

Tick-borne encephalitis

J.F. Valarcher ^{(1,2)*}, S. Hägglund ⁽²⁾, M. Juremalm ⁽¹⁾, G. Blomqvist ⁽¹⁾,
L. Renström ⁽¹⁾, S. Zohari ⁽¹⁾, M. Leijon ⁽¹⁾ & J. Chirico ⁽¹⁾

(1) National Veterinary Institute, SVA, Department of Virology, Immunology, and Parasitology, Travvägen 20, SE-751 89 Uppsala, Sweden

(2) Swedish University of Agricultural Sciences, Host Pathogen Interaction Group, Department of Clinical Sciences, Almas Allé 10, 750 07 Uppsala, Sweden

*Corresponding author: jean-francois.valarcher@slu.se

Summary

Tick-borne encephalitis (TBE), a zoonotic arbovirolosis caused by tick-borne encephalitis virus (TBEV), is an increasing public health concern. Infections result in neurological symptoms in humans and the virus has rapidly expanded to new geographical areas. Three subtypes are currently present in different parts of Europe and Asia. The virus is transmitted by ticks, mainly *Ixodes* spp., between small mammals such as rodents, which serve as virus amplifying hosts. Humans are infected sporadically, either by a tick bite or by ingestion of infected milk or milk products. Other mammals (e.g. ruminants) can also be infected, but most of the time do not show clinical signs. In contrast to rodents, other wild and domestic mammals probably play only a very small direct role in maintaining TBEV in an area, but they might play an important role as hosts in sustaining a large tick population. Therefore, the virus prevalence and the occurrence of TBE can be influenced by several environmental, genetic and behavioural factors associated with the virus, the vectors or the hosts, and understanding these factors is essential for implementation of effective control measures. This article reviews virus characteristics and the epidemiological and clinical aspects of TBEV infections and examines pathogenesis, diagnostic approaches and control measures.

Keywords

Clinic – Control – Diagnostics – Epidemiology – Immune response – Pathogenesis – Tick-borne encephalitis – Virus – Zoonosis.

Introduction

Tick-borne encephalitis (TBE) is an emerging arboviral zoonosis in Europe and Asia that is characterised by severe acute and chronic neurological infections in humans. The disease is caused by a flavivirus (TBE virus [TBEV]), which is transmitted mainly by *Ixodes* spp. ticks from a wild vertebrate host to humans or domesticated animals or through consumption of unpasteurised milk products. The virus is endemic in foci from central and eastern Europe to Siberia, northern China, Japan and South Korea. Between 1990 and 2009, almost 170,000 human clinical cases were reported in Europe and Russia (1). The number of clinical cases varies considerably between years. Whereas vaccination campaigns have resulted in a drastic decrease in the incidence of TBE in the last 15 years in some areas of Europe, the disease has expanded in other areas, such as in Scandinavia, Austria, Germany and Switzerland (1). Due to the growing public concern about this disease, the

European Union (EU) has decided to include TBE on the list of notifiable diseases for humans in the EU (2). The annual fluctuation in the number of clinical cases in endemic areas and the continuous emergence of TBE in new areas provide evidence that different factors affecting vectors and hosts have a great impact on the spread of the disease and its prevalence. Global warming, intercontinental trade and travel, and political changes influencing human behaviour all affect the ecological equilibrium between vector and hosts. Better understanding of the complex epidemiological cycle of TBEV is necessary for the identification, monitoring and control of this virus.

Classification and virology

Tick-borne encephalitis virus belongs to the *Flaviviridae* family and the *Flavivirus* genus. Within this genus, several groups can be distinguished based on the vector and/or the host, and, within each group, species are defined based on

virus characteristics (e.g. genomic, antigenic, geographic, ecologic, vector, host) and associated clinical disease (3).

Tick-borne encephalitis virus is a virus species that belongs to the mammalian tick-borne virus group and the TBEV serocomplex (4) (Fig. 1), which both contain other closely related genetic and antigenic virus species (e.g. Gadgets Gully virus [GGYV], Kadam virus [KADV], Kyasanur or Kyasanur forest disease virus [KFDV], Langat virus [LGTV], Louping ill virus [LIV], Omsk haemorrhagic fever virus [OHFV], Powassan virus [POWV] and Royal farm virus [RFV]). TBEV, LIV and POWV cause encephalitis in humans and animals; OHFV and the KFDV variant Alkhurma

haemorrhagic fever virus (AHFV) cause haemorrhagic fever; while GGYV, LGTV and RFV are non-pathogenic in humans.

Tick-borne encephalitis virus includes three subtypes, also called clusters (Fig. 2):

- the Western European subtype (formerly central European encephalitis virus), which is endemic in rural and forest areas of central, eastern and northern Europe and has *I. ricinus* as its principal tick vector
- the Siberian subtype (formerly West Siberian encephalitis virus), which is endemic in the Ural region, Siberia,

