IMPACT OF NEOSPOROSIS ON BOVINE REPRODUCTION

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INTRODUCTION

Bovine neosporosis is caused by *Neospora caninum* [31], an apicomplexan protozoal parasite closely related to *Toxoplasma gondii*. The parasite was first identified in dogs with encephalomyelitis and myositis [18, 19, 31] and was first associated with an abortion storm in 1987 on a dairy in New Mexico [60]. Since then, bovine neosporosis has emerged as an important worldwide cause of reproductive disease affecting both dairy and beef cattle [4, 32, 33, 34]. Bovine neosporosis has been diagnosed in many countries from six continents. Neosporosis is a major cause of bovine abortion in New Zealand, the Netherlands, the United Kingdom and California in the USA, resulting in significant economic losses. In addition to cattle, natural *N. caninum* infection has been reported in dogs, goats, sheep, horses and deer [4, 32, 33, 34].

NEOSPORA CANINUM: LIFE CYCLE AND MODES OF TRANSMISSION

Like other apicomplexan coccidia, the life cycle of *N. caninum* requires two hosts: an intermediate host and a definitive host. The asexual cycle involves the tachyzoite and the bradyzoite stages of the parasite and occurs in a wide range of intermediate hosts, including cattle, dogs, goats, sheep, horses and deer. The sexual cycle occurs in the definitive host and leads to the production of oocysts. Dogs (*Canis familiaris*) and coyotes (*Canis latrans*) were induced to excrete oocysts in their feces after being fed *N. caninum*-infected murine and/or bovine tissues; they are therefore definitive hosts of *N. caninum* [27, 36, 37, 45, 47]. To date, there are three reported cases of dog naturally excreting oocysts of *N. caninum* [15, 48, 59]. One study reports that dogs fed *N. caninum*-infected bo*vine* fetuses failed to shed oocysts [17].
Cattle may acquire *N. caninum* infection, either by horizontal (postnatal) or vertical (prenatal) transmission. Horizontal infection occurs through ingestion of feed or water contaminated with oocysts shed in the feces of the definitive canine host [26, 36, 67]. Vertical infection involves transplacental transmission of tachyzoites from an infected dam to its fetus during pregnancy [3, 4, 25, 56]. When oocysts are ingested by cattle, sporozoites are released in the gut and become tachyzoites. The rapidly dividing tachyzoites spread through the body, and invade the cells of a variety of organs resulting in tissue damage. The tachyzoite stage is associated with inflammation and necrosis at the site of invasion. In pregnant cattle, tachyzoites can invade the fetus via the placenta. With the onset of the bovine host immune response, tachyzoites revert to the slowly-dividing bradyzoites within tissue cysts. Tissue cysts are surrounded by a thick cyst wall and are mostly found in neural tissues (brain, spinal cord). Tissue cysts elicit minimal inflammatory reaction and can persist in cattle for long periods of time without clinical manifestations. It is assumed that during pregnancy, the bradyzoites in these latent cysts become activated and differentiate into the motile, infective tachyzoite stages which travel via the blood and/or tissues to the placenta and fetus. As a result of this “reactivation” of the parasites, repeat fetal infections can occur in the same persistently infected dam in multiple pregnancies. While some fetal infections lead to

abortion, most result in the birth of live but congenitally infected calves. Congenitally infected heifers can in turn transmit the infection to their future progeny. This cycle of vertical transmission is a major factor in maintaining *N. caninum* infection in cattle herds [3, 20, 25, 51, 56].

Vertical transmission appears to be the predominant route of infection in Europe and the USA with an efficiency rate between 80 and 95% [3, 20, 25, 51, 56, 65, 70], while lower rates (23.5-60%) are found in New Zealand [54, 57]. In endemic herds, the majority of calves born to naturally infected 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, suggesting that the rate of postnatal infection is low. Moreover, the congenitally infected calves have a chronic persistent infection which can be passed on transplacentally to their offspring.

