Strategies to Control Neospora Infection in Cattle

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Neospora caninum is a parasite found to be associated with high rates of abortion in cattle.[1,26] The disease has been diagnosed world-wide and appears to have been present long before it was first suspected in the mid-to-late 1980's.[4,19,33] The close taxonomic and morphologic relationship between *N. caninum* and *Toxoplasma gondii*[13,14] suggest similar life cycles, including involvement of a definitive host[8] and infection by exposure to infected fetuses and placentas.[16]

N. caninum infection in some dairy herds has been found to be quite high, ranging from 36% to 58% in 2 herds extensively evaluated.[24] Although epidemiologic evidence exists for postnatal transmission,[36] the only demonstrated specific mode of infection is congenital,[7,10,11,20-25] which resembles *T. gondii* transmission in mice and rats.[2,15,37] Congenital infection has been found in 78% to 88% of calves born to seropositive cows and accounted for most of the infection in the herds extensively investigated.[24] Risk of abortion for infected cows has been estimated to be twice that of noninfected cows, indicating that half of the abortions in infected cows may be attributable to *N. caninum*.[26]

Considerable speculation exists with respect to whether neospora abortion storms are a consequence of recent exposure to *N. caninum*.[12,18,30-33] In order to maintain an open mind on potential epidemiology of the disease, however, alternative explanations should be considered for neospora abortion epidemics. Theoretically, other conditions or diseases of the dam that act to impair immune surveillance may trigger exposure of a fetus to *Neospora tachyzoites*. Examples of such diseases that could alter immune competence and precipitate abortion of cows already infected with *N. caninum* include infection with bovine viral diarrhea virus (BVDV)[27] or exposure to mycotoxins.[5] When investigating abortion epidemics, therefore, other disease conditions or exposures possibly predisposing to neospora abortion should be considered.

Although little information is available on pathogenesis and specific mechanisms of transmission, the limited knowledge of the epidemiology and theoretical similarity with *T. gondii* offer some strategies for control and prevention. Concepts and approaches proposed here are not unique to control of *N. caninum* infection; they are fundamentally the same as those applied to control of a range of diseases affecting livestock populations.

Herd Diagnostics and Prevalence Information

Herd Prevalence

Estimating prevalence of infection in the herd is an important initial step in developing strategies to control neospora abortion. Knowledge of prevalence is necessary in selecting the most feasible strategies for control. Culling, for example, may be a viable approach if prevalence is low, but not economically realistic if prevalence is high. An estimate of prevalence can be obtained from serology of a randomly selected sample of about 30-50 lactating cows, depending on herd size. Serology should employ a well characterized test, with known ability to classify true negatives (specificity) and true positives (sensitivity). The ELISA for *N. caninum*, which was developed for use in cattle,[25] has reported sensitivity and specificity of 88% and 97%, respectively.

Diagnostics

Necropsy and immunohistochemistry, though less convenient than serologic sampling, provide information on whether the extent and nature of fetal lesions were compatible with neospora infection. Although immunohistochemistry is specific, it is not very sensitive and relies heavily on time-consuming procedures aimed at finding only a few rare lesions. Moreover, diagnosis of neospora infection in an aborted fetus does not necessarily mean the fetus died from the infection. Most, if not all, calves born to infected cows can be expected to be infected congenitally.[24] Consequently, pathologists can expect to find evidence of *N. caninum* infection in normal, nonaborted fetuses[6] and of dual infections with *N.*

caninum and other abortifacients in aborted fetuses.[3,17] For example, diagnosis of *N. caninum* infection in 1 of 3 fetuses would be the expected rate of congenital infection from a herd with an infection prevalence of 33% in the absence of abortion caused by *N. caninum*. In other words, presence of *N. caninum* in a fetus simply may be a reflection of infection prevalence in the herd, and not necessarily indicative of abortion caused by *N. caninum*. Herd serology, therefore, should be used to obtain information on risk of abortion attributable to neospora infection.

