

Neosporosis in dairy cattle: An update from an epidemiological perspective

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Abstract

Our understanding of the epidemiology of bovine neosporosis is advancing rapidly with considerable research activity being facilitated by improving methods. The dynamics of the infection in the known definitive hosts, the dog and the coyote, are being described. Improved procedures for production of oocysts enables the horizontal transmission to intermediate hosts and the subsequent more natural infection process to be studied. Details of the sylvatic cycles, potentially involving other animals in the dairy environment, are also emerging.

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1. Introduction

The protozoal parasite *Neospora caninum* was first observed as a clinical disease of dogs in 1984 [1] and of calves in 1987 [2,3] and as a cause of fetal loss in cattle in 1989 [4]. First isolated and proposed as a new genus and species in 1988 [5], *N. caninum* is now recognized as causing neosporosis, a major cause of bovine fetal loss worldwide. As the subject of much active research, our understanding of the parasite, the host–parasite relationship and the epidemiology of the infection are rapidly advancing although, as the agent was only recently identified, remains incomplete in important respects. An ISI Web of Science® search on the terms “neospora” or “neosporosis” returned almost 1200 indexed papers, 618 including the bovine in some fashion (e.g., terms such as cow, calf, cattle, bovine), with 126 published in 2004. These include extensive

reviews of general and specific aspects of bovine neosporosis [6–14] as well as many primary research papers. With an emphasis on the more recent publications that expand our understanding of neosporosis but not covered in the above reviews, the following are selected from these.

2. Causative organism and life cycle

N. caninum is an Apicomplexa protozoan that is closely related to and in some phases morphologically similar to *Toxoplasma*, *Sarcocystis* and *Hammondia*. However, it is biologically distinct from these other protozoa. The dog [15,16] and the coyote [17] have been shown to be naturally infected definitive hosts, meaning that they may shed oocysts in their feces after consuming tissue cysts from infected intermediate hosts, and the infectivity of such oocysts for the bovine has been demonstrated [18]. The cow and the white-tailed deer [19] are common intermediate hosts; they may be infected by sporulated *N. caninum* oocysts, which in turn produce tissue cysts in the intermediate

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host's body tissues. The infectivity of tissues for definitive hosts has been demonstrated, particularly the placenta from naturally infected intermediate hosts [20]. The development of improved detection techniques is facilitating clarification of transmission cycles of this two-host parasite. For example, a PCR-based method to detect *N. caninum* oocysts in the feces of definitive hosts has been recently developed [21].

3. Fecal shedding

Initial work, particularly by McCallister and coworkers, described the dynamics of fecal oocyst shedding by definitive hosts, although the picture remains incomplete. In a three-period survey of 15 dogs fed beef carcasses, 1 dog shed 378 oocysts per gram on one occasion and was fecal positive but not quantified on another [22]. An experimental study included the feeding of oocysts to calves, later feeding tissues from the infected calves to dogs and sampling their feces daily for 30 d [23]. Eight of the nine dogs fed tissues from infected calves shed oocysts in their feces over a period of 70 d. During this period, oocyst counts in "positive" feces were <1000, 1000–10,000 and >10,000 for 38, 36 and 26% of the time, respectively. On first exposure, dogs fed infective tissues from calves shed greater numbers than those fed infective tissues from mice and young dogs shed greater numbers than adults. In a subsequent study of shedding response to re-exposure, two of these previously shedding dogs did not shed oocysts when fed tissues from the infected calves 8 months after their initial exposure, whereas two of three dogs shed oocysts when exposed a year and a half later [24]. In this study, only the total numbers of oocysts shed, not the daily shedding levels and duration of shedding, were reported. This was unfortunate as point exposures to single bowel movements seem considerably more likely and more difficult to prevent than point exposures to feces accumulated over multiple days. Of note, both of these studies show an inconsistent relationship between the serological and shedding status of dogs, suggesting that serological testing of specific dogs is not a useful prevention tool. In another study, one of four 12-week-old coyotes shed approximately 500 oocysts over 3 d after being fed tissues from infected calves [17]. These findings supported the recommendation that the risk of contamination of cattle feed and water should be minimized. However, considerably more work is indicated on the dynamics of infection in the definitive host (such as the frequency and level of oocyst shedding), and on transmission from definitive to the intermediate hosts.

