ORIGINAL ARTICLE

Suspected tick-borne flavivirus meningoencephalomyelitis in dogs from the UK: six cases (2021)

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Objectives: Tick-borne encephalitis virus and louping ill virus are neurotropic flaviviruses transmitted by ticks. Epidemiologically, tick-borne encephalitis is endemic in Europe whereas louping ill's predominant geographical distribution is the UK. Rarely, these flaviviruses affect dogs causing neurological signs. This case series aimed to describe the clinical, clinicopathological, and imaging findings, as well as the outcomes in six dogs with meningoencephalitis and/or meningomyelitis caused by a flavivirus in the UK in 2021.

Materials and Methods: Observational retrospective case-series study. Clinical data were retrieved from medical records of dogs with positive serological or immunohistochemical results from three different institutions from spring to winter 2021.

Results: Six dogs were included in the study. All dogs presented an initial phase of pyrexia and/or lethargy followed by progressive signs of spinal cord and/or intracranial disease. Magnetic resonance imaging showed bilateral and symmetrical lesions affecting the grey matter of the thalamus, pons, medulla oblongata, and thoracic or lumbar intumescences with none or mild parenchymal and meningeal contrast enhancement. Serology for tick-borne encephalitis virus was positive in five dogs with the presence of seroconversion in two dogs. The viral distinction between flaviviruses was not achieved. One dog with negative serology presented positive immunohistochemistry at *post-mortem* examination. Three dogs survived but presented neurological sequelae. Three dogs were euthanased due to the rapid progression of the clinical signs or static neurological signs.

Clinical Significance: These cases raise awareness of the presence of tick-borne encephalitis as an emergent disease or the increased prevalence of louping ill virus affecting dogs in the UK.

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INTRODUCTION

The *Orthoflavivirus* genus in the *Flaviviridae* family consists of more than 70 positive, single-stranded, enveloped RNA viruses. Many are transmitted by arthropods, such as ticks and mosquitos, and can affect multiple species, including dogs and humans causing a broad spectrum of diseases with common neurological complications (van Leur *et al*., 2021; Thiel et al., 2005).

Tick-borne encephalitis (TBE) is caused by a flavivirus – tick-borne encephalitis virus (TBEV) – that is mainly transmitted via a tick bite, especially *Ixodes ricinus* and *Dermacentor* species (Bogovic & Strle, 2015; Kleeb *et al*., 2021; Phipps & Johnson, 2022). Three different subtypes (European, Siberian and Far-Eastern) have been described in Europe (Phipps & Johnson, 2022), and two recent subtypes (Baikalian and Himalayan) have been described in other parts of the world (Dai *et al*., 2018). The European subtype has an important clinical incidence in Europe, where it presents as an endemic disease in both people and dogs (Bogovic & Strle, 2015; Kaiser, 2008, 2012; Kleeb *et al*., 2021; Pfeffer & Dobler, 2011; Phipps & Johnson, 2022; Riccardi *et al*., 2019; Salat *et al*., 2021; Yoshii, 2019). Around 70% to 98% of human infections are asymptomatic (Kaiser, 2008) and only 20% of dogs present severe disease (Nygren *et al*., 2022). In both humans and animals, the symptomatic cases present a biphasic course, characterised by an initial phase with nonspecific signs such as pyrexia and lethargy followed by the development of severe neurological signs such as altered consciousness, seizures, lower motor neuron paresis or paralysis (especially within the thoracic limbs) and different cranial and spinal nerve dysfunction (Kaiser, 2008; Kleeb *et al*., 2021; Riccardi *et al*., 2019; Salat *et al*., 2021; Yoshii, 2019). Epidemiologically, TBEV affects different species including humans, cattle, dogs, wildlife and small mammals, in which the virus can infect them as primary or reservoir hosts (Kaiser, 2008, 2012; Kleeb *et al*., 2021; Pfeffer & Dobler, 2011; Phipps & Johnson, 2022; Riccardi *et al*., 2019; Yoshii, 2019). Few clinical cases of TBE in dogs have been reported in non-endemic areas such as areas of Southern Europe and Scandinavia (Andersson *et al*., 2020; Pfeffer & Dobler, 2011; Weissenböck & Holzmann, 1997). The incidence of human cases has been gradually increasing in the last decade (Pfeffer & Dobler, 2011; Riccardi *et al*., 2019), reporting sequelae in 20% to 25% of the surviving human patients (Czupryna *et al*., 2018; Nygren *et al*., 2023), and a fatality rate of 2% (Bogovic & Strle, 2015). Scant information is available about survival and sequelae in dogs (Kleeb *et al*., 2021).

Louping ill virus (LIV) is closely related to TBEV and causes a zoonotic disease that shares similarities with TBE, including vector and clinical presentation (Jeffries *et al*., 2014; Reid, 1991). However, LIV primarily affects sheep and red grouse, and its predominant geographical distribution is UK upland areas (Jeffries *et al*., 2014). Similarly, louping ill (LI) rarely affects dogs, although three cases of meningoencephalitis caused by LIV have been previously described by MacKenzie *et al*. (1973), MacKenzie (1982) and Dagleish *et al*. (2018), and they may be underreported. Two cases were diagnosed with LIV, one by mouse inoculation of *post-mortem* examination harvested brainstem and inhibition of virus-induced plaque formation (MacKenzie *et al*., 1973), and the other one by immunolocalization of LIV antigens and genome sequencing (Dagleish *et al*., 2018). The dog that survived was presumptively diagnosed based on antibody seroconversion and compatible clinical signs (MacKenzie, 1982). No cross-sectional imaging was performed in any of these cases.

Although geographic location and primarily affected species are largely different between both diseases, TBEV has recently been detected in ticks (and potentially humans) within the UK, making clinical and serological differentiation difficult due to antibody cross-reactivity between LIV and TBEV (Holding *et al*., 2019, 2020; Pfeffer & Dobler, 2011). This case series describes the clinical, clinicopathological, and imaging features as well as the outcomes of six dogs with meningoencephalomyelitis or meningomyelitis caused by a flavivirus in the UK in 2021.

