



Review

REVIEW ON THE TRANSMISSION MODELS, ABORTION TYPES AND RISK FACTORS ASSOCIATED WITH NEOSPORA CANINUM INFECTION IN COWS

T. Todorova*

Department of Veterinary Microbiology, Infectious and Parasitic Diseases,
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

Neospora caninum is one of the main causes of abortion in cattle worldwide, causing significant economic losses. This review summarizes current knowledge on the epidemiology of bovine neosporosis, transmission routes, abortion types and associated risk factors. Transplacental transmission is identified as the main mechanism maintaining infection in the cattle populations. Transplacental transmission can be endogenously through reactivation of persistent infection in seropositive dams and exogenously following primary infection during pregnancy. Postnatal infection is associated with ingestion of sporulated oocysts shed by definitive hosts, mainly dogs and other canids. *Neospora caninum*-associated abortions may occur in epidemic or endemic types, depending on the route and timing of infection. Persistently infected cows have a significantly higher risk of abortion. Many risk factors influencing infection and abortion have been identified, such as age, herd management practices, presence and behavior of dogs, feed contamination, and coinfections with other pathogens. Understanding these epidemiological aspects is essential for the development of effective prevention and control strategies for bovine neosporosis.

Keywords: cattle, neosporosis, reproductive disorders

INTRODUCTION

Neospora caninum is worldwide spread protozoa, which is one of the main causes of abortion in cattle worldwide (1-4). Due to the economic losses it causes, neosporosis is known as the “billion-dollar disease” (5). The prevalence of the invasion varies widely in different countries.

Long-term serological studies in cattle show that infection with *N. caninum* persists during their entire life (6). In many regions of the world, transplacental transmission represents the main mechanism of infection in cattle (7). In persistently invaded dams, reactivation of the infection during pregnancy is responsible for endogenous transplacental transmission (5). In dams free of the protozoan, postnatal infection during pregnancy, caused by ingestion of

infective oocysts, is responsible for exogenous transplacental transmission. Heifers, originating from transplacentally invaded calves, may transmit the infection to the next generation during pregnancy.

TYPES OF TRANSPLACENTAL TRANSMISSION.

1. Prenatal (transplacental) transmission.

N. caninum is one of the most effective transplacentally transmitted parasites in cattle (6). Evidence for transplacental transmission has been received from natural and experimental studies (6).

Björkman et al., (8) conducted a study, following the family history of animals that showed the presence of antibodies against *N. caninum*, in a dairy cattle herd in Sweden. The authors found that all seropositive animals come from two cows purchased 16 years earlier. Similar studies, demonstrating the influence of prenatal transplacental transmission on the seroprevalence of the generation, have also been conducted in Canada (9), Germany (10), Australia (11), Sweden (12), and Brazil (13).

*Correspondence to: Tereza Todorova, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, 6000, Bulgaria, tel.: +359 42 699 591, E-mail: tereza.todorova@trakia-uni.bg

Anderson et al., (14) conducted a study in California providing convincing evidence, that chronic persistent infection in cows can be transmitted to the offspring through endogenous transplacental transmission. The authors followed the offspring of 25 seronegative and 25 seropositive heifers raised together. The offspring of seronegative heifers were also seronegative, while the offspring of seropositive heifers were congenitally infected. Seven of the congenitally infected calves showed histological lesions caused by *N. caninum*.

Clear evidence for transplacental transmission of *N. caninum* has been obtained through the detection of statistically significant associations between the antibody response in dams and their newborn calves (6).

Several studies have observed that a greater part of newborn calves born from seropositive dams were also seropositive, while a greater proportion of the offspring from seronegative dams were seronegative (6). According to the authors, detection of specific antibodies in precolostral serum proves *in utero* synthesis of antibodies by the fetus and represents an indisputable indicator of transplacental transmission.

In a study conducted at an embryotransfer center by Andreotti et al., (15) low levels of vertical transmission of the infection were identified, ranging from 24% to 50%. Nevertheless, the absence of specific antibodies in calves is not conclusive evidence of absence of infection, as the fetus may have been infected late in pregnancy and antibody synthesis may not have yet begun (6). According to Dubey et al., (6), the hypothesis proposed by McInnes et al., (16), suggesting that transplacentally infected calves may become immunotolerant, is based on unconfirmed detection of parasitic DNA in the serum of seronegative cattle and therefore requires further studies for confirmation. The possibility that a seronegative dam gives birth to a seropositive calf is extremely low. The reason for this may be due to the reduction of antibodies to an undetectable level in the mother (17,12,18). Fioretti et al., (19) conducted a study following three consecutive pregnancies in three cows, aiming to determine the degree of vertical transmission of *N. caninum* and to clarify the period of gestation during which reactivation of persistent infection occurs. The cows included in the study gave birth to clinically healthy but congenitally infected calves during their first

pregnancy. The protozoan has been detected, using biological assays in mice, in the placentas of the three examined cows during each of the three consecutive pregnancies after parturition. The protozoan has been detected post mortem in the brains of all nine calves born clinically healthy from these three cows. The authors found that IgG and IgM titers increased during the third trimester of pregnancy. This increase in antibody titers has been observed during all three pregnancies and in all three cows. IgM titers in calves were elevated after birth, while IgG titers increased after colostrum consumption, which is indicative for late infection. In the same premises, at the same time as the experimental animals, 38 cows were reared, which showed a negative result for antibodies from the beginning to the end of the study. All farm dogs in the area were tested and showed negative results for the presence of antibodies, as well as negative results in attempts to isolate the protozoan. The convincing evidence obtained by the authors give them reason to suggest that *N. caninum* infection can be maintained over several generations and that recurrent persistent infection, rather than a new one, explains *N. caninum* infection in calves.

