MRI Findings in a Rottweiler with Leukoencephalomyelopathy

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A 22 mo old male rottweiler presented with a 1 mo progressive history of general proprioceptive ataxia and upper motor neuron tetraparesis. Neurologic examination was consistent with a lesion affecting the first through fifth cervical spinal cord segments. MRI disclosed bilaterally symmetric hyperintensities on T2-weighted (T2W) images in the crus cerebri and pyramidal tracts of the brain and the dorsal portion of the lateral funiculi of the cervical spinal cord. Fifty days after initial presentation, the dog was euthanized due to disease progression. Pathologic examination of the central nervous system (CNS) revealed a bilaterally symmetric chronic leukoencephalomyelopathy (LEM) consistent with previous reports of LEM in rottweilers. To the authors’ knowledge, this is the first report to describe the MRI characteristics of LEM in the rottweiler. The topography of the changes observed with MRI paralleled the pathologic changes, which were widespread loss of myelin, decreased axon numbers, and astroglial proliferation. Consequently, MRI of the CNS of affected rottweilers may aid in establishing a presumptive antemortem diagnosis of LEM. (J Am Anim Hosp Assoc 2013; 49:255–261. DOI 10.5326/JAAHA-MS-5864)

Introduction

Leukoencephalomyelopathy (LEM) in the rottweiler is a rare degenerative disorder that has been recognized since the early 1980s.1–3 The clinical syndrome manifests as a long-strided gait with the appearance of stiffness and overreaching as the limbs are advanced when walking, consistent with general proprioceptive ataxia and upper motor neuron tetraparesis that begins between 1.5 and 3.5 yr of age.3 Affected dogs initially develop abnormalities in the thoracic limbs, and the thoracic limbs are often more severely affected than the pelvic limbs. Gender predilection has not been reported. Despite a familial relationship among affected dogs, a mode of inheritance has not been determined.1–3 The etiology of LEM remains unknown. Progression of clinical signs occurs over months to up to a year, with most dogs being euthanized due to increased difficulty ambulating.2 Currently, the antemortem diagnosis is based on exclusion of other diseases that result in similar clinical signs, and diagnosis often entails advanced imaging. To the authors’ knowledge, this is the first report to describe the MRI characteristics of LEM in the rottweiler. In the present case, the topography of the lesions observed with MRI exactly paralleled the pathologic changes observed on gross and microscopic examination of the central nervous system (CNS), which consisted of severe myelin loss, decreased axonal numbers, astrogliosis, and astrocitosis. Consequently, MRI of the nervous system may aid clinicians in establishing a presumptive antemortem diagnosis of LEM in rottweilers.

Case Report

A 22 mo old male rottweiler presented to the Veterinary Teaching Hospital, University of Georgia with a 1 mo progressive history of
an abnormal gait. The dog was current on vaccinations and was receiving heartworm preventative monthly. There was no history of other medical problems. Physical examination was normal. On neurologic examination, the dog displayed a longer than normal stride that was characterized by stiffness and overreaching, consistent with general proprioceptive ataxia and upper motor neuron paresis in all four limbs. The gait was characterized by hypermetria resulting in marked overreaching in the thoracic limbs. The dog scuffed the nails on all four feet while walking. Deficits in postural reactions (proprioceptive placing and hopping) were observed in all four limbs. Deficits were worse in the thoracic limbs. Additionally, the left thoracic and pelvic limbs were more affected than the right thoracic and pelvic limbs. Spinal reflexes were normal in the thoracic limbs. In the pelvic limbs, the withdrawal reflexes were normal and patella reflexes were increased bilaterally. Muscle mass and muscular tone were both normal in all four limbs. Cranial nerve examination was normal. There was normal range of motion of the neck. The dog did not appear painful with either manipulation or palpation along the entire vertebral column. Neuroanatomic diagnosis was consistent with a lesion affecting the first through fifth cervical spinal cord segments. Differential diagnoses included cervical vertebra(e) malformation/malarticulation, intervertebral disc disease, fibrotic stenosis, subarachnoid diverticula, neoplasia, LEM, and neuroaxonal dystrophy (NAD).

Hematologic and serum biochemical examinations revealed lymphocytosis (3.3 \times 10^9/\mu L; reference range, 0.4–2.9 \times 10^9/\mu L) and eosinophilia (1.53 \times 10^9/\mu L; reference range, 0–1.3 \times 10^9/\mu L). Urinalysis was normal. Under anesthesia, MRI of the vertebral column from the first cervical vertebra to the third thoracic vertebra and the brain was performed using a 3.0T MR unita and a multichannel phase array spine coil. The following pulse sequences were obtained: T1-weighted fluid-attenuated inversion recovery (T1W FLAIR), T2-weighted (T2W), T2-weighted FLAIR (T2W FLAIR), and T2*-weighted gradient echo (T2*W) images. Additionally, axial and sagittal plane T1W FLAIR images of the vertebral column and the brain were obtained after IV administration (0.1 mmol/kg) of contrast agentb.

In comparison with unaffected white matter of the spinal cord, bilaterally symmetric intra-axial hyperintensities on T2W images were noted in the white matter of the dorsolateral funiculi from the cervicomedullary junction extending contiguously to the level of the sixth to seventh cervical intervertebral disc (Figures 1A, B). In comparison with the unaffected areas of brainstem, bilaterally symmetric intra-axial hyperintensities on T2W images were noted in the pyramids (Figure 1C) and ventral aspect of the crus cerebri of the brain. The lesions also were hyperintense on T2*W and T2W FLAIR images and were isointense on T1W FLAIR images. Abnormal contrast enhancement was not observed in the spinal cord and brain.

