Chapter 62

Infectious Agents: Neospora

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Introduction

Neosporosis is caused by the protozoan parasite Neospora caninum, an obligate intracellular parasite found in dogs and cattle as well as other species. True prevalence is difficult to determine since published reports tend to be specific for a particular region. However, it is estimated that Neospora infects 10–20% of cattle and is responsible for 20% of abortions worldwide.\(^1\) This parasite was first described in dogs having encephalomyelitis in Norway\(^2\) in 1984 and was previously misidentified as Toxoplasma gondii until 1988 when the new species, N. caninum, was described.\(^3\) The first report of bovine abortion associated with Neospora infection was from a New Mexico dairy in the United States in 1989.\(^4\) Both domesticated\(^5\) and wild\(^6\) canids can serve as definitive hosts while cattle,\(^7\) deer,\(^8\) and chickens\(^9\) are intermediate hosts. However, there is some doubt that the red fox is truly a definitive host.\(^10\) Although transmission can occur both horizontally\(^11\) and vertically, vertical transmission is the primary source of infection in cattle.\(^12\) The economic impact of N. caninum is the result of numerous factors including abortion, stillbirth, suffered decreased milk production, increased calving interval, and increased veterinary costs.\(^13\) Global losses are estimated at $1.3 billion and in excess of $600 million in the United States annually.\(^14\)

Epidemiology

Neospora caninum has been identified worldwide and is considered a major cause of abortion in cattle.\(^15\) Globally, 15–20% of dairy cattle and 10–20% of beef cattle have been found seropositive for N. caninum.\(^1\) Surveys in California,\(^16\) the Netherlands,\(^17\) and New Zealand\(^18\) indicate that approximately 20% of all aborted bovine fetuses submitted to diagnostic laboratories tested positive for this infection. The estimates of Neospora infection in US dairy cattle, based on serology, are variable but appear to be in the range 10–20% and ranges from 5 to 98% of cows in individual dairy herds. Many animal species test seropositive for N. caninum but do not exhibit clinical signs.\(^1\) The gold standard for identifying infection is recognition of tissue cysts on histological examination, finding oocysts in feces,\(^6\) or identification of the parasite by immunohistochemical staining\(^2\) or polymerase chain reaction (PCR).\(^2\)

Life cycle

The life cycle of N. caninum is depicted in Figure 62.1. Neospora caninum has a facultative heteroxenous life cycle, meaning that the organism may use more than one host during its life cycle.\(^2\) Unsporulated oocysts (10–14 μm) are shed in the feces of definitive hosts (i.e., dog or wild canid).\(^4\) The oocysts sporulate within 24 hours to the infective form. Each sporulated oocyst contains two sporocysts, and each sporocyst contains four sporozoites. When consumed by cattle or a wide range of other warm-blooded animals which can serve as intermediate hosts, eight sporozoites are released into the gastrointestinal tract for each oocyst consumed.\(^1\) The sporozoites differentiate into tachyzoites (5–7 μm), which subsequently invade the epithelial cells of the gastrointestinal tract. The tachyzoites replicate rapidly via asexual endodyogeny.\(^2\) Endodyogeny is a form of asexual reproduction that involves an unusual process where two daughter cells are produced inside a mother cell, which is then consumed by the offspring prior to their separation.\(^2\) Replication occurs rapidly within cells and tachyzoites may spread hematogenously within mononuclear phagocytes\(^2\) to infect many cell types including neural cells, vascular endothelial cells, myocytes, hepatocytes, renal cells, alveolar macrophages, and placental trophoblasts.\(^2\) Tachyzoites differentiate into bradyzoites which are the slowly replicating encysted stage of the parasite. Each cyst may contain hundreds of bradyzoites.\(^2\) In congenitally infected bovine fetuses and calves, tissue cysts are found in the brain and spinal cord.\(^3\) A few thin-walled cysts have been found in skeletal muscle of two naturally infected 2-day-old calves.\(^3\) Bradyzoites are thought to persist for the duration of the host’s life and be responsible for persistent infection, although tissue cysts have not been observed in histological sections of naturally infected cattle older than 2 months.\(^2\) Bradyzoites are believed to reactivate and...
differentiate back into tachyzoites, thereby establishing persistent infection. Bradyzoites can be differentiated in tissue sections from tachyzoites by labeling with a specific antibody. The cycle continues when tissue cysts shed by the intermediate host (cattle) are consumed by the definitive host (canids).

