PEER REVIEWED

A Review of *Neospora caninum* in Cattle

M.W. Sanderson, *DVM, MS, DACVPM (Epidemiology), DACT*
Department of Clinical Sciences, Kansas State University, Manhattan, KS 66506, Ph 785-532-5700, Fax 785-532-4989, e-mail sandersn@vet.ksu.edu

Abstract

Neospora caninum is a protozoan similar to *Toxoplasma* and *Sarcocystis* species, and has become a commonly recognized cause of bovine abortion. Recent research has expanded our knowledge of the epidemiology of *N. caninum*. Within herds, vertical transmission appears to be the primary method of infection, but horizontal transmission does occur and may be sufficient for maintenance of endemic infection within a herd. The coyote and domestic dog are definitive hosts for *Neospora*, and serologic studies show other wild canids can be infected as well. Herd control of infection may be attained by culling positive animals, preventing entry of positive animals and preventing likely routes of horizontal infection, although the economic value of these interventions requires further research.

Keywords: bovine, neospora, review, abortion

Résumé

Neospora caninum est un protozoaire similaire à *Toxoplasma* et *Sarcocystis* et est de plus en plus reconnu comme une cause d'avortement chez les bovins. Des travaux récents ont permis d'élargir nos connaissances sur l'épidémiologie de *N. caninum*. La transmission verticale semble la voie d'infection la plus courante dans un troupeau mais la transmission horizontale est aussi possible et peut maintenir l'endémisme de l'infection dans un troupeau. Le coyote et le chien sont les hôtes définitifs de *Neospora* et des études sérologiques montrent que d'autres espèces de canidés peuvent aussi être infectées. Le contrôle de l'infection dans un troupeau peut se faire par la réforme des animaux positifs, en prévenant l'entrée d'animaux positifs et en évitant les voies probables d'infection horizontale. Toutefois, la valeur économique de ces approches n'est pas bien établie.

Clinical Syndrome

Neospora caninum is a commonly recognized cause of abortion in dairy[1] and beef cattle,[29,52,53] and increased culling[47] and decreased milk production[48] in dairy cattle. Abortions are most commonly seen from five to six months of gestation,[1] although late-term abortions, weak-born calves and calves born seropositive with no clinical abnormalities may also occur.[19] This pattern of predominantly mid-term abortions is unique among bovine abortion agents. No other clinical signs of disease are apparent in mature animals. Serological evidence of *N. caninum* exposure is common in US beef and dairy herds. Overall individual animal seroprevalence in beef herd surveys has been 18-23%, with individual within-herd seroprevalence ranging from 0 to 67%.[41,44] Herd prevalence has ranged from 55 to 100%.[41,44] In dairy seroprevalence surveys, overall individual animal seroprevalence has been 16-36%, with individual herd seroprevalence ranging from 0 to 100%.[14,33,38] A negative association has been shown between *N. caninum* serostatus and post-weaning weight gain, carcass characteristics and cost of treatment.[5,6] Further studies are needed to validate these results. Risk of abortion is approximately two to seven times higher in seropositive cows compared to seronegative cows.[40,49] Seropositive cows that have aborted previously are approximately five times more likely to have a second abortion, compared to seropositive cows that have not had a previous abortion.[49]

Diagnosis of Disease

A number of diagnostic tests to identify a serologic response to *N. caninum* have been reported in the literature. An enzyme-linked immunosorbant assay (ELISA) test kit is commercially available.[a] A positive serum ELISA indicates previous exposure to *N. caninum* in the cow, but is not sufficient to establish *N. caninum* as the cause of an abortion.[21] Most congenital infections result in a live-born, clinically normal calf,[39] with *N. caninum* lesions and positive immunoperoxidase staining.[50] Presence of fetal lesions consistent with *N. caninum* infection, therefore, does not establish *Neospora* as the cause of abortion, suggesting that some late-term abortions diagnosed as *N. caninum* may be false positives.[50] Serostatus of the individual cow at the time of pregnancy examination has been associated with

