NOTE
Pathology

Concurrent leukoencephalomyelitis and polyneuritis in a Maltese terrier: resembling combined central and peripheral demyelination in humans

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ABSTRACT. A one-year-old male Maltese terrier presented with mild ataxia and disorientation for 4 months. Over time, clinical signs progressed from paraparesis to non-ambulatory tetraparesis, voice change and dysphagia. Histological examination revealed concurrent leukoencephalomyelitis and polyneuritis. Infectious etiologies, including dengue, Japanese encephalitis, Zika, canine distemper, pseudorabies, rabies, toxoplasmosis, neosporosis, leishmaniasis, and encephalitozoonosis, were ruled out by PCR and/or immunohistochemical (IHC) staining. IHC tested on neurological tissues highlighted a heterogeneous population of infiltrating T and B lymphocytes admixed macrophages. Therefore, this case was diagnosed with current leukoencephalomyelitis and polyneuritis, resembling combined central and peripheral demyelination (CCPD), an autoimmune inflammatory demyelinating disease affecting both the CNS and PNS in humans.

KEY WORDS: autoimmune, canine, combined central and peripheral demyelination (CCPD), leukoencephalomyelitis, polyneuritis

In general, demyelinating lesions are limited to either the central nervous system (CNS) or the peripheral nervous system (PNS), and they rarely affect both [4, 15, 20]. Combined central and peripheral demyelination (CCPD) is a diagnosis used to describe a heterogeneous disease or syndrome related to immune-mediated myelin damage affecting both the CNS and PNS in humans [4, 6, 15]. The hallmark lesions of CCPD are concurrent inflammatory demyelination and inflammation of the white matter of the CNS and the PNS [4, 15, 25]. The grey matter of the CNS and PNS is less affected [4]. Previous studies describe the inflammatory demyelinating lesions in the CNS as multifocally and randomly distributed throughout the white matter of the cerebrum, cerebellum, brainstem and spinal cord [4, 6, 25]. Axonal degeneration and astrogliosis in the PNS are also multifocal and may affect a variety of peripheral nerves such as the optic nerve, spinal roots, and sural nerve [4, 6, 25]. The clinical presentation of patients with CCPD is usually varied, but paraparesis is the most common clinical sign at disease onset [4]. Furthermore, CCPD can involve the CNS and PNS simultaneously or arise from either the CNS or the PNS [4, 6, 15]. Previous studies have suggested that CCPD may be triggered by an infection or vaccination [7, 14, 20, 27]. Antibodies against the myelin basic proteins of both the CNS and the PNS have been identified at a higher frequency in human patients with CCPD [4, 15, 16]. However, the underlying mechanism of CCPD is still largely unknown. Up to now, studies on concurrent leukoencephalomyelitis and polyneuritis in animals have been absent in the literature. In the present case report, we describe concurrent leukoencephalomyelitis and polyneuritis with evident autoimmune reactivity in a Maltese terrier, which closely resembling CCPD in humans.

A one-year-old male Maltese presented for medical evaluation for a 4-month history of mild ataxia and disorientation. Over the course of the disease, the clinical signs gradually progressed from paraparesis to non-ambulatory tetraparesis. The dog also had

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evidences of voice change and dysphagia. After initial evaluation, the animal was transferred to the National Taiwan University Veterinary Hospital but died unexpectedly in transit. A full necropsy was performed following the sudden death; however, there were no macroscopic lesions of note.