2. Postnatal (horizontal) transmission.

Ingestion of sporulated *N. caninum* oocysts from the environment is the only proven way of infection in cattle after birth (6). Transmission of the infection between individual animals kept together has not been observed (14). There is no evidence that placentophagy is involved as a mechanism for transmission of the infection (20). There is no conclusive evidence that viable protozoa are present in the excreta and secretions of adult asymptomatic carrier cows (6). Experimental lactogenic infection of calves inoculated with milk containing tachyzoites has been demonstrated (21), but there is no evidence for natural lactogenic transmission of the infection (22-24).

De Yaniz et al., (25) conducted an experimental conjunctival inoculation of high doses of protozoan, which resulted in seroconversion. Moore et al., (26) performed an experiment demonstrating that conjunctival infection does not lead to vertical transmission of the protozoan in pregnant cows, and that natural infection along this route seems unlikely.

Serrano-Martínez et al., (27) reported that intrauterine infection by semen contaminated with 5×10^4 tachyzoites causes persistent

antibody responses in heifers. Although *N. caninum* DNA has been detected in the semen of naturally infected (28-30) and experimentally infected bulls (27), viable organisms, if present, are few and rare. For these reasons, Dubey et al., (6) consider that venereal transmission may be possible, but is unlikely.

EPIDEMIC AND ENDEMIC ABORTION ASSOCIATED WITH *N. caninum*.

Abortions associated with *N. caninum* in cattle may be an epidemic or endemic (sporadic) type (6). Abortions are defined as epidemic when the outbreak is temporary and when 15% of cows at risk abort within 4 weeks, 12.5% of cows abort within 8 weeks, or 10% of cows abort within 6 weeks (6,31-33). If sporadic abortions are observed in the herd for several months or years, the outbreaks are defined as endemic (6).

1. Epidemic abortions caused by exogenous transplacental transmission.

Epidemic abortions are thought to be due to exogenous transplacental transmission—

ingestion of food or water contaminated with sporulated oocysts. (34,35). The detection of IgG antibodies with low antigen-binding activity (avidity) in experimentally infected cattle indicates recent infection (6,36-39,32). Low-avidity IgG antibodies have been detected in most aborted dams from herds with epidemic abortions, indicating that the infection in these animals was recent (40,41,37,42,34,43,32).

2. Endemic abortions caused by endogenous transplacental transmission.

According to Dubey et al., (6), in many herds with a history of endemic abortions due to *N. caninum* (9,8,37,44,23,11,45,46,32,47,48) there is a strong positive correlation between the serological status of the mothers and their adult daughters, i.e. there is evidence that the main route of vertical transmission in these herds is endogenous. (Figure 1)

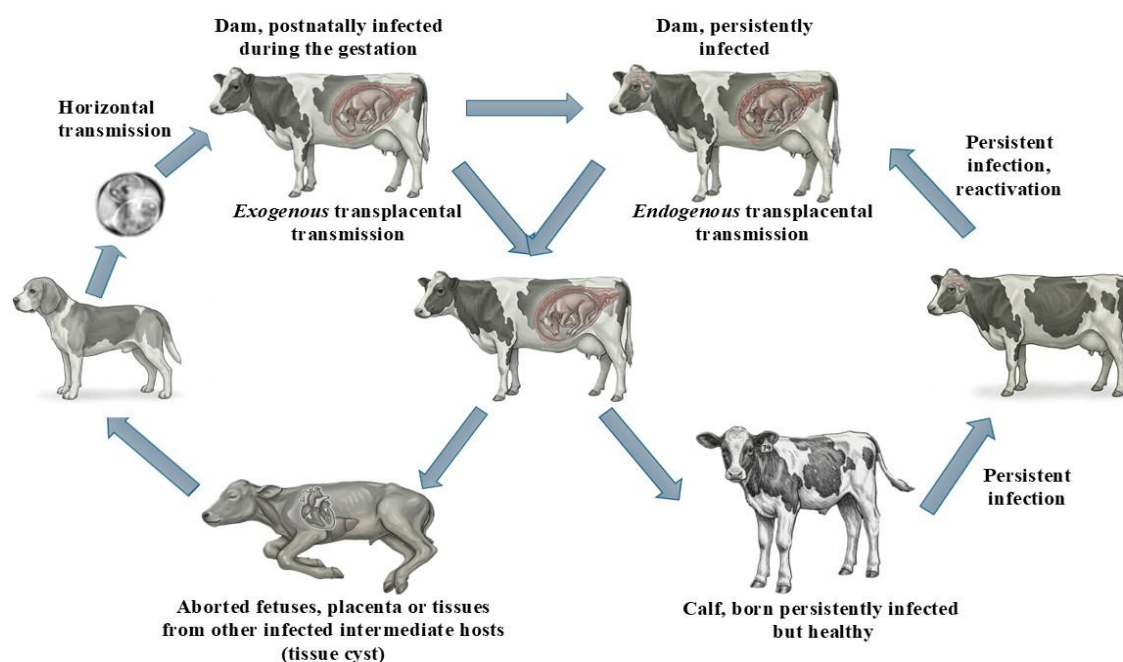


Figure 1. Exogenous and endogenous and transplacental transmission

Cows with persistent infection have a two or more times higher risk of abortion compared to seronegative control animals, which has been proven in a number of studies (18,49-51). A study in Israel, showed that the rate of recurrent abortions in seropositive mothers was about 5 times higher than in seronegative ones (45). Thurmond et al., (52) conducted a study in which it was observed that congenitally infected heifers had an increased risk of abortion during

their first pregnancy, but not in later pregnancies, compared to control seronegative animals. In a similar study in Israel, Mazuz et al., (45), observed that more of the seropositive animals aborted at a late age. According to Dubey et al., (6), it remains unclear if only endogenous or in addition exogenous infection of mothers has caused late-term abortions in Israel.

The mechanism of reactivation of latent *N. caninum* infection is unknown (6). Immunosuppression induced by ingestion of toxic substances, as well as coinfections with other pathogens, has been discussed as a possible mechanism, but is not supported by evidence (6). Some hypotheses include stress factors as a cause of reactivation of persistent infection (6). In herds with confirmed neosporosis but without reproductive disorders, endemic abortions have been observed during episodes of increased rainfall in predominantly dry regions (18,53,54). Based on this, the authors suggest that these abortions are due to the stressful climatic factors, especially in mothers during the second trimester of pregnancy.

