before weaning, do dairy heifer calves have physical 321

contact (nose to nose) with heifers? **No=180, Yes=48**
After separation from the mother, but before weaning, do dairy heifer calves have physical 322

contact (nose to nose) with adult cows? **No=208, yes=20**
What percentages of pre-weaned dairy heifers calves are uniquely identified (e.g. ear 323

tags)?

Mean=98.44, SD= 11.87
Primary location of calving in the summer: **Missing=21** 324

freestall **(2)** tie-stall/stanchion **(11)** loose housing **(35)**

maternity pen **(101)** pasture **(58)**
Primary Location of calving in the winter: **Missing=13** 325

freestall **(6)**

tie-stall/stanchion **(24)**

loose housing **(36)**

maternity pen **(149)**
If maternity pens are used, what is the usual number of cows in the pens at one time? 326

Missing=36

always just a single cow in pen **(124)**

sometimes multiple cows in the pen **(68)**
If multiple cows are in the calving pen at a time, what is the percentage of calvings 327

when multiple cows present:

NA=132, Missing=21, Mean=31.14, SD=35.16
Percentage of placentas partially or fully eaten by: 328

Dogs	[328] **Missing=15** Never **(84)** Sometimes **(88)** often **(41)**	to
Cats	[329] **Missing=13** Never **(92)** Sometimes **(76)** often **(47)**	
Cows	[330] **Missing=5** Never **(24)** Sometimes **(168)** often **(31)**	331
Wild animals	[331] **Missing=17** Never **(115)** Sometimes **(44)** often **(52)**	

Percentage of aborted fetuses partially or fully eaten by: 332

Dogs [332] **Missing=27** Never **(123)** Sometimes **(24)** often **(54)** to
Cats [333] **Missing=25** Never **(131)** Sometimes **(18)** often **(54)**
Wild animals [334] **Missing=24** Never **(105)** Sometimes **(48)** often **(51)** 334

Percentage of cows bred using artificial insemination: 335

Missing=1, Mean=85.32, SD=30.52

Do you use embryo transfer on your farm? **Missing=1, No=179, Yes=48** 336

If YES, number of embryos purchased outside the herd and implanted in the last 12 337 months:

NA=179, Missing=5, Mean=4.32, SD=15.09

If YES, number of embryos collected on farm and implanted in the last 12 months: 338

NA=179, Missing=5, Mean=14.18, SD=16.91

J. Feed, Water and Manure

Do you feed a TMR? **No=97, yes=131** 339

Do you feed greenchop? **No=196, yes=32** 340

How do you store your silage? **Missing=90** 341

tower silo **(34)** bunker silo **(34)** plastic bags/wrap **(64)** none **(6)**

Do dogs, cats or wildlife have access to stored grain? **No=174, yes=54** 342

Do you have an outdoor feed bunk or manger built for heifers? **Missing=1, No=71, yes=156** 343

Do you have an outdoor feed bunk or manger built for milk cows? **Missing=7, No=153, yes=68** 344

Do you have an outdoor feed bunk or manger built for dry cows? **Missing=1, No=82, yes=145** 345

Method of manure removal from milk cow barn. **Missing=77** 346

gutter cleaner **(83)** alley scraper (mechanical or tractor) **(45)** slatted floor **(11)**

removed (with bucket, bulldozer, etc.) as bedded pack **(12)** alley flushed with water **(0)**

Method of storage of manure from milk cow barn: **Missing=71** 347

pit (under barn) **(25)** open pile **(64)** earth lagoon **(41)** concrete lagoon **(18)** other **(9)**

Distance (in feet) from milk cow manure storage area to nearest farm well? 348

Missing=16, Mean=1285.81, SD=3104.98

Distance (in feet) from milk cow manure storage area to stream, lake or pond? 349

Missing=27, Mean=4452.10, SD=10359.88

Do cows have access to a stream, lake or pond? **Missing=1, No=175, yes=52** 350

Which methods are used to dispose of manure on owned or rented land (check all that applies)? 351

injection **Missing=60, No=144, yes=24** 352

spread with surface incorporation (e.g. plowing, disking) **Missing=60, No=34, yes=134** 353

spread without surface incorporation (e.g. plowing, diskig) **Missing=60, No=105, yes=63**

How many days do you wait after applying manure to a field before heifers are allowed to graze the field or be fed green chop from the field? **NA=111, Missing=26, Mean=77.58, SD=104.91** 354

In the last 12 months, what percentage of the grains you fed to heifers was homegrown? **Missing=1, Mean=46.37, SD=45.83** 355

