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Neonatal *Neospora caninum* infections in dogs

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Abstract

Neosporosis was diagnosed ante-mortem in a litter of neonatally-infected dogs. Three pups developed weakness of limbs 7–9 weeks after birth. One of the dogs developed megaesophagus. Treatment with clindamycin improved clinical signs but did not eradicate the parasite. All 3 dogs were euthanized and viable *N. caninum* was isolated from the brains of all 3 dogs. Tissue cysts were found in the brain and muscles of dogs. The dam was bred again. Seven apparently healthy pups were born in the second litter. Six of the 7 pups from the second litter had no demonstrable *N. caninum* antibodies at 32 day of age. The seventh pup had high (1:1280) titer in the *Neospora* agglutination test and the titer remained stable at day 227 when the study was discontinued. The results suggest that the rate of congenital transmission of *N. caninum* decreased in the subsequent pregnancy.

Key words

Neospora caninum, megaesophagus, diagnosis, treatment

Introduction

The protozoan parasite *Neospora caninum* can cause a serious disease in dogs (Bjerkås *et al*. 1984, Dubey *et al*. 1988a, Barber and Trees 1996, Dubey and Lindsay 1996, Dubey *et al*. 1998, Lindsay and Dubey 2000, Dubey 2003a). Most cases of clinical neosporosis reported were in young (<4 months old) dogs, presumably infected congenitally. However, dogs of all ages have died of neosporosis. The most consistent sign of canine neosporosis is paresis of limbs, especially hind limbs. There are several serologic tests that can detect antibodies to *N. caninum* infection in dogs. Indirect fluorescent antibody test (IFAT) is often used to diagnose canine neosporosis. There is no agreement with respect to a diagnostic titer for clinical neosporosis. Barber and Trees (1996) found IFAT titers of 1:800 or higher in 20 dogs with suspected clinical neosporosis and suggested that such high titers may be considered diagnostic because such titers are rare in clinically healthy dogs. There are a very few cases of clinical neosporosis in dogs with serological status verified by demonstration of the parasite. *N. caninum* has been isolated from paralyzed dogs that had a low IFAT titer of 1:50 (Dubey *et al*. 1988b, 1998). We report clinical signs, serological diagnosis, and treatment of neosporosis in littermate dogs.

Materials and methods

Eleven dogs (nos. 1–11) were born on the 19th December 2002 from a 5-year-old purebred chocolate Labrador bitch. This was her first litter. One dog (no. 1) was stillborn and had been dead inside the uterus and the carcass was discarded without necropsy examination. The remaining dogs appeared to be clinically healthy but were not thoroughly examined by the owner until 2 weeks later. At this time, dog no. 2 was found to be small and nursing poorly. Dog no. 2 weighed only 0.5 kg at 3 weeks of age whereas his siblings weighed approximately 2 kg. Dog no. 2 was then hand fed. All other dogs were physically normal until 7 weeks of age when 2 dogs (nos. 3 and 4) developed gait abnormalities. Dog no. 3 developed weakness in 1 pelvic limb. Dog no. 3 was examined at the Madonna Veterinary Clinic (MVC), White Hall, Maryland, when she was 50 days old. She was holding her right pelvic limb in rigid extension, and the stifle joint could not be flexed by the examiner. The dog was injected with 1 mg of dexa-methasone subcutaneously and sent home. The next day the dog was examined further. Abnormalities were not detected on the radiographs of the pelvis and the stifle joint. A neurological problem was suspected and the dog was given 0.25 mg prednisolone orally for 3 days. Dog no. 3 was exam-

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ined again at MVC at 54 days of age. Her stifle joint flexed only to a 90 degree angle. Hematological abnormalities were: increased serum creatinine kinase (1370 U/l, reference range 59–895 U/l), and alkaline phosphatase (140 U/l, reference range 5–131 U/l) activities, increased lymphocytes (8042 cells/µl, reference range 690–4500 cells/µl), eosinophils (1431cells/µl, normal range up to 1200 cells/µl) and increased white blood cells (15.9×10^3 cells/µl, range 4.0–15.5 cells/µl). Differential diagnosis for the neuromuscular dysfunction included inherited myopathy, neosporosis, and toxoplasmosis. Clindamycin (Pharmacia and Upjohn, New Jersey, USA, 50 mg/kg, twice daily) was initially prescribed for 2 weeks while waiting for serological testing results (Table I).

