Disseminated *Neospora caninum* infection in a dog with severe colitis

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Abstract. A 12-y-old spayed female Schipperke dog with a previous diagnosis of inflammatory bowel disease was presented with a 2-mo history of severe colitis. The patient's condition progressed to hepatopathy, pneumonia, and dermatitis following management with prednisolone and dexamethasone sodium phosphate. Colonic biopsies identified severe necrosuppurative colitis with free and intracellular parasitic zoites. Postmortem examination confirmed extensive chronic-active ulcerative colitis, severe acute necrotizing hepatitis and splenitis, interstitial pneumonia, ulcerative dermatitis, myelitis (bone marrow), and mild meningoencephalitis with variable numbers of intracellular and extracellular protozoal zoites. PCR on samples of fresh colon was positive for *Neospora caninum*. Immunohistochemistry identified *N. caninum* tachyzoites in sections of colon, and a single tissue cyst in sections of brain. Administration of immunosuppressive drugs may have allowed systemic dissemination of *Neospora* from the intestinal tract.

Key words: canine; colitis; *Neospora caninum*; neosporosis.

Neospora caninum is an apicomplexan protozoan parasite with a heteroxenous life cycle and worldwide distribution. Canids, including domestic dogs, coyotes, dingoes, and wolves, can be both intermediate and definitive hosts.²⁵ The parasite completes sexual reproduction in the intestine of canids, and infected definitive hosts then may contaminate the environment, including food and water, by shedding unsporulated oocysts in the feces.^{8,9,14,16,23} The organism sporulates in the environment and will eventually form tissue cysts when ingested by an intermediate host. Cattle and other ruminants are the most commonly affected intermediate hosts of domestic veterinary importance, with primary clinical presentations of infertility, abortion, and neonatal mortality.^{3,8,24} Horses and many wildlife species, including rodents, lagomorphs, and birds, have been reported to also be intermediate hosts.^{6,9,25} Ingestion of tissue cysts by the canid host (e.g., from infected aborted material or raw meat of an infected intermediate host) completes the life cycle.⁷ Clinical signs in canids acting as definitive hosts are uncommon. When immunocompetent canids become an intermediate host through ingestion of sporulated oocysts, the organisms eventually form bradyzoite-filled cysts within the central nervous system (CNS) and muscles and can lie dormant with no impact for the life of the host.¹¹

Clinical neosporosis is occasionally observed in immunologically naïve puppies. Neurologic and muscular manifestations are most common. Puppies most consistently become infected postnatally after consumption of infected tissues, but can also become infected by vertical transmission via transplacental migration of tachyzoites from the dam to the

fetus, or potentially by ingestion of contaminated feces.¹⁰ Within the first 6 mo of life, affected pups may experience ascending, progressive paralysis with rigidity and muscle atrophy, mostly of the pelvic limbs, as a result of necrotizing-to-granulomatous encephalitis, myelitis, and myositis.^{2,13,20} In one case, a naturally infected puppy had fibrinohemorrhagic jejunitis and ileitis in an intestinal epitheliotropic form of neosporosis, but colitis was not observed.¹⁹

Clinical signs can also be seen in immunosuppressed adult dogs acting as intermediate hosts. Dogs with quiescent bradyzoite cysts may experience recrudescence that results in multifocal CNS signs and polymyositis. Factors associated with recrudescence are poorly understood but generally involve immunosuppression.²⁵ Cases of myocarditis, pneumonia, dermatitis, and systemic dissemination are uncommon,¹⁶ and have been associated with immunosuppressive therapy, including steroid or chemotherapeutic administration.^{18,20,22} To our knowledge, colitis has not been reported as a significant primary lesion in adult dogs with neosporosis.

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A 12-y-old spayed female Schipperke dog was presented to the Colorado State University Veterinary Teaching Hospital (CSU-VTH; Fort Collins, CO) for evaluation of intractable and progressive large bowel diarrhea of 2-mo duration. The patient had been diagnosed with inflammatory bowel disease 3 y prior via endoscopic biopsies, and the disease had been adequately controlled with budesonide and a low-fat diet. Weeks prior to the onset of her clinical signs, the patient had attended a livestock show. She also had access to areas frequented by wildlife. She had a chronic history of coprophagia, primarily canine feces.

