# *Neospora*: Does it Make Sense to Monitor and Control it?

**John M. Gay,** *DVM, PhD, DACVPM*
*AAHP Field Disease Investigation Unit, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, POB 646610, Pullman, WA, 99164-6610*
*Tel.: +1 509 335 0785; fax: +1 509 335 0880; E-mail address: jmgay@vetmed.wsu.edu*



**Abstract**

Although more research is needed to improve our understanding of the epidemiology of bovine neosporosis, our current knowledge is likely sufficient for developing effective control and monitoring strategies. The decision of whether or not it makes sense to monitor and control the infection in a given herd depends on a complex set of factors that differ between herds and regions. Control likely should be considered in the light of resource limitations, management goals and other problems and opportunities faced by the herd. Several authors have suggested that practitioners should consider performing a cost-benefit analysis on each herd to determine the critical prevalence threshold for undertaking a herd control program and to determine the best strategy. Currently the optimal control method appears to be testing potential replacements for seronegativity prior to their selection, but the optimal strategy will likely change with increased understanding, and as improved vaccines and even therapies emerge.

*Simplicity does not precede complexity, but follows it.*
(Alan Perlis)

**Résumé**

Bien que de plus amples recherches soient nécessaires afin de mieux comprendre l'épidémiologie de la néosporose bovine, l'état actuel des connaissances sur la question suffit sans doute pour élaborer des stratégies efficaces de surveillance et de maîtrise. La décision à savoir s'il vaut la peine ou non de surveiller et de maîtriser l'infection dans un troupeau donné dépend d'un ensemble complexe de facteurs qui diffèrent selon le troupeau et la région. La maîtrise devrait sans doute être étudiée à la lumière des restrictions des ressources, des objectifs de régie et des autres problèmes et occasions auquel le troupeau fait face. Plusieurs auteurs sont d'avis que les praticiens devraient envisager une analyse coûts-avantages pour chaque troupeau afin d'établir le seuil de prévalence critique pour la mise en place d'un programme de maîtrise et pour établir la meilleure stratégie à adopter. À l'heure actuelle, la méthode optimale de maîtrise semble être d'établir la séronégativité lors de la sélection des animaux de remplacement, mais la stratégie optimale va sans doute évoluer en fonction d'une plus grande compréhension de la maladie qui devrait mener à l'élaboration de meilleurs vaccins et de meilleures thérapies.

## Introduction

Does it make sense to monitor and control neosporosis? It depends. In fact, the answer depends on a multitude of interconnected things, many of which are different between herds, across regions and over time. That which makes the most biologic sense often doesn't make the most economic sense; in fact, sometimes it makes the least. Depending on the particular context in which this deceptively simple appearing yes or no question is asked, obtaining the answer requires considering a complex network of interrelated biologic, economic and managerial issues. In most circumstances, answering the question rapidly evolves to answering a sequence of questions, the subsequent questions themselves depending on answers to previous questions and many of the answers being based on vague historical information or uncertain predictions of the future. For a given herd, the answer likely changes over time as the herd situation and market prices change in unexpected ways, and new products and strategies emerge from research. These relationships are sufficiently complex that relying on one's intuition alone, on rules of thumb or on standard practice is likely misleading and potentially dangerous to the herd's economic survival.

The questions requiring consideration range from the biologic to the economic to the managerial to the ethical. What is the within-herd prevalence? If it is unknown, what is the best strategy to determine it to the certainty required for decisions? What is the current impact of the infection on this herd? What are the risk and magnitude of a potential abortion storm? Are cost studies in other herds relevant to this herd? How likely is this infection to be transmitted horizontally in this

herd? Given the scarcity of resources, what are the competing causes of loss for which the cost-benefit ratio for monitoring and control would be higher? What preventive practices against this infection have collateral benefits against other diseases? How should the costs of a intervention common to several infections be apportioned between them? Does the time required to reach a target prevalence fit into management objectives, such as herd dispersal or expansion plans? Does this manager have sufficient ability and resources to implement the control strategy if one were identified? Is it ethical to sell an infected female for replacement purposes?

