

A Novel Spongiform Leukoencephalomyelopathy in Border Terrier Puppies

P. Martin-Vaquero, R.C. da Costa, J.K. Simmons, G.L. Beamer, K.H. Jäderlund, and M.J. Oglesbee

Dog 1 was a 3-week-old male Border Terrier puppy referred to the Neurology and Neurosurgery Service of the Veterinary Medical Center, The Ohio State University, for ataxia and tremors. The signs were first noticed 5 days before presentation when the puppy first attempted to walk on its own. The owners reported that the tremors affected mainly the hindquarters. No improvement or worsening of the tremors had been noted over this period. An injectable corticosteroid (unknown type and dose) had been administered 4 days before referral, with no changes in the clinical signs. The tremors were reported to disappear when the puppy was sleeping. There were 3 other puppies from the same litter, which were reported to be normal, as were the parents. On presentation, the puppy showed severe generalized coarse body tremors (with low frequency and high amplitude), most severe in the hindquarters, which showed a characteristic swinging side-to-side or “rocking horse” movement (as illustrated in the supplementary Video S1). The tremors also involved the head and thoracic limbs, but to a lesser degree, and disappeared when the dog was asleep or at rest. Cerebellar ataxia was noted when the dog was trying to walk. The neurological examination was difficult because of the severity of the tremors, but it showed an absent menace bilaterally (considered normal for a 3-week-old puppy), delayed proprioceptive positioning, and hopping that was slightly hypermetric on all limbs. The remaining cranial nerves, spinal reflexes, cutaneous trunci reflex, and vertebral column palpation were unremarkable. Physical examination was within normal limits. Because the characteristics of the tremors, which were coarse and affected mainly the hindquarters, a diffuse or multifocal CNS disorder such as a congenital disorder of myelination

Abbreviations:

ASPA	aspartocylase
CBC	complete blood count
CNS	central nervous system
CSF	cerebrospinal fluid
GFAP	glial fibrillary acidic protein
MPS	mucopolysaccharides
NAA	N-acetyl aspartate
RBC	red blood cell
WBC	white blood cell

was considered the main differential diagnosis. A cerebellar lesion (infectious or inflammatory cerebellar disease, cerebellar hypoplasia, neonatal cerebellar ataxia) also was considered possible, although less likely, based on the type of tremors noted. A complete blood count (CBC) and biochemical profile showed changes considered normal for a puppy. The owners, who also were the breeders, chose humane euthanasia. A complete necropsy was performed. A cerebrospinal fluid (CSF) sample was collected from the cerebellomedullary cistern immediately after euthanasia. The fluid was colorless and clear, with a total protein of 55 mg/dL (reference range, <25 mg/dL), a white blood cell (WBC) count of 34 cells/ μ L (reference range, <5 cells/ μ L) and a red blood cell (RBC) count of 452 cell/ μ L (reference range, <5 cells/ μ L). On cytology, there were 91% large mononuclear cells, 8% lymphocytes and 1% nondegenerate neutrophils. Occasional ependymal cells were noted and considered an incidental finding. The findings were consistent with a mild-to-moderate nonsuppurative pleocytosis and mild blood contamination.

In addition, a urine sample was collected by cystocentesis immediately after euthanasia and sent to University of Pennsylvania for a metabolic screening panel. Results of the metabolic panel indicated increased aminoaciduria, increased lactate, increased hydroxy-lysine-glycoside, and a strongly positive response to mucopolysaccharides (MPS) spot test. The aminoaciduria was interpreted as an age-related finding. The increased lactate and strong positive MPS spot test were interpreted as suggestive of a defect in intermediary metabolism, possibly consistent with a metabolic disorder.

