

IS THERE AN OUTBREAK OF TICK-BORNE ENCEPHALITIS IN PERNIK DISTRICT, BULGARIA? FOUR CASES REGISTERED FOR A PERIOD OF FOUR YEARS – CLINICAL MANIFESTATIONS AND EPIDEMIOLOGICAL RELATIONS

**Evgeniya Taseva¹, Iva. Christova¹,
Elitsa Panayotova¹, Diana Ilieva²,
Valentina Pavlova²**

¹National reference laboratory of vector-borne infections, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria

² Department of Infectious Diseases, Rahila Angelova Hospital, Pernik, Bulgaria

ABSTRACT

In Europe, the number of tick-borne encephalitis (TBE) cases has been increasing in the last decade, and the number of endemic areas has also been increasing. Bulgaria, located in southeastern Europe, is not in the TBE endemic area. However, sporadic TBE cases have been occasionally detected. The disease has a natural-focal nature, pronounced seasonality, with a vector- *Ixodes* ticks that transmit the infection from animals to humans. TBE is severe infection with specific lesions of the central nervous system (CNS), with residual phenomena and high lethality. The first cases of TBE in Pernik district were registered in 2015. The aim of the study is to consider clinical cases of TBE in Pernik district during period 2017-2020, to look for an epidemiological link between them and to monitor the severity of the infection. We present briefly the most characteristic clinical and laboratory features of four laboratory confirmed cases of tick-borne encephalitis in the last 4 years. The mean age of patients was 56. All four cases were

observed in May, June, and July. They proceeded relatively smoothly with a favourable outcome, without paresis or paralysis of the limbs, without seizures or loss of consciousness. In the first case, no pathological changes in the brain were observed by computed tomography, while in the other three cases multiinfarction encephalopathy, evidence of initial cerebral edema and two porencephalic foci were found. Two of the patients had meningoradicular irritation with positive symptoms of Kerning, Brudzinski and Babinski, while in the other two patients these symptoms were absent. In all cases there was a classic change in the hematogramme: moderate leukocytosis with granulocytosis, and the study of cerebrospinal fluid revealed a slight increase in total protein, moderate pleiocytosis and normal values of sugar and chloride. Only one of the patients was reported to be bitten by a tick, and the other three were most likely infected through food (raw goat's milk). Although isolated, onfirmed cases of TBE in Pernik district, indicate circulation of TBE virus in this region. This is facilitated by a number of factors: climatic changes, activity of the epizootic process in the tick population, different vertebrate species of s in natural foci; presence of a large number of infected goats. Comprehensive measures such as: raising the awareness of clinicians, considering the infection in patients with viral meningitis, intensified screening of raw milk by the Bulgarian Food Safety Agency, future studies of ticks and farm animals for the presence of TBE in this region are needed. This study is a first step in this direction.

Keywords: *tick-borne encephalitis, endemic outbreak, clinical symptoms*

INTRODUCTION

Tick-borne encephalitis (TBE) is a zoonosis, caused by TBE virus (TBEV), a member of the genus *Flavivirus* within the family *Flaviviridae*, that causes fatal encephalitis with severe sequelae in humans (22). TBEV is prevalent over a wide area of Europe and the number of reported TBE cases has been increasing in the past years despite of increased use of TBEV-vaccine not subsidized by the healthcare system across the Europe (20,22). The three main subtypes of TBEV are European, Siberian and Far Eastern (11). They are closely related both genetically and antigenically. Tick-borne encephalitis is the most common arboviral infection in Europe. In Europe alone, 5000-6000 cases have been registered annually in the recent years (5, 21). Most autochthonous cases have been registered in the territories of Lithuania, Estonia and the Czech Republic. In 2016, the Netherlands reported the first two autochthonous cases of tick-borne encephalitis. Two years ago, the number of cases in Switzerland by 20% has been seen.