Transmission

*Neospora* can be transmitted vertically or horizontally with both routes being vital to long-term survival of the parasite. Vertical transmission takes place when tachyzoites migrate transplacentally from dam to fetus during pregnancy. *Neospora caninum* is one of the most efficiently transplacentally transmitted parasites among all known microbes in cattle. The terms “endogenous transplacental transmission” and “exogenous transplacental transmission” have been used to more precisely describe the origin and route of fetal infection (Figure 62.2). Endogenous transplacental transmission occurs in a persistently infected dam when the infection crosses the placenta and enters the fetus. The endogenous form has a higher rate of associated abortion. Exogenous transplacental transmission occurs when a previously noninfected dam ingests infective oocysts while pregnant and her fetus subsequently also becomes infected *in utero*.

Vertical transmission can result in abortion but in most cases the calf is congenitally infected but asymptomatic with no evidence of deleterious effects on subsequent calf health. If the infection occurs during the second or third trimester when immunocompetence of the fetus is greater, the most likely outcome will be a liveborn but persistently infected calf. Once infected, cattle are presumed to be infected for life and females can transmit the infection to successive generations. Although not every pregnancy results in transmission of the disease, it has been reported that the rate of transmission is as high as 75–100%. In the case of exogenous transplacental transmission, the number of oocysts ingested by the dam and stage of gestation influence pregnancy outcome. There is a report indicating that in persistently infected cattle, vertical transmission is more efficient in younger than older cows. Also, transplacental infection may be more likely to occur in dams that were themselves prenatally infected compared with postnatally infected dams. The risk of abortion was positively correlated with *N. caninum*-specific antibodies.

in individual animals\textsuperscript{42} and severity of fetal Neospora-associated lesions also increased with higher maternal sero-positivity.\textsuperscript{43} Cows with high antibody titers (≥400) showed higher vertical transmission frequency (94.8%) than cows with low antibody titers of 25–200 (14.8%).\textsuperscript{44} Occasionally, a seronegative dam will give birth to a seropositive calf.\textsuperscript{45,46} This can occur when the dam has a long-standing infection with low antibody titer and she was wrongly diagnosed as uninfected.\textsuperscript{1}

Horizontal transmission is less common than vertical transmission.\textsuperscript{16} The ingestion of sporulated oocysts from the environment is the only demonstrated natural mode of postnatal infection in cattle (Figure 62.2).\textsuperscript{47,48} Both domestic and wild dogs are frequently implicated in horizontal transmission. This is attributed to canids consuming infected bovine placentas or fetuses and subsequently defecating in the environment where the oocytes are ingested by cattle.\textsuperscript{49} There has been no horizontal cow-to-cow transmission reported at this time.\textsuperscript{37} Experimentally, calves may become infected through ingestion of milk contaminated with tachyzoites\textsuperscript{50,51} and \textit{N. caninum} DNA has been detected in colostrum of seropositive cows.\textsuperscript{32} However, it remains doubtful that lactogenic transmission occurs naturally.\textsuperscript{49}

Since \textit{N. caninum} DNA was reported in fresh and frozen semen from naturally infected bulls,\textsuperscript{39} the possibility of venereal transmission was investigated. Intrauterine inoculation of nine heifers with \textit{10^7} \textit{N. caninum} tachyzoites reacted with seroconversion and a specific interferon (IFN)-γ response. Also, \textit{N. caninum} DNA was demonstrated in the blood and tissues of all nine heifers.\textsuperscript{54} However, the numbers of tachyzoites used to test the venereal route of infection are higher than those previously found in semen of naturally infected bulls, which range from 1 to 10 organisms per milliliter associated with the cell fraction of semen.\textsuperscript{55,56} Therefore, it seems unlikely that venereal transmission is significant under natural conditions.

