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***Neospora caninum* is the leading cause of bovine fetal loss in British Columbia, Canada**

Devon J Wilson^{a,b}, Karin Orsel^c, Josh Waddington^d, Malavika Rajeev^e, Amy R. Sweeny^e, Tomy Joseph^b, Michael E Grigg^{e,*}, and Stephen A. Raverty^{b,*}

^aWestern College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5B4

^bThe Animal Health Center. BC Ministry of Agriculture, 1767 Angus Campbell Road, Abbotsford, British Columbia, V3G 2M3

^cDepartment of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta T2N 4N1

^dGreenbelt Veterinary Services Ltd. 8451 Harvard Place, Chilliwack, British Columbia, V2P 7Z5

^eMolecular Parasitology Section, Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD, USA

Abstract

The protozoan pathogen *Neospora caninum* is recognized as a leading cause of infectious abortions in cattle worldwide. To evaluate the impact of neosporosis on dairy and beef herd production, a retrospective, longitudinal study was performed to identify the impact of neosporosis alongside other causes of fetal abortion in British Columbia, Canada. Retrospective analysis of pathology records of bovine fetal submissions submitted to the Animal Health Centre, Abbotsford, British Columbia, a provincial veterinary diagnostic laboratory, from January 2007– July 2013 identified 182 abortion cases (passive surveillance). From July 2013–May 2014, an active surveillance program identified a further 54 abortion cases from dairy farmers in the Upper Fraser Valley, British Columbia. Of the total 236 fetal submissions analyzed, *N. caninum* was diagnosed in 18.2% of cases, making it the most commonly identified infectious agent associated with fetal loss. During active surveillance, *N. caninum* was associated with 41% of fetuses submitted compared to 13.3% during passive surveillance ($P < 0.001$). Breed of dam was significantly associated with *N. caninum* diagnosis, with a higher prevalence in dairy versus beef breeds, and fetuses of 3–6 months gestational age had the highest prevalence of *N. caninum*. There was no significant association with dam parity. *Neospora caninum* was diagnosed in every year except 2009 and cases were geographically widespread throughout the province. Furthermore, the active surveillance program demonstrates that *N. caninum* is highly prevalent in the Upper Fraser Valley and is a major causal agent of production losses in this dairy intensive region.

*Corresponding authors. Tel.: 1-601-556-3003; Fax: 1-604-556-3010.

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Keywords

Neospora caninum; bovine neosporosis; abortion; fetal loss; British Columbia

1. Introduction

Neospora caninum is recognized as a significant cause of bovine abortions worldwide (Dubey and Schares, 2011). *N. caninum* has a complex life cycle: the definitive canid host sheds highly infectious and stable oocysts in their feces (Dubey et al., 2007) which can be transmitted horizontally to infect a wide variety of intermediate hosts, including cattle, deer, sheep, buffalo, bison, rodents, birds and horses (Dubey and Schares, 2011). Here, the parasite expands asexually to produce a chronic infection harboring infectious cysts that are transmissible to canids. In cattle, it is also efficiently transmitted vertically by transplacental infection, with congenital transmission rates recorded to be 40–95% among calves born to seropositive mothers (Reichel et al. 2014).

