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ORIGINAL RESEARCH

Seropositivity to louping ill virus in dogs in the UK

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Abstract

Background: Louping ill virus (LIV) is a tick-borne flavivirus that can cause fatal meningoencephalomyelitis in dogs. Four dogs with confirmed LIV infection and a case series of dogs with suspected flavivirus infection have been reported in the UK. However, underreporting of LIV infection due to lack of testing is suspected.

Methods: Surplus serum/plasma from 220 dogs was used to determine the seroprevalence of LIV by haemagglutination inhibition (HAI) test. Signalment and environmental factors were investigated for potential correlations with a positive titre (serum dilution of 1:20 or more).

Results: Two hundred and two dogs were suitable for inclusion in the study, nine of which (4.5%) were seropositive. Among the dogs investigated for neurological disease (40/202; 19.8%), six (15%) were seropositive. Ectoparasiticide use approached significance ($p = 0.055$) for being protective against LIV seropositivity.

Limitations: The main limitations were the specificity of the HAI test, the relatively small number of samples, the low number of seropositive dogs, the poor geographical distribution of the samples and the inherent limitations of questionnaire-based research.

Conclusion: The seroprevalence of LIV in the UK dog population appears to be low. However, LIV should be considered in dogs presenting with unexplained acute or subacute progressive neurological clinical signs, especially because of the recent reports of several dogs with clinical flavivirus infections.

INTRODUCTION

Louping ill (LI) is a tick-borne viral disease that has been reported to cause severe and potentially fatal neurological disease in dogs in the UK.[1–4] Although the number of cases reported in dogs to date is small, as LI primarily affects sheep and red grouse, underreporting in dogs is suspected due to a lack of awareness and, therefore, specific diagnostic testing. The aetiological agent, louping ill virus (LIV), is a neurotropic, enveloped, single-stranded, positive-sense RNA virus from the genus *Orthoflavivirus* and is a member of the tick-borne encephalitis virus (TBEV) complex. LIV is transmitted by the sheep tick *Ixodes ricinus* and was, historically, endemic to the British Isles, with livestock cases mostly occurring in upland areas.[5] In recent decades, geographical spread of the virus has been recognised, and LIV infection in sheep has been reported in non-upland areas of the UK as well as in Norway,[6] Denmark,[7] Russia[8] and Spain.[9] LIV can cause clinical infections in humans,[10] although this is extremely rare, and can infect and cause fatal disease in many other species, including cattle,[11] goats,[12] roe and red deer,[13,14] alpacas,[15] llamas[16] and pigs.[17]

In sheep, LI causes an acute biphasic febrile disease followed by the development of neurological clinical signs, including paralysis, ataxia, tremors and generalised hyperexcitability. Most sheep born in LIV endemic areas seroconvert in their first year of life, suggesting that subclinical infection followed by recovery occurs.[18] In dogs, LI appears to have a similar clinical presentation, and to date, LIV meningoencephalomyelitis has been confirmed in four reported cases in the UK.[1–4] However, given the small number of reports of clinical cases of LI in dogs and the lack of specificity of the clinical signs, LI may be easily overlooked by clinicians who are unaware or unfamiliar with this disease. Moreover, the possible occurrence of subclinical LI in dogs is unknown.[19] A recent case series reported six dogs with meningoencephalomyelitis and pyrexia that were diagnosed with suspected flavivirus infection in the UK.[20] This finding

raises concern of a possible increase in the incidence of flavivirus infections in dogs in the UK.[21]

The diagnosis of LI is based upon a history of known recent exposure to ticks, pyrexia, neurological signs, serological evidence of immunoglobulin (Ig) M and/or IgG antibodies to LIV (especially if seroconversion is demonstrated) and, definitively, histological examination of the brain using specific immunohistochemistry or identification of the agent using the reverse transcriptase polymerase chain reaction (RT-PCR).[5] Due to the short viraemic phase characteristic of this disease, PCR of blood or cerebrospinal fluid is rarely useful for the diagnosis.[5] Seroconversion and discrimination between IgG and IgM antibody titres can provide an indication of the stage of infection.[22] A recent description of magnetic resonance imaging findings suggestive of viral meningoencephalitis in British dogs has been published,[20] which may help increase the suspicion of flavivirus infection.

