Neosporosis in cattle

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Abstract

Neospora caninum is an obligate intracellular coccidian parasite of cattle and dogs that infects 10-20% of all cattle worldwide and causes up to 20% of bovine abortions. *N. caninum* cycles at low levels between the definitive host, canids, and a wide range of intermediate hosts, cattle being the most important. Abortion is the only clinical sign observed in adult cattle. *N. caninum* most commonly causes an endemic increase in a herd's annual abortion rate and infrequently causes epidemic abortion storms. Rarely, congenitally infected calves are born with neurological disease. Most infected calves are born clinically normal but with a titer. The parasite persists within cattle herds through vertical transmission via transplacental infection of successive pregnancies of chronically infected dams and by infrequent horizontal transmission. All infected cows are at increased risk of abortion. Abortion diagnosis is challenging because *Neospora* may be present but not the cause. Ruling out other causes of abortion, detecting the characteristic fetal lesions, or establishing an association between abortion and infection within the herd is important. Control options are limited. No antiparasitic drugs are approved or economical for treatment or prevention. Although a killed vaccine is available commercially, it has questionable efficacy. In many instances, tolerating the infection is the most economical option. Test-and-cull strategies are often cost-prohibitive. For reducing a herd infection rate, precolostral testing of heifer calves and excluding those with titers from breeding is usually an economical option.

Introduction

Neospora caninum is an apicomplexan intracellular parasite, first described in 1984, that is a leading cause of bovine abortion worldwide.[1,2] It is closely related to and for many years was misdiagnosed as *Toxoplasma gondii*. Domestic dogs and some wild canids are the definitive hosts. Many species, including dogs, serve as intermediate hosts, cattle being the most important (figure 1).[2]

N. caninum is transmitted vertically (dam to fetus) remarkably efficiently with up to 95% of calves born to infected dams congenitally infected themselves. Vertical transmission is the primary mechanism by which the parasite maintains itself within cattle herds.[2,3] *In utero* infection appears key to establishing a chronic infection capable of infecting successive pregnancies of the infected dam.[4] *N. caninum* appears highly tuned to the physiology of bovine pregnancy. Experimental feeding of oocysts to cows does not reliably produce infection. It appears that horizontal transmission between cattle or between cattle and canids is uncommon and thus less important.

Although estimated losses to the beef and dairy industries are at hundreds of millions of dollars, effective and economical control strategies for neosporosis are lacking.[5] Understanding the adaptations that make *N. caninum* a successful parasite and a difficult control target will help practitioners to diagnose and manage this ubiquitous and expensive parasite.

Prevalence

Since its recognition in 1984, *N. caninum* has been detected in cattle populations worldwide. Numerous seroprevalence surveys have documented specific antibody responses in cattle, canids, and a plethora of likely intermediate hosts, both wild and domestic (figure 2).[6] Studies employing molecular techniques have demonstrated *N. caninum* DNA in neural tissues of various animals.[2] While, these techniques document widespread exposure to and likely infection with *N. caninum,* they do not confirm infection or disease. The gold standard for demonstrating infection includes identifying tissue cysts histopathologically, identifying oocysts in feces, and isolating viable parasites. The successful isolations of viable parasites are limited to cattle, sheep, water buffalo, bison, white-tailed deer, and dogs.[6] Oocyst shedding has been detected in only a few naturally infected dogs.[6] Infrequently tissue cysts have been observed histopathologically in fetuses, calves, and experimentally infected mice but not in adult cattle.[7]

For complete *N. caninum* epidemiology, see Dubey et al.[2] Worldwide, approximately 15 to 25% of dairy cattle are seropositive and approximately 10 to 20% of beef cattle. Some regions approach 50-60% seropositivity and some herds approach 100%. Seropositivity among domestic dogs varies more widely among regions, ranging from 5% to 60% positive.[2]

A survey in northwestern U.S. found 24% (N=2585) of beef cows seropositive to *N. caninum*, with 100% of herds infected.[8] A study including 20 eastern and midwestern states and Puerto Rico found 16% of beef and dairy cows were seropositive with 90% of herds infected.[9] Only 7% of 1,077 canine serum samples from pet dogs across 35 states submitted on suspicion of neosporosis were seropositive.[10] Due to the difficulty of definitively diagnosing *N. caninum* as causing an abortion, estimating the proportion of abortions it causes is challenging. A study of a large number of fetuses submitted from California cattle attributed approximately 20% of bovine abortions to *N. caninum*.[2]

