Neosporosis in Cattle

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Introduction

Neosporosis is a newly recognized protozoal infection that has a worldwide distribution and is reported to be a major cause of abortion in North America, Europe and New Zealand. An important feature of the disease is that the parasite can be maintained in cattle as a chronic, apparently lifelong, infection which can be passed on to the fetus during pregnancy. In some cows this fetal infection may result in an abortion. However, most infected cows give birth to a healthy, but congenitally infected calf. A heifer calf that is born congenitally infected is capable of transmitting the infection on to the next generation when she becomes pregnant, thus maintaining the infection in the herd. Diagnosis of the infection is assisted through the examination of aborted fetuses and serologic testing of cattle.

This paper is intended to provide the bovine practitioner with current information about neosporosis in cattle. Several review papers and book chapters are available for additional information.[1,3,23,30,55]

The protozoan parasite *Neospora caninum* was first identified in dogs with encephalomyelitis and myositis.[13,14,21,22] It has been associated with disease in various species of livestock including cattle, sheep, goats and horses. In cattle, the parasite has been isolated from aborted fetuses and congenitally infected calves.[10,23] The pathogenic potential of these isolates has been confirmed by experimental infection of pregnant cattle resulting in fetal death and/or congenitally infected calves.[19,23] These isolates have been used in the development of a variety of serodiagnostic tests.[12,16,20,23,24,29,37,39,41,45,58,61]

Bovine neosporosis has emerged as an important reproductive disease since its first association with an abortion storm in 1987 on a dairy in New Mexico.[46] Numerous reports of *Neospora* abortion have confirmed this infection as a significant cause of abortion, particularly among dairy cattle.[2,7,18,23,25,26,36,45,46,47,48,55] It has a worldwide distribution, having been diagnosed in many countries from six continents, and is prevalent throughout North America. Bovine neosporosis is probably not a new disease, but rather a newly recognized one. Retrospective studies in California have confirmed that the parasite has been endemic since 1984, and a decade earlier the infection was identified retrospectively in a stillborn calf in Australia.[23] Surveys in California, the Netherlands and New Zealand indicate that approximately 20% of all aborted bovine fetuses submitted to the diagnostic laboratory are diagnosed with this infection. In abortion submissions from dairy herds with a history of *Neospora* abortion, the proportion of *Neospora* infection in aborted fetuses is as much as 44%.[4] Since fetal infection can result in either fetal death or the birth of a live congenitally infected calf, there are questions about what factors determine the clinical outcome of fetal infection and what are the appropriate methods to diagnose *Neospora* abortion. This will be discussed.

Clinical Presentation

There are no clinical signs in cows that abort due to *Neospora* infection. The aborted fetuses are usually autolyzed with no gross lesions and placentas are not retained. Abortions have been diagnosed in both heifers and cows from three months' gestation to term, but the majority of *Neospora* abortions occur in the second trimester of pregnancy. This pattern of mid-gestation abortion is distinctive from other diagnosed causes of infectious abortion in dairy cattle which tend to occur later in gestation. Whether *Neospora* infection can cause reproductive problems in the first trimester of gestation is unknown. Fetal mummification has been associated with *Neospora* outbreaks. *Neospora* abortions occur throughout the year.

Neospora infections associated with abortion and congenital infections have been reported in both dairy and beef cattle. There are more reports attributing significant numbers of abortions in dairy cattle, particularly those in dry-lot dairies. The reasons for this are not certain. It is likely that the mid-gestation fetuses typical of *Neospora* abortion would more easily be found and submitted for diagnosis in the dry-lot dairy environment. Alternatively, it may be that the environment of the dry-lot dairy is more conducive to the spread and transmission of this disease. The serologic evidence of *Neospora caninum* infection in dairy herds in the U.S. ranges from 5% to 98%. Overall, the seroprevalence in the U.S. is estimated to be approximately 45% in dairy cattle and about 5% in beef cattle, based on submissions to the California Veterinary Diagnostic Laboratory