Cytology and protein analysis of cerebrospinal fluid obtained from the cerebellomedullary cistern were normal. Based on the signalment, history, the neurologic examination, and abnormalities observed on MRI, a presumptive diagnosis of LEM was made; however, infiltrative disease, such as neoplasia or infectious/noninfectious inflammatory myelitis, could not be excluded. Consequently, the dog was administered prednisonec (0.5 mg/kg per os q 24 hr). After 10 days, no improvement was noted. Fifty days after initial presentation, the dog was presented for humane euthanasia. The owner stated that the dog could no longer walk without falling down; however, if allowed to either run or walk fast, the dog would not fall as often.

**FIGURE 1** Transverse plane T2-weighted (T2W) MRI of the second cervical vertebral (A) and fourth cervical vertebral (B) spinal cord segments, the medulla oblongata (C) and corresponding gross spinal cord specimen from the fourth cervical vertebra (D) from a 22 mo old male rottweiler with general proprioceptive ataxia and upper motor neuron tetraparesis. There are bilaterally symmetrical, hyperintense lesions in the dorsolateral funiculi of the spinal cord (arrows in A and B). Images were acquired 50 days after initial presentation. A, B: Insets are from the MRI performed on initial presentation. In the caudal medulla oblongata, symmetrical hyperintensities also are observed in the pyramids of the medulla oblongata (arrows in C). At the level of the fourth cervical vertebra, the hyperintensities corresponded to bilaterally symmetric opaque foci (arrows) on gross sections (D).
Prior to euthanasia and with owner consent, MRI of the cervical vertebral column and brain was performed to determine if the lesions had progressed. Imaging was performed as previously described with the same unit, with the exception that the brain was imaged with the dog in sternal recumbency using an extremity coil to provide improved image quality. The previously identified lesions were present, and new lesions were not identified. Subjectively, the lesions in the white matter of the dorsal aspect of the lateral funiculi were larger. The lesions in the brain were unchanged in size. Following MRI, the dog was euthanized and immediately necropsied.

At necropsy, gross lesions were restricted to the cervical spinal cord and brainstem. Opaque white, well-demarcated, bilaterally symmetric foci were noted in the white matter in the dorsal aspect of the lateral funiculi of the cervical spinal cord (Figures 1D, 2A) and the pyramidal tracts of the medulla oblongata. The crus cerebri were grossly normal. Brain, spinal cord, and representative tissue samples of internal organs were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Selected sections of the brain and spinal cord were stained with luxol fast blue.

Immunohistochemistry was performed with monoclonal antibodies against neurofilament (1:8,0000), glial fibrillary acidic protein (GFAP, 1:8,000), myelin basic protein (MBP, 1:2,000), and canine distemper virus. Also samples from the affected dorsolateral region of the lateral funiculus of the first cervical spinal cord segment were removed at necropsy and fixed in 2% (para)formaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer for transmission electron microscopy. Following dehydration in graded alcohols, the tissues were embedded in epon-araaldite. Thin sections were made and stained with lead citrate and uranyl acetate.

On low power magnification, the white matter of the affected areas observed on MRI showed a marked pallor consistent with loss of myelin (Figures 2B, C). Microscopically, lesions were confined to the white matter of the brain and spinal cord. The most severe lesions were located in the cervical spinal cord, but lesions extended caudally into the thoracic spinal cord and rostrally into the brainstem. Within the cervical spinal cord, the lesion affected the white matter in the dorsal portion of the lateral funiculi in the area of the dorsal spinocerebellar, lateral corticospinal, reticulospinal, and rubrospinal tracts. A subpial rim of normal white matter was always preserved except at the first cervical spinal cord segment where the lesion extended to the pia. In the brain, the pyramidal tracts, crus cerebri, area of medial lemniscus, caudal cerebellar peduncle, trapezoid body, area of the spinal tract of the trigeminal nerve, and area of the optic tracts were most severely affected. The white matter of the cerebellar folia was also multifocally affected. The parenchymal portions of the oculomotor nerves in the mesencephalon were mildly affected.

Within the affected white matter, myelin and axon loss was observed. The myelin and axonal loss was replaced by prominent astrogliosis and astrocytosis with numerous GFAP-positive astrocytic processes and gemistocytic astrocytes in the affected regions. Many normal appearing axons were present within the area of severe demyelination. The degree of myelin loss exceeded the loss of axons. Degenerative axonal changes were relatively mild.

**FIGURE 2** Transverse gross and microscopic sections of the spinal cord at the level of second cervical vertebra from the 22 mo old male rottweiler in Figure 1 reveal lesions involving the white matter. A: Similar to Figure 1D, gross transverse section of the cervical spinal cord at the level of the second cervical vertebra, bilaterally symmetric white foci are also evident in the dorsal area of the lateral funiculi (arrows). B: On a low power magnification of a transverse section of the cervical spinal cord, there are bilaterally symmetric areas of pallor in the lateral funiculi (arrows). Hematoxylin and eosin staining, bar = 2 mm. Inset of B: A transverse section of the cervical spinal cord from banked tissue from an age matched control dog. Bar = 2 mm. Note the uniform staining of the white matter. C: The bilaterally symmetric areas of pallor in the dorsal areas of the lateral funiculi indicate loss of myelin (arrows). Luxol fast blue staining, bar = 2 mm. Inset of C: A transverse section of the cervical spinal cord from banked tissue from an age matched control dog. Note the uniform staining of the white matter. Bar = 2 mm. D: The loss of myelin is replaced by gliosis with numerous gemistocytic astrocytes (arrows). In the affected areas, vessels are prominent with hypertrophy of the endothelial cells (arrowheads). Hematoxylin and eosin staining, original magnification ×400, bar = 40 mm.
digestion chambers were rare, and few axonal spheroids were seen. In affected areas, vessels were mildly thickened with prominent endothelial cells and increased cellularity of the perivascular space (Figure 2D).

