Case Report  Rapport de cas

Treatment of canine pediatric Neospora caninum myositis following immunohistochemical identification of tachyzoites in muscle biopsies

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Abstract — A 7-week-old Irish wolfhound was evaluated for an abnormal hind limb gait. Quadriceps muscle atrophy was pronounced and patellar reflexes were absent bilaterally. *Neospora caninum* myositis was diagnosed by histopathologic and serologic examination and immunohistochemical staining of muscle. Substantial clinical improvement was noted after 18 weeks of treatment with clindamycin.

Résumé — Traitement de la myosite pédiatrique à *Neospora caninum* à la suite de l’identification immunohistochimique de trachyzoïtes dans des biopsies de muscles. Un Irish wolfhound âgé de 7 semaines a été évalué pour une démarche anormale au niveau des membres postérieurs. L’atrophie du quadriceps était prononcée et le réflexe patellaire était absent des 2 côtés. *Neospora caninum* a été diagnostiqué par examen histopathologique et sérologique et par coloration immunohistochimique des muscles. Une amélioration clinique marquée a été constatée après 18 semaines de traitement à la clindamycine.

A 7-week-old, female, Irish wolfhound was referred to the Western College of Veterinary Medicine (WCVM) for hind limb gait evaluation. An abnormal gait was first observed at 4 wk of age. The puppy had been treated as a “swimmer” by the referring clinic and placed in hobbles for 2 wk. Her condition had neither progressed nor improved. The other 7 puppies in the litter were normal.

Case description

Upon presentation, the puppy was bright, alert, and active, but with abnormal hind limb posture and gait. She stood with hind limbs excessively abducted and rotated outwards, and walked with both stifles flexed. Orthopedic examination was normal and no pain was detected on palpation of muscles, joints, bone, or vertebral column. A neurological evaluation revealed normal conscious proprioception and postural reactions. There was severe bilateral atrophy of the quadriceps muscles, bilateral absence of patellar reflexes, and a weak right cranial tibial nerve reflex. Funduscopic examination was normal.

A complete blood (cell) count (CBC) revealed a moderate regenerative anemia (hematocrit 0.230 L/L; reference range, 0.370 to 0.550 L/L, reticulocytes 9.4%) with microcytosis (mean cell volume 47.2 fL; reference range, 60.0 to 77.0 fL) and hypochromasia (mean cell hemoglobin concentration 305.0 g/L; reference range, 320 to 360 g/L). Biochemical profile abnormalities included decreased urea (0.7 mmol/L; reference range, 3.0 to 10.5 mmol/L) and albumin (28 g/L; reference range, 29 to 38 g/L), and increased creatine kinase (768 U/L; reference range, 0 to 300 U/L). Elevations in phosphorus (2.37 mmol/L; reference range, 0.82 to 1.87 mmol/L) and alkaline phosphatase (245 U/L; reference range, 12 to 106 U/L) were attributed to the age of the dog.

The puppy was anesthetized for thoracic, pelvic limb, and spinal radiographs, as well as abdominal ultrasonography and cerebrospinal fluid (CSF) collection. Radiographs of the thorax, pelvis, hips, hind limbs, and abdomen were interpreted as normal. Spinal radiographs indicated slight dorsoventral undulation through the lumbar region, thought to be a normal variation in skeletal maturity. Ultrasonographs revealed prominent and enlarged hepatic portal veins, suggesting possible portal hypertension. Cytologic evaluation and protein determination of CSF collected from the cerebellomedullary cistern were normal.

Liver function was evaluated. Pre- and postprandial bile acids were measured and an ammonia tolerance test was performed. The bile acids were normal (pre < 5 μmol/L; reference range, 0 to 10 μmol/L; post 15 μmol/L; reference range, 0 to 20 μmol/L), and the postprandial ammonia sample was only slightly increased (87 μmol/L; reference range, 0 to 80 μmol/L), suggesting that significant portosystemic shunting was unlikely.

The puppy was subsequently reanesthetized for a myelogram, electromyography, and muscle and nerve biopsies. The myelogram was normal. Results from electromyographic examination...
of the quadriceps, pectineus, gracilis, and adductor muscles showed bilateral spontaneous depolarization, consistent with either myopathy or neuropathy. Results from electromyographic examination of the semimembranosus, biceps femoris, triceps, and temporalis muscles were normal. Biopsies were taken from the right quadriceps, pectineus, and biceps femoris muscles, and a branch of the femoral nerve.