**Pathogenesis of abortion**

Neosporosis is primarily a disorder of the fetoplacental unit that occurs subsequent to maternal parasitemia resulting either from exogenous or endogenous infection.\textsuperscript{23} In the case of exogenous infection, the ingested oocytes excyst in the small intestine and presumably release sporozoites which parasitize the maternal intestinal epithelium and transform into tachyzoites which, in turn, multiply in the mesenteric lymph nodes.\textsuperscript{23} The tachyzoites are released into the blood and have been detected in the leukocyte fraction\textsuperscript{27} of naturally infected cattle. The parasitemia results in dissemination throughout the body including the gravid uterus. The endogenous (vertical) route of transmission is more common.\textsuperscript{23,57} It is suspected that latent infections are reactivated due to the immunosuppression associated with mid-gestation pregnancy.\textsuperscript{58} It is also reported that endogenous transplacental transmission is more likely in cattle that were themselves infected \textit{in utero}.\textsuperscript{59,60}

The mechanism by which \textit{N. caninum} actually causes abortion is a topic of ongoing investigation and multiple hypotheses have been proposed. First, since the parasite is able to establish itself in the caruncular septum before crossing to the fetal placental villi,\textsuperscript{61,62} the resultant placental damage may result in fetal death due to placental insufficiency.\textsuperscript{21} Placental insufficiency, because it causes fetal stress

![Figure 62.2 Transmission of bovine neosporosis. Oocysts are produced by the canine definitive host and their subsequent ingestion by a susceptible pregnant cow leads to infection of the fetus (exogenous transplacental transmission). Liveborn infected heifer calves would be expected to remain infected into adulthood when they, in turn, may pass infection to their fetus (endogenous transplacental transmission). Spread of \textit{N. caninum} in this second way is the principal route whereby the parasite is propagated in a herd. Adapted with permission from Dubey JP. Neosporosis in cattle. Vet Clin North Am Food Anim Pract 2005;21:473–483.](image-url)
and release of adrenocorticotropic, could also cause premature delivery of either viable or compromised calves. Second, infection by *N. caninum* stimulates cell-mediated immune responses associated with cytokine release from the damaged placenta, resulting in fetal rejection by the maternal immune system culminating in fetal expulsion. Finally, the placental damage may result in endometrial prostaglandin (PG)F₂α release causing luteolysis and abortion as has been demonstrated in goats with toxoplasmosis. However, cows with high *Neospora* antibody titers have higher plasma progesterone levels throughout gestation than seronegative cows or cows with low antibody titers; in a study in which exogenous progesterone was administered during the mid-gestation period, the risk of abortion increased dramatically in cows with high levels of antibodies against *N. caninum*. It seems most likely that all three of the proposed mechanisms play a part in inducing *Neospora*-associated abortion in cattle.

The first-trimester fetus seems particularly susceptible to damage by invading *N. caninum* since it is not immunocompetent and unlikely to survive infection. In the middle trimester the fetus may be able to mount an immune response to *N. caninum* challenge, although the response may not be robust enough to preclude fetal death. By the third trimester the fetus is capable of mounting an immune response of sufficient magnitude that it is likely to survive *N. caninum* infection, leading to birth of clinically normal but infected calves. In beef cows experimentally infected with *N. caninum* at various times during gestation by either subcutaneous or intravenous routes, transplacental infection rate increased with gestational age and cows infected during later gestation generally delivered normal but infected calves.

In cows experimentally infected with *N. caninum* the most severe lesions are found in the placenta and the fetal brain. Lesions consist of a nonsuppurative inflammatory response. The associated inflammatory cells have been shown capable of producing INF-γ, which suggests fetal death may be more a consequence of the maternal immune response rather than direct effects of the parasite. At the time of placental invasion the parasite also enters the fetal bloodstream with a predilection for the central nervous system, where it is located perivascularly, and in younger fetuses causes widespread destruction of neuropil with very little inflammatory response. When fetuses later in gestation become infected with *N. caninum* the tissue reaction is more moderate, with areas of necrosis restricted to small foci surrounded by intense inflammatory infiltrate in brain tissue and sometimes, mild meningitis. *Neospora caninum* fetal infection also causes characteristic lesions of inflammation and necrosis in tissues such as liver and heart.