Abortion and stillbirth result in significant production and economic loss in the beef and dairy industries (De Vries, 2006; Jonker, 2004). The extent to which transmission occurs by exogenous exposure to oocysts versus transplacental infection from a chronically infected seropositive dam is necessary information to manage the spread of this disease among cattle (Basso et al., 2010; Dubey et al., 2007). Dogs, coyotes, wolves and dingoes are the known definitive hosts of *N. caninum*, but experimentally infected canids do not appear to shed substantial numbers of oocysts (Dubey and Schares, 2011). Due to the apparent low fecundity of the *N. caninum* sexual cycle, it is thought that the primary driver of the high transmission rates in cattle herds is vertical transmission (Barr et al., 1990; Cardoso et al., 2012; Morales et al., 2001; Pare et al., 1996). However, recent studies have shown significant variation in transplacental infection rates (Haddad et al., 2005), and in some cases, there is evidence of exogenous transmission and post-natal seroconversion among herds where *N. caninum* circulates endemically (Basso et al., 2010; Dijkstra et al., 2001; Hall et al., 2005). Furthermore, the efficiency of vertical transmission decreases with parity (or the number of pregnancies post-infection) and the occurrence of sporadic, clustered outbreaks of abortions (epidemic pattern) suggest that exogenous exposure to the oocyst stage of the parasite (Anderson et al., 2000; Bartels et al., 2006) may be more significant than previously envisaged (Dubey and Schares, 2006; Thurmond and Hietala, 1997). Ultimately, to determine the exact transmission dynamics of *N. caninum* associated fetal loss, a robust assay and reliable baseline data in a region endemic with disease needs to be developed in order to evaluate the extent to which each transmission cycle contributes to the spread of disease. Such baseline data is important to begin to understand how the parasite circulates in nature, the impact it has economically, and the best control methods that could be adopted in order to limit disease (Reichel et al., 2014).

Despite the significant contribution of *N. caninum* to reproductive losses, a definitive diagnosis of *N. caninum* infection remains a major challenge requiring multiple diagnostic approaches (Dubey and Schares, 2006). Historically, immunohistochemistry (IHC) has been utilized because it is very specific, however, it is an insensitive method of identification so

many positive diagnoses are missed (Jenkins et al., 2002). Recently, the sensitivity and specificity of PCR methods have improved diagnosis (Ortega-Mora et al., 2006), but because transplacental infection does not always result in fetal loss, no diagnosis can be made on congenitally infected but clinically normal calves (Dubey and Schares, 2011; Sager et al., 2001).

In Canada, seroprevalence ranges from 5–25% among beef and dairy cattle (Haddad et al., 2005; Pruvot et al., 2014). However, few studies have investigated the extent to which *N. caninum* infection causes fetal abortion in Canada. Two studies that utilized pathology and/or IHC methodologies reported an abortion rate of 2–5% (Alves et al., 1996; Khodakaram-Tafti and Ikede, 2005). In British Columbia, Canada, little is known about the prevalence and impact of this important production pathogen (Haddad et al., 2005). Our aim was to 1) establish a baseline dataset that measures the relative impact of *N. caninum* associated fetal loss compared to other agents associated with infection, and 2) determine the risk factors associated with infectious loss. To do this, we evaluated a suite of veterinary diagnostic and pathologic tools including fetal pathological examination, PCR, IHC and serology in combination with epidemiologic data to identify etiologies associated with fetal submissions to the Animal Health Centre over a 7 year period (Abbotsford, British Columbia).

2. Methods

2.1 Fetal Submissions and Database Search

The Animal Health Centre, Abbotsford, BC is the only veterinary laboratory in the province performing gross post-mortems. It is the only American Association of Veterinary Laboratory Diagnosticians (AAVLD)-accredited veterinary diagnostic laboratory in the province, and it accepts the majority of case material from veterinarians and producers in BC. Pathology records of bovine fetuses submitted from January 1, 2007 – June 1, 2014 were retrieved through a database search (Vetstar Animal Disease Diagnostic System (VADDS); Advanced Technology Corporation, Ramsey, New Jersey, USA) with “bovine fetus” and “abortion” as search criteria. Data extracted from the pathology reports was entered into Microsoft Access and Excel (Microsoft Corporation, Redmond, Washington, USA). In addition to routine submissions, as part of Agriculture and Agri-food Canada’s Growing Forward-2 Initiative, dairy farmers in the Upper Fraser Valley Region were actively recruited from July 1, 2013 to May 16, 2014 to submit all of their aborted fetuses, placenta, milk and serum from the dam (active surveillance) for diagnostic work-up. The active surveillance program was initiated by personal communications with farms by neighboring veterinary practices, clinic newsletters, email, and seminar/client meetings.