Although contact with dog feces potentially containing *N. caninum* oocysts and consumption of raw or undercooked beef potentially containing tissue cysts likely provide ample exposure opportunity for humans, the evidence supporting human infection is limited and inconclusive. One U.S. study using a low positive titer threshold found 7% seropositivity to *N. caninum* among healthy blood donors.[11] The majority of titer positive people were negative for antibodies against *T. gondii,* indicating that cross-reactivity with this pathogen is unlikely. A Brazilian study found 18% and 38% seropositivity among neurologic and AIDS patients, respectively, but found rates similar to the U.S. study among healthy adults and newborns.[12] In contrast to the U.S. study, nearly all the *Neospora* positive samples were also positive for toxoplasma, suggesting that cross-reactivity and/or polyclonal immune stimulation may have occurred. A low positive threshold of 1:80 or 1:100 was used compared to the higher ones used for cattle and dogs (1:320 and 1:200, respectively). Three serological surveys in England, France, and Denmark found little or no evidence for *Neospora* infection in humans.[13-15] Taken together, these data appear to support *N. caninum* exposure but not infection. Further studies to detect parasites or parasite DNA, most likely to be observed among immunocompromised individuals, are indicated.

Economic impact

Monetary losses from neosporosis are due to abortions and increased culling risk. Losses may result from decreased milk production, increased services per conception, increased calving interval, and increased veterinary costs, but authors differ on these. *Neospora*-induced abortion is estimated to cost the California cattle industry $35 million dollars annually.[2] Global losses, based on ten countries with sufficient published data, are estimated at $1.3 billion dollars annually.[5] The US dairy industry is estimated to lose $546 million per year, an annual loss of $12,200 to the average dairy farm.[5] The US beef industry is estimated to lose $111 million per year, an annual loss of $100 to the average beef producer.[5] The impact to an individual producer can be devastating when abortions occur as epidemics in which more than10% of a herd aborts in a short period.[5]

Life cycle

A schematic presentation of *Neospora caninum* life cycle is given in figure 3. *Neospora caninum* is a member of the Coccidia class containing the related genera *Eimeria, Isospora, Cryptosporidium, Sarcocystis,* and *Toxoplasma*, all having a facultative heteroxenous life cycle. Unsporulated 10-14 µm diameter oocysts are shed in the feces of the definitive host and have been found in the feces of dogs, coyotes, dingoes, and wolves.[16-18] Oocysts are highly resistant to environmental temperatures and common chemicals. Treatments that inactivate cysts include 100˚C for one minute or 10% sodium hypochlorite for one hour, with less severe treatments failing to inactivate all cysts.[19] Within 24 hours, the oocysts sporulate to the infective form. Each sporulated oocyst contains two sporocysts, each of which contains four sporozoites. When ingested by an intermediate host, eight sporozoites are released into the gut for each oocyst consumed.[4] Cattle, sheep, goats, horses, deer, dogs, and other animals may serve as intermediate hosts, but cattle are the most frequent and economically important.[2]

Sporozoites differentiate into tachyzoites, which parasitize small intestinal epithelial cells. Tachyzoites, 5-7 µm long and 1-2 µm wide, replicate rapidly by asexual endodyogeny, forming two

daughter cells within the parent cell.[1] These replicate in various tissues, including neural cells, vascular endothelial cells, myocytes, hepatocytes, renal cells, alveolar macrophages, maternal caruncular septal cells, and placental trophoblasts,[7] and disseminate hematogenously, presumably within mononuclear phagocytes. *N. caninum* DNA has been demonstrated in the leukocyte fraction but not the serum of blood from naturally infected cows.[20] Parasitemia is difficult to detect directly, suggesting that it may be of short duration and may wax and wane.

Tachyzoites differentiate into slowly-replicating bradyzoites, which are found within thick-walled, 100 µm cysts in the brain and spinal cord and rarely in muscle tissue. Each cyst may contain hundreds of bradyzoites.[4] Bradyzoite cysts are thought to persist for the life of the host and to re-activate and differentiate back into tachyzoites, thereby establishing persistent infection. Tissue cysts have been detected in congenitally infected calves and fetuses but not adult cattle.[7] Viable *N. caninum* have been isolated from the brains of adult cows that produced infected calves.[21] The parasite has been consistently isolated from placental tissue of congenitally infected dams at calving. Bradyzoite cysts may be present because dogs fed placental tissue from infected cows reliably shed oocysts.[22,23] Because bovine placental tissues are often readily available in the environment, they are likely are the principal infection source for dogs and other canids.