MATERIAL AND METHODS

This observational retrospective multi-centre case-series study was approved by the Research Ethics Committee of the School of Veterinary Medicine of the University of Glasgow.

The medical records from three UK-based veterinary referral hospitals were searched for dogs presenting with neurological signs and either (1) positive serum antibodies to TBEV and/or LIV, or (2) positive immunohistochemical staining of *post-mortem* tissues for TBEV and/or LIV. Data retrieved included signalment, known or suspected tick exposure, vaccination status and specific information on products/measures used for tick prevention, feeding practices, geographic location and travel history, dog's lifestyle, physical and neurological examination, clinicopathological and diagnostic imaging findings, treatment and outcome, neurological sequelae in the surviving dogs, and *post-mortem* examination findings where available.

Results of haematology, serum biochemistry, serum antibodies against *Toxoplasma gondii* and *Neospora caninum* (indirect immunofluorescence), rapid antigen enzyme-linked immunosorbent assay (ELISA) for *Angiostrongylus vasorum*, rapid antigen (IgG) ELISA for *Dirofilaria immitis*, *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum* and *A. platys*, and *Borrelia burgdoferi*, PCR in cerebrospinal fluid (CSF) for *T. gondii*, *N. caninum* and canine distemper virus (CDV) as well as CSF analysis (total cell count, nucleated cell count and protein levels) were included. Other tests were performed in some cases: urinalysis and urine culture, urine organic acid metabolic screen (NHS Cambridge University Hospitals, Blood Sciences, Cambridge, UK), abdominal and cardiac ultrasound examinations, and CSF bacterial cultures.

Magnetic resonance imaging (MRI) was obtained with three different 1.5 Tesla scanners (Gyroscan ACS NT, Philips Medical System; Canon Vantage Elan, Canon Medical Systems

Europe B.V., and Intera 1.5T, Phillips Healthcare, Amsterdam, Netherlands). Imaging protocols were different for each referral centre; however, all of them included T2w and T1w pre- and post-gadolinium sequences.

Where performed, the following specific diagnostic results for TBEV and LIV were included in results; these varied between patients but included serological analysis for TBEV and LIV in serum and CSF performed by ELISA (IgM and/or IgG) techniques for TBEV (Laboklin, Manchester, UK) and by hemagglutination inhibition for LIV (Moredun Research Institute, Penicuik, UK). It was not possible to perform virus neutralizations due to small sample size. Genetic testing included RT-PCR in CSF, *post-mortem* formalin-fixed and paraffin-embedded from brain and cardiac tissues for TBEV (Laboklin, Manchester, UK), and qRT-PCR for LIV (Moredun Research Institute, Penicuik, UK). *Post-mortem* examination and immunohistochemistry were performed in the brain and heart for TBEV when available. Virus isolation was attempted on A549 Npro cells as per Hilton *et al*. (2006), with plaque or immunofocus assays carried out on the same cells (6 days for cultures). Sequencing data is available in the Short Read Archive (NCBI SRA accession: PRJNA910132).

Next-generation sequencing was performed in clinical samples or cell culture samples following attempts to isolate the virus, using an untargeted sequencing approach (G-Meta method) as per Thomson *et al*. (2016), followed by sequencing on a NextSeq Illumina sequencer. A subset of these libraries was subsequently enriched using a pan-viral probe capture design, the VirCapSeq-VERT Capture Panel (https://sequencing.roche.com/content/dam/rochesequence/worldwide/resources/brochure-vircapseq-vert-capture-panel-SEQ1000117.pdf), and sequenced on a MiSeq Illumina sequencer. Next-generation sequencing data were processed using a standard metagenomic pipeline which involved quality filtering, depleting reads that map to the dog genome, de novo assembly, and classification of the contigs against the NCBI non-redundant database.

Follow-up information was obtained on patient re-evaluation or telephone updates with the owners of the animals that survived. Descriptive statistics are presented as median, range, and percentages.

RESULTS

Six dogs were included in the study: three entire males, two neutered males, and one entire female. Breeds included Labrador retriever (3/6), Cocker spaniel (1/6), Rottweiler (1/6) and Crossbreed (1/6) with a median age of 3.5 years old (range 1 to 5). Four dogs lived in Scotland, one in North East England, and one in South East England. Although all dogs were reported to be on schedule for vaccination, ectoparasite and endoparasite treatments, information regarding specific drugs and intervals was missing in one dog. The specific treatments and intervals are summarised in Table 1. Three dogs were reported to have a recent tick exposure, within 4 weeks of presentation, as owners found single ticks. No history of travelling was reported for any of the dogs, and all of them were fed with a commercial diet.

The duration of the clinical signs before referral ranged from one to 21 days (median of 5 days). Five dogs received different medications at their local practices, including probiotics, antibiotics (amoxicillin-clavulanic, clindamycin, metronidazole), anti-emetics (maropitant), anti-epileptic (phenobarbital, diazepam) and anti-inflammatory drugs (meloxicam, dexamethasone in two dogs). Although all dogs presented with pyrexia at some point during the clinical course, only four dogs were pyretic on admission (median 39.5°C, range 38.5 to 40.7). All dogs presented with acute or subacute progressive neurological signs. The neuroanatomical localisation was defined as multifocal for three dogs, cerebellum for one dog, C6-T2 for one dog and L4-S1 spinal cord segment for one dog. Five dogs presented with non-ambulatory tetraparesis with lower motor neuron signs affecting the thoracic limbs in three dogs, and additional severe flaccidity of the head and neck in one dog. One dog presented non-ambulatory paraparesis with lower motor neuron signs affecting only the right pelvic limb which progressed to paraplegia. Other neurological findings were observed in the dogs, such as erratic behaviour (4/6), facial hyperaesthesia (4/6), inconsistent bilateral menace responses (3/6), generalised tonic–clonic seizures (2/6), spinal hyperaesthesia (2/6), intermittent swaying movements of the head (2/6), proprioceptive (1/6) and cerebellar (1/6) ataxia, reduced vestibulo-ocular reflex (1/6), mild bilateral miosis (1/6) and intermittent bilateral medial strabismus (1/6). Signalment and clinical and neurological findings for each dog are summarised in Table 1.