While vertical transmission appears to be the major way that cattle become infected with *N. caninum* in endemic herds, there is serologic evidence that cows that have aborted during an epidemic probably acquired the infection after birth based on the lack of association between the seropositivity of dams and daughters [65]. In addition, the pattern of abortion outbreaks in epidemic neosporosis is suggestive of a point source exposure with acquired infection [14, 30, 32, 46, 61]. Another study reports a high rate of seroconversion for *N. caninum* in a dutch dairy herd, involving 47% of seronegative animals within a period of 6 months [28]. In this herd, a low avidity antibody (indicating a recent infection) and a lack of association between the serological status of daughters and mothers are consistent with a point source exposure. Interestingly, the high rate of seroconversion was not associated with an increase in abortions, indicating that mass seroconversion in dairy herds may go unnoticed [28]. In endemically infected herds which have been sampled more extensively, there is serologic evidence that a low level of postnatal infection from unknown sources, apparently does occur [16, 25, 39, 52, 56, 66]. This is consistent with results from mathematical modeling studies which suggest that *N. caninum* infection would disappear from cattle herds over time if vertical transmission was the only route of infection [35].

There is epidemiological evidence which suggests that dogs play a role in the transmission of neosporosis. Several studies report an association between the herd seroprevalence of *N. caninum* infection and the presence and number of dogs at the herd [29, 49, 53, 55, 71]. A spatial analysis study in Texas beef cattle herds reveals that seropositivity for *N. caninum* was associated with cattle density and abundance of coyotes and/or grey foxes (*Urocyon cinereoargenteus*), suggesting a possible sylvatic transmission cycle of the infection [8].

CLINICAL PRESENTATION

There are no signs of clinical illness in cows that abort due to *N. caninum* infection. The aborted fetuses are usually autolyzed with no gross lesions and placentas are not retained. Abortions have been diagnosed from three months of gestation to term, but the majority of *N. caninum* abortions occur in the second trimester of pregnancy. This pattern of mid-gestation abortion is distinctive from other diagnosed causes of infectious abortion in dairy cattle which tend to occur later in gestation. Whether *N. caninum* infection can cause reproductive problems in the first trimester of gestation is unknown. Fetal mummification has been associated with *N. caninum* outbreaks. *N. caninum* abortions occur throughout the year and have been reported in both dairy and beef cattle.

Cattle with serologic evidence of infection have an increased risk of abortion [50, 52, 63, 68, 70]. In a California study, seropositive congenitally infected cows had a 7.4 increased risk of abortion in their first pregnancy. The risk in the second pregnancy was considerably lower, though this may have been influenced to some degree by selective culling of aborting cows from the first pregnancy [63]. In the Netherlands, a 3-fold increase in abortion risk was associated with seropositive cows, when compared to seronegative herdmates [70]. A case-control study involving over 1000 randomly selected cows in England and Wales showed that seropositive cows were 3.5 times mmore likely to abort than seronegative cows[25]. In addition to abortion and congenital infection, *N. caninum* infection may cause reduced milk production and shortened production life based on a study of seropositive cows which produced less milk and were culled earlier than seronegative herdmates [38, 62, 64]. The milk yield of *N. caninum*-infected, non-aborting cows was shown to be reduced by over 4% in first lactation dairy cows[65]. Seropositive beef cattle have an increased risk of abortion and stillbirth, increased risk of culling for poor reproductive performance, reductions in postweaning weight gain and feeding efficiency [7, 9].

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 which persists for years. In investigations of two California dairies with endemic *N. caninum* abortions, the annual abortion rate attributable to neosporosis in these herds was estimated to be 10.6% and 17.3% [65]. 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 [61]. An apparent mixture of these patterns may be observed in some herds that have experienced a prolonged history of sporadic cases of *N. caninum* abortion and occasional outbreaks of abortions attributable to *N. caninum*. Point-source infections are most likely the cause for *N. caninum*-induced abortion outbreaks in cattle, whereas an increased annual abortion rate may be a consequence of vertical transmission.

In most instances, cows that abort a *N. caninum* infected fetus will have either additional abortions [2] or infected fetuses in subsequent pregnancies [11, 32]. The clinical outcome of these subsequent pregnancies is variable but a seropositive cow that has an abortion has a 5.7 greater risk of abortion in the subsequent pregnancy [63].

An uncommon manifestation of fetal *N. caninum* infection is the birth of a clinically affected full-term calf which exhibits variable neurological signs manifested as limb dysfunctions, ranging from mild proprioceptive defects to complete paralysis. Microscopically there is a multifocal protozoal encephalomyelitis which may be particularly localized in the spinal cord gray matter [11, 32]. The majority of calves that acquire a *N. caninum* infection during gestation are born clinically normal. These calves will have a high precolostral antibody titer to *N. caninum* which is useful in detecting *in utero* infection. A high percentage, 80% to over 95%, of calves born to seropositive cows are congenitally infected based on serology [51, 56, 66]. These clinically normal, congenitally infected calves are important in maintaining the infection in the herd.

