Ticks and Tick-borne Diseases 14 (2023) 102226

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

First clinical case of tick-borne encephalitis (TBE) in a dog in Greece

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ARTICLE INFO

Keywords:
Canine
Europe
Ixodes ricinus
Neurological signs
Tick-borne disease
Tick-borne encephalitis virus

ABSTRACT

Tick-borne encephalitis virus (TBEV) causes tick-borne encephalitis (TBE), affecting human health in Europe and Asia. Reports on canine clinical cases of TBE are rare, although dogs are used as sentinels for assessing human health risks. The first canine clinical TBE case in Greece is reported in this case report. The dog had a history of tick infestation and displayed neurological symptoms, particularly tetraparesis, neck hyperalgesia, and a sudden behavior change. Serum samples were obtained and examined in a commercial ELISA to detect anti-TBEV specific IgG and IgM antibodies. The dog tested seropositive for both IgG and IgM, and based on its history and compatible clinical signs, the diagnosis of TBE infection was reached. The prognosis was initially poor, and treatment included the administration of fluids, corticosteroids, and antibiotics, followed by physical therapy. After a 10-day hospitalization, the dog had a much better prognosis. This case highlights that TBEV does emerge in new locations, increasing human and animal infection risk. Veterinarians should include TBE in their differential diagnosis of canine patients with a history of tick infestations, progressive neurological symptoms, and abnormal behavior.

1. Introduction

Tick-borne encephalitis virus (TBEV) (*Flaviviridae*) is a flavivirus that causes tick-borne encephalitis (TBE) (Dultz and Goldhammer, 2021; Salat and Ruzek, 2020). Ticks, particularly *Ixodes ricinus* (Linnaeus, 1758), are the primary vector of the European strain of TBEV. In contrast, *Ixodes persulcatus* (Schulze, 1930) carries the more severe Siberian and Far Eastern subtypes of the tick-borne encephalitis virus (Lindhe et al., 2009; Weissenböck et al., 1998). Transmission of the virus to humans and animals, including dogs, occurs during the tick-feeding process (Dultz and Goldhammer, 2021; Salat and Ruzek, 2020). According to the World Health Organization, in Europe alone, human infection cases with TBE can reach up to 12,000 in a year (World Health Organization, 2021). A lesser-known transmission route is the ingestion of unpasteurized infected ruminant milk or milk products (Salat and Ruzek, 2020). In vector-competent tick species, the virus can be transmitted between progressive life cycle stages (transstadial transmission) or to the offspring of the engorged female ticks (transovarial transmission). Together with ticks, rodents such as mice and rats play a crucial role in preserving TBEV in nature through a continuous cycle of tick transmission between viremic and naïve rodents (Salat and Ruzek, 2020). In dogs, the first confirmed case of natural TBEV infection was reported in 1972 in Switzerland (Pfeffer and Dobler, 2011).

In Greece, *I. ricinus* is considered the primary and sole vector of TBEV, but no studies have been conducted on the TBEV circulating strains (Efstratiou et al., 2021). Antibodies against this virus have been detected in humans from different prefectures of Greece, particularly Northern Greece, including Xanthi and other neighbouring regions (Efstratiou et al., 2021). This area is endemic to different tick species, including *Rhipicephalus sanguineus sensu lato, R. turanicus, R. camicasi, R. bursa, Hyalomma scupense, Hy. marginatum, Hy. dromedarii, Dermacentor marginatus, Haemaphysalis parva, H. concinna*, and *I. ricinus* (Efstratiou et al., 2021; Latrofa et al., 2017). Tick infestation prevalence reaches up to 85% for rural dogs in Xanthi, with *R. sanguineus* as the most prevalent tick species (Latrofa et al., 2017). There are also reports of *I. ricinus,* the natural host of TBEV (Salat and Ruzek, 2020), collected either from dogs or the environment in Xanthi (Latrofa et al., 2017; Papadopoulos et al., 1996; Pavlidou et al., 2008). Furthermore, many reports indicate the high prevalence of tick-borne pathogens in dogs from Greece, including *Ehrlichia* and *Anaplasma* species (Angelou et al., 2019; Latrofa et al., 2017). Moreover, in one older study in dogs in Greece, TBE seropositivity varied from 0.97% to 8.6% (Chambouris et al., 1989), but no

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https://doi.org/10.1016/j.ttbdis.2023.102226
Received 13 March 2023; Received in revised form 28 June 2023; Accepted 1 July 2023
Available online 5 July 2023
1877-959X/

clinical case has been described until now. This current study describes the first clinical case of TBE in a dog in Greece.