To better assess myelin content and axonal changes, banked tissue samples of the cervical spinal cord from an age matched control dog free of neurologic disease were used for comparison. In the affected dog, immunohistochemistry for MBP confirmed partial to total loss of myelin in the white matter of affected areas with patchy staining of the remaining white matter and naked axons compared to the control dog. (Figures 3A, B) Immunohistochemistry for neurofilament demonstrated decreased numbers of axons. (Figures 3C, D) Multiple axons had irregular profiles and many were smaller. Only a few axons were larger than in the control dog. In the affected dog, immunohistochemistry for GFAP revealed the abnormal neuroparenchyma to consist mostly of gemistocytic astrocytes as well as astrocytic processes (Figures 3E, F) Immunohistochemistry for canine distemper virus was negative.

Ultrastructurally, affected spinal cord white matter contained oligodendroglia, a small number of small naked axons, small numbers of myelinated axons, and hypertrophied astrocytic processes (Figure 4A). Myelinated axons were some of the larger axons present and were typically irregularly shaped. The myelin sheaths had extensive splitting of the lamellae and lack of compaction. Schwann cells with similarly myelinated axons were present near vessels indicating Schwann cell remyelination (Figure 4B).

Discussion
The clinical and pathologic features of the dog described in this report are consistent with a diagnosis of LEM as previously described in rottweilers.1,3 A definitive diagnosis of LEM requires histopathology. In this case, MRI precisely identified the affected areas, allowing representative transverse sections to be obtained for macroscopic, histopathologic, and ultrastructural examination. In the case reported here, bilaterally symmetric, continuous hyperintensities on T2W images were identified in the same topography as reported for the most severe lesions in LEM in rottweilers.1–3 Moreover, the lesions observed on MRI in the present case correlated with the degenerative changes in the white matter observed histologically, which included severe loss of myelin and astrogliosis. Those findings were similar to findings

![FIGURE 3](image-url) "Microscopic sections of the demyelinated area of dorsolateral lateral funiculus of the first cervical vertebral spinal cord segment from the affected rottweiler compared with the white matter of a similar area of the cervical spinal cord from banked tissue samples from an age matched control dog free of neurologic disease using a variety of immunohistochemical stains. A: Immunohistochemical stain for myelin basic protein (MBP). In the bilaterally symmetric areas of pallor in the cervical spinal cord, there is severe myelin loss around axons with minimal punctuate positive remnants of myelin. MPB with fast red chromogen/hematoxylin counterstain, original magnification ×1000. B: For comparison, similar location of the cervical spinal cord from a normal dog stained for MBP with fast red chromogen/hematoxylin counterstain, original magnification ×1000. C: Immunohistochemical stain for neurofilament reveals decreased axon numbers. Diaminobenzidine (DAB) chromogen/hematoxylin counterstain, original magnification ×1000. D: For comparison, similar location of the cervical spinal cord from a normal dog stained for neurofilament as in panel C, original magnification ×1000. E: Immunohistochemical stain for glial fibrillary acidic protein (GFAP). Myelin loss is replaced by astrocytosis and gemistocytic astrocytes. DAB chromagen/hematoxylin counterstain, original magnification ×1000. F: For comparison, similar location of the cervical spinal cord from a normal dog stained for glial fibrillary acidic protein (GFAP) as in panel E. DAB chromagen/hematoxylin counterstain, original magnification ×1000. Bar = 15 mm."
Previous microscopic investigations in the case using immunohistochemistry (i.e., antibodies against MBP, neurofilament, and GFAP) and electron microscopy corroborated severe myelin loss, astrogliosis/astrocytosis, as well as demonstrating decreased numbers and degeneration of axons. In addition, minimal abortive attempts of oligodendroglial processes and Schwann cell remyelination were present ultrastructurally. Interestingly, Schwann cells have not been observed in the spinal cord in previously reported cases of LEM. Although typically excluded from the CNS, Schwann cell invasion into the spinal cord can occur in a variety of conditions, including primary myelin disorders as well as focal compressive and concussive processes. Although typically excluded from the CNS, Schwann cell invasion occurs at transition zones where the peripheral nervous system interfaces with the CNS, such as the dorsal and ventral root entry zones and near blood vessels. Consistent with this, Schwann cells in the present case were observed near blood vessels.

Given the MRI characteristics and topography of the lesions observed in the dog reported here, many of the differential diagnoses for clinical signs referable to the first through fifth cervical spinal cord segments can be eliminated from consideration. Other important degenerative nervous system disorders may affect rottweilers. Those degenerative diseases must be considered in the differential diagnoses. NAD is a neurodegenerative disease that presents in young rottweilers as a chronic, progressive, general proprioceptive ataxia and upper motor neuron paresis of all four limbs. Pathologically, NAD results in axonal swellings (spheroids) throughout the CNS gray matter, except the cerebral cortex. The spheroids are primarily localized in the distal regions and axon terminals of afferent fibers entering sensory nuclei in the spinal cord, brainstem, and diencephalon. Cerebellar atrophy may also be appreciated in more chronic cases. Although not described in rottweilers with NAD, the MRI findings have been documented in one papillon with NAD. At 3 mo of age, no abnormalities were detected; however, at 6 mo of age, diffuse atrophy of the cerebrum, cerebellum, and brainstem were seen in the affected dog. In humans with NAD, the most consistent MRI abnormality observed is a hyperintense cerebellum on T2W and T2 FLAIR images. To the authors’ knowledge, reports detailing imaging studies involving encephalomyelopathy and polyneuropathy in the rottweiler have not been published. Of the available MRI data for LEM and NAD in the dog, no similarities are seen. Encephalomyelopathy and polyneuropathy has also been reported in young rottweilers. Signs consist of ataxia and paresis involving all four limbs, as well as laryngeal and pharyngeal dysfunction. Although initial signs share similarities with LEM, dogs with encephalomylopathy and polyneuropathy are typically younger and, with disease progression, signs reflect neuromuscular dysfunction.