ADDRESS FOR CORRESPONDENCE:

Evgeniya Taseva, PhD
Department of Microbiology
National Centre of Infectious and Parasitic Diseases
44A, Gen. N. Stoletov Blvd.
1233 Sofia, Bulgaria,
e- mail: evgenia_taseva@yahoo.com,
Tel. +359 2832 91 12

TBE is a focal infection. The sick person is not a source of infection. Different species of vertebrates, mainly rodents (*Apodemus*, *Myodes*, *Microtus*, etc.) serve as natural reservoir of the virus in nature and *Ixodes* ticks are vectors. The main tick vector in Europe is *Ixodes ricinus*, and for Russia and the Far East – *Ixodes persulcatus* (13). The virus is transmitted to humans by the bite of an infected tick or by consuming most often of raw goat's milk (3).

Global climate changes affect the epidemiology of vector-transmitted infections. The increased average temperatures in winter have expanded the northern boundary of the range of vectors' distribution to the north. Bulgaria is not an endemic region for tick-borne encephalitis. Climatic changes in our country have contributed to the spread of the main TBE vectors *Ixodes ricinus* ticks, and created real opportunities for contracting the infection. In Bulgaria, single TBE cases have been described since the first one in 1953 (23). Individual cases in humans were described in 1961 (1), 1966 (2) and 2006 (15). The disease is almost unknown to us. It is associated with the consumption of raw goat's milk. In 2003, P. Manolov and G. Katsarov detected a case of TBE in a 57-year-old man in Varna district after consumption of raw goat's milk (15). Between 2009 and 2012, five more cases of TBE were described, one of which was fatal (7,17).

Laboratory diagnosis of TBE, based on serological complement fixation assay, was introduced in Bulgaria in the 1970s. Since then, single case reports of presumed TBE have been reported, but these lack reliable microbiological confirmation. Modernization of the laboratory diagnostic approach allowed targeted detection of patients with tick-borne encephalitis in Bulgaria. Beginning in 2009, the National Reference Laboratory of Vector-Borne infections has introduced reliable laboratory diagnosis methods for TBE, based on polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) (9).

TBE virus infections are mostly asymptomatic. Only in 2015, the first cases of TBE in Pernik district were registered (a 51-year-old man and a 64-year-old woman with neurological manifestations, with detected specific anti-TBEV antibodies of IgM and IgG classes by ELISA). A link has been established between age and severity of the infection (12) and both patients were diagnosed with viral encephalitis. Both cases originated from Pernik region – near border with Serbia and near the districts of Kyustendil and Sofia, where we found relatively high levels of TBEV seroprevalence among healthy individuals - 2% and 3.03%, respectively (8,17).

The two patients lived in the same household. They had no data of tick bites. We assume that of the infection was associated with consumption of raw

milk and / or other dairy products. The occurrence of two cases of severe TBE, within half a year, among the total of 225 studied patients (or 0.9%) again suggested that the virus is clearly not so rare in the country, and is a cause of some neuroinfections in Bulgaria (8).

The aim of the study is to consider the clinical cases of TBE in Pernik district during the last 4 years, to look for an epidemiological link between them and to monitor the severity of the infection.

MATERIAL AND METHODS

Four patients with tick-borne encephalitis from Pernik district were observed. Methods for epidemiological observation and follow-up, clinical-laboratory, diagnostic imaging, virological and serological tests were employed.

ELISA commercial kits (Euroimmun, Germany) were used to detect TBEV specific IgM and IgG antibodies. Serum samples were diluted 1: 100 in serum diluent. Peroxidase-labeled anti-human IgM or IgG goat conjugate from was used. Optical density of each well was read on an ELISA reader BIOTEK Elx 800 at a wavelength of 450 nm. The results were calculated and interpreted according to the instructions:

< 0.9 u / ml - negative result

0.9 - 1.1 u / ml - limit result

> 1.1 u / ml - positive result.

Serum samples from the four patients with clinical evidence of tick-borne encephalitis were investigated. The samples were drawn on day 7 of the disease. Three of the patients lived in the town of Pernik and one in the town of Breznik, Pernik district.

RESULTS

We briefly present the most characteristic clinical and laboratory features of the four laboratory confirmed cases of tick-borne encephalitis in the last 4 years.