2.2 Routine Diagnostic Work-up

Upon presentation, fetuses were examined for external abnormalities. The sex, weight and crown-to-rump length (CRL) were recorded, and a standard suite of tissues was harvested for histologic analysis and ancillary diagnostic studies (Kirkbride, 2012). If specific lesions or inflammatory infiltrate was identified by the pathologist consistent with an infectious process, immunohistochemistry (IHC) and histochemical stains were used to demonstrate or

confirm the presence of the following pathogens, including *N. caninum* (mouse anti-*N. caninum* monoclonal antibody (VMRD)), bovine herpesvirus I (BHV-1), and intralesional bacteria and fungi. Aerobic bacterial culture of lung, placenta, and stomach content was part of the standard fetal work protocol. Additional tests were conducted as directed by the assigned pathologist, including aerobic and anaerobic culture and pathogen identification by polymerase chain reaction (PCR), tissue culture (TC), serum neutralization test (SN) or fluorescent antibody tests (FAT). Specialized diagnostics for other viruses, protozoa or bacteria were completed upon request of the pathologist. The majority of neosporosis cases possessed myocardial lesions and epicarditis, and lesions were typically observed in the heart, brain, and liver. Molecular diagnosis of neosporosis was done using the *N. caninum*-specific primer pair Np2I/Np6 (Yamage et al., 1996) for cases submitted before July 2013. After this period, ApiITS1 primers that target a region within the 110-copy ribosomal SSU rDNA gene array followed by DNA sequencing of the positive amplicon (Gibson et al., 2011) was performed due to the increased sensitivity and specificity of this method. A diagnosis of neosporosis was made if any tissue (brain, diaphragm, heart, liver, lung, muscle, placenta, pooled tissues, or tongue) tested PCR positive for *N. caninum*. A commercially available competitive inhibition ELISA test (*N. caninum* Antibody Test Kit, cELISA, VMRD Inc., Pullman, WA, USA) was used to test dams for *N. caninum* antibodies, but this test was not routinely requested.

2.3 Diagnostic Criteria and Case Definitions

We analyzed data from all fetal submissions associated with fetal loss, including abortions (a fetus which was less than 260 days old or of unknown age, $n = 212$) and stillborn cases (260 days old to full term, $n = 22$). Fetal age of submissions ranged from 3 months to near term abortions and stillbirths. Age was based on crown-to-rump length (CRL) and owner submitted information when CRL measurements were not available (Forar et al., 1996; McGeady et al., 2013). CRL was maximized to agreement between submitted gestation length and measured CRL with fetal age in days correlated to the CRL (range in cm) as follows; 60 (0–15), 90 (15–27), 120 (27–40), 150 (40–52), 180 (52–70), 210 (70–87), 260 (>87).

The cause of fetal death was classified based on the pathologists' interpretation, which reflected gross and histologic examinations and review of results from ancillary diagnostics tests. We diagnosed *N. caninum* based on histopathology and an additional positive ancillary diagnostic test (IHC, PCR, or serology) or PCR testing alone. Fetal loss was assigned to one of the following five categories: confirmed infectious (an etiological agent was diagnosed), inflammatory (lesions indicative of infectious processes were observed but no infectious agent was diagnosed), nutritional (histologic lesions were observed indicative of iodine, vitamin A or vitamin E/Se deficiency), developmental (gross malformations or developmental lesions were observed and no pathogens were identified) or idiopathic (no gross or histologic lesions or significant pathogens were identified).