4. Transmission following oral exposure

Two experiments exposing pregnant cattle orally to oocysts were recently reported. In one, three cows challenged with 600 oocysts at 10 weeks of gestation gave birth to uninfected calves [25]. When euthanized 4 months after calving, the cows were seronegative, although their brains were PCR-positive. In a subsequent experiment, 17 of 19 (89%) cows in various stages of gestation given oral doses ranging from 1500 to 115,000 oocytes, became seropositive [26]. Transplacental infection occurred in 6 of the 17 fetuses and 1 aborted due to neosporosis. The authors concluded that the odds of transplacental infection increase with larger oocyte doses and advancing gestation. It was noteworthy that the lowest oocyst dose resulted in a congenital infection, and a single abortion was associated with a mid-range dose. These findings suggest that the relationship between oral oocyst exposure, as would occur with a herd point exposure from feed contamination, and reproductive consequences, such as an abortion storm, is complex.

5. Venereal transmission

Limited initial evidence suggests that semen from infected bulls may be a potential vector for venereal transmission of bovine neosporosis. Fifteen of 102 (15%) semen samples from 5 of 8 naturally infected bulls were *N. caninum* DNA PCR-positive over a 22-week period, with an estimated parasite load of 1 to 10 parasites/mL [27]. None of the PCR-positive semen samples were bioassay positive, although the authors note that this may have been due to the toxic effects of semen and to the low parasite numbers. In another experiment by the same group, all of nine heifers administered 10^7 tachyzoites by intrauterine inoculation of artificially contaminated semen responded serologically and six had *N. caninum* DNA PCR-positive tissues when necropsied 36 d post-insemination [28]. Considerably more work remains to be done to determine whether or not natural transmission via the venereal route represents an important risk.

6. Vertical transmission

Although the relative importance of each potential route of infection has not been well established, vertical transmission remains most likely for the individual dairy cow in typical farm situations. In a prospective study of a 240-cow California dairy herd involving monthly sampling of pregnant cows and pre-colostral

sampling of their offspring, the risk of vertical transmission from a seropositive dam was 88% (76–96% CI) and the relative risk of fetal loss was 1.8 [29]. In a prospective study based on pre-colostral testing in four European dairy herds that had previously experienced abortion outbreaks, the overall risk of vertical transmission from a seropositive dam was 92% (81–98% CI) and the relative risk of fetal loss was 3.13 (1.71–5.73 CI) [30]. In a cross-sectional study of a small closed European herd, 14 of 15 (93%; 68–99% CI) descendants of seropositive cows were seropositive [31]. In a cross-sectional serological study of cattle over 6 months of age in 23 Quebec dairy herds, based on reported cow pedigrees the overall risk of vertical transmission from a seropositive dam was 44% (36–53% CI) [32]. In a cross-sectional serological study of cattle over 5 months of age in 20 Costa Rican dairy herds, the probability that a daughter of a seropositive dam was seropositive was 60% (52–66% CI) [33]. Based on mathematical modeling, the latter authors concluded that in these herds the probability of vertical and horizontal transmission was 0.84 and 0.22, respectively. In a cross-sectional seroprevalence survey in an Australian dairy herd, the probability that a direct descendant of a seropositive dam was seropositive was 90% (55–99% CI) [34]. The estimates of the probability of vertical transmission varied fairly widely, depending upon testing procedures, whether descendant sampling was pre-colostral or later, when the dam was sampled, herd seroprevalence, previous outbreak status and other factors. As indicated by the width of the 95% CI, due to differences in sample sizes the precision of these estimates also varied considerably.

7. Role of embryo transfer

Embryo transfer using seropositive donors and seronegative recipients to prevent vertical transmission has been evaluated in several studies [35,36]. In the largest study, 50 progeny from seropositive donors transferred to seronegative recipients using the International Embryo Transfer Society (EITS) embryo handling procedure were free of infection (0–5.8% CI) although 6 of 8 progeny transferred to seropositive recipients were not [35]. In another study, 6 seropositive dams gave birth to 13 seropositive heifers following natural service and, although the serostatus of only 4 recipients was known, embryo transfer using the IETS procedure resulted in the birth of 14 seronegative heifers [36]. A study employing a more routine embryo washing technique was also performed, but the numbers were too small to be particularly useful (0–84% CI) [37].