DIAGNOSIS

Diagnosing *N. caninum*-induced abortion will require the assistance of a veterinary diagnostic laboratory. The preferred samples in cases of abortion include one or more aborted fetuses submitted with placenta and sera from the dam. Histologic lesions in fetal tissues 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. 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 and focal nonsuppurative interstitial pneumonia [1, 10]. Confirmatory diagnosis of *N. caninum* infection relies on identification of the tachyzoite and tissue cyst stages of the parasite in fetal tissues by immunohistochemistry using antibodies raised against *N. caninum* antigens, and on detection of *N. caninum*-specific DNA by polymerase chain reaction PCR [40, 41, 42].

An accurate diagnosis of *N. caninum* abortion can be achieved if the diagnostician takes into consideration, prior to establishing the infection as the cause of the abortion: 1) the gestational age and postmortem condition (autolyzed), 2) the presence of compatible disseminated inflammatory lesions, 3) the presence of detectable parasites with immunohistochemistry or parasite DNA by PCR, and 4) the lack of other abortifacients. Conversely, a *N. caninum* infected aborted fetus with mild focal lesions, (usually consisting of focal encephalitis in late term fetuses), may have an incidental *N. caninum* infection and other causes for the abortion should be investigated.

A variety of serologic tests are available to assist in the diagnosis of neosporosis [21, 42]. These include the indirect fluorescent antibody test (IFAT), the modified agglutination test and a number of enzyme-linked immunosorbent assays (ELISA). The assays utilize *N. caninum* tachyzoites or specific derived antigens. Laboratories utilizing any of the serologic tests for *N. caninum* should establish appropriate cut-off titers using sera from known infected and noninfected cattle. A single serum sample from an individual cow may not accurately reflect her infection status since titers in known positive cattle fluctuate and may fall below the cut-off value for some period of time [24].

Neospora caninum serology is effective in detecting elevated antibodies in the serum of congenitally infected calves, or aborted infected fetuses six months or more in gestation. IFAT was shown to be the most accurate method to detect fetal antibodies [12, 69]. However, a negative fetal *N. caninum* IFA 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. 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. In addition to its use in the routine abortion screen in individual abortion cases, the ELISA test is used on a herd basis. The test can be used to estimate herd seroprevalence of *N. caninum* infection, to investigate the association between seropositivity and abortion, and to estimate the extent of herd infection attributable to congenital infection.

Assessment of the avidity index of *N. caninum* antibodies using an avidity ELISA has made it possible to differentiate recent (low avidity) from chronic (high avidity) infections [22, 58].

ECONOMIC IMPACT

The major economic impact of bovine neosporosis is associated with abortion. In California, neosporosis is the most important cause of abortion in dairy cattle and the second most frequently diagnosed cause of abortion in beef cattle [4]. A recent survey of bovine abortions cases received from January 1998 to July 2003 at three veterinary diagnostic laboratories reveals that *N. caninum*-abortions were the largest category (50.5 %) of bovine abortion diagnosed in California and exceed all other combined diagnosed cases. The annual cost of *N. caninum*-associated abortions to the California dairy industry was conservatively estimated in 1997 at $35 million [13].
Additional costs are related to reduced milk production and premature culling in dairy cattle [38, 62, 64]. A recent prospective study involving 565 Holstein cows reports a 3 to 4% decrease in milk production or 800 lb/seropositive cow for a typical 305-day lactation which represents a loss of $128/cow [38]. Studies with beef cattle in Texas show that in addition to abortion, *N. caninum* infection is associated with significant reductions in postweaning weight gain, carcass weight and feeding efficiency, costing the Texas beef industry at least $ 37 million in 2001 [7, 9].
In New Zealand, where horizontal transmission is considered an important route of *N. caninum* infection, the average annual loss associated with abortion outbreaks was estimated at NZ$ 24 million at a national and NZ$ 6,800 at an individual farm level [5].