MRI has been used in other white matter disorders. In spongy degeneration of the CNS in Labrador retrievers, MRI disclosed symmetrical, hyperintense lesions on T2W images, which correlated with degenerative white matter lesions detected during gross and histologic examination. Recently, a novel LEM in two leonbergers was described. As in the present case, the bilaterally symmetric hyperintensities on T2W images were observed in the dorsolateral funiculi of the spinal cord; however, lesions were restricted to the second cervical spinal cord segment in one dog and to the second to fourth cervical spinal cord segments in the other dog. Additionally, lesions were not noted on MRI of the brain in affected leonbergers. MRI of globoid cell leukodystrophy of the West Highland white terrier revealed bilaterally symmetrical, hyperintense lesions on T2W images, which included the corpus callosum, centrum semiovale, internal capsule, corona radiate, and cerebellar white matter. Symmetrical contrast enhancement was observed in the corpus callosum, internal capsule, and corona radiata on T1W postcontrast images.
Demyelinating lesions have been found to correlate well with areas of hyperintensity on T2W images in canine distemper. Dysmyelination in the English springer spaniel and Portuguese water dog secondary to GM1 gangliosidosis was correlated with a mild hyperintensity of the corona radiata on T2W images.

The etiology of LEM remains obscure, and the pathogenesis still remains to be determined. Bilaterally symmetric degenerative lesions in the CNS are usually due to nutritional, metabolic, or toxic causes. It is possible that LEM is a result of an inborn error of metabolism. In humans, numerous primary disorders of myelin occur secondary to inborn errors of metabolism. In two dogs with LEM, biochemical analysis of peripheral blood leukocytes for evidence of lysosomal storage defects was evaluated for activity of β-galactosidase, β-hexosaminidase, β-hexosaminidase A, aryl sulphatase A, acid phosphatase, β-glucuronidase, α-mannosidase, α-fucosidase, β-glucocerebrosidase, β-galactocerebrosidase, and sphingomyelinase). All evaluations were normal.

Based on immunohistochemical and ultrastructural studies in those cases, the lesions are clearly demyelinating with simultaneous, although inadequate and abnormal, remyelination. The question remains whether the defect in LEM involves either a primary disorder of oligodendrocytes (primary demyelination) or loss of myelin secondary to primary changes in the axons (axonopathy). Oevermann et al. (2008) hypothesized that LEM in the Leonberger was a consequence of an alteration in the intimate relationship between the oligodendrocyte and the neuron so myelin was produced, but not stable, and the topography of the lesion reflected the population of neurons affected. In humans, there is a close relation between myelin and axon, where axonal pathology may precede demyelination. Interestingly, in this case, not only was myelin loss observed, but also decreased axonal diameters and irregularity in axonal shape. That finding could be related to either loss or disorganization of the neurofilaments, and thus, impaired cytoskeletal organization and axonal transport in dogs with LEM. Minimal attempts at remyelination were seen in this case, some of which was due to Schwann cells migrating into the spinal cord, suggesting disruption of the glia limitans formed by astroglia, which, as a consequence of disruption, allowed Schwann cells to enter the CNS and promote remyelination. That could indicate that the Schwann cells were compensating for some form of oligodendrocyte/neuronal dysfunction that caused demyelination; however, there was also evidence of remyelination by oligodendroglia, albeit abnormal. Although speculative, given the more severe pathologic involvement of myelin than axons observed histologically and ultrastructurally in the case reported herein, the underlying disease process likely represents a leukodystrophy rather than an axonal disorder with secondary demyelination.

In the present case, MRI was helpful in establishing a presumptive antemortem diagnosis of LEM. Although unknown, it is possible that either early in the course of the disease or if clinical signs are mild, lesions may not be detected with MRI. The factors that contribute to lesion conspicuity likely include the degree of myelin loss, number of affected axons (and their diameter), and number of glial cells in the white matter. Additionally, the sophistication of the MRI unit also may play a role. In the present case, the lesions in the white matter of the dorsal aspect of the lateral funiculi were larger after 50 days. Therefore, a repeat MRI should be recommended in cases where there is a high index of suspicion yet lesions are not observed.

**Conclusion**

Understanding the correlation between lesion characteristics and topography on MRI and histologic findings can refine the diagnostic approach categorizing different morphologic changes observed in the CNS. The ability to establish an accurate presumptive antemortem diagnosis of LEM has many implications. Although not established, given the occurrence of LEM in a specific breed, the rottweiler, a hereditary basis is suspected. The ability to identify affected individuals and remove them from the breeding pool is imperative. Also, owners of affected dogs could be provided an accurate prognosis as rottweilers with LEM are typically euthanized within 1 yr of diagnosis.

**FOOTNOTES**

- Signa HDx; GE Healthcare, Milwaukee, WI
- Magnevist; Berlex Laboratories, Wayne, NJ
- Prednisone; West-Ward Pharmaceutical Corp., Eatontown, NJ
- Neurofilament antibody; Biogenex, San Ramon, CA
- Glial fibrillary acidic protein antibody; Abcam, Cambridge, MA
- Myelin basic protein antibody; Abcam, Cambridge, MA
- Canine distemper virus antibody; VMDR, Pullman WA

**REFERENCES**


Magnetic resonance imaging and genetic investigation of a case of rottweiler leukoencephalomyelopathy

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Abstract

Background: Leukoencephalomyelopathy is an inherited neurodegenerative disorder that affects the white matter of the spinal cord and brain and is known to occur in the Rottweiler breed. Due to the lack of a genetic test for this disorder, post mortem neuropathological examinations are required to confirm the diagnosis. Leukoencephalopathy with brain stem and spinal cord involvement and elevated lactate levels is a rare, autosomal recessive disorder in humans that was recently described to have clinical features and magnetic resonance imaging (MRI) findings that are similar to the histopathologic lesions that define leukoencephalomyelopathy in Rottweilers. Leukoencephalopathy with brain stem and spinal cord involvement is caused by mutations in the DARS2 gene, which encodes a mitochondrial aspartyl-tRNA synthetase. The objective of this case report is to present the results of MRI and candidate gene analysis of a case of Rottweiler leukoencephalomyelopathy to investigate the hypothesis that leukoencephalomyelopathy in Rottweilers could serve as an animal model of human leukoencephalopathy with brain stem and spinal cord involvement.