The cycle repeats when tissue cysts are consumed by the definitive host. The sexual stages that lead to oocyst formation have not been demonstrated but are presumed to occur in the intestinal epithelial cells of dogs and other definitive hosts.[4]

Transmission

Cattle become infected with *N. caninum* either by ingestion of sporulated oocysts postnatally (horizontal transmission) or congenitally by transplacental migration of tachyzoites from an infected dam to her fetus (vertical transmission). Some evidence suggests that the immune responses provoked by horizontally- and vertically-acquired infections are different.[2,24] Cattle are apparently often infected for life, regularly or intermittently transmitting the infection to successive pregnancies.[22] The majority of natural infections are thought to result from vertical transmission, which occurs with 75% to 100% efficiency.[5] Horizontal cow to cow transmission does not appear to occur.[2]

Horizontal transmission

Evidence for horizontal transmission comes from observed abortion patterns, avidity-ELISA serological studies, mathematical models, and risk-factor analyses. Because vertical transmission risk is less than 100%, some horizontal transmission must occur to maintain the parasite within the herd. Otherwise, within a herd prevalence would decline until the infection was eliminated.[25] Neospora-associated abortions occur in two patterns: in concentrated epidemic outbreaks or as a higher endemic annual herd abortion rate. The occurrence of epidemics, or "abortion storms," suggests a point-source; broad herd exposure to infective material and provides evidence of horizontal transmission.[7] Avidity-ELISA, based on an infected animal's immunoglobulin antigen binding affinity increasing with time post-infection, has been applied to herds after epidemic Neospora abortions. Bjorkman et al described a beef herd abortion outbreak in which at the time of the outbreak seropositive cows had low-avidity antibody responses, suggesting that many of the infections were recent and were the result of horizontal transmission of *N. caninum*.[26] The mean avidity of seropositive cows increased over the subsequent three years, suggesting the onset of chronic infections. This study also documented that calves born seronegative had a low risk of seroconversion and that vertical transmission occurs with 85% efficiency.

Observational studies of bovine neosporosis risk factors have frequently implicated dogs in *Neospora* transmission, with their presence on farms, their number, their behavior (e.g., feeding on placentas and defecating near feeds), and their density in the region all being associated with increased risk.[2] Coyote abundance in an ecological area was identified as a risk factor in a Texas study.[27] A French study identified using pond water as a drinking water source as a risk factor, suggesting that oocysts may be disseminated in water.[2] These studies indicate the importance of continuing horizontal *Neospora*

oocyst transmission from definitive hosts for maintaining the parasite in cattle populations, despite the larger role played by transplacental vertical transmission.

Other routes of vertical and horizontal transmission are thought not to occur or to be rare. Milk and colostrum containing added *Neospora caninum* tachyzoites infected calves experimentally, but this is not thought to occur naturally to any significant extent.[2] Similarly, *N. caninum* DNA was identified in an infected bull's semen and semen containing added tachyzoites infected heifers but large numbers were needed and no evidence suggest that venereal transmission occurs naturally.[2]

The outcome of horizontal infection depends on the fetal gestational age in pregnant dams and presumably on the dose and virulence of the *Neospora* strain involved. A non-pregnant animal will mount an immune response that either causes the parasite to form bradyzoite tissue cysts and become dormant or eliminates it.[28] The parasite may or may not cross the placenta in the pregnant animal. If the dam's immune response quickly controls the infection, transmission may not occur and the calf is born healthy and uninfected. If transplacental transmission occurs, the outcome depends on fetal gestational age, which determines its immunocompetence.[4] The fetus may be killed, in which case resorption, mummification, abortion, or stillbirth occurs, depending on gestational age. It may survive to birth but be born with neurologic disease or develop it within four weeks of birth. It may be infected but mount an immune response that controls the infection and be born clinically normal but persistently infected. In all these scenarios, most dams are presumed to remain persistently infected.[4] This is not well-established and some evidence suggests that horizontal transmission may not always result in persistent infection.

Vertical transmission

Vertical, or transplacental, transmission, the predominant mode in cattle, is divided into endogenous and exogenous forms. The possible outcomes for the developing fetus are the same for both. Exogenous transplacental transmission occurs when tachyzoites cross the placenta and infect the fetus after a maternal parasitemia initiated by a naïve dam consuming infective oocysts. This form is primarily associated with epidemic abortion outbreaks. Endogenous transplacental transmission occurs when the maternal parasitemia is initiated by the recrudescence of a persistent infection acquired before that pregnancy. This form is associated with an increased endemic abortion rate.[29] It is believed to most commonly occur in the second or third trimesters when the fetus is more immunocompetent, usually resulting in the birth of a healthy but infected calf.[4] The risk of endogenous transplacental transmission decreases with increasing parity,[30] suggesting that cows may eventually develop sufficient immunity to reduce recrudescence and transplacental transmission.