Histologically, all muscle biopsies contained multiple focal infiltrates of lymphocytes, plasma cells, and macrophages. The normal mosaic pattern of muscle fiber types was present and intramuscular nerve branches were normal in appearance. No abnormalities of myelin or axons were observed within the femoral nerve biopsy, but there was increased cellularity within the supporting tissues. The histological findings supported a diagnosis of multifocal lymphohistiocytic myositis. No organisms were observed within myofibers; however, given the age of onset and clinical signs, an infectious cause (such as *Toxoplasma gondii* or *Neospora*) was considered most likely and prompted treatment for protozoal disease. Clindamycin (Antirobe; Pharmacia Animal Health, Orangeville, Ontario) 12 mg/kg bodyweight (BW), PO, q8h, was administered. Physiotherapy was also performed to help prevent muscle tie-down or contracture of the muscles in the hind limbs.

Serum and CSF samples were negative for *Toxoplasma gondii* antibodies by hemagglutination testing, using a commercially available kit (Wampole Laboratories; Princeton, New Jersey, USA). Indirect immunoperoxidase testing was performed to measure antibodies against *Neospora* spp. in the puppy’s serum. Briefly, serial dilutions of test and control serums were applied to multi-chambered glass slides coated with *Neospora* tachyzoites (VRMD; Pullman, Washington, USA). The slides were then incubated for 30 min in a humid chamber, rinsed, and then had antisera specific to either canine IgG or IgM conjugated to the enzyme horseradish peroxidase (Cappel Research Organon Teknika, Durham, North Carolina, USA) applied to each well. The slides were then incubated in a humid chamber, rinsed, and had a peroxidase substrate, di-aminobenzadine (DAB), applied to each well, followed by 5 min incubation. Light microscopy was used to determine the last dilution of the patient’s serum that stained the *Neospora* tachyzoites. There was no evidence of serum IgM antibodies; however, the titer of IgG antibodies specifically binding *Neospora* was high (≥ 1/800), suggesting an active infection with a *Neospora* sp. Formalin fixed biopsies of quadriceps muscle were tested immunohistochemically for *Neospora* sp. or *Toxoplasma* sp. antigen by using an avidin biotin complex immunoperoxidase technique with rabbit polyclonal antisera (1). The antisera to *Toxoplasma gondii* (Biogenex Laboratories, San Ramon, California, USA) was used on serial sections of tissues at dilutions of 1/1600 and 1/3200 and the antisera to *Neospora* (obtained from Dr. D. Lindsay, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA) was used at dilutions of 1/10000 and 1/20000. The specificity of these antisera in this assay has been described previously (2,3). The results were negative for *T. gondii*, but positive for a *Neospora* sp. In several areas within the lesional muscle there was staining of small clusters of organisms morphologically consistent with *Neospora* spp.

Two of the asymptomatic littermate pups and the dam were tested for serum antibodies to *Neospora* spp. The bitch was seropositive (> 1:50), although she had not shown any clinical signs of neosporosis. One puppy was seronegative and the other had a titer of 1:200. The 7 littermate puppies and the bitch were not treated and did not subsequently develop clinical signs of neosporosis.

Clindamycin treatment along with physiotherapy and massage of the clinically affected puppy was continued for 18 wk. Following 4 wk of treatment, the puppy’s gait was much improved. Her quadriceps muscles were stronger and she was able to take individual steps. One year later, the owners reported that her gait was 90% normal; the only residual clinical sign was occasional scuffing of the toenails on her left hind limb, and a slightly stiff appearance to her hind limbs when running. The dog had no other medical problems attributable to the *Neospora* sp. infection. She was euthanized at 9 y of age for unrelated causes.

**Discussion**

*Neospora caninum* is a protozoan parasite that causes meningoencephalitis, myositis, and polyradiculoneuritis in dogs throughout the world, as well as being an important cause of abortion in cattle (4,5). Domestic dogs and coyotes are the only documented definitive hosts, shedding unsporulated oocysts in the feces following ingestion of bradyzoites (4,6). Naturally occurring intermediate hosts include dogs, cattle, sheep, goats, horses, and deer (4). Since bradyzoites are found within tissue cysts in the central nervous system (CNS) and the skeletal muscle of intermediate hosts, transmission can occur through ingestion of infected tissues. Transplacental infection is believed to be the most common route of transmission in dogs, including occasional reports of transplacental transmission to successive litters by an asymptomatic bitch (4,5,7).