Haemagglutination inhibition (HAI) is a serological test generally used for the detection of LIV antibodies, although other tests, such as the plaque reduction neutralisation test (PRNT), are available at specialised facilities. The HAI assay is based on the ability of LIV to cause agglutination of erythrocytes and the capacity of anti-LIV antibodies to prevent the virus from haemagglutinating.[23] Until recently, HAI was considered to be specific for LIV in the UK, as LIV was the only tick-borne flavivirus found in this country. However, in 2019, TBEV, another member of the TBEV complex, was detected in ticks in eastern England,[24] although there have been no reports of other flaviviruses, such as West Nile virus or Usutu virus, in the UK, despite positive diagnoses in animals in Europe.[19] Since then, one probable and two confirmed cases of autochthonous human tick-borne encephalitis (TBE) caused by TBEV infection have been reported in the UK.[25,26] Both LIV and TBEV are antigenically closely related viruses,[27] and antibody cross-reactivity could result in samples from patients with either LIV or TBEV being positive in the HAI test, representing a diagnostic challenge.[28,29] Both viruses can cause meningoencephalomyelitis in dogs, and RT-PCR of infected neural tissue followed by Sanger sequencing is necessary to establish a definitive aetiological diagnosis in live animals, which may not be achieved.[20]

Although LI is considered to be a rare disease in dogs, there is growing evidence of flavivirus infections in both dogs[20] and humans[25] in the UK. However, to date, no reports exist with respect to the seroprevalence of LIV and distribution of LI in the UK dog population. We hypothesise that the occurrence of LI in dogs is underreported, and dogs living in more rural environments where sheep are present and with a history of tick bites will have a higher risk of exposure to the virus. The aims of this study were to investigate the incidence of seropositivity of LIV infection in the UK dog population and to examine signalment and environmental lifestyle factors associated with a positive antibody titre. Additionally, given the recent emerging cases of flavivirus infections in this country, we aimed to increase awareness of LI within the veterinary community.

MATERIALS AND METHODS

This was a multicentre prospective study undertaken between September 2021 and October 2022. During the study period, blood samples collected for clinical purposes from dogs presented to two referral hospitals – the Internal Medicine Service at the Vets Now Referral Hospital (VNR) and the Queen Mother Hospital for Animals at the Royal Veterinary College (RVC) – and two primary care clinics – Millcroft Veterinary Group (MVG) and Moorgate Vets (MV) – were considered for inclusion. Dogs with a minimum of 300 µL surplus serum or plasma samples and whose owners completed a questionnaire (Supporting Information) were enrolled in the study. The data gathered from the questionnaire included signalment (sex, neutering status, age and breed), the status of the dog (pet, rescue, working dog or stray), client's home postcode, type of outdoor access (rural only, urban only, rural with urban access or urban with rural access), history of overseas travel (if travelled, specifying where and when), observation of ticks on the dog in the last year and the use of acaricides (specifying, when known, the product used and the last time of administration). The medical records of the dogs included in the study were reviewed, and the reasons for blood sampling (pre-anaesthesia checks, health checks or disease investigations) were recorded. The main clinical signs of dogs presented for disease investigation were reviewed and categorised into two groups: dogs with neurological signs (e.g., behavioural abnormalities, ataxia, paresis, seizures and spinal pain) and dogs without neurological signs.