Host immune response

The immune response to *N. caninum* is characterized by a strong pro-inflammatory type 1 helper cells (Th-1) response that promotes a specific cell-mediated immune response. Cattle appear to have some innate immunity to *N. caninum,* as ntural killer (NK) cells have been shown to be able to lyse infected fibroblasts in culture.[31] Natural killer cells also secrete IFN-γ, a cytokine that plays an important role in a Th-1 response. IFN-γ causes type 0 helper (Th-0) cells to differentiate into Th-1 cells, and suppresses type 2 helper (Th-2) cells that would otherwise promote a humoral response and down-regulate the cell-mediated response. Type 1helper cells then secrete more IFN-γ, creating a positive reinforcement loop. IFN-γ has also been shown to inhibit parasite growth in cell culture and may play a role in causing the parasite to switch from the tachyzoite to the bradyzoite stage.[31] Type 1 helper cells coordinate a specific immune response that is mediated by CD4+ cytotoxic T-cells, which lyse infected host cells by a perforin-granzyme pathway.[32] Although humoral immunity plays a lesser role in *N. caninum* control, host antibodies generated against proteins on the tachyzoite surface block parasite entry into host cells.[33]

Immunomodulation during pregnancy

The immune system changes of pregnancy may be significantly related to the vertical transmission of *N. caninum*. A fetus represents a genetic foreign body to its dam, being essentially a

semi-allogeneic tissue graft. To prevent the maternal immune system from rejecting the fetus, the cytokine balance at the materno-fetal interface is slightly tipped to favor "beneficial" Th-2 regulatory cytokines, including interleukin 10 and TGF-β.[31] Th-2 cytokines inhibit cell-mediated immunity and promote a humoral immune response. Innes et al observed a pregnancy-specific down-regulation of the maternal cell-mediated immune response, evidenced by decreased responsiveness of circulating lymphocytes to antigen that occurred regardless of *N. caninum* infection status.[34] This decrease occurred between gestation weeks 12 and 18 after which the response slowly increased back to previous levels by gestation week 38,[34] corresponding to the typical timing of *Neospora*-induced abortions, which mainly occur at three to 8 months gestation (12-32 weeks).[4]

Host-parasite interactions

N. caninum vertical transmission appears to correspond with this pregnancy-associated immunomodulation. When pregnant cows were experimentally infected, the likelihood of exogenous transplacental transmission increased if the infection occurred later in gestation when the maternal cell-mediated response was down-regulated.[31] In naturally infected dams, the endogenous transplacental transmission is associated with a transient increase in circulating *Neospora*-specific antibody.[31] This increase is seen earlier in dams that abort and later in dams that give birth to congenitally infected calves. Recrudescence later in pregnancy is beneficial to parasite survival because congenitally infected heifer calves are more likely to be produced that are capable of passing the infection on to their offspring.[3] The mechanism by which *N. caninum* senses pregnancy-related immune down-regulation is unknown.

In utero exposure may be required for persistent *N. caninum* infections to be capable of infecting offspring.[4] Research suggests that post-natal infection may not result in persistent infections while congenital infection increases the risk of endogenous transplacental transmission. The protective quality of the immune response from *in utero* exposure may be inferior to that from post-natal exposure.[29] This suggests that *in utero* exposure produces immune tolerance somewhat similar to that which occurs with bovine viral diarrhea in which the congenitally infected animal's immune system cannot mount a response equivalent to that of a post-natally exposed animal.[2] Due to fetal immune system immaturity, *in utero* exposure may be more likely to result in persistent infections characterized by bradyzoite tissue cysts than does postnatal exposure.[7]

Evidence suggests that post-natal exposure rarely results in persistent infection capable of endogenous transmission to subsequent pregnancies. Three studies have shown that naïve cattle experimentally infected six or ten weeks prior to pregnancy do not transmit the parasite to their fetuses, even when challenged by *N. caninum* in mid-gestation.[34-36] Williams et al infected naïve cows with a low-virulence strain ten weeks prior to insemination and at 70 days of gestation administered an intravenous fetopathic challenge dose of 10^7 live tachyzoites to this and a control group.[34] In the treatment group, all six calves were born healthy with no signs of *N. caninum* infection while in the control group 82% (9 of 11) of the fetuses died.[35] This study also demonstrated that cattle fail to become persistently infected after a post-natal challenge and that they develop a protective immune response against future challenge.