For any disease, the first questions are biologic ones: do we have the technical means to reduce disease occurrence or impact? Do we know enough about the epidemiology of the disease, and is that evidence of sufficient strength to develop plausible interventions against the disease to minimize the likelihood of its reintroduction into a herd? If we do, then the questions become primarily economic. If control involves a significant up-front investment requiring a multi-year payback, such as a major facility change or herd culling, is there sufficient evidence that the interventions work, that benefits will exceed costs in the long run?

Recent extensive reviews, selected from a range of sources, summarize our current understanding of the biological aspects of neosporosis infection and its diagnosis and control.[3-7, 18] For this parasite, the answers to many of the important questions are a qualified "yes". We likely have a sufficiently sound understanding of the key features of neosporosis epidemiology to develop reasonably plausible interventions against continuation of the infection status quo in a given herd. We know the major routes of transmission (vertical), the primary reservoirs (infected cows) and the key definitive hosts of concern (dogs and related canids) in herds. We know that for all intents and purpose, once infections are established they are lifelong. We have tests of sufficient performance that, when properly applied, detect infected cattle with reasonable accuracy, recognizing that false negatives and positives do occur but in limited numbers and circumstances. Plausible strategies to reduce herd prevalence are reasonably easy to devise. But we have little evidence that these interventions are effective over the long run from a technical perspective, and virtually none from an economic perspective.

For example, knowing that approximately three-quarters of offspring born to infected dams are infected via *in utero* transmission, we could apply several strategies to reduce vertical transmission to a negligible level. We can establish the infection status of the adults and older replacements in a herd with good, but not perfect, reliability through a one-time blood test. In situations of attended calvings, we can establish the status of the offspring that are potential replacements by drawing a pre-colostral blood sample and submitting it for serological testing. A positive test establishes the status of both the offspring and the dam reasonably reliably. Recent research suggests the reverse, a negative offspring test, is not as reliable for either dam or offspring.[13] Alternatively and likely more reliably, we could establish the status of potential replacements by bleeding them after their colostral titers have declined. Knowing that the risk of transmission via embryo transfer is very low to non-existent, vertical transmission from an infected dam with a valuable genome could be almost eliminated by transferring her embryos into titer-negative recipients.

Some essential biological information is clearly missing, components of our understanding will change with further research and some recent findings require further verification and illustrate the need for further research. As this is a very active research area with several hundred papers being published per year, regularly updating oneself through PubMed searches or resources such as Cornell Consultant is likely wise. Considerable work is being done on vaccine development, with one recent finding that immunization with a live form of the agent blocks vertical transmission on subsequent experimental challenge.[19] An example of study requiring independent verification is one suggesting that seronegative cattle and their fetuses may have freely circulating *N. caninum* DNA as detected by nested PCR, and thus are silently infected.[13] Another is the finding of organism DNA in colostrum, possibly (but unlikely) complicating colostrum management on dairies.[14] The body site in which the parasite resides in the chronically infected dam is unknown. Some have hypothesized that differences in infection impact between herds may be due to strain differences, but this hypothesis remains to be examined thoroughly. The background rate of horizontal transmission appears to be approximately on the order of one event per 100 cow-years, but to date little empirical evidence supports this estimate and the risk factors affecting the rate are unclear. Further, recent experimental evidence suggests that horizontal transmission is unlikely to establish persistent infections in the exposed dam, but is capable of establishing congenital infections in her fetus if it is exposed at certain stages of gestation.[12]

Our knowledge of definitive host oocyst shedding patterns and of oocyst environmental survival characteristics is inadequate. A better understanding of the risk that infected cattle present for horizontal transmission to herdmates is needed. Of particular concern are those scenarios that result in horizontal transmission to a large number of herdmates, because such exposures are suspected to be one of the causes of abortion storms. Other risk factors for such storms remain unknown. Rate of introduction from the sylvatic cycle and

associated reservoirs is unknown, as are the factors determining this rate. How secure must feed be from fecal contamination by vermin to significantly reduce this risk? How is feed security best assessed? Does reducing the likelihood of feed serving as a vehicle for fecal-oral transmission of infectious agents pay, particularly when combined with other potential infections transmitted in the same manner? Pieces of the information puzzle that are likely important are currently missing, and some initial experimental findings need strengthened through replication by independent investigators.