(Hietala, personal communication 1999). The estimated seroprevalence in dairy cattle in England and Wales is 6% (Trees, Vet. Rec. letter, 1998) and a similar level of seroprevalence was identified in New Zealand cattle (Reichel, N. Z. Vet. J, letter, 1998). A survey of Canada beef cattle found a seroprevalence of 30%.[57]

Cattle with serologic evidence of infection have an increased risk of abortion.[35,43,52,57,60] In a California study, seropositive congenitally infected cows had a 7.4 increased risk of abortion in their first pregnancy. The risk in the second pregnancy was considerably lower, though this may have been influenced to some degree by selective culling of aborting cows from the first pregnancy.[52] In the Netherlands, a 3-fold increase in abortion risk was associated with seropositive cows and compared to seronegative herdmates.[60] In addition to abortion and congenital infection, *Neospora* infection may cause reduced milk production and shortened production life based on a study of seropositive cows which produced less milk and were culled earlier than seronegative herdmates.[50,51] Seropositive beef cattle have an increased risk of abortion and stillbirth, in addition to increased risk of culling for any reason and culling for poor reproductive performance.[57]

Two patterns, endemic abortion and epidemic abortion, have been described in association with neosporosis in herds of cattle.[55] In the endemic pattern of abortion, the herd experiences an elevated abortion rate of greater than 5% per year which persists for years. In investigations of two California dairies with endemic *Neospora* abortions, the annual abortion rate attributable to neosporosis in these herds was estimated to be 10.6% and 17.3%.[53] The epidemic pattern of abortion is less common and is characterized by abortions in a high proportion of pregnant cattle over a relatively brief period of time. In some instances, over 30% of pregnant cattle have aborted due to neosporosis within several months.[48] An apparent mixture of these patterns may be observed in some herds that have experienced a prolonged history of sporadic cases of *Neospora* abortion and occasional outbreaks of abortions attributable to *Neospora*.

In most instances, cows that abort a *Neospora*-infected fetus will have either additional abortions,[4,28] or infected fetuses in subsequent pregnancies.[9,23] The clinical outcome of these subsequent pregnancies is variable but a seropositive cow that abortions has an estimated 2-fold to 5.7-fold greater risk of abortion in subsequent pregnancies.[35,52]

An uncommon manifestation of fetal *Neospora* infection is the birth of a clinically affected full-term calf which exhibits variable CNS signs manifested as limb dysfunctions, ranging from mild proprioceptive defects to complete paralysis. Microscopically there is a multifocal protozoal encephalomyelitis which may be particularly localized in the spinal cord gray matter.[9,23]

The majority of calves that acquire a *Neospora* infection during gestation are born clinically normal. These calves will have a high precolostral antibody titer to *Neospora caninum* which is useful in detecting *in utero* infection. A high percentage, 80% to over 90%, of calves born to seropositive cows are congenitally infected based on serology.[9,42,45,55] As will be discussed in the section on transmission, these clinically normal, congenitally infected calves are important in maintaining the infection in the herd.

Diagnosis

The confirmation of *Neospora* infection as the cause of abortion will require the assistance of a veterinary diagnostic laboratory. The preferred samples in cases of abortion include 1 or more aborted fetuses submitted with placenta and serum from the dam. The aborted fetus is usually autolyzed with serosanguinous fluid accumulation in body cavities. Rarely there are subtle gross lesions, consisting of pale white foci in the skeletal muscles or the heart. Histologic lesions consist of widespread nonsuppurative infiltrates. The most diagnostically significant lesions are found in the brain and consist of scattered foci of nonsuppurative cellular infiltrates with occasional foci of necrosis. Protozoa are not usually seen on routinely stained slides. Other histologic lesions that are consistently found include nonsuppurative epicarditis and/or myocarditis, focal nonsuppurative myositis and nonsuppurative portal hepatitis, frequently with focal hepatic necrosis and focal nonsuppurative interstitial pneumonia.[3,7]

Immunohistochemistry using antibodies raised against *Neospora caninum* antigens is an effective method employed by many diagnostic laboratories to identify the tachyzoite and tissue cyst stages of the parasite in fetal tissues. *Neospora* immunohistochemistry is most successful on sections of fetal brain, although the parasites also are frequently present in the lung, kidney and skeletal muscle. Immunohistochemistry has been successfully employed to diagnose *Neospora* infections in mummified fetuses, although the autolytic state of these fetuses diminishes the diagnostic accuracy.