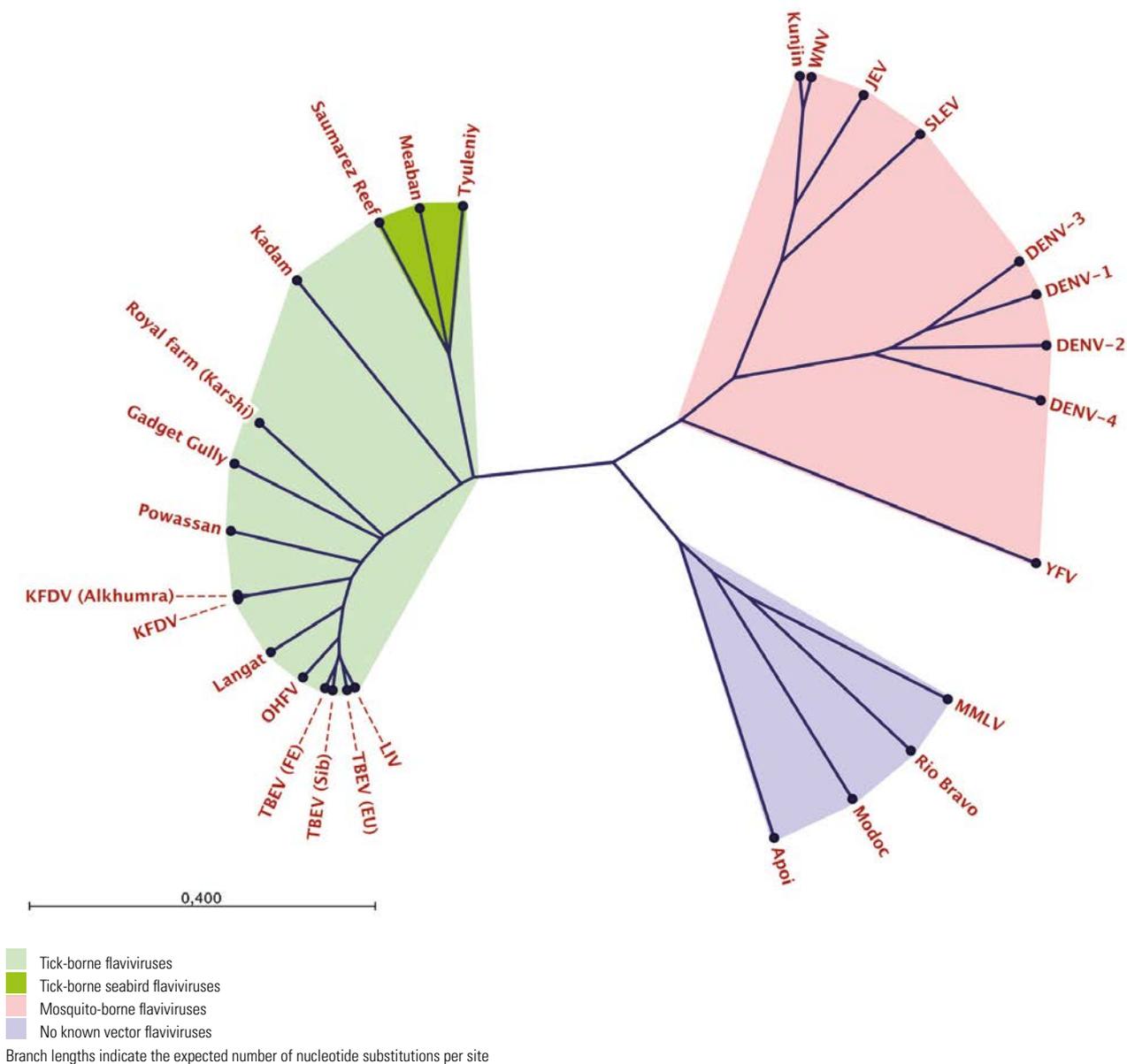


Fig. 1
Unrooted maximum likelihood phylogeny from complete polyprotein sequences of representative flaviviruses

A guide tree was constructed using the Unweighted Pair-Group Method Using Arithmetic Mean (UPGMA) and the Whelan and Gold (WAG) substitution rate model was used. The robustness of the tree was assessed using 1,000 bootstrap replicates. All branches were supported by more than 90% of the replicates except the Gadgets Gully node (74%) and the Saumarez Reef/Meaban node (42%)

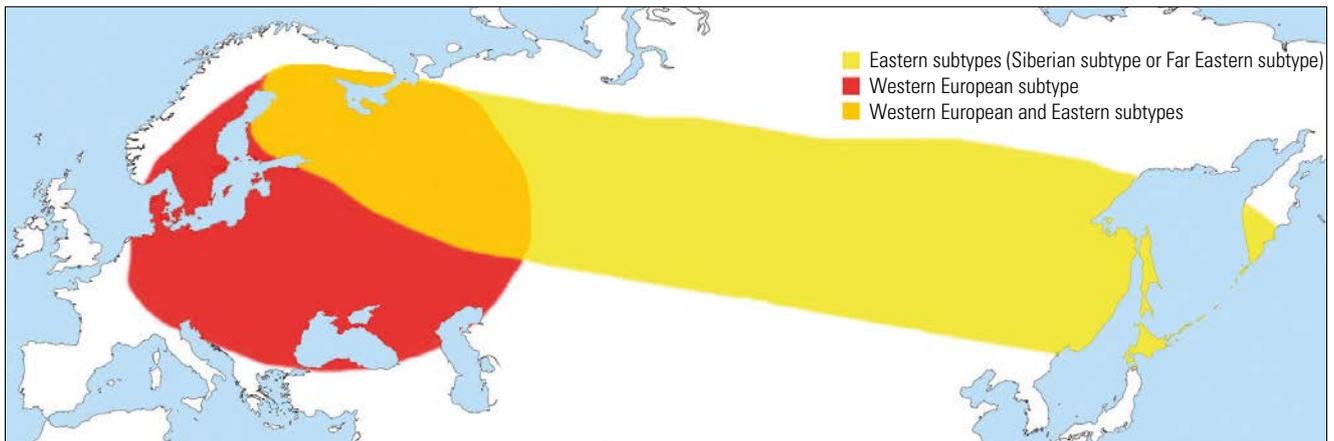


Fig. 2
Geographical distribution of Western and Eastern subtypes of tick-borne encephalitis virus

far-eastern Russia and some areas in north-eastern Europe and has *I. persulcatus* as its principal tick vector

– the Far Eastern subtype (formerly Russian spring-summer encephalitis virus), which is endemic in far-eastern Russia and in forest regions in China and Japan and has *I. persulcatus* as its principal tick vector.