Based on our current knowledge of neosporosis epidemiology, the following range of prevention and control options or some variation are being recommended:[2,6]

- General biosecurity and hygiene measures:
  - Prevent fecal contamination of livestock feed and water by dogs and other canid definitive hosts
  - Properly dispose of placentas, fetal membranes, aborted fetuses and calf carcasses so that dogs and other carrion eaters cannot consume materials that are potentially infective for definitive hosts
  - Control rodents and other vermin around feed and housing facilities so they cannot attract definitive hosts or serve as intermediate hosts
  - Keep dogs, particularly pregnant bitches, litters or young puppies, and other canids away from livestock areas and particularly livestock feed areas
- Individual measures after testing and identifying seropositive dams:
  - Cull outright upon testing positive or after aborting
  - Exclude progeny as replacements or test prior to selection as a replacement
  - Breed dairy breeds with semen from beef bulls to reduce the risk of seropositive animals aborting
  - Transfer embryos from dams with valuable genomes to seronegative recipients
  - Future chemotherapy of dam or their offspring
- Vaccination

"But knowing how doesn't mean that we should" (Dr. xxx, AABP-L). The next questions are managerial and economic. What is the basis for evaluating potential intervention strategies? Are the criteria economic or non-economic, and over what time frame? Establishing freedom from the infection for non-economic reasons when cost is not a consideration is relatively straightforward. Test the herd and cull the seropostive. But for most herds, the criterion of ultimate concern is an economic one. What is the desired herd "state"? Reduced ongoing economic loss from endemic disease? Reduced risk of heavy economic loss from a future abortion storm? In some herds the infection appears to have no adverse consequences on production or reproduction. Reliably estimating prevalence is difficult enough in a single herd; reliably estimating economic effect of the endemic form is even more difficult.

Several recent papers address the control and monitoring decision from the economic perspective, but under considerably different scenarios. Because of the complexity of these scenarios and the differences between them, the following represents only a superficial summary. Larson and coworkers used a five-year dynamic Missouri farm profitability simulation model to estimate the effects of four strategies in beef cow-calf herds with starting seroprevalences of 10%, 30%, 50% and 70%.[11] During a period of relatively high feeder calf prices, mean return to fixed assets was reduced by 1.3% at a seroprevalence of 10%, up to a reduction of 8.1% at a seroprevalence of 70%. During a period of low feeder prices, mean return was reduced by 22% at a seroprevalence of 10% and by 30% at 70%. The four control strategies ranged from none, culling cows that did not produce a live calf post pregnancy diagnoses, herd testing and culling test-positive cows with replacement by test-negative heifers, and herd testing with heifers from seropositive dams being ineligible as replacements. The strategy of culling cows not producing a live calf after pregnancy diagnosis was found ineffective. During periods of high or low feeder prices, the least profitable strategy was test and cull while the most profitable strategy was to select replacement heifers only from seronegative dams. Under this strategy, in five years the herd prevalence declines to approximately half the initial value. As vertical transmission was modeled as 100% efficient but evidence suggests that it is not this high in many herds, a viable alternative strategy might be to test replacement candidates sometime after the disappearance of any colostral titers, such as during the selection process. The modeling results are sufficiently complicated that they are difficult to summarize succinctly with just three variable factors (initial herd prevalence, period of high feeder calf prices vs. period of low feeder calf prices, control strategy). Thus, determining the best strategy when the other factors differ from those used in the model, such as those associated with reproductive performance, feed costs and market values, appears virtually impossible without re-running the model itself. Further details about the model, such as whether it would be appropriate for use in modeling specific herd situations, are not provided in this or any referenced papers. This model also did not include the potential for horizontal transmission from sources outside the herd or from infected animals remaining within the herd, and did not include the risk of abortion storms.