RISK FACTORS ASSOCIATED WITH INFECTION.

Knowledge of the risk factors leading to infection with *N. caninum* or to the occurrence of abortions in herds is important for the development and application of control measures for bovine neosporosis (6).

1. Age.

With increasing age and/or number of pregnancies, the seroprevalence in both beef and dairy cattle breeds also increases, suggesting that postnatal infection through horizontal transmission of *N. caninum* is of particular importance (6). Sanderson et al., (55) investigated the seroprevalence of *N. caninum* and associated risk factors among 2,585 cows in 55 herds. Blood samples were collected from beef cows and calves located in five northwestern states of the USA. The authors collected information related to animal husbandry and feeding practices, as well as vaccination programs implemented in cattle farms. The overall seroprevalence was 24%, and the seroprevalence within the herd ranged from 3 to 67%. The authors found that herds managed under a combination of indoor and grazing systems show lower seroprevalence than herds managed under year-round indoor systems. Cows under 3 years of age had higher percentage inhibition values using ELISA (competitive inhibition assay, CI) than cows over 6 years of age.

The influence of age on seroprevalence in dairy cattle may vary among different regions (6). In Spain, Bartels et al., (56), found that seroprevalence in cattle increased with age, while in Sweden the results were opposite. In another study in Spain, Eiras et al., (57) found

that seroprevalence increased with age in beef and dairy cattle. Bartels et al., (56) hypothesized that age as a risk factor may be influenced by the risk of oocyst ingestion, the way animals are reared (year-round indoor or indoor-grazing) and management practices, such as selective culling of seropositive animals.

Non-selective culling of animals in a herd with high seroprevalence may result in a positive relationship between age and prevalence if the population from which new heifers are imported has a lower seroprevalence than the herd itself (6). This effect (lower prevalence in younger age groups) is further amplified by the fact that the proportion of transplacental transmission is often below 100% (6). According to the authors, relapses during pregnancy may have additionally contributed to the higher seroprevalence observed in older animals.

2. Definitive hosts (including dogs, coyotes, and wolves).

Most epidemiological studies on dairy herds indicate that the presence of farm dogs is a risk factor for seropositivity in cattle (6). According to the authors, studies in which positive correlations between the seroprevalence of *N. caninum* in dogs and cattle have been observed (58-60) further confirm that the presence of *N. caninum* in cattle is associated with infection in dogs.

Not only the presence of free-ranging dogs on farms, but also the possibility of these dogs to have direct contact with the herd, have been identified as risk factors (61, 62). Dijkstra et al., (63) performed a comparative study between 12 dairy herds with confirmed postnatal infection with *N. caninum* and 21 negative herds. In 92% of the farms with evidence of postnatal infection, defecation of farm dogs along the feeding alleys was observed, whereas in the control farms the proportion was significantly lower (24%). The same trend was observed for dog defecation in grass silage - 75% of postnatally infected farms and 19% of control farms - as well as in maize silage - 50% of postnatally infected farms and 10% of control farms. Based on the data from this study, Dubey et al., (6) assumed that fecal contamination in the feeding area is more closely associated with postnatal infection in cows than contamination of feed during storage.

Comparing the feeding habits of dogs originating from farms with confirmed

postnatal infection and control farms is found that dogs from infected farms are fed with raw meat, placentas, fetuses, uterine secretions, colostrum, or milk, which indicate that these biological materials may represent sources of infection for dogs (61, 63, 64). Dijkstra et al., (65) experimentally confirmed that placenta, but not colostrum, is a source of infection for dogs.

No oocyst shedding was observed in dogs fed with naturally infected bovine fetuses (66). Dubey et al., (6) suggest that the obtained results were influenced by the degree of fetal autolysis, since death of the protozoan is directly associated with death of host cells. For this reason, viable tachyzoites can be detected only extremely rarely in autolyzed tissues (5). Dijkstra et al., (67) provide convincing evidence that newly introduced dogs have a higher risk of transmitting the infection compared with local dogs. According to Dubey et al., (6), these findings can be explained by the fact that dogs do not shed (or shed only a very small number of) oocysts even after repeated feeding with infected tissues (65,68,69). Another explanation proposed by Dubey et al., (6) is that young dogs (10–14 weeks of age) shed more oocysts than older dogs (2–3 years of age) (68).

Stray dogs living in areas near farms, as well as farm dogs, are at high risk for spreading the infection (6). In addition to dogs, coyotes (70) and gray wolves (71) are also confirmed definitive hosts of *N. caninum*. In areas with high species diversity of wild canids (coyotes and wolves), natural foci of infection (in which the protozoan circulates between wild ruminants and wild canids) may be important for maintaining infection in domestic animals (cattle and dogs) (72).

3. Other intermediate hosts.

Not only cattle, but also other intermediate hosts of *N. caninum*, can be a source of infection for dogs and wild canids (6). The presence of *N. caninum* DNA in naturally infected mice and rats suggests that these animals may represent important sources of infection for definitive hosts (6). In a study conducted in France, it was found that the presence of rabbits and/or ducks on the farms is a potential risk factor associated with seropositivity in dairy cattle (73). The presence of poultry on the farm is stated as a potential risk factor for seroprevalence in the studied dairy herds in Mexico (74). In the Netherlands, Bartels et al., (75) discussed the

potential role of poultry as mechanical carriers of oocysts of *N. caninum*, after involving them as a risk factor for the occurrence of abortions associated with the protozoan in dairy herds.