Three other Border Terrier puppies, dogs 2–4, had a similar history and presentation. They were presented to one of the authors (KHJ) at the Swedish University of Agricultural Sciences, Uppsala, Sweden (dogs 2 and 3) and the Norwegian School of Veterinary Science in Oslo, Norway (dog 4). These puppies were two 6-week-old female littermates and one 5-week-old

From the Department of Veterinary Clinical Sciences (Martin-Vaquero, da Costa) and the Department of Biosciences, College of Veterinary Medicine (Simmons, Beamer, Oglesbee), The Ohio State University, Columbus, OH; and the Department of Companion Animal Clinical Sciences, Norwegian School of Veterinary Science, Oslo, Norway (Jäderlund). This work was performed at the College of Veterinary Medicine, The Ohio State University, Columbus, OH. This article was presented in part as a Research Report at the 2011 American College of Veterinary Internal Medicine (ACVIM) Forum, Denver, Colorado.

Corresponding author: P. Martin-Vaquero, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp Street, Columbus, OH 43210; e-mail: martin-vaquero.1@osu.edu

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female dog. The clinical course had been slowly progressive since onset at 15–28 days of age in these puppies. Physical and neurologic examination findings were consistent with those described for dog 1. A CBC and a biochemical profile in dog 4 were considered normal for the age of the puppy. No diagnostic tests were pursued for dogs 2 and 3. Dogs 2, 3, and 4 were euthanized on the day of presentation and complete necropsies were performed. Littermates and parents of the puppies were reportedly normal.

Pedigrees were available for all affected puppies. A common ancestor, a male born in 1975, was found 6–8 generations back in both the paternal and maternal lines of all affected dogs (Fig 1).

At necropsy, no macroscopic abnormalities were identified in any puppy. Histologic evaluation of the CNS in dog 1 identified a spongiform change affecting the white matter with diminished myelin content and no microgliosis (Fig 2B). These changes were most severe within the cerebellum, brainstem, and spinal cord with mild changes in the thalamus and cerebral hemispheres. In the spinal cord, all white matter tracts were affected, with increased severity in the ventral funiculi. In the brainstem, the ventral white matter tracts also were most severely affected. Decreased myelin content was confirmed with Luxol fast blue staining, and the presence of intact axons was demonstrated with Bielschowsky staining (Fig 3B–C). A 4-week-old Gordon Setter was used as control (Figs 2A

and 3A). Compared with the control, there was an equivocal increase in GFAP-positive astroglial fibers (Fig 3D, control not shown). The diminished myelin staining together with the absence of microglial responses were interpreted as consistent with a spongy degeneration of the white matter or spongiform leukoencephalomyelopathy. The presence of intact axons and lack of microgliosis indicated a primary myelin defect in the absence of clinically relevant demyelination. Morphologically, oligodendrocytes appeared to have normal nuclei and soma. No clinically relevant microscopic lesions were noted in the skeletal muscle, nerves, and internal organs. Cerebrum, cerebellum, and brainstem also were histologically evaluated from dogs 2, 3 and 4. Dogs 2, 3, and 4 had lesions consisting of decreased myelin content and spongiform change in the cerebellum, similar to dog 1. In dogs 2 and 4, other lesions included moderate-to-severe spongiform degeneration and hypomyelination in the interpeduncular nucleus, brainstem, and cerebrum. These changes were most pronounced in the cerebellum, interpeduncular nucleus and brainstem, with only mild-to-moderate spongiform changes throughout the cerebral sections. In dog 4, severe spongiform changes were present in the white matter of the cranial spinal cord; more caudal segments were not investigated. Additional lesions in dog 3 were marked hypomyelination within the brainstem and mild scattered foci of hypomyelination in the cerebrum. Similar to dog 1, the brainstem lesions were most severe in the ventral white matter tracts. Spinal cord was not available for evaluation in dogs 2 and 3.