2.4 Data Analysis

Based on available data for each case, we categorized fetal submissions by breed (Beef: Charolais, Hereford, Angus, Brahma vs. Dairy: Holstein, Jersey, Ayershire, Brown Swiss,

Crossbred), dam parity (heifers: first pregnancy vs. multiparous dams: cows that had been calved at least once before), and gestational age (3–6 months vs. greater than 6 months). *N. caninum* diagnoses were compared between these separate groups by chi-squared tests. All statistical analyses were performed in Prism (Version 6, GraphPAD Software, San Diego, CA). Additionally, we compared the frequency of histological lesions observed across different tissue sites (adrenal, brain, kidney, liver, muscle, heart, and other tissue sites combined) for *Neospora caninum* cases ($n = 43$). We also analyzed the number of *N. caninum* cases diagnosed by month and year. To examine the spatial distribution of submissions and *N. caninum* occurrence, fetal specimens were linked to a farm location and farming region as presented in the BC Holstein directory 2013–2014 (British Columbia Holstein-News, 2013/2014) or openly available on Google Maps. Distribution maps were constructed in R 3.01 (R Core Development Team) using the package RgoogleMaps (2015) with a 5% randomization added to farm location GPS coordinates. Occurrence data for beef farms was not presented due to limited data availability.

3. Results

3.1 *Neospora caninum* is the leading infectious agent associated with bovine abortion

Between April 16, 2007 and May 16, 2014, 237 bovine fetuses were submitted to the AHC from across British Columbia, Canada. One case was excluded from the study due to missing observations. In total, 146 (61.9%) cases of fetal loss were attributable to an infectious process (Table 1). Of these, we classified 55 (23.3%) as inflammatory, where histopathological evidence suggested an infectious process but no causative pathogen was identified. Ninety-three (39.4%) were diagnosed as a confirmed infectious case where the infectious agent was identified. Among these cases, *N. caninum* ($n = 43$) was the primary agent diagnosed, representing 46.2% of infectious cases and 18.2% of all cases. Other pathogens identified included bacterial ($n = 36$), viral ($n = 10$), and fungal ($n = 2$) agents (Table 1). Fetal loss due to nutrition ($n = 6$) or reproductive development ($n = 4$) was limited. We classified 80 (33.9%) cases as idiopathic, as there was no pathologic findings or pathogens identified. Focused active surveillance and recruitment from dairy farmers in the Upper Fraser Valley (UFV) resulted in 54 fetal submissions in 2013 and 2014, and approximately 41% ($n = 22$) of these were diagnosed as *N. caninum* compared to 13.3% ($n=4$ out of 30 total submissions from the UFV) during the routine surveillance period, a significant increase in diagnoses ($\chi^2 = 6.778$, $p=0.009$).

3.2 Diagnostics and Histopathological Findings for *N. caninum* cases

Of the 43 *N. caninum* cases, the pathologist noted lesions indicative of *N. caninum* infection alongside the positive PCR diagnostic result for 37 cases; 6 diagnoses were made on PCR results alone. For the *N. caninum* cases, lesions were most commonly observed in the heart (68.9%, $n=31$), liver (60.0%, $n=27$), and brain (46.7%, $n=21$) (data not shown). More specifically, myocarditis was the most commonly associated lesion type presenting in 29/31 cases with lesions in the heart. For all other sites (adrenal, kidney, lung, muscle, placenta, thymus, and spleen), lesions were observed in less than 25% of *N. caninum* cases. For 14 cases, lesions indicative of *N. caninum* infection were identified but *N. caninum* was not detected by an ancillary diagnostic test ($n=12$) or no ancillary diagnostics were done ($n=2$).

3.3 Risk factors for *N. caninum*

Breed data was submitted for 218 cases, with 180 identified as dairy breeds (Holstein = 173, other = 7) and 38 identified as beef breeds. *N. caninum* was diagnosed more commonly in dairy fetuses (20.0 %) compared to beef fetuses (5.3%) ($\chi^2 = 4.73$, $p = 0.03$). Gestational age was also significantly associated with *N. caninum* infection, with positive diagnosis occurring in 26.9% of fetuses between the ages of 3–6 months versus 14.2% of fetuses older than 6 months ($\chi^2 = 5.21$, $p = 0.02$). The parity of the dam was known in 55 cases, and categorized as born from heifers or multiparous cows. *N. caninum* was diagnosed in 28.6% of fetuses born from heifers versus 33.3% of fetuses born from multiparous cows, and the difference was not significant ($\chi^2 = 0.024$, $p = 0.88$).