Although these results are a guide, applying them to a specific herd situation likely should be done with caution.

Reichel and Ellis developed a decision tree model to determine the cost/benefit of control options for average-sized (300-cow) Australian dairies.[16] The control measures evaluated were status quo (do nothing), herd test and cull, a hypothetical treatment of infected cows that cost NZ$569 per cow and annual vaccination of the adult herd at NZ$10 per cow. A treatment efficacy of 90% was assumed and a vaccine efficacy of 50%. The risk of an abortion storm was established at 10% for the term of the model, the risk of horizontal transmission at 0.01 event per cow-year and no production effects were included. Evaluations were done under a variety of "best case", "average case" and "worst case" scenarios for risks and costs of events such as endemic abortion and abortion storms. The optimum solution varied with scenario severity, herd seroprevalence and length of planning horizon. Under the "average" scenario with a five-year planning horizon, up to a seroprevalence of 20% the optimal solution was to do nothing. Above 20%, the optimal solution was vaccination. Unfortunately, the authors did not consider the strategy of herd testing with heifers from seropositive dams being ineligible as replacements. Given the vaccine efficacy and cost assumed by the authors, at the test cost of NZ$10 used by the authors such a strategy would appear very competitive. The authors note that constructing a "local" decision tree to evaluate control options is prudent when dealing with other conditions such as in other countries.

Hasler and coworkers evaluated control options in two steps, both of which are well described.[8,9] First, they developed a dynamic deterministic simulation model to evaluate the infection dynamics of different control strategies in Swiss dairy herds and then developed a Monte Carlo spreadsheet simulation model to evaluate the economic costs, using the outputs of the first model as inputs for the second. They compared five main scenarios of doing nothing (the status quo with 12% seroprevalence), herd testing and culling, herd testing with heifers from seropositive dams being ineligible as replacements, a hypothetical treatment of potential replacements from all dams or just seropositive dams that cost Euro $3.80 per calf and annual vaccination of the adult herd at Euro $5 per cow. Both treatment and vaccine efficacies were assumed to be 60%, and testing was priced at Euro $41.50 per head. They used an endemic abortion risk of fourfold over baseline, a vertical transmission efficiency of 90% and derived an "unknown" introduction risk of approximately 0.001 events per cow year and a within-herd risk for a horizontal transmission risk of approximately 0.004 events per cow year. The two control strategies with positive benefit-cost ratios and positive net present values were therapeutic treatment of potential replacements and herd testing with heifers from seropositive dams being ineligible as replacements, the hypothetical treatment having the greatest economic benefit.

Because of the complex relationships between the factors and the differences in these factors between herds, several authors have recommended that a cost-benefit analysis specific for the herd be performed as part of this decision making process.[2,6,16] As developing and validating these models is time intensive, practitioners are more likely to benefit from using existing models, entering the specific factors for a herd much like the use of nutrition programs, rather than developing their own. As these models are complex and this complexity will likely increase as the important risk factors in the epidemiology of this infection and its effects are better understood, their underlying structure will likely change with time.

The preceding presumes that the infection has been identified as being present in a herd at a prevalence above a threshold of concern, or as being associated with sufficiently severe consequences that control options are being considered. Whether a herd of unknown status should be monitored for the infection and if so, how requires considering another set of questions. The first question is, why is monitoring being considered? Of what use will the information be if a low prevalence is discovered? How high is too high, and what will be done if it is above that level?

Although the modeling results above suggest that merely determining presence of the infection in a herd is not sufficient, doing so is reasonably straightforward. The first question is the best animals to sample to detect presence of the infection. The answer is those that are more likely to have it if it is present in the herd, such as cows found open after being confirmed pregnant or those observed aborting. The next question is how many to sample. The answer is enough to have the desired likelihood of at least one being found test-positive. An easy, "back of the envelope", "hood of the truck" way to determine this number is by determining the probability that all "n" samples are test-negative for a given prevalence "p" (between 0 and1) in the group from which the samples are being selected. Assuming that the test has high specificity and sensitivity (> 0.9), this probability is approximately $(1 - p)^n$. The probability of detecting the infection, if is present in that group, is then approximately $1 - (1 - p)^n$, which can be calculated on most calculators by trying different sample size guesses iteratively. For example, if one wanted a 95% chance of detecting the infection if it was present in 25% of the animals experiencing fetal loss problems in a herd, approximately 11 samples would be required: $1 - (1 - 0.25)^{11} = 0.96$, ignoring the effect of herd size. More direct formulas are available in resources such as Cannon and Roe[1] and Noordhuizen and coauthors.[15]