An epidemiologic approach can be used to assess whether abortion is attributable to *N. caninum*. The diagnostic strategy should include serology of a sample of aborted and nonaborted cows. The purpose of such serology is to determine if the proportion of seropositivity in aborted cows is larger than the proportion in nonaborted cows. If neospora contributed to the abortion problem, then the proportion of seropositive aborted cows should be significantly higher than the proportion of seropositive nonaborted cows. The difference in proportions can be assessed statistically by use of a Chi Square or Fisher exact test. At least an equal number of nonaborted cows as aborted cows should be selected randomly from cows that were pregnant and at risk of having an abortion diagnosed during the abortion problem. If the seropositivity rate is not significantly higher for aborted than for nonaborted cows, then no evidence would exist for neospora involvement in the abortion problem.

If aborted cows have a higher rate of seropositivity than nonaborted cows, an additional calculation can be done to estimate the extent to which neospora may have contributed to the problem. An estimate using the odds ratio assesses whether the relative risk of abortion in neospora-infected cows is compatible with that expected for endemic neospora abortion, which can be expected to be a magnitude of about 2,[26] or whether it is much higher, as would be expected for an epidemic of neospora abortion (see example and Table 1). Knowledge of whether or not neospora abortion is endemic or epidemic in the herd may be helpful in planning control strategies assuming the mode of transmission of *N. caninum* differs for the two patterns of occurrence. Cows aborting a neospora infected fetus on dairies experiencing endemic neospora abortion were more likely to be infected congenitally, compared to cows aborting during an epidemic where recent exposure was hypothesized.[35]

An additional use of serology is to estimate the extent of congenital infection, which has been demonstrated to be the single most important mode of transmission in some herds,[24] and accounted for 98% of infections in heifers less than 2 years of age.[36] Two approaches can be taken, one requiring precolostral blood sampling and the other requiring an estimation of colostral antibody decay. Precolostral serology is the best approach to determine whether or not a calf was infected *in utero*. If blood cannot be collected precolostrally, antibody concentration of calves sampled between birth and 5 months of age can be compared to normal neospora antibody levels determined from a population of known

EXAMPLE (Table 1):
Serology interpretation is provided for a hypothetical case involving 17 aborted heifers to determine whether *N. caninum* was associated with abortion, and, if so, to estimate the strength of the association. The rate of seropositivity of 76% in aborted heifers is significantly higher than that of 35% in nonaborted heifers (p = 0.02) (Table 1). The low chi Square p-value of 0.02, indicates the distribution of seropositivity in aborted compared to nonaborted heifers was real, and not likely to have occurred by chance. Evidence of a strong association is given by the odds ratio (OR) of 6.1, and the exclusion of 1.0 in the 95% confidence limits. Weak associations would be expected to have OR <2-3, with 95% confidence limits that may include 1.0. The strength of the association between *N. caninum* and abortion in this hypothetical case would be compatible with an epidemic where the risk of abortion associated with *N. caninum* (6-fold) was substantially more than that for endemic abortion (2-fold). The lower p-value and narrower confidence interval of the OR for the sample with 46 controls demonstrate how confidence is improved when more controls are sampled.

Table 1. Distribution of seropositivity of *N. caninum* for a hypothetical abortion case, where 17 aborted heifers were tested and either 23 (A) or 46 (B) nonaborted control heifers were tested.

	Aborted	Nonaborted
	A	
Seropositive	13 (76%)	8 (35%)
Seronegative	4 (24%)	15 (65%)
Total	17	23
Chi-Square (Yates) p = 0.02		
OR (95% confidence interval) = 6.1 (1.25, 33.1)		
	B	
Seropositive	13 (76%)	16 (35%)
Seronegative	4 (24%)	30 (65%)
Total	17	46
Chi-Square (Yates) p = 0.008		
OR (95% confidence interval) = 6.1 (1.5, 29.1)		

noninfected calves. If the concentration is above that expected for noninfected calves, the calf would be considered infected. Calves can be blood sampled after 5 months of age when neospora colostral antibodies are no longer detectable, and a positive test would be indicative of either congenital or postnatal infection.