Blood samples were centrifuged to obtain serum or plasma, which was then stored at −18°C prior to analysis. Subsequently, samples were shipped frozen and analysed at the Moredun Research Institute. Serological analysis for LIV was performed by HAI test for total anti-LIV immunoglobulins following the protocol previously described by Reid and Doherty.[22] Test serum or plasma was initially pre-absorbed with kaolin (20 minutes at 4°C, followed by goose blood overnight) to remove non-specific inhibitors. Sera or plasma were then diluted (base 2 titration, starting from 1:10) and added to a standardised amount of virus antigen in a 96-well plate format and incubated at 4°C overnight for a second time. Lastly, a standardised amount of goose blood was added the following day and the presence or inhibition of haemagglutination was recorded. The titre of positive samples was recorded as the last dilution in which inhibition of haemagglutination was observed (Figure S1). A titre greater than 1:10 was considered positive and was the outcome of interest. A titre of 1:10 was considered an equivocal result, and a titre below 1:10 was considered negative. The medical records of the seropositive dogs that presented for disease investigation were reviewed retrospectively to

obtain the clinician's final diagnosis and progression and short-term follow-up information.

Statistical analyses were conducted using a generalised linear regression with binomial error and logistic link.[30] All analyses were conducted in R (R Core Team, 2023).[31] Samples showing partial inhibition in the control wells were considered invalid and excluded from statistical analyses. The variables analysed were signalment and lifestyle factors, including the status of the dogs, type of outdoor access, overseas travel history, ticks seen, acaricide use, reasons for blood sampling and sample type. To assess the association between each of the studied variables and LIV seropositivity, a univariate logistic regression model was used for each covariate. A *p*-value of 0.05 or less was considered statistically significant.

RESULTS

Study population

Two hundred and twenty dogs were enrolled in the study. Eighteen dogs (8.2%) were excluded after performing serological analyses as their results were considered invalid due to partial inhibition of haemagglutination. Therefore, a total of 202 samples gave valid results and were subjected to statistical analysis. Of these, 83 (41.1%) of the dogs were female (68.7% neutered) and 119 (58.9%) were male (65.5% neutered). The median age at the time of blood collection was 7.62 years (range 0.31–14.08 years). The breeds represented were mainly purebred (160/202; 79.2%).

Regarding lifestyle, 191 dogs were pets (94.6%), eight were originally rescued dogs (4%), three were working dogs (1.5%) and none were stray animals. All owners of historically rescued dogs considered them as pets at the time of the study. However, they were considered a separate group both because they have an uncertain history of risk and because they possibly included both rehomed animals (pets) and animals awaiting rehoming (non-pets). Forty-nine dogs (24.3%) had rural access only, 62 dogs (30.7%) had urban access only, 29 dogs (14.4%) had primarily rural with some urban access and 59 dogs (29.2%) had primarily urban with some rural access. The outdoor access of three dogs (1.5%) was unknown. Figure 1 shows the locations of all 202 dogs included in the study for statistical analysis, as determined by the client's postcode.

Of the 202 dogs included in the analysis, 19 (9.4%) had a history of travelling outside the UK. The overseas travel history of two dogs (1%) was unknown. The owners of 197 (97.5%) dogs answered the question regarding the presence or absence of ticks on their dogs. Of these, 31 (15.3%) reported observing ticks on their dogs and 166 (82.2%) reported that no ticks were observed. Ectoparasiticide had been administered to 24 (77.4%) of the dogs on which ticks were observed and to 91 (54.8%) dogs on which no ticks were seen. The overall use of acaricides was 58.4%.

Sample collection

Overall, 97 samples were obtained from VNR, 89 from RVC, 10 from MVG and *six* from MV, meaning that 92.1% of the samples were obtained from a referral population and 7.9% from first-opinion practice. Serum samples were included from 131 dogs (64.9%) and plasma samples from 71 dogs (35.1%). The reasons for sampling were pre-anaesthesia checks (n = 10, 5%), health checks (n = 18, 8.9%) and disease investigations (n = 174, 86.1%).

Serology

Nine of the 202 (4.5%) dogs had a positive titre (1:20 titre [n = 7], 1:40 titre [n = 1] and 1:80 titre [n = 1]), 22 (10.9%) dogs had an equivocal titre of 1:10 and the remaining 171 (84.7%) dogs were seronegative. Table 1 summarises the signalment and the studied variables (status of the dog, type of outdoor access, overseas travel history, ticks observed on the dog and use of tick prophylaxis) for the seropositive, equivocal and seronegative dogs.