CONTROL AND PREVENTION

Currently, there is no effective method for control of bovine neosporosis. Although various antimicrobial agents have been tested against *N. caninum in vitro* there is currently no known method whereby an infected cow can be cleared of the infection. One explorative study reports that calves experimentally infected with *N. caninum* tachyzoites and treated with Toltrazuril-sulfone (Ponazuril) for six consecutive days starting one day after infection, did not have detectable parasite DNA, had reduced fever and significantly lower *N. caninum* antibodies, when compared to non-treated controls [43].
A major method of *N. caninum* transmission in herds is through the infection of fetuses in cattle that are chronically infected. These chronically infected cows can be identified based on their serologic titers or from a history of previous *N. caninum* 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 *N. caninum* abortion can be made with the knowledge that there is a higher risk of repeat abortion in these animals [50, 63]. 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 [50, 52, 63, 68, 70]. In addition, seropositive heifers have been shown to have a reduced milk production [64]. In both dairy and beef cattle, epidemiological studies have found that seropositive cattle have an increased rate of culling for a variety of reasons [62, 68]. However, culling seropositive cows or heifers may not be economically viable in herds with high seroprevalence. Embryo transfer into seronegative recipients has been shown to be effective in preventing vertical transmission [6, 23, 44].
There are no proven methods available to prevent postnatal infection. However, based on the evidence that dogs and coyotes can be definitive hosts [37, 47] and the association between dogs on farms and the seroprevalence in the herd [29, 49, 53, 55, 72], 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.

NeoGuard®, a USDA-approved *Neospora caninum* vaccine manufactured by Intervet Inc., has been shown to reduce abortions in healthy, pregnant heifers challenged with *N. caninum* (Technical bulletin 2).

REFERENCES

1- Anderson ML, Barr BC, Conrad PA. 1994 Protozoal causes of reproductive failure in domestic ruminants. Vet Clin North America. Food Animal Practice, 10, 439-461.

2- Anderson ML, Palmer CW, Thurmond MC, et al. 1995 Evaluation of abortions in cattle attributable to neosporosis in selected dairy herds in California. J Am Vet Med Assoc, 207, 1206-1210.

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

4- Anderson ML, Andrianarivo AG, Conrad PA. 2000 Neosporosis in cattle. Animal Reproduction Science, 60-61, 417-431.

5- Antony A, Williamson NB. 2001 Recent advances in understanding the epidemiology of *Neospora caninum* in cattle. NZ Vet J 49, 42-47.

6- Baillargeon P, Fecteau G, Paré J, et al. 2001 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.

7- Barling KS, McNeill JW, Thompson JA, et al. 2000 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.

8- Barling KS, Sherman M, Peterson MJ, et al. 2000 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.

9- Barling KS, Lunt DK, Snowden KF, et al. 2001 Association of serologic status for *Neospora caninum* and postweaning feed efficiency in beef steers. J Am Vet Med Assoc 219, 1259-1262.

10- Barr BC, Anderson ML, Dubey JP, Conrad PA. 1991 *Neospora*-like protozoal infections associated with bovine abortions. Vet Pathol 28,116.

11- Barr BC, Conrad PA, Breitmeyer R, et al. 1993 Congenital *Neospora* infection in calves born from cows that had previously aborted *Neospora*-infected fetuses: four cases (1990-1992). J Am Vet Med Assoc, 202, 113-117.

12- Barr BC, Anderson ML, Sverlow KW, Conrad PA. 1995 Diagnosis of fetal *Neospora* infection with the *Neospora* indirect fluorescent antibody test (IFAT). Vet Rec 137, 611-613.

13- Barr BC, Bjerkas I, Buxton D, Conrad PA, et al. 1997 Neosporosis - Report of the International *Neospora* Workshop. Compendium On Continuing Education For the Practicing Veterinarian, 19, S120-144.

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

15- Basso W, Venturini L, Venturini MC, et al. 2001 First isolation of *Neospora caninum* from the feces of a naturally infected dog. J Parasitol 87, 612-618.

16- Bergeron N, Fecteau G, Paré J, Martineau R, Villeneuve A. 2000 Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Québec. Can Vet J 41, 464-467.

17- Bergeron N, Fecteau G, Villeneuve A, Girard C, Paré J. 2001 Failure of dogs to shed oocysts after being fed bovine fetuses naturally infected by *Neospora caninum*. Vet Parasitol 97,145-152.

18- Bjerkås I, Mohn SF, Presthus J. 1984 Unidentified cyst-forming sporozoan causing encephalomyelitis and myositis in dogs. Z. Parasitenkd 70, 271-274.

19- Bjerkås I, Dubey JP. 1991 Evidence that *Neospora caninum* is identical to the *Toxoplasma*-like parasite of Norwegian dogs. Acta Vet Scand 32, 407-410.

20- Björkman C, Johansson O, Stenlund S, Holmdahl OJM, Uggla A. 1996 *Neospora* species infection in a herd of dairy cattle. J Am Vet Med Assoc, 208, 1441-1444.