First case Patient with viral encephalitis. A 26-year-old man was admitted to the Clinic of Infectious Diseases of "Rahila Angelova" Hospital in Pernik on June 5, 2017 with complaints of fever, headache, noise and light-provoked irritation, nausea, vomiting, unstable gait, dating from 5-6 days and general fatigue. Physical examination found that the patient was afebrile, adequate, dehydrated and intoxicated. Neurological status showed strong neck rigidity and positive symptoms of Kernig, Brudzinski and of Babinski on the right side. The headache persisted for 4 days. The patient did not report a tick bite. Diagnosis at admission was unspecified viral encephalitis.

Laboratory tests showed leukocytosis - up to 12.0×10^9 g/l with an increase of granulocytes (88.4%). CSF examination showed in total protein increase - 0.584 g/l (slightly elevated), and increased number of cells (42). There were no pathological findings

from radiography of the lungs and ultrasound of the abdominal organs. Diffuse and focal changes in the brain parenchyma were not detected by computed tomography examination of the head.

Candida albicans was isolated by microbiological cultivation. ELISA for TBEV antibodies detected 5.7 IU IgM and 1.6 IU IgG antibodies in the first serum sample of the patient (norm up to 1.1 IU).

Treatment with ceftriaxone and dexamethasone was started. The patient was discharged after complete clinical recovery, with persisting astheno-adynamic syndrome. At the control clinical examination 30-days later, no abnormalities in neurological status were found.

Second case *Viral meningoencephalitis in a patient with hypertensive heart.* An 86-year-old man was admitted in the infectious ward of "Rahila Angelova" Hospital in Pernik on June 17, 2019. He complained of headache, fever with chills, slow speech and difficult moving. The body temperature has been increased for 5 days, dizziness and nausea appeared. The patient reported repeated tick bites in a village where ticks were abundant. He was admitted to the ward in impaired general condition, fever - 38°, adequate and dehydrated. Neurological status revealed unstable Romberg symptom, positive symptoms of Kernig and Brudzinski bilaterally, and positive symptom of Babinski on the left.

Laboratory tests showed mild anemia - hemoglobin 128 g/l, leukocytosis - up to 11.1×10^9 g/l with increased level of polymorphonuclear leukocytes and lymphopenia (Lym -12.9%). A lumbar puncture was performed on 17 June. Cerebrospinal fluid examination showed normal of total protein and glucose values, erythrocytes - 4-5, Pandi reaction - opalescence. Microbiological examinations of throat and nasal secretions, blood culture and uroculture did not give growth.

The X-ray showed a reinforced picture of the lung on the left paracardial area. Against this background, an inflammatory-infiltrative process could not be ruled out. Computed tomography of the head showed demyelination of the white matter of vascular type in the area of corona radiata and centrum semiovale. Signs of multiinfarction encephalopathy were present.

Serological testing showed antibodies of significant values against tick-borne virus encephalitis - IgG -2.05 IU and IgM - 7.96 IU (positive results above 1.1 IU). After treatment with ceftriaxone, cyprinol and dexamethasone, the patient's condition gradually improved, he became afebrile, the toxo-infectious and astheno-adynamic syndromes and meningo-radicular reactions disappeared.

Third case *A case of unspecified viral encephalitis.* It concerns a 41-year-old man. He was admitted for

treatment in the infectious ward at "Rahila Angelova" Hospital in Pernik on May 23, 2020. For 3 days he had headache, high fever (39° C) with chills, nausea, vomiting and general weakness.

At admission, he was in a damaged general condition, febrile (37.8° C), adequate, dehydrated, with acute vesicular respiration. Neurological status revealed an unstable symptom of Romberg.

Laboratory tests found leukocytosis - up to 14.8×10^9 /l with lymphopenia (Lym-8.5%) and granulocytosis (granulocytes-87.6%). On May 27, lumbar function was performed. Examination of the cerebrospinal fluid showed a significant increase of total protein (0.912 g/l), erythrocytes - $3-4 \times 10^{12}$ /l, reactions of Pandi, Rivalta and Pavlovich - opalescence, 84 cells. Microbiological examination of secretions from the throat, nasopharynx and ears did not give growth.