The use of pathology and immunohistochemistry on aborted fetuses to establish a diagnosis of *N. caninum* as the cause of an abortion has been questioned since the fetus can be infected and not abort due to the infection.[54] The answers to what determines whether a fetus will live or die and how to accurately diagnose the cause of abortion requires an understanding of the parasite and fetal ontogeny. *Neospora caninum* is a relatively weak pathogen that is adapted to cattle and utilizes vertical transmission: in the birth of a live infected calf, rather than abortion, helps maintain the infection in the herd.

Immune-competent animals or fetuses that become infected are likely to develop subclinical infection or overcome the infection. In cattle, fetal immune competence is first detectable at about 120 to 150 days gestation. Fetuses that acquire the infection prior to sufficient development of an immune response become overwhelmed with disseminated infection, resulting in death. These deaths occur in fetuses primarily through the sixth month of gestation, after which the number of *Neospora caninum*-induced fetal deaths is greatly reduced. These disseminated inflammatory lesions occur in the brain, lungs, heart, liver, kidney, muscles, placenta and other organs. Fetal death is presumed to be primarily related to the placental and heart lesions. A lack of fetal resistance to disseminated infection is responsible for the predominance of abortions and fetal mummification that occurs in mid-gestation.

To increase the likelihood of an accurate diagnosis of *Neospora* abortion, the diagnostician must take into consideration the following factors prior to establishing the infection as the cause: gestational age and postmortem condition (autolyzed); presence of compatible disseminated inflammatory lesions; presence of detectable parasites with immunohistochemistry; and lack of other abortifacients. Conversely, a *Neospora caninum*-infected aborted fetus with mild focal lesions (usually consisting of focal encephalitis in late-term fetuses) may have an incidental *Neospora* infection, and other causes for the abortion should be investigated.

A variety of serologic tests are available to assist in the diagnosis of neosporosis. These include the indirect fluorescent antibody test (IFA), the modified agglutination test, and a number of enzyme-linked immunosorbent assays (ELISA).[12,16,20,24,26,27,28,29,37,39,41,58,61] The assays utilize *Neospora caninum* tachyzoites or specific derived antigens. The specificity and sensitivity of the various serologic tests are comparable, depending on the minimum antibody titer that has been established as the cut-off for a positive result. Laboratories utilizing any of the serologic tests for *Neospora* should establish appropriate cut-off titers using sera from known infected and noninfected cattle. In some tests, the positive cut-off titer has been selected based on the antibody titer in a cow that has aborted an infected fetus. Thus, this cut-off may not be the most appropriate for the serologic diagnosis of a chronic infection in cattle which vary in age and pregnancy status.[45]

A single serum sample from an individual cow may not accurately reflect her infection status, since titers in known positive cattle fluctuate and may fall below the cut-off value for some period of time. In rare instances, cows that abort a *Neospora*-infected fetus may not have a significantly elevated titer. Also, elevated titers at the time of abortion may decline over several months following abortion.[20] A portion of congenitally infected heifers with a history of positive titers, which had infected their fetuses, had titers below the cut-off at the time of parturition.[5] There is no conclusive evidence to demonstrate that a seropositive cow can revert to a consistently seronegative status.

