Parasite Epidemiology and Control 10 (2020) e00160

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

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

Clinical and laboratory findings in tick-borne encephalitis virus infection

Nicole Barp [a], Alessandro Trentini [b], Mariachiara Di Nuzzo [a], Valeria Mondardini [c], Ermenegildo Francavilla [c], Carlo Contini [a,*]

[a] Dept Medical Sciences, Section of Infectious Diseases, University of Ferrara, Italy
[b] Dept Biomedical Sciences, Section of Biochemistry, Molecular Biology and Medical Genetics, University of Ferrara, Italy
[c] Infectious Diseases, Belluno Medical Hospital "San Martino", Ulss1 Dolomiti, Italy

ARTICLE INFO

Article history:
Received 14 March 2020
Received in revised form 17 May 2020
Accepted 13 June 2020

Keywords:
Tick-borne encephalitis
Tick-borne encephalitis virus (TBEV)
Monophasic course
Biphasic course
Neurological/neurocognitive sequelae
Abnormal laboratory data

Purpose: Tick-Borne Encephalitis (TBE), a disease caused by Tick-Borne Encephalitis Virus (TBEV), is emerging in Italy. This study aimed to characterize the epidemiological, clinical, laboratory, imaging and electroencephalogram characteristics in Belluno, North-East Italy.

Results: 76% were males, mean age 53 years; 50% did not report tick bite. 72% had a biphasic course, 42% a monophasic one, 8 cases of abortive TBE. Mostly no specific symptoms were observed, together with neurological signs and symptoms. None died, but 35% had sequelae at the one-month follow-up. Men had a higher risk of having neurological/neurocognitive sequelae; paresthesia or tremors were associated independently with sequelae. In terms of laboratory data, thrombocytopenia, neutropenia and lymphocytosis were associated with the first phase ($p < .01$), while monocytosis, lymphocytopenia, high levels of ESR and CRP with the second ($p < .05$). Other abnormal laboratory data were observed: high levels of transaminases, bilirubin, GGT, fibrinogen, amylase, LDH, CPK and electrolyte disorders. Most of the liquor showed pleocytosis and increased protein levels. No specific findings characterized imaging; electroencephalogram mainly reported general and focal anomalies in the temporal lobe.

Conclusions: Although patients have not reported a tick bite, TBEV infection should be considered for diagnosis. Usually no specific symptoms are reported along with neurological signs and symptoms. The biphasic course is more often described than the monophasic course; abortive TBE is sometimes present. Paresthesia and tremors are independently associated with neurological/neurocognitive sequelae; men have a higher risk of having sequelae. The first phase is probably associated with thrombocytopenia, neutropenia and lymphocytosis; the second with monocytosis, lymphocytopenia, high levels of CRP and ESR. Electrolyte disorders, high levels of transaminases, GGT, bilirubin, CPK, LDH, fibrinogen and amylase may characterize TBEV infection.

* Corresponding author at: Department of Medical Sciences, Section of Infectious Diseases and Dermatology, University S. Anna Hospital, Ferrara, 44124 Ferrara, Italy.
E-mail address: cnc@unife.it. (C. Contini).

https://doi.org/10.1016/j.parepi.2020.e00160

1. Introduction

Tick-Borne Encephalitis (TBE) is a human viral infectious disease, caused by Tick-Borne Encephalitis Virus (TBEV), a member of Flaviviridae family. Descriptions of diseases compatible with TBE appeared in Austria in the early 1930s; however, TBEV was initially isolated in Russia in 1937 (Riccardi et al., 2019; World Health Organization, 2019; Zlobin et al., 2017) and in the Central Europe (Bohemia) in 1948 (Daniel et al., 2011; Daniel et al., 2018; Riccardi et al., 2019).

Hard ticks of the family *Ixodidae* act both as vector and reservoir. The main hosts are small rodents, while man is an accidental host. The infection usually follows tick bites or, rarely, the consumption of raw milk from infected animals. Three main virus subtypes are described: European or Western Tick-Borne Encephalitis Virus (TBEV-Eu), Siberian Tick-Borne Encephalitis Virus (TBEV-Sib), and Far Eastern Tick-Borne Encephalitis Virus (TBEV-FE). Additional subtypes lineage have also been proposed: strain 178–179, strain 886–884 (named Baikalian TBEV, TBEV-Bk) and Himalayan TBEV (identified in rodent *Marmota himalayana* in China) (Ecker et al., 1999; Ruzek et al., 2019; Demina et al., 2010; Kozlova et al., 2018; Dai et al., 2018).

Features of TBE described in this study are characteristic of regions where only the TBEV-Eu subtype is endemic; in regions with the predominance of the TBEV-Sib and TBEV-FE, the clinical course of TBE is slightly different.

Clinically, the infection may occur with the monophasic (with or without neurologic symptoms) or biphasic course (about two-thirds of patients), characterized by the first stage with non-specific symptoms and the second one with additional neurological involvement. Symptomatic infection without central nervous system (CNS) involvement is defined "abortive TBE" (Bogovič et al., 2010; Kaiser, 2008; Lindquist and Vapalahti, 2008). As regards of laboratory test, during the initial phase of TBE, leukopenia as well as thrombocytopenia are found in about 70% of patients, while abnormal liver function test results are relatively rare (Bogovič et al., 2010). During the second phase, elevated white blood cell count may be present, C-reactive protein (CRP) concentration and erythrocyte sedimentation rate (ESR) may be elevated, especially in long-lasting severe cases. Analysis of cerebrospinal fluid (CSF) usually shows pleocytosis and a moderately raised protein level (Bogovič et al., 2010; Kaiser and Holzmann, 2000; Kaiser, 2008; Lindquist and Vapalahti, 2008).

The fatality rate from infection with TBEV-Eu is <2%, but the incidence of sequelae may vary between 40% and 50%, with long-term or even permanent neurologic symptoms. Less is known about the risk of contracting sequelae (Bogovič et al., 2018; Haglund and Günther, 2003).

Actually, TBE is endemic in different Asiatic and European regions (World Health Organization, 2019); as far as Italy is concerned, TBE is endemic in three North-Eastern areas: Belluno, Trentino-Alto Adige and Friuli Venezia-Giulia. As Belluno is the most affected area, we aimed to investigate clinical manifestations and abnormal laboratory data during TBEV infection in this province; moreover, we analysed the correlation between abnormal laboratory data and the phase of the disease during the biphasic course and the predictive factors of neurological/neurocognitive sequelae.

2. Materials and methods

A retrospective analysis of 148 TBEV infection cases from both urban and rural areas of Belluno province admitted to the Department of Infectious Disease of S. Martino Hospital in Belluno, Italy, was performed between June 2000 and April 2019. TBEV infection diagnosis was based on serological parameters consisting in IgM or IgG and IgM positivity.

Medical data extracted from all patient medical records, were patient's age, gender, vaccination coverage for TBEV, consciousness of tick bite, Charlson Comorbidity Index (CCI), type of course (biphasic or monophasic), clinical manifestations, one-month follow up sequelae, CSF analysis, complete blood count cell analysis, electrolyte disorders, levels of CRP, ESR, fibrinogen, amylase, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), transaminase, bilirubin, gamma-glutamyltransferase (GGT).

Not all data were available for all patients, decreasing the case numbers in particular analyses.