Case presentation: A two-and-a-half-year-old male purebred Rottweiler was evaluated for generalised progressive ataxia with hypermetria that was most evident in the thoracic limbs. MRI (T2-weighted) demonstrated well-circumscribed hyperintense signals within both lateral funiculi that extended from the level of the first to the sixth cervical vertebral body. A neurodegenerative disorder was suspected based on the progressive clinical course and MRI findings, and Rottweiler leukoencephalomyelopathy was subsequently confirmed via histopathology. The DARS2 gene was investigated as a causative candidate, but a sequence analysis failed to identify any disease-associated variants in the DNA sequence.

Conclusion: It was concluded that MRI may aid in the pre-mortem diagnosis of suspected cases of leukoencephalomyelopathy. Genes other than DARS2 may be involved in Rottweiler leukoencephalomyelopathy and may also be relevant in human leukoencephalopathy with brain stem and spinal cord involvement.

Keywords: Rottweiler, DARS2, LBSL, White matter disease, Progressive ataxia
Background

Rottweiler leukoencephalomyelopathy (LEM) was initially recognised in the US as a cause of chronic progressive ataxia with insidious onset in Rottweilers between 1.5 and 4 years of age [1]. The clinical and pathological characteristics of this disease entity were further defined in subsequent reports originating from Australia, the Netherlands and the UK, which described 16 pathologically confirmed cases (of 22 total cases described in the literature) and suggested an autosomal recessive pattern of inheritance [2-6]. In these reports, Rottweiler LEM presented as a distinctive neurodegenerative disorder restricted to the lateral and dorsal funiculi of the cervical spinal cord and spinal tracts of the trigeminal nerve, pyramids, caudal cerebellar peduncles, cerebellar medulla and optic tracts that showed a sharp demarcation between abnormal and normal white matter and occasional microcavitation in the centre of the lesion. Clinically, affected dogs exhibit progressive ataxia with hypermetria and subtle postural reaction deficits. Thus far, the ante mortem diagnosis of LEM in Rottweilers has been based on clinical suspicion and the exclusion of other diseases of the cervical spinal cord, e.g., compression/instability, neoplasia and inflammation. To date, there have been no magnetic resonance imaging (MRI) studies or genetic investigations of this disease entity.

Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) is a neurodegenerative disease in humans with clinical features and MRI findings that are surprisingly similar to the histopathologic lesions of LEM in Rottweilers. Specifically, these patients exhibit slow progressive spasticity and ataxia, MRI findings of selective involvement of the brain stem and spinal tracts in both lateral funiculi and dorsal columns and changes in the cerebral and cerebellar white matter. Spinal cord involvement with MR signal intensity changes has also been reported in other leukodystrophies in humans, e.g., adult onset autosomal dominant leukodystrophy with autonomic features, Alexander’s disease, vitamin B12 deficiency myelopathy and sporadic cases of adult onset lysosomal leukodystrophies [7-11]; however, a very distinct and well-demarcated pattern of signal intensity change is considered to be most characteristic of LBSL. In LBSL, high levels of lactate are frequently demonstrated in brain lesions using magnetic resonance (MR) spectroscopy; this finding suggests a respiratory chain defect, but lactate is rarely elevated in the blood or cerebrospinal fluid (CSF) [12,13]. To date, all human cases of LBSL have been found to be caused by mutations in the DARS2 gene, which encodes mitochondrial aspartyl-tRNA synthetase [14,15].

To investigate the hypothesis that LEM in Rottweilers could represent a possible animal model of LBSL, MRI results and DARS2 gene integrity were investigated in a single, affected dog.

Case presentation

A two-and-a-half-year-old male purebred Rottweiler was referred for further investigation of progressive ataxia. The dog had been placed in an animal shelter 8 weeks prior to the study. Unfortunately, no pedigree data were available, and we were unable to ascertain whether inbreeding had occurred. At the time of shelter placement, the dog had already been ataxic, and the ataxia progressed during the subsequent 8 weeks. Haematologic and serum biochemical analyses, thoracic and abdominal radiographs, and echocardiography had been performed prior to referral, and the findings were unremarkable.

Physical examination showed excessive wearing of the nails on all four limbs, particularly of the thoracic limbs. A neurological examination showed severe generalised ataxia with hypermetria of the thoracic (prolonged protraction and overreaching action with limb extension) and pelvic limbs. Additionally, difficulties in rising, intermittent crossing of the thoracic limbs, and a wide-based stance of all limbs were observed. The postural reactions (wheelbarrowing with and without neck extension, hopping, and proprioceptive positioning) were delayed, and the thoracic limbs were more severely affected than the pelvic limbs. A supplemental movie file shows these findings in more detail (see Additional file 1). The spinal reflexes (extensor carpi radialis, thoracic and pelvic limb flexor, patellar, cranial tibial, gastrocnemius, cutaneous trunci, and perineal) were all normal. The mentation and cranial nerve function, including vision, were unimpaired, but an inconsistent menace response was observed; this was attributed to the lack of cooperation by the dog but could also indicate a cerebellar lesion. Palpation of the head and spine and neck flexion and extension did not elicit any signs of pain. There was no evidence of tremor or uncoordinated movements of the head. The findings of the neurological examination were most consistent with a cervical myelopathy (C1-C5 spinal cord segments) involving the spinocerebellar tracts, although a cerebellar lesion could not be ruled out completely. The differential diagnoses included several breed-related neurodegenerative disorders: neuronal vacuolation and spinocerebellar degeneration, neuroaxonal dystrophy, LEM, cervical spondylomyelopathy and arachnoid diverticula [16-20].