21- Björkman C, Uggla A. 1999 Serological diagnosis of *Neospora caninum* infection. Int J Parasitol 29, 1497-1507.

22- Björkman C, Naslund K, Stenlund S, Maley SW, Buxton D, Uggla A. 1999 An IgG avidity ELISA to discriminate between recent and chronic *Neospora caninum* infection. J Vet Diagn Invest 11,41-44.

23- Campero CM, Moore DP, Lagomarsino H, et al. 2003 Serological status and abortion rate in progeny obtained by natural service or embryo transfer from *Neospora caninum*-seropositive cows. J Vet Med B Infect Dis Vet Public Health 50, 458-460.

24- Conrad PA, Sverlow KW, Anderson ML, et al. 1993 Detection of serum antibody responses in cattle with natural or experimental *Neospora* infections. J Vet Diagn Invest 5, 572-578.

25- Davison HC, Otter A, Trees AJ. 1999 Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infections in dairy cattle. Int J Parasitol, 29, 1683-1689.

26- De Marez T, Liddell S, Dubey JP, et al. 1999 Oral infection of calves with *Neospora caninum* oocysts from dogs: humoral and cellular immune responses. Int J Parasitol 29, 1647-1657.

27- Dijkstra T, Eysker M, Schares G, Conraths FJ, Wouda W, Barkema HW. 2001 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.

28- Dijkstra T, Barkema HW, Bjorkman C, Wouda W. 2002 A high rate of seroconversion for *Neospora caninum* in a dairy herd without an obvious increased incidence of abortions. Vet Parasitol 109, 203-211.

29- Dijkstra T, Barkema HW, Eysker M, Hesselink JW, Wouda W. 2002 Natural transmission routes of *Neospora caninum* between farm dogs and cattle. Vet Parasitol 105, 99-104.

30- Dijkstra T, Barkema HW, Hesselink JW, Wouda W. 2002 Point source exposure of cattle to *Neospora caninum* consistent with periods of common housing and feeding and related to the introduction of a dog. Vet Parasitol 105, 89-98.

31- Dubey JP, Carpenter JL, Speer CA, Topper MJ, Uggla A. 1988 Newly recognized fatal protozoan disease of dogs. J Am Vet Med Assoc 192, 1269-1285.

32- Dubey JP, Lindsay DS. 1996 A review of *Neospora caninum* and neosporosis. Vet Parasitol, 67, 1-59.

33- Dubey JP. 1999 Recent advances in *Neospora* and neosporosis. Vet Parasitol, 84, 349-367.

34- Dubey JP. 2003 Review of *Neospora caninum* and neosporosis in animals. Korean J Parasitol 41, 1-16.

35- French NP, Clancy D, Davison HC, Trees AJ. 1999 Mathematical models of *Neospora caninum* infection in dairy cattle: transmission and options for control. Int J Parasitol 1999 29,1691-1704.

36- Gondim LF, Gao L, McAllister MM. 2002 Improved production of *Neospora caninum* oocysts, cyclical oral transmission between dogs and cattle, and in vitro isolation from oocysts. J Parasitol 88, 1159-63.

37- Gondim LFP, McAllister MM, Pitt WC, Zemlicka DE 2004 Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. Int J Parasitol 34, 159-161.

38- Hernandez J, Risco C, Donovan A. 2001 Association between exposure to *Neospora caninum* and milk production in dairy cows. J Am Vet Med Assoc 219, 632-635.

39- Hietala SK, Thurmond MC. 2001 Postnatal *Neospora caninum* transmission and transient serologic responses in two dairies. Int J Parasitol 31, 209-215.

40- Ho MS, Barr BC, Marsh AE, et al. 1996 Identification of bovine *Neospora* parasites by PCR amplification and specific small-subunit rRNA sequence probe hybridization. J Clin Microbiol 34, 1203-1208.

41- Ho MS, Barr BC, Rowe JD, et al. 1997 Detection of *Neospora* sp. from infected bovine tissues by PCR and probe hybridization. J Parasitol 83, 508-514.

42- Jenkins M, Baszler T, Björkman C, Schares G, Williams D. 2002 Diagnosis and seroepidemiology of Neospora caninum-associated bovine abortion. Int J Parasitol 32, 631-636.

43- Kritzner S, Sager H, Blum J, Krebber R, Greif G, Gottstein B. 2002 An explorative study to assess the efficacy of Toltrazuril-sulfone (Ponazuril) in calves experimentally infected with *Neospora caninum*. Ann Clin Microbiol Antimicrob1, 4.