Representative tissue samples from the cerebrum, cerebellum, brainstem, spinal cord, tongue, thyroid glands, larynx, trachea, lungs, heart, diaphragm, gastrointestinal tract, spleen, liver, both adrenal glands, urogenital tract, sciatic nerves, and skeletal muscle were collected for histological review. Tissues were routinely processed, sectioned at 5 μm, and stained with hematoxylin and eosin (HE) or Luxol fast blue (LFB). A broad spectrum of infectious etiologies were investigated by immunohistochemical (IHC) staining and polymerase chain reaction (PCR), including dengue virus, Japanese encephalitis virus, Zika virus, canine distemper (CD) virus, pseudorabies (PR) virus, rabies virus, *Toxoplasma gondii*, *Neospora caninum*, *Leishmania* spp., and *Encephalitozoon cuniculi*. Immunohistochemistry proceeded by deparaffinizing tissue sections of the cerebrum, cerebellum, brainstem, spinal cord, and sciatic nerves. Tissues were then heated in retrieval solution (Trilogy, Cell Marque, Rocklin, CA, U.S.A.) at 121°C in an autoclave for 15 min (for IHC of CDV) or treated with 100 μg/m*l* of proteinase K (Roche, Mannheim, Germany) in 0.6 mol/*l* of Tris (pH 7.5)/0.1% CaCl$_2$ for 15 min (for IHC of rabies virus). Subsequently, tissue sections were incubated in 2.5% normal goat serum in Tris-buffered saline (TBS) solution for 30 min at room temperature, and then incubated with antibodies against CDV (diluted 1:500; clone DV2-12; Santa Cruz Biotechnology, Dallas, TX, U.S.A.) and rabies virus (diluted 1:3,000; anti-nucleoprotein polyclone rabies antibody, kindly provided by Dr. Satoshi Inoue, National Institute of Infectious Disease, Tokyo, Japan) at room temperature for 1 hr. The sections were then treated with 3% hydrogen peroxide followed by peroxidase-conjugated secondary antibodies for 60 min at room temperature. After exposure to 2% diaminobenzidine for 3 min, the slides were counterstained with hematoxylin for 30 sec. IHC stainings for CD11d (diluted 1:50; clone CA18.3C6; P.F. Moore, University of California, Davis, CA, U.S.A.), CD18 (diluted 1:50; clone CA16.3C10; P.F. Moore, University of California), CD3 (diluted 1:400; clone F7.2.38; Dako, Glostrup, Denmark), CD79a (diluted 1:200; clone HM57; Abgent, San Diego, CA, U.S.A.), glial fibrillary acidic protein (GFAP; diluted 1:250; clone GA5; Novocastra Laboratories, Newcastle upon Tyne, U.K.), and major histocompatibility complex class II (MHCII; diluted 1:400; clone TAL.1B5; Dako) were performed in an automated system (BondMax, Leica Microsystems Inc., Bannockburn, IL, U.S.A.). Internal controls from the normal spleen and cerebrum from the Maltese were positive for the aforementioned antibodies and provided evidence of appropriate target cell staining.

DNA and RNA were extracted from the formalin-fixed, paraffin-embedded cerebrum, cerebellum, and sciatic nerves using an AllPrep DNA/RNA FFPE kit, (Qiagen, Valencia, CA, U.S.A.). RNA reverse transcription was performed immediately with the QuantiTect® Reverse Transcription kit (Qiagen). Polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR) using primer sets targeting dengue virus [22], Japanese encephalitis virus [21], Zika virus [8], canine distemper virus [9], rabies virus [11], pseudorabies virus [5], *T. gondii* [13], *N. caninum* [19], *Leishmania* spp. [17], and *E. cuniculi* [12] were performed. In addition, the primer set targeting canine glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH; GenBank accession no. AB038240.1 and DQ403060.1) was designed by using Primer3 (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) to provide quality control for the extracted DNA/RNA products. The results of PCR and RT-PCR were positive for the GAPDH gene confirming adequate DNA/RNA extraction. In addition, positive controls for all tested infectious diseases were prepared from previously tested positive clinical samples and synthetic DNA sequences were included in all PCR tests. The primer sets used in this case report are listed in Supplementary Table 1.

Histological evaluation of the CNS revealed multifocal to coalescing foci of white matter demyelination in the cerebrum and thalamus that are highlighted as pale-staining areas by Luxol fast blue (LFB) staining (Fig. 1A and 1B). Demyelination lesions are characterized by marked reactive astrogliosis with infiltration of the neuropil by mononuclear inflammatory cells (Fig. 1C and 1D). In the cerebellum, brainstem, and spinal cord, demyelination was less prominent and perivascular infiltration of mononuclear inflammatory cells predominated the pathological changes (Fig. 1E and 1F). The examined cranial nerves, including the optic and trigeminal nerves, were histologically unremarkable.