abortion risk,[52] but has not been consistently associated with pregnancy status.[44,52] Timing of infection or recrudescence may be important in determining the outcome of *N. caninum* infection.[39] One study examined timing of experimental infection of cows following intravenous administration of 10^7 tachyzoites. Of six cows infected nine weeks prior to breeding, all produced normal, seronegative calves at term, although it was not certain that infection was established in the dams. Experimental infection of six cows at 10 weeks of gestation resulted in abortion three weeks later in five of six cows, while infection at 30 weeks of gestation resulted in birth of clinically normal, but seropositive and congenitally infected calves in six of six cows.[54] Gondim *et al*[24] inoculated cows orally with varying numbers of oocysts (1500-115,000) at varying times of gestation ranging from 70 to 176 days. They concluded that increasing numbers of oocytes and exposure later in pregnancy increases risk for fetal infection. Only one cow out of the 19 inoculated aborted her fetus, but 17 of 19 cows became seropositive. The live-born calves were euthanized, and based on histologic and immunohistologic evidence, six calves were determined to be infected. The differences between the two studies may be due to the different routes of exposure. The first study used intravenous inoculation, and therefore may more closely match tissue cyst recrudescence and intravenous transmission to the fetus. The second oral inoculation may more closely approximate oral exposure and horizontal transmission. In both cases a likely natural exposure dose is unknown, so specific application to production enterprises is difficult.

Reservoir and Transmission of *N. caninum* in Cattle

The life cycle of *N. caninum* and associated risk factors for bovine infection have not been completely defined. A seropositive test result indicates previous exposure to *N. caninum,* and once a bovine is infected they remain infected. Periodic recrudescence of infection may stimulate increased immunity and result in transmission to the fetus. Vertical transmission[10,15,39] is common. Estimates of the efficiency of vertical transmission from dam to fetus range from 44%[8] to over 90%,[15,39,45] and is apparently higher in herds with a high prevalence of seropositive animals.[45] Available evidence indicates horizontal transmission also occurs,[34] although at low rates of approximately one to eight infections per 100 cows per year.[15,28,40] Colostrum inoculated with *N. caninum* tachyzoites has also been shown to transmit infection to calves experimentally,[51] but shedding of *Neospora* tachyzoites in bovine colostrum or milk has not been shown, so the significance of this study is unknown. Dogs[31,35] and coyotes[26] are definitive hosts for *N. caninum* and widespread seropositivity has been established in domestic and wild canids, including foxes and coyotes.[4,11,32] Deer may also be intermediate hosts[20] and could be a source of infection for dogs and wild canids. Feeding brains from naturally infected deer to dogs resulted in two of four dogs shedding oocysts.[25] Infection in cattle resulting from ingestion of oocysts from the feces of a definitive host, such as dogs or wild canids, has been demonstrated,[16,24] and has been hypothesized as a route of horizontal transmission within herds.[36] Recently reported data from an outbreak investigation has supported the role of the domestic dog in horizontal transmission.[37] Abortions due to *N. caninum* may be a source of infection for dogs or wild canids. In one study, ingestion of naturally infected bovine placenta by dogs resulted in shedding of oocysts in their feces.[17] In a separate study, dogs did not shed oocysts in their feces when fed naturally infected fetuses, so additional factors beyond exposure may be important.[9] Increased seroprevalence on ranches has been associated with increased cow density,[7,44] with increased wild canid density[7] and with the presence of dogs on the farm or ranch.[55] While both vertical and horizontal transmission are possible, vertical transmission appears to be the predominant route, and infection can be maintained over multiple generations in a herd without significant horizontal transmission.[23,45]