Tick-borne encephalitis was first described in 1931 in Austria (5) and first isolated in 1937 in Russia (6). Like other flaviviruses, TBEV is a spherical, enveloped, positive-sense, single-stranded RNA virus. It is approximately 50 nm in diameter (3). The capsid is formed by a single structural protein (protein C) and contains a non-segmented RNA genome of approximately 11,000 nucleotides, which codes for three structural proteins and seven non-structural proteins. The virus envelope that surrounds the capsid contains two viral structural proteins: protein M (membrane), which is derived from a cleaved precursor (PrM), and protein E (envelope). Protein E is the main component of the viral surface and plays a key role in post-infection protection by inducing virus-neutralising antibodies (7). The virus life cycle and the roles of different viral proteins are extensively reviewed by Mansfield *et al.* (2009) (8).

Antigenic and genetic variability

The degree of genetic and antigenic variability is low between TBEV strains. In a comparative study of gene sequences coding for protein E, the maximum degree of genetic variation between strains within subtypes was 2.2% at the amino-acid level and a maximum difference of 5.6% was detected between the three subtypes, which is in the range of variation reported for other flaviviruses (9, 10). Antigenically, protein E is also very conserved within

and among subtypes, leading to a very close antigenic relatedness and the induction of cross-protection among subtypes (11).

Epidemiology

Tick-borne encephalitis virus is endemic in central, northern and eastern Europe, Russia, and the Far East, including Mongolia, the northern part of China and Japan (12). The virus has been reported in 28 countries around the world and the highest incidence of clinical cases is reported in the Baltic countries, Slovenia and the Russian Federation (13, 14). However, the disease incidence in some of these countries is low because a large part of the population is vaccinated (e.g. Austria) or because TBE is present only in limited areas (14). In countries where the virus is present, the distribution of the cases of clinical disease is very patchy. The risk of being infected following a tick bite varies from 1/200 up to 1/1,000, depending on in which geographic area the bite occurred (15).

Worldwide, the number of clinical cases of TBE is estimated to be between 10,000 and 12,000 a year (16) and in Europe more than 3,000 human TBE cases are hospitalised each year (17). Moreover, a large proportion of TBEV infections are not diagnosed (18). Of the 30 countries of the European Union/European Free Trade Association, 20 have implemented a surveillance system for TBE. Eighteen of these countries carry out comprehensive surveillance and 16 list TBE as a notifiable disease (19). In general, there has been an increase in the number of human clinical cases during recent years; however, strong fluctuations and decreases in clinical disease have also been observed (13, 20). This may be explained by the simultaneous spread of the virus into new geographical areas due to changes that favour tick or host reservoirs,

increased exposure to infected ticks due to socioeconomic and political changes leading to changes in outdoor leisure habits, or economic constraints resulting in increased harvesting of wild vegetables and fruits (e.g. mushrooms and berries) (13, 21, 22).

According to Jaenson *et al.* (2012), an increased incidence of clinical cases of TBE in Sweden between 2009 and 2012 was probably the result of a temporary change in the ecosystem. A large population of roe deer supported the maintenance of a large tick population. Following harsh winters, the roe deer population decreased and simultaneously the bank vole population, which is a reservoir of TBEV, strongly increased. This host became an important feed source for immature ticks, which, subsequently, increased the probability of TBEV infection. Finally, the unusually warm and humid weather in the summer and autumn of 2011 favoured the transmission to humans who were spending more time outdoors.

More than 14 species of ticks can be infected by TBEV, but *I. ricinus* (Fig. 3) and *I. persulcatus* are the principal vectors of the Western European subtype and the Siberian and Far Eastern subtypes, respectively (12). However, in Finland, the Western European subtype has also been detected in *I. persulcatus* (23) (Fig. 2). Other ticks have also been identified as vectors of TBEV. In the Republic of Korea, *Haemaphysalis longicornis*, *H. flava* and *I. nipponensis* are potential vectors, since 0.06%, 0.17%, and 2.38% of these ticks, respectively, were infected by the Western European subtype of TBEV (24). Furthermore, 10.8% of *Dermacentor reticulatus* were also found to be infected with TBEV in eastern Poland (25).

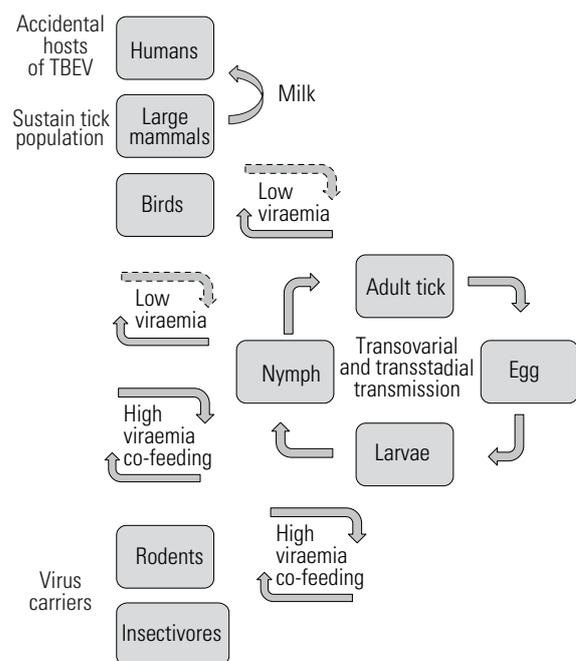
The prevalence of infected ticks varies between regions. In Europe, the TBEV prevalence in *I. ricinus* varies between 0.1% and 5% in unfed ticks and increases with stage development (1, 26). In some foci (e.g. Slovakia and the



Fig. 3
Engorged adult tick (*Ixodes ricinus*)
Source: Anders Lindström/SVA property

Baltic countries), the prevalence has reached 26.6% in *I. ricinus* and more than 37% in *I. persulcatus* in certain years (27, 28). In Hungary, on the other hand, TBEV prevalence in *I. ricinus* ticks was 0.08% for unfed nymphs and 0.78% for feeding larvae (29).

