
Neosporosis: A Newly Recognized Cause Of Abortion In Dairy Cattle

By Patricia A. Conrad, DVM, PhD
Mark L. Anderson, DVM, PhD
Bradd C. Barr, DVM, PhD
*Department of Pathology,
Microbiology & Immunology,
and the California Veterinary Diagnostic Laboratory
School of Veterinary Medicine
University of California,
Davis, CA 95616-8739
916-752-1385
fax 916-752-3349*

Neospora is a newly recognized genus that was first identified as a *Toxoplasma*-like protozoa in dogs with encephalomyelitis and myositis¹⁵ and later shown to be the same *Neospora caninum* parasite that was isolated from a litter of puppies in the United States^{14,24,27,29}.

The genus designation *Neospora* has since been applied to a similar protozoal parasite identified in livestock^{9,51}. In 1991, the first bovine *Neospora* isolates were obtained from aborted fetuses and these isolates have been maintained in continuous cell culture¹⁸. Whether the canine isolate (*Neospora caninum*) and bovine isolate represent identical or different species is not known. At present, the infection in livestock is most appropriately referred to as a *Neospora* species.

Neospora have morphologic similarities to *Toxoplasma gondii*, but can be antigenically differentiated by immunohistochemistry.¹² By light microscopy, *Neospora* can only be differentiated from *Toxoplasma* in the tissue cyst stage where *Neospora* often has a thicker cyst wall. In addition, there are distinct ultrastructural differences between *Toxoplasma gondii* and *Neospora*.^{12,39}

Infection due to *Neospora* has been reported in various species of livestock, including cattle^{1,5,8,9,11,12,20,25,28,30,33,35,38,41,43,46,49,50,51,52,56} sheep^{26,31} goats^{11,23} horses³⁴ and deer⁵⁵. Although only recently recognized, bovine neosporosis has emerged as an important reproductive disease. Since its first association with an abortion storm in 1987 in a dairy in New Mexico⁵¹ there have been reports of *Neospora* abortions in California and the Midwest which have confirmed this infection as a significant cause of abortion, particularly among dairy cattle^{1,5,9,41,49}. Retrospective studies in California suggest that the parasite has been endemic since at least 1985⁶.

In California, 18-19% of all aborted bovine fetuses submitted to the California Veterinary Diagnostic Laboratory System (CVDLS) are diagnosed with this infection^{5,9}. In dairy cattle submissions from California, the proportion of *Neospora* abortion is even higher, 24.4%⁶. In a Midwest survey, *Neospora* infection was identified in 2.7% of all cattle abor-

tions and was the largest cause of abortion in dairy cattle submissions.⁴¹

Bovine neosporosis has a worldwide distribution and has been diagnosed in the United States,³⁸ Canada,^{*}³⁸ Mexico,¹ Britain,^{20,44,46} Netherlands,⁵⁶ Denmark,² Australia,^{25,42} New Zealand,⁵² South Africa,³⁵ and Japan^{*,43}. Within the United States 28 states have reported cases including Alabama,³⁸ Arizona,^{*,41} California,^{3-6,8-12} Colorado,^{*,49} Georgia,³⁸ Idaho,^{*} Illinois,^{*} Indiana,²⁸ Iowa,⁴¹ Kansas,^{*} Maryland,^{*,33} Michigan,^{*} Minnesota,^{*,41} Missouri,^{*} Montana,^{*} Nebraska,³⁸ New Mexico,⁵¹ New York,^{*} North Dakota,^{*} Ohio,^{*} Oklahoma,^{*} Texas,^{*,41} South Dakota,⁴¹ Utah,^{*} Virginia,^{*} Washington,^{48,50} West Virginia^{*} and Wisconsin^{*,41}.

Pathogenesis:

The pathogenic potential of bovine *Neospora* sp. has been confirmed by experimental infection of pregnant cattle resulting in fetal death and in the birth of an in utero exposed or congenitally infected calf³. *Neospora caninum* from dogs also has been experimentally inoculated into pregnant cattle and sheep, resulting in transplacental fetal infection in a cow and abortion in sheep^{31,32}.

The natural route of infection and life cycle of *Neospora* is unknown, but similarities to other apicomplexan coccidia, particularly *Toxoplasma*, suggests that postnatal infection probably is acquired through oral ingestion of coccidial oocysts shed from an unidentified carnivorous definitive host. Attempts are being made to identify the definitive host of bovine *Neospora*. To date, dogs, cats, rats and mice have been screened for *Neospora* coccidia following experimental infection with bovine *Neospora* sp. (Conrad P and Barr B, unpublished data, 1992) and dogs, cats, and raccoons have been screened for *N. caninum* coccidia following experimental infections with *N. caninum*³⁸, but no fecal oocysts identified as *Neospora* have been identified in these species. Tachyzoites and tissue cysts are the two morphologic forms currently identified.