Dog no. 4 also developed pelvic limb stiffness and was squatting 1 day after dog no. 3 developed limb weakness. Dog no. 4 was examined at MVC at 54 days of age. This dog had the same signs as dog no. 3 and was struggling to walk on all 4 limbs. Dog no. 4 was treated similarly to dog no. 3. Both dogs were given oral clindamycin (50 mg/kg) for a total of 62 days until euthanasia. Both dogs were euthanized with pentobarbital when they were 116 days old because they were not improving clinically despite treatment.

Dog no. 2 did not develop any clinical signs of illness until 9 weeks of age, when he started to fall over. His condition worsened over the following 2 days. Treatment with oral clindamycin was then started immediately and continued for 14 days. The dog's physical condition dramatically improved after 3 days of treatment. The dog weighed 5.5 kg when 64 days old. He was brought to MVC again when he was 3 months old because of vomiting, dysphagia, and restlessness. He was treated with clindamycin for 14 days, along with fluid therapy. A week later he was diagnosed with megaesophagus, which was identified by thoracic radiography. Treatment was instituted with injectable clindamycin (50 mg/kg, twice daily) and the treatment continued until euthanasia. The dog showed some clinical improvement but was euthanized because of deterioration in his condition when the dog was 4.5 months old. Dogs nos. 5 to 11 were sold before *Neospora* testing but apparently remained clinically healthy. Retrospectively, blood sera were obtained from dogs nos. 5–7 when they were 99 days old.

Sera from dogs were examined for antibodies to *N. caninum* using the indirect fluorescent antibody test (IFAT, Dubey *et al*. 1988b, cut-off 1:25), direct *Neospora* agglutination test (NAT, Romand *et al*. 1998, cut-off 1:25), and by competitive inhibition ELISA (Baszler *et al*. 1996, 2001; % inhibition, kit controls: negative = 0, positive = 70.5). Sera from dogs were also examined for antibodies to *Toxoplasma gondii* using the modified agglutination test (MAT, Dubey and Desmonts 1987, cut–off 1:25). For bleeding schedule see Table I. Sera from all dogs from all bleedings were examined in each test at the same time to avoid day-to-day variation.

Complete necropsy examinations were performed on all 3 dogs and their tissues were examined histologically and for isolation of *N. caninum* as described (Dubey *et al*. 2004).

Results

All 3 dogs had antibodies (NAT 1:100 or higher) to *N. caninum* at the time of necropsy. Antibodies to *T. gondii* were not found in 1:25 dilution of serum from any of the 3 dogs (Table I). The NAT titers of dogs nos. 5–7 at 99 day of age were negative at 1:25 dilution.

The dam was rebred to the same sire and 7 healthy pups (nos.12–18) were born 18 months after the birth of the first infected litter. The NAT titer of the dam before, during, and after birth remained at 1:160. The dam and the pups were serologically tested by the use of NAT, 32 days after birth. Six of the 7 pups were seronegative at 1:20 dilution of the serum and 1 pup (no. 12) and the dam had a NAT titer of 1:160. The NAT titers of the pup no.12 remained stable at 1:1280 at 51, 93, and 227 day of age. Pup no. 12 was hysterectomyzed at 227 day of age and thus we could not study congenital infection in its progeny.

Table I. Serological examination for *Neospora caninum* and *Toxoplasma gondii*

Dog no.	Age (days)	*N. caninum*			*T. gondii*
		NAT	IFAT	ELISA	MAT
2	77	400	800	90.8	<25
	113	400	400	89.7	<25
3	77	800	3200	87.6	<25
	106	400	400	82.7	<25
4	77	400	800	91.7	<25
	106	100	200	89.2	<25
Dam	113[a]	50	100	90.4	<25
	276[a]	100	Not done	86.2	<25

[a]Day after parturition.

Lesions were confined to the brain and muscles. The encephalitis was characterized by multifocal gliosis and, perivascular infiltration with mononuclear cells and minimal necrosis of neural tissue. Among the 3 dogs, encephalitis was most severe in dog no. 4. In all 3 dogs, lesions were more severe in the cerebrum than in the midbrain. Intact, degenerating, and ruptured tissue cysts were seen associated with lesions. Tissue cysts and bradyzoites had ultrastructural features of *N. caninum* and they stained positively with BAG-1 antibodies to *T. gondii* (Dubey *et al*. 2004). A few intact tissue cysts were present in neural tissue without any inflammation.