Seven of the nine seropositive dogs (77.8%) were classified as having some rural access. The remaining two dogs had urban access only. The proportion of equivocal and seronegative dogs with rural access was slightly lower (68.2% and 67.2%, respectively). The locations of the seropositive and equivocal dogs are shown in Figure 2. Eight of the nine seropositive dogs (88%) lived in southeastern England, and none of these dogs had a history of overseas travel. Ticks had not been observed on any of the seropositive dogs according to the owners' recollections, and only one dog had received ectoparasiticide (Table 1). The use of acaricides, as reported by the dogs' owners, was n = 1 (11.1%) in seropositive dogs, n = 16 (72.7%) in equivocal dogs and n = 98 (57.3%) in seronegative dogs.

Clinical presentation

Among the overall study population, 40 dogs (19.8%) presented with neurological signs, 134 dogs (66.3%) had non-neurological signs and 28 dogs (13.9%) had no clinical signs at the time of sampling. Table 2 shows the signalment, clinical signs and the presumptive diagnoses of the nine seropositive dogs. The presumptive diagnosis was either based on clinician opinion, imaging findings or a combination of both.

Six of the nine seropositive dogs (66.7%) had neurological signs, which represented 15% of all dogs under investigation for neurological disease. In contrast, three of 162 dogs (1.8%) without neurological signs were seropositive. In the seropositive group, two dogs had a presumptive diagnosis of meningoencephalitis of unknown origin (MUO); one of which was diagnosed 4 years prior to enrolment in this