2. Clinical case description

In October 2022, a three-year-old 20 kg male intact mongrel dog from Xanthi (41°08′5.57″ N 24°53′16.80″ E) was referred from a first-opinion veterinarian to a private veterinary clinic for a second opinion. The dog presented neurological symptoms and a sudden behavior change (did not recognize its owners and stumbled into walls) the week before referral. Based on the owner's history, the dog received insufficient ectoparasite protection, and engorged ticks had been observed and removed from the dog in the past weeks. Furthermore, the owners reported that symptoms appeared suddenly and were progressively worsening. Upon a detailed physical and neurological examination by the clinical veterinarians, the dog had a fever of 41.3 °C and displayed anorexia, depression, general apathy, and tetraparesis. Moreover, myoclonus, decreased consciousness, reduced head sensitivity, paralysis of the facial nerve, nystagmus, miosis, neck hyperalgesia, and stiffness were also present. An abscess in the abdominal area due to an embedded foxtail was also discovered, that was then surgically opened, drained, and cleaned, and the foxtail was removed.

Blood samples were collected into an EDTA tube and a tube without anticoagulant. A complete blood count (CBC) was carried out with the EDTA blood (scil Vet abc Plus hematology analyzer, Scil Animal Care Company GmbH, Viernheim, Germany), and the only abnormal findings were mild leukocytosis (12.7 × 1000/μl) and lymphopenia (0.9 × 1000/μl), reduced hemoglobin (12.2 g/dl) and hematocrit (37.4%) values.

Serum was obtained from the tube without anticoagulant and using a commercial immunochromatographic test kit (VetExpert Rapid CaniV-4 Leish Test, Anicell, Greece), the dog tested seropositive for *Ehrlichia canis* and negative for *Leishmania infantum*. Based on the dog's history and clinical signs, TBE was placed at the top of the differential diagnosis. Therefore, the rest of the serum was transported to a specialized veterinary diagnostic laboratory (LABOKLIN GMBH & CO.KG, Bad Kissingen, Germany). Once there, the serum was used in a commercially available ELISA, Immunozym FSME All-Species ELISA (PROGEN Biotechnik GmbH, Heidelberg, Germany), to detect specific anti-TBEV IgG and IgM antibodies. The dog was seropositive for IgM and IgG, with IgM antibody levels at 39.53 LE (technical-artificial units) with a cut-off value of 25 LE and IgG at 126.85 U/ml (cut-off value: 63 U/ml). According to the manufacturer's recommendation, an IgM value greater than 25 is considered positive and a value greater than 30 LE indicates an acute TBE infection. Considering the dog's history, seropositivity for anti-TBEV specific IgM and IgG antibodies, and combined with the compatible clinical signs, the diagnosis of TBE infection was confirmed. The prognosis was initially poor because the dog was not eating and displayed severe neurological symptoms.

To let the dog rest, provide intensive nursing care, and better monitor its progress, the owners agreed to hospitalize it in the veterinary clinic for ten days. Food and water were provided *ad-libitum*. Symptomatic treatment included the administration of the following drugs. Fluid therapy (sodium chloride 0.9%) and corticosteroids (dexamethasone and prednisolone) were administered at anti-inflammatory doses to reduce CNS inflammation and fever. Antibiotics were used to prevent secondary bacterial infections (clindamycin), treat the abdominal abscess (amoxicillin) and ehrlichiosis (doxycycline). Finally, physical therapy was suggested to the owners so that the dog regains normal physical movements and motor functions. Considering Day 0, the day of hospitalization, the following dosage regimens were used for each drug. On Day 0, sodium Chloride 0.9% (NaCl 1000 ml, Bioser, Greece) was administered intravenously at 2 mg/kg/h from Day 0 until Day 3. Dexamethasone (Rapidexon® 2 mg/ml, Eurovet Animal Health BV, The Netherlands) at a dose of 0.2 mg/kg was administered intravenously twice a day from Day 0 to Day 2. Consequently, prednisolone (Hedylon® 5 mg/tablet, Livisto, Spain) at a dose of 0.5 mg/kg, administerial orally from Day 2 to Day 7, then reduced to a dose of 0.25 mg/kg from Day 7 to Day 12 and finally at a dose of 0.125 mg/kg, from Day 12 to Day 17. Clindamycin (Zodon® 264 mg/tablet, Ceva Hellas, Greece) was administered orally at a dose of 11 mg/kg, twice a day, from Day 0 to Day 7, and simultaneously Amoxicillin (Synulox® Dog 500 mg/tablet, Zoetis Hellas, Greece) administered orally at a dose of 20 mg/kg, twice a day. Finally, for treating ehrlichiosis, Doxycycline (Doxycare® 200 mg/tablet, Animalcare, Greece) was administered orally at 10 mg/kg once a day from Day 0 until Day 28.

On Day 2, the dog started slowly eating again on its own. By the end of the hospitalization on Day 10, the dog showed slow but constant improvement: neck pain and stiffness mainly had resolved, the dog had regained full consciousness, its movement on all four limbs had improved, and appetite had mainly returned to normal. The owners continued the drug administration and initiated physical therapy on their dog in a specialized rehabilitation center, where the dog made a full recovery.