Pregnant congenitally infected animals challenged at mid-gestation with a fetopathic infection were also immune to the challenge dose and did not abort. Later in gestation their persistent infection recrudesced and transmitted to their fetus, just as would have been expected with no intervention.[29] This suggests that they were tolerant of their own infections but not of the new strains or that their bradyzoite cyst burden developed before they were completely immune-competent. The persistent cysts are apparently able to reactivate quietly enough and to disseminate to the placenta before maternal immune control.

McCann et al orally infected naïve, pregnant cows and followed them through subsequent pregnancies.[24] All five calves or fetuses were congenitally infected but not those of subsequent pregnancies. No signs of recrudescence appeared in the dams' serology, indicating that they had not become persistently infected.[24]

In a herd that experienced a mass seroconversion without increased abortions, Dijkstra et al observed endogenous transmission in subsequent pregnancies.[28] Nine infected daughters were born to dams that seroconverted before their insemination dates, suggesting that persistent infection with subsequent endogenous transplacental transmission occurred due to the post-natal exposure.[28] This interpretation depends on the nine dams being correctly classified as naïve based on serological test results and on the nine offspring not having seroconverted after birth but before they were tested at about four to six months of age after maternal antibody waned. The circulating antibody level of persistently-infected animals is known to periodically dip below the threshold of serological tests.[7] More research is indicated to clarify this question, which has direct management implications.

Mechanism of abortion

Neosporosis is primarily a disease of the placenta and the fetus, the only clinical sign observed in naturally infected adult cattle being abortion.[4] *N. caninum* is an example of a parasite that is highly adapted to persist within its host with minimal morbidity.

Several abortion mechanisms are hypothesized. Parasite replication and the fetal response may destroy enough fetal tissue to kill the fetus outright or parasite replication and the maternal response may destroy enough placental tissue to kill the fetus by insufficient oxygenation/nutrition. Damage to the placenta may release maternal prostaglandins that cause luteolysis and subsequent abortion. The strong Th-1 type immune response stimulated by the parasite may disrupt the normal immunologic environment of the fetal-placental interface, leading to maternal immune rejection of the fetus.[4] The maternal inflammatory infiltrate with NK cells, cytotoxic T-cells, and cells containing mRNA for IFN- γ surrounding areas of fetal villous necrosis present in the placenta of aborting dams support this hypothesis.[37] Relatively small parasite numbers in the placenta might tip the balance towards a damaging maternal immune response that causes immune rejection of the pregnancy.

Diagnosis

Because *Neospora* is prevalent in cattle populations and may be present without causing abortion, definitive abortion diagnosis is difficult and depends on serology, molecular techniques, and histopathology. Ruling out other causes of abortion is important. Dubey and Schares developed a diagnostic strategy that relies on the subjective impression of the histopathologist and/or on statistical analysis of herd serology.[7]

Gross lesions are not usually present in the aborted fetus and they are commonly autolyzed or mummified.[7] Pale white foci may be observed in the heart or skeletal muscles and very small, pale to dark necrotic foci may be observed in the brain.[7] Hydrocephalus has been reported.[38]

The first laboratory submissions should include sera from aborted dams, fetal fluids, and tissues, including placenta if available. Maternal serum and fetal fluids (e.g., serum, pleural, and peritoneal fluids) may contain *Neospora* specific antibody detectable by serological assays that include competitive and indirect ELISA and IFAT.[3] Before acquiring and submitting samples, consult with the laboratory regarding the specimens they prefer, the tests they offer, and their diagnostic performance. Because *Neospora* may be present as a bystander, a positive serological result on a fetal or maternal sample indicates *Neospora* may be associated with the abortion but does not provide a definitive diagnosis.[3] Because most dams aborting due to *Neospora* are antibody-positive, a negative result on maternal serum indicates that *Neospora* was unlikely the cause.[7] Rarely, infected calves are born to seronegative dams, likely due to fluctuating antibody levels in the dam.[39] In contrast, a negative result on fetal submissions does not rule out *Neospora*, because autolysis, immature fetal immunocompetence, or a short interval between infection and death may lead to false negative serological results.[7]

Polymerase chain reaction (PCR)-based assays are available to detect parasite DNA in fetal tissues, amniotic fluid, milk, semen, or dog feces. These tests are interpreted much like serology, positive results requiring the presence of lesions incompatible with life or a clear statistical association between abortions and test results.[7]