Microscopic evaluation of the PNS was performed on the ventral and dorsal spinal nerve roots at the cervical, thoracic, lumbar, and sacral regions. Multifocally surrounding and infiltrating the nerve bundles were moderate numbers of lymphocytes, plasma cells and fewer macrophages (Fig. 2A). The inflammatory components were more prominent in the lumbar and sacral root segments than gradually diminished moving cranially to the thoracic and cervical regions. The sciatic nerve and nerve fascicles around the larynx and adrenal glands had infiltration of mononuclear inflammatory cells that were associated with digestion chambers containing axonal fragments (Fig. 2B), which was consistent with a diagnosis of polyneuritis.

Immunohistochemical staining of the demyelinated foci in the CNS showed a predominance of positive intracytoplasmic staining for CD3 (T lymphocytes; Fig. 3A), admixed with fewer intracytoplasmic positive staining CD79a cells (B lymphocytes; Fig. 3B) and CD18 cells (macrophages; Fig. 3C). Extensive positive membranous staining for MHCII was observed in affected areas denoting expression of receptors for antigen presentation [1, 10, 18, 24, 29]. Although all cells types can conditionally express MHC II, the distribution of staining suggests the presence of elevated expression in tissue macrophages, lymphocytes, microglial cells, astrocytes, and endothelial cells (Fig. 3D and 3E) [1, 10, 24, 29]. Marked reactive astrogliosis in the demyelinating foci was demonstrated by positive cell body and cell process staining using GFAP hypertrophied astrocytes with overlapping astrocyte processes (Fig. 3F) [23]. In the spinal roots and sciatic nerve sections, infiltrating inflammatory CD3-positive cells and CD79a-positive cells were observed. In addition to the intracytoplasmic