Computed tomography examination of the head revealed no evidence of focal changes in the brain parenchyma and that part of the arachnoid spaces of the hemispheres were not clearly visualized. Abdominal ultrasound showed no changes in the abdominal organs. An ophthalmological examination revealed initial hypertensive angiopathy. Computed tomography showed data of initial cerebral oedema and ophthalmoscopy showed data of papilledema.

On May 28, a cerebrospinal fluid test for enteroviruses was performed giving a negative result. On June 1, anti-TBEV antibodies were established by a serological test IgG -1.98 IU and IgM - 5.76 IU (positive results above 1.1 IU).

After treatment with ceftriaxone, dexamethasone and tavanic, the patient was discharged with improvement. Toxinoinfectious and meningo-radicular symptoms resolved, but astheno-adynamic syndrome was persisting.

Fourth case *Febrile disease with mild neurological symptoms.* It was about a 72-year-old man who fell ill on July 17, 2020 with a body temperature of 37° C, general fatigue, headache, dizziness and unstable walk. After treatment with ciprofloxacin, he did not improve and on July 20 was admitted in the infectious ward of Rahila Angelova Hospital in Pernik.

The examination revealed that the patient was afebrile, in a moderately settled general condition, relaxed, dehydrated. Auscultation revealed bilaterally vesicular respiration with dry wheezing. Neurological status showed an unstable symptom of Romberg and missing symptoms of Kernig and Brudzinski. The patient did not report a tick bite.

Clinical laboratory tests showed lymphopenia (Lym 10.6%) and granulocytosis (granulocytes 84.9%). Microbiological examinations of throat and nasal secretions, blood culture and uroculture were negative. Lumbar puncture performed on 21 July 2020 showed no increase of the total protein in CSF,

no xanthochromia and erythrocytes, the reactions of Pavlovich, Pandi and Rivalta were negative. In the following days, normalization of the values of lymphocytes and granulocytes was observed.

Computed tomography examination of the head in the left cerebral sphere showed two temporal and periventricular porencephalic foci. No diffuse demarcation changes were found in the brain of the parenchyma. No changes were observed in the brain parenchyma, part of the arachnoid spaces of the hemispheres was not visualized clear. Computed tomography of the brain showed lacunar strokes. A

small pleural effusion was observed by computed tomography of the abdomen.

A serological test on July 23 detected anti- TBEV antibodies - IgG -2.01 IU and IgM - 4.16 IU (positive results above 1.1 IU). Therapy was performed with ceftriaxone, dexamethasone and cyprinol. The patient responded to the applied therapy and was discharged with improvement.

The summarized results from medical history and from radiography of internal organs and computed tomography of the head in the four cases of TBE are presented in Table 1.

Table 1. Comparative data from the medical history and diagnostic imaging in the four cases of TBE

patient, gender, age	L.S., male, 26 old	I.D., male, 86 old	A.M., male, 41 old	V.S., male, 72 old
medical history data				
date of illness	June 5, 2017	June 17, 2019	May 23, 2020	July 20, 2020
fever /how many days/	+	+ /5 days/	+ /3 days/	+
headache	+ / had in May	+	+	+
chills	-	+	+	-
speech delay	-	+	-	-
sensitivity to light	+	-	-	-
nausea, vomiting	+	+	+	-
unstable gait	+	+	-	-
tick bite	-	+	-	-
consumption of raw milk	+	-	+	+
concomitant diseases	-	hypertonic heart	-	-
objectively				
contact, adequacy	+	+	+	+
breathing	vesicular without wheezing	wet wheezing	vesicular without wheezing	dry wheezing
neck rigidity	+	-	-	-
symptoms of Kernig, Brudzinski	+ / bilaterally/	+ / bilaterally/	-	-
symptom of Babinski	+ / in right/	+ / in left/	-	-
symptom of Romberg	-	unstable	unstable	unstable
ultrasound diagnosis of internal organs				
abdominal ultrasound	normal status	pyelonephritis, aortic calcification	normal status	pyelonephritis, globus vesicalis
computed tomography of head				
data	changes in the brain parenchyma were not detected	demyelination of the white matter of vascular type	changes in the brain parenchyma were not detected	two temporal and periventricular porencephalic foci
conclusion	without pathological changes	multiinfarction encephalopathy	initial cerebral edema	lacunar strokes

IS THERE AN OUTBREAK OF TICK-BORNE ENCEPHALITIS IN PERNIK DISTRICT, BULGARIA? FOUR CASES...