MRI was performed in five dogs, including the head (4/5), cervical (3/5) and/or thoracolumbar vertebral column (1/5). The imaging findings are summarised in Table 2. The lesions affected mainly the grey matter of different parts of the central nervous system (CNS) such as the ventral horns of the thoracic intumescence (3/5), thalamus, pons, and medulla oblongata (3/5), basal nuclei (1/5), mesencephalic periaqueductal grey matter (1/5) and T7 to L4 spinal cord segments (1/5). The lesions appeared bilateral and symmetrical, with poorly defined margins and intramedullary/intra-axial T2W hyperintense and T1W isointense in four dogs. One dog presented bilateral T2W hyperintensities in the piriform lobes. There was none (3/5) or mild (2/5) contrast enhancement within the central nervous system parenchyma and meninges (Fig 1). Additional imaging findings included multifocal T2W hyperintensities with mild contrast enhancement within the epaxial cervical (1/5) and psoas (1/5) muscles. MRI of the brain was unremarkable in one dog who presented lesions within the cervical spine.

Haematology and serum biochemistry were available for all dogs. All but one dog demonstrated mild non-regenerative anaemia (median 32.5%, range 30 to 35.4). One dog demonstrated ventricular tachycardia on an electrocardiogram, therefore, cardiac troponin was measured, revealing marked elevation (>50 ng/mL, range 0 to 0.11). A total of seven CSF collections from the six dogs were performed: five from the cerebellomedullary cistern only and two from the lumbar cistern in the same dog. Lumbar CSF collection was repeated in dog 4 three weeks after the initial diagnosis due to deterioration after immunosuppressive

Table 1. Summary of signalment and clinical and neurological findings of the affected dogs

	Breed	Age (Y)	Gender	Area	Ectoparasites prevention	Tick bite	Temp (°C) *	Neurological signs	Neurolocalisation	Other findings
1	Cocker spaniel	4	MN	Inverness-shire (Scotland)	Fluralaner Q3 months Milbemycin oxime/ praziquantel Q1 month	Yes	38.6	Non-ambulatory tetraparesis with marked flaccidity of the neck. Absent PR and reduced withdrawal reflexes in TLs. Normal reflexes in PLs. Subjectively bilateral miosis	C6-T2 spinal cord segments	Severe hypoventilation for 48 hours due to reduced thoracic muscle compliance
2	Labrador retriever	1	FE	Aberdeenshire (Scotland)	Not recorded	No	40.5	Erratic behaviour, vestibular ataxia which evolved to non-ambulatory tetraparesis. Absent PR and reduced withdrawal reflexes in TLs. Facial hyperesthesia, intermittent bilateral sway of the head, bilateral inconsistent menace responses	Cerebellum	Low body condition
3	Crossbreed	5	ME	Lanarkshire (Scotland)	Afolxolone Q3 months	No	40.1	Erratic behaviour, generalised tremors, cerebellar ataxia which evolved to non-ambulatory tetraparesis. Absent PR in all limbs, reduced spinal reflexes and muscle tone (TLs>PLs). Hyperaesthesia upon cervical manipulation	Multi-focal (C6-T2+forebrain)	Bilateral elbow discomfort
4	Labrador retriever	3	MN	Northumberland (North England)	Imidacloprid/ moxidectin Q1 month Praziquantel Q3 months	No	38.5	Initial ambulatory paraparesis with severe LMN in right PL. Deterioration after immunosuppression: non-ambulatory paraparesis, absent movement, PR in right pelvic limbs. Reduced reflexes and muscle tone in PLs. Severe lumbar pain	L4-S1 spinal cord segments	–
5	Labrador retriever	4	ME	Fife (Scotland)	Febantel/ pyrantel pamoate/ praziquantel Q3 months Fluralaner Q3 months	Yes	40.1	Non-ambulatory tetraparesis. Absent PR in all limbs. Facial hyperesthesia, bilateral inconsistent menace responses, reduced oculocephalic reflex. Partial seizures evolved to status epilepticus	Multi-focal (forebrain+brainstem)	–
6	Rottweiler	1	ME	Buckinghamshire (South East England)	Milbemycin oxime/ praziquantel Q1 month	Yes	39	Generalised tonic–clonic seizures and episodes of aggression. Progression to non-ambulatory tetraparesis with erratic behaviour and facial hyperesthesia. Mildly reduced PR in the PLs. Normal spinal reflexes. Intermittent bilateral medial strabismus	Multi-focal (forebrain+brainstem)	Ventricular tachycardia, hypotension, adipsia (hypodipsic), hypernatraemia

FE Female entire, LMN Lower motor neuron, ME Male entire, MN Male neutered, TLs Thoracic limbs, PLs pelvic limbs, PR postural reactions, Y years
*Temperature upon admission of the dogs