Recently *N. caninum* has been identified intermittently in low numbers (1-10 parasites/mL) in the semen of seropositive, naturally infected bulls.[22] Subsequently, a study examined transmission by frozen-thawed semen artificially contaminated with 10^6-10^7 *N. caninum* tachyzoites using a total of eight cows (four exposed and four controls). None of the four exposed cows became pregnant, and only one of the four control heifers; only one of the exposed cows mounted a transient immune response.[12] In contrast, another study utilized 18 primiparous heifers (nine exposed and nine control). Exposed heifers were inseminated with semen contaminated with 10^7 *N. caninum*. All nine exposed heifers seroconverted, and *N. caninum* DNA was found in blood of all nine head and in brain, lung, liver and uterine horn of several. At necropsy 36 days after insemination, one of nine exposed heifers had viable embryos, and six of nine controls had viable embryos.[46] While these data suggest that *N. caninum* may be transmitted in semen and play a role in early embryonic death, other explanations should be considered. Both studies utilized inoculation doses in semen four to five orders of magnitude above currently recognized natural levels. The significance of these results is uncertain in the epidemiology of *N. caninum* infection, but bears further research.

Prevention and Control

While criteria to establish critical control points

for *N. caninum* are not yet clear, some recommendations can be made. Ideally, imports should only come from herds with records of excellent reproductive performance to decrease the risk of importing a problem animal. Seropositive cows do appear more likely to have an abortion,[40,49] but are unlikely a significant risk to the herd until calving, when an infected calf may be born or a canine infected by placental tissues. Mathematical modeling of *N. caninum* infection in dairy herds suggests that culling seropositive cattle is an effective method of control to reduce prevalence of infection within a herd.[23] Culling alone, however, was not sufficient to reduce prevalence to zero in the models without changes to minimize horizontal transmission as well.[23] According to these disease models, horizontal transmission alone can maintain low-level endemic infection in the herd.[23] A plan to identify and cull positive animals from the herd as well as quarantine and testing to identify and exclude seropositive imports may be useful, but methods to control horizontal transmission would likely also be necessary. Clearly, if *Neospora* is already common in the herd, quarantine and testing of imported cattle alone will be ineffective in controlling disease. The results of these models[23] are consistent with observational field studies, however further observational and experimental research is needed to confirm their findings before clear recommendations can be made.

The economic value of control strategies has been examined in beef[30] and dairy herds.[42] A five-year economic simulation model in beef herds evaluated varying seroprevalences and control strategies.[30] Endemic *N. caninum* infection in the herd decreased return to fixed assets, and this effect grew with increasing within-herd prevalence. No intervention and three possible control strategies were modeled: 1) culling females that experienced mid-term abortion or a stillborn calf; 2) testing the whole herd, selling seropositive females and purchasing seronegative replacements; and 3) testing the whole herd and excluding female offspring of positive cows. Control strategy 3 showed the most favorable return to fixed assets over the five-year period.[29] The authors commented that strategy 3 halted introductions of infected replacements without incurring the costs of substantial culling in the herd. A dairy economic decision model based on New Zealand and Australian dairy systems found a strategy of no intervention to be most economical at low-to-moderate herd seroprevalence (11-31%, depending on the cost of abortions). As herd seroprevalence increased up to 15-33% (again depending on the cost of abortions), vaccination with a 50% efficacious vaccine was the most economic alternative.[42] The differences in conclusion of the two models may reflect both significant differences in dairy vs. beef management as well as differences in the model assumptions, and indicate a need for greater understanding of the epidemiology of *N. caninum* to inform modeling, prevention and control efforts.

Models also suggest that control of environmental sources of horizontal transmission of *N. caninum* infection may be necessary to eradicate disease from a herd.[23] If canine feces are a significant source of infection for cows or calves, then control measures to minimize exposure may be prudent. Placentas and fetuses should be collected and disposed of as promptly as possible. Dogs and wild canids should be prevented from consuming placenta and fetal tissues from abortions. Cattle feed sources should be protected so that dogs and wild canids do not have access to them.