2.1. Statistical analysis

The Chi-squared test was used to investigate the correlation between abnormal laboratory data and the stage of the disease during the biphasic course. In addition, in order to identify the predictive factors of neurological/neurocognitive sequelae, binary logistic regression was performed using SPSS 25.0 (IBM) software. The data were analysed using either Fisher's exact test or Mann-Whitney's test, as appropriate. The values $P < .05$ were considered significant.

3. Results

3.1. Epidemiology

The analysis of the distribution of TBEV infection cases in the Province of Belluno in the 2006–2018 interval is shown in Fig. 1.

3.2. Baseline characteristics

Of the 148 cases, 112 (76%) were male and 36 (24%) female, average age 53 years (13–81 years). Tick bites were reported by 74 (50%) patients. 145 (98%) had not received vaccination against TBEV, while 3 subjects (2%) had received one, two or three doses of vaccination without booster vaccine (one case each). As regards CCI, in 32% patients it was 0, in 26% it was 1, in 22% it was 2, in 13% it was 3, in 4% it was 4, in 2% it was 5, while it was 6 and 9 in 1%.

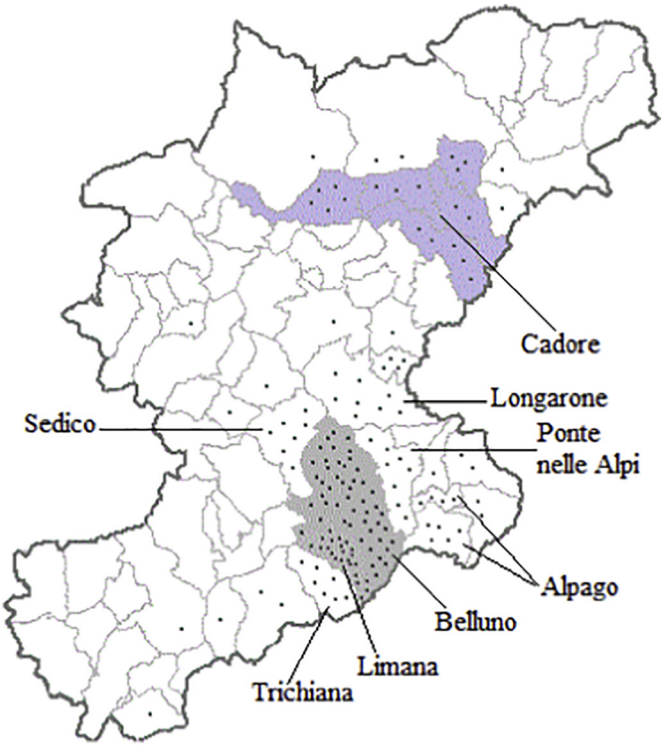

Fig. 1. Distribution of TBEV infection cases (n. 166) in the province of Belluno in the interval 2006–2018 (scattered dots correspond to number of cases). Limana and Belluno have the majority of cases and constitute an epidemic outbreak together with Trichiana, Alpago, Ponte nelle Alpi, Longarone and Sedico; the Cadore area seems to constitute another epidemic outbreak.

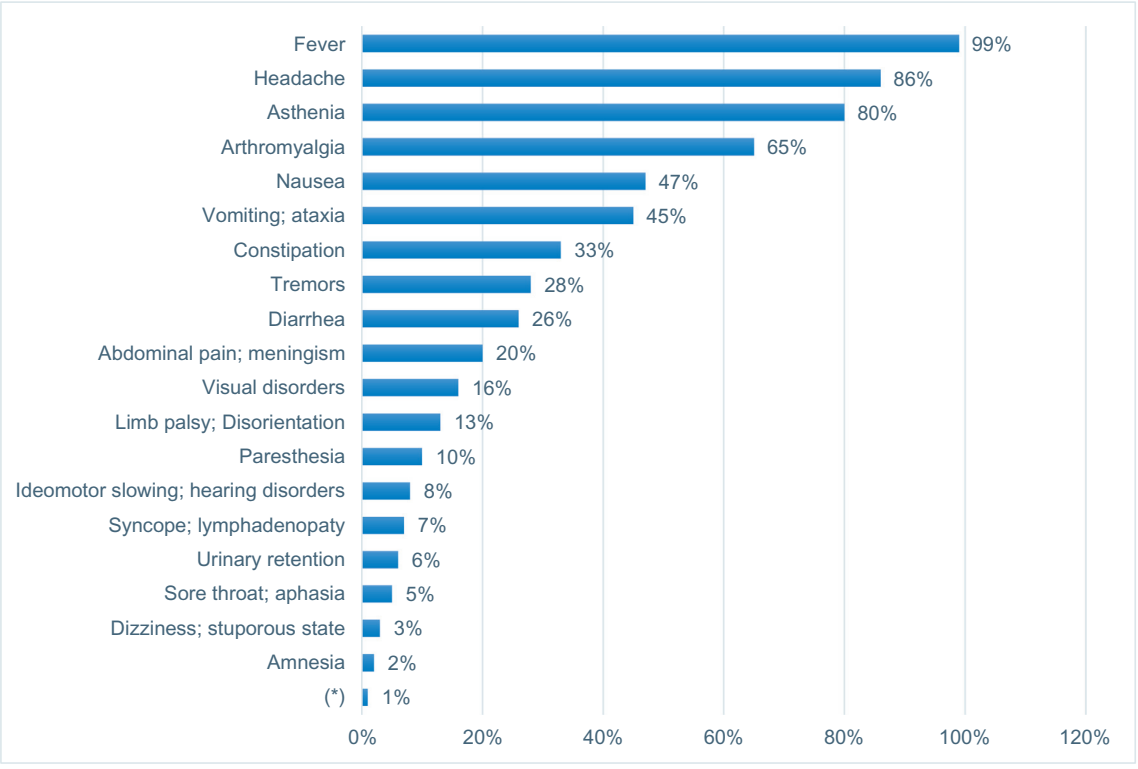

Fig. 2. Clinical manifestations observed in all patients during TBE. (*) = Facial nerve palsy; fasciculations; clonia; bradycardia; hiccups; hypotension; akinesia.