Neospora serology is effective in detecting elevated *Neospora* antibodies in the serum of congenitally infected or *in utero*-exposed calves.[9,23] In addition, serology may be useful in establishing the diagnosis in aborted fetuses, since infected fetuses 6 months or more in gestation may have elevated *Neospora* antibody titers. Recent work suggests that the *Neospora caninum* IFA test is the most accurate method to detect fetal antibodies.[11,59] However, a negative fetal *Neospora* IFA titer does not rule out the possibility of infection, and a positive titer does not prove that this infection was the cause of the abortion.

An ELISA for detection of *Neospora* antibodies has been developed and is used for routine diagnostic testing at the California Veterinary Diagnostic Laboratory as part of the bovine abortion serologic panel.[41,55] This procedure is rapid, inexpensive and has excellent sensitivity (88.6%) and specificity (96.5%). Cutoff values have been established for the ELISA by which the probability of infection can be estimated in cattle. In the individual aborting cow, a positive serology result does not prove that the abortion was due to neosporosis, but it can assist the diagnosis. In addition to its use in the routine abortion screen in individual abortion cases, the ELISA test is used on a herd basis. The test can be used to estimate herd seroprevalence of *Neospora* infection, investigate the association between seropositivity and abortion, and estimate the extent of herd infection attributable to congenital infection. It isn't clear whether the titer in the individual cow can be used to predict the outcome of an ongoing pregnancy, but cows with high antibody levels at 180 and 210 days' gestation were less likely to abort than cows with low antibody titers at these times.[43]

Transmission

The ways cattle acquire *Neospora* infection are under investigation. Forms of the parasite that have been identified in infected fetuses and calves are the tachyzoite and tissue cyst stages. Tachyzoites can spread through the body and invade the cells of a variety of organs. The tachyzoite stage is associated with inflammation and necrosis at the site of invasion. The tissue cyst stage, containing multiple bradyzoites surrounded by a thick cyst wall, is found in neural tissues. The tissue cyst elicits minimal inflammatory reaction and can persist for long periods of time.

There are several ways cattle may acquire *Neospora* infection, either by horizontal (postnatal) infection or by vertical transmission of the infection transplacentally during pregnancy. The horizontal

transmission model is based on the fact that *Neospora* is similar and related to other apicomplexan coccidia, such as *Toxoplasma gondii* and *Sarcocystis* spp. The life cycle of this family of protozoa parasites requires two hosts: a carnivorous predator is the definitive host and a prey species is the intermediate host. For example, in toxoplasmosis, the cat is the definitive host and can acquire the infection through ingestion of parasites contained in the prey species. *Toxoplasma gondii* parasites undergo sexual replication in the intestine of the cat and oocysts are shed in the feces. These *Toxoplasma gondii* oocysts are capable of infecting a wide variety of animal species (intermediate hosts). Among livestock species, toxoplasmosis is a significant cause of abortion in sheep and goats, though not in cattle.

The taxonomic and morphologic similarities between *Neospora* and *Toxoplasma* support the hypothesis that the cow may be infected through oral ingestion of coccidial oocysts shed from a carnivorous definitive host. The identification of this proposed definitive host has been difficult. Previous studies on dogs, cats, rats, mice, raccoons and various bird species had confirmed no role for these species in transmission of *Neospora* to cattle.[6,23] However, recently McAllister, et al were successful in transmitting *Neospora* infection from experimentally infected mice to puppies, which shed oocysts in their feces.[34] Experimental transmission of the infection from the dog to cattle has not yet been reported, but the results of this study strongly suggest that the dog is a definitive host. There is also epidemiological evidence which suggests that dogs may play a role in the transmission of neosporosis. Paré, et al found an association between the herd seroprevalence of *Neospora* infection and the presence and number of dogs at the herd.[44]

Although there are close similarities between *Neospora* and *Toxoplasma,* there are differences between neosporosis in cattle and toxoplasmosis in sheep. In sheep toxoplasmosis, when the infection is acquired during pregnancy, the ewe seroconverts to the parasite and fetal infection and/or abortion may occur. In subsequent pregnancies the ewe is resistant to infection. However, neosporosis in pregnant cattle differs because the cow does not need to acquire *Neospora* infection or seroconvert during pregnancy for her fetus to become infected. Unlike toxoplasmosis, cows that abort in one pregnancy are susceptible to repeat infection and are at an increased risk of repeat abortion.