staining for CD3 and CD79a, there were numerous MHCII-positive antigen presenting cells such as macrophages, lymphocytes, Schwann cells and endothelial cells (Figs. 4 and 5). Myofibers composing the cricothyroid and quadriceps femoris muscle groups were atrophied and replaced by fibrous connective tissue or adipose tissue.
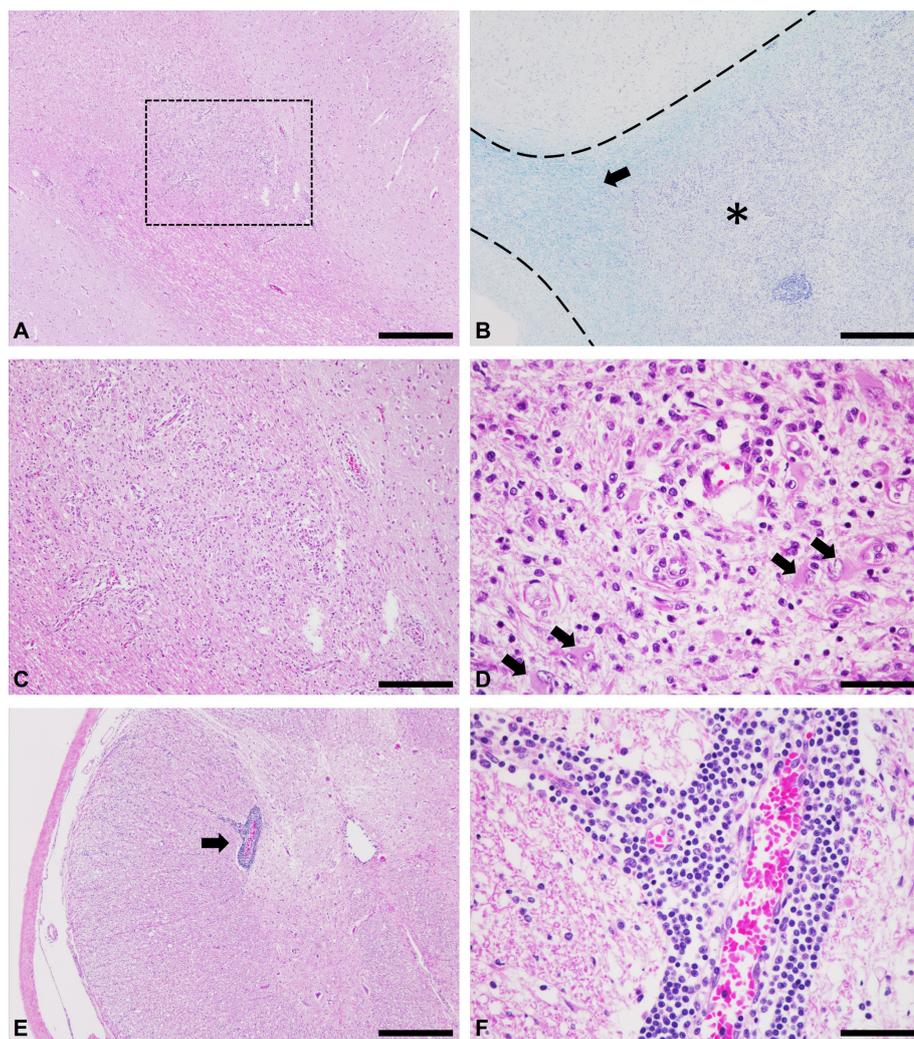
Fig. 1. Photomicrographs of microscopic changes in the cerebrum. (A) The box outlines a focal area of hypercellularity. H&E, bar=500 μm. (B) A Pale-staining area of the cerebral white matter with increased numbers of mononuclear inflammatory cells is present (*). Note normal staining of the cerebral white matter (arrow). The two black dashed lines delineates the outer border of cerebral white matter. LFB stain, bar=500 μm. (C) Higher magnification of area inside black dashed rectangle in Fig. 1A. There are astrogliosis and inflammatory infiltrates expanding the white matter. H&E, bar=250 μm. (D) Higher magnification of Fig. 1C. There are low numbers of infiltrating mononuclear inflammatory cells admixed with scattered gemistocytic astrocytes (arrows). H&E, bar=50 μm. (E) Low magnification photomicrograph of the lumbar spinal cord. Perivascular cuffing is noted in the white matter (arrow). H&E, bar=500 μm. (F) Higher magnification of Fig. 1E. The perivascular cuffing shows a predominance of mononuclear inflammatory cells. H&E, bar=50 μm.
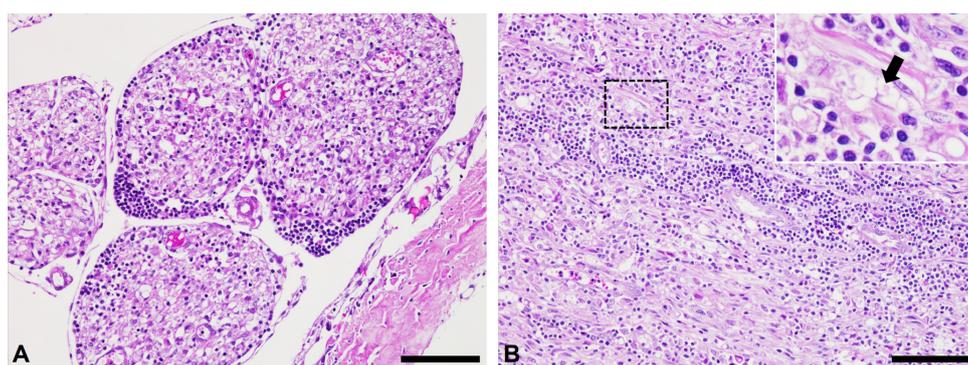

Fig. 2. Photomicrographs of peripheral nervous system changes. (A) Lymphocyte-predominant inflammation infiltrating the spinal nerve roots at lumbar region. H&E, bar=100 μm. (B) Lymphocyte-predominant mononuclear inflammatory cells infiltrate the sciatic nerves in association with digestion chambers (inset: arrow). H&E, bar=100 μm.