8. Sylvatic cycles

Evidence of sylvatic cycles of this parasite are emerging, including documentation of natural infections in a number of wild species [9,38], some of which are potentially found in the dairy farm environment. As noted above, coyotes have been observed shedding oocysts from natural infections [19]. Experimental transmission of *N. caninum* between wild and domestic animals has been demonstrated and a seroprevalence survey with an indirect fluorescent antibody test found positive titers in 39% of 164 gray wolves, 11% of 113 coyotes, 26% of 193 white-tailed deer, and 13% of 61 moose, all animals at various locations in the USA [39]. Previous seroprevalence surveys based on the *N. caninum* agglutination test found positive titers in 40% of 400 white-tailed deer in Illinois [40] and in 48% of 305 white-tailed deer in the southeastern USA [41]. Considerably more work is needed to determine the consequences of these sylvatic cycles on domestic cattle that are exposed to them, whether by direct contact, or via contaminated feed or water.

References

- [1] Bjerkaas I, Mohn SF, Presthus J. Unidentified cyst-forming sporozoan causing encephalomyelitis and myositis in dogs. *Z Parasitenkd* 1984;70:271–4.
- [2] O'Toole D, Jeffrey M. Congenital sporozoan encephalomyelitis in a calf. *Vet Rec* 1987;121:563–6.
- [3] Parish SM, Maag-Miller L, Besser TE, Weidner JP, McElwain T, Knowles DP, et al. Myelitis associated with protozoal infection in newborn calves. *J Am Vet Med Assoc* 1987;191:1599–600.
- [4] Thilsted JP, Dubey JP. Neosporosis-like abortions in a herd of dairy cattle. *J Vet Diagn Invest* 1989;1:205–9.
- [5] Dubey JP, Carpenter JL, Speer CA, Topper MJ, Uggla A. Newly recognized fatal protozoan disease of dogs. *J Am Vet Med Assoc* 1988;192:1269–85.
- [6] Anderson ML, Andrianarivo AG, Conrad PA. Neosporosis in cattle. *Anim Reprod Sci* 2000;60–61:417–31.
- [7] Dubey JP. Neosporosis in cattle. *Vet Clin North Am Food Anim Pract* 2005;21:473–83.
- [8] Dubey JP. Recent advances in Neospora and neosporosis. *Vet Parasitol* 1999;84:349–67.
- [9] Dubey JP. Review of *Neospora caninum* and neosporosis in animals. *Korean J Parasitol* 2003;41:1–16.
- [10] Dubey JP, Lindsay DS. A review of *Neospora caninum* and neosporosis. *Vet Parasitol* 1996;67:1–59.
- [11] Haddad JP, Dohoo IR, VanLeewen JA. A review of *Neospora caninum* in dairy and beef cattle—a Canadian perspective. *Can Vet J* 2005;46:230–43.
- [12] Innes EA, Wright S, Bartley P, Maley S, Macalodow C, Esteban-Redondo I, et al. The host-parasite relationship in bovine neosporosis. *Vet Immunol Immunopathol* 2005;108:29–36.
- [13] Jenkins M, Baszler T, Bjorkman C, Schares G, Williams D. Diagnosis and seroepidemiology of *Neospora caninum*-associated bovine abortion. *Int J Parasitol* 2002;32:631–6.