On initial presentation to the CSU-VTH (day 0), the dog had frequent episodes of large bowel diarrhea. Temperature, pulse, and respirations were within normal limits. The dog was quiet, but alert and responsive, and had a mildly decreased body condition score of 4 of 9. The patient had slightly tacky mucous membranes and was moderately dehydrated. No other significant abnormalities were noted. *Giardia* ELISA (Ova and parasite, *Giardia* ELISA; Antech Diagnostics), parvovirus PCR (FastPanel canine GI PCR panel; Antech Diagnostics), fecal cultures (Fecal culture; Antech Diagnostics), and multiple fecal flotations (in-house modified double-centrifugal fecal flotation technique with Sheather sugar solution) were negative. Thoracic radiographs were normal.

The patient was hospitalized for work-up and treatment. Budesonide was discontinued and replaced by prednisolone (1 mg/kg/d PO q12h) and then intravenous dexamethasone sodium phosphate (0.2 mg/kg IV q24h) on day 1 with the goal of controlling a presumptive exacerbation of the patient's severe inflammatory bowel disease. Serial venous blood gas analysis (d 1–4) revealed persistent hyperlactatemia and eventual acidemia (see below). Within 12–24 h (d 2) of starting dexamethasone sodium phosphate, the patient developed pyrexia (39.4°C [102.9°F]) and hyporexia progressing to anorexia. Multifocal erythematous skin lesions were found upon clipping the coat. Given the pyrexia, enrofloxacin (10 mg/kg IV q24h) and metronidazole (10 mg/kg IV q12h) were initiated to treat a presumptive bacterial etiology. Dexamethasone sodium phosphate was tapered to 0.05 mg/kg IV q12h. Abdominal ultrasound was performed (d 2), and notable findings included markedly thickened small and large intestines and hepatomegaly with multiple hypoechoic nodules, the largest measuring 7 mm diameter.

On d 3, the patient was lethargic, inappetent, and persistently pyrexia. Metronidazole was discontinued, and ampicillin–sulbactam (25 mg/kg IV q8h) was initiated. Dextrose supplementation was started because of hypoglycemia (4.11 mmol/L [74 mg/dL]). Maropitant (1 mg/kg IV q24h) was continued for anti-emesis, and pantoprazole (1 mg/kg IV q12h) was added. A complete blood count (d 3) revealed a mixed inflammatory and stress leukogram characterized by a normal leukocyte count ($11 \times 10^9/L$, RI: $4.5\text{--}15.0 \times 10^9/L$), a normal neutrophil count ($10 \times 10^9/L$, RI: $2.6\text{--}11 \times 10^9/L$) with a significant left shift (band neutrophils $1.2 \times 10^9/L$, RI:

$0\text{--}0.2 \times 10^9/L$), and moderate lymphopenia ($0.5 \times 10^9/L$, RI: $1\text{--}4.8 \times 10^9/L$). Neutrophils were noted to have moderate toxic change. A biochemistry panel (d 4) revealed hypoalbuminemia (11.7 g/L, RI: 30–43 g/L), low normal globulin (19 g/L, RI: 15–32 g/L), and hypocholesterolemia (2.0 mmol/L, RI: 3.6–10.2 mmol/L [78 mg/dL, RI: 130–300 mg/dL]), suggestive of protein-losing enteropathy. Elevated activities of alkaline phosphatase (29.9 μ kat/L [1,788 U/L, RI: 15–140 U/L]), alanine aminotransferase (86.6 μ kat/L, RI: 0.17–1.5 μ kat/L [5,186 U/L, RI: 10–90 U/L]), aspartate aminotransferase (33.7 μ kat/L, RI: 0.25–0.75 μ kat/L [2,020 U/L, RI: 15–45 U/L]), and creatine kinase (159 μ kat/L, RI: 0.83–4.59 μ kat/L [9,530 U/L, RI: 50–275 U/L]) supported severe concurrent hepatocellular and muscular injury. Metabolic acidosis (bicarbonate 5.5 mmol/L, RI: 15–25 mmol/L [5.5 mEq/L, RI: 15–25 mEq/L]) with respiratory compensation (pCO₂ of 2.81 kPa [21.1 mm Hg]) was identified with acidemia (pH 7.16). A coagulation panel (d 4) revealed prolonged prothrombin time (36.2 s, RI: 7.4–9.4 s) and partial thromboplastin time (28.9 s, RI: 9.8–13.3 s), and elevated fibrin degradation products (>20 mg/L, RI: 0–4 mg/L) and d-dimers (14.6 nmol/L [2.67 μ g/mL, RI: 0.03–0.4 μ g/mL [RI: 0.16–2.19 nmol/L]) consistent with disseminated intravascular coagulation.