Primary disorders of central nervous system (CNS) myelin formation and/or maintenance include dysmyelinating diseases (leukodystrophies), hypomyelinating disorders, and spongiform (spongy) degenerations of myelin.^{1–4} In leukodystrophies, there is production of defective myelin that cannot be maintained.^{1–3} Hypomyelinating disorders are characterized by a decreased myelin production.^{1–3} Spongiform or spongy degenerations include diseases where there is vacuolation of myelin.^{3–5} Although infrequently seen, several forms of these myelin disorders have been described in various dog breeds.^{1,2,5–19}

We report a disorder of myelin affecting the CNS white matter consisting of a unique combination of spongy degeneration and decreased myelination in 4 Border Terrier puppies. To our knowledge, such a condition has not been previously reported in this breed. The combination of decreased myelin combined with spongiform white matter changes in the absence of microglial responses suggests a complex pathogenesis affecting both the capacity of oligodendrocytes to synthesize myelin and stability of the myelin that is formed. In congenital hypomyelinating diseases, oligodendrocytes are decreased in number or may be quantitatively normal, but functionally incompetent.¹ Spongy degeneration of white matter is a descriptive classification used when tissues exhibit decreased myelin staining in association with vacuolar changes.⁵ Because the molecular basis for the myelin disorder

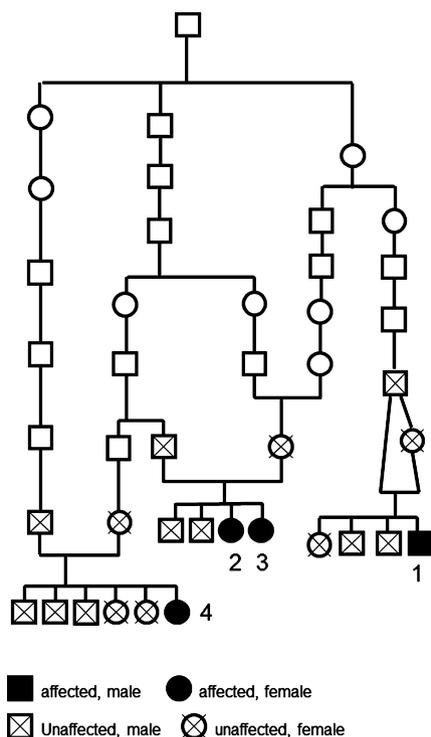


Fig 1. Pedigree of the relationship among 3 Border Terrier litters containing puppies diagnosed with a spongiform leukoencephalomyelopathy. The pedigree is simplified, showing only the shortest and straightest lineages leading from the dam and sire of the affected dogs back to a common ancestor.

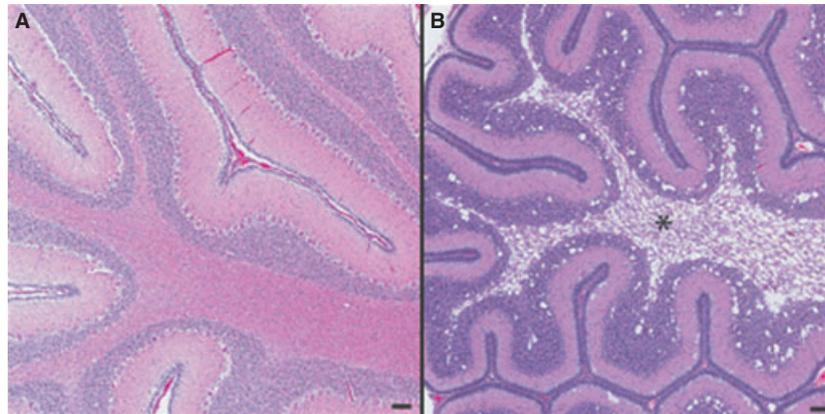


Fig 2. Photomicrographs of cerebellum from the age-matched Gordon Setter control dog (A; 3.7 \times) and dog 1 (B; 4 \times). The asterisk shows marked spongiform degeneration and hypomyelination within the cerebellar white matter tracts. The bar represents 100 microns (Hematoxylin and Eosin staining).

described here is currently unknown, the authors chose to use the term spongiform leukoencephalomyelopathy instead of spongy degeneration. This term currently is used in both human and veterinary literature when the cause of white matter myelin change cannot be determined.³⁻⁵