Table 2. Summary of imaging and clinicopathological of the affected dogs

	MRI findings	CSF results	HCT (%)	Other findings
1	Bilateral, symmetrical, and diffuse T2w hyperintense and T1w isointense lesions in thalami, pons, medulla oblongata and ventral horn grey matter of the cervical spine. No contrast enhancement	Lymphocytic pleocytosis (TNCC: 36 cells/μL; proteins: 48 mg/dL)	32.5	Moderate leucocytosis (WBC 20.9×10^9) *Toxoplasma* and *Neospora* serologies: negative 4Dx SNAP test: negative
2	Bilateral, diffuse and symmetrical T2w hyperintense lesions at the grey matter of C5-7 spinal cord segments and thalamus. Mild contrast-enhancement parenchyma and brain leptomeninges Multi-focal T2w hyperintensities in left epaxial muscle C2	Lymphocytic pleocytosis (TNCC: 145 cells/μL; proteins: 118.3 mg/dL). Sterile culture	33	Mild leukopenia (WBC 5.03×10^9) *Toxoplasma*, *Neospora* and *Angiostrongylus vasorum* serologies: negative 4Dx SNAP test: negative
3	Bilateral, symmetrical and diffuse T2w hyperintense lesions in the grey matter of the C5-6 spinal cord. No contrast enhancement Normal MRI of the brain	Lymphocytic pleocytosis (TNCC: 8 cells/μL; proteins: 48 mg/dL) Sterile culture	35.4	Moderate leukopenia (WBC 4.1×10^9) Mildly increased methylmalonate acid in urine *Toxoplasma*, *Neospora* and *Angiostrongylus vasorum* serologies: negative 4Dx SNAP test: negative
4	Extensive, diffuse, and slight right lateralisation T2w hyperintense and T1w isointense lesion at T7 to L4 spinal cord segments. Mild contrast enhancement. Faint contrast enhancement from L1 to L4 spinal nerves and adjacent muscles	Initial CSF: mixed pleocytosis (TNCC: 830 cells/μL; proteins: 1329 mg/dL) Second CSF: mixed pleocytosis (TNCC: 38 cells/μL; proteins: 105 mg/dL)	31.7	*Toxoplasma* and *Neospora*: negative
5	–	–	30	–
6	Bilateral, diffuse, symmetrical intra-axial T2w and FLAIR hyperintense and T1w isointense lesions in the thalamus, pons, medulla oblongata and basal nuclei. No contrast enhancement. Mild enlargement and bilateral T2w and FLAIR hyperintensity in piriform lobes (suspected postictal)	Lymphocytic pleocytosis (TNCC: 220 cells/μL; proteins 66 mg/dL)	50.1	Cardiac troponin I: >50 ng/L (RI: 0.00 to 0.11) Urinalysis and culture: negative *Toxoplasma* and *Neospora* serologies: negative 4Dx SNAP test: negative *Toxoplasma*, *Neospora* and distemper virus PCRs: negative

MRI Magnetic resonance imaging, FLAIR Fluid attenuation inversion recovery, HCT Haematocrit, TNCC Nucleated cell count, T1w T1-weighted sequence, T2w T2-weighted sequence

treatment. Mild to moderated mixed or lymphocytic pleocytosis was observed in all dogs (total nucleated cell count 8 to 430 cells/μL, protein concentration 48 to 1329 mg/dL). Serum serologies for *T. gondii* (IgG and IgM) and *N. caninum* (IgG) (5/6), rapid antigen/antibody ELISA from serum or complete blood for *D. immitis*, *E. canis*, *E. ewingii*, *A. phagocytophilum* and *Anaplasma platys*, and *B. burgdoferi* (4/6), rapid antigen ELISA from serum for *A. vasorum* antigen (2/6) and PCR for *T. gondii*, *N. caninum* and CDV in CSF (1/6) were negative in all dogs tested. Aerobic and anaerobic bacterial cultures of CSF yielded no growth for the two tested dogs. Urinalysis was normal and urine culture yielded no growth in one dog. Urine organic acids metabolic screen revealed a non-clinically significant mild increase in methylmalonic acid in one dog (60.1 μmol/mmol creatinine, range 0 to 10). The clinicopathological findings for each dog are summarised in Table 2.

Further serological studies in serum (5/6) and CSF (1/6) were conducted for TBEV and/or LIV considering the clinical and MRI similarities with previously reported dogs with confirmed TBEV by Weissenböck *et al.* (1998), Beckmann *et al.* (2014), and Beckmann *et al.* (2015), and showed suspected active disease in presence of seroconversion (Salat *et al.*, 2021). Dogs 1 to 5 had presence of serum antibodies for TBEV (IgM and/or IgG). Antibodies were absent in CSF for dog 6. Dogs 1 to 3 presented serum antibodies for LIV and dogs 1 and 3 showed seroconversion in the fourth and second weeks from diagnosis, respectively. CSF samples in dogs 1, 2 and 5 and paraffin-embedded formalin-fixed brain and heart in dog 6 were submitted for qRT-PCR for LIV. Two CSF samples from dogs 2 and 6 were submitted for TBEV PCRs. All PCRs samples yielded negative or inconclusive results. A *post-mortem* examination of dog 6 revealed severe lymphocytic and histiocytic meningoencephalitis with neuronal degeneration and, necrosis, gliosis, and glial nodule formation (Fig 2). Immunohistochemistry was positive in neurons and axons of the cortex for flavivirus and, based on this distribution, TBEV infection was suspected in this dog (Weissenböck *et al.*, 1998). Next-generation sequencing was performed in clinical samples (dogs 2 to 4) or cell culture samples following attempts to isolate the virus (dog 1). A subset of these libraries, corresponding to dogs 1 to 3, was subsequently enriched using a pan-viral probe capture design (VirCapSeq-VERT Capture Panel). The number of reads for each sample following trimming and the number of contigs produced by the de-novo assembly is indicated in (Table S1). However, viral sequencing for differentiation of RNA from the CNS or following attempts to isolate the virus in cell culture did not give conclusive information on the nature of the virus, probably because viraemia had passed. All serological, genetic, and immunohistochemistry results are summarised in Table 3.

Four dogs survived to discharge (1 to 14 days, mean 6 days of hospitalisation), and three dogs were alive at the time of writing,