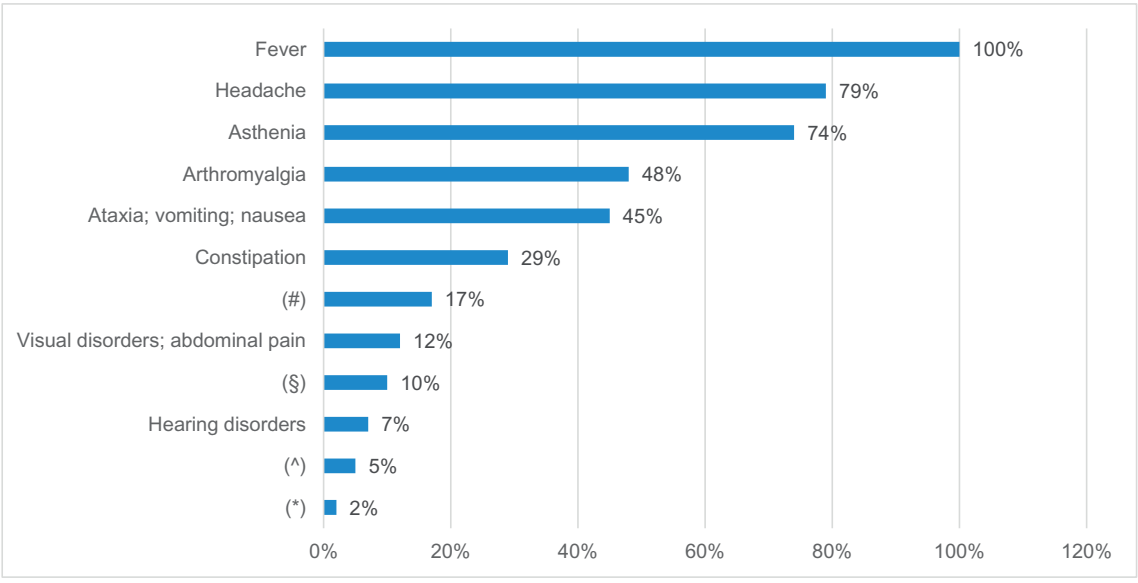

Fig. 3. Clinical manifestations observed in patients during the monophasic course. (*) = Hypotension; akinesia; amnesia; stuporous state; aphasia. (^) = Dizziness; sore throat; lymphadenopathy; syncope; paresthesia (§) = Urinary retention; ideomotor slowing; diarrhea; (#) = Limb palsy; disorientation; meningism; tremors.

3.3. Clinical findings

106 (72%) cases had a biphasic course, while 42 (28%) had a monophasic course; among patients with monophasic course, 8 cases (5% of all cases, 19% of cases with monophasic course) had abortive TBE. Figs. 2, 3, 4 and 5 show the clinical manifestations observed during infection.

3.3.1. TBEV-Lyme co-infection was diagnosed in 4 patients

None died from TBEV infection, but 52 (35%) had neurological (33/148, 22%), neurocognitive (11/148, 7%) or non-specific (46/148, 31%) sequelae. Figs. 6, 7 and 8 show the sequelae observed at the one-month follow-up. Binary logistic regression revealed

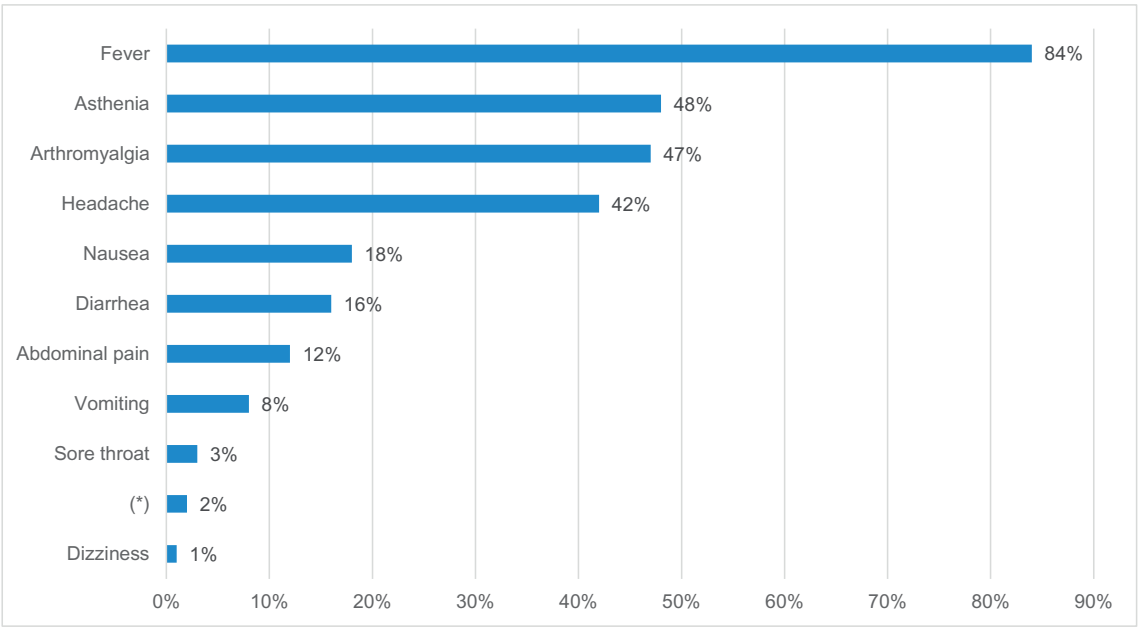

Fig. 4. Clinical manifestations observed in patients during the first phase of the biphasic course. (*) = Urinary retention; syncope; hearing disorders; visual disorders; constipation.

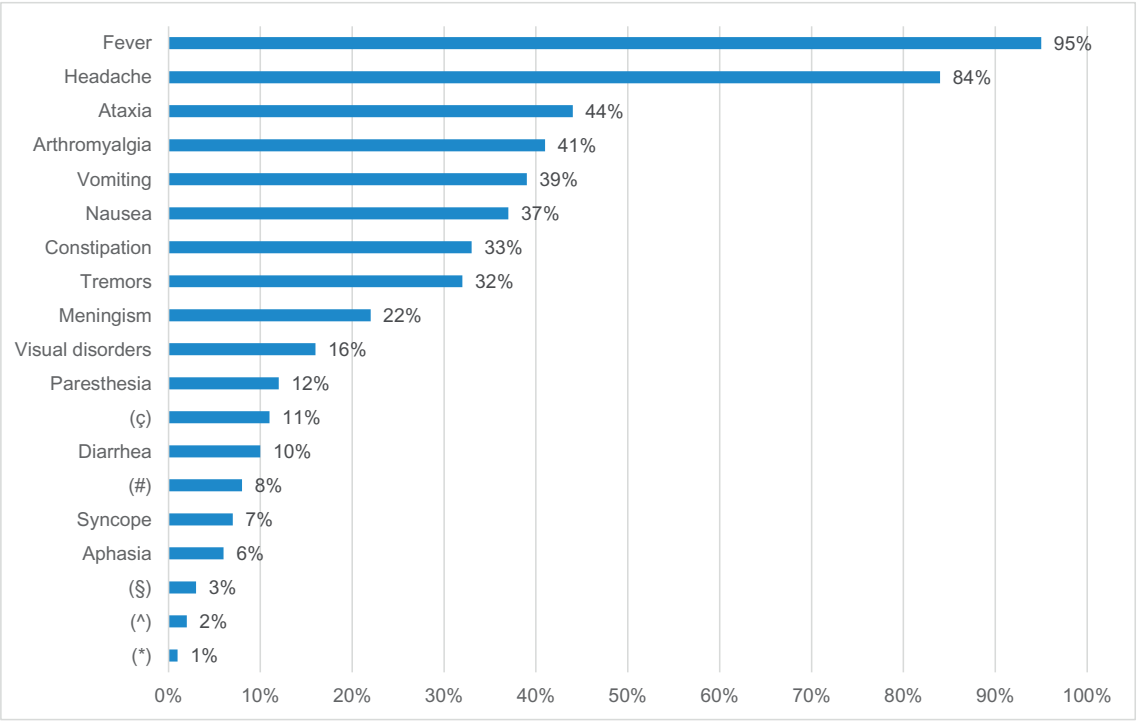

Fig. 5. Clinical manifestations observed in patients during the second phase of the biphasic course. (*) = Fasciculations; clonia; bradycardia; hiccups. (^) = Amnesia; facial nerve palsy; dizziness; (§) = Stupors state; sore throat; urinary retention; (#) = Lymphadenopathy; hearing disorders; ideomotor slowing; (ç) = Disorientation; limb palsy; abdominal pain.

that men had a 5-fold higher risk of neurological/neurocognitive sequelae (OR: 5017, 95%CI: 1199-20,987). In addition, paresthesia or tremors were independently associated with neurological/neurocognitive sequelae, causing a 9-fold higher risk of having these sequelae (R.O. 9679, 95%CI: 4066-23,038). Age, course type (monophasic or biphasic), leukocytosis, elevated CRP, transaminases, bilirubin and GGT levels were not associated with neurological/neurocognitive sequelae ($p > .05$).