The summarized results from laboratory tests in the four cases of TBE are presented in Table 2.

Table 2 Comparative data from laboratory results in the four cases of TBE

patient, gender, age	L.S., male, 26 old	I.D., male, 86 old	A.M., male, 41 old	V.S., male, 72 old
results from a clinical laboratory /blood/				
haemoglobin	146 g/l /normal/	128 g/l /mild anemia/	153 g/l /normal/	134 g/l /slightly lowered/
leukocytes	12.0 x 10 ⁹ g/l /high/	11.6 x 10 ⁹ g/l / / slightly elevated/	14.8x10 ⁹ g/l /high/	8.7 8x10 ⁹ g/l / normal/
lymphocytes	8.4% /low/	12.9% /low/	8.5% /low/	10.6% /low/
granulocytes	88.4% /high/	76.3% /slightly elevated/	87.6% /high/	84.9% /high/
results from a clinical laboratory / cerebrospinal fluid/				
total protein	0.584 g/l / slightly elevated/	0.330 g/l /normal/	0.912 g/l /high/	0.530 g/l / slightly elevated/
reactions of Pavlovich, Pandi and Rivalta	Pandi opalescens	Pandi opalescens	Pavlovich, Pandi and Rivalta - opalescens	negative
cells in cubic ml	42 /elevated/	4 /normal/	84 /elevated/	2 /normal/
sugar	3.02 mmol/l / normal/	3 mmol/l /normal/	5 mmol/l /slightly elevated/	3.44 mmol/l / normal/
chlorides	114 mmol/l / normal/	115.6 mmol/l / normal/	120 mmol/l /normal/	115 mmol/l / normal/
erythrocytes	6-7	4-5	3-4	-
microbiological results				
serological test ELISA for TBE	IgM -5.7 IU +/- IgG - 1.6 IU +/-	IgG -2.05 IU +/- IgM - 7.96 IU +/-	IgG -1.98 IU +/- IgM - 5.76 IU +/-	IgG -2.01 IU +/- IgM - 4.16 IU +/-
cerebrospinal fluid test for enteroviruses	-	-	negative	-
microbiological examinations of throat and nasal secretions	<i>Candida albicans</i>	without growth	without growth	without growth
blood culture	-	-	-	without growth
uroculture	-	-	-	without growth

DISCUSSION

According to data published in Europe, 2/3 of TBE cases are caused by an European viral subtype (4). In the four cases described in this paper, the classic two-phase course of the disease was observed. The average age of the patients was 56 years. There are studies on the relationship between age and severity of the disease. The symptoms of TBE in children are milder than in adults, with meningitis in 97% of cases. In adults, meningoencephalitis and meningoencephalomyelitis account for 49.26% of cases. Nausea and vomiting are common in children, while neurological manifestations are common in adults (14).

In our study, only in the first case no pathological brain changes were detected. In the remaining three cases, computed tomography revealed: multiinfarction encephalopathy, evidence of initial cerebral edema and two porencephalic foci. In the first officially reported TBE case in Bulgaria for the last 10 years (in 2009), atypical course was observed without evidence of meningitis or encephalitis (6). Cases of TBE occur usually in the warm months - between April and November. All four cases were observed in May, June and July. All four cases proceeded relatively smoothly with a favourable outcome, without paresis or paralysis of the limbs, without seizures and loss of consciousness.

A recent study in Serbia reported that 50% of patients had meningoencephalitis, that presents with varying degrees of disorder of consciousness and with other neurological disorders: ataxia (100%), paralysis of the limbs (60%), speech impairment and tremor (60%) (19). Except for tick bites, infection is possible by consuming contaminated unpasteurized milk and dairy products from infected domestic animals, especially goats (16).