A cautionary note - determining that the infection is present in a group of animals showing non-specific signs of the disease does not mean the infection is the cause of the signs. Reasoning from the results of a case series, such as submitting samples only from the animals experiencing a non-specific clinical sign (such as pregnancy loss), can be very misleading with infections like this one that can occur predominately in a subclinical form with little impact. Generating evidence for a causal relationship requires further steps involving some form of comparison between affected and unaffected animals, such as a case-control study between animals experiencing the signs and those not. Concluding that such an infection is the cause of the problem when the prevalence is similar (within sampling variation) in unaffected animals results in overlooking the actual cause, which may be preventable. To avoid potential pitfalls in constructing, executing and interpreting such concurrent comparison studies, consult specialized texts[10,15,17] or the more general veterinary epidemiology texts. Attempting to estimate herd prevalence from such a group so selected is most likely folly; actual herd prevalence is likely considerably lower if the infection is associated with the clinical signs. Because of biological variability, the sample sizes required to detect economically important effects are often quite large.

The question of whether or not the infection prevalence is above or below an action threshold is a more expensive one to answer than whether the infection is present or not. For example, some have suggested that this critical threshold is a 15% prevalence, but as noted above others recommend that this should be determined for each herd. What are the potential costs of a false-positive mistake (undertaking a control program when the true prevalence is below the action threshold for the herd) vs. a false-negative mistake (not undertaking a control program when the true prevalence is above the action threshold)? To guide decisions, the sample size must be large enough to obtain a prevalence estimate of sufficient precision (a sufficiently narrow confidence interval) so as to reduce such mistakes to an acceptable level. If the sample size is too small, the estimated infection prevalence may be well on one side of the critical threshold for the herd but one of the 95% confidence interval bounds well on the other, leaving open the question about whether the infection is likely a problem or not. Table 1 shows the prevalence and 90% confidence interval limits, based on exact rather than Wald methods, adjusted for serum

**Table 1.** Adjusted infection prevalence and 90% confidence interval limits for 97% sensitive and 97% specific serum ELISA test.