3.4. Laboratory features

3.4.1. Full blood count examination

The full blood count was analysed in 147 cases. Figs. 9, 10, 11 and 12 show the blood count changes observed during the period of clinical onset. During the biphasic course, the Chi-squared test revealed that thrombocytopenia, neutropenia and lymphocytosis were significantly associated with the first phase ($p < .01$), while monocytosis and lymphocytopenia, with the second phase ($p < .05$).

3.4.2. CRP and ESR

High CRP levels were observed in 71/142 (50%) cases: 22/41 (54%) in the monophasic course, 3/22 (14%) in the first phase and 46/101 (46%) in the second.

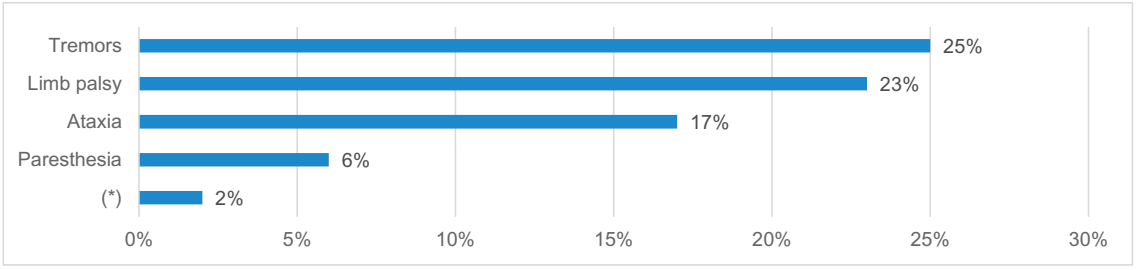

Fig. 6. Neurological sequelae observed at one-month follow-up (*) = Akinesia; hiccups; visual disorders.

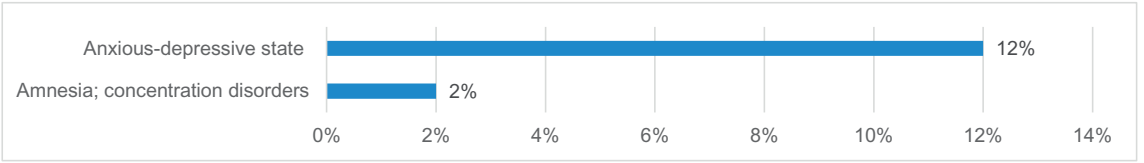

Fig. 7. Neurocognitive sequelae observed at one-month follow-up.

High levels of ESR were found in 55/110 (50%): 16/31 (52%) in the monophasic course, 3/18 (17%) during the first phase and 37/76 (49%) in the second phase. The chi- Squared test showed that both CRP and ESR were significantly associated with the second phase ($p < .05$).

3.4.3. CSF features

CSF was performed for 58 patients. Pleocytosis and raised protein levels were found in 79% each, while 22% had positive Pandy reaction. As for immunoglobulin, 36% had both IgG and IgM and 28% only IgG.

3.5. Serological analysis

During the monophasic course, at the first detection of serum antibodies, 38/42 (90%) had IgG and IgM positivity, 1/42 (2%) only IgM positivity, while 3/42 (7%) were completely negative (positive afterwards).

As for the biphasic course, during the first phase, serum antibody detection was performed for 13 patients: in 2 cases it was positive for IgG and IgM and completely negative in the other cases. During the second phase, the onset of clinical manifestations was always accompanied by positive results for both IgG and IgM, unlike 2 cases: one of these had only IgM, the other was completely negative, while later on it was positive.

3.6. Other laboratory findings

High levels of bilirubin, GGT or transaminase levels were found in 118/145 (81%) cases. In particular, 36% had hyperbilirubinemia, 23% had high levels of GGT, 21% had high ALT and 14% AST levels. During the monophasic course 39% (16/41) had hyperbilirubinemia, 22% (9/41) had high GGT levels, 20% (8/41) high ALT levels and 15% (6/41) high AST levels. In the first phase of the biphasic course 3% (1/32) had hyperbilirubinemia, 22% (7/32) high GGT levels, 25% (8/32) high ALT levels and 28% (9/32) high AST levels; the second phase was characterized by hyperbilirubinemia in 34% (35/104), high GGT levels in 20% (21/104), high ALT levels in 17% (18/104) and high AST levels in 7% (7/104).

Electrolyte disorders were observed in 63/140 (45%) cases: they showed hyponatremia (32%), hypochloraemia (31%) and hypokalaemia (18%). Fibrinogen was increased in 37/91 (41%): 6/24 (25%) in the monophasic course, 0 during the first phase and 31/65 (48%) in the second phase. High levels of amylase were observed in 9/90 (10%): 2/28 (7%) in the monophasic course, 3/13 (23%) in the first phase and 6/56 (11%) in the second phase. The increase of CPK was reported in 22/76 (29%): 8/18 (44%) in the monophasic course, 5/11 (45%) in the first phase and 9/54 (17%) in the second one.

LDH was increased in 11/90 (12%): 3/20 (15%) during the monophasic course, 2/14 (14%) in the first phase and 6/61 (10%) in the second one. Electrolyte disorders and high levels of bilirubin, GGT, ALT, AST, fibrinogen, amylase, LDH and CPK were not significantly associated with a specific phase of the biphasic course ($p > .05$).

3.6.1. Imaging findings

Computed tomography (CT) was performed in 57 patients; 13 of them (23%) had CT abnormalities. On the other hand, Magnetic Resonance Imaging (MRI) abnormalities were found in 20/45 (44%). Both CT and MRI showed no specific findings.

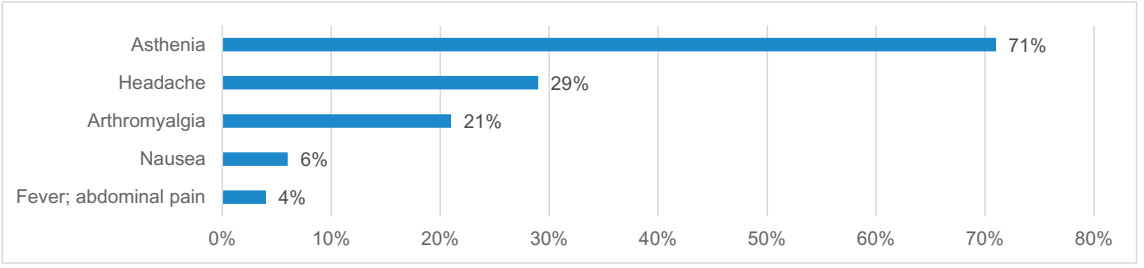

Fig. 8. Non-specific sequelae observed at one-month follow-up.