The susceptibility of poultry to *N. caninum* is still under debate (6). According to Dubey et al., (6), although antibodies against the protozoan have been detected in chickens (76-78) and DNA has been isolated from seropositive chickens (76,79), there is still no evidence of successful isolation of viable *N. caninum* from chickens. Although experimental infection of chicken embryos is possible (80-82) and this material has induced oocyst shedding in dogs (81), there is no evidence that chickens are important natural intermediate hosts of *N. caninum* (6). These results require further investigations on the susceptibility of other domestic animals to *N. caninum* infection, such as rabbits, ducks, chickens and other poultry, as well as wild animals, and whether these potential intermediate hosts could be a risk for infection of definitive hosts (6).

4. Oocyst contamination (including grazing and feed).

Oocyst-contaminated pastures, feed and drinking water are considered potential sources of postnatal infection in cattle (6). In Italy (83) and in the northwestern United States (55), it has been reported that cattle managed under indoor–grazing system exhibited lower seroprevalence compared with cattle kept under year-round indoor conditions. According to Dubey et al., (6), although domestic and wild canids have free access to pastures, oocyst contamination caused by them may be too low to represent a significant risk of infection, or the oocysts may not survive during the summer months if they are very hot and dry. There is still no information about climatic conditions under which *N. caninum* oocysts can survive in the environment (6).

Barling et al., (84), found that the use of round hay feeders in beef cattle farms in Texas is a risk factor influencing the seroprevalence. This risk factor is explained with the fact that cows often give birth, abort, or expel their placentas near these feeders. The authors suggest that fecal contamination from canids that have been fed with infected materials may concentrate near the feeders, because they are rarely moved.

Seroepidemiological study in 15 dairy cattle farms in Puerto Rico revealed that on-farm feed production, lack of artificial insemination, and access of domestic and wild animals to facilities

or to livestock feed were directly associated with seroprevalence in cows (85). Since farm dogs play a major role in the life cycle of *N. caninum*, contamination of feed with oocysts can occur, especially when it is produced on the farm (6). Farm-stored silage is considered one of the feeds with the highest potential for contamination with oocysts of the protozoan (86).

5. Feeding with colostrum or milk.

It is experimentally confirmed that calves up to 1 week of age developed a specific antibody response against the protozoa after being fed with colostrum and/or milk replacer containing 10^7 *N. caninum* tachyzoites (NCLivB1) (22). In the same study, it was shown that feeding newborn calves from seronegative mothers with colostrum from seropositive mothers did not result in infection of the calves. Since *N. caninum* DNA has been detected in the milk of seropositive cows (87,88), the question of whether lactogenic transmission of the protozoan is possible remains under discussion (6).

6. Coinfections.

In Spain, González-Warleta et al., (89), found that coinfection with *Toxoplasma gondii* increased the risk of seropositivity to *N. caninum* in dairy cows. According to Dubey et al., (6), common risk factors that may favor postnatal infections with coccidia could have contributed to these results. According to the authors, this finding is unexpected, due to the differences in the life cycles and ways of transmission of the protozoa: *N. caninum* (mainly vertical) and *T. gondii* (mainly horizontal).

Statistically significant association between the presence of antibodies to *N. caninum* and Bovine Viral Diarrhea Virus (BVDV) is documented in Sweden (90). These results suggest that the risk factors for the spreading of BVDV in cattle, such as high stocking density and frequent purchase of new animals, lead to increasing the risk of *N. caninum* infection (6). Rinaldi et al., (91) investigated the seroprevalence of *N. caninum* and bovine herpesvirus 1 (BHV-1) in 948 grazing cattle from 81 farms. Blood samples from these animals were tested for antibodies against *N. caninum* and BHV-1 using ELISA and neutralization tests, respectively. Seropositive for *N. caninum* animals were found in 63 (77.8%) and for BHV-1 in 80 (98.8%) of the farms studied. Coinfection was found in 62

(76.5%) of the farms. Of the 948 bovine serum samples, 303 (32.0%) were positive for antibodies against *N. caninum* and 735 (77.5%) against BHV-1. Presence of antibodies against both *N. caninum* and BHV-1 have been found in 256 (27.0%) of the animals. The results of the logistic regression showed that seropositivity for BHV-1 was a risk factor for seropositivity against *N. caninum*, and seropositivity for *N. caninum* was a risk factor for seropositivity against BHV-1.

CONCLUSIONS

The review of literature shows that Bovine neosporosis, caused by *Neospora caninum*, is worldwide distribution. Transplacental (vertical) transmission is the main mechanism for maintaining infection in herds, but postnatal (horizontal) infection by ingestion of oocysts from definitive hosts plays a key role in the introduction of the protozoan and the occurrence of epidemic abortions. Analysis of epidemiological data indicates that the risk of abortion is significantly higher in persistently infected cows, where reactivation of the infection during pregnancy leads to endogenous transmission to the fetus. The identified risk factors- including the presence of dogs on farms, their access to placentas and feed, as well as coinfections with other pathogens (such as BVDV and BHV-1), highlight the complexity of the disease.

Although breed-specific comparisons are limited, several studies report differences in seroprevalence between dairy and beef cattle populations, suggesting possible breed- or production-related variation in susceptibility to *N. caninum* infection. Further investigations are necessary to estimate if there are cow breeds with higher resistance to the infection.

REFERENCES

1. Dubey, J. P., & D. D. Lindsay, A review of *Neospora caninum* and neosporosis. *Veterinary parasitology*, 67, 1-59, 1996.
2. Hemphill, H., & Gottstein, B., A European perspective on *Neospora caninum*. *International Journal for Parasitology*, 30, 877-924, 2000.
3. Dubey, J. P., Neosporosis in cattle. *Journal of Parasitology*, 89, 542-556, 2003a.
4. Dubey, J. P., Review of *Neospora caninum* and neosporosis in animals. *Korean Journal of Parasitology*, 41, 1-16, 2003b.
5. Dubey, J. P., Buxton, D., Wouda, W., Pathogenesis of bovine neosporosis. *J. Comp. Pathol.* 134, 267-289, 2006.