The differential diagnoses for canine leukoencephalomyelitis and polyneuritis include 1) infectious diseases, such as dengue virus [22], Japanese encephalitis virus [21], Zika virus [8], canine distemper virus [9], rabies virus [11], pseudorabies virus [5], *T. gondii* [13], *N. caninum* [19], *Leishmania* spp. [17], and *E. cuniculi* [12]; 2) non-infectious diseases, such as granulomatous meningoencephalitis (GME), Greyhound meningoencephalitis (GhME), necrotizing meningoencephalitis (NME), necrotizing encephalitis (NE) and acute polyneuritis (AP); and 3) autoimmune response against the myelin protein with/without a previous
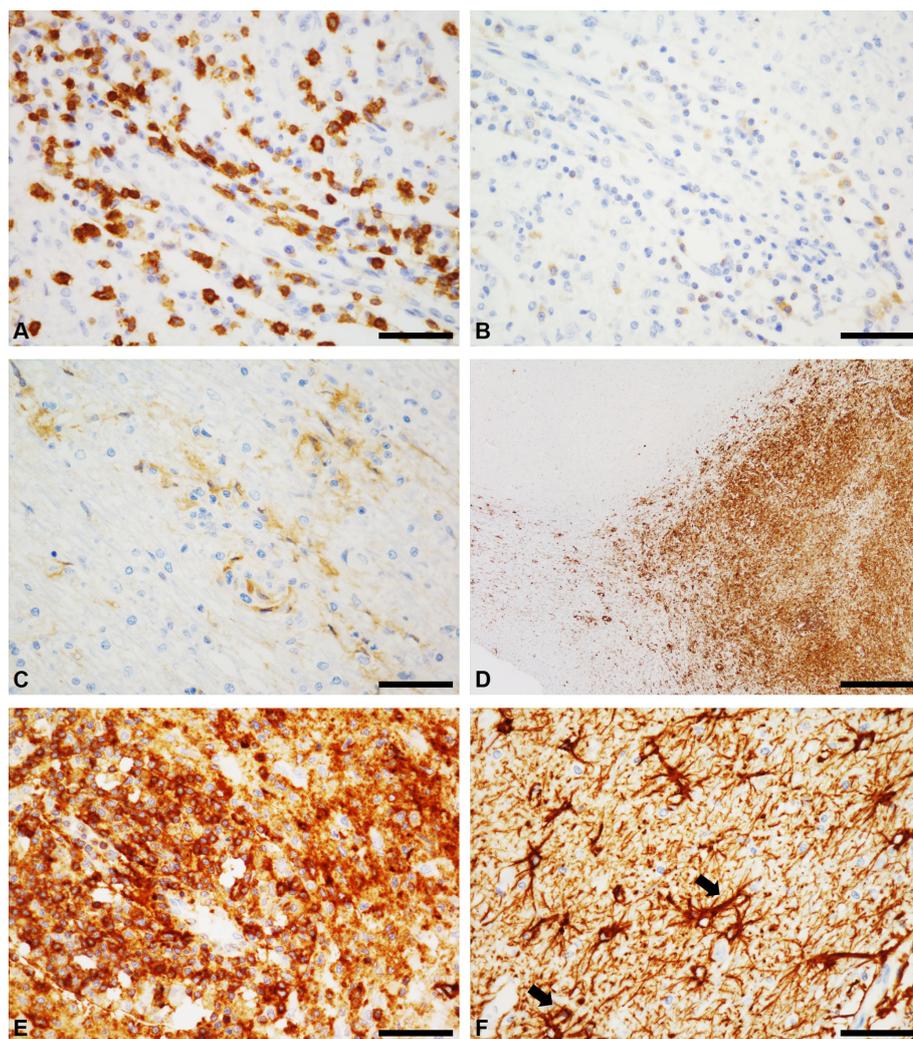
Fig. 3. Photomicrograph of inflammatory cells infiltrating the cerebral white matter. (A) CD3-positive cells, (B) CD79a-positvie cells, and (C) CD18-positvie cells. (D) Diffuse and intense positive membranous staining for major histocompatibility complex class II (MHCII) are noted in the demyelinating foci of the cerebral white matter. Note the adjacent regions only show weak, scattered positive membranous staining. Bar=500 μm. (E) Higher magnification of Fig. 3D. There are abundant MHCII-positive cells. Bar=50 μm. (F) Reactive astrogliosis, characterized by disruption of individual astrocyte domains by overlapping of astrocyte processes (arrows), is demonstrated by glial fibrillary acidic protein (GFAP) staining. Bar=50 μm.