Fetal tissues may be examined histopathologically for characteristic lesions and stained immunohistochemically to identify parasites in tissue sections. Lesions are most commonly observed in the brain, spinal cord, heart, and liver, but may be present in other tissues.[7, 40.41] The most characteristic lesions are multifocal encephalitis, myocarditis, and periportal hepatitis, with tachyzoites demonstrable immunohistochemically in up to 85% of brains.[40] When lesions are present and associated with *N. caninum*, the pathologist must decide whether the severity of lesions is incompatible with life and, if so, concluding that *N. caninum* was the cause of the abortion is reasonable.[7]

Herd-level serology and epidemiology provide additional evidence for diagnosing *Neospora*-induced abortion, which is useful when serology and histopathology are inconclusive. Gather information about herd reproduction and abortion occurrence timing to establish the abortion pattern, which also has direct control implications. A herd experiencing a *Neospora*-associated abortion storm (abortions began recently and occurred over a time course of eight to ten weeks) was likely recently exposed to infective oocysts, most likely in the feed or water.[7] A herd experiencing *Neospora*-associated abortions sporadically over a longer time course suggests a high level of persistent infection with vertical transmission and possibly the presence of other factors that increase abortion risk, such as mycotoxins in moldy feed.[3,7] Once the duration of abortion is established, the 'dams at risk' can be determined, meaning all dams pregnant and therefore at risk of aborting during the period when the abortions occurred. If the first round of serology and histopathology are inconclusive, serologically test sufficient samples of 'dams at risk,' randomly selecting both aborting and non-aborting dams, and determine if a positive association exists between presence of *N. caninum* antibodies and abortion. A statistically significant positive association provides strong evidence for *Neospora*-associated abortion.[3,7]

Serological testing can also determine carrier status for embryo recipient selection, for test-and-cull control strategies, and to aid in purchasing decisions. Using a lower test cut-off and therefore higher sensitivity is desirable.[7] Verify with the laboratory that the test and protocol being used have been validated for determining infection status. Bulk milk can be tested with ELISA to detect those herd with a within herd seroprevalence greater than 15%, which are herds likely to benefit from a control program.[42]

Control

Developing a *Neospora*-related abortion control strategy starts with a cost-benefit analysis for the farm in question. Due to regional differences in *N. caninum* epidemiology and in how economic losses are counted, researchers differ on the most cost-effective means of control.[2] Control strategies include no intervention, improving farm biosecurity, vaccination, test-and-cull, test-and-exclude-from-breeding, and use of beef bull semen to reduce abortions.

Where herd seropositivity is low, the most cost-effective option may be to do nothing. A decision-tree analysis of the Australia and New Zealand dairy industries determined that doing nothing was the most economical option for within-herd seroprevalence up to 18 to 21% and that vaccination with an efficacious product was the more economical option at higher prevalences.[43] No protection is provided against epidemics; in the model it was assumed that only one abortion storm was likely to occur in the time frame considered, followed by an increased risk of sporadic abortion.

Farm biosecurity may be important in controlling bovine neosporosis, particularly for uninfected and low seroprevalence herds. Maintaining a closed herd is the best way to avoid introducing the parasite. Although avoiding the introduction of infected replacements into negative herds is important, occasional false negative results on individual animal tests are likely because antibody levels wax and wane. A better approach is to buy only from herds that are test-negative or that have an ongoing testing strategy.[44] To reduce the risk of horizontal transmission, farm access to dogs and other canids should be minimized. Dogs and other canids should not be allowed to feed on aborted fetuses, placentas, or dead stock or to defecate in feed bunks or feed storage areas and should be excluded from the premises.[2]

No pharmaceuticals are approved for treating bovine neosporosis but several compounds have shown promise in research trials. The coccidiostat toltrazuril has been shown to block exogenous transplacental transmission in mice infected early in gestation.[45] Congenitally infected calves treated with the compound had stronger humoral immune responses to the parasite at four to six months of age than

did untreated controls.[46] The related compound ponazuril given to calves one day following experimental infection prevented them from developing an infection detectable by PCR.[47] Treatment expense and lengthy withholding times may make treatment uneconomical for all but exceptionally valuable cattle.[2]