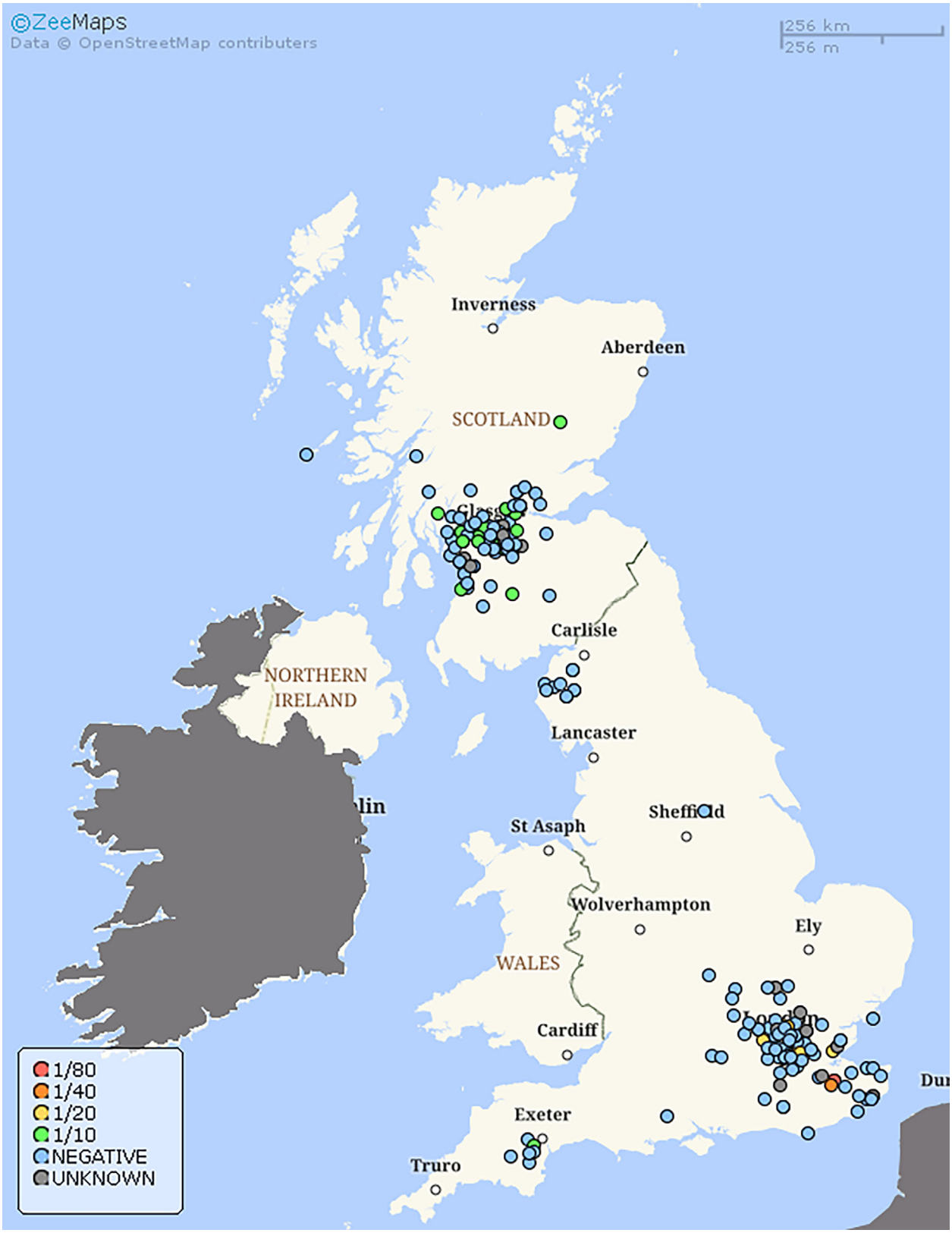

FIGURE 1 Location of the dogs enrolled in the study, as determined by their owner's postcode. "Unknown" refers to samples that were excluded from the statistical analysis due to the presence of partial inhibition of haemagglutination in the haemagglutination inhibition (HAI) test

study. This dog's sample was collected when it presented for immunosuppressive treatment of ongoing MUO. None of the seropositive dogs, including the two dogs with MUO and the dog diagnosed with idiopathic epilepsy, was reported to have progressive neurological disease and none died between the time of sampling and the time of writing.

In comparison, there was one dog (4.5%) in the equivocal group and 33 dogs (19.3%) in the seronegative group that had neurological signs. The only dog

TABLE 1 Characteristics of the seropositive, equivocal and seronegative dogs, including their signalment and the studied variables (status of the dog, type of outdoor access, overseas travel history, observance of ticks on the dog and use of tick prophylaxis)

Variables	Seropositive dogs		Equivocal dogs		Seronegative dogs	
Sex, *N* (%)						
Female	2 (22.2)		9 (40.9)		72 (42.1)	
Male	7 (77.8)		13 (59.1)		99 (57.9)	
Age (years), median (range)	8.0 (0.6–12.0)		8.7 (1.3–14.0)		8.0 (0.3–14.1)	
Breed, *N* (%)						
Purebreed	7 (77.8)		17 (77.3)		136 (79.5)	
Crossbreed	2 (22.2)		5 (22.7)		35 (20.5)	
Status of the dog, *N* (%)						
Pet	9 (100)		22 (100)		160 (93.6)	
Rescue	0 (0)		0 (0)		8 (4.6)	
Working dog	0 (0)		0 (0)		3 (1.8)	
Stray dog	0 (0)		0 (0)		0 (0)	
Type of outdoor access, *N* (%)						
Rural	1 (11.1)		8 (36.4)		40 (23.4)	
Urban	2 (22.2)		7 (31.8)		53 (31)	
Rural–urban	2 (22.2)		1 (4.5)		26 (15.2)	
Urban–rural	4 (44.4)		6 (27.3)		49 (28.7)	
Unknown	0 (0)		0 (0)		3 (1.7)	
Overseas travel, *N* (%)						
Yes	0 (0)		3 (13.6)		16 (9.4)	
No	9 (100)		19 (86.4)		153 (89.5)	
Unknown	0 (0)		0 (0)		2 (1.2)	
Presence of ticks and use of tick prophylaxis, *N* (%)						
Yes	0 (0)		6 (27.3)		25 (14.6)	
	Of which, acaricide use:					
	Yes	N/A	Yes	3 (50.0)	Yes	21 (84.0)
	No	N/A	No	3 (50.0)	No	4 (16.0)
No	9 (100)		16 (72.7)		141 (82.5)	
	Of which, acaricide use:					
	Yes	1 (11.1)	Yes	13 (81.2)	Yes	77 (54.6)
	No	8 (88.9)	No	3 (18.8)	No	64 (45.4)
Unknown	0 (0)		0 (0)		5 (2.9)	

with neurological clinical signs in the equivocal group was presented for investigations of seizures and was ultimately diagnosed with a brain mass compatible with a brain tumour by computed tomography, but this was not investigated further.

