An 8-year-old Kentucky Mountain Saddle gelding was examined for dysuria. The horse was clinically normal when purchased 7 months before examination, and at this time was dewormed and vaccinated for influenza, rhinopneumonitis, eastern and western encephalomyelitis, West Nile encephalomyelitis, tetanus, and rabies. Tail rubbing and dysuria were noted 1 month before presentation. Upon palpation of the epaxial and gluteal muscles, the horse trembled repeatedly and moved away from the examiner. Three weeks later the horse developed marked hyperesthesia of the tail, dropped the penis often, and dribbled urine without posturing. The horse was treated for cystitis with trimethoprim and sulfadiazine PO for 3 days with no beneficial response. Because of concerns of urine voiding, the urinary bladder was catheterized and emptied before referral.

Physical examination did not reveal abnormalities. Neurologic abnormalities included subtly decreased tongue tone, markedly decreased to absent tail, anal, and perineal tone, hypoesthesia to anesthesia of the tail, anal sphincter and perineal region, penile paresis, urinary incontinence, anesthesia and hypotonicity over the left gluteal and semimembranosus muscles, and hyperesthesia over the right semimembranosus muscle to tuber ischium. The horse also had apparent left-sided atrophy of the gluteal and semimembranosus muscles, and hyperesthesia and anhidrosis over the left sphincter and perineal region, penile paresis, urinary incontinence, anhidrosis and hypotonicity over the left gluteal and semimembranosus muscles and hyperesthesia over the right semimembranosus muscle to tuber ischium. 

Results of lumbosacral cerebrospinal fluid (CSF) revealed moderate mixed (primarily lymphocytic) pleocytosis of 20 total nucleated cells/μL (reference interval 1–5/μL) with 19% nondegenerate neutrophils, 72% lymphocytes, and 9% monocyte/macrophages. There was marked lymphoid reactivity with a few granular lymphocytes admixed with many small lymphocytes, reactive lymphocytes, and plasma cells. Macrophages were mildly vacuolated and rarely erythrophagia was noted. A few mitotic figures were seen. The CSF protein concentration (56 mg/dL) was within reference values (<80 mg/dL). From the CSF immunofluorescent antibody tests for *Sarcocystis neurona* and *Neospora hughesi*, and m-capture ELISA for antibodies to West Nile virus were negative. Polymerase chain reaction (PCR) assays for respiratory pathogens influenza A, herpes virus 1 and 4, equine arteritis virus, *S. neurona* and *N. hughesi*, and PCR assays for equine herpes virus 1 and West Nile virus were negative. There was no growth on culture media for bacteria and fungi.

A transrectal ultrasound examination with an 8.5 MHz curvilinear microconvex transducer revealed enlargement and diffusely mottled, hypoechoic appearance of the extradural sacral nerve roots as they exited the ventral sacral foramina and also iliac lymphadenopathy (Fig 1).

From the Department of Medicine and Epidemiology (Aleman), The William R. Pritchard Veterinary Medical Teaching Hospital (Katzman), Department of Surgical and Radiological Sciences (Vaughan), Department of Pathology, Microbiology and Immunology, the School of Veterinary Medicine; University of California, Davis, Davis, CA (Hodges, Crabbs, Christopher, Higgins), Department of Pathology, University of California, San Diego, CA (Shelton).

Corresponding author: Monica Aleman, MVZ, PhD, Dipl. ACVIM; Department of Medicine and Epidemiology, Tupper Hall 2108, One Shields Avenue, University of California, Davis, CA 95616; e-mail: mraleman@ucdavis.edu.

Submitted October 9, 2008; Revised December 15, 2008; Accepted January 16, 2009.

Copyright © 2009 by the American College of Veterinary Internal Medicine

10.1111/j.1939-1676.2009.0285.x
lar nerve branches within this muscle. Specifically, the sacrocaudalis dorsalis lateralis muscle was selected based on ease of collection with minimal adverse residual effects. After collection, biopsy tissue was flash-frozen in isopentane precooled in liquid-nitrogen, and stored at −80 °C or immersion fixed in 10% formalin until further processing for histological, histochemical, and immunohistochemical evaluation. In frozen muscle biopsy sections, terminal intramuscular nerve branches contained intense lymphocytic and histiocytic cell infiltration that almost obliterated the nerve architecture with sparing of myofibers (Fig 2A). Excessive epi- and perineurial fibrosis was confirmed with the modified Gomori trichrome stain (Fig 2B). A predominant infiltration of macrophages was evident with acid phosphatase stain and confirmed with immunophenotypic identification of CD11a⁺ and c⁺ ([1 : 10 dilution], Gift from Dr Peter F. Moore, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, Davis, CA) immunoreactive macrophage populations (not shown). Immunophenotyping of cellular infiltrates in frozen sections identified immunoreactive CD8⁺ ([1 : 10 dilution], Gift from Dr Jeffrey Stott, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, Davis, CA) cytotoxic T lymphocytes (CTL, Fig 2C) and rare CD4⁺ helper T-lymphocytes ([1 : 10 dilution], Gift from Dr Peter F. Moore). There were also admixed some populations of immunoreactive CD20⁺ plasma cells and fewer CD3⁺ T lymphocytes ([1 : 10 dilution], Gift from Dr Peter F. Moore). Supportive of nerve fiber damage, a pattern of neurogenic atrophy was identified in the frozen muscle biopsy sections as shown by angular atrophy involving both muscle fiber types, and some hypertrophic or split fibers (Fig 2D). Thus the provisional diagnosis of polyneuritis equi was made based on clinical, ultrasound, CSF analysis, and histological demonstration of extensive cellular infiltrations in intramuscular nerve branches.