Vertical transmission of *Neospora* through generations of cattle appears to be the major method by which *Neospora* infection is maintained in herds.[5,15,40,42,43,45,53,55,60] In a Swedish study, the role of congenital transmission of neosporosis was supported by evidence of the familial distribution of seropositive cattle through successive generations. In a German study, 93% of the descendants of seropositive cows were also seropositive, indicating that vertical transmission was the major method of transmission of infection in the herds examined.[45] In California dairies, several serologic studies also offer evidence of vertical transmission. In endemic herds, the majority of calves born to seropositive cows have serologic evidence of congenital infection. In addition, the rate of seropositivity in the herd is not associated with the age of the cow, suggestive that the rate of acquired infection after birth is low.

There is additional pathologic and serologic evidence which indicates that these congenitally infected calves have a chronic persistent infection which can be passed on transplacentally to their offspring. In a survey of heifer calves in a known *Neospora* dairy herd, heifer calves with serologic evidence of congenital exposure were compared with serologically negative cohorts. The two groups were similar until calving, at which time all the offspring of the seropositive heifers had elevated *Neospora* titers, all seronegative heifers had serologically negative calves, and there was no evidence of seroconversion to *Neospora* among the negative heifers.[5] A portion of the calves were necropsied, and the calves from seropositive heifers had histologic lesions in the brain and spinal cord consistent with congenital *Neospora* infection. Protozoa were identified by immunohistochemistry. The calves from seronegative heifers had no lesions or other findings suggestive of congenital *Neospora* infection.

While vertical transmission appears to be the major way that cattle become infected with *Neospora* in endemic herds, there is serologic evidence that cows that have aborted during an epidemic probably acquired the infection after birth based on analysis of the seropositivity of dams and daughters.[53] In addition, the pattern of abortion outbreaks in epidemic neosporosis is suggestive of a point-source exposure with acquired infection.[23,32,36,48] However, there has been no confirmation that the cattle aborting in an epidemic acquired the infection during their pregnancy. In endemically infected herds which have been sampled more extensively, there is serologic evidence that a low level of postnatal infection from unknown sources apparently does occurs.[45,55]

Control and Prevention

A major method of *Neospora* transmission in herds is through the infection of fetuses in cattle that are chronically infected. These infected cows can be identified based on their serologic titers or from a history of *Neospora* abortion or congenital infection. With this knowledge, control of the infection could be focused on reducing the number of infected cows in the herd and limiting the introduction of infected replacement cattle into the herd. Culling decisions concerning cows that have had a confirmed *Neospora* abortion can be made

with the knowledge that there is a higher risk of repeat abortion in these animals.[35,52,] Seropositive cows also have a greater risk of abortion, and there is a very high probability of congenital infection in their calves.[35,43,52,57,60] Seropositive heifers also have been shown to have reduced milk production.[51] Epidemiological studies in both dairy and beef cattle have found that seropositive cattle have an increased rate of culling for a variety of reasons.[50,57]

Although various antimicrobial agents have been tested against *Neospora caninum* in vitro, there is no known method whereby an infected cow can be cleared of the infection.[23]

A provisional killed vaccine has recently become available for *Neospora* but there is no information on its efficacy in reducing fetal infection or abortion in an infected cow or in preventing postnatal infection in a non-infected cow.

There are no proven methods available to prevent postnatal infection, as details of the life cycle are still incomplete. However, based on experimental evidence that the dog can be a definitive host[34] and the association between dogs on the dairy and seroprevalence in the herd,[44] it would be prudent to take measures to reduce the potential for this type of transmission. The removal of all potentially infected tissues, such as aborted fetuses and placentas from the environment, that might serve as a source of infection for susceptible hosts would be advisable. In addition, fecal contamination of feed and water sources by other animals should be minimized.

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