PEER REVIEWED

Review: *Neospora caninum*-induced Abortion in Cattle

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Abstract

Neospora caninum is a protozoal parasite implicated as a leading cause of abortion in some dairy and beef herds. The bovine is an intermediate host for the parasite, and therefore *N. caninum* is not transmitted horizontally from infected individuals in the herd to non-infected individuals. Transmission can take place vertically from dam to fetus during pregnancy when tachyzoites from maternal tissues enter the bloodstream and cross the placenta to cause fetal infection. Once infected, an animal is considered to be infected for life and a female will pass the infection to a high percentage of her offspring. Although fetal infection in some herds is common, abortion following fetal infection is a fairly rare event, with risk of abortion being greatest in the first pregnancy following infection and decreasing thereafter. Vertical transmission of the parasite results in an endemic abortion pattern within family lines. *N. caninum* can also be passed from the definitive host, the canine, to cattle, presumably via contamination of feed or water with oocyst-infected feces. Canines become infected by consuming *N. caninum*-infected tissue. Horizontal transmission of the parasite results in an epidemic abortion pattern which can cause a high percentage of susceptible females to abort. Laboratory tests are able to identify maternal, fetal, or calf infection with the parasite, but because of the low percentage of abortions in infected dams in endemically-infected herds, predictive value of *N. caninum* infection is low for abortion causation, and statistical tests to attribute risk are needed to infer abortion causation.

Résumé

Neospora caninum est un parasite protozoaire qui représente une cause majeure d'avortement dans certains troupeaux laitiers et de boucherie. Le bovin est un hôte intermédiaire du parasite et par conséquent *N. caninum* n'est pas transmis horizontalement d'un animal infecté à des animaux sains du même troupeau. La transmission peut être verticale de la mère au fœtus durant la gestation lorsque les tachyzoïtes provenant des tissus maternels circulent dans le sang et traversent le placenta causant ainsi l'infection du fœtus. Suite à l'infection, un animal est considéré atteint pour la vie et une vache transmettra l'infection à plusieurs de ses veaux. Bien que l'infection fœtale soit assez fréquente dans certains troupeaux, l'avortement suite à l'infection fœtale est rare et se produit le plus souvent durant la première gestation suivant l'infection devenant plus rare par la suite. La transmission verticale du parasite engendre des patrons endémiques d'avortement dans des lignées familiales. *N. caninum* peut aussi être transmis à partir de l'hôte définitif, le chien, au bovin probablement par la contamination de la nourriture ou de l'eau par des fèces contenant des oocystes. Les chiens deviennent infectés en mangeant des tissus infectés par *N. caninum*. La transmission horizontale du parasite engendre un patron endémique d'avortement qui peut impliquer un grand nombre de vaches à risque. Les tests de laboratoire permettent de détecter l'infection chez les fœtus, les vaches et les veaux. Toutefois, en raison du faible pourcentage d'avortement chez les vaches d'un troupeau infecté endémiquement, la valeur prédictive d'une infection causée par *N. caninum* est faible comme cause d'avortement et des tests statistiques pour attribuer le risque sont nécessaires pour inférer la cause d'avortement.

Neosporosis

Neospora caninum is a protozoal parasite considered a leading cause of abortion in some dairy and beef herds. *N. caninum* is morphologically similar to other apicomplexa protozoal parasites of importance to veterinary medicine, *Toxoplasma gondii* and *Sarcocystis* sp. This parasite was first recognized as a neuromuscular disease in dogs in 1984, and was first reported as a cause of abortion in cattle in the US in 1989.[8,28] However, a later retrospective study indicated that *N. caninum* abortion is not a new or recently emerging infection in cattle.[29] Bovine neosporosis has been identified in many parts of the world, including North America, Europe, Asia, Africa, Australia and South America.[12] A survey of beef herds in the northwest US indicated an overall seroprevalence of 23%, and a within-herd seroprevalence ranging from 2.5 to 67% (median 19%).[25] A study of Texas feeder cattle revealed that 54 of 92 (57.8%) consignments had at least one seropositive animal and 131 of 1,009 (13%) calves were seropositive.[3] *N. caninum* has also been shown to elicit an antibody response in sheep, goats, deer, horses, coyotes, foxes and Australian dingoes from natural infection, and many other species from experimental infection.[2,12,18]