**Clinical signs**

*Neospora caninum* infection causes abortion in both beef and dairy cattle. Abortion may occur from 3 months of gestation to term but typically occurs at 5–6 months of gestation. Possible outcomes of infection include fetal death followed by resorption, mummification, autolysis, stillbirth, born with clinical signs, or born clinically normal but persistently infected. Fetuses dying in utero between 3 and 8 months are usually expelled with evidence of moderate autolysis while those dying before 5 months of gestation are more likely to mummify. Experimental infection of pregnant cows at various points during gestation showed that parasitemia during the first 10 weeks resulted in fetopathy and resorption of the fetal tissue 3 weeks after infection. Infection at 30 weeks of gestation resulted in birth of asymptomatic congenitally infected calves. The period in between appears as most likely for *N. caninum*-induced abortion.

In *Neospora*-infected calves clinical signs have been reported only in animals less than 2 months of age. Infected calves may be born underweight, unable to rise, and with neurologic signs. Hindlimbs and/or forelimbs may be flexed or hyperextended and the calf may be ataxic, have diminished patellar reflexes, and loss of conscious proprioception. Exophthalmia has been observed in some cases.

Within herds, *Neospora*-associated abortions may be sporadic, enzootic, or endemic. In comparison with endemic abortion, epidemic abortion storms are less common. It appears that most sporadic and endemic abortions in cattle due to neosporosis are the result of reactivation of chronic infections because *Neospora*-seropositive cows are at twofold to threefold higher risk of abortion than seronegative cows. Reactivation of a chronic *Neospora* infection may occur as a result of factors that cause immunosuppression. We have observed a *Neospora*-associated abortion storm in a dairy herd concomitant with an outbreak of coli-form mastitis. In herds with endemic *Neospora*-associated abortions there is often a positive correlation between serostatus of the dam and her daughters, indicating that the major route of transmission is vertical.

Epidemic abortion outbreaks (abortion storms) have been defined as those in which over 10–15% of at-risk cows abort within a 4–8 week period. There is epidemiological evidence that epidemic *Neospora*-associated abortions may be caused by the postnatal infection of naive cattle via exposure to oocyst-contaminated feed or water, with up to 57% of pregnant dairy cows aborting over a few weeks to months. However, in an epidemiological investigation of abortion storms in the Netherlands, the authors concluded that the abortion storms appeared to be induced by factors causing recrudescence of *N. caninum* infection in chronically infected animals rather than being the result of a recent introduction.

**Diagnosis**

Definitive diagnosis of neosporosis is challenging and relies on numerous diagnostic techniques. There are serologic assays available to test the maternal serum and fetal fluids (serum, fluid from body cavities) for *Neospora*-specific antibodies. Serology of fetal fluids is useful after 5 months of gestation as the fetus becomes immunocompetent but sensitivities reported varied from 50 to 84%. Immature fetal immunocompetence and a short interval between infection and death can all lead to a false-negative result. Finding the antibodies does not definitively confirm *Neospora* as the cause of abortion since clinically normal calves may have congenital antibodies.

Serology of the dam can aid in diagnosis of *Neospora*-associated abortion. Sera obtained within 2 weeks of abortion generally have the highest titers and then titers
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decline and may be undetectable 60 days after abortion.88,90 Seropositive cows do not display detectable clinical signs of infection and must deliver healthy congenitally infected calves. Therefore, seropositivity has a high predictive value for congenital infection in cows but not for abortion91 (although there is an increased probability of abortion of twofold to threefold compared with seronegative cows92). High antibody levels persist for prolonged periods in some cows90 or may increase again in subsequent gestations.89 Seronegativity in a mother cow or heifer does not exclude N. caninum-associated abortions.92

PCR has also been utilized to detect parasite DNA,93 but again it is not always found.28 In a Swiss study, N. caninum was detected by PCR in the brains of 21% of all aborted fetuses. Microscopic lesions indicative of cerebral protozoal infection were detected in 84% of PCR-positive fetal brains.92

Histopathology of fetal tissues can be examined to identify characteristic lesions and parasites.74 Multifocal inflammation and necrosis of the heart, brain, liver, and skeletal muscle are common lesions and tachyzoites are found primarily in the brain.18,89 Lesions can be found in the placenta but are not particularly valuable.89 Even in grossly autolyzed or mummified fetuses it is possible to find suspect lesions, especially in the brain.89 On necropsy, gross lesions are not frequently seen.