In most of the cases, between 70 and 98%, TBEV infections are asymptomatic (4). The incubation period varies from 2 to 28 days, usually 7-14 days. As a rule, in the case of alimentary infection, the incubation period is short, usually 3-4 days (10). In this study, only one of the patients reported a tick bite, and the rest were most likely infected through food, as symptoms developed rapidly after 3-4 days. All four cases occur with initial toxoinfectious syndrome, severe headache and adynamy.

Two of the patients had positive symptoms of Kerning, Brudinski and Babinski, while the other two did not have these symptoms but an unstable Romberg symptom was found. Three of the patients developed meningeal form, while in the fourth, such data was not detected by the computed tomography (probably because he was the youngest one and without concomitant diseases).

All four cases followed a classic change in the hematogramme: moderate leukocytosis with granulocytosis. Examination of CSF revealed clear fluid and slightly increased total protein, moderate pleocytosis, and normal sugar and chlorides values. The diagnosis of all four cases was confirmed by serological examination and detection of significantly increased anti-TBEV antibody values. Because of the possibility of another etiology of CNS infection and especially due to the seasonal character of other viruses, infections with other pathogens such as West Nile Virus (WNV), Herpes Simplex Virus 1 (HSV), and Zoster Virus (VZV) were excluded by serological analysis of the blood.

The district of Pernik is characterized by extremely mountainous terrain, which favors breeding of goats. On the territory of Pernik, there are numerous farms for goat milk production, which predetermines its intensified consumption. Increased control by the Bulgarian Food Safety Agency (BFSA) is needed to prevent the consumption of infected raw milk.

The confirmed TBE cases in Bulgaria during these 4 years are only in Pernik district. Although single, these cases showed TBE virus circulation in this region. The fact that there are two cases in 2020 that have not been confirmed in other areas, indicates the presence of some source of infection. The reasons for this can be complex: climate change, activity of the epizootic process in the tick population, different species of vertebrates in natural foci; presence of a large number of goats infected with the virus and increased consumption of raw, unpasteurized goat's milk. In addition, the Pernik Valley with an altitude between 700 m and 850 m is a prerequisite for the formation of high-risk biotopes with high humidity and moderate temperatures favourable for development of *Ixodes* ticks.

A more in-depth study of the area, examination of a representative number of ticks for the presence of TBEV, and sampling of animals is needed. Given the fact that patients who develop neurological manifestations represent a small proportion of those infected, it can be predicted that the number of people infected with TBEV in Bulgaria is many times higher. It is important to develop clinicians' clinical thinking, as the risk of infection is often underestimated and the outcome can be fatal to the patient if misdiagnosed. TBE should be sought in patients with various manifestations of CNS infection.

CONCLUSIONS

Although TBE cases are reported only sporadically due to lack of sufficient testing and / or reporting, TBEV circulates in Pernik region, causing human infections by tick bites or consumption of unpasteurized milk. In our country, Tick-borne encephalitis remains still underrecognized among the cases of viral encephalitis. Active clinical thinking about a possible CNS infection caused by the tick-borne encephalitis virus, supported by optimized laboratory diagnostics, would help to clarify the actual state of the problem in our country.

ACKNOWLEDGEMENTS

The study was supported by the National Science Fund, Bulgaria, Project № KP-06-M33/3 /13.12.2019.

REFERENCES

1. Andonov P., Rusakiev M., Hristova T., Prodromov A., Petkov P., Penchev S., Grancharov K., Naturalhearth from the group of ticks encephalitis in the Eastern Old Mountain, Hygiene, 1961, 6: 23-26.

IS THERE AN OUTBREAK OF TICK-BORNE ENCEPHALITIS IN PERNIK DISTRICT, BULGARIA? FOUR CASES...