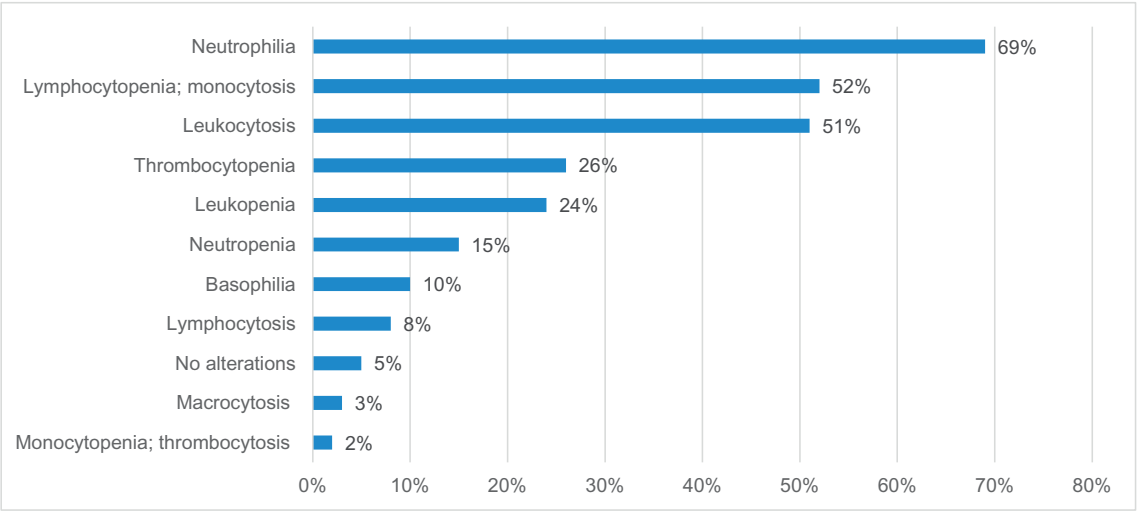

Fig. 9. Changes of the blood count during the time of clinical manifestations in all cases.

3.6.2. Electroencephalogram (EEG)

EEG was performed in 126 patients; 87/126 had different abnormalities: generalized abnormalities in 57/126 (66%), focal abnormalities in the temporal lobe in 30/126 (34%), frontal lobe in 26/126 (30%), occipital lobe in 15/126 (17%) and parietal lobe in 6/126 (7%). At one-month follow up, 27/55 (49%) patients still had EEG abnormalities.

4. Discussion

Belluno, a district in the North-East of Italy, is an ecological area that favours the spread of *Ixodes ricinus* and the maintenance of the transmission cycle of tick borne pathogens, including TBEV. Humid climate and availability of hosts are particularly suitable for the development of ticks, which have a particularly high density in this region compared to the rest of the country (Da Rold et al., 2018).

From 1994 (first human TBEV infection) to April 2019, 223 cases were reported in this city; in the 2006–2018 interval, the annual incidence was found to be 6/100.000 cases. Fig. 1 shows the distribution of TBEV infection cases in the province of Belluno in the 2006–2018 interval. In some rural areas, ticks are found in backyards and people are at high risk of being bitten.

In Italy only TBEV-Eu subtype is endemic: in regions with the predominance of the TBEV-Sib and TBEV-FE, clinical features are slightly different.

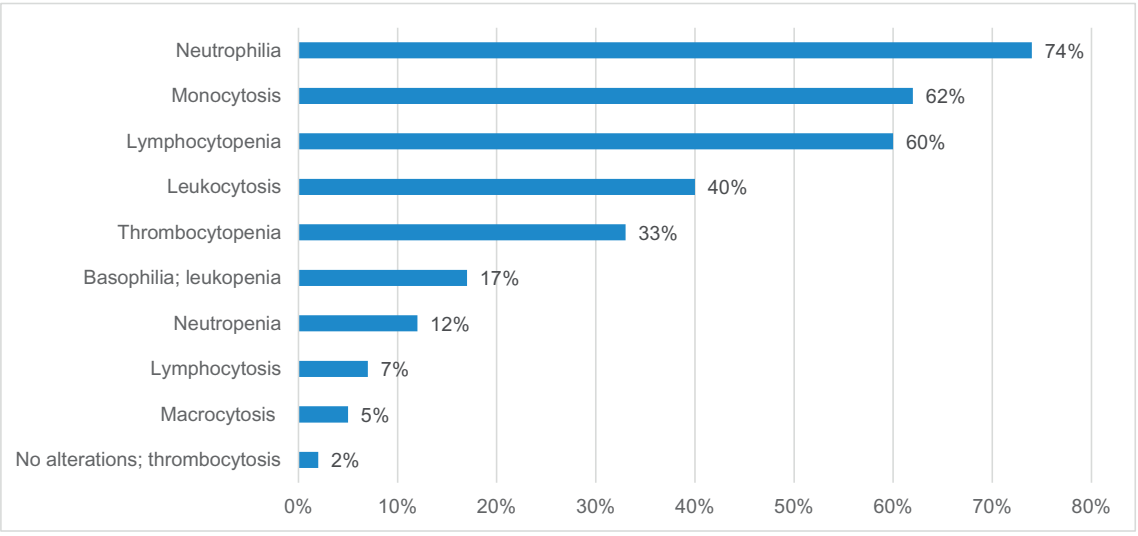

Fig. 10. Changes of the blood count during the time of clinical manifestations during the monophasic course.

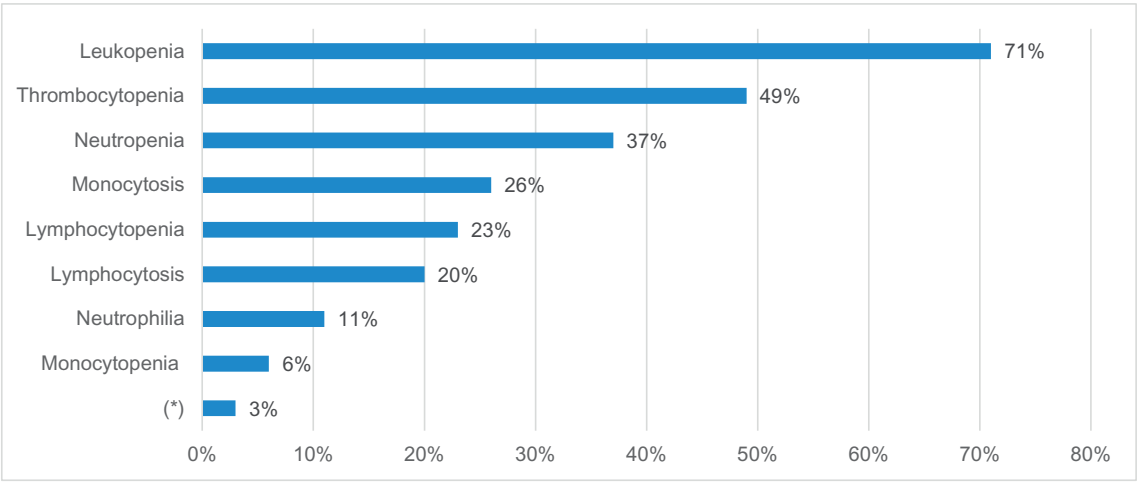

Fig. 11. Changes of the blood count during the time of clinical manifestations during the first phase of the biphasic course. (*) = No alterations; thrombocytosis; leukocytosis; basophilia; macrocytosis.