To positively identify parasites associated with lesions, immunohistochemistry is commonly employed.17,31 In one study, in which N. caninum tachyzoites were identified by immunohistochemistry performed on tissues taken from calves with confirmed neosporosis, 85% of the brains were positive, 14% of the hearts, and 26% of the livers.18 Significant differences between epizootic and sporadic abortion cases with regard to positivity on immunohistochemical examination were found only in the liver, where tachyzoites were more frequently found and in higher numbers in epizootic cases than from sporadic cases.31 One must bear in mind that positive results of any diagnostic tests without the presence of life-threatening lesions are insufficient for a definitive diagnosis.28

In investigations of herds in which multiple abortions occurred, a tentative diagnosis can be supported by cross-sectional or herd serology in which the N. caninum status of aborting and nonaborting cows are compared to determine if there is an association between serologic status and abortions.90,95 In the case of an abortion storm, a suggested protocol would be to immediately take blood samples from all animals at risk, then take post-abortion samples to determine if seroconversion occurred.89

Control/prevention

A number of approaches have been employed to control neosporosis in cattle herds. These include improving farm biosecurity, test and cull programs, test and exclude from breeding, and artificially inseminating with beef semen. Biosecurity may prove beneficial to avoid introducing the parasite into a closed herd not already infected with Neospora.26 Farm management practices to reduce N. caninum infection can include (i) minimizing fecal contamination of cattle feed or water by canids,1 (ii) prompt removal of aborted bovine fetuses and fetal membranes; and (iii) limiting the introduction of infected cattle into the herd and culling infected animals. If employing the test and cull method of control, every animal must be serologically tested and those that test positive removed from the herd. Test-negative animals should be periodically retested since titers tend to wax and wane and there is a possibility of horizontal transmission resulting in new infections. This approach can be devastating depending on how many in the herd test positive. Notwithstanding the risk of horizontal transmission, a more economic approach would be to test each animal and exclude the daughters of seropositive cows from the replacement pool. If a cow is very valuable there is always the opportunity to preserve the genetics by embryo transfer into a seronegative dam.97

It is possible to reduce the risk of Neospora abortions in dairy cattle by inseminating seropositive dams with beef breed semen. This is effective because crossbreed pregnancies have a more robust placenta with higher levels of peripartum pregnancy-associated glycoprotein, which may have protective value.98 Beef breeds in general, and the Limousin breed in particular, are more resistant to Neospora infection than are dairy breeds.99 Beef cow-calf herds that manage their cows on range for summer grazing have lower seroprevalence than those that do not, while increased seroprevalence is associated with higher winter stocking density.99

These control methods might not be economically or practicably feasible on dairies or beef cattle operations.100 Buying replacements originating from seronegative herds can mitigate the risk of introducing infected replacements into a negative herd.101 General management efforts to reduce stress from concurrent disease, environmental and social stress, and providing an adequate wholesome ration may reduce immunosuppression and hence abortion rates.

There is accumulating evidence that some N. caninum-infected cows can develop a degree of protective immunity against abortion and/or congenital transmission, indicating that immunoprophylaxis to prevent abortion or congenital transmission is a feasible goal.65 A commercial vaccine is not currently available. In the future, vaccine development is likely to depend on identification of specific Neospora genes that may enable the production of genetically engineered vaccines.

Treatment

Currently, there are no approved practical chemotherapeutic compounds useful for treatment of bovine neosporosis.16 Various antimicrobial agents have been tested in vitro102–104 or in vivo in mice,105 but there is no known drug that can be used to clear N. caninum infection in adult cattle. However, when toltrazuril was administered to congenitally infected newborn calves the infection was eliminated, suggesting that vertical transmission in subsequent generations may be reduced by treatment of calves soon after birth.106

References


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