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## Neosporosis in Dairy Cattle

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Bovine Neosporosis, a disease caused by the protozoan parasite, *Neospora caninum*, is now recognized throughout the world, including in Japan. Neosporosis is the major identified cause of abortion in many regions, including California. A critical feature of the disease is that the infection is maintained in cattle as a chronic, persistent infection that is efficiently passed on to the fetus during pregnancy. While fetal infection in pregnant cattle may result in abortion, most of the infected fetuses do not abort but rather acquire a congenital infection. A congenitally infected heifer calf is capable of transmitting the infection onto the next generation when she becomes pregnant, thus maintaining the infection in the herd.

### Clinical presentation

The primary manifestation of disease due to neosporosis is the abortion of mid gestation, autolyzed fetuses with no other signs of clinical

illness in aborting cattle. The vast majority of abortions occur in the second trimester of pregnancy, 4 to 6 months gestation. Mummification has been associated with *Neospora* infection in younger fetuses. In rare instances a fetal *Neospora* infection may result in a full-term calf with paresis or paralysis due to encephalomyelitis. However, the majority of the pregnancies that acquire *Neospora* infection do not terminate in abortion. It is estimated that 80% to 90% of pregnancies of seropositive (infected) cows produce apparently normal calves that are congenitally infected based on serology. These congenitally infected calves have an important role in maintaining the infection in the herd and are at an increased risk of abortion.

*Neospora* abortions occur in heifers and cows throughout the year and occur in both dairy and beef cattle, although there are more reports of abortions in dairy cattle. Two patterns of abortion, endemic and epidemic, may occur. In the endemic pattern of abortion, the herd experiences a persistent elevated abortion rate of

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greater than 5% per year. 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. A mixture of these patterns may be observed. Cattle aborting due to *Neospora* infection are not immune in subsequent pregnancies but rather have an increased risk of abortion or congenital infection.

### Diagnosis

Several methods are available to diagnose neosporosis as a cause of abortion. The preferred samples in cases of abortion include one or more aborted fetuses submitted with placenta and sera from the dam. The aborted fetuses are 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 cysts 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. The presumptive diagnosis of protozoan infection can usually be made on the basis of histologic lesions. Immunohistochemistry using antibodies to *Neospora* is an effective method to identify the parasites in fetal tissues. *Neospora* immunohistochemistry is most successful on sections of fetal brain and kidney.

The identification of *Neospora* infection in an aborted fetus is not sufficient to establish that the infection was responsible for the abortion because there are additional considerations to be made before an abortion can be attributed to *Neospora* infection. The infection in cattle is

widespread and, during pregnancy, the majority of infected dams will infect their fetuses. However, most infected fetuses do not die and abort as a result of the infection. It is critical that the diagnosis of abortion take in consideration additional factors beyond evidence of infection. Whether an infected fetus survives or dies may depend on various factors such as the dose and the time of the infection in relation to the immune competence of the fetus. Fetal infection primarily through the sixth month of gestation, prior to sufficient development of an immune response can result in an overwhelming infection. Disseminated inflammatory lesions in the brain, lungs, heart, liver, kidney, muscles, placenta and other organs are present in fetuses that die and are aborted. An accurate diagnosis of *Neospora* abortion requires the diagnostician to consider, prior to establishing the infection as the cause of the abortion, the gestational age and postmortem condition (autolyzed), the presence of compatible disseminated inflammatory lesions, the presence of detectable parasites with immunohistochemistry, and the lack of other abortifacients. A *Neospora caninum* infected aborted fetus that only has 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.

Serologic tests can assist the diagnosis of neosporosis. The specificity and sensitivity of the various serologic tests depend on the antibody titer that has been established as the cut-off for a positive result. Laboratories utilizing serologic tests for *Neospora* should establish appropriate cut-off titers using sera from known infected and noninfected cattle. A positive cut-off titer selected based on the antibody titer in a cow that has aborted an infected fetus may not be appropriate for serologic diagnosis of a chronic infection in cattle of different ages and pregnancy status. A single serum sample from an individual cow may not accurately reflect her infection status since titers fluctuate and may fall below the cut-off

value for a period of time. In rare instances, cows that abort a *Neospora* infected fetus may not have a significantly elevated titer. Also, previously elevated titers at abortion may decline over several months following abortion. There is no conclusive evidence to demonstrate that a serologic positive cow can revert to a consistently seronegative status.

An enzyme-linked immunosorbent assay (ELISA) for detection of *Neospora* antibodies is used for routine diagnostic testing at CAHFS as part of the bovine abortion serologic panel. This procedure is rapid, inexpensive and consistent with excellent sensitivity and specificity. 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. *Neospora* serology is effective in detecting elevated *Neospora* antibodies in the serum of congenitally infected or *in utero* exposed calves. In addition, serology may be useful in establishing the diagnosis in aborted fetuses, since some infected fetuses may have elevated *Neospora* antibody titers. However, a negative fetal *Neospora* 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. The ELISA test can be used on a herd basis to estimate herd seroprevalence and to determine the proportion of abortions attributable to *Neospora* infection by comparing the titers among aborting and non-aborting herdmates.

### Transmission

The transmission of *Neospora* infection is complex and remains under investigation. There are several ways that cattle may acquire *Neospora* infection, by horizontal (postnatal) infection by infective oocysts or by vertical transplacental transmission of the infection during pregnancy.

In bovine neosporosis, cattle are intermediate hosts; therefore, the forms of the parasite identified in fetuses and calves are the tachyzoite and tissue cyst stage. The tachyzoites spread through the body and invade cells in a variety of organs causing damage at the site of invasion. The tissue cyst stage, containing multiple bradyzoites surrounded by a thick cyst wall, is primarily found in neural tissues and elicits a minimal inflammatory reaction but can persist for long periods of time. The definitive host sheds the oocyst stage following an coccidian intestinal infection.

The proposed model of horizontal transmission is that the bovine intermediate host acquires the infection through ingestion of oocysts shed by the definitive host. This is similar to other apicomplexan coccidia, such as *Toxoplasma gondii* and *Sarcocystis* species. The dog has been identified as a definitive host for bovine neosporosis from experimental and epidemiological evidence. However, *Neospora* infection in cattle differs from other apicomplexan coccidia infections because cattle don't need to acquire the infection during pregnancy for her fetus to become infected. Many infected pregnancies are the result of vertical transmission in chronically infected cattle that acquired their infection congenitally from their dams. This vertical transmission through generations of cattle is a major method by which *Neospora* infection is maintained in herds. In endemically infected herds, there is serologic evidence that a low level of postnatal infection occurs from unknown sources but vertical transmission appears to be the major route of infection. In epidemics of *Neospora* abortion, there is serologic evidence that aborting cows probably acquired the infection after birth. Certainly the pattern of abortions in epidemics is suggestive of a point source exposure with acquired infection, but there is scant evidence that the pregnant cattle aborting in an epidemic acquired the infection during the pregnancy.

**Control:**

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 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 and there is a very high probability of congenital infection in the calves born to these cows.

A killed vaccine is available for *Neospora* but

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

There are no proven methods available to prevent postnatal infection. However, based on the experimental and epidemiological evidence that the dog can be a definitive host, 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.