Median age is consistent with epidemiological European data. Men were more likely to become infected with TBEV than women, as observed in other EU countries; however, the ratio male/female (3:1) was higher than European data (1.5:1) (Beauté et al., 2018). Probably this was caused by Belluno's culture: men often visit rural area for work (e.g. woodcutters, shepherds, farmers, foresters), mushrooms picking, hunting, sporting activities (e.g. mountain-biking, trekking). Patients had a low CCI score: good health probably allows more outdoor activities, causing exposure to the risk of being bitten. CCI is one of the most used scoring system for comorbidities in research, considering the number and severity of 19 predefined comorbid conditions (age, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular accident or transient ischemic attack, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, hemiplegia, chronic kidney disease, solid tumor, leukemia, lymphoma, AIDS); this score can be used to predict short and long-term outcomes (e.g. hospitalization duration, physical function and mortality rates) (Charlson et al., 1987).

While 98% did not have receive any TBEV vaccination dose, 3 cases received one, two or three doses without booster (one case each): an incomplete vaccination program does not completely protect humans from TBEV infection (Ruzek et al., 2019).

Even if patients do not report the presence of ticks in their history, tick diseases should always be considered. In fact, 50% ignore tick-bite, probably due to the modulation of pain reflexes and itching of the active molecules in tick-saliva. In addition, a long lasting tick bite is not necessary for TBEV transmission.

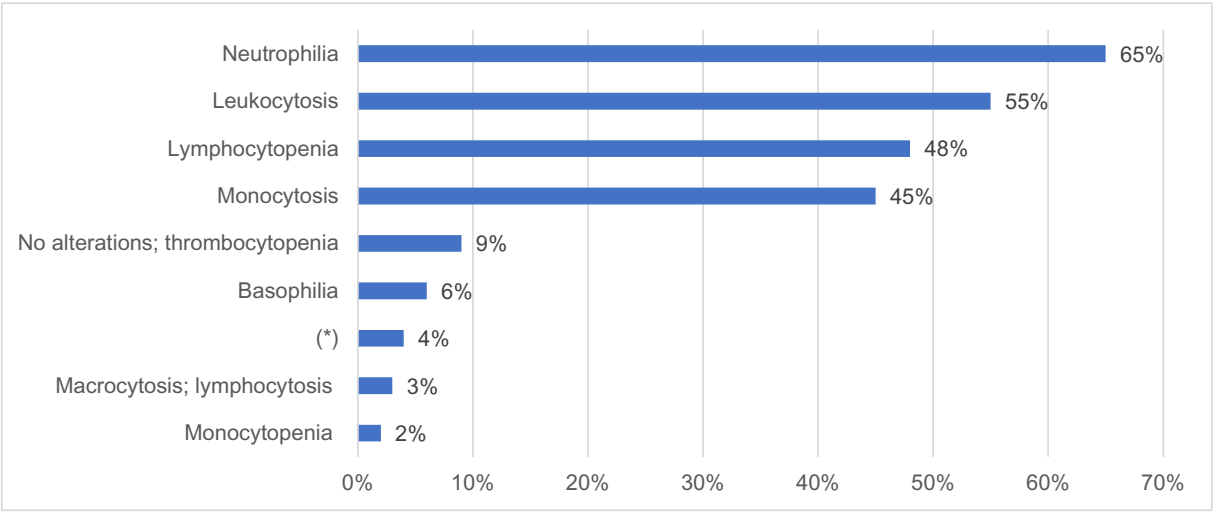

Fig. 12. Changes of the blood count during the time of clinical manifestations during the second phase of the biphasic course. (*) = Thrombocytosis; neutropenia; leukopenia.

The serological diagnosis of TBEV infection is based on positive IgM or IgG and IgM antibodies. However, during the first phase and the beginning of the second phase or the monophasic course, the serological analysis may be negative; in this context it must be repeated after a few days (Ruzek et al., 2019).

According to the literature, and with regard to clinical features, the biphasic course has been reported more often than the monophasic course (Bogovič and Strle, 2015); moreover, abortive TBE was identified as 5% more than other reports (<2%) (Lotric-Furlan et al., 2002). Abortive TBE is not often reported because of difficulty of diagnosis, as it is characterized only by non-specific symptoms such as fever, asthenia, headache, arthromyalgia.

Raising the awareness of the population of Belluno about tick-borne disease could perhaps allow a more in-depth diagnosis: even if people have mild symptoms after a probable tick bite, they should consult their doctor so that a specific serological analysis for TBEV can be carried out. This is the presumed reason why abortive TBE is more identified in Belluno (Barp et al., 2019a).

The diagnosis of TBEV infection can be difficult, since symptoms are not characteristic as observed in Fig. 2. The most frequent neurological symptoms were ataxia (45%), tremors (28%), meningism (20%), visual disturbances (16%) and limb paralysis (13%). Ataxia and limb paralysis were observed more than reported in other studies, while meningism was lower (Czupryna et al., 2011).

During the biphasic course, the first phase was mostly characterized by flu-like syndrome; 9 patients presented neurological symptoms during this phase including vision and hearing disorders, urinary retention, meningism, dizziness and tremors. The first phase is usually defined without any CNS involvement (Bogovič and Strle, 2015; Gritsun et al., 2003; Haglund and Günther, 2003; Kaiser, 2012; Ruzek et al., 2019); however, it is rarely characterized by neurologic sign and symptoms, as described in some case-reports (Dorko et al., 2018). As regards the second phase, neurologic sign and symptoms have always been present; cranial nerve involvement was reported as visual and hearing disturbances, dizziness and facial palsy.

The monophasic course was also characterized by no-specific and neurological sign and symptoms; limb palsy and disorientation were more frequent than in the biphasic course (Barp et al., 2019a).

According to literature data (35–58%) (Czupryna et al., 2018), at one-month follow up, 35% had no specific sequelae, mostly no-specific such as asthenia, headache and arthromyalgia. Instead, neurological sequelae (22%) were mainly characterized by tremors, limb palsy and ataxia. In contrast with other findings, dysphagia and hearing disorders were not observed in this study (Bogovič et al., 2010; Haglund and Günther, 2003). Neurocognitive sequelae, reported in 7%, were mostly characterized by anxious-depressive syndrome. Unlike other previous reports, neurocognitive sequelae were less frequent, probably due to underestimation: follow-up was only performed after one month, but different sequelae, mainly neurocognitive, may appear after several months from the infection (Czupryna et al., 2018).

Some studies reported age, CSF protein and leukocyte concentration, CSF albumin index, CSF IgG index, presence of ataxia or palsy, abnormal MRI and TBE-Lyme co-infection as risk factor predisposing to sequelae development (Bogovič et al., 2018; Czupryna et al., 2018; Haglund et al., 1996; Kaiser, 1999; Karelis et al., 2012; Mickiene et al., 2002). However, other studies have not found these risk factors (Günther et al., 1997). In our study the risk of neurological/neurocognitive sequelae is about 5 times higher for men than for women (OR: 5.017, 95%CI: 1.199–20.987), while paresthesia or tremors were independently associated with neurological/neurocognitive sequelae (higher risk than 9 times, O.R. 9.679, 95%CI: 4.066–23.038). Instead, age, course type (monophasic or biphasic), leukocytosis, elevated CRP, transaminase, bilirubin and GGT levels were not associated with neurological/neurocognitive sequelae ($p > .05$).