3.4 Spatiotemporal Patterns of *N. caninum* Cases

The proportion of *N. caninum* diagnoses peaked in the autumn months, (September – November; 53%, $n=23$) and represented the majority of cases associated with fetal loss ($\chi^2 = 11.26$, $p = <0.001$) compared to all other months combined (December – August; 47%, $n=20$). No *N. caninum* diagnoses were made in May or August. *N. caninum* cases occurred every year except 2009. Submissions were collected from six regions in British Columbia (Central, Lower, and Upper Fraser Valley, Richmond Delta, North Okanagan, and Vancouver Island). The majority of diagnoses were in the Fraser River Valley where the most intensive production occurs, but in general, the occurrence of *N. caninum* infection was widespread throughout the province (Figure 1).

4. Discussion

In this largely retrospective, longitudinal study, *N. caninum* was identified as the leading infectious agent associated with fetal loss, with 18.2% of cases diagnosed as primary *N. caninum* infection. This is significantly higher than previously reported for Canada, although this is likely due to differences in the sensitivity of the diagnostic techniques utilized as previous studies primarily used IHC, a diagnostic method with significantly lower sensitivity than PCR (Alves et al., 1996; Khodakaram-Tafti and Ikede, 2005). Studies in California, New Zealand, and the Netherlands have shown similar rates of *N. caninum* associated abortions as we found in this study, with ~20% of bovine fetal abortion due to neosporosis (Anderson et al., 2000). Importantly, the active surveillance program dramatically improved diagnostic success, with 41% of cases diagnosed as neosporosis. We presume that the immediate responses from farmers after they detected a fetal abortion led to higher-quality submissions (i.e. fetuses that were submitted immediately after abortions or were stored properly) for detection by the PCR methodology used.

In our estimate, we included cases where PCR testing alone was positive for *N. caninum* since a definitive diagnosis of *N. caninum* infection is a major challenge (Jenkins et al., 2002). However, it is also important to note that *N. caninum* DNA has previously been identified in congenitally infected but clinically normal calves, which indicates that the detection of *N. caninum* DNA in an aborted fetus does not necessarily indicate causality. However, the lack of identification of other pathogens associated with fetal loss in the PCR

alone positive cases strongly argues for the *N. caninum* infection as the primary or contributing factor that led to the fetal loss.

Overall, the majority of cases diagnosed as *N. caninum* had histologic lesions that were characteristic of protozoal infection, with lesions noted in the heart, brain, and liver, with myocarditis especially characteristic of diagnosed cases. These lesions and lesion sites have been shown previously to be highly associated with *N. caninum* infection (Anderson et al., 1990; Collantes-Fernández et al., 2006; Ortega-Mora et al., 2006; Otter et al., 1997). Twelve cases that had inflammatory lesions suggestive of *N. caninum* infection were identified, but these cases were negative on PCR testing. False negatives are not uncommon for a variety of reasons, including PCR test performance as a result of the quality and type of tissues submitted, or whether the tissue sampled contained parasite DNA (Ortega-Mora et al., 2006). While these cases are likely due to *N. caninum*, whether other closely related protozoa were causal agents, such as *Sarcocystis* spp. or *Toxoplasma gondii*, both of which have similar patterns of pathogenesis and cause fetal loss (Jenkins et al., 2002), was not systematically addressed in this study.