44- Landmann JK, Jillella D, O'Donoghue PJ, McGowan MR. 2002 Confirmation of the prevention of vertical transmission of *Neospora caninum* in cattle by the use of embryo transfer. Aust Vet J 80, 502-503.

45- Lindsay DS, Dubey JP, Duncan RB. 1999 Confirmation that the dog is a definitive host for *Neospora caninum*. Vet Parasitol 82, 327-333.

46- McAllister MM, Huffman EM, Hietala SK, Conrad PA, Anderson ML, Salman MD. 1996 Evidence suggesting a point source exposure in an outbreak of bovine abortion due to neosporosis. J Vet Diagn Invest 8, 355-357.

47- McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM. 1998 Dogs are definitive hosts of *Neospora caninum*. Int J Parasitol 28, 1473-1478.

48- McGarry JW, Stockton CM, Williams DJ, Trees AJ. 2003 Protracted shedding of oocysts of *Neospora caninum* by a naturally infected foxhound. J Parasitol 89, 628-30.

49- Otranto D, Llazari A, Testini G, et al. 2003 Seroprevalence and associated risk factors of neosporosis in beef and dairy cattle in Italy. Vet Parasitol 118, 7-18.

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

51- Paré J, Thurmond MC, Hietala SK. 1996 Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. Can J Vet Res, 60, 133-139.

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

53- Paré J, Fecteau M, Fortin M, et al. 1998 Seroepidemiologic study of *Neospora caninum* in dairy herds. J Am Vet Med Assoc 213, 1595-1598.

54- Patitucci AN, Charleston WAG, Alley MR, et al. 1999 Serological study of a dairy herd with a recent history of *Neospora* abortion. N Z Vet J 47, 28-30.

55- Sanchez GF, Morales SE, Martinez MJ, Trigo JF. 2003 Determination and correlation of anti-*Neospora caninum* antibodies in dogs and cattle from Mexico. Can J Vet Res 67,142-145.

56- Schares G, Peters M, Wurm R, et al. 1998 The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analysed by serological techniques. Vet Parasitol, 80, 87-98.

57- Schares G, Conraths FJ, Reichel MP. 1999 Bovine neosporosis: comparison of serological methods using outbreak sera from a dairy herd in New Zealand. Int J Parasitol 29,1659-1667.

58- Schares G, Barwald A, Staubach C, et al. 2002 p38-avidity-ELISA: examination of herds experiencing epidemic or endemic *Neospora caninum*-associated bovine abortion. Vet Parasitol 106, 293-305.

59- Slapeta JR, Modry D, Kyselova I, et al. 2002 Dog shedding oocysts of *Neospora caninum*: PCR diagnosis and molecular phylogenetic approach. Vet Parasitol 109,157-67.

60- Thilsted JP, Dubey JP. 1989 Neosporosis-like abortions in a herd of dairy cattle. J Vet Diagn Invest 1, 205-209.

61- Thornton RN, Gajadhar A, Evans J. 1994 *Neospora caninum* abortion epidemic in a dairy herd. N.Z. Vet J. 42, 190-191, 1994

62- Thurmond MC, Hietala SK. 1996 Culling associated with *Neospora caninum* infection in dairy cows. Am J Vet Res 57, 1559-1562.

63- Thurmond MC, Hietala SK. 1997 Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. Am J Vet Res, 58,1381-1385.

64- Thurmond MC, Hietala SK. 1997 Effect of *Neospora caninum* infection on milk production in first-lactation dairy cows. J Am Vet Med Assoc 210, 672-674.

65- Thurmond MC, Hietala SK, Blanchard PC. 1997 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.

66- Thurmond MC, Hietala SK. 1999 *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.

67- Trees AJ, McAllister MM, Guy CS, McGarry JW, Smith RF, Williams DJ. 2002 *Neospora caninum*: oocyst challenge of pregnant cows. Vet Parasitol 109,147-154.

68- Waldner CL, Janzen ED, Ribble CS 1998 Determination of the association between *Neospora caninum* infection and reproductive performance in beef herds. J Am Vet Med Assoc 213, 685-690.

69- Wouda W, Dubey JP, Jenkins MC. 1997 Serological diagnosis of bovine fetal neosporosis. J Parasitol 83,545-547.

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

71- Wouda W, Dijkstra T, Kramer AM, et al. 1999 Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. Int J Parasitol 29, 1677-1682.