Unlike clinical and CSF characteristics, other abnormal laboratory data are not so often considered in literature (Barp et al., 2019b). Complete blood count usually exhibits neutrophilia (69%), monocytosis (52%), lymphocytopenia (52%) and leukocytosis (51%). In our study, during the first phase, thrombocytopenia, neutropenia and leukocytosis were frequently observed ($p < .01$), unlike other studies that reported only leukopenia and thrombocytopenia. The second phase was associated with monocytosis and lymphocytopenia ($p < .05$), while others reported only neutrophil leukocytosis (Bogovič and Strle, 2015; Lotric-Furlan and Strle, 1995). High levels of CRP and ESR (50% each) were significantly associated with the second phase ($p < .05$).

Compared to the literature, levels of transaminase, GGT, bilirubin, CRP, ESR and electrolyte disorders, were observed more frequently (Bogovič and Strle, 2015; Grygorczuk et al., 2002; Ruzek et al., 2019). Electrolyte disorders were present in 45%, particularly hyponatremia (32%), hypochloraemia (31%) and hypokalaemia (18%) probably caused by fever with sweating or diarrhea. Syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is sometimes described during TBEV infection, but no patient presented SIADH in this study (Czupryna et al., 2014).

In contrast to most epidemiological and clinical studies conducted so far, we found some abnormal laboratory data such as high levels of fibrinogen (41%), CPK (29%), LDH (12%) and amylase (10%). As to why these alterations in laboratory tests are sometimes observed, some assumptions are made. Fibrinogen is probably more produced during the acute phase of TBEV infection (as in other acute infections); instead high levels of LDH could be caused by cellular damage during viral infection. CPK levels show possible muscle damage in some patients; a correlation between high CPK levels and the presence of arthromyalgia has been hypothesized but has not been found ($p > .05$). Finally, high levels of amylase are probably caused by salivary gland involvement during TBEV infection.

The one-month's follow up revealed more EEG abnormalities than sequelae: although patients had no neurological/neurocognitive symptoms, they could present abnormalities in EEG.

5. Conclusion

Although patients have not reported tick bites in their history and have received almost a dose of TBEV vaccination, the diagnosis of TBEV infection should always be considered in rural or endemic areas. In addition, although the serological analysis may be negative at the beginning, it should be repeated after a few days if the diagnostic doubt persists. Sensitization of population could permit more diagnosis of abortive TBE.

Abnormal laboratory data, together with clinical features, can help clinicians in diagnosing and monitoring time. Paresthesia and tremors are independently associated with neurological/neurocognitive sequelae.

The first phase is associated with thrombocytopenia, neutropenia and lymphocytosis, while the second phase is associated with monocytosis, lymphocytopenia and high levels of CRP and ESR. Electrolyte disorders, high levels of transaminases, GGT, bilirubin, CPK, LDH, fibrinogen and amylase have been also observed. The EEG is mostly abnormal, while the temporal lobe is more affected.

A limitation of our retrospective study is the lack of different laboratory, imaging and EEG results from some patients and clinical data on the presence of sequelae after one-month follow-up. A larger sample will be needed to confirm the laboratory data and to analyse the images and EEG results. In addition, EEG data should not be considered totally objective due to neurologists' EEG interpretation.

Declaration of Competing Interest

None.

Acknowledgments

The authors would like to thank S. Martino Hospital in Belluno, particularly the Department of Infectious Disease.

References

Barp, N., Di Nuzzo, M., Maritati, M., Mondardini, V., et al., 2019a. Epidemiological and clinical issues of Tick Borne Encephalitis Virus infection in Italy (poster n. 0055). Presented in part at 29th ESCMID, Amsterdam, Netherlands (Personal Communication(a)).

Barp, N., Di Nuzzo, M., Trentini, A., Maritati, M., et al., 2019b. Significance and value of laboratory data in Tick-Borne Encephalitis Virus infection (TBEVi): a North-Eastern Italy Experience (poster P17). Presented in part at International Symposium on Tick-Borne Pathogens and Disease 2019, Vienna (personal communication(b)).

Beauté, J., Spiteri, G., Warns-Petit, E., Zeller, H., 2018. Tick-borne encephalitis in Europe, 2012 to 2016. Euro Surveill. 23 (45). https://doi.org/10.2807/1560-7917.ES.2018.23.45.1800201.

Bogovič, P., Strle, F., 2015. Tick.borne encephalitis: a review of epidemiology, clinical characteristics, and management. World J. Clin. Cases 3 (5), 430–441. https://doi.org/10.1016/j.tmaid.2010.05.01110.12998/wjcc.v3.i5.430.

Bogovič, P., Lotric-Furlan, S., Strle, F., 2010 Jul. What tick-borne encephalitis may look like: clinical signs and symptoms. Travel Med. Infect. Dis. 8 (4), 246–250. https://doi.org/10.1016/j.tmaid.2010.05.011.

Bogovič, P., Stupica, D., Rojko, T., Lotrič-Furlan, S., Avšič-Županc, T., Kastrin, A., Lusa, L., Strle, F., 2018. The long-term outcome of tick-borne encephalitis in Central Europe. Ticks Tick Borne Dis. 9 (2), 369–378. https://doi.org/10.1016/j.ttbdis.2017.12.001.

Charlson, M.E., Pompei, P., Ales, K.L., 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J. Chronic Dis. 40, 373–383. https://www.ncbi.nlm.nih.gov/pubmed/3558716.

Czupryna, P., Moniuszko, A., Pancewicz, S.A., Grygorczuk, S., Kondrusik, M., Zajkowska, J., 2011. Tick-borne encephalitis in Poland in years 1993-2008–epidemiology and clinical presentation. A retrospective study of 687 patients. Eur. J. Neurol. 18 (5), 673–679. https://doi.org/10.1111/j.1468-1331.2010.03278.x.

Czupryna, P., Moniuszko, A., Garkowski, A., Pancewicz, S., Guziejko, K., Zajkowska, J., 2014. Evaluation of hyponatraemia in patients with tick-borne encephalitis–a preliminary study. Ticks Tick Borne Dis. 5 (3), 284–286. https://doi.org/10.1016/j.ttbdis.2013.11.005.

Czupryna, P., Grygorczuk, S., Krawczuk, K., Pancewicz, S., Zajkowska, J., Dunaj, J., Matosek, A., Kondrusik, M., Moniuszko-Malinowska, A., 2018. Sequelae of tick-borne encephalitis in retrospective analysis of 1072 patients. Epidemiol. Infect. 146 (13), 1663–1670. https://doi.org/10.1017/S0950268818002005.

Da Rold, G., Ravagnan, S., Soppelsa, F., Porcellato, E., Soppelsa, M., Obber, F., Citterio, C.V., Carlin, S., Danesi, P., Montarsi, F., Capelli, G., 2018. Ticks are more suitable than red foxes for monitoring zoonotic tick-borne pathogens in northeastern Italy. Parasit. Vectors 11 (1), 137. https://doi.org/10.1186/s13071-018-2726-7.

Dai, X., Shang, G., Lu, S., Yang, J., Xu, J., 2018. A new subtype of eastern tick-borne encephalitis virus discovered in Qinghai-Tibet plateau, China. Emerg. Microb. Infect. 7 (1), 74. https://doi.org/10.1038/s41426-018-0081-6.

Daniel, M., Benes, C., Danielová, V., Kríz, B., 2011. Sixty years of research of tick borne encephalitis—a basis of the current knowledge of the epidemiological situation in Central Europe. Epidemiol. Mikrobiol. Imunol. 60 (4), 135–155. https://www.ncbi.nlm.nih.gov/pubmed/22324243.