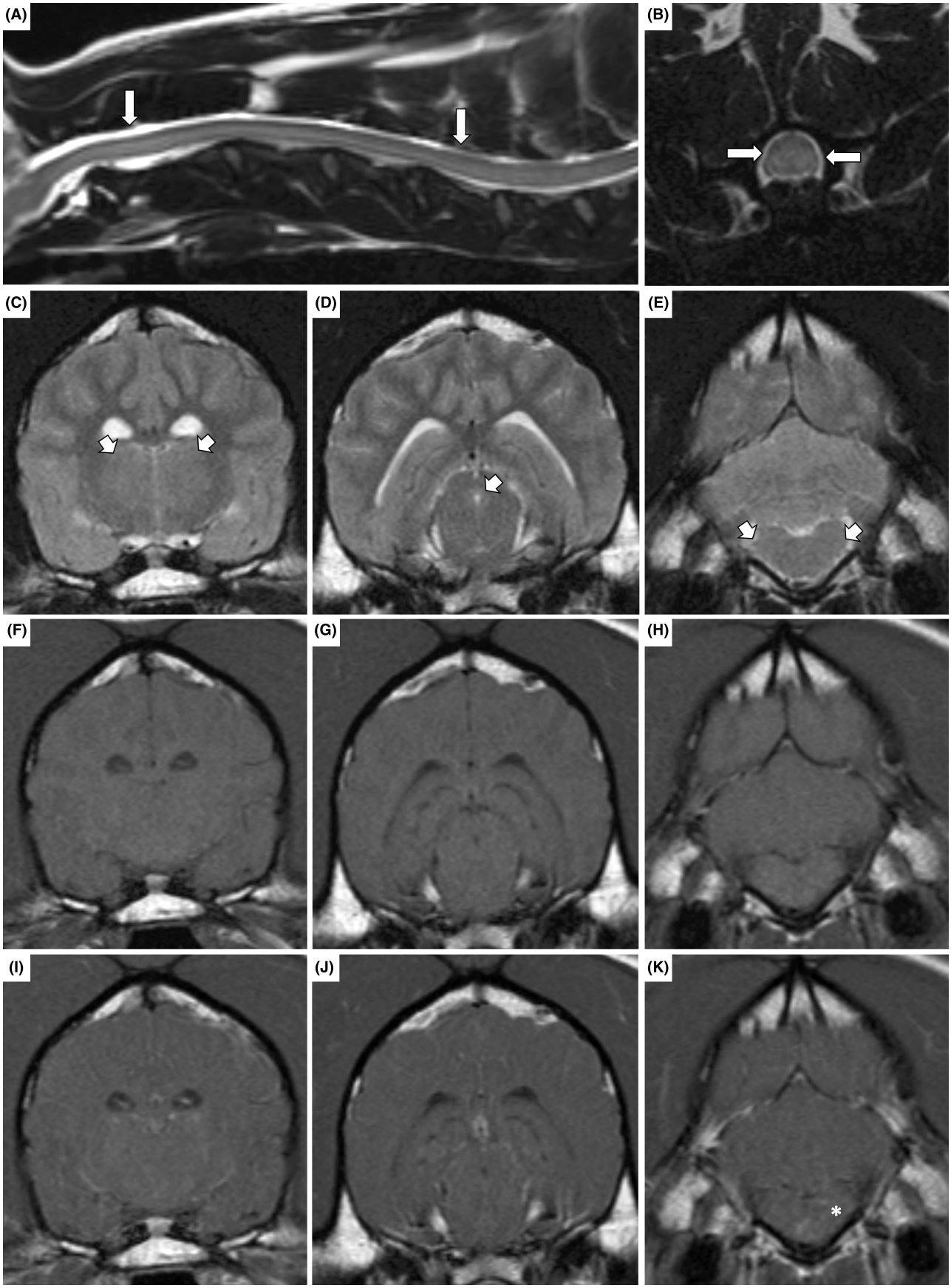

FIG 1. (Legend on next page)

FIG 1. (A,B) Sagittal and transverse sequences of magnetic resonance imaging (MRI) of the cervical spine of dog 1. (C-K) Transverse sequences of the MRI of the brain of dog 2 in T2-weighted (C, D, E), T1-weighted (F, G, H) and T1-weighted post-contrast (I, J, K). (B) T2w transverse sequence at the level of C6 vertebral body, showing bilateral symmetrical intramedullary hyperintensities (arrows) of the ventral horn of the grey matter. (A) Sagittal T2w MRI sequence of the same dog, showing diffuse intramedullary hyperintensities from the middle vertebral body of C5 to the caudal endplate of the C7 vertebral body (arrows). (C) Bilateral symmetrical diffuse intramedullary hyperintensities affecting both thalami, periaqueductal grey matter and dorsomedial area of the medulla oblongata (arrowheads). (D, E) The lesion in the T2w sequences appeared isointense in T1w with mild meningeal contrast enhancement and diffuse and patchy contrast enhancement within the medulla oblongata (asterisk)

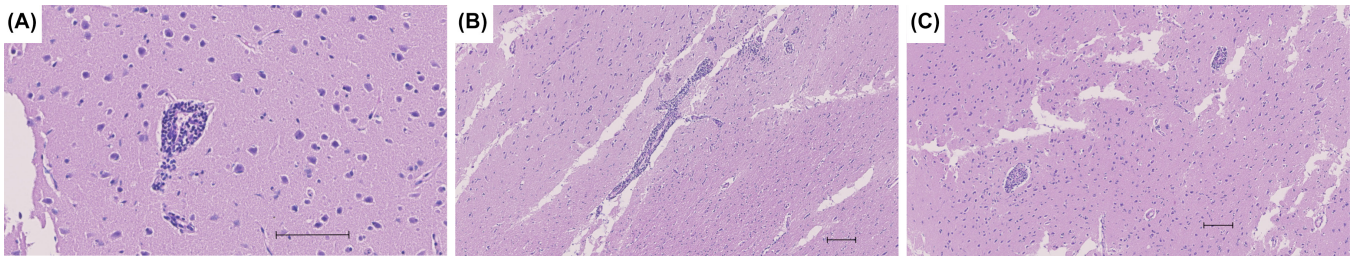

FIG 2. Cerebrum (×3, dog 6). Multi-focally through the section Virchow-Robins space is expanded by high numbers of lymphocytes and plasma cells, and there are multi-focal glial nodules within grey matter. Tissues are negatively affected by autolysis and freeze–thaw artefact. Haematoxylin and eosin. Bar=100 µm

Table 3. Summary of serologies, genetic and *post-mortem* examination results for LIV and TBEV for the affected dogs

	LIV*		TBEV†		PCR		Virus isolation/ identification
	IgM	IgG	IgM (LE)	IgG (U/mL)	LIV	TBEV	LIV/TBEV
1	1:40	1:10	–	306.6	Negative RT-PCR (CSF)	–	Negative next-generation sequencing and cell culture
4-weeks post-diagnosis	1:320	1:40	–	–			
2	1:320	1:10	18.83	177	Negative RT-PCR (CSF)	Negative PCR (CSF)	Negative next-generation sequencing
3	–	–	47	97.52	–	–	–
2-weeks post-diagnosis	1:5220	1:320	–	–			
4	–	–	–	50.7‡	–	–	Negative next-generation sequencing
5	–	–	14.9	171.9	Negative RT-PCR (CSF)	–	–
6	–	–	–	Negative§	Negative RT-PCR (brain and cardiac tissue)	Negative PCR (CSF)	Positive immunohistochemistry in PEFF for TBEV