Statistical analysis results

No signalment or lifestyle covariate was found to be statistically significant at the 5% level. However, given the small number of cases where seroconversion was demonstrated, this study had a poor power to identify any covariate associated with seropositivity. The authors therefore feel justified in presenting the results for acaricide use as the only covariate that had a *p*-value of less than 0.1 and is therefore worthy of further study. Acaricide use demonstrated an indication that it might be reducing the risk of LIV seropositivity, with a log odds ratio of −1.165 (95% confidence interval 0.095–2.425, *p*-value 0.055). Table 1 shows the relationship between observation of ticks and prophylactic use of acaricide.

DISCUSSION

This is the first study evaluating the seroprevalence of LIV in UK dogs, and in this cohort, 4.5% were seropositive and 10.9% had equivocal serological results. These results suggest that exposure to the virus, as denoted by serological response, exists in UK dogs, although at a relatively low prevalence. Nevertheless, due to lack of awareness, LIV in dogs is likely to be under-reported or underdetected, and the true seroprevalence may well be higher. To date, only a few cases of LIV infection have been documented in dogs,[1–4] all of which were in the British Isles. Among the four confirmed cases,

FIGURE 2 Location of the seropositive dogs (haemagglutination inhibition [HAI] titre ≥1/20) and dogs with equivocal (HAI titre = 1/10) serological results

three dogs developed severe meningoencephalitis with fatal outcome, and one dog survived after an acute progressive encephalomyelitis, although neurological sequalae were observed long term.[2] In a recently published case series of six dogs with neurological disease due to suspected flavivirus infection, all dogs presented with clinical signs similar to the cases reported previously; an initial phase of pyrexia followed by behavioural abnormalities, progressive ataxia and paresis.[20] Three of these dogs were seropositive by the LIV HAI test, and two of them showed seroconversion at 2 and 4 weeks after diagnosis, indicating

TABLE 2 Signalment, presenting clinical signs and presumptive diagnoses of the nine seropositive dogs

Animal	Age (months)	Sex	Breed	HAI result	Presenting clinical signs	Presumptive diagnosis
Case 1	108	MN	Chihuahua	1/20	Ambulatory paraparesis with pelvic limb proprioceptive ataxia	Peripheral nerve sheath tumour (right T8–T9)
Case 2	108	FE	French bulldog	1/20	C1–C5 myelopathy and right facial neuropathy	Meningoencephalitis of unknown origin (diagnosed 4 years prior to sampling). Dog currently on cytarabine treatment
Case 3	96	MN	Crossbreed	1/80	Acute progressive painful T3–L3 myelopathy	T11–T12 intervertebral disc extrusion
Case 4	31	FN	French bulldog	1/20	Cluster seizures	Idiopathic epilepsy (tier II)
Case 5	120	MN	Golden retriever	1/20	Seizures	Left olfactory bulb extra-axial mass lesion (suspected meningioma)
Case 6	7	ME	French bulldog	1/20	Blindness with absent menace response and reduced PLRs bilaterally	Meningoencephalitis of unknown origin
Case 7	74	MN	Shih tzu	1/20	Mild right forelimb lameness with no neurological deficits	Suspected primary hyperparathyroidism due to parathyroid nodule
Case 8	144	MN	Border collie	1/40	Tachypnoea and pyrexia	Bronchopneumonia with recurrent pleuritis and subpleural sterile abscess PCR-positive for *Mycoplasma* spp.
Case 9	50	MN	Crossbreed	1/20	Acute vomiting and regurgitation	Acute gastroenteritis and aspiration pneumonia