- (in bulgarian).
2. Andonov P, Rusakiev M. Natural hearth of ticks encephalitis from a sick child, *Epidem., microb., and inf. bol.*, 1966, 3: 128-138. (in bulgarian).
 3. Balogh Z, Ferenczi E, Szeles K, Stefanoff P, Gut W, Szomor KN, Takacs M, Berencsi G. Tick-borne encephalitis outbreak in Hungary due to consumption of raw goat milk. *J Virol Methods*, 2010, 163,; 481–485.
 4. Bogovic P, Franc Strle Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management *World J Clin Cases*, 2015, 16; 3(5): 430-441.
 5. Christie L. The global burden of bacterial and viral zoonotic infections. *Clin Microb Inf* 17, 2011; 326–330.
 6. Christova I, L. Andonova, M. Slavova, E. Taseva, T. Gladnishka, I. Trifonova, V. Ivanova, Atypical case of tick-borne encephalitis, *Probl. Inf. Parasit. Dis*, 37, 2009, 2, 13-14.
 7. Christova I., I. Dikov, L. Andonova, E. Taseva, N. Kalvachev, Sh. Popal, R. Mikhailova, M. Pishmisheva, I. Baltadzhiev, M. Slavova, K. Plochev, Four cases of tick-borne encephalitis, *Science Infectious Diseases and Parasitology*, 2011, 1, 43-45. (in bulgarian).
 8. Christova I., Panayotova I., Tchakarova S., Taseva E., Trifonova I., Gladnishka T., A nationwide seroprevalence screening for West Nile virus and Tick-borne encephalitis virus in the population of Bulgaria, *Journal of Medical Virology*, 2017; 89(10):1875-1878.
 9. Christova I. TBE in Bulgaria. Chapter 12b. In: Dobler G, Erber W, Bröker M, Schmitt HJ, eds. *The TBE Book*. 3rd ed. Singapore: Global Health Press; 2020.
 10. Dumpis U, Crook D, Oksi J. Tick-borne encephalitis. *Clin Infect Dis*, 1999; 28:882-890.
 11. Ecker, M., Allison, S. L., Meixner, T. & Heinz, F. X. Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. *J Gen Virol*, 1999, 80, 179–185.
 12. Kaiser R: Presented at 7th annual meeting of ISW-TBE in Vienna, 2005.
 13. Kaiser R. Tick-borne encephalitis. *Infect Dis Clin North Am*, 2008; 22: 561-575.
 14. Krawczuk K., Czupryna P., Pancewicz S., Ołdak E., Moniuszko-Malinowska A., Comparison of tick-borne encephalitis between children and adults—analysis of 669 patients, *J of NeuroVirology*, 2020; 26(4):565-571.
 15. Manolov P, Katsarov B. A case of tick-borne encephalitis after consummate of raw goat's milk. *Infectology*, 2006, 2: 37-38. (in bulgarian).
 16. Mansfield K., Johnson N., Phipps L., Stephenson J., Fooks A., and Solomon T., Tick-borne encephalitis virus – a review of an emerging zoonosis. *J. Gen. Virol.* 2009, 90:1781–1794.
 17. Mohareb E. , I Christova , A Soliman , R Younan , T Kantardjiev, Tick-borne encephalitis in Bulgaria, 2009 to 2012, *Eurosurveillance*, 2013, 18, (46), 1-4.
 18. Panayotova E., Study of the distribution of some Bunya and Flaviviruses in Bulgaria, dissertation, 2016. (in bulgarian).
 19. Poluga J., Barac., Katanic N., Rubino S. at all, Tick-borne encephalitis in Serbia: A case series, *J Infect Dev Ctries*, 2019; 13(6):510-515.
 20. Sormunen JJ, Klemola T, Hänninen J, Mäkelä S, Vuorinen I, Penttinen R, Sääksjärvi IE, Vesterinen EJ The importance of study duration and spatial scale in pathogen detection-evidence from a tick-infested island. *Emerg Microbes Infect*, 2018, 7: 189.
 21. Suss J. Tick-borne encephalitis in Europe and beyond – the epidemiological situation as of 2007. *Eurosurveillance* 13, 2008; pii 8916.
 22. Yoshii K. Epidemiology and pathological mechanisms of tick-borne encephalitis. *J Vet Med Sci*, 2019, 81: 343-347.
 23. Vaptsarov I., Tarpomanov A., Spasov Z., Nikov D., Dragiev M. Reversible (two-wave) meningoencephalitis in Southern Bulgaria, *Modern. Medic.*, 1964, 2: 86-103.(in bulgarian).