CSF Cerebrospinal fluid, IgG Immunoglobulins G, IgM Immunoglobulins M, LIV Louping ill virus, PCR Polymerase chain reaction, PEFF Paraffin-embedded formalin-fixed, RT-PCR Real-time polymerase chain reaction, TBEV Tick borne encephalitis virus
*Hemagglutination inhibition test. Moredun Research Institute, Bush Loan, UK. Reference ranges: 1:20 is considered positive; <1:20 equivocal result
†ELISA. Laboklin, Manchester, UK. Reference ranges: IgM<25 LE; IgG<63 U/mL
‡ELISA technique but different reference range: IgG<20 U/mL
§Serology performed in CSF

1 year and a half after diagnosis. Dog 5 developed status epilepticus after admission and the owner elected humane euthanasia. Dog 6 presented fair control of the seizures but subsequently developed abnormal behaviour, fluctuating non-ambulatory tetraparesis, head tremors, lethargy and pyrexia. This dog continued deteriorating, developing hypodipsia, hypernatraemia and ventricular tachycardia 5 days after admission. Therefore, the owners elected humane euthanasia. Dog 4 was re-admitted 3 weeks after the initial diagnosis of suspected immune-mediated meningomyelitis; he experienced neurological deterioration presumptively caused by the initial immunosuppressive treatment with cytosine arabinoside and cyclosporine. Treatment included a variety of short courses of corticosteroids, antibiotics and an intensive physiotherapy and nursing care programme (Table 4). Five dogs received an initial immunosuppressive dose of dexamethasone at 0.15 to 0.3 mg/kg (1 to 7 days, mean 3 days), followed by prednisolone at immunosuppressive or anti-inflammatory doses (0.5 to 2 mg/kg) in four dogs, decreased over a period from 3 to 8 weeks. Five dogs received different antibiotics: two dogs received a single dose of amoxicillin-clavulanic 15 mg/kg, two dogs had clindamycin 12 mg/kg twice a day for 3 and 7 days, respectively, and one dog received metronidazole 10 mg/kg twice a day for 7 days.

One dog was euthanased due to a static neurological condition and owners' concerns about signs of non-specific generalised discomfort 3 weeks after diagnosis. Three dogs showed neurological improvement within 2 to 3 months from the diagnosis. All were ambulatory but presented mild to moderate sequelae such as moderate paresis of the cervical musculature causing weakness of the head, mild paresis of the thoracic limbs, mild paresis and proprioceptive ataxia of the pelvic

Table 4. Summary of treatment, outcome and sequelae of the affected dogs

	Hospitalisation (days)	Treatment	Outcome	Sequelae
1	14	Corticosteroids: • Dexamethasone 0.3 mg/kg for 72 hours • Prednisolone (4-week course) 0.5 mg/kg for 2 weeks, 0.25 mg/kg for 1 week, and 0.125 mg/kg for 1 week Amoxycillin-clavulanic 15 mg/kg once a day Omeprazole 1 mg/kg twice a day for 4 weeks Lactulose 3 mL four times a day for 2 weeks Vitamin complex once a day for 6 weeks	Good Ambulatory with weakness in cervical muscles 3 months after diagnosis	Moderate paresis of the neck and ataxia in TLs
2	8	Corticosteroids: • Dexamethasone 0.3 mg/kg for 7 days • Prednisolone (3-week course) 2 mg/kg for 2 weeks, 1 mg/kg for 1 week Clindamycin (12 mg/kg) twice a day for 7 days Paracetamol 15 mg/kg three times a day	Euthanasia 3 weeks after diagnosis. Absence of neurological improvement and unspecific signs of discomfort	–
3	14	Corticosteroids: • Dexamethasone 0.3 mg/kg for 48 hours • Prednisolone (3-week course) 1 mg/kg for 7 days, 0.5 mg/kg for 5 days, 0.25 mg/kg for 5 days, 0.125 mg/kg for 5 days Metronidazole 10 mg/kg twice a day for 7 days Probiotics once a day for 3 weeks	Good Ambulatory 2 months after diagnosis	Moderate paresis and reduced muscle tone of the TLs
4	6 (1st) 10 (2nd)	Prednisolone (8-week course): • Initial immunosuppression: 2 mg/kg for 7 days, 1 mg/kg for 2 weeks • After deterioration: 2 mg/kg for 7 days, halving dose every 7 days for 5 weeks Cytosine arabinoside (200 mg/m^2 over 8 hours; 2 cycles separated by 3 weeks) Ciclosporin 5 mg/kg twice a day for 5 weeks from deterioration	Good. Ambulatory 2 months after diagnosis	Moderate paresis of right PL
5	1	Dexamethasone 0.5 mg/kg once Amoxycillin-clavulanic 15 mg/kg once a day Midazolam 0.3 mg/kg once Phenobarbital 3 mg/kg once Levetiracetam 60 mg/kg once	Euthanasia after deterioration from admission	–
6	5	Levetiracetam 30 mg/kg three times a day Clindamycin 12.5 mg/kg twice a day Dexamethasone 0.15 mg/kg once a day for 2 days Lidocaine 2 mg/kg iv followed by 20 µg/kg/minute	Euthanasia 5 days after diagnosis due to severe neurological deterioration, ventricular tachycardia, hypotension and hypernatraemia	–

TLs Thoracic limbs, PLs Pelvic limbs, iv Intravenous

limbs, and occasional mild generalised body tremors. At the time of writing this report, the three dogs continue receiving physiotherapy and improving. All treatments and outcomes are summarised in Table 4. Find videos about the progression of the dogs in Video S1.