Vaccination

A study of the immunologic response to an experimental vaccine has been published,[2] but in a subsequent study efficacy was not demonstrated following experimental challenge.[3] Recently, a vaccine for *N. caninum* has received full approval and is commercially available in the US.[b] An anecdotal report of a decrease in abortions in a dairy herd following vaccination has been published, however there was no control group and the criteria for allocation of cows to different vaccination schedules was not clear.[13] In a field trial involving the same vaccine in 876 cows in 25 Costa Rican dairy herds, vaccinated cows had an 11% abortion rate and unvaccinated cows a 21% abortion rate (vaccine efficacy 46%).[43] Cows were eligible to be enrolled in the trial at two months' gestation. For each cow enrolled in the vaccinated group, a control cow was selected and matched on herd age and breed. Criteria for allocation and method of allocation were not specified. The authors reported marked differences in the vaccine effect between herds, but it was not clear from the methods whether the potential non-independence associated with the multiple herds was statistically controlled for. No testing was done to confirm *N. caninum* as the cause of any abortions or for other potential causes of abortion. A Kaplan-Meier time-to-abortion analysis indicated that the difference in abortion risk between the vaccinated and control groups occurred between 150 and 200 days of gestation, when the rate of abortion in vaccinated cows was significantly lower. This pattern is consistent with the expected mid-term abortion risk of *Neospora*.[43] A New Zealand field trial utilized 2240 pregnant cows in five dairy herds selected for the trial based on a documented history of abortion rates above 8% and the confirmed presence of *N. caninum*.[27] Cows were randomized to initial vaccination or placebo at 25-45 days into gestation, with boosters three to four weeks later. The analysis appropriately accounted for herd effects. The effect of vaccine varied between herds and with the pre-vaccination *N. caninum* serostatus of the cow. The overall vac-

cine efficacy estimate for the study was 24.6%, but was not significant. A significant vaccine efficacy of 54% was found in one herd.[27]

Available vaccination studies are consistent with a modest effect of vaccine in reducing abortion risk in some herds, however substantial uncertainty remains. Additional well designed and implemented studies are needed to clarify the significance and magnitude of the vaccine effect. Well characterized vaccine efficacy is critical for implementation of an appropriate management decision regarding vaccine use. A vaccination program could complicate differentiation of naturally infected animals from vaccinates and limit identification and culling options. The relative merits of vaccination and culling, as well as an appropriate integration of the two, is not yet clear. Clearly, more research on the value of culling and the effectiveness of vaccination is required.

Conclusion

Substantial research progress has been made in our knowledge of *Neospora caninum* in cattle and recent reviews are available.[18] Additional questions remain, however, regarding diagnosis, transmission, critical risk factors and times, and vaccine efficacy. Additional research efforts are needed to further understand the epidemiology and ecology of this disease and to formulate cost effective prevention protocols.

Acknowledgement

No external funding was used for preparation of this manuscript.