3. Discussion

The seroprevalence of TBEV in the human population of Xanthi was evaluated to be 2.9% in one study in 2007, confirming that TBEV is present in the specific area in both humans and dogs (National Public Health Organization, 2021; Pavlidou et al., 2007). Importantly, a total of six autochthonous cases of TBE infections have been reported (National Public Health Organization, 2022) in humans in Greece, one of which in 2021 was from a 56-year-old livestock farmer in Xanthi (National Public Health Organization, 2021). The livestock farmer was bitten by a tick and hospitalized one week later with mild TBE symptoms, eventually making a full recovery. Regarding the other five cases, one was in 2014 in Eastern Macedonia, one in 2015 in Peloponnese, and three more cases in 2021, two of which were in Central Macedonia with an epidemiological connection and the last one in Thessaly (National Public Health Organization, 2021). All six cases were from rural/mountainous areas of northern, central, and southern Greece, involving livestock farmers and shepherds (National Public Health Organization, 2021). According to the Greek National Public Health Organization, people living in the countryside and rural or mountainous areas (i.e., farmers, shepherds), where ticks are present, have a higher risk of acquiring the infection (National Public Health Organization, 2021).

TBE is usually acute in dogs but can also be chronic (Dultz and Goldhammer, 2021), with most infected dogs being asymptomatic (Salat and Ruzek, 2020; Weissenböck et al., 1998). If symptoms appear, they arise mainly from the central nervous system (CNS) (Salat and Ruzek, 2020), such as quadriparesis, cognitive disorders, increased pain sensitivity, miosis, cervical spine flexion and nystagmus among others (Pfeffer and Dobler, 2011; Weissenböck et al., 1998). Although severe symptoms are uncommon (Salat and Ruzek, 2020), in some cases, the infection with TBEV can prove fatal (Pfeffer and Dobler, 2011; Weissenböck et al., 1998). The prognosis improves dramatically if the dog lives through the first seven days of infection (Leschnik et al., 2002).

Reaching a conclusive diagnosis requires a combination of tests and clinical and laboratory findings. TBE is typically underdiagnosed, since many clinical veterinarians, are unaware or ill-informed about the disease (Dultz and Goldhammer, 2021; Pfeffer and Dobler, 2011). According to the European center for Disease Prevention and Control (ECDC) (European Center for Disease Prevention and Control, 2023), for a human TBE case to be confirmed, the following clinical and laboratory criteria must be fulfilled. "Clinical Criteria: The person must exhibit clinical signs associated with inflammation of the CNS. Laboratory Criteria: At least one of the following five: TBE-specific IgM AND IgG antibodies in the blood, TBE-specific IgM antibodies in cerebrospinal fluid (CSF), seroconversion or four-fold increase of TBE-specific antibodies in paired serum samples, detection of TBE viral nucleic acid in a clinical specimen, or isolation of TBE virus from a clinical specimen.

Although there is no established case definition for TBE in canines, laboratory confirmation is typically based on the detection of antibodies (Pfeffer and Dobler, 2011). Veterinarians should consider the condition in their differential diagnosis if the dog exhibits abnormal behavior or acute neurological symptoms quickly worsen, as described above (Salat and Ruzek, 2020). Consequently, anti-TBEV immunoglobulins must be isolated from the dog's serum or CSF. Alternatively, the TBEV can be identified in the dog's blood or CSF via molecular analysis (Dultz and Goldhammer, 2021; Salat and Ruzek, 2020; Weissenböck et al., 1998). Ante-mortem detection of the virus with PCR is difficult and impractical since most dogs are no longer viraemic when they are brought to the vet (Andersson et al., 2020; Salat and Ruzek, 2020). Therefore, diagnosis in live dogs usually depends on the dog's history, a compatible clinical picture, and the identification of specific TBEV immunoglobulins in the serum which is conclusive (Pfeffer and Dobler, 2011; Salat et al., 2021; Salat and Ruzek, 2020; Weissenböck et al., 1998), as in the current case. In more detail, an increase of IgG levels from serum samples taken 14 days apart or a positive IgM titre prove infection with TBEV in the acute phase (Pfeffer and Dobler, 2011). As regards laboratory examinations, in the acute phase, the CBC is usually unremarkable, with only a few dogs showing decreased leukocytes and lymphocytes and increased monocyte counts (Salat et al., 2021; Salat and Ruzek, 2020). In contrast, in the chronic phase of the disease, leukocytosis may be observed (Leschnik et al., 2002). Concerning other diseases in the differential diagnoses, rabies, and canine distemper have similar clinical manifestations and should be included in the differential list (Weissenböck et al., 1998). In our case, the dog was fully vaccinated for both viral diseases, and therefore they were excluded from the differential list. Furthermore, West Nile Virus antibodies can cross-react with TBEV antibodies in an ELISA test. However, in a recent study investigating the cross-reaction between the two viruses in ELISA, when applying a more stringent cut-off value of 126 U/ml, any sample above this value was truly positive for TBEV (Könenkamp et al., 2022), as in the current case. Nevertheless, we cannot exclude the possibility of a mixed infection with both viruses.