DISCUSSION

The present study describes six dogs with meningoencephalomyelitis or meningomyelitis associated with a tick-borne flavivirus in the UK during 2021. These cases shared similar clinical signs, ranging from initial pyrexia and lethargy to behavioural abnormalities, progressive ataxia and paresis. Although not all dogs presented with pyrexia on admission, this was present in all dogs at some point in the course of the disease (before or after admission). Four dogs presented lower motor neuron signs, including severe flaccidity of the cervical muscles in one of these dogs. Two dogs did not show lower motor neuron signs but presented generalised tonic seizures and abnormal behaviour. In the literature, symptomatic cases present a biphasic appearance with an initial unspecific period of pyrexia, anorexia and lethargy lasting a maximum of 10 days followed by the development of the neurological signs (Kaiser, 2008, 2012; Kleeb *et al.*, 2021; Pfeffer & Dobler, 2011; Riccardi *et al.*, 2019; Yoshii, 2019). Although no pathognomonic signs have been associated with TBEV infections, the presence of severe neck flaccidity has been previously described by Horger *et al.* (2012), Beckmann *et al.* (2015), and Kleeb *et al.* (2021) and raises the suspicion of extensive cervical myelopathy from which differential diagnoses are limited and unlikely to be caused by other more common disorders such as intervertebral disc degeneration (Argent *et al.*, 2022).

In the present study, three owners reported evident tick exposure. The main mechanism of transmission for both TBEV and LIV is through a tick bite, mainly by *I. ricinus* and *Dermacentor* species (Jeffries *et al.*, 2014; Kleeb *et al.*, 2021; Leschnik *et al.*, 2002; Pfeffer & Dobler, 2011; Reid, 1991). However,

other mechanisms of transmission have been reported such as ingestion of unpasteurized milk or contact with biological fluids from infected animals (Jeffries *et al.*, 2014, Kleeb *et al.*, 2021, Leschnik *et al.*, 2002, Pfeffer & Dobler, 2011). Various wild animals act as reservoirs for these viruses and can show high tick infestation rates (Jeffries *et al.*, 2014). The presence of reservoir hosts (rodents and small mammals) could explain the development of the disease in dogs living in urban areas without direct access to livestock, as tick transmission is likely as mentioned by Jeffries *et al.* (2014). Nonetheless, tick exposure could have been missed by the owners, as previously reported for dogs with TBE, and animals can show signs of disease once ticks have completed their feeding (Kaiser, 2008, 2012; Kleeb *et al.*, 2021; Pfeffer & Dobler, 2011; Riccardi *et al.*, 2019; Yoshii, 2019). These clinical entities have a seasonal presentation in correlation with the tick's biological activity with increased odds for tick-borne transmittable diseases during early to late spring and autumn (Beckmann *et al.*, 2015; Jeffries *et al.*, 2014; Kleeb *et al.*, 2021). All dogs presented between spring to winter as previously described in other studies. Further investigations are warranted to correlate the presence of TBE and/or LI in dogs in the UK with other factors such as climate change or changes in life patterns of dog owners (increased access to the countryside during the lockdowns of the COVID-19 pandemic).

MRI was obtained in five dogs. No imaging was performed in one dog as euthanasia was elected by the owners due to the rapid progression of the clinical signs. Although MRI findings differed between dogs, they showed similar features characterised by bilateral, symmetrical and diffuse lesions affecting the grey matter of different parts of the CNS. Additionally, there was no or only a mild degree of contrast enhancement within the parenchyma and/or meninges. These findings resembled MRI characteristics previously reported in dogs with confirmed TBE in Central Europe (Beckmann *et al.*, 2015), where all dogs but one showed symmetrical and bilateral affecting the grey matter of the hippocampus, basal nuclei, thalamus, brainstem, and ventral horn of the spinal cord with none or mild contrast enhancement. One dog from the present study showed asymmetrical lesions affecting the thoracolumbar spinal cord segments which have been described in previous studies with dogs with TBE (Beckmann *et al.*, 2015; Kleeb *et al.*, 2021). On histopathological examination, both TBEV and LIV infections show neuronophagia and gliosis throughout the grey matter of the brainstem, cerebellum and ventral horn of the spinal cord (Horger *et al.*, 2012; Sykes & Tipold, 2023), described as non-suppurative meningitis (Kaiser, 2008, Kaiser, 2012, Kleeb *et al.*, 2021, Pfeffer & Dobler, 2011, Riccardi *et al.*, 2019, Yoshii, 2019). Similar imaging characteristics have been found in humans infected with TBEV, but the cerebellum is frequently affected when compared to dogs (Horger *et al.*, 2012). Comparison between TBE and LI imaging findings is not possible due to the scant published reports in dogs in which MRI was not part of the diagnosis (Dagleish *et al.*, 2018; MacKenzie, 1982; MacKenzie *et al.*, 1973). As previously discussed, LIV affects mainly sheep and grouse and, to the authors' knowledge, cross-sectional imaging has never been obtained for these species (Jeffries *et al.*, 2014; Reid, 1991). An MRI was performed for suspected meningoencephalitis caused by LIV in a human patient with refractory status epilepticus, showing no major abnormalities and, therefore, unclear diagnosis (Walkington *et al.*, 2013).