Ticks are vectors as well as reservoirs of TBEV and they can carry the virus throughout their life (Fig. 4). Therefore, the virus can be transmitted by larvae, nymphs or adult ticks. However, the nymph seems to be the most important stage for virus transmission due to the large population of this stage (15). Tick saliva contains analgesic, anti-inflammatory and anti-coagulant substances that enable the tick to feed without being noticed (30). The virus is transmitted to ticks by feeding on infected hosts or by co-feeding close to an infected tick (probably a major route). It can also be transmitted transovarially from an infected female to egg (occurs at low frequency) (17) and, possibly, through sexual interaction (1). The maintenance of TBE in an area



TBEV: tick-borne encephalitis virus

Fig. 4

Transmission and maintenance of tick-borne encephalitis virus in the tick vector and its hosts

Ticks become infected with TBEV mainly while feeding at early developmental stages (larvae, nymph) on an infected rodent or insectivore, either through co-feeding beside an infected tick or through the presence of high levels of virus in the blood of the animal. The virus persists throughout the succeeding developmental stages of the tick and transmits transovarially from adult to egg. Whereas large mammals and birds sustain infected and susceptible tick populations, these animals are poor TBEV transmitters due to low levels of viraemia. However, although tick bites remain the major source of human infection, unpasteurised milk products from infected ruminants may transmit TBEV to humans

necessitates a host population that has a high and prolonged viraemia, which favours tick infection, or a host population that maintains a large number of ticks. Transmission between ticks through co-feeding is particularly important for maintaining the virus when host viraemia is slight or of short duration, or when the host has TBEV-specific immunity (31, 32).

The transmission of TBEV by ticks is closely linked to the tick biology and is also dependent on the ecosystem. For example, the life cycle of *I. ricinus* lasts two to six years and each stage takes on average one year (15). Tick activity (development, feeding, movement, etc.) starts when the temperature reaches greater than 5°C and there is a sufficient degree of humidity (92% is optimal). In temperate climate zones this occurs in spring or the beginning of the summer, depending on the latitude, and goes on until the end of the summer or the autumn. Peak activity occurs at the beginning and at the end of this period (33). In some warmer areas (e.g. the southern Mediterranean region) ticks are mainly active between November and January (34). *Ixodes ricinus* can feed on more than 300 different species of wild and domestic mammals, birds and reptiles (15). This diversity of potential hosts allows maintenance of the tick population in a large range of environmental conditions.

Among host vertebrates, susceptibility to TBEV varies from species to species. Some have a high viraemia and thus play a major role in the transmission of TBEV to ticks; for example:

- rodents (*Apodemus*, *Myodes*, *Microtus*, *Micromys*, *Pitimus*, *Arvicola*)
- insectivores, e.g. hedgehogs (*Erinaceus europaeus*) and moles (*Talpa europea*)
- wild carnivores, e.g. young foxes (*Vulpes*) (15).

Some rodent species, such as the red vole (*Myodes rutilus*), can serve as a reservoir host by means of a prolonged viraemia and possibly by vertical virus transmission to offspring (35, 36). Moreover, the bank vole (*Myodes glareolus*) can carry live virus in its brain for five to six months (37, 38).

Other animal species with low or absent viraemia following TBEV infection will participate indirectly in the maintenance of the virus by hosting ticks (e.g. bats, cattle, goats, roe deer, chamois, hares, pigs and some birds) (12, 39). In a limited number of cases some accidental hosts (e.g. humans, sheep and dogs) can develop high viraemia, but since these cases are limited they do not play an important epidemiological role in TBEV transmission (40). The seroprevalence in dogs and horses is nonetheless high in certain areas and sometimes clinical signs can be observed following infection (41, 42, 43, 44, 45, 46). It is not clear whether TBEV infects cats, despite their regular contact with both ticks and infected rodents. Cats seem to be quite resistant

to a closely related virus, the Powassan virus (47). Birds may be infected, but their role as a reservoir is unclear (48, 49). Nevertheless, the virus might be spread across long distances by infected ticks carried on birds or by infected birds themselves (49, 50, 51).

Humans are incidental hosts. Infection may occur via the bite of an infected tick or through consumption of unpasteurised TBEV-infected milk, since the virus is highly resistant to the acidity of the stomach (52). Raw milk and cheese made of unpasteurised milk can be sources of transmission (27, 53, 54, 55, 56, 57). This type of transmission has been reported in Albania, Hungary, Latvia, Lithuania, Poland, Russia and Slovakia, but not in western Europe. In cattle and small ruminant herds the seroprevalence can vary between 1.1% and 42.8% (58). Even though the viraemia is low and of short duration, virus can be detected in milk up to 18 days post infection (59). In some countries where TBE is present (e.g. Sweden), the increasing number of small-scale producers who sell unpasteurised milk and milk products presents an increased risk of virus transmission. Anecdotal reports of other transmission routes have been summarised by Suss (1).

To identify TBEV-infected areas, different vertebrate species (such as ruminants, dogs, foxes and rodents) have served as sentinels and have been monitored for presence of TBEV-specific antibodies, either in sera (15, 58, 60) or in milk (Valarcher *et al.*, in preparation). These methods are preferable compared to virus detection in ticks since the prevalence of infected ticks is often low.

Clinical signs

Tick-borne encephalitis virus is a major cause of infection of the central nervous system (CNS) in humans and can cause clinical disease in all ages, but infection is often more severe in adults and the elderly (17, 61). The clinical symptoms of TBEV infection are biphasic (12, 17, 18). The incubation time is between two and 28 days, with an average of seven days. The viraemic phase, which lasts between two and eight days, is characterised by flu-like symptoms with a slight temperature increase, headache, tiredness, aching back and limbs and nausea. Thereafter, there follows a period without clinical symptoms and, in one-third of patients, a second phase of disease occurs, characterised by a sudden onset of fever. During this stage the virus spreads to the CNS and causes anorexia, fever, headache, vomiting, photophobia and sometimes sensory changes, visual disturbances, paresis, paralysis, or even coma. Other symptoms include hyperkinesia of the limbs and face muscles, lingual tremor, convulsions, and paresis of the respiratory muscles. Death might occur as soon as one week after the onset of clinical disease.