Strategies to Control Congenital Infection

So far congenital infection is the only demonstrated specific means of transmission of *N. caninum.*[23,24] Although epidemiologic evidence indicates that postnatal transmission can take place,[36] congenital transmission may be the most common, if not the only, means of transmission in some herds.[24,36]

Reducing infection in the herd

The most straightforward approach to reduce congenital transmission in a herd is to remove infected cows. The rationale for culling is that most infected cows can be expected to give birth to an infected calf[24] and congenital infection appears to be lifelong.[36] If initial serology of a sample of cows suggested a sufficiently low prevalence to justify culling, the remaining females in the herd could be tested and the positive cows culled. Alternatively, if logistics and cost dictate a more conservative and gradual approach to control, dams and daughters of cows that aborted and/or that were known to be infected can be tested. For those who do not want to cull solely on serology, serologic status of a cow may become only one of many criteria to be considered in culling.

In deciding how much weight to place on infection as a culling criterion, consideration should be given to the herd infection prevalence, an expected 2-fold increase in the risk of abortion for infected cows, a cost per abortion of at least $500,[34] and the expected net benefit to be gained. The cost of neospora abortion may not outweigh the loss of milk production and/or genetic value.

Minimizing infected replacements

Congenital infection also can be reduced by permitting only seronegative females as replacements, which would include heifer calves born to known infected cows. Herds engaged in embryo transfer should use only seronegative recipients, and a heifer that was the product of embryo transfer should not be purchased if the recipient was seropositve. Compared to culling, the approach of using only seronegative replacements may be a less costly alternative for long-term elimination of infection.

Strategies to Control Postnatal Transmission

Although epidemiologic evidence exists for postnatal transmission,[34] definitive hosts and specific mechanisms of transmission remain unknown. The strategies proposed here for the control of postnatal transmission are based on theoretical possibilities, using *T. gondii* as a model, and employing concepts commonly practiced in livestock disease prevention. Such strategies would be expected to help reduce postnatal transmission regardless of the animal species ultimately demonstrated to act as a definitive host.

Minimizing transmission from theoretical definitive hosts

The objective in minimizing postnatal transmission via a presumptive definitive host is to break a cycle of transmission, which would include reducing exposure of a definitive host to infected tissue (eg. placenta or aborted fetus), and reducing exposure of cattle to feces of a definitive host. Theoretically, a definitive host of *N. caninum* may be any carnivore or omnivore that became infected with *N. caninum* after eating infected tissue. Such hosts might include, but not be limited to, birds, dogs, cats, rats, mice, or coyotes. At some time after the parasite undergoes development in the intestinal epithelium, oocysts may be released into the feces of the definitive host. Cattle would become infected if they consumed feces of a definitive host that contained an infective dose of oocysts.

Specific measures to control such transmission would include removal of fetuses, dead calves, and placentas that could serve as a source of infection for a definitive host. Exposure of cows to feces of theoretical definitive hosts could be reduced by maximizing rodent control, minimizing the number of cats and dogs cohabitating the herd, covering feed and commodities to prevent contamination by bird feces, and removing feces from bulk feed areas, feed bunks, and aprons.

Minimizing transmission from infected tissues

Using a model of *T. gondii,*[16] cattle theoretically could become infected after licking or eating tissues infected with *N. caninum.* Such transmission could take place when a noninfected cow or calf ingests infected tissues or fluids expelled from an infected cow, such as at calving or abortion. If the toxoplasma theory holds for neospora, it is conceivable that some abortion storms attributable to *N. caninum* infection could result from exposure to a fetus or placenta aborted from an infected heifer or cow. Strategies to prevent this type of transmission would include prompt disposal of fetuses and afterbirth, use of individual calving pens, and segregation of positive and negative cows at calving and perhaps while pregnant.

Regardless of the effectiveness of these measures to control postnatal transmission, however, they should not be expected to prevent congenital transmission.

Summary

Even though little is known about the disease, knowledge of the potential for extensive congenital infection and the assumption of a similar life cycle to *T. gondii* offer some general guidelines to those who do not want to wait for definitive results before pursuing prevention and control.

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