Abbreviations: FE, female entire; FN, female neutered; HAI, haemagglutination inhibition; ME, male entire; MN, male neutered; PCR, polymerase chain reaction; PLR, pupillary light reflex.

a recent infection. However, a final diagnosis of LIV infection was not definitively confirmed as these dogs also tested positive in an ELISA for TBEV,[20] and sequencing of viral nucleic acids was not achieved in order to differentiate LIV from TBEV.[20]

Determining the true prevalence of LIV exposure is challenging for several reasons, one being the serological cross-reactivity with other flaviviruses, especially TBEV. In the present study, the HAI test for anti-LIV immunoglobulins was used as this diagnostic test is commonly used in the UK for the diagnosis of LI in sheep and red grouse, the main species affected.[5] Titres above 1:640 are considered to indicate recent infection, and assessment of IgM and IgG titres using heated and unheated serum for the HAI test can discriminate between a recent LIV infection and historical exposure.[22] In our study population, none of the seropositive cases had a titre above 1:640; therefore, differentiation between IgM and IgG was not performed. None of the seropositive dogs were considered to have LI after the diagnostic workup, nor did they develop any progressive neurological deterioration. The prevalence of seropositivity in our population was higher in patients with neurological signs; however, most of these patients had other neurological diseases unrelated to LIV infection (e.g., intervertebral disc disease or neoplastic disease). Therefore, this seropositivity may represent historical LIV exposure, as occurs in sheep, where lifelong seropositivity may occur after infection.[18] For the cohort of dogs with an equivocal result, this may be due to an unexplained reactivity in the HAI assay, which has been observed previously in some naive animals. A small proportion of samples showed partial inhibition of haemagglutination, described as an empty red circle in the control wells. This finding represents a sample-specific phenomenon, the cause of which is unknown.

Clinical cases of LI in sheep and red grouse are predominantly seen in upland areas of Scotland, Ireland, northern England and Wales.[5] In contrast, this study found the majority of seropositive dogs were in southeastern England, an area not known for LIV infection,[5] although it should be noted that the dataset analysed contained a high degree of geographical bias. This finding may suggest that these cases could have been exposed to TBEV, representing a potential sentinel for human TBE. TBEV is not endemic to the UK; however, a notable increase in the incidence of TBE in humans has been observed in various European countries. The European subtype of TBEV is predominantly vectored by *I. ricinus*, which is the same tick species involved in LIV transmission, and this tick is highly prevalent in the UK.[32] While this subtype of TBEV primarily affects humans in central Europe, new foci have been discovered recently in the UK.[24] TBEV can affect dogs, with reported seroprevalence ranging from 0% to 53.6% in dogs with neurological clinical

signs.[33] As in humans, TBE in dogs may manifest with severe, often fatal, neurological disease due to meningoencephalitis, meningomyelitis or both.[34] However, despite high seroprevalence rates in some areas, dogs rarely develop clinical disease. Until the recent incursion of TBEV in the UK, differences in the geographical distribution of LIV and TBEV were used to differentiate between these two infections. Due to climate change leading to new patterns of tick distribution[35] and the growing international movement of dogs, the incidences of both LIV and TBEV may be increasing in this[20] and other countries.[36] Therefore, both of these viruses should be considered in the differential diagnosis of dogs in the British Isles with known tick exposure presenting with a history of lethargy and/or pyrexia and an acute or subacute progressive meningoencephalomyelitis. Additionally, although immune-mediated diseases are the most common causes of central nervous system disease in dogs in England,[37] as MUO and viral meningoencephalitis share common clinical and even histopathological features,[38] LIV and TBEV meningoencephalitis should not be neglected from the differential diagnoses.

The second aim of this study was to explore potential risk factors associated with LIV seropositivity in dogs. Associations between a positive LIV antibody titre and the dog's signalment, life history and acaricide use were examined. No signalment or lifestyle variables were found to be statistically significant, but due to the low number of seropositive cases, this study was underpowered for valid statistical analysis and precluded determining the definitive effect of any of the covariates considered. A correlation between acaricide use and being seronegative to LIV approached significance, highlighting a potential protective effect of acaricide use against exposure to LIV.