The occurrence of fetal loss due to neosporosis was geographically widespread, and the observed clustering of cases in the Upper Fraser Valley was likely due to the high number of submissions in this region after the active surveillance program was initiated. Other factors significantly associated with *N. caninum* diagnosis were 1) time of year, with more infections occurring in the Fall; 2) breed, with dairy fetuses more frequently diagnosed with fetal abortion due to *N. caninum* than beef breeds; and, 3) gestational age, with the greatest proportion of *N. caninum* fetuses aborted between 3–6 months. These results are in line with most studies of *N. caninum* abortion risk, in which higher rates have previously been found in dairy versus beef herds globally (Reichel et al., 2013) and the majority of fetuses aborting were between 5–7 months of gestation (Dubey and Schares, 2006). In our study the majority of cases submitted were from dairy breeds, and in general data on *N. caninum* prevalence in beef operations are scarce compared to dairy cattle globally (Reichel et al., 2013). Parity was not significantly associated with *N. caninum* diagnosis, and different studies have identified increasing dam age/parity as both a risk factor and a protective factor for *N. caninum* abortions (Thurmond and Hietala, 1997; Wouda et al., 1999) potentially due to a difference in epidemiologic patterns of *N. caninum*-associated abortion where increasing dam age is associated with increased epidemic abortions but decreased endemic abortions (Collantes-Fernández et al., 2006; Dubey et al., 2007). Whether environmental contamination of water or silage with oocysts shed from canid definitive hosts has contributed to the high incidence of fetal loss as a result of *N. caninum* infection could not be ascertained from this study. Certainly, recent studies have shown that abortion risk is associated with the presence or proximity of dogs (Dubey and Schares, 2011; Pruvot et al., 2014). Future work should be done to examine the incidence rate of *Neospora* infection in wild and domestic canids that co-associate with cattle in the Upper Fraser Valley, in addition to developing a serological test that identifies dams that are acutely infected by the presence of IgM and low-avidity IgG antibodies.

5. Conclusions

In our study, *N. caninum* was the most commonly diagnosed infectious etiology associated with bovine fetal loss, representing a significant cause of economic loss to the dairy industry in British Columbia, Canada. Furthermore, we found that *N. caninum* was geographically and temporally widespread, suggesting that this pathogen circulates endemically in this region. Focused active surveillance significantly increased the success rate of making a definitive diagnosis and it identified the Upper Fraser Valley as a region with high occurrence of fetal loss due to *N. caninum* infection. Future targeted surveillance of dairy farms in this region, including determining whether infection is the result of endogenous or exogenous transmission, and the identification of risk factors associated with *N. caninum* infection and associated fetal loss will provide critical insight into how this pathogen is being maintained in cattle populations and will help to elucidate optimal control strategies to manage this high-impact production pathogen.

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References

- Alves D, McEwen B, Hazlett M, Maxie G, Anderson N. Trends in bovine abortions submitted to the Ontario Ministry of Agriculture, Food and Rural Affairs, 1993–1995. *Can Vet J.* 1996; 37:287–288. [PubMed: 8705972]
- Anderson M, Andrianarivo A, Conrad P. Neosporosis in cattle. *Anim Reprod Sci.* 2000; 60:417–431. [PubMed: 10844212]
- Anderson ML, Blanchard PC, Barr BC, Hoffman RL. A survey of causes of bovine abortion occurring in the San Joaquin Valley, California. *J Vet Diagn Investig.* 1990; 2:283–287. [PubMed: 2095280]
- Barr BC, Anderson ML, Blanchard PC, Daft BM, Kinde H, Conrad PA. Bovine fetal encephalitis and myocarditis associated with protozoal infections. *Vet Pathol.* 1990; 27:354–361. [PubMed: 2238388]
- Bartels CJ, Arnaiz-Seco JI, Ruiz-Santa-Quitera A, Bjorkman C, Frossling J, von Blumroder D, Conraths FJ, Schares G, van Maanen C, Wouda W, Ortega-Mora LM. Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. *Vet Parasitol.* 2006; 137:17–27. [PubMed: 16417972]
- Basso W, Schares S, Minke L, Bärwald A, Maksimov A, Peters M, Schulze C, Müller M, Conraths FJ, Schares G. Microsatellite typing and avidity analysis suggest a common source of infection in herds with epidemic *Neospora caninum*-associated bovine abortion. *Vet Parasitol.* 2010; 173:24–31. [PubMed: 20609521]
- Cardoso JMS, Amaku M, dos Santos Araújo AJU, Gennari SM. A longitudinal study of *Neospora caninum* infection on three dairy farms in Brazil. *Vet Parasitol.* 2012; 187:553–557. [PubMed: 22309800]
- Collantes-Fernández E, Arnaiz-Seco I, Burgos BM, Rodríguez-Bertos A, Aduriz G, Fernandez-Garcia A, Ortega-Mora L. Comparison of *Neospora caninum* distribution, parasite loads and lesions between epidemic and endemic bovine abortion cases. *Vet Parasitol.* 2006; 142:187–191. [PubMed: 16884852]