Three morphological stages of *N. caninum* have been identified. Two asexual stages of development occur in the intermediate host, tachyzoite and bradyzoite, and one sexual stage, oocyst, occurs in the definitive host.[22] Tachyzoites actively penetrate cells and divide rapidly until they lyse the host cell, then the newly released tachyzoites infect neighboring cells. They are found intracellularly in many tissues of intermediate hosts and can cross the placenta to cause fetal infection. Bradyzoites are the slowly dividing, dormant phase found only in tissue cysts in the CNS, peripheral nerves and retina of intermediate hosts.[13] Oocysts are the sexual stage found in the feces of the definitive carnivorous host. At this time, dogs are the only proven definitive host for *N. caninum*.[19,22] However, dogs exposed to *N. caninum*-infected tissue do not always seroconvert or excrete oocysts. In one trial, zero of nine young dogs excreted oocysts, seroconverted, or had clinical signs compatible with *N. caninum* after being fed bovine fetuses and placenta naturally infected by *N. caninum*.[7] In another trial, three of three dogs fed placental cotyledonary tissue from *N. caninum* seropositive cows shed *N. caninum* oocysts following the first exposure to the parasite, but subsequent feeding of *N. caninum*-infected placenta to the same three dogs did not result in renewed shedding of oocysts.[11]

Vertical transmission of the parasite from an infected dam to her fetus is considered to be the predominant route of transmission of *N. caninum* in cattle herds, with horizontal transmission from feed or water contaminated by oocysts in feces from a definitive host being a fairly rare event. An infected dam will pass the parasite to the fetus in most (80%), but not necessarily all subsequent pregnancies.[10,23,30,37] Once a bovine is infected with the parasite, it is considered to be infected for life. Despite fluctuations in titers, there is no conclusive evidence to show that serologically positive cows can revert to a consistently seronegative status.[1]

Infected heifer calves that enter the herd will subsequently pass the infection onto most of their offspring.[37] Calves infected *in utero* are typically born alive and without apparent clinical signs.[23,37] The primary clinical sign of *N. caninum* infection in cattle is abortion, which usually occurs at 5 to 6 months of gestation.[1] Aborted fetuses may be autolyzed or mummified.[13] Clinical signs have also been reported in calves that were infected *in utero* and survived to birth that include ataxia, decreased patellar reflex, loss of conscious proprioception, flexed or hyperextended limbs, being underweight, or unable to rise.[13]

Abortion in an infected herd may manifest itself with either an endemic or epidemic pattern. Vertical transmission (dam to daughter) results in sporadic but persistent abortions in infected herds. In beef herds, Waldner found that cows seropositive for *N. caninum* were at significantly greater risk of abortion (odds ratio = 5.7) or having a stillborn calf (odds ratio = 2.8) compared to seronegative cows.[35] Reports from dairy herds also indicate an increased risk of abortion for congenitally infected heifers and cows versus seronegative females.[30,31]

Whether an infected cow aborts due to *N. caninum*-induced fetal death, carries an infected calf to term, or does not infect the fetus is probably determined by a combination of maternal and fetal immune responses.[1] $CD4^+$ T cells, interferon gamma (IFNγ) and macrophages have all been found to significantly inhibit multiplication of *N. caninum* tachyzoites *in vitro*, indicating that cell-mediated immune mechanisms may play an important role in reducing multiplication of *N. caninum* and resulting parasitemia within the host.[14] Evidence suggests that inflammatory responses, such as those induced by IFNγ, at the interface between the placenta and fetus are likely to result in abortion.[24] In mice, these normal responses to infection are decreased during gestation in order to protect pregnancy in the face of inflammation.[9] Therefore, it is theorized that a decrease in a *N. caninum*-infected dam's immune response during pregnancy may cause reactivation of encysted bradyzoites and recrudescence of the infection, resulting in parasitemia of the pregnant animal and in many cases, infection of the fetus.[14]

The timing of *N. caninum* parasitemia during gestation, as well as the quantity and duration of the parasitemia, probably affect the clinical outcome. Parasitemia that occurs earlier in gestation and lasts longer is more likely to damage the fetus than late or short-lived parasitemia.[14] Laboratory analysis has also shown that individual *N. caninum* isolates differ in some DNA segments and in growth rate *in vitro*. These differences may contribute to the range of pathology seen in cattle infected with the parasite.[26]