Another limitation of this study was the use of owner-completed questionnaires, as they are susceptible to recall bias with respect to the observation of ticks on their pets and the use of acaricides. Although the predominant route of LIV infection is via a bite from an infected tick, there are other potential routes of infection, such as the ingestion of raw meat from infected sheep and consumption of unpasteurised sheep/goat milk (as for TBEV). However, information allowing assessment of such risk factors was not available. Furthermore, the specificity of the LIV HAI test is under discussion, considering recent reports of TBEV cases in ticks and humans in the UK[24,26] and the serological cross-reactivity of these two viruses.[39] While an ELISA is commercially available for TBEV, as well as a flavivirus multispecies ELISA, an LIV-specific ELISA is not yet available. However, due to known cross-reactivity among flaviviruses,[39] the results of the TBEV ELISA should be considered with caution. Serological differentiation between TBEV and LIV is difficult, but could be achieved using PRNT; however, this test is not readily available to practitioners since it requires specialised facilities (CL3/BSL3). Of note, none of the seropositive dogs in this study had a history of overseas travel, which potentially suggests these dogs had a lower probability of exposure to TBEV. Conversely, southeastern England has been recognised as being focally endemic for TBEV and is not a typical site for LIV.[5] The dogs sampled in this area could therefore plausibly have been exposed to TBEV. The detailed national distribution of TBEV is unknown. An alternative hypothesis would be that recent and ongoing changes in climate (e.g., warmer average summer temperatures) are impacting tick population dynamics throughout the UK, which in turn can impact tick-borne disease transmission and, consequently, LIV and TBEV prevalence. This was a multicentre study; however, the study population obtained was mostly centred in two locations associated with two referral hospitals. As a result, this sample of the dog population may not have been representative of the wider dog population in the UK. Furthermore, the working dog population was not fully captured. A larger scale study covering a broader area of the UK is necessary to further evaluate the risk factors for exposure to LIV in dogs. This would also provide a better understanding of the clinical impact this pathogen may have in this species.

In conclusion, this study sheds light on the seroprevalence of flaviviruses in dogs in the UK. Even if all the seropositivity was due to LIV alone, the LIV seroprevalence in the UK dog population appears to be low. However, it is higher than we would have expected, given that flavivirus infections are rarely considered as a diagnosis in dogs by veterinary surgeons in the UK. LIV and TBEV infections should be considered in the differential diagnosis for dogs presenting with acute or subacute progressive neurological signs, particularly if accompanied by pyrexia, in light of the recent report of several dogs with clinical flavivirus infection in the UK.[20] An improved understanding of LIV seropositivity in dogs, and whether this is confounded by exposure to TBEV, may aid clinical decision making for dogs presenting with a history of lethargy and/or pyrexia with progressive neurological signs due to meningoencephalomyelitis.

AUTHOR CONTRIBUTIONS

Sample analysis, data collation and curation, preparation and editing and revision of the manuscript: Iris Elgueta. *Sample curation and analysis*: Kayleigh Allen. *Patient recruitment and data collation*: Theofanis Liatis. *Patient recruitment and data collation*: Verónica Gonzalo-Nadal. *Sample curation and analysis*: Eleanor Laming. *Funding acquisition, experimental design and editing and revision of the manuscript*: Mark P. Dagleish. *Patient recruitment, experimental design, data collation and curation, preparation and editing and revision of the manuscript*: Pauline M. Jamieson. *Statistical analysis and preparation of the manuscript*: Giles Innocent. *Funding acquisition, experimental design, patient recruitment preparation and editing and revision of the manuscript*: Mara S. Rocchi.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

Informed consent was obtained from owners prior to inclusion, and ethical approval was sought and granted by the Moredun Research Institute's Animal Welfare and Ethical Review Body before study commencement. The funding body (Dogs Trust) was informed of the ethical review board decision and also approved the study.

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SUPPORTING INFORMATION

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

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