A follow-up laboratory examination revealed mild eosinophilia (1.51 × 10³ eosinophilic granulocytes/μl; reference range: 0.04 - 0.6 × 10³/μl) and unremarkable serum biochemical results. The dog was subsequently anesthetised for further examination of the cervical spine and brain using MRI and CSF analysis. Electrodiagnostic examination was scheduled as a supplemental examination to investigate the presence of additional lesions in the peripheral nerves. Magnetic resonance imaging was performed using a 1.5 T magnetic resonance unit. The brain imaging protocol
utilised sagittal, dorsal and transverse T2-weighted (TR/TE 5190/108 ms) and T1-weighted (TR/TE 386/13 ms) sequences and transverse FLAIR (TR/TE/TI 9110/122/2500 ms) and gradient echo (TR/TE 1000/28 ms) sequences. The spinal imaging protocol included sagittal and dorsal T2-weighted (TR/TE 2880/111 ms) and T1-weighted (TR/TE 623/1 ms), transverse T2-weighted (TR/TE 3290/99 ms) and T1-weighted (TR/TE 651/12 ms) and sagittal STIR (TR/TE/TI 3310/61/140 ms) sequences. The sagittal and dorsal spinal sequences were performed from C1 to T3 (vertebral body), and the transverse sequences used C1 to C7 (vertebral body). Gadolinium (0.1 mmol/kg; 0.045 mmol/lb) was administered intravenously, and post-contrast transverse T1-weighted sequences of the brain and dorsal and sagittal T1-weighted sequences of the spine were acquired. Descriptions of intensity referred to normal appearance of grey matter. The spinal MRI studies showed bilateral symmetrical hyperintensities in the region of both lateral funiculi on transverse T2-weighted images (Figure 1).

The lesions were most visible on the transverse sections; they appeared well demarcated and ovoid and extended from the level of the first to the sixth cervical vertebral body (Figure 2). In T1-weighted plain images, the lesions were isointense, and no contrast enhancement was observed. MRI studies of the brain failed to reveal any abnormalities.

Routine CSF analysis (cisterna cerebellomedullaris) with leukocyte (0/µl; reference range 0-5/µl) and erythrocyte counts (4/µl), CSF cytology and protein measurements (0.18 g/l; reference range 0-0.3 g/l) were unremarkable, as were the lactate concentrations in the CSF (1.6 mmol/l; reference range 0.2-3.1 mmol/l [21,22]) and serum (1.0 mmol/l; reference range 1.1-
3.3 mmol/l [22]). No abnormal spontaneous activity pattern was observed during electromyographic recordings using a concentric needle electrode in the anesthetised dog. The tibial and ulnar motor nerve conduction velocity, tibial nerve F-waves and repetitive nerve stimulation were within established laboratory reference ranges.

Considering the progressive clinical course and the MRI lesion pattern, a neurodegenerative disorder predominantly involving the cervical spinal cord white matter with a bilateral and symmetrical distribution was suspected. Due to the existing severe neurological signs, the progressive deterioration and the poor prognosis, the dog was euthanised.

A complete necropsy was performed, and it confirmed Rottweiler LEM. Significant lesions were confined to the central nervous system. Macroscopic examination revealed bilateral, symmetrical lesions restricted to the dorsal aspect of the lateral funiculi of the cervical spinal cord segments. In transverse sections, these lesions appeared as well-demarcated, whitish, opaque discoloured areas (Figure 3). No gross changes were observed in the brain. Formalin-fixed and paraffin-embedded tissue samples of the brain and spinal cord were sectioned at 5 μm and stained using haematoxylin-eosin and Luxol Fast Blue for histological examination. Histologically, the cervical spinal cord (from C2 to C6 (vertebral body)) exhibited severe, bilaterally symmetrical funicular disruption of the inner dorsal part of the lateral funiculus, including the rubrospinal tract, the innermost layer of the dorsal spinocerebellar tract and the dorsal aspects of the lateral fasciculus proprius. Upon low-power inspection, the lesion was characterised by a severe loss of myelin staining; at high-power, the lesion resembled a dense core of non-myelinated white matter with extensive astrocytosis and astrogliosis with the occasional observation of bizarre cells surrounded by a rim of spongiotic white matter with fibre degeneration, resorptive lesions, vascular prominence and mild-to-marked angiocentric lymphohistiocytic infiltration. The adjacent cervical grey matter appeared hypoplastic in both the ventral and dorsal horns, but there were no further histomorphological changes. Another severe white matter lesion identified in the cerebellar roof showed focal, bilaterally symmetric tissue necrosis, macrospongiosis due to interlamellar myelin sheath oedema (ballooning) and severe intralesional astrogliosis and astrocytosis accompanied by fibrillary astrogliosis and gemistocytes at the margins. A moderate vascular prominence with endothelial hyperplasia was again observed both in the intra- and perilesional areas.