The current cases exhibited a decreased myelin staining that could represent disruption of normal structure or diminished formation (ie, hypomyelinogenesis). Congenital hypomyelinating diseases have been described in several canine breeds, including Springer Spaniels, Chow Chows, Samoyeds, Dalmatians, Lurchers, Weimaraners, and Bernese Mountain dogs.^{1,2,7-14} Wide variation exists in the distribution and severity of the myelin deficiency, as well as the characteristics of the glial changes noted among the different breeds.^{2,10} The majority of canine hypomyelinating diseases manifest as whole body tremors that are noticeable from birth or appear during the first few weeks of life.^{2,7-14} The characteristic features and age of onset of the body tremors noted in the Border Terriers are similar to those of previously described hypomyelinating diseases in dogs. However, the spongiform appearance of the Border Terrier leukoencephalomyelopathy differs from other hypomyelinating disorders. The presence of intact axons confirms that the diminished myelin formation is a primary process and not secondary to neuronal defects.

Congenital spongy degeneration of the CNS white matter has been described in Labrador Retrievers, Shetland Sheepdogs, Australian Cattle Dogs, a Samoyed puppy, and a Silky Terrier puppy.^{1,5,6,15-18} Congenital tremor with spongy degeneration of the CNS gray matter also has been described in Malinois crossbred puppies.¹⁹ The age at the onset of signs in Border Terriers (before 1 month of age in all puppies), as well as the type and distribution of spongiform lesions, is most similar to the Samoyed puppy previously described.¹⁵ Labrador Retrievers ranged from 4 to 6 months of age when signs were first noticed and these dogs suffered from intermittent episodes of extensor rigidity and opisthotonus as part of their clinical

presentation, which differ from the disorder described here.^{16,17} Unlike the Border Terrier spongiform leukoencephalomyelopathy reported here, the spongiform disorder of the Silky puppy presented with myoclonia of the paravertebral muscles and the spongiform changes did not involve the spinal cord.¹⁸ A spongiform leukoencephalomyelopathy of Australian Cattle Dogs and Shetland Sheepdogs also presented with diffuse vacuolation of the white matter of the brain and spinal cord, similar to that noted on Border Terriers.^{5,6} However, the Shetland Sheepdogs showed more severe spinal cord vacuolation in the dorsal funiculi, rather than the ventral funiculi as noted in the Border Terriers. Also, the Shetland Sheepdogs and Australian Cattle Dogs suffered from clinical signs, such as seizures or opisthotonus, which were not present in the Border Terriers described here.^{5,6}

In people, spongy degeneration of the white matter of the brain is the neuropathologic hallmark of the leukodystrophy known as Canavan's disease.^{5,20,21} This is an autosomal recessive disorder in which a mutation in the aspartocylase (ASPA) enzyme gene results in a deficiency of this enzyme, and causes an increase in its substrate N-acetyl aspartate (NAA) and a decrease in acetate, which is a product of NAA breakdown. Acetate is considered necessary for myelin synthesis, and this deficiency is thought to disturb myelination.²⁰ The main histopathologic findings in Canavan's disease include spongy degeneration and hypomyelination of the brain and cerebellum.^{20,21} Only dog 1 was screened for the presence of amino acid and organic acid anomalies. The results in this puppy were suggestive of a possible error of metabolism, but no further analyses were pursued and concentrations of NAA were not measured in this puppy.

Cerebrospinal fluid analysis of dog 1 indicated a moderate lymphocytic pleocytosis. No evidence of inflammation was noted at necropsy to correlate with these CSF findings. In humans, known differences exist between neonatal and adult CSF, with neonates having higher WBC counts than adults.²² In a study investigating cerebellomedullary cistern CSF from 10 normal

