THIS ARTICLE IS SPONSORED BY THE
MINNESOTA DAIRY HEALTH CONFERENCE.

UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION

ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

Neospora Infection in Dairy Cattle

Mark Anderson

California Animal Health and Food Safety Laboratory, UC Davis

Neosporosis is a newly recognized coccidian parasite that has a worldwide distribution and is a major cause of abortion in dairy cattle. An important feature of the disease is that the parasite can be maintained in cattle as a chronic, apparently lifelong, infection that 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 onto 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 for evidence of infection. Since the first report of *Neospora* abortion in 1987 on a dairy in New Mexico (Thilsted, 1989), there have been numerous reports that have confirmed this infection is a significant cause of abortion. 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. The estimates of *Neospora* infection in U.S. dairy cattle based on serology is variable but appears to be in the range of 10 to 20% and range from 5% to 98% of cows in individual dairy herds.

Fetal *Neospora* infection has a variable outcome. *Neospora* infected cattle that abort have no signs of clinical illness other than abortion and the placentas are not retained. The majority of the abortions occur during the second trimester of pregnancy and the fetus is autolyzed. Abortions may occur throughout the year in both heifers and cows. Fetal mummification has been associated with *Neospora* outbreaks. Two patterns, endemic abortion and epidemic abortion, have been described in association with neosporosis in herds of cattle. In the endemic pattern of abortion, the herd experiences an elevated abortion rate of greater than 5% per year that persists for years. 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. A mixture of these patterns may be observed in some herds. In most instances, cows that abort a *Neospora* infected fetus will have either additional abortions or infected fetuses in subsequent pregnancies. The clinical outcome of these subsequent pregnancies is variable but, in one dairy, a seropositive cow that had an abortion had a 5.7 greater risk of abortion in the subsequent pregnancy (Thurmond 1997). An uncommon manifestation of fetal *Neospora* infection is the birth of a clinically affected full-term calf that exhibits variable CNS signs manifested as limb dysfunctions, ranging from mild proprioceptive defects to complete paralysis due to a multifocal protozoal encephalomyelitis. The majority of calves that acquire a *Neospora* infection during gestation are born clinically normal except that they have precolostral antibody titers to *Neospora caninum*.

The confirmation of *Neospora* infection as the cause of abortion requires veterinary diagnostic laboratory assistance. The preferred samples in cases of abortion include one or more aborted fetuses submitted with placenta and sera from the dam. The diagnosis is based on the presence of the typical pathologic lesions, identifying the parasite and other serologic and microbiologic tests to eliminate other causes. 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. There are widespread histologic lesions in most organs including the brain, heart, lung, liver, kidney and skeletal muscles. 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. Immunohistochemistry using antibodies raised against *Neospora caninum* antigens is a method employed by many diagnostic laboratories to identify the tachyzoite and tissue cyst stages of the parasite in fetal tissues.

A variety of serologic tests are available to assist in the diagnosis of neosporosis including the indirect fluorescent antibody test, the modified agglutination test, and a number of enzyme-linked immunosorbent assays. 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. Enzyme-linked immunosorbent assays (ELISA) for detection of *Neospora* antibodies are widely used because the procedure is rapid, inexpensive and consistent. Cutoff values have been established by which the probability of infection can be estimated in cattle (Pare 1994). A new avidity ELISA test can help evaluate the chronicity of the infection and provide information about routes of transmission within the herd (Bjorkman, 2003).

Neospora serology can be used as part of the routine abortion screen, to estimate herd infection rate, to assess the proportion of abortions that can be attributed to neosporosis and to evaluate routes of transmission (Thurmond 1997, 1999; Dijkstra, 2002). In the individual aborting cow titers usually are at a peak at the time of abortion. A positive serology result does not prove that the abortion was due to neosporosis but it can assist the diagnosis. In the non-aborting cow, a single serum sample may not accurately reflect her infection status since titers in known positive cattle can fluctuate and may fall below the cut-off value. There is no conclusive evidence to demonstrate that a serologic positive cow can revert to a consistently seronegative status. In rare instances, cows that have a *Neospora* infected fetus may not have a significantly elevated titer and previously elevated titers may decline over several months following abortion or parturition. In pregnant cattle it isn't clear whether titers in the individual cow can be used to predict the outcome of a specific ongoing pregnancy.

Currently there is active investigation of the ways by that cattle can acquire *Neospora* infection. The two forms of the parasite identified in infected fetuses and calves are the tachyzoite and tissue cyst stage. 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 predominately found in neural tissues. The tissue cyst elicits minimal inflammatory reaction and can persist for long periods of time. There are several ways that 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 rare in cattle.