Endnotes

[a] HerdChek®, IDEXX, Westbrook, MA
[b] Neoguard™, Intervet Inc., Millsboro, DE

References

1. Anderson ML, Blanchard PC, Barr BC, Dubey JP, Hoffman RL, Conrad PA: *Neospora*-like protozoan infection as a major cause of abortion in California dairy cattle. *J Am Vet Med Assoc* 198:241-244, 1991.
2. Andrianarivo AG, Choromanski L, McDonough SP, Packham AE, Conrad PA: Immunogenicity of a killed whole *Neospora caninum* tachyzoite preparation formulated with different adjuvants. *Int J Parasitol* 29:1613-1625, 1999.
3. Andrianarivo AG, Rowe JD, Barr BC, Anderson ML, Packham AE, Sverlow KW, Choromanski L, Loui C, Grace A, Conrad PA: A POLYGEN™-adjuvanted killed *Neospora caninum* tachyzoite preparation failed to prevent foetal infection in pregnant cattle following i.v./i.m. experimental tachyzoite challenge. *Int J Parasitol* 30:985-990, 2000.
4. Barber JS, Gasser RB, Ellis J, Reichel MP, McMillan D, Trees AJ: Prevalence of antibodies to *Neospora caninum* in different canid populations. *J Parasitol* 83:1056-1058, 1997.
5. Barling KS, Lunt DK, Snowden KF, Thompson JA: Association of serologic status for *Neospora caninum* and postweaning feed efficiency in beef steers. *J Am Vet Med Assoc* 219:1259-1262, 2001.
6. Barling KS, McNeill JW, Thompson JA, Paschal JC, McCollum FT, Craig TM, Adams LG: Association of serologic status for *Neospora caninum* with postweaning weight gain and carcass measurements in beef calves. *J Am Vet Med Assoc* 217:1356-1360, 2000.
7. Barling KS, Sherman M, Peterson MJ, Thompson JA, McNeill JW, Craig TM, Adams LG: Spatial associations among density of cattle, abundance of wild canids, and seroprevalence to *Neospora caninum* in a population of beef calves. *J Am Vet Med Assoc* 217:1361-1365, 2000.
8. Bergeron N, Fecteau G, Pare J, Martineau R, Villeneuve A: Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Quebec. *Can Vet J* 41:464-469, 2000.
9. Bergeron N, Fecteau G, Pare J, Villeneuve A, Girard C, Pare J: Failure of dogs to shed oocysts after being fed bovine fetuses naturally infected by *Neospora caninum*. *Vet Parasitol* 97:145-152, 2001.
10. Bjorkman C, Johansson O, Stenlund S, Holmdahl OJM, Uggla A: *Neospora* species infection in a herd of dairy cattle. *J Am Vet Med Assoc* 208:1441-1443, 1996.
11. Buxton D, Maley SW, Pastoret PP, Brochier B, Innes EA: Examination of red foxes (*Vulpes vulpes*) from Belgium for antibody to *Neospora caninum* and *Toxoplasma gondii*. *Vet Rec* 141:308-309, 1997.