In the last 12 months, what percentage of the roughages you fed to heifers was homegrown? **Missing=1, Mean=90.37, SD=24.42** 356

In the last 12 months, what percentage of the grains you fed to cows was homegrown? **Missing=1, Mean=43.75, SD=44.47** 357

In the last 12 months, what percentage of the roughages you fed to cows was homegrown? **Missing=1, Mean=90.26, SD=23.40** 358

Origin of drinking water by season; fill in for each group of animals on your farm: 359

1 - Surfaced water (stream, pond or lake) to

2 - Well water 366

3 – Municipal water

Source of water in the...	Open heifers	Bred heifers	Dry cows	Milking cows
WINTER	359 **Missing=71** **1=8,** **2=142,** **3=7**	360 **Missing=70** **1=8,** **2=143,** **3=7**	361 **Missing=69** **1=8,** **2=144,** **3=7**	362 **Missing=70** **1=8,** **2=143,** **3=7**
SUMMER	363 **Missing=89** **1=31,** **2=103,** **3=5**	364 **Missing=93** **1=45,** **2=86,** **3=4**	365 **Missing=90** **1=34,** **2=100,** **3=4**	366 **Missing=80** **1=19,** **2=122,** **3=7**

How often is equipment that holds manure (e.g. bucket, spreader) also used to handle feed fed to 367

heifers? **NA=2, Missing=2**

regularly (at least weekly) **(37)** occasionally (less than once a week) **(43)** not a practice **(144)**

How often is equipment that holds manure (e.g. bucket, spreader) also used to handle feed fed to 368

cows? **NA=2, Missing=1**

regularly (at least weekly) **(35)** occasionally (less than once a week) **(39)** not a practice **(151)**

Do heifers less than 12 months of age share a feed bunk with adult cattle? **No=212, Yes=16** 369

Do heifers less than 12 months of age share a water trough with adult cattle? **No=176, Yes=52** 370

K. Prevalence of disease

Please fill in the table based on the last 12 months, Give your best estimate: (for disease monitoring, do not include animals tested as part of this research project) 371 to 386

	Clinical Cases (sick animals)	Disease Monitoring (sick and healthy animals)	
	Number of animals with the disease problem	Number of animals tested (blood, milk or fecal test)	Number of animals with positive tests results
BVD	371 **Missing=18 Mean=0.26, SD=2.23**	372 **Missing=58 Mean=0.14, SD=0.97**	373 **NA=164 Missing=58 Mean=0.17, SD=0.41**
Leukosis	374 **Missing=18 Mean=0.93, SD=7.62**	375 **Missing=19 Mean=2.02, SD=14.81**	376 **NA=191 Missing=19 Mean=7.94, SD=24.33**
Johne's Disease	377 **Missing=58 Mean=0.11, SD=0.53**	378 **Missing=11 Mean=4.76, SD=17.40**	379 **NA=138 Missing=12 Mean=1.09, SD=1.80**
Neosporosis	380 **Missing=16 Mean=0.62, SD=3.94**	381 **Missing=8 Mean=0.82, SD=2.22**	382 **NA=163 Missing=9 Mean=1.18, SD=2.80**
Retained afterbirth (> 24 hrs)	383 **Missing=15 Mean=6.34, SD=10.65**		
Abortion less than 4 months	384 **Missing=52 Mean=1.84, SD=4.29**		
Abortion 4 to 7 months	385 **Missing=19 Mean=1.09, SD=2.43**		
Abortion greater than 7 months	386 **Missing=19 Mean=0.60, SD=1.76**		

In the LAST 5 YEARS, how many cattle have been diagnosed with Johne's disease by: 387 to 391

	Number of animal tested	Number of positives
Fecal test	387 **Missing=7** **Mean=1.15,** **SD=13.46**	388 **NA=213** **Missing=7** **Mean=2.12,** **SD=3.36**
Blood test	389 **Missing=10** **Mean=1.48,** **SD=6.80**	390 **NA=193** **Missing=11** **Mean=0.79,** **SD=0.93**
Veterinary diagnosis		391 **Missing=12** **Mean=0.08,** **SD=0.46**

IN The LAST 12 MONTHS, how many of your CULLED COWS showed chronic diarrhea, normal 392

appetite and weight loss that did not respond to treatment? **Missing=10, Mean=0.34, SD=1.43**

What is done with apparently healthy cows that have a positive Johne's disease test? 393

Missing=202

immediately shipped **(6)** slaughtered at end of lactation **(4)**

kept on farm but handled differently **(5)** nothing **(11)**

Any other Johne's Disease's Control procedure? Describe. 394