Abortion is most likely to occur during the first pregnancy following infection, with future pregnancies less likely to result in abortion. Thurmond and Hietala report that in a herd of Holstein dairy cows, 95% of calves born to infected cows were infected with *N. caninum in utero*, and 13.1% of seropositive heifers aborted during their initial pregnancy, compared to 1.9% for seronegative heifers.[31] When that same group of animals was evaluated the following year during the first lactation, congenitally infected cows aborted 19.2% of first pregnancies, whereas seronegative cows aborted 11.5% of first pregnancies. As this group of cows was followed to the second lactation, 14.3% of congenitally infected cows aborted and 7.7% of seronegative cows aborted the first pregnancy during the second lactation.[31] Analysis of attributable risk indicate that the percentage of abortions attributable to *N. caninum* in congenitally infected cows is 84% for the initial pregnancy as a heifer, 41% for the first pregnancy of the first lactation, and 0% for the initial pregnancy during the second lactation.[31] This suggests that some level of protective immunity is developed against abortion in the face of infection. Innes *et al* provided additional evidence for development of some protective immunity when they found that experimental exposure of cows to *N. caninum* tachyzoites six weeks prior to mating provided protection against vertical transmission of the parasite if the cows were exposed to *N. caninum* tachyzoites during mid-gestation.[15]

Most epidemiologic studies indicate that vertical transmission is the major route of transmission within a herd; however, questions still persist concerning the route of entry of *N. caninum* into a herd. While purchased females can introduce the infection into a herd with subsequent generations of those additions being infected and seropositive, this route cannot infect animals already present in the herd because the disease is not contagious. Herd epidemics of neosporosis have occurred in closed herds, and in these and other cases, horizontal transfer by cattle ingesting infective oocysts excreted in the feces of carnivorous definitive hosts that contaminate feed or water is the most likely route of entry.[20,21] In cases of point source, horizontal transmission of the parasite into a herd, an epidemic pattern of abortion (abortion storm) is likely to be encountered.[21,33] An abortion storm consistent with horizontal exposure to an infectious agent occurred in a herd of 208 pregnant beef cows.[20] Seventy-nine percent of the cows were seropositive to *N. caninum* on day 14 of the outbreak and 21% (43/208) of the herd aborted.[20] In a dairy herd with presumptive horizontal exposure to *N. caninum*, 18% (66/360) of the cohort of cows between 110 and 240 days of gestation aborted, and 100% of a subset of 79 cows from that cohort were seropositive to *N. caninum* on day 18 of the outbreak.[21]

In cases of horizontal exposure to *N. caninum*, prior exposure to the organism appears to provide some protection against abortion. McAllister *et al* reported that during an abortion outbreak due to point-source horizontal transfer of *N. caninum*, cows with serologic evidence of previous exposure to *N. caninum* were significantly less likely to abort or give birth prematurely compared to cows with evidence of recent, primary infections.[20] Therefore, both endemic and epidemic patterns of abortion may occur within the same herd following horizontal *N. caninum* transmission. When exposed to a point source of infective oocysts, seropositive animals are at least partially protected from an abortion epidemic but continue to experience an endemic abortion problem, and seronegative animals are likely to experience an abortion storm.[1]

Diagnostic Testing and Strategies

Indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) are the serologic tests used for diagnosis of *N. caninum* infection. Positive serologic tests from pre-colostral blood samples may identify congenitally infected calves; however, lack of anti-*N. caninum* antibodies in pre-colostral sera from fetuses or calves does not rule out *N. caninum* infection. Antibody production by the fetus is dependent on the stage of gestation at which infection is developed, the intensity of exposure and the length of time between infection and abortion or birth.[36] Serologic evidence of exposure to *N. caninum* does not constitute a diagnosis of the parasite as the cause of an abortion. Remember that most *N. caninum*-infected fetuses survive to term and are clinically normal, so finding evidence of *N. caninum* infection in an aborted fetus does not rule out other causes of abortion.

Although fetal or pre-colostral serology is a strong indicator of exposure to the parasite, care should be taken in the interpretation of maternal serology.[14] Antibody titers in naturally infected cows can vary considerably between cows and may fluctuate considerably throughout pregnancy.[27] Despite this fluctuation, a negative serology test from the blood of an aborting cow is good evidence that *N. caninum* is not the cause of abortion, but a positive sample only indicates exposure.