The degree of myositis varied in 3 dogs. The myositis in gastrocnemius was characterized by interstitial fibrosis, fragmentation and basophilia of myocytes and mononuclear cell infiltration. In dog no. 2 there was non-suppurative myositis involving the gastrocnemius but protozoa were not seen. Tissue cysts without any inflammation were seen in the masseter muscle of dog no. 2. There was also severe esophagitis in dog no. 2 and tissue cysts were seen in myocytes. In dog no. 3 myositis was confined to gastrocnemius but protozoa were not seen. In dog no. 4 the myositis involved the diaphragm, ab-

dominal muscles, quadriceps, and gastrocnemius; tissue cysts were seen in all of these muscles but not in gastrocnemius. Protozoa in muscles stained positively with *N. caninum* polyclonal antibodies and BAG-1 antibodies, indicating they were bradyzoites (Dubey *et al*. 2004); only a few tissue cysts were present and we were unable to locate them in thin sections for transmission electron microscopy. These intramuscular tissue cysts had thin (<0.5 µm thick) cyst walls.

Neospora caninum was isolated by bioassays in mice from all 3 dogs (Dubey *et al*. 2004).

Discussion

In the present study, lesions were confined to the brain and skeletal muscle and tachyzoites were not found. The absence of lesions and parasites in the spinal cord was surprising, contrary to previous reports (Dubey and Lindsay 1996). One of the 3 littermates in the present study (dog no. 2) developed megaesophagus. Megaesophagus has been reported in 2 of 27 cases of clinical neosporosis (Barber and Trees 1996). In the present study the diagnosis was confirmed at necropsy and *N. caninum* was demonstrated histologically in the esophageal musculature.

Treatment with clindamycin has been reported to improve clinical recovery in naturally infected dogs with neurological signs (Barber and Trees 1996). However, rarely has there been an opportunity to verify parasiticidal effects in animals naturally infected with *N. caninum* (Dubey *et al*. 1995, 1998). In the present study, dogs had been treated with clindamycin for approximately 2 months and they had *N. caninum* tissue cysts but no demonstrable tachyzoites. Clindamycin affects multiplication of *N. caninum* tachyzoites but is thought to have little or no effect on bradyzoites (Lindsay *et al*. 1994).

Diagnosis of neosporosis during life is difficult. In the present case, the age of dogs (nursing), simultaneous infection in littermates, and limb paralysis aroused suspicion of *N. caninum* infection. Finding of antibodies to *N. caninum* and not *T. gondii* helped diagnosis. Antibodies were detected by all 3 serological tests used (NAT, IFAT, ELISA). All 3 clinically affected dogs had IFAT and NAT titers of 1:400 or higher which were much higher than a cut-off value of 1:25. In previous reports low antibody titers (IFAT 1:50) were found in dogs that died of confirmed neosporosis (Dubey *et al*. 1988b, 1998). Therefore, even low titers in young dogs with paresis should be taken into consideration for diagnosis. The cut-off values for IFAT and NAT for the diagnosis of *N. caninum* in dogs have not been determined.

In the present study, only 3 of 11 littermate dogs had evidence of *N. caninum* infections. In most cases of neonatal neosporosis, clinical signs are not apparent until 5–7 weeks after birth. These data suggest that *N. caninum* is transmitted from the dam to the neonates towards terminal stages of gestation or postnatally via milk. As yet, there is no evidence of in utero transmission of *N. caninum* infection in naturally-infected dogs but the transplacental transmission has been demonstrated in experimentally infected dogs (Dubey and Lindsay 1989, Cole *et al*. 1995).

Neospora caninum is one of the most efficiently transplacentally transmitted parasites in cattle (Dubey 2003b). However, this may not apply to non-bovine hosts of *N. caninum*. According to Barber and Trees (1998) vertical transmission of *N. caninum* in dogs is highly variable and not likely to persist in the absence of horizontal infection. Results of the present study support this conclusion. In the present study at least 3 of 11 pups of the first litter had clinical neosporosis and in the second litter only 1 of 7 pups was infected. This investigation had to be discontinued because the dam was run over by a car and the seropositive female pup was hysterectomyzed. It is noteworthy NAT titers remained stable for several months in the neonatally-infected pup no. 12; its 6 littermates were seronegative at 1:20 serum dilution. Because all 7 pups were nursed by the same dam and the dam was seropositive it is assumed that pups would have acquired *N. caninum* antibodies from colostrum and that these antibodies had disappeared by day 32 in six pups. However, we cannot prove this point because the pups were not bled before day 32. These findings are important when making a decision when breeding chronically *N. caninum* infected dogs.