The horse was euthanized and necropsied 7 days after admission because of financial constraints, progression of neurologic deficits, and guarded to poor prognosis. On macroscopic examination, most intra- and extradural spinal nerves of the cauda equina that exited the intervertebral foramen caudal to S1 were thickened, firm, and diffusely white (Fig 3). Sacral lymph nodes were bilaterally enlarged. The bladder wall was markedly distended and red containing abundant thick, pasty and gritty brown yellow material consistent with sabulous cystitis and likely secondary to bladder atony. There were no obvious macroscopic abnormalities in the remaining nervous system. Bacterial and fungal cultures, acid fast stain, and immunohistochemical reactivity to West Nile virus, equine herpes virus 1, Neospora spp., and S. falcata and S. neurona antigens on the affected tissue were all negative.

Microscopic lesions consisted of severe necrotizing pyogranulomatous inflammatory cell infiltrate that was centered in, and frequently effaced, individual nerve fascicles of both intradural and extradural spinal nerves of the cauda equina (Fig 4A). There was profound epi- and perineurial fibrosis. A continuum of lesions occurred with early lesions in fascicles characterized by a predominantly lymphocytic infiltrate that was eventually replaced with necrosis and abundant neutrophils and macrophages as the lesion progressed (Fig 4B). Multinucleated synecytial cells were common. There was also an intense dorsal root ganglioneuritis. In spinal nerves distal to their gross involvement, moderate nerve fiber loss and regenerating axonal clusters indicated both degeneration and attempts at regeneration (not shown). Immunohistochemical findings in frozen cauda equina included profound infiltration with macrophages, moderate to marked infiltration with CTL, mild infiltration of B-lymphocytes, and rare helper T-lymphocytes in perineurial and endoneurial regions (not shown). There was no microscopic evidence of any cranial or other spinal nerve involvement.

PNE is an uncommon neurologic disease of undetermined cause characterized by progressive, insidious granulomatous inflammation of peripheral nerves.2–14
Results of CSF cytological analysis in PNE are variable and nonspecific ranging from mild mixed mononuclear to primarily neutrophilic pleocytosis, with normal to increased protein concentrations.5,15 The predominance of lymphocytes and marked lymphoid reactivity in the present case with reactive lymphocytes and plasma cells was supportive of a humoral response to inflammation such as that observed with viral encephalitis, chronic protozoal and rickettsial infections, and immune-mediated diseases.16 Because relevant infectious etiologies were ruled out in this horse, immune-mediated disease was considered the most likely explanation for the CSF findings. These cytologic findings correlated closely with the inflammatory infiltrates seen in intramuscular nerve branches in the initial muscle biopsy specimen and in the cauda equina at necropsy. In addition, ultrasonographic findings in affected extradural sacral nerve roots were supportive of a diagnosis of PNE. Although serum anti-P2 myelin antibodies have been found in some cases of PNE9,17; these antibodies lack specificity for PNE9,15 and were not investigated in this horse.

This report describes biopsy of a skeletal muscle innervated by nerves arising from the cauda equina with immunohistochemical abnormalities supportive of an antemortem diagnosis of PNE. This study further characterized T-lymphocytes subpopulations with predominant CD8+ CTL and rare CD4+ helper T lymphocytes, which has not been described previously. Immunochemistry of both frozen and formalin-fixed tissue demonstrated that various macrophage subpopulations and CTL were the predominant cell types in this case of PNE. Immunoreactive CD3+ T lymphocytes were individually scattered and fewer than the focal aggregates of CD20+ immunoreactive B-lymphocytes and plasma cells in formalin-fixed tissue. The presence of B-lymphocytes and plasma cells suggest a local production of antibodies. Early attempts at nerve regeneration were detected, suggesting that enhanced regeneration could be possible if the inflammatory response is controlled early in the course of the disease.

Fig 2. Sacrocaudalis dorsalis lateralis muscle biopsy. (A) Intramuscular nerve branches showed severe mixed inflammatory cell infiltration and fibrosis on hematoxylin and eosin. (B) Intramuscular nerve branch stained with modified Gomori trichrome showing fibrosis and absence of pink myelin staining. (C) Notice abundant red stain (chromogen: amino ethyl carbazole) uptake indicating positive immunohistochemical staining results for T-cytotoxic lymphocytes (CD8+) infiltrating intramuscular nerve branch. (D) A neurogenic pattern of myofiber atrophy was evident with the myofibrillar ATPase reaction at pH 9.8 (note angular atrophy of both fiber types with type 1 fibers light pink and type 2 dark brown [no stain used]).

Fig 3. Cauda equina collected postmortem from the horse with polyneuritis equi. Note thickened intra- and extradural spinal nerve roots. The spinal cord is not shown. CR, cranial, CD, caudal, scale bar = 1 cm.
To date a confirmed diagnosis of PNE has only been possible through postmortem evaluation of the cauda equina and other peripheral nerves. In this report, we demonstrate the value of the muscle biopsy and other ancillary testing to reach an antemortem diagnosis. The muscle biopsy allowed the identification of massive cellular infiltrations into intramuscular nerve branches, a finding that has not been previously reported in PNE. Such cellular infiltrations into terminal intramuscular nerve branches have been described in experimental models of autoimmune neuritis in mice.18 The presence of CD8+ T-cells in excess of CD4+ T-cells is consistent with underlying immune-mediated disease.18 The combination of a muscle biopsy with the neurological examination, CSF evaluation, ultrasonography should enable a definitive clinical diagnosis of PNE to be reached premortem and allow early intervention and treatment of the disease.

Acknowledgments

The authors thank Dr Bruce Daniels for the referral of this case, Ms Colette Williams and Mr John Doval for technical assistance, and Ms Diane Naydan for her immunocytochemical expertise.

Footnote

* Labvision (1:10 dilution), Freemont, CA

References