Unfortunately, there is no etiological treatment, and only supportive measures are recommended (Dultz and Goldhammer, 2021). Medications can be used to manage pain and fever, while antibiotics can help prevent secondary bacterial infections (Salat and Ruzek, 2020). During the rehabilitation period, corticosteroids can be used to treat clinical signs but not during the acute phase because they can extend viral multiplication and worsen CNS lesions (Pfeffer and Dobler, 2011; Salat and Ruzek, 2020). In addition, physiotherapy is crucial in regaining lost motor functions and movements (Salat and Ruzek, 2020).

Prevention is based on avoiding tick bites using a licensed veterinary product (i.e., tick repellents or acaricides) (Salat and Ruzek, 2020). It is worth noting that ticks can transmit TBEV as soon as they start feeding on the host's blood because TBEV is found in their saliva (Leschnik et al., 2013).

One limitation of the current study is that there was no confirmation of TBE infection with a serum neutralization assay. Neutralization tests represent an important method that can be used to detect specific neutralizing anti-TBEV antibodies and support the disease diagnosis (Salat et al., 2021). In an acute TBE infection, PCR can be also employed to confirm the disease, but as demonstrated in experimentally infected dogs, viraemia lasts only three days (Salat et al., 2021). In the current case, the dog exhibited symptoms for more than a week before being referred to the second veterinary practice and therefore a PCR test would have been negative.

Seroprevalence studies on anti-TBEV specific antibodies in dogs have been carried out in various countries, with seropositivity ranging from 0.35% in Belgium (Roelandt et al., 2011) to 42.7% in Germany (Salat and Ruzek, 2020). Interestingly, dogs can be sentinel animals in certain areas, especially in regions with a small number of TBE infections (Csángó et al., 2004; Levanov et al., 2016; Lindhe et al., 2009).

This first clinical case of canine TBE in Greece provides solid evidence that the disease is a threat to both dogs and humans in Greece. Only a few other clinical cases of canine TBE infections are reported in the available literature, mainly from Germany, Switzerland, Sweden, and Austria (Pfeffer and Dobler, 2011).

In the future, new climatic conditions and animal movements are expected to facilitate the expansion and establishment of new tick species among European countries and facilitate the emergence of TBEV in new locations, increasing the infection risk for humans and animals alike (Levanov et al., 2016; Roelandt et al., 2011; Salat and Ruzek, 2020). Regarding public health, TBEV can cause encephalomyelitis in human patients, which can prove fatal (Salat and Ruzek, 2020). Although currently, no TBEV vaccine exists for dogs or other animals, a novel TBD virus vaccine candidate was recently tested for veterinary use with success in preventing death in mice and viraemia in sheep (Salát et al., 2018). If licensed, it could represent an additional prevention measure for TBE in dogs (Salat et al., 2021).

4. Conclusions

The current work constitutes the first clinical case of TBE in a dog in Greece and highlights the clinical aspects and diagnostic steps required to identify the infection in dogs. Veterinarians should be aware of TBE and place the disease in their differential diagnosis in canine cases with a history of tick infestations, presenting progressive neurological symptoms and abnormal behavior. Lastly, updated canine seroprevalence studies in Greece are required to estimate the TBEV circulation in certain areas as a baseline for public health awareness.

Funding

This study was partially funded by VIRBAC HELLAS (payment of publication fees).

Institutional review board statement

Ethical review and approval were waived for this study because it is a case report. All treatments and procedures for collecting samples from the dog were performed by licensed veterinarians adhering to a high standard (best practice) of veterinary care and in accordance with the national laws and regulations on animal welfare.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

CRediT authorship contribution statement

Georgios Sioutas: Methodology, Project administration, Visualization, Software, Writing – review & editing, Writing – original draft, Resources, Data curation, Investigation. **Kyriaki Tsakou:** Methodology, Writing – review & editing, Investigation, Formal analysis. **Chousein Top:** Methodology, Writing – review & editing, Investigation, Formal analysis. **Frans Jongejan:** Methodology, Validation, Writing – review & editing, Data curation. **Elias Papadopoulos:** Conceptualization, Visualization, Writing – review & editing, Supervision, Resources, Investigation, Project administration, Validation, Methodology, Data curation, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interests.

Data availability

No data was used for the research described in the article.

Acknowledgments

The authors would like to thank the dog owner for their interest in

helping his animal and assisting in diagnosis by providing a detailed history.

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