6. J.P. Dubey, A. Hemphill, R. Calero-Bernal, G. Schares, Neosporosis in animals, CRC, Boca Raton, FL, USA, 2017.
7. Dubey, J. P., Schares, G., Ortega-Mora, L. M., Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev.* 20, 323–367, 2007.
8. Björkman, C., Johansson, O., Stenlund, S., Holmdahl, O. J. M., Uggla, A., *Neospora* species infection in a herd of dairy cattle. *J. Am. Vet. Med. Assoc.* 208, 1441–1444, 1996.
9. Bergeron, N., Fecteau, G., Paré, J., Martineau, R., Villeneuve, A., Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Québec. *Can. Vet. J.* 41, 464–467, 2000.
10. Schares, G., Peters, M., Wurm, R., Bärwald, A., Conraths, F. J., The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analysed by serological techniques. *Vet. Parasitol.* 80, 87–98, 1998.
11. Hall, C. A., Reichel, M. P., Ellis, J. T., *Neospora* abortions in dairy cattle: Diagnosis, mode of transmission and control. *Vet. Parasitol.* 128, 231–241, 2005.
12. Frössling, J., Uggla, A., Björkman, C., Prevalence and transmission of *Neospora caninum* within infected Swedish dairy herds. *Vet. Parasitol.* 128, 209–218, 2005.
13. de Magalhães, V. C. S., de Oliveira, U. V., Costa, S. C. L., Santos, I. A., Pereira, M. J. S., Munhoz, A. D., Transmission paths of *Neospora caninum* in a dairy herd of crossbred cattle in the northeast of Brazil. *Vet. Parasitol.* 202, 257–264, 2014.
14. Anderson, M. L., Reynolds, J. P., Rowe, J. D., Sverlow, K. W., Packham, A. E., Barr, B. C., Conrad, P. A., Evidence of vertical transmission of *Neospora* sp. infection in dairy cattle. *J. Am. Vet. Med. Assoc.* 210, 1169–1172, 1997.
15. Andreotti, R., Barros, J. C., Pereira, A. R., Oshiro, L. M., Cunha, R. C., Neto, L.F.F., Association between seropositivity for *Neospora caninum* and reproductive performance of beef heifers in the Pantanal of Mato Grosso do Sul, Brazil. *Rev. Bras. Parasitol. Vet.* 19, 119–123, 2010.
16. McInnes, L. M., Ryan, U. M., O’Handley, R., Sager, H., Forshaw, D., Palmer, D. G., Diagnostic significance of *Neospora caninum* DNA detected by PCR in cattle serum. *Vet. Parasitol.* 142, 207–213, 2006.
17. Cavalcante, G. T., Monteiro, R. M., Soares, R. M., Nishi, S. M., Alves Neto, A. F., Esmerini, P. O., Sercundes, M. K., Martins, J., Gennari, S. M., Shedding of *Neospora caninum* oocysts by dogs fed different tissues from naturally infected cattle. *Vet. Parasitol.* 179, 220–223, 2011.
18. López-Gatius, F., López-Béjar, M., Murugavel, K., Pabón, M., Ferrer, D., Almería, S., *Neospora*-associated abortion episode over a 1-year period in a dairy herd in North-east Spain. *J. Vet. Med. B* 51, 348–352, 2004a.
19. Fioretti, D. P., Pasquai, P., Diaferia, M., Mangili, V., Rosignoli, L., *Neospora caninum* infection and congenital transmission: Serological and parasitological study of cows up to the fourth gestation. *J. Vet. Med. B* 50, 399–404, 2003.
20. Schares, G., Rauser, M., Söndgen, P., Rehberg, P., Bärwald, A., Dubey, J. P., Edelhofer, R., Conraths, F. J., Use of purified tachyzoite surface antigen p38 in an ELISA to diagnose bovine neosporosis. *Int. J. Parasitol.* 30, 1123–1130, 2000.
21. Uggla, A., Stenlund, S., Holmdahl, O. J. M., Jakubek, E. B., Thebo, P., Kindahl, H., Björkman, C., Oral *Neospora caninum* inoculation of neonatal calves. *Int. J. Parasitol.* 28, 1467–1472, 1998.
22. Davison, H. C., Guy, C. S., McGarry, J. W., Guy, F., Williams, D. J. L., Kelly, D. F., Trees, A. J., Experimental studies on the transmission of *Neospora caninum* between cattle. *Res. Vet. Sci.* 70, 163–168, 2001.
23. Dijkstra, T., Barkema, H. W., Eysker, M., Wouda, W., Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. *Int. J. Parasitol.* 31, 209–215, 2001a.
24. Trees, A. J., Guy, F., Low, J. C., Roberts, L., Buxton, D., Dubey, J. P., Serological evidence implicating *Neospora* species as a cause of abortion in British cattle. *Vet. Rec.* 134, 405–407, 1994.
25. de Yaniz, M. G., Moore, D. P., Odeón, A. C., Cano, A., Cano, D. B., Leunda, M. R., Campero, C. M., Humoral immune response in pregnant heifers inoculated with *Neospora caninum* tachyzoites by conjunctival route. *Vet. Parasitol.* 148, 213–218, 2007.
26. Moore, D. P., Alvarez-García, G., Chiapparrone, M. L., Regidor-Cerrillo, J., Lischinsky, L. H., de Yaniz, M. G., Odeón, A. C., Ortega-Mora, L. M., Campero, C. M., *Neospora caninum* tachyzoites inoculated by the conjunctival route are not vertically transmitted in pregnant cattle: A descriptive study. *Vet. Parasitol.* 199, 1–7, 2014.