Figure 3 Pathological lesions in the brain (A, B) & spinal cord (C, D). The most severe white matter lesions were observed in the cerebellum (A: asterisk) and cervical spinal cord (C: framed area). Macroscopic examination revealed bilateral, symmetrical lesions in the lateral funiculi of the cervical cord segments only. In transverse sections, these lesions appeared as well-demarcated, whitish, opaque discoloured areas (C: framed area). The cerebellar lesions spared the fibres adjacent to the roof nuclei (A: arrow). Nuclear degeneration was most severe in the raphe nuclei (B) and medial vestibular nuclei (not shown). Note the extensive juxtaneuronal vacuolisation (B: asterisk). The affected spinal cord segments show demyelination, astrogliosis and astrocytosis (D: white arrowhead) with gemistocytes (D: black arrowheads). Within the grey matter, hypoplasia of the dorsal and ventral horn (C: black arrow) is evident. Scale bars: A: 1.5 cm; B: 100 μm; C: 2 mm; D: 35 μm.
Necrotic areas exhibited macrophage-mediated resorption (Figure 3).

Similar demyelinating lesions were observed in the pyramids and caudal cerebellar peduncles and – to a lesser extent – in the medial lemniscus, optic tracts, crura cerebri and subcortical white matter. Lesions in the central visual pathways projected to the optic nerves and manifested as the degeneration of multiple fibres. Further brain stem changes included macrocavitary degeneration of the raphe nuclei and medial vestibular nuclei associated with mild gliosis and axonal spheroids. Immunohistochemical staining for canine distemper virus was negative. A mild diffuse endoneurial hypercellularity was observed in the preganglionic aspects of the dorsal roots of the cervical spine. Both the radial and common peroneal nerve presented with a mild dropout of myelinated fibres, as denoted by enlarged subperineurial spaces with myoid replacement oedema, reduced endoneurial area and decreased myelinated nerve fibre density that was associated with a mild expansion of the endoneurial collagenous matrix. Residual large A (alpha)-type myelinated fibres showed myelin ovoids, consistent with stage II – III Wallerian degeneration, and abundant internodal and paranodal inner and outer myelin loops due to the moderate axonal atrophy of the respective fibres.

Due to the phenotypic similarities between human LBSL patients and LEM-affected Rottweilers, the DARS2 gene was investigated as a candidate for canine LEM. Genomic DNA was extracted from blood collected in tubes containing EDTA using DNeasy blood spin columns (Qiagen). For the DARS2 mutation analysis, suitable PCR products were amplified using AmpliTaq Gold 360 (Life Technologies). The PCR products were resequenced after rAPid alkaline phosphatase (Roche) and exonuclease I (New England Biolabs) treatment and resequenced after rAPid alkaline phosphatase (Roche) and exonuclease I (New England Biolabs) treatment. The sequence data were analysed using Sequencer 4.9 software (GeneCodes). The sequences of all 17 coding exons and flanking intron sequences of the DARS2 gene from the affected Rottweiler were identical to a canine reference gene sequence (CanFam3 assembly; http://genome.ucsc.edu).

**Conclusion**

Magnetic resonance imaging has become the primary tool for the ante mortem diagnosis of white matter disease in humans due to its high sensitivity for detecting changes in white matter. Decreased myelin and elevated water content is revealed by increased T1 and T2 relaxation times, with a consequent reduction in signal intensity in T1-weighted images and increased signal intensity in T2-weighted images [23]. Thus, the pattern of MRI changes is very helpful in defining disease because it reveals the distribution of histopathologic changes [24,25].

At present, there are few case reports describing the use of MRI for the diagnosis of canine and feline neurodegenerative diseases. T2-weighted hyperintensities of white brain matter were evident in cats with GM2 gangliosidosis [26,27] and in a West Highland white terrier with globoid cell leukodystrophy [28]. Increased signal was also evident in T2-weighted images of the spinal cord of Leonberger dogs with leukoencephalomyelopathy [29]. Dogs with GM2 gangliosidosid displayed T2-weighted hyperintensities in the region of the caudate nucleus and atrophy of the cerebrum and cerebellum [30,31]. MRI of Papillon dogs with neuroaxonal dystrophy [32] and Scottish Terriers with hereditary cerebellar degeneration demonstrated atrophy only and failed to detect changes in white matter [33].

MRI of the cervical spine may be used to support the clinical diagnosis of LEM in Rottweilers. A similar MRI pattern has been described in Leonberger dogs with LEM [29]. Interestingly, however, brain lesions were not detected using MRI in the Leonberger dogs or in the case reported here, although histological analyses showed that the optic tracts and particularly the cerebellar medulla were significantly affected in both breeds [2,29]. It is possible that the white matter lesions in the brain were less advanced than those in the spinal cord at the time of imaging, and improved imaging protocols may be required for the visualisation of brain lesions. These protocols may include smaller slice thicknesses and the application of sequences other than conventional T1- and T2-weighted imaging, e.g., diffusion tensor imaging, magnetisation transfer imaging or MR spectroscopy [34]. It is also questionable whether the pathological changes in these regions have sufficiently altered the physics of the tissue to induce changes visible with a 1.5 T clinical scanner.