viral infection/vaccination [2, 4, 7, 28]. In this case, none of abovementioned infectious diseases were detected by IHC staining or PCR. For the non-infectious diseases, dogs with GME usually have disseminated angiocentric lesions in the white matter and meninges. NME and GhME in dogs generally causes lesions in the meninges, cerebral cortex, and subcortical white matter [2, 26, 28]. Most importantly, GME, NME, and GhME do not cause PNS lesions making these differential diagnoses less likely in the present case. Furthermore, the lesions of NE are characteristically found in the white matter, but the PNS should not be affected [2, 28]. Alternatively, AP is an autoimmune disease of the PNS and the CNS usually remains intact [2, 28]. The lesion distributions of NE and AP are partially consistent with our case. Although concurrent NE and AP could not be completely ruled out, thus possibility was less likely because NE is a species specific disease and AP is associated with raccoon biting [2, 28](Table 1). Therefore, it is presumed that the current case may share the same pathogenesis of autoimmune reactivity against myelin antigens, which is similar to CCPD in humans.

Based on the disease progression, CCPD is classified into 1) simultaneous CNS and PNS involvement, 2) CNS involvement preceding PNS involvement, or 3) PNS involvement preceding CNS involvement [4, 15, 16]. In this case, mild ataxia and disorientation were initially reported and could be associated with the demyelinating foci and marked astrogliosis in the thalamus and cerebral white matter [3]. Subsequently, tetraparesis ascended upwards from paraparesis with voice change and dysphagia were described and likely associated with the lesions in the PNS. Therefore, the current disease process is suspected to have begun in the CNS, with secondary PNS involvement. The clinical and histological presentation of the current case is similar to human CCPD, which usually presents with paraparesis at disease onset [4]. The underlying pathogenesis of the CCPD-like disease in the current animal is still undetermined, but in humans, antibodies directly against neurofascin (NF)-155 and NF-186 proteins have been identified with higher frequency in patients diagnosed with CCPD [4, 15, 16]. NF186 and NF155 are respectively nodal and paranodal proteins expressed on the axon of both the CNS and the PNS. Therefore, it is proposed that the NF155- and NF186-specific autoimmune response may be important for the development of CCPD in humans [4, 15, 16]. Concurrent leukoencephalomyelitis and polyneuritis in animals have not been reported in the literature. Further investigation into the underlying mechanisms, especially the interactions between infectious agents or vaccination and host immunity, is warranted in CCPD-like cases to identify the true cause of demyelination in the CNS and PNS.
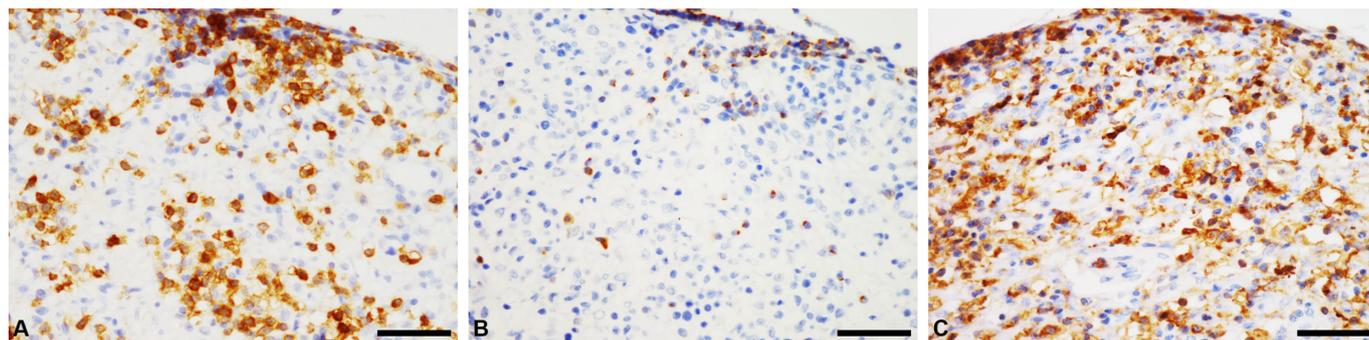
Fig. 4. Photomicrograph of infiltrating inflammatory cells in the spinal nerves. (A) CD3-positive cells and (B) CD79a-positive cells. Numerous cell types are major histocompatibility complex class II (MHCII)-positive (C). Bar=50 μm.
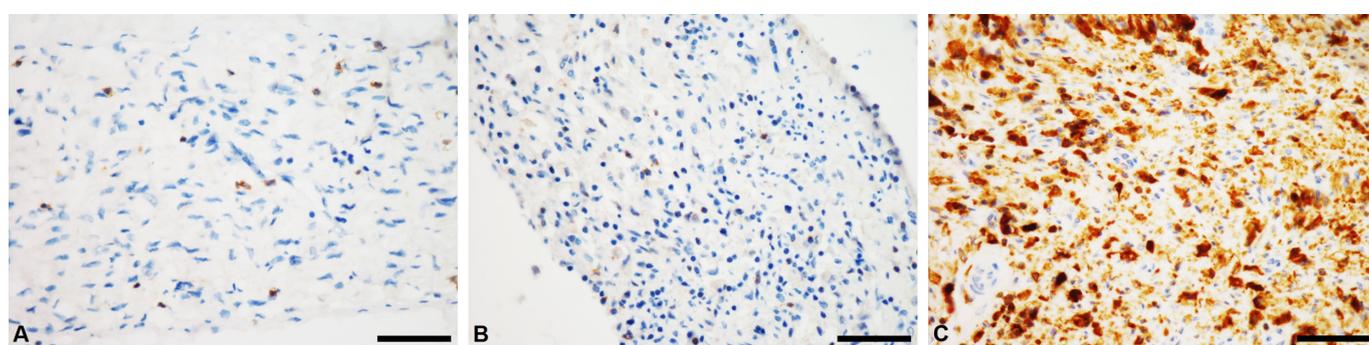