Diagnosis of *N. caninum* infection in aborted fetuses is usually based upon detection of the parasite in aborted fetal tissue fixed in neutral-buffered 10% formalin that has characteristic histopathologic lesions. The parasite is most commonly detected with immunohistochemistry (IHC); however, some laboratories use polymerase chain reaction (PCR) because of its increased sensitivity compared to IHC.[6,14] Fetal brain is the most consistently affected organ, but heart and liver are also commonly affected.[13]

Similar to the evidence obtained from serology, indications of fetal infection only confirm infection, not causation. While IHC and PCR testing for *N. caninum* have fair to good sensitivity and specificity for identifying infected animals (IHC sensitivity = 0.45, specificity = 0.99;[32] PCR sensitivity = 1.0, specificity = 0.94)[6], the positive predictive value for using IHC or PCR to identify the cause of individual abortion during an endemic problem is only 0.08 (92% false positive) or 0.18 (82% false positive), respectively, because many non-aborting animals are also infected.[6,32] The negative predictive value of IHC or PCR testing for *N. caninum* during an abortion endemic is 0.92 (8% false negatives) and 1.0 (0% false negatives), respectively.[6,32] The positive predictive value would be expected to increase and the negative predictive value to decrease during an abortion epidemic due to *N. caninum*.[32]

Strong evidence for *N. caninum* being the cause for an abortion endemic would be demonstration of the parasite in a high percentage of aborted fetuses tested, and evidence of increased risk of abortion in seropositive cows compared to seronegative cows. To establish increased risk of abortion in seropositive cows, the entire herd or a cross-section is used to collect samples for serology from both aborting cows and non-aborting pregnant cows. A Chi-square or Fisher exact test is used to determine whether or not the proportion of seropositive aborting cows differs from the proportion of seronegative aborting cows.

Testing dogs in contact with a herd for infection with *N. caninum* does not appear to be helpful in the control of the disease. Not all dogs that excrete oocysts after experimental infection seroconvert, and many dogs that have been experimentally infected have shed very small numbers of oocysts, making diagnosis by examination of the feces very difficult.[5,17,22]

Control of Vertical Transmission

Vertical transmission of *N. caninum* from dam to fetus is very efficient (>80%) and because few infected fetuses are aborted, the infection can be maintained in the herd over long periods of time without reintroduction of the parasite through purchase of infected animals or horizontal exposure to infective oocysts. There are no proven control methods for the prevention or treatment of neosporosis. Suggested control measures for vertically transmitted neosporosis focus on strategies to reduce the number of congenitally infected animals retained in the herd by culling seropositive animals.[1] In addition, a vaccine has recently been licensed for use in the US, but published field trials demonstrating its efficacy for decreasing the risk of infection from vertical transmission or decreasing the risk of abortion is not available. In addition, use of the vaccine may complicate or eliminate the ability to utilize a test and cull strategy, because the diagnostician may not be able to differentiate between antibody responses elicited by natural infection and vaccination.

When evaluating control strategies to reduce the number of congenitally infected animals in the herd, Larson *et al* showed that the best economic return over a 5-year horizon for beef herds with *N. caninum* seroprevalence ranging from 10% to 70% was to serologically test the herd and exclude the daughters of test-positive dams as replacements (i.e. sell as feeders). This was economically superior to a strategy of culling of all seropositive animals and purchasing seronegative bred replacements.[16] The ethical constraints to sell seropositive animals for slaughter only, and hence cull cow price, limits the economic return for the test and replace strategy because of the large difference in purchase price of seronegative replacements and cull cow prices.

Herds with a biosecurity plan to exclude *N. caninum* should test all purchased replacement females and only allow test-negative individuals into the herd. Herds that do not have any testing or control measures in place to monitor or reduce *N. caninum* prevalence may not benefit from a pre-purchase testing strategy alone.

The commercially available vaccine is likely to provide somewhat less than 100% prevention of vertical transfer of the parasite from an infected cow to her fetus. If field trials demonstrate an efficacy close to 100%, the economic result should be similar to that found by identifying all seropositive females and removing their daughters from the replacement pool. The advantage to the vaccination strategy would be that more heifer calves would be potential replacements. The disadvantages are that the cost of vaccination would be borne every year, and it is unclear whether serologic testing could be used to identify infected cows in herds vaccinated against the parasite. Because actual vaccine efficacy to prevent vertical transmission is not estimated at this time, this strategy cannot be compared against other control strategies until field trials indicate the level of protection that can be expected.