- De Vries A. Economic value of pregnancy in dairy cattle. *Journal of dairy science*. 2006; 89:3876–3885. [PubMed: 16960063]
- Dijkstra T, Barkema HW, Eysker M, Wouda W. Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. *Int J Parasitol*. 2001; 31:209–215. [PubMed: 11239942]
- Dubey J, Schares G, Ortega-Mora L. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev.* 2007; 20:323–367. [PubMed: 17428888]
- Dubey JP, Schares G. Diagnosis of bovine neosporosis. *Vet Parasitol*. 2006; 140:1–34. [PubMed: 16730126]
- Dubey JP, Schares G. Neosporosis in animals--the last five years. *Vet Parasitol*. 2011; 180:90–108. [PubMed: 21704458]
- Gibson AK, Raverty S, Lambourn DM, Huggins J, Magargal SL, Grigg ME. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. *PLoS Negl. Trop. Dis*. 2011; 5:e1142. [PubMed: 21629726]
- Haddad JP, Dohoo IR, VanLeeuwen JA. A review of *Neospora caninum* in dairy and beef cattle--a Canadian perspective. *Can Vet J*. 2005; 46:230–243. [PubMed: 15884645]
- Hall CA, Reichel MP, Ellis JT. *Neospora* abortions in dairy cattle: diagnosis, mode of transmission and control. *Vet Parasitol*. 2005; 128:231–241. [PubMed: 15740860]
- Jenkins M, Baszler T, Björkman C, Schares G, Williams D. Diagnosis and seroepidemiology of *Neosporacanium*-associated bovine abortion. *Int J Parasitol*. 2002; 32:631–636. [PubMed: 11943234]
- Jonker FH. Fetal death: comparative aspects in large domestic animals. *Animal reproduction science*. 2004; 82–83:415–430.
- Khodakaram-Tafti A, Ikede BO. A retrospective study of sporadic bovine abortions, stillbirths, and neonatal abnormalities in Atlantic Canada, from 1990 to 2001. *Can Vet J*. 2005; 46:635–637. [PubMed: 16152720]
- Morales E, Trigo F, Ibarra F, Puente E, Santacruz M. Neosporosis in Mexican dairy herds: lesions and immunohistochemical detection of *Neospora caninum* in fetuses. *J Comp Pathol*. 2001; 125:58–63. [PubMed: 11437517]
- Ortega-Mora LM, Fernandez-Garcia A, Gomez-Bautista M. Diagnosis of bovine neosporosis: Recent advances and perspectives. *Acta Parasitol*. 2006; 51:1–14.
- Otter A, Jeffrey M, Scholes SFE, Helmick B, Wilesmith JW, Trees AJ. Comparison of histology with maternal and fetal serology for the diagnosis of abortion due to bovine neosporosis. *Vet Rec*. 1997; 141:487–489. [PubMed: 9402719]
- Pare J, Thurmond MC, Hietala SK. Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. *Can J Vet Res*. 1996; 60:133. [PubMed: 8785719]
- Pruvot M, Kutz S, Barkema H, De Buck J, Orsel K. Occurrence of *Mycobacterium avium* subspecies paratuberculosis and *Neospora caninum* in Alberta cow-calf operations. *Prev Vet Med*. 2014; 117:95–102. [PubMed: 25277685]
- Reichel MP, Ayanegui-Alcérreca MA, Gondim LF, Ellis JT. What is the global economic impact of *Neospora caninum* in cattle--the billion dollar question. *Int J Parasitol*. 2013; 43:133–142. [PubMed: 23246675]
- Reichel MP, McAllister MM, Pomroy WE, Campero C, Ortega-Mora LM, Ellis JT. Control options for *Neospora caninum*--is there anything new or are we going backwards? *Parasitology*. 2014; 141:1455–1470. [PubMed: 24667014]
- Sager H, Fischer I, Furrer K, Strasser M, Waldvogel A, Boerlin P, Audige L, Gottstein B. A Swiss case-control study to assess *Neospora caninum*-associated bovine abortions by PCR, histopathology and serology. *Vet Parasitol*. 2001; 102:1–15. [PubMed: 11705647]
- Thurmond MC, Hietala SK. Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. *Am J Vet Res*. 1997; 58:1381–1385. [PubMed: 9401685]
- Wouda W, Bartels CJM, Moen AR. Characteristics of *Neospora caninum*-associated abortion storms in dairy herds in the Netherlands (1995 to 1997). *Theriogenology*. 1999; 52:233–245. [PubMed: 10734391]