- [14] Moore DP. Neosporosis in South America. *Vet Parasitol* 2005;127:87–97.
- [15] Lindsay DS, Dubey JP, Duncan RB. Confirmation that the dog is a definitive host for *Neospora caninum*. *Vet Parasitol* 1999;82:327–33.
- [16] McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM. Dogs are definitive hosts of *Neospora caninum*. *Int J Parasitol* 1998;28:1473–8.
- [17] Gondim LF, McAllister MM, Pitt WC, Zemlicka DE. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. *Int J Parasitol* 2004;34:159–61.
- [18] De Marez 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* 1999;29:1647–57.
- [19] Vianna MC, Sreekumar C, Miska KB, Hill DE, Dubey JP. Isolation of *Neospora caninum* from naturally infected white-tailed deer (*Odocoileus virginianus*). *Vet Parasitol* 2005;129:253–7.
- [20] Dijkstra T, 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* 2001;31:747–52.
- [21] Hill DE, Liddell S, Jenkins MC, Dubey JP. Specific detection of *Neospora caninum* oocysts in fecal samples from experimentally-infected dogs using the polymerase chain reaction. *J Parasitol* 2001;87:395–8.
- [22] McGarry JW, Stockton CM, Williams DJ, Trees AJ. Protracted shedding of oocysts of *Neospora caninum* by a naturally infected foxhound. *J Parasitol* 2003;89:628–30.
- [23] Gondim LF, Gao L, McAllister MM. Improved production of *Neospora caninum* oocysts, cyclical oral transmission between dogs and cattle, and in vitro isolation from oocysts. *J Parasitol* 2002;88:1159–63.
- [24] Gondim LF, McAllister MM, Gao L. Effects of host maturity and prior exposure history on the production of *Neospora caninum* oocysts by dogs. *Vet Parasitol* 2005;134:33–9.
- [25] Trees AJ, McAllister MM, Guy CS, McGarry JW, Smith RF, Williams DJ. *Neospora caninum*: oocyst challenge of pregnant cows. *Vet Parasitol* 2002;109:147–54.
- [26] Gondim LF, McAllister MM, Anderson-Sprecher RC, Bjorkman C, Lock TF, Firkins LD, et al. Transplacental transmission and abortion in cows administered *Neospora caninum* oocysts. *J Parasitol* 2004;90:1394–400.
- [27] Ferre I, Aduriz G, Del-Pozo I, Regidor-Cerrillo J, Atxaerandio R, Collantes-Fernandez E, et al. Detection of *Neospora caninum* in the semen and blood of naturally infected bulls. *Theriogenology* 2005;63:1504–18.
- [28] Serrano E, Ferre I, Osoro K, Aduriz G, Mateos-Sanz A, Martinez A, et al. Intrauterine *Neospora caninum* inoculation of heifers. *Vet Parasitol* 2006;135(3–4):197–203.
- [29] Pare J, Thurmond MC, Hietala SK. *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. *J Parasitol* 1997;83:82–7.
- [30] Wouda W, Moen AR, Schukken YH. Abortion risk in progeny of cows after a *Neospora caninum* epidemic. *Theriogenology* 1998;49:1311–6.
- [31] Schares G, Peters M, Wurm R, Barwald A, Conraths FJ. The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analysed by serological techniques. *Vet Parasitol* 1998;80:87–98.
- [32] 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* 2000;41:464–7.
- [33] Romero JJ, Frankena K. The effect of the dam-calf relationship on serostatus to *Neospora caninum* on 20 Costa Rican dairy farms. *Vet Parasitol* 2003;114:159–71.
- [34] Hall CA, Reichel MP, Ellis JT. *Neospora* abortions in dairy cattle: diagnosis, mode of transmission and control. *Vet Parasitol* 2005;128:231–41.
- [35] Baillargeon P, Fecteau G, Pare J, Lamothe P, Sauve R. 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* 2001;218:1803–6.
- [36] Campero CM, Moore DP, Lagomarsino H, Odeon AC, Castro M, Visca H. 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* 2003;50:458–60.
- [37] Landmann JK, Jillella D, O'Donoghue PJ, McGowan MR. Confirmation of the prevention of vertical transmission of *Neospora caninum* in cattle by the use of embryo transfer. *Aust Vet J* 2002;80:502–3.
- [38] Rosypal AC, Lindsay DS. The sylvatic cycle of *Neospora caninum*: where do we go from here? *Trends Parasitol* 2005; 21:439–40.
- [39] Gondim LF, McAllister MM, Mateos-Pinilla NE, Pitt WC, Mech LD, Nelson ME. Transmission of *Neospora caninum* between wild and domestic animals. *J Parasitol* 2004;90:1361–5.
- [40] Dubey JP, Hollis K, Romand S, Thulliez P, Kwok OC, Hungerford L, et al. High prevalence of antibodies to *Neospora caninum* in white-tailed deer (*Odocoileus virginianus*). *Int J Parasitol* 1999;29:1709–11.
- [41] Lindsay DS, Little SE, Davidson WR. Prevalence of antibodies to *Neospora caninum* in white-tailed deer, *Odocoileus virginianus*, from the southeastern United States. *J Parasitol* 2002;88:415–7.