References

Barber J.S., Trees A.J. 1996. Clinical aspects of 27 cases of neosporosis in dogs. *Veterinary Record*, 139, 439–443.

Barber J.S., Trees A.J. 1998. Naturally occurring vertical transmission of *Neospora caninum* in dogs. *International Journal for Parasitology*, 28, 57–64.

Baszler T.V., Adams S., Vander-Schalie J., Mathison B.A., Kostovic M. 2001. Validation of a commercially available monoclonal antibody-based competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to *Neospora caninum* in cattle. *Journal of Clinical Microbiology*, 39, 3851–3857.

Baszler T.V., Knowles D.P., Dubey J.P., Gay J.M., Mathison B.A., McElwain T.F. 1996. Serological diagnosis of bovine neosporosis by *Neospora caninum* monoclonal antibody-based competitive inhibition enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology*, 34, 1423–1428.

Bjerkås I., Mohn S.F., Presthus J. 1984. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. *Zeitschrift für Parasitenkunde*, 70, 271–274.

Cole R.A., Lindsay D.S., Blagburn B.L., Sorjonen D.C., Dubey J.P. 1995. Vertical transmission of *Neospora caninum* in dogs. *Journal of Parasitology*, 81, 208–211.

Dubey J.P. 2003a. Review of *Neospora caninum* and neosporosis in animals. *Korean Journal of Parasitology*, 41, 1–16.

Dubey J.P. 2003b. Neosporosis in cattle. *Journal of Parasitology*, 89, Suppl., S42–S56.

Dubey J.P., Carpenter J.L., Speer C.A., Topper M.J., Uggla A. 1988a. Newly recognized fatal protozoan disease of dogs. *Journal of the American Veterinary Medical Association*, 192, 1269–1285.

Dubey J.P., Desmonts G. 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Veterinary Journal*, 19, 337–339.

Dubey J.P., Dorough K.R., Jenkins M.C., Liddell S., Speer C.A., Kwok O.C.H., Shen S.K. 1998. Canine neosporosis: clinical

signs, diagnosis, treatment and isolation of *Neospora caninum* in mice and cell culture. *International Journal for Parasitology*, 28, 1293–1304.

Dubey J.P., Hattel A.L., Lindsay D.S., Topper M.J. 1988b. Neonatal *Neospora caninum* infection in dogs: Isolation of the causative agent and experimental transmission. *Journal of the American Veterinary Medical Association*, 193, 1259–1263.

Dubey J.P., Lindsay D.S. 1989. Transplacental *Neospora caninum* infection in dogs. *American Journal of Veterinary Research*, 50, 1578–1579.

Dubey J.P., Lindsay D.S. 1996. A review of *Neospora caninum* and neosporosis. *Veterinary Parasitology*, 67, 1–59.

Dubey J.P., Metzger Jr. F.L., Hattel A.L., Lindsay D.S., Fritz D.L. 1995. Canine cutaneous neosporosis: clinical improvement with clindamycin. *Veterinary Dermatology*, 6, 37–43.

Dubey J.P., Sreekumar C., Knickman E., Miska K.B., Vianna M.C.B., Kwok O.C.H., Hill D.E., Jenkins M.C., Lindsay D.S., Greene C.E. 2004. Biologic, morphologic, and molecular characterisation of *Neospora caninum* isolates from littermate dogs. *International Journal for Parasitology*, 34, 1157–1167.

Lindsay D.S., Dubey J.P. 2000. Canine neosporosis. *Journal of Veterinary Parasitology*, 14, 1–11.

Lindsay D.S., Rippey N.S., Cole R.A., Parsons L.C., Dubey J.P., Tidwell R.R., Blagburn B.L. 1994. Examination of the activities of 43 chemotherapeutic agents against *Neospora caninum* tachyzoites in cultured cells. *American Journal of Veterinary Research*, 55, 976–981.

Romand S., Thulliez P., Dubey J.P. 1998. Direct agglutination test for serologic diagnosis of *Neospora caninum* infection. *Parasitology Research*, 84, 50–53.

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