Clinical Presentation:

Although congenital *Neospora* infections have been diagnosed in most of the domestic livestock

species, cattle are the only livestock species in which there is sufficient information available concerning the natural infection to describe its clinical features. There are no signs of clinical illness 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 3 months gestation to term^{5,9,33}. Whether *Neospora* infection can cause reproductive problems in the first trimester of gestation is unknown. A majority (78%) of *Neospora* abortions occur between 4-6 months gestation and this pattern of mid-gestation abortion is distinctive from other diagnosed causes of infectious abortion in dairy cattle which tend to occur later in gestation⁶.

While *Neospora* infections occur in both dairy and beef cattle, most reports attributing significant numbers of abortions to this infection have been associated with dairy cattle, particularly those in drylot dairies^{1,3-6,8,9,41,49,51}. This apparent disparity between beef and dairy cattle is not thought to represent breed susceptibility, since beef cattle have been shown to be susceptible to experimental infection¹³ and both congenital infections and abortions due to *Neospora* have been documented in beef breeds^{*,28,30,33,48}.

It is probable that the environment of the drylot dairy is more conducive to the spread and transmission of this disease. Cattle in drylot dairies are densely populated and fed a variety of harvested feeds and commodities which are frequently stored on or around the dairy prior to being mixed and fed. These feeding practices offer many opportunities for fecal contamination, either on the dairy or in the crop in the field, of any of these individual ration components which would then be mixed and fed, efficiently exposing much of the herd. This pattern of increased exposure and disease associated with

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intensive management mimics factors affecting the incidence of *Toxoplasma* abortion in sheep flocks.

Neospora infection has been identified throughout California in over a third of the dairy herds submitting aborted fetuses to the CVDLS^{6,9}. The herd incidence of abortion due to this infection can be quite variable.

Rare sporadic cases may occur in some dairies with a nominal abortion rate. However, explosive outbreaks of *Neospora* abortion may occur.

A well documented example involved a group of 147 drylot dairy heifers in which 27 (18%) aborted during a six-week period and all fetuses examined (17) were diagnosed with *Neospora* infection (Reynolds J, personal communication, Nov. 1993). In some instances, up to 5% of pregnant cattle have aborted due to neosporosis within one to three months. Annual herd abortion rates up to 30% have been reported in dairies with *Neospora* abortions and these abortions may continue to occur over a period of several years^{*,1,49}. Over a one-year period of time, all aborted fetuses available on 26 selected California dairies were collected and submitted to the CVDLS for diagnosis. A total of 266 abortions were submitted, of which 113 (42.5%) were confirmed *Neospora* abortions from 19 dairies (73%)⁷. In addition to abortion, fetal mummification has been associated with *Neospora* outbreaks^{*,49}. *Neospora* abortions occur throughout the year but there is possibly a small increased risk of abortion during the late fall and winter⁵³.

Bovine fetal *Neospora* infection does not always produce fetal death resulting in abortion or stillbirth. Fetal infection may result in the birth of live full-term congenitally affected calves^{11,12,20,25,28,30,33,44,48}. Central nervous system infection and damage in these calves results in highly variable clinical signs which are often limited to 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. However, calves may have serologic and histopathologic evidence of in utero *Neospora* exposure or congenital infection with no obvious signs of clinical postnatal disease. A consistent finding in these calves is a high precolostral antibody titer to *Neospora* which are useful in detecting in utero exposed or congenitally infected calves¹¹. In a survey of calves on a dairy with a previous history of *Neospora* abortions, 67/189 newborn calves (35%) had serologic evidence of in utero *Neospora* infection, with no evidence of increased morbidity or mortality in these calves⁴⁷. The apparent wide variability in clinical presentation of these in utero exposed or congenitally infected calves may be due to multiple factors, including the age and immune development of the fetus at the time of exposure to *Neospora*, as well as the distribution of the lesions in the central nervous system.

Evidence is accumulating that cows that abort a *Neospora*-infected fetus may have additional infected fetuses in subsequent pregnancies. Barr and colleagues identified 5 calves born to 4 cows with *Neospora* abortions in the previous pregnancy. In all calves there was serologic and histopathologic evidence of congenital infection¹¹.

Repeat abortions can also occur. In a survey of abortions in drylot dairies in California, two confirmed *Neospora* abortions were identified in 4 of 41 cows in which information concerning other pregnancies was available⁷. It is not known whether these repeat transplacental infections are the result of a release of parasites from tissue cysts in the dam or from reinfection of the dam from the environment. However, there is recent evidence that suggests that chronic persistent infections do occur. In a survey of heifer calves in a known *Neospora* dairy herd, 25 calves with serologic evidence of congen-

ital exposure were compared with 25 serologically negative cohorts. The two groups were similar until pregnancy and calving. At the present time 20 of the 50 heifers have calved. All calves born to heifers with an history of congenital *Neospora* exposure have had elevated *Neospora* titers (8 of 8) and all negative heifers have had serologically negative calves (12 of 12) (Mark Anderson and Jim Reynolds, unpublished data, 1995).

The results suggest both that a chronic latent infection can occur with *Neospora* and, most interestingly, that there is vertical transmission of this disease through generations of cattle with little obvious clinical symptoms.

Diagnosis:

The confirmation of a suspect *Neospora* infection will require the assistance of a veterinary diagnostic laboratory. The preferred samples in cases of abortion include one or more aborted fetuses submitted with placenta and sera 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.⁹ The presumptive diagnosis of protozoal infection can usually be made on the basis of histologic lesions. Immunohistochemistry using antibodies to *Neospora caninum*³⁷ or the bovine *Neospora* isolate^{12,13} is an effective method to identify *Neospora* in fetal tissues and establish a definitive diagnosis.

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Recently, a monoclonal antibody against *Neospora caninum* has been developed which can also be used to detect infection in aborted fetuses¹⁷. *Neospora* immunohistochemistry is most successful on sections of fetal brain, although the parasites are also frequently present in the lung, kidney and skeletal muscle (Anderson M., unpublished data, 1994). Immunohistochemistry has been successfully employed to diagnose *Neospora* infections in mummified fetuses although the autolytic state of these fetuses diminishes the diagnostic accuracy (Anderson M, Barr B, unpublished data, 1994).

Diagnosis of congenital *Neospora* infection in calves on the basis of necropsy and histopathology may be difficult due to the variability of histologic lesions and numbers of parasites present¹². The most characteristic lesions are in the spinal cord, consisting of a multifocal nonsuppurative myelitis. In some cases thick-walled tissue cysts may be present within neurons. However, these tissue cysts may be extremely rare in many in utero exposed calves, making it difficult to establish a diagnosis on the basis of *Neospora* immunohistochemistry alone.

Neospora serology, utilizing an indirect fluorescent antibody (IFA) test, has proven effective in detecting elevated *Neospora* antibodies in the serum of congenitally infected or in utero exposed calves^{12,19}. In addition, the *Neospora* IFA test may also be useful in establishing the diagnosis in aborted fetuses, since most infected fetuses older than 5 months gestation have elevated *Neospora* antibody titers. However, just as with *Toxoplasma* infections, a negative fetal *Neospora* IFA titer does not rule out the possibility of infection.

While serology is an effective diagnostic tool in identifying *Neospora* infection in aborted fetuses and in utero exposed calves, the use of *Neospora* IFA for serodiagnosis in the adult cow may be less reliable. A significant portion (22%) of cows aborting a *Neospora* infected fetus had *Neospora* IFA titers that were within 2 dilutions of titers in presumed noninfected cattle¹⁹. In addition, within 2-5 months following abortion, the previously elevated titers in cows aborting a *Neospora* infected fetus

may drop to levels similar to noninfected cattle¹⁹. Laboratories utilizing this test must establish appropriate cut-off titers using standardized sera and should attempt to confirm their positive results by the identification of parasites in aborted fetuses.

Control And Prevention:

At present, there are no proven methods available for the control, prevention or treatment of bovine neosporosis as there is insufficient information on the biology of this parasite, including the mode of transmission, on which to base specific recommendations. However, it is prudent to remove all potentially infected tissues, such as aborted fetuses and placentas from the environment, that might serve as a source of infection for susceptible hosts. In addition, fecal contamination of feed and water sources by other animals should be minimized. It is apparent that fecal contamination of the environment or feeds of cattle is extremely common since virtually all cattle are infected with *Sarcocystis cruzi* through ingestion of coccidia from a canidae definitive host. As is the case with toxoplasmosis, development of an effective *Neospora* vaccine will be extremely difficult. At present, no culling recommendations can be offered for cows that have a *Neospora* abortion. Although repeat abortions or repeat congenital infections might occur in these animals, there is insufficient information available to estimate their future reproductive performance.

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notes