Bradyzoites within tissue cysts in infected dogs normally remain quiescent when host immunity is adequate. Lesions develop when tachyzoites multiply rapidly within cells, causing rupture (8). The puppy described in this report was most likely infected transplacentally, based on her age and the presence of a positive *N. caninum* antibody titer in the dam. It is unknown why this particular puppy developed clinical neosporosis while its littermates remained normal. The source of infection for this puppy’s dam is uncertain, as she was fed a commercial dog food and was not in contact with animal carcasses.

Adult dogs with neosporosis can develop a variety of neurologic signs, depending on the site of inflammation within the nervous system. Multi-focal CNS involvement is most common with seizures, head tilt, ataxia, paresis, and paralysis (4,5,7,9). Myositis, myocarditis, hepatitis, dermatitis, ocular lesions, interstitial pneumonia, and pancreatitis have also been reported (10,11).

Most cases of clinical neosporosis are reported in dogs aged < 6 mo, where the infection can be more focal (4,5,10–13). Affected puppies initially have inflammation limited to muscles and nerve roots of the hind limbs. Typical signs of early pediatric neosporosis include progressive hind limb paresis, muscle atrophy, and loss of patellar reflexes. Over time, lower motor
neuron damage and quadriceps muscle atrophy and fibrosis will lead to rigid hyperextension of the hind limbs. The infection occasionally becomes more generalized in puppies, with meningo-encephalomyelitis leading to multi-focal CNS signs or multiple organ involvement with systemic signs or myocarditis (4, 5, 7, 9–11, 14).

Clinical and neurologic findings may suggest infection with *N. caninum* in a puppy; however, further testing is required to confirm this diagnosis. Serum creatine kinase may occasionally be elevated due to muscle necrosis (4, 10). Serologic testing for canine neosporosis is useful; however, many seropositive dogs remain clinically normal, and some clinically affected dogs have negative titers. A seroepidemiologic study reported that 50% of puppies born to seropositive bitches were seropositive; however, only 25% of puppies born to seropositive bitches developed neospora-like clinical signs (7). Further, maternal antibodies may be passed from a dam to offspring, causing false-positive serologic results; but by 32 d of age, antibodies are not detected in uninfected pups (4). Seroprevalence in apparently healthy adult dogs varies with geographic location, ingestion of raw beef, and roaming lifestyle (4). Indirect fluorescent-antibody testing (IFAT) is the criterion-referenced standard for measuring serum antibodies against *Neospora caninum*, but enzyme-linked immunosorbent assays (ELISA) have also been used (4). In the present report, a modification of the IFAT method was used to detect *Neospora* antibodies. A multiplex polymerase chain reaction assay has recently been developed for detection of *N. caninum* DNA in canine biological samples, but false negative results are reported when organisms encyst and embed deep within CNS tissues (4). Cerebrospinal fluid from dogs with neospora meningoencephalomyelitis typically will be mildly inflammatory and have low levels of CSF antibodies against *N. caninum*. Cytologic detection of *N. caninum* tachyzoites in CSF cytologic smears is, however, uncommon (5). Definitive diagnosis of neosporosis requires identification of organisms in a muscle or CNS biopsy, using immunohistochemical (IHC) staining.

There are few reports of antemortem diagnosis and successful treatment of adult and pediatric neosporosis (10, 14, 15). Current treatment protocols are largely extrapolated from recommendations for treatment of canine toxoplasmosis. Clindamycin, sulfadiazine, and pyrimethamine, alone or in combination, have been recommended (4, 10, 13, 14). Corticosteroids have been shown to worsen clinical disease in dogs with neosporosis, so they should not be administered (8, 13).

The prognosis for recovery in puppies with *N. caninum* myositis depends on the chronicity and severity of the clinical signs. The prognosis improves if therapy is started early. The development of rigid hyperextension of the hind limb is associated with a poor prognosis, as it generally indicates irreversible muscle damage and fibrosis (10). To reduce the chance of illness, some have recommended that all puppies in a litter should be treated as soon as the diagnosis has been made in 1 puppy (4, 14).

This case illustrates the potential for substantial clinical improvement and long-term remission following early diagnosis and treatment of pediatric *N. caninum* infection in puppies. (cv)

**References**