Repeat thoracic radiographs were performed given increased respiratory rate and effort, and showed a bronchointerstitial pattern. Blood cultures (in-house, collected in brain–heart infusion bottles, plated on Columbia agar plates, incubated at 37°C, 5% CO₂, and monitored for growth for 2 wk), serum *Pythium* titers (*Pythium insidiosum* ELISA; *Pythium* Serology Laboratory, Department of Pathobiology, College of Veterinary Medicine, Auburn University), *Toxoplasma* serology,²¹ and *Heterobilharzia* fecal PCR⁴ were negative.

Blind endoscopic colonic biopsies were acquired (d 4). Post-procedure, a nasoesophageal feeding tube was placed. Colonic biopsy results showed chronic-active necrosuppurative colitis with many intraepithelial, intraendothelial, intrahistiocytic, and free zoites. The patient became more tachypneic and then dyspneic. Re-check thoracic radiographs showed progression to an unstructured interstitial-to-alveolar pattern. The patient was placed in an oxygen cage. Acidemia worsened later in the day (d 4) to pH 6.98, and respiratory compensation ceased (pCO₂ 5.92 kPa [44.5 mm Hg]). A plasma transfusion was initiated to supplement clotting factors and provide oncotic support. Magnesium and bicarbonate supplementation were also initiated. Despite intensive care, the patient arrested on d 4.

At postmortem examination, the carcass was mildly and diffusely icteric, and there was erythema at sites of hair clipping with patchy areas of suffusive hemorrhage. The walls of the small and large intestines were 2–4 times normal thickness. The mucosal surface of the colon was severely ulcerated, with surface mats of fibrin (Fig. 1). The liver was soft, homogeneously pale-brown, and mildly enlarged. All liver

Figures 1–4. Systemic neosporosis in a domestic dog. **Figure 1.** Severe multifocal-to-coalescing necrosuppurative colitis. **Figure 2.** Large numbers of free and intracellular zoites are present within the smooth muscle of the colon. H&E. **Figure 3.** Both free and intracellular zoites in the colon show strong immunoreactivity. mAb 6g7, *N. caninum* immunohistochemistry. **Figure 4.** A single bradyzoite tissue cyst in a section of cerebrum, strong immunoreactivity. mAb 6g7, *N. caninum* immunohistochemistry.

lobes had disseminated pale-tan necrotic foci of 3–5 mm diameter. Similar foci were identified throughout the lungs, which were diffusely mottled pink and purple. The lungs were slightly firm, and sections floated just below the surface when placed in 10% neutral-buffered formalin. The spleen was mildly enlarged with a meaty consistency. Femoral bone marrow was diffusely red. There was a unilateral, regionally extensive, ulcerated, and crusting lesion on one pinna.

Representative tissues were fixed in 10% neutral-buffered formalin and processed routinely for histopathology. Sections of colon were severely ulcerated with large areas of necrosis and transmural histiocytic-to-suppurative inflammation, with tachyzoites free in the tissues and within mural leiomyocytes, macrophages, endothelial cells, and remaining epithelial cells (Fig. 2). Zoites were 2–3 μ m long by 1–2 μ m wide, crescent-shaped, with a central round basophilic

nucleus, and perinuclear clearing extending to both tapered ends. The colonic wall was markedly thickened by fibroplasia. Sections of liver had large random necrotic foci with tachyzoites both free in the tissue and within hepatocytes and macrophages. Within sections of lung, alveolar septa were multifocally necrotic with infiltrates of neutrophils, histiocytes, and rare tachyzoites. The skin of the ear was severely ulcerated, and the dermis was expanded by lymphocytes, plasma cells, and macrophages with large numbers of free, intrahistiocytic, and intraepithelial tachyzoites. Lower numbers of tachyzoites were present within the spleen and bone marrow with multifocal necrosis. Sections of tongue, heart, and urinary bladder had mild multifocal myocyte degeneration and mineralization, but no organisms were identified. The meninges were mildly expanded by low numbers of lymphocytes and plasma cells.