Yamage M, Flechtner O, Gottstein B. Neospora caninum: Specific oligonucleotide primers for the detection of brain "cyst" DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). J Parasitol. 1996; 82:272–279. [PubMed: 8604096]

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Highlights

- *Neospora caninum* is the leading cause of bovine abortion in British Columbia, Canada.
- Diagnoses increased more than two fold during active surveillance.
- *Neospora caninum* was diagnosed throughout the study period.
- Cases were geographically widespread in British Columbia, Canada.

Fig. 1.

Neosporosis Distribution in British Columbia, Canada. Distribution of *N. caninum* infection among 183 bovine cases submitted in British Columbia, Canada during both the passive surveillance and active surveillance programs across 90 farms. Cases shown represent samples submitted between January 2007 and July 2014 for which geographical information was available. The distribution map represents dairy-breed samples only due to lack of geographical information for the majority of beef-breed cases. Each symbol represents a single farm location; Symbols are proportionate in size to the square root of the number of samples submitted per farm. Shading of the symbol reflects the proportion of submissions positive for *N. caninum* for each location as indicated in the key above. Detailed distribution insets represent cases highlighted by the red boxes for areas **A**) (Vancouver Island), **B**) (Fraser River Valley Region), and **C**) (Okanagan Region). Additional dairy submissions not shown in the figure insets are highlighted by red circles on the map. Distribution maps were constructed using R(Version 3.0.1) with a 5% randomization added to farm location GPS coordinates. Base map data ©2015 Google.

Table 1

Diagnoses of fetal submissions to the Animal Health Center, British Columbia, Canada from 2007–2014

Diagnosis	% of Total	No. of Cases
<i>Neospora caninum</i>	18.2	43
Histopathology and PCR		34
Histopathology and IHC		1
Histopathology and Serology		2
PCR		6
Bacterial	15.3	36
<i>Trueperella pyogenes</i>		9
Mixed bacterial		6
<i>Leptospira spp.</i>		3
<i>Streptococcus spp.</i>		3
<i>Escherichia coli</i>		2
<i>Listeria monocytogenes</i>		2
<i>Pseudomonas aeruginosa</i>		2
<i>Salmonella dublin</i>		2
<i>Staphylococcus spp.</i>		2
<i>Bacillus licheniformis</i>		1
<i>Histophilus somni</i>		1
<i>Mycoplasma bovis</i>		1
<i>Ureaplasma diversum</i>		1
<i>Acinetobacter spp.</i>		1
Viral	4.2	10
Bovine Herpes Virus-1 (BHV-1)		8
Bovine Viral Diarrhea Virus		2
Fungal	0.1	2
Inflammatory	23.3	55
Lesions indicative of <i>N. caninum</i>		14
Lesions indicative of unknown infectious agent		41
Idiopathic	33.9	80
Developmental	1.7	4
Nutritional	2.5	6
Total		236