An adjuvanted killed tachyzoite vaccine (Bovilis Neoguard®, Merck Animal Health, Millsboro, DE) is commercially available. Field efficacy data suggest that up to a 25-60%, reduction in abortions may be possible but negative effects have been detected in some herds. In a Costa Rican field trial Bovilis Neoguard® reduced abortion incidence by approximately half.[48] In a New Zealand field trial the vaccine had a statistically significant effect in one of five herds where it reduced abortions by about 60% but had little effect in the others and may have increased early embryonic death risk.[49] Vaccination results in seroconversion that cannot be differentiated from infection, eliminating the use of test-and-cull strategies in vaccinated animals. Research on a subunit vaccine is underway. Immunization with a surface protein involved in host cell attachment and invasion stimulated a humoral immune response that reduced transplacental transmission in mice.[33]

A protective immune response to vaccination is possible in non-congenitally infected animals. Infection with Nc-Nowra (Australian isolate), a naturally attenuated strain, before gestation protected 100% of fetuses from a lethal dose of a virulent strain delivered on gestation day 70. All fetuses were born uninfected and no parasite DNA was detectable in the vaccinated dams.[35,50] Congenitally infected dams did not abort after a mid-gestation challenge but their chronic infection later recrudesced and infected their calves.[29] This suggests that producing a protective vaccination response is much more difficult in congenitally infected animals. Live vaccines present other challenges, including difficulty of production, stability, and concerns over reversion to virulence.[50]

Several approaches to *Neospora* control use whole herd serological testing to determine infection status of every animal. Test-and-cull is the most straightforward but is not economical in most circumstances.[51] A more economical approach is to test the herd, identify the daughters of seropositive cows, and exclude them from the replacement pool. Two computer models identified this as the most economical approach, reducing herd prevalence by half within five years.[51,52] These models do not account for any horizontal transmission.

Embryo transfer technology can preserve the genetics of high genetic merit but seropositive animals. Embryos transferred following the International Embryo Transfer Society protocol into seronegative dams remain uninfected, regardless of donor infection status.[53,54]

Dairy operations can reduce *Neospora* abortions by inseminating seropositive dams with beef breed semen. Crossbreed pregnancies have a more robust placentation with higher levels of peripartum pregnancy associated glycoprotein-1 (PAG-1), which is a marker of placental/fetal well-being. High PAG-1 may represent a placental mechanism reducing maternal rejection, which is increased in crossbreed pregnancy because of increased genetic distance between dam and fetus.[55] Beef breeds in general and the Limousin breed in particular are more resistant to *N. caninum*. In a study conducted in Holstein-Friesians using semen from four beef breeds, abortion rates were reduced by half, and rates in low-titer dams bred to Limousin bulls were the same as those in seronegative animals.[55] Using beef semen also has the advantage of producing hybrid calves that are easily identified for removal and have a higher value for beef production.

Determining the serostatus of each animal at birth is valuable for selection decisions and ultimately for effective and economical control of *Neospora*-induced abortion. Dubey et al and McCann et al note that post-natal infections do not or rarely lead to persistent infections characterized by recrudescence and transmission during repeat pregnancies.[2,24] If animals born seronegative are infected post-natally, they are unlikely to abort due to *Neospora* or transmit the infection transplacentally more than once. Thus, nothing is gained by culling post-natally infected dams after they have aborted or have seroconverted. Congenitally infected animals should be identified and excluded from breeding, or bred only with beef semen in the case of dairy animals. If an attenuated live vaccine becomes available, vaccine costs could be reduced by not vaccinating congenitally infected animals identified through precolostral screening because vaccination is unlikely to be of benefit in them.[29] Elimination of congenitally infected females through precolostral screening and vaccination of naïve heifers before

breeding with an attenuated live vaccine may dramatically reduce losses associated with *Neospora caninum*. More research will be needed to clarify the differences between congenital versus post-natally acquired neosporosis with respect to endogenous transplacental transmission and the efficacy of live vaccines.

Conclusion

Neospora caninum is a highly successful, ubiquitous, intracellular parasite of cattle that is thought to be responsible for up to 20 % of all bovine abortions worldwide, incurring a global annual loss estimated at $1.3 billion. It persists within cattle populations primarily by evading its host's immune system in thick-walled cysts until, by an unknown mechanism, it senses the conditions of second to third trimester pregnancy and recrudesces to cross the placenta and infect a new fetal host. It is challenging to diagnose and control; indeed, there is no indication that our efforts have reduced its prevalence since its discovery.[7] The most successful control efforts involve vaccinating naive animals before gestation with live, naturally attenuated strains of the parasite, and identifying congenitally infected female progeny by precolostral serological testing to exclude them from becoming replacement animals.