27. Serrano-Martínez, E., Ferre, I., Osoro, K., Aduriz, G., Mota, R. A., Martínez, A., del-Pozo, I., Hidalgo, C. O., Ortega-Mora, L. M., Intraurine *Neospora caninum* inoculation of heifers and cows using contaminated semen with different numbers of tachyzoites. *Theriogenology* 67, 729–737, 2007.
28. Caetano-da-Silva, A., Ferre, I., Collantes-Fernández, E., Navarro, V., Aduriz, G., Ugarte-Garagalza, C., Ortega-Mora, L. M., Occasional detection of *Neospora caninum* DNA in frozen extended semen from naturally infected bulls. *Theriogenology* 62, 1329–1336, 2004.
29. Ferre, I., Aduriz, G., del-Pozo, I., Regidor-Cerrillo, J., Atxaerandio, R., Collantes-Fernández, E., Hurtado, A., Ugarte-Garagalza, C., Ortega-Mora, L. M., Detection of *Neospora caninum* in the semen and blood of naturally infected bulls. *Theriogenology* 63, 1504–1518, 2005.
30. Ortega-Mora, L. M., Ferre, I., del Pozo, I., Caetano da Silva, A., Collantes-Fernández, E., Regidor-Cerrillo, J., Ugarte-Garagalza, C., Aduriz, G., Detection of *Neospora caninum* in semen of bulls. *Vet. Parasitol.* 117, 301–308, 2003.
31. Moen, A. R., Wouda, W., Mul, M. F., Graat, E. A. M., van Werven, T., Increased risk of abortion following *Neospora caninum* abortion outbreaks: A retrospective and prospective cohort study in four dairy herds. *Theriogenology* 49, 1301–1309, 1998.
32. Schares, G., Bärwald, A., Staubach, C., Söndgen, P., Rauser, M., Schröder, R., Peters, M., Wurm, R., Selhorst, T., Conraths, F. J., p38-avidity-ELISA: Examination of herds experiencing epidemic or endemic *Neospora caninum*-associated bovine abortion. *Vet. Parasitol.* 106, 293–305, 2002.
33. Wouda, W., van den Ingh, T. S. G. A. M., van Knapen, F., Sluyter, F. J. H., Koeman, J. P., Dubey, J. P., *Neospora* abortus bij het rund in Nederland. [Bovine abortion due to *Neospora* in The Netherlands]. *Tijdschr. Diergeneesk.* 117, 599–602, 1992.
34. McAllister, M. M., Björkman, C., Anderson-Sprecher, R., Rogers, D. G., Evidence of 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, M. M., Wallace, R. L., Björkman, C., Gao, L., Firkins, L. D., A probable source of *Neospora caninum* infection in an abortion outbreak in dairy cows. *Bovine Practitioner* 39, 69–74, 2005.
36. Aguado-Martínez, A., Álvarez-García, G., Arnaiz-Seco, I., Innes, E., Ortega-Mora, L. M., Use of avidity enzyme-linked immunosorbent assay and avidity Western blot to discriminate between acute and chronic *Neospora caninum* infection in cattle. *J. Vet. Diagn. Invest.* 17, 442–450, 2005.
37. Björkman, C., McAllister, M. M., Frössling, J., Näslund, K., Leung, F., Uggla, A., Application of the *Neospora caninum* IgG avidity ELISA in assessment of chronic reproductive losses after an outbreak of neosporosis in a herd of beef cattle. *J. Vet. Diagn. Invest.* 15, 3–7, 2003.
38. Björkman, C., Álvarez-García, G., Conraths, F. J., Mattsson, J. G., Ortega-Mora, L. M., Sager, H., Schares, G., *Neospora caninum* IgG avidity tests: An interlaboratory comparison. *Vet. Parasitol.* 140, 273–280, 2006.
39. Domingues, L. M., Machado, R. Z., Tinucci Costa, M., Carvalho, C. S., Costa, A. J., Malheiros, E. B., Canine toxoplasmosis: A comparative evaluation of the detection of anti-*Toxoplasma gondii* antibodies by the indirect immunoenzymatic assay (ELISA) and the indirect immunofluorescence reaction (IIF). *Rev. Bras. Parasitol. Vet.* 7, 79–85, 1998.
40. Aguado-Martínez, A., Álvarez-García, G., Fernández-García, A., Risco-Castillo, V., Arnaiz-Seco, I., Rebordosa-Trigueros, X., Navarro-Lozano, V., Ortega-Mora, L. M., Usefulness of rNcGRA7- and rNcSAG4-based ELISA tests for distinguishing primo-infection, recrudescence, and chronic bovine neosporosis. *Vet. Parasitol.* 157, 182–195, 2008.
41. Basso, W., Schares, S., Minke, L., Bärwald, A., Maksimov, A., Peters, M., Schulze, C., Müller, M., Conraths, F. J., Schares, G., Microsatellite typing and avidity analysis suggest a common source of infection in herds with epidemic *Neospora caninum*-associated bovine abortion. *Vet. Parasitol.* 173, 24–31, 2010.
42. Jenkins, M. C., Caver, J. A., Björkman, C., Anderson, T. C., Romand, S., Vinyard, B., Uggla, A., Thulliez, P., Dubey, J. P., Serological investigation of an outbreak of *Neospora caninum*-associated abortion in a dairy herd in southeastern United States. *Vet. Parasitol.* 94, 17–26, 2000.