12. Canada N, Sofia Meireles C, Ferreira P, Correia da Costa JM, Rocha A: Artificial insemination of cows with semen *in vitro* contaminated with *Neospora caninum* tachyzoites failed to induce neosporosis. *Vet Parasitiol* 139:109-114, 2006.
13. Choromanski L, Zimmerman J, Rodgers S: Evaluation of the field performance of the first commercial *Neospora* vaccine in dairy cattle. *Proc Am Assoc Bov Pract* 34:149, 2001.
14. Davison HC, French NP, Trees AJ: Herd-specific and age-specific seroprevalence of *Neospora caninum* in 14 British dairy herds. *Vet Rec* 144:547-550, 1999.
15. Davison HC, Otter O, Trees AJ: Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infections in dairy cattle. *Int J Parasitol* 29:1683-1689, 1999.
16. DeMarez T, Liddell S, Dubey JP, Jenkins MC, Gasbarre L: Oral infection of calves with *Neospora caninum* oocysts from dogs: humoral and cellular immune responses. *Int J Parasitol* 29:1647-1657, 1999.
17. Dijsktra TH, Eysker M, Schares G, Conraths FJ, Wouda W, Barkema HW: Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. *Int J Parasitol* 31:747-752, 2001.
18. Dubey JP: Neosporosis in cattle. *Vet Clin North Am Food Anim Pract* 21:473-483, 2005.
19. Dubey JP: Neosporosis in cattle: biology and economic impact. *J Am Vet Med Assoc* 214:1160-1163, 1999.
20. Dubey JP, Hollis K, Romand S, Thulliez P, Kwok WCH, Hungerford L, Anchor C, Etter D: High prevalence of antibodies to *Neospora caninum* in white tailed deer (*Odocoileus virginianus*). *Int J Parasitol* 29:1709-1711, 1999.
21. Dubey JP, Jenkins MC, Adams DS, McAllister MM, Anderson-Sprecher R, Baszler TV, Kwok OCH, Lally NC, Bjorkman C, Uggla A: Antibody responses of cows during an outbreak of neosporosis evaluated by indirect fluorescent antibody test and different enzyme linked immunosorbant assays. *J Parasitol* 83:1063-1069, 1997.
22. Ferre I, Aduriz G, delPozo I, Regidor Cerrillo J, Atxaerandio R, Collantes Fernandez E, Hurtado A, Ugarte Garagalza C, OrtegaMora LM: Detection of *Neospora caninum* in the semen and blood of naturally infected bulls. *Therio* 63:1504-1518, 2005.
23. French NP, Clancy D, Davison HC, Trees AJ: Mathematical models of *Neospora caninum* infection in dairy cattle: transmission and options for control. *Int J Parasitol* 29:1691-1704, 1999.
24. Gondim LF, McAllister MM, Anderson-Sprecher RC, Bjorkman C, Lock TF, Firkins LD, Gao L, Fischer WR: Transplacental transmission and abortion in cows administered *Neospora caninum* oocysts. *J Parasitol* 90:1394-1400, 2004.
25. Gondim LF, McAllister MM, Mateus-Pinilla NE, Pitt WC, Mech LD, Nelson ME: Transmission of *Neospora caninum* between wild and domestic animals. *J Parasitol* 90:1361-1365, 2004.