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Figure 1. Animals reported as seropositive for *Neospora caninum*

Australian Dingoes	Bali cattle
Beef cattle	Bison
Black tailed deer	Camel
Capybara	Caribou
Cat	Chicken
Coyote	Dairy Cattle
Dog	Egyptian mongoose
Eurasian badger	Eurasian Lynx
European Lynx	Feral cat
Follow deer	Fox
Gaur	Grey wolf
Hare	Iberian Lynx
Jaguar	Kuril harbor seal
Leopard	Lion
Llama	Moose
Mouflon	Mouse
Mule deer	North American opossum
Panda	Pine martin
Pole cat	Puma
Rabbit	Rat
Raven	Red deer
Red panda	Roe deer
Sea otter	Spanish Ibex
Sparrow	Stone martin
Tiger	Vietnam sika deer
Vole	Water buffalo
Water vole	White Rhinoceros
White tailed deer	Yak

Figure 2. Countries reported seroprevalence of bovine neosporosis in the last 5 years

Algeria
Argentina
Australia
Brazil
Canada
China
Czech Republic
Egypt
Germany
Greece
Iran
Latvia
Mexico
New Zealand
Norway
Pakistan
Peru
Philippines
Romania
Spain
Sweden
Thailand
Turkey
United Kingdom
United States of America
Vietnam

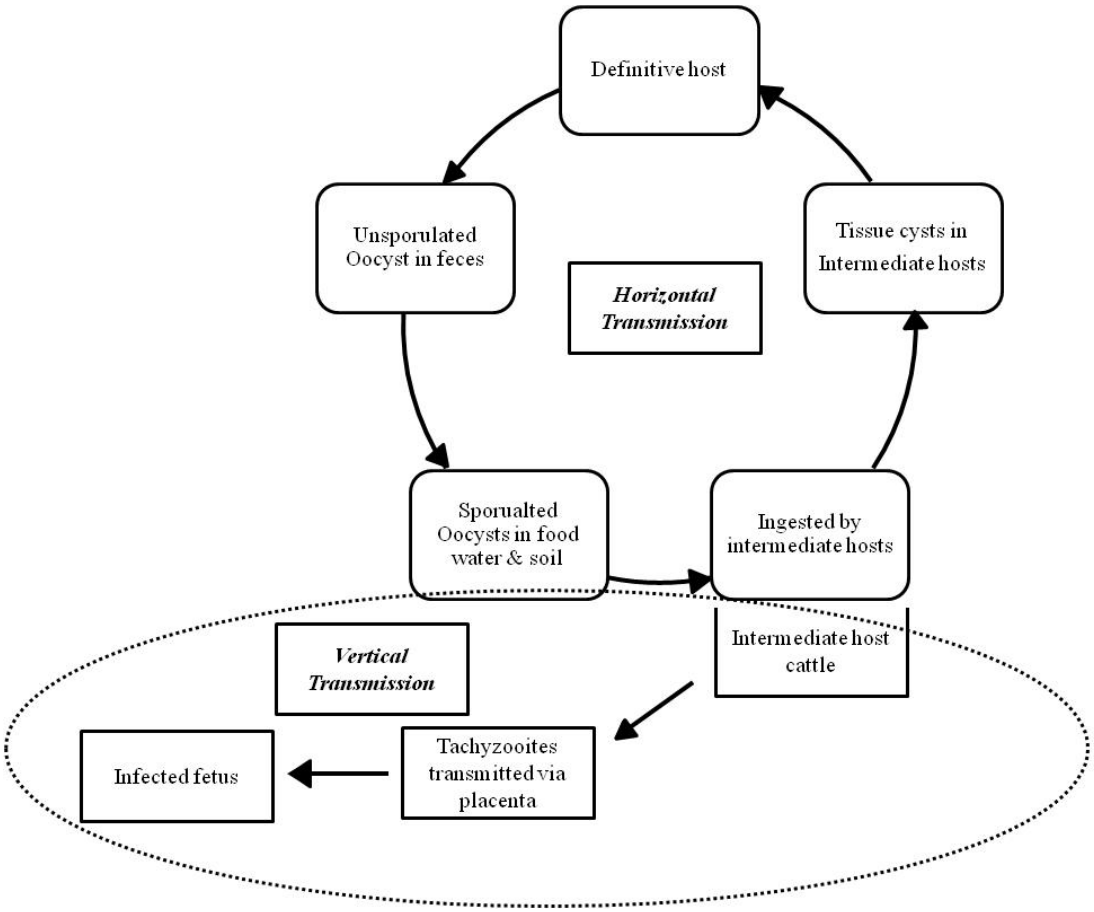

Figure 3. Life cycle of *Neospora caninum*