43. Sager, H., Hüssy, D., Kuffer, A., Schreve, F., Gottstein, B., Mise en évidence d'un cas de "abortion storm" (transmission transplacentaire exogène de *Neospora caninum*) dans une exploitation de vaches laitières: une première en Suisse. [First documentation of a *Neospora*-induced "abortion storm" (exogenous transplacental transmission of *Neospora caninum*) in a Swiss dairy farm]. *Schweiz. Arch. Tierheilkd.* 147, 113–120, 2005.
44. Cardoso, J. M. S., Amaku, M., Araújo, A. J. S., Gennari, S. M., A longitudinal study of *Neospora caninum* infection on three dairy farms in Brazil. *Vet. Parasitol.* 187, 553–557, 2012.
45. Mazuz, M. L., Fish, L., Reznikov, D., Wolkomirsky, R., Leibovitz, B., Savitzky, I., Golenser, J., Shkap, V., Neosporosis in naturally infected pregnant dairy cattle. *Vet. Parasitol.* 205, 85–91, 2014.
46. Sawada, M., Kondo, H., Tomioka, Y., Park, C. H., Morita, T., Shimada, A., Umemura, T., Isolation of *Neospora caninum* from the brain of a naturally infected adult dairy cow. *Vet. Parasitol.* 90, 247–252, 2000.
47. Thurmond, M. C., Hietala, S. K., Blanchard, P. C., 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, 1997a.
48. Wouda, W., Moen, A. R., Schukken, Y. H., Abortion risk in progeny of cows after a *Neospora caninum* epidemic. *Theriogenology* 49, 1311–1316, 1998.
49. López-Gatius, F., Pabón, M., Almería, S., *Neospora caninum* infection does not affect early pregnancy in dairy cattle. *Theriogenology* 62, 606–613, 2004b.
50. Pabón, M., López-Gatius, F., García-Ispuerto, I., Bech-Sabat, G., Nogareda, C., Almería, S., Chronic *Neospora caninum* infection and repeat abortion in dairy cows: A 3-year study. *Vet. Parasitol.* 147, 40–46, 2007.
51. Paré, J., Thurmond, M. C., Hietala, S. K., *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. *J. Parasitol.* 83, 82–87, 1997.
52. Thurmond, M. C., Hietala, S. K., Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. *Am. J. Vet. Res.* 58, 1381–1385, 1997b.
53. López-Gatius, F., García-Ispuerto, I., Santolaria, P., Yániz, J. L., López-Béjar, M., Nogareda, C., Almería, S., Relationship between rainfall and *Neospora caninum*-associated abortion in two dairy herds in a dry environment. *J. Vet. Med. B* 52, 147–152, 2005.
54. Yániz, J. L., López-Gatius, F., García-Ispuerto, I., Bech-Sabat, G., Serrano, B., Nogareda, C., Sanchez-Nadal, J. A., Almería, S., Santolaria, P., Some factors affecting the abortion rate in dairy herds with high incidence of *Neospora*-associated abortions are different in cows and heifers. *Reprod. Dom. Anim.* 45, 699–705, 2010.
55. Sanderson, M. W., Gay, J. M., Baszler, T. V., *Neospora caninum* seroprevalence and associated risk factors in beef cattle in the northwestern United States. *Vet. Parasitol.* 90, 15–24, 2000.
56. Bartels, C. J. M., Arnaiz-Seco, J. I., Ruiz-Santa-Quiteria, A., Björkman, C., Frössling, J., von Blumröder, D., Conraths, F. J., Schares, G., van Maanen, C., Wouda, W., Ortega-Mora, L. M., Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. *Vet. Parasitol.* 137, 17–27, 2006.
57. Eiras, C., Arnaiz, I., Álvarez-García, G., Ortega-Mora, L. M., Sanjuán, M. L., Yus, E., Diéguez, F. J., *Neospora caninum* seroprevalence in dairy and beef cattle from the northwest region of Spain, Galicia. *Prev. Vet. Med.* 98, 128–132, 2011.
58. Ghalmi, F., Dramchini, N., China, B., Risk factors for abortion in cattle herds in Algeria. *Vet. Rec.* 165, 475–476, 2009.
59. Guimarães, J. S., Souza, S. L. P., Bergamaschi, D. P., Gennari, S. M., Prevalence of *Neospora caninum* antibodies and factors associated with their presence in dairy cattle of the north of Paraná state, Brazil. *Vet. Parasitol.* 124, 1–8, 2004.
60. Moore, D. P., Campero, C. M., Odeón, A. C., Posso, M. A., Cano, D., Leunda, M. R., Basso, W., Venturini, M. C., Späth, E., Seroepidemiology of beef and dairy herds and fetal study of *Neospora caninum* in Argentina. *Vet. Parasitol.* 107, 303–316, 2002.
61. Bruhn, F. R. P., Daher, D. O., Lopes, E., Barbieri, J. M., da Rocha, C. M. B. M., Guimarães, A. M., Factors associated with seroprevalence of *Neospora caninum* in dairy cattle in southeastern Brazil. *Trop. Anim. Health Prod.* 45, 1093–1098, 2013.