Control of Horizontal Transmission

Exposure of cattle to feed or water contaminated by feces of infected dogs or possibly wild canids shedding oocysts is the most likely source of horizontal transfer of *N. caninum*. All potentially infected tissues (placenta, aborted fetuses, dead infected juveniles or adults) should be removed from the environment and disposed so that carnivores cannot eat them. In addition, feed and water sources should be designed and managed to reduce the chance of fecal contamination by dogs and other potential definitive canid hosts. Decreasing the number of infected cows in the herd and in surrounding herds by using one of the methods described for controlling vertical transmission will reduce the number of placenta, aborted fetuses and carcasses that have *N. caninum* tachyzoite-infected tissue.

In a survey of beef herds in the northwest US, increased *N. caninum* seroprevalence was associated with higher winter stocking density.[25] Because the disease is not contagious, the relationship between higher stocking density and seroprevalence is probably due to more cows coming into contact with the same contaminated feed or water compared to herd exposure when animals are spread over a greater area. A survey of Texas herds showed similar results in that increased risk of exposure to *N. caninum* was associated with increased density of beef cattle and with increased abundance of wild canids (gray foxes and coyotes).[4]

Economic Effect

One study showed that first lactation dairy cows seropositive for *N. caninum* yielded an average of 2.5 lb (1.13 kg)/cow/day less milk than seronegative first lactation cows.[30] If milk production could be negatively affected by *N. caninum* infection, weaning weights of beef calves could be negatively affected by the serologic status of their dams. However, Waldner reported that 64 calves with pre-colostral antibodies to *N. caninum* had an average weaning weight that was not different from 36 seronegative calves (660 vs 666 lb; 300 vs 303 kg).[34] A survey of feeder cattle in Texas found a link between being seropositive for *N. caninum* and average daily gain during the feedlot phase of production. Seropositive calves gained 0.11 lb (0.05 kg) /day less than seronegative calves.[3] This observation merits controlled trials to test whether *N. caninum* serologic status impacts post-weaning growth performance.

Reproductive efficiency, as measured by percentage of exposed cows that wean a calf, is decreased in herds with more abortions. Income is reduced in herds with poorer reproductive efficiency because fewer calves are sold per exposed cow. In addition, herds with a higher culling rate, and hence a higher number of replacement heifers, have added cost associated with developing more replacements and reduced income due to selling fewer market heifers. Larson *et al* report that in a modeled beef herd, return to fixed cost in herds with endemic *N. caninum* abortion losses was reduced by 22.2% (10% seroprevalence) to 29.9% (70% seroprevalence) during a 5-year period of relatively low feeder calf prices and low profit, and by 0.3% (10% seroprevalence) to 8.1% (70% seroprevalence) during a 5-year period of relatively high feeder calf prices and high profit when compared to a non-infected herd.[16]

The economic impact of a *N. caninum* abortion epidemic due to horizontal transmission of infected oocysts can be substantial. It is calculated as simply the total annual cost for each cow that aborts plus expected profit above all costs for each calf not weaned. Assuming no further horizontal exposure, in subsequent years an endemic pattern of abortion will occur in infected cows with a reduction in income related to the prevalence of infection.[16]

Conclusions

Neospora caninum-induced abortion can be a diagnostic and control challenge. In an abortion epidemic, serologic or histopathologic evidence of the parasite's presence is highly suggestive of causation. However, in endemically infected herds, abortion causation is not confirmed by evidence of the parasite's presence in aborted fetuses or aborting females. Because few females that are persistently infected with the parasite actually abort, especially after their second pregnancy, the parasite can be maintained in a herd indefinitely, causing overall reduced reproductive efficiency, particularly among heifers.

Once a herd is diagnosed as having endemic abortion losses due to *N. caninum* infection, veterinarians have several management strategies available to control losses. The most promising strategy is to identify serologically positive animals and sell heifer calves from these individuals as feeders. In addition, a recently available strategy is to utilize the commercially available vaccine with the assumption that abortion losses will decrease and prevalence of persistently infected females will decrease over time.

Because *N. caninum* infection in cattle herds is potentially economically devastating, prevention must be emphasized. In areas shared by infected cattle and canids, veterinarians should institute biosecurity measures to prevent the parasite from contacting client herds. The high cost and long-term negative impact of horizontal *N. caninum* exposure make it imperative that veterinarians who serve cattle clients institute a feed storage protocol that decreases the likelihood for fecal contamination from canids and other animals. In addition, every effort should be made to eliminate contact of canids and other wild animals with aborted fetuses, stillborn calves, placental tissue, and infected juvenile and adult carcasses.

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