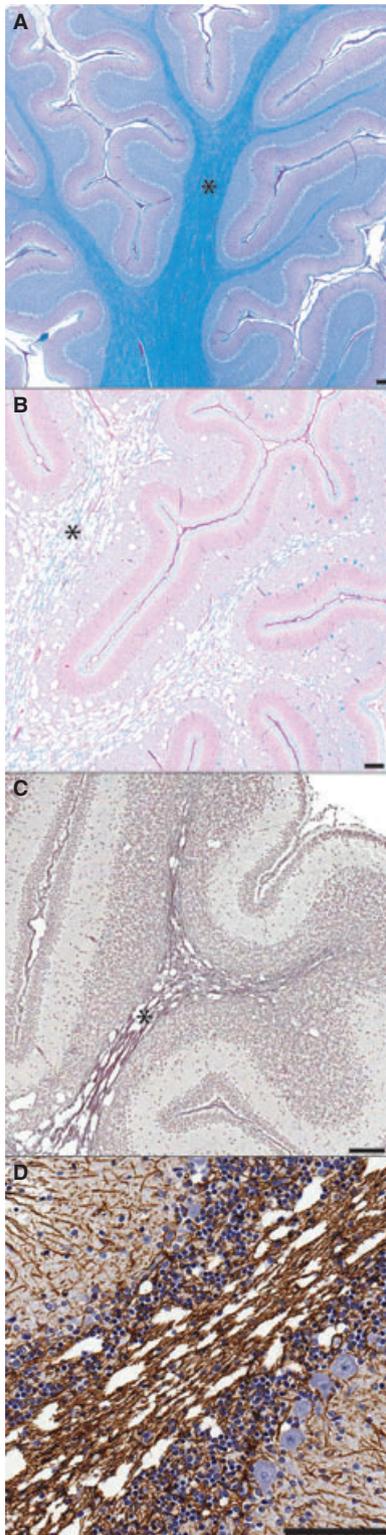


Fig 3. Photomicrographs of cerebellum from the age-matched control with Luxol fast blue staining (A; 1.6 \times) and cerebellum from dog 1 with Luxol fast blue staining (B; 4 \times), Bielschowsky staining (C; 8 \times), and glial fibrillary acidic protein (GFAP) (D; 20 \times). Photomicrographs B and C show lesions of hypomyelination with intact axonic processes, respectively (asterisks). The GFAP stain demonstrates an equivocal increase in GFAP positive astrocytic fibers (D). All bars represent 100 microns.

puppies ranging from 4 to 10 weeks of age, it was noted that the younger puppies had significantly higher WBC on the CSF than older ones. Hence, the results of the CSF in dog 1 may have been consistent with a normal variation for the age of the puppy.²³

The neurologic signs noted during the examination of these Border Terrier puppies correlate well with the microscopic lesions. The spongiform change and reduced myelin staining of the white matter were most severe in the cerebellum, brain stem and spinal cord. In contrast, the low myelin content of the cerebral cortex may be age-appropriate and was largely independent of the spongiform change. In the spinal cord, the ventral funiculi were more severely affected, similar to the hypomyelinating disorder of Weimaraner dogs.^{1,11}

The congenital nature of the defect and uniformity of both the clinical presentation and the changes within the CNS in the Border Terriers reported here suggest a hereditary basis for the disease. In addition, a common male ancestor was identified in the maternal and paternal lines of all affected puppies. Taken together, an inherited condition is strongly suspected. An autosomal recessive mode of inheritance could fit with the appearance of the disease in this group of dogs, similar to the hypomyelinating condition of the Bernese mountain dogs.¹⁴ Many of the congenital myelin disorders in dogs are believed to be inherited.¹ In the Springer Spaniels hypomyelination, an X-linked mode of inheritance has been identified.^{1,2,7} In the case of the Shetland Sheepdogs and Australian Cattle Dogs with spongiform leukoencephalomyelopathy, a maternal mode of inheritance was suggested, and the disease was associated with a missense mutation in the mitochondrial genome.⁵

This report presents the first description of a disorder of myelin affecting Border Terrier puppies, which was characterized clinically by severe generalized coarse tremors, primarily affecting the hindquarters. Histologically, this disorder was characterized as a spongiform leukoencephalomyelopathy with a combination of diffuse reduced myelin content of the brain and spinal cord white matter in association with a spongiform change. Some hypomyelinating conditions such as the one seen in Weimaraner,¹¹ stabilize and improve over time. Because only week-old puppies were studied here, the long-term progression of this disease is currently unknown.

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Conflict of Interest: The authors have no conflict of interest to disclose.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1. Puppy 1 with severe generalized coarse body tremors. Note the characteristic swinging side-to-side or “rocking horse” movements, most severe in the hindquarters.

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