In 10–20% of patients with severe disease, chronic neuropsychiatric or nervous sequelae are observed, such as depression, lack of concentration, or paresis of the face or limbs due to chronic myelitis or encephalitis (8).

Animals show varying degrees of clinical signs following TBEV infection. Whereas most wild animals are asymptomatic (e.g. red vole, *Myodes rutilus*), some may develop encephalitis (e.g. bank vole, *Myodes glareolus*). Monkeys (e.g. *Macaca sylvanus*) can develop clinical signs and lesions similar to those observed in humans (13, 62), but in livestock, such as cattle, sheep, goats and pigs, clinical signs are only rarely observed (40). Although sparse, reports exist of TBE in horses with clinical signs including poor general condition, anorexia, ataxia, sudden cramps, seizures, and paralysis of the neck and shoulder muscles (41, 43). Dogs will also occasionally develop fever and clinical signs from infection of the CNS. Infections with TBEV are probably underdiagnosed in this animal species (44, 63).

Immune response

Mammalian host cells have several defence strategies against viral infections. At the local site of infection TBEV induces an innate and adaptive immune response. However, this virus has developed different strategies to block the host innate immune response allowing efficient replication of the virus in the primary infected cells (64). For example, the protein NS5 appears to be a type I interferon (IFN) antagonist that acts, possibly, by inhibiting the JAK/STAT signalling pathway, as demonstrated by Langkat virus, which makes the infected cells resistant to type I IFN (65, 66). In addition, the double-stranded TBEV RNA is located in intracellular membrane vesicles of infected cells, which delays the induction of type I IFN (66). Nevertheless, proinflammatory cytokines such as tumour necrosis factor (TNF) alpha, interleukin (IL)-1 alpha and IL-6 are detected in the cerebrospinal fluid (CSF) after infection, followed by low levels of IL-10 (67, 68). Several chemokines with an attractive effect on lymphocytes have also been detected in the CSF (69, 70) and might be at the origin of the immunopathogenic mechanisms associated with TBEV infection (71).

Adaptive immune responses are detectable in blood and CSF and consist of TBEV-specific neutralising and non-neutralising immunoglobulin (IgM and IgG antibodies (72), as well as CD4+ T lymphocytes (protein E and C-specific) and CD8+ T lymphocytes (73, 74, 75). Tick-borne encephalitis virus-specific neutralising antibodies have an important role in protection against the high viraemia required for passage of the virus across the blood–brain barrier into the CNS, while the cellular immune

responses are required for virus clearance from the CNS. However, these cellular responses might also contribute to pathology, as described below.

Pathogenesis

After a tick bite, TBEV replicates first in dermal cells and then in Langerhans cells, macrophages and neutrophils (31). Recognition of the virus by the innate immune system leads to migration of dendritic cells (DC) to the primary site of infection. These cells are activated after TBEV infection and bring the virus to a regional lymph node via the lymphatic system. Similarly, when TBEV is ingested in milk, replication occurs, probably initially in epithelial gut cells, before transmission to DC (76).

After a new phase of replication in DC, macrophages and, possibly, T lymphocytes (76, 77), viraemia occurs and the virus spreads to other organs, in particular the reticulo-endothelial system (spleen, liver and thymus). High virus titres are necessary for the virus to pass through the blood–brain barrier (78). Thus, TBEV is characterised by a low neuro-invasiveness (79). Nonetheless, in patients with insufficient titres of TBEV-specific neutralising antibodies to prevent CNS infection, neurons are clearly the targets of the infection and virus replication causes nervous lesions and symptoms due to an inflammatory process, cellular lysis, necrosis and apoptosis, as well as cellular dysfunction (12, 79).

The infection of neurons leads to migration of microglia and TBEV-specific T lymphocytes to the CNS and, more specifically, to the grey matter. The cytotoxic T-lymphocyte response is essential for virus clearance but may also lead to immunopathogenesis by targeting infected neurons (71, 75). In human lethal cases, meningitis and poliomyelitis are localised in the spinal cord, brainstem and cerebellum and are associated with inflammatory and lymphocytic cell infiltrations (79).

Diagnosis

In humans, clinical suspicion needs to be confirmed by laboratory diagnosis since the clinical symptoms are not specific enough to diagnose TBEV (61). During the first clinical phase, the virus and its RNA can be detected by isolation in cell culture and by reverse-transcription polymerase chain reaction (RT-PCR), respectively, in the blood or in the CSF (80, 81, 82, 83). Multiplex PCRs have also been developed to distinguish TBEV subtypes (84). In later stages of the disease, the virus may no longer

be detectable in body fluids (80, 85), but rather may be detected in tissues collected post mortem (72, 81).

At the end of the first clinical phase, in the second clinical phase when the virus has been neutralised, or retrospectively, testing for TBEV-neutralising antibodies or TBEV-specific IgM and IgG antibody is preferred (80). IgM antibodies appear in sera between zero and six days after the occurrence of nervous signs, which corresponds to two to three weeks after the infection. These antibodies peak four to five weeks after the infection and are absent after seven weeks (80, 86). IgM can also persist for a long time after vaccination and this needs to be taken into account when making a diagnosis (80, 87). TBEV-specific IgG antibodies start to appear at the same time as IgM antibodies, reach a plateau after seven to eight weeks (87) and often persist for life. Both IgM and IgG antibodies appear ten days later in CSF than in serum.

In animals, except dogs and horses, TBE diagnosis is usually not performed, since clinical signs following infection have not been frequently reported. However, commercial indirect enzyme-linked immunosorbent assays (ELISAs) for detection of TBEV-specific IgG and IgM antibodies are available in veterinary medicine. These assays can be used to detect TBEV-specific antibodies in many species due to cross-antibody recognition by protein G (88). The cut-off in these tests varies between species and has been defined for horses, cattle, sheep, goats, pigs, mice, dogs, rabbits and monkeys based on virus-neutralising titres in control sera (88).