26. Gondim LF, McAllister MM, Pitt WC, Zemlicka DE: Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *Int J Parasitol* 34:159-161, 2004.
27. Heuer C, Nicholson C, Russell D, Weston J: Efficacy of vaccination against *Neospora caninum* for the prevention of abortion in New Zealand dairy cattle. *19th International Conf World Assoc Advancement Vet Parasitol*. New Orleans, USA, 2003.
28. Hietala SK, Thurmond MC: Postnatal *Neospora caninum* transmission and transient serologic response in two dairies. *Int J Parasitol* 29:1669-1676, 1999.
29. Hoar BR, Ribble CS, Spitzer CC, Spitzer PG, Janzen ED: Investigation of pregnancy losses in beef cattle herds associated with *Neospora* sp infection. *Can Vet J* 37:364-366, 1996.
30. Larson RL, Hardin DK, Pierce VL: Economic considerations for diagnostic and control options for *Neospora caninum*-induced abortions in endemically infected herds of beef cattle. *J Am Vet Med Assoc* 224:1597-1604, 2004.
31. Lindsay DS, Dubey JP, Duncan RB: Confirmation that the dog is a definitive host for *Neospora caninum*. *Vet Parasitol* 82:327-333, 1999.
32. Lindsay DS, Kelly EJ, McKown RD, Stein FJ, Plozer J, Herman J, Blagburn BL, Dubey JP: Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in coyotes (*Canis latrans*) and experimental infections of coyotes with *Neospora caninum*. *J Parasitol* 82:657-659, 1996.
33. Mainar-Jaime RC, Thurmond MC, Berzal-Herranz B, Hietala SK: Seroprevalence of *Neospora caninum* and abortion in dairy cows in northern Spain. *Vet Rec* 145:72-75, 1999.
34. McAllister MM, Bjorkman C, Anderson-Sprecher R, Rogers DG: Evidence of a point source exposure to *Neospora caninum* and protective immunity in a herd of beef cows. *J Am Vet Med Assoc* 217:881-887, 2000.
35. McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM: Dogs are definitive hosts for *Neospora caninum*. *Int J Parasitol* 28:1473-1478, 1998.
36. McAllister MM, Huffman EM, Hietala SK, Conrad PA, Anderson ML, Salman MD: Evidence suggesting a point source exposure in an outbreak of bovine abortion due to neosporosis. *J Vet Diagn Invest* 8:355-357, 1996.
37. McAllister MM, Wallace RL, Bjorkman C, Gao L, Firkins LD: A probable source of *Neospora caninum* infection in an abortion outbreak in dairy cows. *Bov Pract* 39:69-74, 2005.
38. Pare JP, Fecteau G, Fortin M, Marsolais G: Seroepidemiologic study of *Neospora caninum* in dairy herds. *J Am Vet Med Assoc* 213:1595-1598, 1998.
39. Pare JP, Thurmond MC Hietala SK: Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. *Can J Vet Res* 60:133-139, 1996.
40. Pare JP, 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.
41. Quintanilla-Gozalo A, Pereira-Bueno J, Tabares E, Innes EA, Gonzalez-Paniello R, Ortega-Mora LM: Seroprevalence of *Neospora caninum* infection in dairy and beef cattle in Spain. *Int J Parasitol* 29:1201-1208, 1999.
42. Reichel MP, Ellis JT: If control of *Neospora caninum* infection is technically feasible does it make economic sense? *Vet Parasitol* 142:23-34, 2006.
43. Romero JJ, Perez E, Frankena K: Effect of a killed whole *Neospora caninum* tachyzoite vaccine on the crude abortion rate of Costa Rican dairy cows under field conditions. *Vet Parasitol* 123:149-159, 2004.
44. Sanderson MW, Gay JM, Baszler TV: *Neospora caninum* seroprevalence and associated risk factors in beef cattle in the northwestern United States. *Vet Parasitol* 90:15-24, 2000.
45. Schares G, Peters M, Wurm R, Barwald A, Conraths FJ: The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analysed by serologic techniques. *Vet Parasitol* 80:87-98, 1998.
46. Serrano E, Ferre I, Osoreo K, Aduriz G, Mateos-Sanz A, Martinez A, Atxaerandio R, Hidalgo CO, Ortega-Mora LM: Intrauterine *Neospora caninum* inoculation of heifers. *Vet Parasitol* 135:197–203, 2006.
47. Thurmond MC, Hietala SK: Culling associated with *Neospora caninum* infection in dairy cows. *Am J Vet Res* 57:1559-1562, 1996.
48. Thurmond MC, Hietala SK: Effect of *Neospora caninum* infection on milk production in first lactation dairy cows. *J Am Vet Med Assoc* 210:672-674, 1997.
49. Thurmond MC, Hietala SK: Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. *Am J Vet Res* 58:1381-1385, 1997.
50. Thurmond MC, Hietala SK, Blanchard PC: Predictive values of fetal histopathology and immunoperoxidase staining in diagnosing bovine abortion caused by *Neospora caninum* in a dairy herd. *J Vet Diagn Invest* 11:90-94, 1999.
51. Uggla A, Stenlund S, Holmdahl OJM, Jakubek EB, Thebo P, Kindahl H, Bjorkman C: Oral *Neospora caninum* inoculation of neonatal calves. *Int J Parasitol* 28:1467-1472, 1998.
52. Waldner CL, Janzen ED, Ribble CS: Determination of the association between *Neospora caninum* infection and reproductive performance in beef herds. *J Am Vet Med Assoc* 213:685-690, 1998.
53. Waldner CL, Janzen ED, Henderson J, Haines DM: Outbreak of abortion associated with *Neospora caninum* infection in a beef herd. *J Am Vet Med Assoc* 215:1485-1490, 1999.
54. Williams DJL, Guy CS, McGarry JW, Guy F, Tasker L, Smith RF, MacEachern K, Cripps PJ, Kelly DF, Trees AJ: *Neospora caninum*-associated abortion in cattle: the time of experimentally-induced parasitaemia during gestation determined foetal survival. *Parasitol* 121:347-358, 2000.
55. Wouda W, Dijkstra TH, Kramer AMH, van Maanen C, Brinkhof JMA: Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. *Int J Parasitol* 29:1677-1682, 1999.