PCR on sections of colon was positive for *Neospora caninum*,²⁷ and negative for *Toxoplasma gondii*¹⁷ and *Leishmania* spp. (*Leishmania* PCR; Vector Borne Disease Diagnostic Laboratory, North Carolina State University). Formalin-fixed, paraffin-embedded sections of colon, skeletal muscle, heart, tongue, bladder, and brain were examined by immunohistochemistry (IHC) utilizing murine monoclonal antibody (*N. caninum* IHC antibody mAb 6g7).⁵ Organisms within the colon showed strong positive reactivity (Fig. 3), and a single tissue cyst was identified within the parenchyma of the cerebrum (Fig. 4). Organisms were not identified in the other tissues examined by IHC.

Systemic neosporosis is rare in dogs and is usually limited to naïve puppies or recrudescence in immunocompromised adult dogs acting as intermediate hosts. Affected dogs usually have signs attributed to inflammation in the CNS and/or skeletal muscles with rare multisystemic dissemination. Our case is a unique form of recrudescence adult canine neosporosis that began as primary colitic neosporosis with secondary dissemination. The dog's primary initial clinical sign was large bowel diarrhea of 2-mo duration, and the chronicity and severity of the colitis at postmortem examination suggests that this was the primary site of initial tropism by the organism; at autopsy, all other injured tissues had more acute inflammation. Previous inflammatory bowel disease and the chronic administration of budesonide may have predisposed to enteric infection by altering mucosal immune defenses.^{15,28} The switch from budesonide, which has local enteric immunosuppressive effects, to systemic glucocorticoids may have contributed to dissemination of the parasite, but it is possible that budesonide alone was sufficient. A case has been described of disseminated *Strongyloides* infection following budesonide monotherapy.¹⁵

Successful treatment of neosporosis may depend on early intervention with appropriate antimicrobials (usually a combination of clindamycin, sulfonamides, and pyrimethamine).¹ This requires early definitive diagnosis, which can be challenging, especially when an atypical presentation occurs. After ingesting tissue cysts, dogs may shed oocysts for a few days or weeks, but dogs that are presented with clinical signs typically are not shedding oocysts, and fecal flotations are negative, as in our case.²⁶ Elevations in titers via indirect immunofluorescence assays may help confirm the diagnosis, but measurements can vary greatly between laboratories, and there does not appear to be a correlation between titer level and severity of clinical disease.¹² Biopsy for histopathology combined with PCR or IHC may be the most useful technique for confirmation of clinical suspicion but requires more invasive tissue sampling and may require days to process.¹² By the time biopsy results were available in our case, the patient was decompensating.

N. caninum infection should be a differential diagnosis for primary large bowel diarrhea in the presence of severe

necroulcerative and histiocytic colitis in immunosuppressed dogs. This novel presentation is important to consider, given that diagnosis is difficult, and prognosis may be guarded because of the potential for widespread dissemination and death. The original infection route for our patient is unknown. Possibilities include: 1) activation of a latent infection secondary to ingestion of infected raw meat or placental tissues,¹⁰ 2) activation of a latent infection secondary to transplacental infection,¹⁰ or 3) ingestion of sporulated oocysts by coprophagia of canid feces or contaminated food or water.^{1,10} Although the risk of adult-onset canine neosporosis is low, this risk can be minimized by preventing dogs from contacting oocysts in potentially contaminated environments and by preventing coprophagia.^{1,10} Neosporosis is one of several reasons not to feed dogs raw or undercooked meat of intermediate host species, which could be contaminated with tissue cysts. Ingestion of placentas from intermediate hosts should also be prevented;^{10,24} although this route of exposure results in definitive host status and not intermediate host status, it results in transmission of the organism to cattle and other susceptible intermediate hosts and contributes to environmental contamination.¹⁰

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