Cross-reactivity of antibodies is a problem in humans (89). Following infection by or immunisation against other flaviruses (e.g. yellow fever virus, Japanese encephalitis virus or dengue virus), IgG antibody cross-reactions can occur in the ELISA (80). Therefore, a significant increase of TBEV-specific antibodies needs to be detected at a two-week interval or the results need to be confirmed by a virus-neutralising test that is more specific (80). Alternatives to indirect ELISAs (e.g. immune complex ELISA) have been observed to be highly specific and useful for avoiding misinterpretations of the immunity of people exposed to various flaviviruses (90). In animals, cross-reactions occur between TBEV-specific antibodies and West Nile virus in a competitive ELISA, and, moreover, antibodies directed against looping-ill virus can neutralise TBEV (58).

Treatment and prevention

No specific treatment is available against TBEV infection (91). Antiviral therapy is not used as treatment when neurological signs are observed since the virus has already disappeared (61). Specific immunoglobulin therapy post

TBEV exposure has been tried, but was stopped after a report of aggravated clinical signs following administration (92, 93). Therefore, when clinical signs are observed in humans, treatment is mainly symptomatic and based on non-steroidal anti-inflammatory therapy (61). For patients in a coma or with breathing difficulties, reanimation treatments are implemented (17, 61).

The control of TBEV is mainly based on the prevention of infection by preventing transmission from ticks or food products (e.g. pasteurisation of milk) and by active immunisation of populations at risk. To be cost-effective, targeted control measures need to be based on knowledge of the virus prevalence. This can be achieved by searching for the presence of the virus in ticks; however, the virus prevalence in ticks is often low and such studies are therefore expensive (94). Another possibility is to determine seroprevalence in the host species, such as goats, sheep (94, 95), dogs (96) or wildlife (97), but this requires extensive collection of samples since the seroprevalence can be very low. A promising strategy is to analyse bulk tank milk of dairy herds for the presence of TBEV-specific antibodies. Attempts to control TBE by decreasing the tick population using dichlorodiphenyltrichloroethane (DDT) were made in the former Union of Soviet Socialist Republics (USSR). However, not only did this practice pose risks to public health and the environment, it was also unsuccessful (98). A safer and more efficient method of prevention is to wear protective clothing, use a tick repellent and check for the presence of ticks and remove them (12, 99).

In a risk zone, active immunisation by vaccination is the best way to prevent disease (14, 89). There are at least five commercial inactivated vaccines on the market in Europe: two in Germany and Austria (each of which exists in an adult and a junior formulation), two in Russia and one in China (100). In Europe, vaccines contain one European strain (Neudofl or K23 isolated from the *I. ricinus* tick in 1971 and 1975, respectively). In Russia, they contain Far Eastern strains (Sofjin isolated from a patient brain in 1937 or strain 205 isolated from *I. persulcatus* in 1973), and in China the vaccine contains a Far Eastern strain (isolated from a patient brain in 1953) (100). The vaccines are produced with similar methods: the virus is propagated in cell culture (e.g. primary embryo fibroblasts), inactivated in formalin or formaldehyde, and the purified antigens are adjuvanted with aluminium hydroxide. Naive patients need three to four vaccine injections to be fully immunised and vaccination needs then to be repeated every three to five years according to the type of vaccine and the age of the patient (89).

Whereas adverse reactions to European vaccines directed against Western TBEV are now limited to local reactions (redness/pain) and sometimes fever, more adverse reactions are observed with the Russian vaccines (89).

Previously, strong adverse reactions were similarly observed against European vaccines, including fever and, in some cases, convulsions, nausea and headache (101). These complications were associated with the induction of TNF alpha and IL-1 beta and are avoided by using human instead of bovine serum albumin, increasing the concentration of sucrose as stabiliser and by formulating a junior vaccine that contains only half the quantity of antigens compared to the adult formulation (101, 102). Western TBEV vaccines have been shown to have a good safety profile and induce good protection that can last at least five years after the fourth injection (89).

Due to the low degree of genetic variation between TBEV, vaccines based on European and Far Eastern strains induce good heterologous protection even though the vaccine strains were collected quite a long time ago (11, 103). Cross-protection against Siberian strains by vaccines containing Far Eastern or European strains seems to occur (104), but can vary from optimal to partial protection depending on antigenic differences and vaccine composition (e.g. payload and adjuvant), which necessitates regular evaluation (100).

Vaccination can give very good results when applied following an appropriate protocol. Indeed, in Austria, which had the highest TBE incidence in central Europe before implementation of mass vaccination in 1981, the incidence

is now estimated to be 0.88 per 100,000 inhabitants, and 86% of the population is vaccinated (105). Furthermore, vaccine efficacy is high because 96–99% of people who are regularly vaccinated, and even 90% of those who are irregularly vaccinated, are protected (20). It is believed that mass vaccination in Austria has prevented around 4,000 cases of TBE and 15 to 30 deaths (20).

No vaccines are currently available on the market for animals.

Acknowledgements

The authors would like to thank Rickard Wolrath of the Swedish National Veterinary Institute (SVA) for his help in producing the map in Figure 2.