The taxonomic and morphologic similarities between *Neospora* and *Toxoplasma* support the hypothesis that the cow may be infected horizontally through the oral ingestion of coccidia oocysts shed from a carnivorous definitive host. The identification of this proposed definitive host has been difficult in that initial studies on dogs, cats, rats, mice, raccoons, and various bird species had found no confirmation of a role for these species. However, recently McAllister et al and later Lindsay et al were successful in transmitting *Neospora* infection from experimentally infected mice to puppies that shed oocysts in their feces. The shedding of oocysts in dogs fed infected mouse tissues was relatively low but subsequent studies utilizing infected placentas and experimentally infected calf tissues have produced a higher yield of fecal oocysts (Dijkstra, 2002). Experimental infection of dogs with aborted fetuses has not yet been successful. Calves have been successfully infected with oocysts obtained from experimentally infected dogs (Uggla 1998) although at the present time there are no reports of similar experiments in pregnant cattle resulting in abortion.

There is epidemiological evidence that suggests that dogs have a role in the transmission of neosporosis. Pare' et al found an association between the herd seroprevalence of *Neospora* infection and in the presence and number of dogs at the herd. Bartels et al in the Netherlands and Mainar-Jaine et al in Spain have also found a significant association between herd seroprevalence and the presence of dogs in the dairy. Dijkstra et al has found evidence linking the introduction of dogs into a previously infected dairy with the subsequent post-natal transmission of the infection to other cattle in the dairy. In another publication, Dijkstra et al found that dairies with evidence of post-natal infection had more observed placental consumption and defecation by dogs in feeds than was observed in dairies with no evidence of post-natal transmission. An implication of these observations is that it is possible that previously uninfected dogs may promote the spread of the infection among the herd when they are introduced into an infected dairy. Studies in several countries have found that rural or farm dogs have higher seroprevalence to *Neospora caninum* than dogs from an urban environment, a finding that may reflect more opportunity for exposure to infectious material from cattle in this group.

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 and 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 during pregnancy for her fetus to become infected. A pre-existing chronic infection can infect the fetus when pregnancy occurs. In addition, unlike toxoplasmosis, cows that abort in one pregnancy are susceptible to repeat infection and are at an increased risk of repeat abortion.

An important feature of bovine neosporosis is that the parasite can be maintained in cattle as a chronic infection that 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 onto the next generation when she becomes pregnant, thus maintaining the infection in the herd. Vertical transmission of *Neospora* through generations of cattle appears to be the major method by which *Neospora* infection is maintained in herds. In a Swedish study by Bjorkman et al, 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, Schares et al found 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. In California dairies, several serologic studies also offer evidence of vertical transmission (Pare 1994 and Thurmond 1997). 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 that indicates that these congenitally infected calves have a chronic persistent infection that can be passed on transplacentally to their offspring. In a survey of heifer calves in a known *Neospora* dairy herd (Anderson 1997), 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. 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 and 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 (Thurmond 1997; Dijkstra 2003). In addition, the pattern of abortion outbreaks in epidemic neosporosis is suggestive of a point source exposure with acquired infection (McAllister 1996). In endemically infected herds that have been sampled extensively, there is serologic evidence that a low level of postnatal infection may occur. In Dutch dairy herds, serologic studies that evaluated the age distribution of seropositive animals and the daughter-mother serologic relationships, found evidence of both horizontal and vertical transmission in among individual herds (Dijkstra, 2003).

Control of this infection must take in account that 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 from their serologic titers or from a history of previous *Neospora* abortion or congenital infection. With this knowledge, control of the infection could be focused on reducing the numbers 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. Seropositive cows also have a greater risk of abortion (3 to 7 times greater risk of abortion than seronegative cattle, (Wouda 1998, Moen 1998 and Thurmond 1999). There is a high probability of congenital infection (80-90%) in calves born to these cows. There are conflicting studies in regard to whether seropositive cattle have reduced milk production. In two published studies, reduced milk production was observed in seropositive cattle while in another study no such association was identified. In both dairy and beef cattle, epidemiological studies have found that seropositive cattle have an increased rate of culling for a variety of reasons (Thurmond 1999 and Waldner 1997).

One recommendation for control in herds utilizing embryo transfer procedures is to insure that all recipient cows are seronegative. This can be an effective method to prevent vertical transmission as demonstrated by Baillargeon et al (2001) and later Landmann et al (2002). They showed that fetal transmission occurred in accordance with the infection status of the recipient cow and was not influenced by the infectious status of the donor cow. Embryos implanted into infected recipients became infected in a similar proportion to natural pregnancies while an embryo from an infected donor cow could be implanted into a noninfected recipient to prevent vertical transmission.

Various antimicrobial agents have been tested against *Neospora caninum in vitro* and have been used to treat clinical infections in dogs. Recently, Toltrazuril-sulfone (ponazuril) has been shown to have efficacy in experimental *Neospora* infections in calves (Kritzner, 2002). However, it has not been confirmed to be of use in naturally infected cattle and currently, there is no proven chemotherapeutical method whereby an infected cow can be cleared of the infection. A killed vaccine (NeoGuard, Intervet Inc) has recently become available for *Neospora* but there is no information on its efficacy in regard to 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. However, based on the experimental evidence that the dog can be a definitive host and the association between dogs on the dairy and the seroprevalence in the herd, 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.

References

Anderson, ML, Reynolds JP, Rowe JD et al. Evidence of vertical transmission of *Neospora* infection in dairy cattle. J Am Vet Med Assoc, 210:1169-1172, 1997.