Daniel, M., Danielová, V., Fialová, A., Malý, M., Kříž, B., Nuttall, P.A., 2018. Increased relative risk of tick-borne encephalitis in warmer weather. Front. Cell. Infect. Microbiol. 8, 90. https://doi.org/10.3389/fcimb.2018.00090.

Demina, T.V., Dzhioev, Y.P., Verkhozina, M.M., Kozlova, I.V., Tkachev, S.E., Plyusnin, A., Doroshchenko, E.K., Lisak, O.V., Zlobin, V.I., 2010. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. J. Med. Virol. 82 (6), 965–976. https://doi.org/10.1002/jmv 21765.

Dorko, E., Hockicko, J., Rimárová, K., Bušová, A., Popad'ák, P., Popad'áková, J., Schréter, I., 2018. Milk outbreaks of tick-borne encephalitis in Slovakia 2012–2016. Cent. Eur. J. Public Health 26 (Suppl), S47–S50. https://doi.org/10.21101/cejph.a5272.

Ecker, M., Allison, S.L., Meixner, T., Heinz, F.X., 1999. Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. J. Gen. Virol. 80 (Pt 1), 179–185. https://www.ncbi.nlm.nih.gov/pubmed/9934700.

Gritsun, T.S., Lashkevich, V.A., Gould, E.A., 2003. Tick-borne encephalitis. Antivir. Res. 57 (1–2), 129–146. https://www.ncbi.nlm.nih.gov/pubmed/12615309.

Grygorczuk, S., Mierzynska, D., Zdrodowska, A., Zajkowska, J., Pancewicz, S., Kondrusik, M., Swierzbinska, R., Pryszmont, J., Hermanowska-Szpakowicz, T., 2002. Tick-borne encephalitis in North-Eastern Poland in 1997-2001: a retrospective study. Scand. J. Infect. Dis. 34 (12), 904–909. https://www.ncbi.nlm.nih.gov/pubmed/12587623.

Günther, G., Haglund, M., Lindquist, L., Forsgren, M., Sköldenberg, B., 1997. Tick-bone encephalitis in Sweden in relation to aseptic meningo-encephalitis of other etiology: a prospective study of clinical course and outcome. J. Neurol. 244 (4), 230–238. https://www.ncbi.nlm.nih.gov/pubmed/9112591.

Haglund, M., Günther, G., 2003. Tick-borne encephalitis-pathogenesis, clinical course and long-term follow-up. Vaccine. 21 (Suppl. 1), S11–S18. https://www.ncbi.nlm.nih.gov/pubmed/12628810.

Haglund, M., Forsgren, M., Lindh, G., Lindquist, L., 1996. A 10-year follow-up study of tick-borne encephalitis in the Stockholm area and a review of the literature: need for a vaccination strategy. Scand. J. Infect. Dis. 28 (3), 217–224. https://www.ncbi.nlm.nih.gov/pubmed/8863349.
Kaiser, R., 1999. The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994-98: a prospective study of 656 patients. Brain. 122 (Pt 11), 2067–2078. https://www.ncbi.nlm.nih.gov/pubmed/10545392.
Kaiser, R., 2008. Tick-borne encephalitis. Infect. Dis. Clin. N. Am. 22 (3), 561–575. https://doi.org/10.1016/j.idc.2008.03.013.
Kaiser, R., 2012. Tick-borne encephalitis: clinical findings and prognosis in adults. Wien. Med. Wochenschr. 162 (11−12), 239–243. https://doi.org/10.1007/s10354-012-0105-0.
Kaiser, R., Holzmann, H., 2000. Laboratory findings in tick-borne encephalitis–correlation with clinical outcome. Infection. 28 (2), 78–84. https://www.ncbi.nlm.nih.gov/pubmed/10782392.
Karelis, G., Bormane, A., Logina, I., Lucenko, I., Suna, N., Krumina, A., Donaghy, M., 2012. Tick-borne encephalitis in Latvia 1973-2009: epidemiology, clinical features and sequelae. Eur. J. Neurol. 19 (1), 62–68. https://doi.org/10.1111/j.1468-1331.2011.03434.x.
Kozlova, I.V., Demina, T.V., Tkachev, S.E., Doroshchenko, E.K., Lisak, O.V., Verkhozina, M.M., Karan, L.S., Dzhioev, Y.P., Paramonov, A.I., Suntsova, O.V., Savinova, Y.S., Chernoivanova, O.O., Ruzek, D., Tikunova, N.V., Zlobin, V.I., 2018. Characteristics of the Baikal subtype of tick-borne encephalitis virus circulating in Eastern Siberia. Acta Biomed. Sci. 3 (4), 53–60. https://doi.org/10.29413/ABS.2018-3.4.9.
Lindquist, L., Vapalahti, O., 2008. Tick-borne encephalitis. Lancet. 371 (9627), 1861–1871. https://doi.org/10.1016/S0140-6736(08)60800-4.
Lotric-Furlan, S., Strle, F., 1995. Thrombocytopenia–a common finding in the initial phase of tick-borne encephalitis. Infection. 23 (4), 203–206. https://www.ncbi.nlm.nih.gov/pubmed/8522376.
Lotric-Furlan, S., Avsic-Zupanc, T., Strle, F., 2002 Jul 31. An abortive form of tick-borne encephalitis (TBE)–a rare clinical manifestation of infection with TBE virus. Wien. Klin. Wochenschr. 114 (13–14), 627–629. https://www.ncbi.nlm.nih.gov/pubmed/12422615.
Mickiene, A., Laiskonis, A., Günther, G., Vene, S., Lundkvist, A., Lindquist, L., 2002. Tickborne encephalitis in an area of high endemicity in Lithuania: disease severity and long-term prognosis. Clin. Infect. Dis. 35 (6), 650–658. https://www.ncbi.nlm.nih.gov/pubmed/12203160.
Riccardi, N., Antonello, R.M., Luzzati, R., Zajkowska, J., Di Bella, S., Giacobbe, D.R., 2019. Tick-borne encephalitis in Europe: a brief update on epidemiology, diagnosis, prevention, and treatment. Eur. J. Intern. Med. 62, 1–6. https://www.ncbi.nlm.nih.gov/pubmed/30678880.
Ruzek, D., Avšič Županc, T., Borde, J., Chrdle, A., Eyer, L., Karganova, G., Kholodilov, I., Knap, N., Kozlovskaya, L., Matveev, A., Miller, A.D., Osolodkin, D.I., Överby, A.K., Tikunova, N., Tkachev, S., Zajkowska, J., 2019. Tick.Borne encephalitis in Europe and Russia: review of pathogenesis, clinical features, therapy, and vaccines. Antivir. Res. 164, 23–51. https://www.ncbi.nlm.nih.gov/pubmed/30710567.
World Health Organization, 2019. International Travel and Health. Tick-Borne Encephalitis. https://www.who.int/ith/diseases/tbe/en/.
Zlobin, V.I., Pogodina, V.V., Kahl, O., 2017. A brief history of the discovery of tick-borne encephalitis virus in the late 1930s (based on reminiscences of members of the expeditions, their colleagues, and relatives). Ticks Tick Borne Dis. 8 (6), 813–820. https://doi.org/10.1016/j.ttbdis.2017.05.001.