| 90% | N = 40 | | | N = 30 | | | N = 20 | | | N = 10 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| number positive | Lower Bound | Adjusted Prevalence | Upper Bound | Lower Bound | Adjusted Prevalence | Upper Bound | Lower Bound | Adjusted Prevalence | Upper Bound | Lower Bound | Adjusted Prevalence | Upper Bound |
| 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0.07 | 0 | 0 | 0.21 |
| 1 | 0 | 0[a] | 0.06 | 0 | 0[a] | 0.10 | 0 | 0.02 | 0.17 | 0 | 0.07 | 0.36 |
| 2 | 0 | 0.02 | 0.11 | 0 | 0.04 | 0.16 | 0.01 | 0.07 | 0.25 | 0.03 | 0.18 | 0.49 |
| 3 | 0.01 | 0.05 | 0.15 | 0.02 | 0.07 | 0.21 | 0.03 | 0.13 | 0.32 | 0.08 | 0.29 | 0.59 |
| 4 | 0.02 | 0.07 | 0.18 | 0.03 | 0.11 | 0.25 | 0.06 | 0.18 | 0.38 | 0.15 | 0.39 | 0.69 |
| 5 | 0.04 | 0.10 | 0.22 | 0.05 | 0.15 | 0.29 | 0.09 | 0.23 | 0.44 | 0.22 | 0.50 | 0.78 |
| 6 | 0.05 | 0.13 | 0.25 | 0.08 | 0.18 | 0.34 | 0.13 | 0.29 | 0.50 | | | |
| 7 | 0.07 | 0.15 | 0.28 | 0.10 | 0.22 | 0.38 | 0.17 | 0.34 | 0.55 | | | |
| 8 | 0.09 | 0.18 | 0.31 | 0.13 | 0.25 | 0.41 | 0.21 | 0.39 | 0.60 | | | |
| 9 | 0.11 | 0.21 | 0.34 | 0.16 | 0.29 | 0.45 | 0.26 | 0.45 | 0.65 | | | |
| 10 | 0.13 | 0.23 | 0.37 | 0.19 | 0.32 | 0.49 | 0.30 | 0.50 | 0.70 | | | |
| 11 | 0.15 | 0.26 | 0.40 | 0.21 | 0.36 | 0.53 | | | | | | |
| 12 | 0.17 | 0.29 | 0.43 | 0.24 | 0.39 | 0.56 | | | | | | |
| 13 | 0.20 | 0.31 | 0.46 | 0.28 | 0.43 | 0.59 | | | | | | |
| 14 | 0.22 | 0.34 | 0.48 | 0.31 | 0.47 | 0.63 | | | | | | |
| 15 | 0.24 | 0.37 | 0.51 | 0.34 | 0.50 | 0.66 | | | | | | |
| 16 | 0.26 | 0.39 | 0.54 | | | | | | | | | |
| 17 | 0.29 | 0.42 | 0.56 | | | | | | | | | |
| 18 | 0.31 | 0.45 | 0.59 | | | | | | | | | |
| 19 | 0.34 | 0.47 | 0.61 | | | | | | | | | |
| 20 | 0.36 | 0.50 | 0.64 | | | | | | | | | |

[a] Note: The adjusted prevalence is zero because the specificity of the test is less than 100%.

ELISA test performance (97% sensitivity, 97% specificity) and ignoring sampling fraction for the number of test positives from sample sizes of 10, 20, 30 and 40. The 90% confidence intervals are less conservative (narrower) than 95% confidence limits.

Currently, the best way to establish herd infection prevalence is by testing individuals from a randomly selected sample of the herd members at risk of the condition (infection), excluding those in late pregnancy or immediately postpartum because of depressed antibody levels during those periods. Although milk-based ELISA tests perform sufficiently well on individual milk samples that they can be used in reliably determining infection prevalence in a dairy herd, they currently don't perform well on bulk-tank milk samples in most circumstances. Because bulk-tank milk is much more easily obtained than samples from individual animals, particularly of serum, considerable work is under way to improve the analytical performance of milk ELISAs and to better understand how to apply the procedure to bulk-tank milk samples, such as repeated sampling over time.

If the herd is raising its own replacements, a logical place to begin is testing the potential replacements both to establish within-herd prevalence and to begin the selection process if that prevalence is above the herd's critical threshold. Unless the herd is experiencing an unusually high horizontal transmission frequency during youngstock rearing, a test-positive result from a replacement also establishes her dam's status as well. Because vertical transmission is not 100% efficient, the converse (a negative test establishing her dam's status) is not true. As long as dam-to-offspring identification is maintained, not testing the offspring from dams tested prior to their selection as replacements would not be a perfect strategy, but would minimize control costs. The process of testing replacements could continue until a majority of the cows in the mature herd were tested negative prior to their selection as replacements, offspring from the balance of untested dams not being considered as replacements.

## Conclusion

Ongoing research will better define the epidemiology of bovine neosporosis, improve test performance and application, increase vaccine efficacy and determine the benefit of different control and prevention strategies. This better understanding and new tools will likely improve the strategies available to control the infection in beef and dairy herds. Because of the complex relationships between the factors involved in the determining the benefits of alternative control strategies and their differences between herds, practitioners would benefit from access to decision modeling software into which they can enter herd-specific values for the important variables. At present, the economically optimal control strategy appears to be selecting replacements on the basis of their seronegativity in those herds with infection prevalence above a critical threshold.

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