Serology yielded positive results in all but one dog for TBEV and the three tested dogs for LIV. Additionally, two dogs with positive LIV antibodies presented seroconversion 2 and 4 weeks after diagnosis. These results were consistent with a recent infection rather than simple exposure. Two of them presented substantially increased IgG antibodies for TBEV in addition to compatible clinical and imaging findings, and a recent tick exposure in one dog. Dog 6 presented negative antibodies for TBEV in CSF, but the diagnosis was achieved on *post-mortem* examination and immunohistochemistry antigen demonstration in the CNS. Importantly, the differences between TBEV and LIV IgM and IgG antibodies were insufficient to provide a final diagnosis or the identity of the infecting virus. Cross-reactivity has been highly recognised between different flaviviruses, leading to positive serological results for LIV and TBEV infections (Gilbert *et al.*, 2020), which is likely to explain the positive results obtained for both diseases in the present study. However, other possibilities should be considered. Firstly, LIV seropositive results could have been obtained in dogs without clinical disease living in an endemic area such as Scotland (Jeffries *et al.*, 2014, Reid, 1991) as well for TBEV (Salat *et al.*, 2021). Secondly, viral recombination between LIV and TBEV has been reported in Europe (Bertrand *et al.*, 2012; Uzcategui *et al.*, 2012), but a recent genomic study in LIV has found no detectable signal of recombination (Clark *et al.*, 2020). The recent detection of TBEV in the UK (Holding *et al.*, 2019) and TBEV in dogs in other non-endemic European areas (Andersson *et al.*, 2020; García-Bocanegra *et al.*, 2018; Pfeffer & Dobler, 2011; Weissenböck & Holzmann, 1997) has added the possibility of recombination between the two agents, but this is an unlikely explanation. Other flaviviruses were not tested, *e.g.* West Nile virus (WNV), due to the different arthropods and geographical distribution of this virus (García-Bocanegra *et al.*, 2018). However, WNV has been detected as an emergent virus in non-endemic areas, challenging the diagnosis for different species because of the cross-reactivity with related flaviviruses (García-Bocanegra *et al.*, 2018; Magouras *et al.*, 2022). Neuropathological findings were similar to those previously reported in dogs with TBE (Kleeb *et al.*, 2021; Weissenböck *et al.*, 1998). Tick-borne flaviviruses present a tropism for neurons that are subsequently damaged and undergo neurophagia. Moreover, lymphocytic and plasmacytic inflammatory perivascular infiltration are frequently encountered. Immunohistological demonstration of TBEV is a useful method for diagnosis in dogs compared to humans as in the latter TBEV immunohistochemistry confirmation is only achieved in fatal cases with relatively short courses of the disease (Gelpi *et al.*, 2005; Weissenböck *et al.*, 1998). However, this technique is not specific; therefore, it could result in cross-reactivity with other flaviviruses as serum antibody testing.

Next-generation sequencing and PCR-based analysis were performed on CSF and brain and/or cardiac samples with negative or inconclusive results in the four tested dogs. This finding is not surprising as the presence of the TBEV in blood and CSF has been reported as insignificant once the initial viremic phase is finished (Kaiser, 1999, 2012; Marriott *et al.*, 2006; Veje *et al.*, 2018). A recent study by Alnefelt *et al.* (2021) has demonstrated the utility of CSF antibody analysis compared to PCR in TBEV and LIV infections. However, in the present study, a dog showed negative TBEV serology and PCR in CSF, but positive immunohistochemistry on *post-mortem* examination of the brain. The absence of antibodies in both serum and CSF samples, and positivity in *post-mortem* immunohistochemistry might be due to the hyperacute progression of the clinical signs (dog 6).

Three dogs are still alive at the time of writing more than 1 year after diagnosis. Four of the dogs survived to discharge but one dog was euthanased 3 weeks after diagnosis as she presented no further improvement. All the surviving dogs presented some degree of paresis affecting the neck, thoracic or pelvic limbs. One dog persisted with paresis of the cervical muscles 1 year after diagnosis but he was able to maintain his head in a rasing position. The survival rates for TBEV and LIV infections vary between species. In human cases diagnosed with TBE, a low fatality rate has been reported, though this depends on the subtype (Phipps & Johnson, 2022), as well as post-recovery sequelae in 20% to 25% of the surviving patients (Czupryna *et al.*, 2018; Nygren *et al.*, 2023). The mortality rate of dogs infected with TBE is higher than the observed in human cases, accounting for 33% of the dogs during the first four months from diagnosis (Kleeb *et al.*, 2021). Mortality rates in sheep and grouse with LIV are variable, ranging from 5% to 60% and up to 80%, respectively (Jeffries *et al.*, 2014; Reid, 1975). Information about mortality rates in other species with LIV infection is scant, but fatal cases have been reported in dogs, cattle, and humans (MacKenzie, 1982; MacKenzie *et al.*, 1973; Walkington *et al.*, 2013). Long-term sequelae have been identified in 17% of the dogs diagnosed with TBE in which motor dysfunction was the main complaint (Kleeb *et al.*, 2021). The prognosis and presence of paresis as the main post-recovery sequelae in the dogs from this study are very similar to the previous studies. Dogs were reported to be functional from 2 to 3 months after diagnosis despite the presence of mild to moderate paresis. This finding corresponds with the previous studies in which the mean recovery time was 3 months (Beckmann *et al.*, 2015; Kleeb *et al.*, 2021; Pfeffer & Dobler, 2011).

The present case series has some limitations due to its retrospective nature. Diagnostic protocols were not standardised through the different referral hospitals. Therefore, complete LIV and TBEV serological studies were unavailable for all dogs, and only two dogs had repeated antibody testing after the initial diagnosis. The presence of seroconversion would help in the certainty of the diagnosis in the surviving dogs. However, a recent study by Salat *et al.* (2021) has also described the presence of seroconversion in experimentally infected dogs without clinical signs, and, therefore, serological results should be analysed in conjunction with the clinical signs. In addition, CSF antibodies would have been a useful test as recently reported by Alnefelt *et al.* (2021). Only one of the deceased dogs underwent *post-mortem* examination, limiting the diagnosis in the other two dogs. The major limitation is the lack of differentiation between TBEV and LIV due to cross-reactivity in serology, but we can state that tick-borne flavivirus antibodies were detected in the presented dogs.

In conclusion, the dogs in the present study showed clinical, clinicopathological, and imaging findings compatible with meningoencephalomyelitis or meningomyelitis suspected to be caused by a tick-borne flavivirus. All but one dog presented positive antibodies for LIV and/or TBEV but negative results for PCR methods in the CSF. Serological differentiation between both viruses is challenging. However, these cases raise awareness of the presence of TBE in the UK, or the increased number of LIV affecting dogs in the UK, an endemic area for LI but with low prevalence in animals apart from its primary hosts. Therefore, tick-borne viral diseases should be considered as a possible differential in dogs within the UK when clinical, clinicopathological and imaging findings are compatible. Further studies are warranted to understand the epidemiology of both viruses and their increased presence within the UK.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Data availability statement

The data is available on request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Number of reads for each sample following trimming and the number of contigs produced by de-novo assembly.

Video S1. Recovery of dog 1.