Many inherited white matter diseases and associated genetic defects have been described in humans [35]. Leukoencephalopathies may be characterised as lysosomal or peroxisomal disorders, mitochondrial disorders, methylation cycle disorders, organic acidemias or amino acid disorders or as leukoencephalopathy associated with calcification, hypomyelination, abnormal lipid metabolism, vasculopathy or muscular dystrophy. Many distinct entities, e.g., Alexander’s disease, adult onset autosomal dominant leukoencephalopathy, vanishing white matter disease and adult polyglucosan encephalopathy, have also been recognised. A vast number of genetic defects are currently associated with these conditions, and many more remain to be elucidated; the molecular cause remains unknown in ~50% of affected humans [35]. The lesion distribution and MRI appearance of LBSL are considered unique and diagnostic in humans; consequently, only a single candidate gene was examined in the present study [36].
Leukoencephalopathy with brain stem and spinal cord involvement is a rare, autosomal recessive disorder that typically manifests in childhood or adolescence. The diagnosis of LBSL in humans is based on clinical presentation and is characterised by a slowly progressive cerebellar ataxia, spasticity, dorsal column dysfunction and a highly characteristic pattern of abnormalities observed using MRI and spectroscopy. Typical MRI findings include a combination of high T2-weighted signal changes in the cerebral white matter accompanied by the selective involvement of the brain stem and spinal cord tracts (the entire length of the pyramidal tracts with the additional involvement of cerebellar connections and the intraparenchymal and mesencephalic parts of the trigeminal nerve) [13,37]. MR spectroscopy demonstrates an elevation in lactate levels in the abnormal white matter of almost all of the affected human patients. These findings led researchers to assume that the disease was a mitochondrial disorder, which was subsequently confirmed by the discovery of various mutations in the DARS2 gene, which encodes mitochondrial aspartyl-tRNA synthetase [14,38]. As demonstrated by multiple case reports of LBSL in humans, normal CSF and blood lactate concentrations, as were noted in the case reported herein, do not exclude a mitochondrial disorder as the underlying cause of leukoencephalomyelopathy. Thus, further investigations should utilise MR spectroscopy to investigate the possible mitochondrial origin of Rottweiler LEM.

The diagnosis of mitochondrial disorders faces specific difficulties due to the complex genetics of these conditions. Mitochondrial disorders may occur due to mutations in mitochondrial genes or mutations in nuclear proteins, with mitochondrial tRNA representing a hot spot for mutations. Heteroplasmy, i.e., the simultaneous presence of mutated and normal RNA/DNA in the cell, is a characteristic feature of mitochondrial disorders. The degree of heteroplasmy varies between tissues in the same organism, which is considered a critical factor in the manifestation of mitochondrial disease in specific tissues [36,39]. Finally, we investigated the coding region of the canine DARS2 gene as a candidate causative gene for LEM, and no mutation was found. At this time, we cannot rule out the possibility of variants in the promoter or intronic regions that could affect DARS2 expression. More comprehensive DNA sequencing approaches, such as the use of next-generation technologies for whole-exome or whole-genome resequencing, may enable the identification of the causative mutation of Rottweiler LEM. A recent study identified the causative mutation of canine neonatal cerebellar cortical degeneration in SPTBN2 (genome-wide mRNA sequencing) using only a single case of this neurodegenerative disease [40].

Further limitations of our case report include the lack of pedigree analysis and brain lactate MR spectroscopy measurements and the failure of MR to demonstrate the involvement of the cerebrum despite the pathology observed in histological sections. Another limitation is that the comparison of the pathologies of these diseases in dogs and humans is limited by the paucity of case data from both. To date, there is only one short description of the pathology of LBSL in humans which has shown spongy white matter degeneration, rarefaction of the neuropil, macrophage infiltration and an increased number of astrocytes in the white matter of the brain and axonal degeneration of the peripheral nerves [25]. Spinal cord changes have not been noted, but it is unknown whether this part of the CNS was sampled and investigated. In dogs with LBSL-like changes, the neuroanatomical mapping of CNS lesions is more precise [2,29]. Clinical and pathological findings emphasise cerebellar and spinal changes, although the microscopic white matter damage is far more widespread and extends from the lower brain stem to the subcortical white matter. Consistent with the fibres affected, Gamble et al. discovered secondary grey matter changes in connected brain stem nuclei, such as the accessory cuneate nucleus, nucleus gracilis, nucleus cuneatus and nucleus of the dorsal spinocerebellar tract [1]. However, the involvement of multiple independent centres and tracts is compatible with multisystemic degeneration, as has been shown in Leonbergers and Rottweilers (discussed above) [2,29]. We also discovered macrovacuolar nuclear degeneration in the Rottweiler, which has not previously been described in dogs. Vacuole formation in LBSL patients was predominantly perineuronal and was therefore dissimilar to the neuronal vacuolation and spinocerebellar degeneration observed in Rottweiler dogs [16]. This degeneration merits further examination to investigate the relationship between neurons and astrocytes in the subcortical grey matter. It also remains to be established whether this manifestation causes the white matter pathology or whether it is an additional, co-existing disorder that is distinct from the breed-specific neurodegenerative disorders described above.

In summary, magnetic resonance imaging revealed leukodystrophic lesions in the lateral funiculi of the cervical spinal cord; these findings will assist in the ante mortem diagnosis of future cases of suspected LEM in Rottweilers. Further investigations should utilise MR spectroscopy to investigate the possible mitochondrial origin of Rottweiler LEM. Although LEM is similar to LBSL based on its clinical features and imaging results, we were unable to identify a coding or splice site mutation in the canine DARS2 gene in our case, suggesting that other genes may be involved in Rottweiler LEM and potentially also in human LBSL.
Additional files

Additional file 1: Movie of a Rottweiler with confirmed leukoencephalomyelopathy. The movie shows the severe generalised ataxia with hypermetria of the thoracic (with prolonged protraction, overreaching action and limb extension) and pelvic limbs. The postural reactions were delayed; and the thoracic limbs were more severely affected than the pelvic limbs.

Additional file 2: Sequencing methods and primers.

Abbreviations
CSF: Cerebrospinal Fluid; FLAIR: Fluid-Attenuated Inversion Recovery; LEMS: Leukoencephalomyelopathy; MR: Magnetic Resonance; MRI: Magnetic Resonance Imaging; Ms: Millisecond; TE: Time to Echo; TR: Time to Repetition.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
KH was responsible for data collection and interpretation and for drafting the manuscript. KM performed the necropsy and histopathology and finalised the version to be published. All authors have approved the final manuscript. AF contributed to data collection, helped draft the manuscript. BR contributed substantially to the acquisition of data used in the genetic study. BR contributed substantially to the acquisition of data used in the manuscript. AF contributed to data collection, helped draft the manuscript and finalised the version to be published. All authors have approved the final manuscript.

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