62. Gharekhani, J., Haddadzadeh, H., Bahonar, A., Prevalence of immunoglobulin G (IgG) antibody to *Neospora caninum* in dairy cattle of Hamedan province, west of Iran. *Vet. Res. Forum* 5, 149–152, 2015.
63. Dijkstra, T., Barkema, H. W., Eysker, M., Hesselink, J. W., Wouda, W., Natural transmission routes of *Neospora caninum* between farm dogs and cattle. *Vet. Parasitol.* 105, 99–104, 2002a.
64. VanLeeuwen, J. A., Haddad, J. P., Dohoo, I. R., Keefe, G. P., Tiwari, A., Scott, H. M., Risk factors associated with *Neospora caninum* seropositivity in randomly sampled Canadian dairy cows and herds. *Prev. Vet. Med.* 93, 129–138, 2010.
65. Dijkstra, T., Eysker, M., Schares, G., Conraths, F. J., Wouda, W., Barkema, H. W., 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, 2001b.
66. Bergeron, N., Fecteau, G., Villeneuve, A., Girard, C., Paré, J., Failure of dogs to shed oocysts after being fed bovine fetuses naturally infected by *Neospora caninum*. *Vet. Parasitol.* 97, 145–152, 2001.
67. Dijkstra, T., Barkema, H. W., Hesselink, J. W., Wouda, W., 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, 2002b.
68. Gondim, L. F. P., McAllister, M. M., Gao, L., Effects of host maturity and prior exposure history on the production of *Neospora caninum* oocysts by dogs. *Vet. Parasitol.* 134, 33–39, 2005.
69. Schares, G., Heydorn, A. O., Cüppers, A., Conraths, F. J., Mehlhorn, H., *Hammondia heydorni*-like oocysts shed by a naturally infected dog and *Neospora caninum* NC-1 cannot be distinguished. *Parasitol. Res.* 87, 808–816, 2001.
70. Gondim, L. F. P., McAllister, M. M., Pitt, W. C., Zemlicka, D. E., Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *Int. J. Parasitol.* 34, 159–161, 2004a.
71. Dubey, J. P., Jenkins, M. C., Rajendran, C., Miska, K., Ferreira, L. R., Martins, J., Kwok, O. C. H., Choudhary, S., Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. *Vet. Parasitol.* 181, 382–387, 2011.
72. Gondim, L. F. P., McAllister, M. M., Mateus-Pinilla, N. E., Pitt, W. C., Mech, L. D., Nelson, M. E., Transmission of *Neospora caninum* between wild and domestic animals. *J. Parasitol.* 90, 1361–1365, 2004b.
73. Ould-Amrouche, A., Klein, F., Osdoit, C., Mohamed, H. O., Touratier, A., Sanaa, M., Mialot, J. P., Estimation of *Neospora caninum* seroprevalence in dairy cattle from Normandy, France. *Vet. Res.* 30, 531–538, 1999.
74. Sierra, R. C., Medina-Esparza, L., Parra, M. R., García-Vázquez, Z., Cruz-Vázquez, C., Risk factors associated with *Neospora caninum* antibody seroprevalence in dairy cattle in Aguascalientes, Mexico. *Rev. Mex. Cienc. Pecu* 2, 15–24, 2011.
75. Bartels, C. J. M., Wouda, W., Schukken, Y. H., Risk factors for *Neospora caninum*-associated abortion storms in dairy herds in the Netherlands (1995–1997). *Theriogenology* 52, 247–257, 1999.
76. Costa, K. S., Santos, S. L., Uzêda, R. S., Pinheiro, A. M., Almeida, M. A. O., Araújo, F. R., McAllister, M. M., Gondim, L. F. P., Chickens (*Gallus domesticus*) are natural intermediate hosts of *Neospora caninum*. *Int. J. Parasitol.* 38, 157–159, 2008.
77. Ibrahim, H. M., Seroprevalence of *Neospora caninum* antibodies in chicken samples from Delta Egypt using a recombinant NcSAG1 protein-based ELISA. *Egypt. J. Immunol.* 20, 29–37, 2013.
78. Martins, J., Kwok, O. C. H., Dubey, J. P., Seroprevalence of *Neospora caninum* in free-range chickens (*Gallus domesticus*) from the Americas. *Vet. Parasitol.* 182, 349–351, 2011.
79. Gonçalves, I. N., Uzêda, R. S., Lacerda, G. A., Moreira, R. R. N., Araújo, F. R., Oliveira, R. H. M., Corbellini, L. G., Gondim, L. F. P., Molecular frequency and isolation of cyst-forming coccidia from free ranging chickens in Bahia State, Brazil. *Vet. Parasitol.* 190, 74–79, 2012.
80. Bahrami, S., Rezaie, A., Boroomand, Z., Namavari, M., Ghavami, S., Embryonated pigeon eggs as a model to investigate *Neospora caninum* infection. *Lab. Anim.* Doi: 10.1177/0023677216652373, 2016.
81. Furuta, P. I., Mineo, T. W. P., Carrasco, A. O. T., Godoy, G. S., Pinto, A. A., Machado, R. Z., *Neospora caninum* infection in birds: Experimental infections in chicken and embryonated eggs. *Parasitology* 134, 1931–1939, 2007.
82. Khodakaram-Tafti, A., Mansourian, M., Namavari, M., Hosseini, A.,

- Immunohistochemical and polymerase chain reaction studies in *Neospora caninum* experimentally infected broiler chicken embryonated eggs. *Vet. Parasitol.* 188, 10–13, 2012.
83. Otranto, D., Llazari, A., Testini, G., Traversa, D., di Regalbono, A. F., Badan, M., Capelli, G., Seroprevalence and associated risk factors of neosporosis in beef and dairy cattle in Italy. *Vet. Parasitol.* 118, 7–18, 2003.
 84. Barling, K. S., McNeill, J. W., Paschal, J. C., McCollum, F. T. III, Craig, T. M., Adams, L. G., Thompson, J. A., Ranch-management factors associated with antibody seropositivity for *Neospora caninum* in consignments of beef calves in Texas, USA. *Prev. Vet. Med.* 52, 53–61, 2001.
 85. Martins, A. A., de Zamproga, T. O., Lucas, T. M., da Cunha, I. A. L., Garcia, J. L., da Silva, A. V., Frequency and risk factors for infection by *Neospora caninum* in dairy farms of Umuarama, PR, Brazil. *Arq. Ciên. Vet. Zool.* 15, 137–142, 2012.
 86. McAllister, M. M., Diagnosis and control of bovine neosporosis. *Vet. Clin. Food Anim.* 32, 443–463, 2016.
 87. Moskwa, B., Cabaj, W., Pastusiak, K., Bien, J., The suitability of milk in detection of *Neospora caninum* infection in cows. *Acta Parasitol.* 48, 138–141, 2003.
 88. Moskwa, B., Pastusiak, K., Bien, J., Cabaj, W., The first detection of *Neospora caninum* DNA in the colostrum of infected cows. *Parasitol. Res.* 100, 633–636, 2007.
 89. González-Warleta, M., Castro-Hermida, J. A., Carro-Corral, C., Cortizo-Mella, J., Mezo, M., Epidemiology of neosporosis in dairy cattle in Galicia (NW Spain). *Parasitol. Res.* 102, 243–249, 2008.
 90. Björkman, C., Alenius, S., Emanuelsson, U., Uggla, A., *Neospora caninum* and bovine virus diarrhoea virus infections in Swedish dairy cows in relation to abortion. *Vet. J.* 159, 201–206, 2000.
 91. Rinaldi, L., Pacelli, F., Iovane, G., Pagnini, U., Veneziano, V., Fusco, G., Cringoli, G., Survey of *Neospora caninum* and bovine herpes virus 1 coinfection in cattle. *Parasitol. Res.* 100, 359–364, 2007.

TODOROVA T.