Anderson M, Andrianarivo A, Conrad P. Neosporosis in cattle. Animal Reproduction Science Vol 60-61, pp 417-431, 2000.

Baillargeon P, Fecteau G, Pare J et al. Evaluation of the embryo transfer procedure proposed by the International Embryo Transfer Society as a method of controlling vertical transmission of *Neospora caninum* in cattle. J Am Vet Med Assoc 218:1803-1806, 2001

Bartels CJM, Wouda W, Schukken YH. Risk factors for *Neospora caninum*- associated abortion storms in dairy herds in the Netherlands (1995 to 1997). Theriogenology 52:247-257, 1999.

Bjorkman C, Johansson O, Stenlund S et al. *Neospora* species infection in a herd of dairy cattle. J Am Vet Med Assoc 208:1441-1444, 1996.

Bjorkman C, McAllister MM, Frossling J et al. Application of the Neospora caninum IgG avidity ELISA in assessment of chronic reproductive losses after an outbreak of neosporosis in a herd of beef cattle. J Vet Diagn Invest 15(1): 3-7, 2003

Dijkstra, T, Barkema, HW, Hesselink JW et al. Point source exposure of cattle to *Neospora caninum* consistent with periods of common housing and feeding and related to the introduction of a dog. Veterinary Parasitology, 105,89-98, 2002

Dijkstra, T, Barkema, HW, Eysker, M et al. Natural transmission routes of Neospora caninum between farm dogs and cattle, Veterinary Parasitology, 105, 99-104, 2002

Dijkstra, T, Eysker, M, Schares, G et al. Dogs shed Neospora caninum oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with Neospora caninum tachyzoites, International Journal for Parasitology, 31, 747-752, 2001

Dubey JP, Recent advances in Neospora and neosporosis. Veterinary Parasitology 84(1999) 349-367

Jenkins M, Baszler T, Bjorkman C et al. Diagnosis and seroepidemiology of Neospora caninum-associated bovine abortion. Int J Parasitol 32(5):631-6, 2002

Kritzner S, Sager H, Blum J et al. An explorative study to assess the efficacy of Toltrazuril-sulfone (Ponazuril) in calves experimentally infected with Neospora caninum. Ann Clin Microbiol Antimicrob 18;1(1):4, 2002

Landmann JK, Jillella D, O'Donoghue PJ et al. Confirmation of the prevention of vertical transmission of Neospora caninum in cattle by the use of embryo transfer. Australian Vet J 80:502-503, 2002

Mainar-Jaime RC, Thurmond MC, Berzal-Herranz B et al. Seroprevalence of Neospora caninum and abortion in dairy cows in northern Spain. Vet Rec 145:72-5, 1999

McAllister MM, Huffman EM, Hietala SK et al. Evidence suggesting a point source exposure in an outbreak of bovine abortion due to neosporosis. J Vet Diagn Invest 8:355-357, 1996

McAllister MM, Dubey JP, Lindsay DS et al. Dogs are definitive hosts of *Neospora caninum.* International Journal for Parasitology 28:1473-1478, 1998

Moen AR, Wouda W, Mul MF et al. Increased risk of abortion following *Neospora caninum* abortion outbreaks: a retrospective and prospective cohort study in four dairy herds. Theriogenology 49:1301-1309, 1998

Pare' J, Thurmond MC, Hietala SK. Congenital *Neospora* infection in dairy cattle. Vet Rec 134:531-532, 1994.

Pare' J, Hietala SK, Thurmond MC. An enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of *Neospora* infection in cattle. J Vet Diagn Invest 7:352-359, 1995.

Pare' J, Thurmond MC, Hietala SK. *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. J Parasitol. 83:82-87, 1997

Pare' J, Fecteau G, Fortin M et al. Seroepidemiologic study of Neospora caninum in dairy herds. J Am Vet Med Assoc 213:1595-8, 1998.

Schares G, Peters M, Wurm R et al. The efficiency of vertical transmission of Neospora caninum in dairy cattle analyzed by serologic techniques. Veterinary Parasitology 80:87-98, 1998

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

Thurmond MC, Hietala SK, Blanchard PC. Herd-based diagnosis of *Neospora caninum*-induced endemic and epidemic abortion in cows and evidence for congenital and postnatal transmission. J Vet Diagn Invest 9:44-49, 1997.

Thurmond MC and Hietala SK. *Neospora caninum* infection and abortion in cattle. In Howard and Smith eds. Current Veterinary Therapy: Food animal practice, 1999, W.B Saunders, pp 425-431

Uggla A, Stenlund OJ, Jakubek EB et al. Oral Neospora caninum inoculation of neonatal calves. International Journal for Parasitology 28:1467-72, 1998

Waldner CL, Jansen ED, Ribble CS. Determination of the association between Neospora caninum infection and reproduction performance in beef herds. J Am Vet Med Assoc 213:685-90, 1998

Wouda W, Moen AR, Schukken YH. Abortion risk in progeny of cows after a *Neospora caninum* epidemic. Theriogenology 49:1311-1316, 1998