Encéphalite transmise par les tiques

J.F. Valarcher, S. Hägglund, M. Juremalm, G. Blomqvist, L. Renström, S. Zohari, M. Leijon & J. Chirico

Résumé

L'encéphalite transmise par les tiques est une arbovirose zoonotique due au virus de l'encéphalite transmise par les tiques (TBEV), qui suscite une attention croissante en santé publique. L'infection humaine se traduit par des symptômes neurologiques. Le virus s'est rapidement propagé et a conquis de nouvelles aires de distribution. Trois sous-types sont actuellement présents dans différentes régions d'Europe et d'Asie. Les vecteurs sont des tiques, principalement du genre *Ixodes*, qui transmettent l'infection entre petits mammifères, surtout des rongeurs, qui font office d'hôtes amplificateurs. L'infection humaine est sporadique et survient suite à la morsure d'une tique ou à l'ingestion de lait ou de produits laitiers contaminés, l'homme vivant souvent à proximité de mammifères infectés mais généralement asymptomatiques (par exemple des ruminants). Contrairement aux rongeurs, les autres mammifères domestiques ou sauvages ne jouent qu'un rôle direct mineur

dans la persistance du virus de l'encéphalite transmise par les tiques, mais en tant qu'hôtes ils contribuent à maintenir les effectifs élevés des populations de tiques. La prévalence du virus et l'incidence de l'encéphalite transmise par les tiques sont donc conditionnées par plusieurs facteurs environnementaux, génétiques et comportementaux associés au virus, aux vecteurs ou aux hôtes, qu'il est essentiel de bien comprendre afin de concevoir des mesures de contrôle efficaces. Les auteurs examinent les caractéristiques du virus ainsi que les aspects épidémiologiques et cliniques des infections par le virus de l'encéphalite transmise par les tiques et décrivent la pathogénie de la maladie, les méthodes diagnostiques utilisées et les mesures de contrôle applicables.

Mots-clés

Clinique – Contrôle – Diagnostic – Encéphalite transmise par les tiques – Épidémiologie – Pathogénie – Réponse immune – Virus – Zoonose.



Encefalitis transmitida por garrapatas

J.F. Valarcher, S. Hägglund, M. Juremalm, G. Blomqvist, L. Renström, S. Zohari, M. Leijon & J. Chirico

Resumen

La encefalitis transmitida por garrapatas, que es una arbovirosis zoonótica, suscita crecientes problemas de salud pública. El virus causante de esta infección provoca síntomas neurológicos en el ser humano y viene extendiéndose con rapidez a nuevas áreas geográficas. Ahora mismo hay tres subtipos presentes en distintas partes de Europa y Asia. Las garrapatas, principalmente del género *Ixodes*, son el vector de transmisión del virus entre pequeños mamíferos, como roedores, que ejercen de anfitrión amplificador. Esporádicamente se produce la infección humana, ya sea por picadura de una garrapata o por ingestión de leche o productos lácteos infectados, puesto que otros mamíferos (como los rumiantes) también pueden resultar infectados, aunque pocas veces muestren signos clínicos. A diferencia de los roedores, otros mamíferos salvajes y domésticos tienen seguramente muy poca intervención directa en el mantenimiento del virus en una zona, pero en cambio podrían cumplir una función importante como hospedadores para sostener una población cuantiosa de garrapatas. De ahí que la prevalencia del virus y la aparición de encefalitis transmitida por garrapatas puedan verse influidas por varios factores de tipo ambiental, genético y de comportamiento correlacionados con el virus, los vectores o los hospedadores, factores que por lo tanto es esencial conocer y entender para aplicar medidas eficaces de lucha. Los autores pasan revista a las características del virus y los rasgos epidemiológicos y clínicos de la infección y examinan su patogénesis, así como los métodos de diagnóstico y las medidas de lucha contra ella.

Palabras clave

Clínica – Control – Diagnóstico – Encefalitis transmitida por garrapatas – Epidemiología – Patogénesis – Respuesta inmunitaria – Virus – Zoonosis.



References

- Suss J. (2011). – Tick-borne encephalitis 2010: epidemiology, risk areas, and virus strains in Europe and Asia: an overview. *Ticks Tick Borne Dis.*, **2** (1), 2–15.
- Amato-Gauci A. & Zeller H. (2012). – Tick-borne encephalitis joins the diseases under surveillance in the European Union. *Eurosurveillance*, **17** (42), 1–2.
- Simmonds P., Becher P., Colett M., Gould E., Heinz F., Meyers G., Monath T., Pletnev A., Rice C., Stiasny K., Thiel H., Weiner A. & Bukh J. (2012). – *Flaviviridae*. In *Virus taxonomy* (A. King, M. Adams, E. Carstens & E. Lefkowitz, eds). Elsevier Academic Press, Waltham, Massachusetts, 1003–1020.
- Calisher C.H. & Gould E.A. (2003). – Taxonomy of the virus family *Flaviviridae*. *Adv. Virus Res.*, **59**, 1–19.
- Schneider H. (1931). – Über epidemische akute 'Meningitis serosa'. *Wien. Klin. Wochenschr.*, **44**, 350–352.
- Gould E.A. & Solomon T. (2008). – Pathogenic flaviviruses. *Lancet*, **371** (9611), 500–509.
- Heinz F.X. & Stiasny K. (2012). – Flaviviruses and flavivirus vaccines. *Vaccine*, **30** (29), 4301–4306.
- Mansfield K.L., Johnson N., Phipps L.P., Stephenson J.R., Fooks A.R. & Solomon T. (2009). – Tick-borne encephalitis virus: a review of an emerging zoonosis. *J. Gen. Virol.*, **90** (8), 1781–1794.
- 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**, 179–185.
- Gould E.A., Moss S.R. & Turner S.L. (2004). – Evolution and dispersal of encephalitic flaviviruses. *Arch. Virol. Suppl.*, **18**, 65–84.
- Holzmann H., Vorobyova M.S., Ladyzhenskaya I.P., Ferenczi E., Kundi M., Kunz C. & Heinz F.X. (1992). – Molecular epidemiology of tick-borne encephalitis virus: cross-protection between European and Far Eastern subtypes. *Vaccine*, **10** (5), 345–349.
- Dumpis U., Crook D. & Oksi J. (1999). – Tick-borne encephalitis. *Clin. Infect. Dis.*, **28** (4), 882–890.
- Suss J. (2008). – Tick-borne encephalitis in Europe and beyond: the epidemiological situation as of 2007. *Eurosurveillance*, **13** (26), pii: 189116.
- World Health Organization (WHO) (2011). – Vaccines against tick-borne encephalitis. WHO position paper: recommendations. *Vaccine*, **29** (48), 8769–8770.
- Suss J. (2003). – Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine*, **21** (Suppl. 1), S19–S35.
- Zavadská D., Anca I., Andre F., Bakir M., Chlibek R., Cizman M., Ivaskeviciene I., Mangarov A., Meszner Z., Pokorn M., Prymula R., Richter D., Salman N., Simurka P., Tamm E., Tesovic G., Urbancikova I. & Usonis V. (2013). – Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). *Hum. Vaccin. Immunother.*, **9** (2), 362–374.
- Lindquist L. & Vapalahti O. (2008). – Tick-borne encephalitis. *Lancet*, **371** (9627), 1861–1871.
- Bogovic P., Lotric-Furlan S. & Strle F. (2010). – What tick-borne encephalitis may look like: clinical signs and symptoms. *Travel Med. Infect. Dis.*, **8** (4), 246–250.
- European Centre for Disease Prevention and Control (ECDC) (2012). – Epidemiological situation of tick-borne encephalitis in the European Union and European Free Trade Association countries. Available at: www.ecdc.europa.eu/en/publications/Publications/TBE-in-EU-EFTA.pdf (accessed on 21 May 2014).
- Kunze U. (2013). – Tick-borne encephalitis: a notifiable disease. Report of the 15th Annual Meeting of the International Scientific Working Group on Tick-Borne Encephalitis (ISW-TBE). *Ticks Tick Borne Dis.*, **4** (5), 363–365.
- Randolph S.E. (2008). – Tick-borne encephalitis incidence in Central and Eastern Europe: consequences of political transition. *Microbes Infect.*, **10** (3), 209–216.
- Daniel M., Kriz B., Danielova V., Valter J. & Kott I. (2008). – Correlation between meteorological factors and tick-borne encephalitis incidence in the Czech Republic. *Parasitol. Res.*, **103** (Suppl. 1), S97–S107.
- Jaaskelainen A.E., Tonteri E., Sironen T., Pakarinen L., Vaheri A. & Vapalahti O. (2011). – European subtype tick-borne encephalitis virus in *Ixodes persulcatus* ticks. *Emerg. Infect. Dis.*, **17** (2), 323–325.
- Yun S.M., Song B.G., Choi W., Park W.I., Kim S.Y., Roh J.Y., Ryou J., Ju Y.R., Park C. & Shin E.H. (2012). – Prevalence of tick-borne encephalitis virus in ixodid ticks collected from the Republic of Korea during 2011–2012. *Osong Public Health Res. Perspect.*, **3** (4), 213–221.
- Wojcik-Fatla A., Cisak E., Zajac V., Zwolinski J. & Dutkiewicz J. (2011). – Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* and *Dermacentor reticulatus* ticks collected from the Lublin region (eastern Poland). *Ticks Tick Borne Dis.*, **2** (1), 16–19.
- Pettersson J.H., Golovljova I., Vene S. & Jaenson T.G. (2014). – Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* ticks in northern Europe with particular reference to southern Sweden. *Parasit. Vectors*, **7**, 102.

27. Labuda M., Eleckova E., Lickova M. & Sabo A. (2002). – Tick-borne encephalitis virus foci in Slovakia. *Int. J. Med. Microbiol.*, **291** (Suppl. 33), 43–47.
28. Bormane A., Lucenko I., Duks A., Mavtchoutko V., Ranka R., Salmina K. & Baumanis V. (2004). – Vectors of tick-borne diseases and epidemiological situation in Latvia in 1993–2002. *Int. J. Med. Microbiol.*, **293** (Suppl. 37), 36–47.
29. Pinter R., Madai M., Vadkerti E., Nemeth V., Oldal M., Kemenesi G., Dallos B., Gyuranecz M., Kiss G., Banyai K. & Jakab F. (2013). – Identification of tick-borne encephalitis virus in ticks collected in southeastern Hungary. *Ticks Tick Borne Dis.*, **4** (5), 427–431.
30. Sauer J.R., McSwain J.L., Bowman A.S. & Essenberg R.C. (1995). – Tick salivary gland physiology. *Annu. Rev. Entomol.*, **40**, 245–267.
31. Labuda M., Austyn J.M., Zuffova E., Kozuch O., Fuchsberger N., Lysy J. & Nuttall P.A. (1996). – Importance of localized skin infection in tick-borne encephalitis virus transmission. *Virology*, **219** (2), 357–366.
32. Labuda M., Kozuch O., Zuffova E., Eleckova E., Hails R.S. & Nuttall P.A. (1997). – Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology*, **235** (1), 138–143.
33. Kriz B., Benes C., Danielova V. & Daniel M. (2004). – Socio-economic conditions and other anthropogenic factors influencing tick-borne encephalitis incidence in the Czech Republic. *Int. J. Med. Microbiol.*, **293** (Suppl. 37), 63–68.
34. Estrada-Peña A., Bouattour A., Camicas J.L. & Walker A.R. (2004). – Biology of ticks and methods of identification. In *Ticks of domestic animals in the Mediterranean region: a guide to identification of species* (EU ICA4-Ct-2000-30006). University of Zaragoza, Spain.
35. Bakhvalova V.N., Dobrotvorsky A.K., Panov V.V., Matveeva V.A., Tkachev S.E. & Morozova O.V. (2006). – Natural tick-borne encephalitis virus infection among wild small mammals in the southeastern part of western Siberia, Russia. *Vector Borne Zoonotic Dis.*, **6** (1), 32–41.
36. Bakhvalova V.N., Potapova O.F., Panov V.V. & Morozova O.V. (2009). – Vertical transmission of tick-borne encephalitis virus between generations of adapted reservoir small rodents. *Virus Res.*, **140** (1–2), 172–178.
37. Tonteri E., Kipar A., