Neosporosis: A Newly Recognized Cause Of Abortion In Dairy Cattle

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Neospora is a newly recognized genus that was first identified as a *Toxoplasma*-like protozoa in dogs with encephalomyelitis and myositis[15] 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 culture[18]. 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] horses[34] and deer[55]. 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 Mexico[51] 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 1985[6].

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 abortions 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 calf[13]. 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 gestation[6].

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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 infection[13] 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 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 winter[53].*

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 calves[11]. 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 calves[47]. 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 infection[11].

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 available[7]. 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 congenital 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).

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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.

Recently, a monoclonal antibody against Neospora caninum has been developed which can also be used to detect infection in aborted fetuses[17]. 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 present[12]. 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 cattle[19]. 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 cattle[19]. 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.

References:

**: Anderson, ML, Barr BC, Conrad PA: Unpublished Neospora immunohistochemistry results from submissions to California Veterinary Diagnostic Laboratory, 1995.*

1. Abbitt B, Craig TM, Jones LP, et al. Protozoal abortion in a herd of cattle concurrently infected with Hammondia pardalis. J Am Vet Med Assoc 203:444, 1993.

2. Agerholm JS, Barr BC. Bovine abortions associated with Neospora in Denmark. Dansk Veterinaer Tidsskrift [Danish Veterinary J], in press, 1995.

3. Anderson ML, Barr BC, Conrad PA, et al: Bovine Protozoal Abortions in California. The Bovine Practitioner 26:102, 1991.

4. Anderson, ML, Blanchard, PC, Barr BC, et al: A survey of causes of Abortion in San Joaquin Valley, California. J Vet Diagn Invest 2:283, 1990.

5. Anderson ML, Blanchard PC, Barr BC, et al: Neospora-like protozoan infection as a major cause of abortion in California dairy cattle. J Am Vet Med Assoc 198:241, 1991.

6. Anderson ML, Picanso J, Thurmond M et al. Epidemiological investigations of bovine protozoal abortion in California dairy herds. Proc XVII World Buiatrics Cong and XXV Am Assoc Bovine Pract Conf 2:74, 1992.

7. Anderson, ML, Palmer C, Thurmond M, et al. Prospective survey of Neospora abortions in selected California dairies. J Am Vet Med Assoc, accepted for publication, 1994.

8. Barr BC, Anderson ML, Blanchard PC, et al: Bovine fetal encephalitis and myocarditis associated with protozoal infection. Vet Pathol 27:354, 1990.

9. Barr BC, Anderson ML, Dubey JP, et al: Neospora-like protozoal infections associated with bovine abortions. Vet Pathol 28:116, 1991.

10. Barr BC, Anderson ML, Woods LW, et al. Neospora-like protozoal infections associated with abortions in goats. J Vet Diagn Invest 4:365, 1992.

11. Barr BC, Conrad PA, Brietmeyer R, et al: Congenital Neospora infection in calves born from cows that had previously aborted Neospora-infected fetuses: Four cases (1990-1992). J Am Vet Med Assoc 202:113, 1993.

12. Barr BC, Conrad PA, Dubey JP, et al: Neospora-like encephalomyelitis in a calf: pathology, ultrastructure, and immunoreactivity. J Vet Diag Invest 3:39, 1991.

13. Barr BC, Rowe JD, Sverlow KW, BonDurant BH, Ardans AA, Oliver MN, Conrad PA. Experimental reproduction of bovine fetal Neospora infection and death with a bovine Neospora isolate. J Vet Diagn Invest, in press, 1994.

14. Bjerkas I & Dubey JP: Evidence that Neospora caninum is identical to the Toxoplasma-like parasite of Norwegian dogs. Acta Vet Scand 32:407, 1991.

15. Bjerkas I, Mohn SF, Presthus J. Unidentified cystforming sporozoan causing encephalomyelitis and myositis in dogs. Z. Parasitenkd 70:271, 1984.

16. Buxton D, Finlayson J: Experimental infection of pregnant sheep with Toxoplasma gondii: pathological and immunological observations on the placenta and foetus. J Comp Path 96:319, 1986.

17. Cole RA, Lindsay DS, Dubey JP, et al. Detection of Neospora caninum in tissue section using a murine monoclonal antibody. J Vet Diagn Invest 5:579, 1993.

18. Conrad PA, Barr BC, Sverlow KW, et al: In vitro isolation and characterization of a Neospora sp. from aborted bovine fetuses. Parasitology 106:239, 1993.

19. Conrad PA, Sverlow KW, Anderson ML, et al. Detection of serum antibody responses in cattle with natural or experimental Neospora infections. J Vet Diagn Invest 5:572, 1993.

20. Dubey JP. Congenital neosporosis in a calf. Vet Rec 125:486, 1989.

21. Dubey JP: A review of Neospora caninum and Neospora-like Infection in animals. J Protozool Res 2:40, 1992.

22. Dubey JP: Toxoplasma, Neospora, Sarcocystis, and other tissue cyst-forming coccidia of humans and animals. In Kreier JP:Parasitic Protozoa, ed 2. New York, Academic Press Inc, 1993.

23. Dubey JP, Acland HM, Hamir AN. Neospora caninum (Apicomplexa) in a stillborn goat. J Parasitol 78:532, 1992.

24. Dubey JP, Carpenter JL, Speer CA, et al: Newly recognized fatal protozoan disease of dogs. J Am Vet Med Assoc 192:1269, 1988.

25. Dubey JP, Hartley WJ, Lindsay DS. Congenital Neospora caninum infection in a calf with spinal cord anomaly. J Am Vet Med Assoc 197:1043, 1990.

26. Dubey JP, Hartley WJ, Lindsay DS, et al: Fatal congenital Neospora caninum infection in a lamb. J. Parasitol 76:127, 1990.

27. Dubey JP, Hattel AL, Lindsay DS, et al: Neonatal Neospora caninum infection in dogs: Isolation of the causative agent and experimental transmission. J Am Vet Med Assoc 193:1259, 1988.

28. Dubey JP, Janovitz EB, Skowronek AJ. Clinical neosporosis in a 4-week-old Hereford calf. Vet Parasitol 43:137, 1992.

29. Dubey JP, Koestner A, Piper RC. Repeated transplacental transmission of Neospora caninum in dogs. J Am Vet Med Assoc 197:857, 1990.

30. Dubey JP, Leathers CW, Lindsay DS. Neospora caninum-like protozoan associated with fatal myelitis in newborn calves. J Parasitol 75:146, 1989.

31. Dubey JP, Lindsay DS. Neospora caninum induced abortion in sheep. J Vet Diagn Invest 2:230, 1990.

32. Dubey JP, Lindsay DS, Anderson ML et al. Induced transplacental transmission of Neospora caninum in cattle. J Am Vet Med Assoc 201:709, 1992.

33. Dubey JP, Miller S, Lindsay DS, et al: Neospora caninum- associated myocarditis and encephalitis in an aborted calf. J Vet Diagn Invest 2:66, 1990.

34. Dubey JP, Porterfield ML. Neospora caninum (Apicomplexa) in an aborted equine fetus. J Parasitol 76:732, 1990.

35. Jardine JE, Last RD: Neospora caninum in aborted twin calves. Journal of South Africa Veterinary Association 64:101, 1993.

36. Jerret IV, McOrist S, Waddington J, et al: Diagnostic studies of the fetus, placenta and maternal blood from 265 bovine abortions. Cornell Vet 74:8, 1984.

37. Lindsay DS, Dubey JP. Immunohistochemical diagnosis of Neospora caninum in tissue sections. Am J Vet Res 50:1981, 1989.

38. Lindsay DS, Dubey JP, Cole RA, et al: Neospora-induced protozoal abortions in cattle. Comp Cont Ed Pract Vet 15:882, 1993.

39. Lindsay DS, Speer CA, Toivio-Kinnucan MA, et al: Use of infected culture cells to compare ultrastructural features of Neospora caninum from dogs and Toxoplasma gondii. Am J vet Res 54:103, 1993.

40. McCausland IP, Badman RT, Hides S, Slee KJ: Multiple apparent Sarcocystis abortion in four bovine herds. Cornell Vet 74: 146, 1984.

41. Nietfeld JC, Dubey JP, Anderson ML, et al: Neospora-like protozoan infection as a cause of abortion in dairy cattle. J Vet Diagn Invest 4:223, 1992.

42. Obendorf D and Mason R. Neospora caninum infection detected in a bovine aborted foetus. Aust Sac Vet Path Report 28:36, 1990.

43. Ogino H, Watanabe E, Watanabe S, et al. Neosporosis in the aborted fetus and newborn calf. J Comp Path 107:231, 1992.

44. O'Toole JM, Jeffrey M. Congenital sporozoan encephalomyelitis in a calf. Vet Rec 121:563, 1987.

45. O'Toole D, Jeffrey M, Challoner D, et al: Ovine myeloencephalitis-leukomyelomalacia associated with a Sarcocystis-like protozoan. J Vet Diagn Invest 5:212, 1993.

46. Otter A, Griffiths IB, Jeffrey M: Bovine Neospora caninum abortion in the U.K. (letter) Vet Rec 133:375, 1993.

47. Pare' J, Hietala SK, Thurmond MC. Precolostral titers to the Neospora-like agent in dairy calves and effect of congenital infection on preweaning health. (Abstract) Conference of Research Workers in Animal Diseases, Chicago, 52, November 1993.

48. Parish SM, Maag-Miller L, Basser TE, et al: Myelitis associated with protozoal infection in newborn calves. J Am Vet Med Assoc 191:1599, 1987.

49. Rogers DG, McCullough MS, Shain WS, et al. Endemic protozoal abortions in a dairy cow herd. Agri-practice 14:16, 1993.

50. Shivaprasad HL, Ely R, Dubey JP. A Neospora-like protozoan found in an aborted bovine placenta. Vet Parasitol 34:145, 1989.

51. Thilsted JP, Dubey JP. Neosporosis-like abortions in a herd of dairy cattle. J Vet Diagn Invest 1:205, 1989.

52. Thornton RN, Thompson EJ, Dubey JP. Neospora abortion in New Zealand cattle. New Zealand Veterinary Journal 39:129, 1991.

53. Thurmond, MC, Anderson ML, Picanso J: Estimation seasonality of bovine protozoal abortion with a variable and unknown population at risk. (Abstract) 72nd Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, November 11-12, 1991.

54. Vickers M, Brooks HV: Suspected Sarcocystis infection in an aborted bovine foetus. NZ Vet J 31:166, 1983.

55. Woods LW, Anderson ML, Swift PK, Sverlow KW. Systemic neosporosis in a California black-tailed deer (Odocoileus hemionus columbianus). J Vet Diagn Invest 6:508-510, 1994.

56. Wouda W, Visser IJR, Van Knapen F: Bovine protozoal abortion (letter) Vet Rec 130(13:279, 1992.

notes