Fig. 5. Photomicrograph of infiltrating inflammatory cells in the sciatic nerves. (A) low numbers of CD3-positive cells and (B) CD79a-positive cells. Numerous cell types are major histocompatibility complex class II (MHCII)-positive (C). Bar=50 μm.

Table 1. Differential diagnoses of canine leukoencephalomyelitis and polyradiculoneuritis and the results of investigation in the current case

Etiology	Lesion distribution					Results of Investigations	
	Central nervous system			Peripheral nervous system		IHC stainings	PCR
	Meninges	Grey matter	White matter	Ganglion	Nerve fiber		
Dengue			Variable			N.A.	-
Japanese encephalitis			Variable			N.A.	-
Zika			Variable			N.A.	-
Canine distemper	Y	Y	Y	N	N	-	-
Pseudorabies	Y	Y	Y/N	Y	N	N.A.	-
Rabies	Y	Y	Y/N	N	N	-	-
Toxoplasmosis	Y	Y	Y	Y/N	Y	N.A.	-
Neosporosis	Y	Y	Y	Y/N	Y	N.A.	-
Leishmaniasis	Y	Y	Y	Y/N	N	N.A.	-
Encephalitozoonosis	Y	Y	Y	N	N	N.A.	-
GME	Y	Y/N	Y	N	N		
NME	Y	Y	Y/N	N	N		
NE	N	Y/N	Y	N	N		
GhME	Y	Y	N	N	N		
Acute polyneuritis	N	N	N	N	Y		
This case	N	N	Y	N	Y		

IHC=immunohistochemical, GME=granulomatous meningoencephalomyelitis, GhME=Greyhound meningoencephalitis, NME=necrotizing meningoencephalitis, NE=necrotizing encephalitis, PCR=polymerase chain reaction, Y=lesions can be found, Y/N=lesions may or may not be found; N=lesions are usually not found; N.A.=not available.

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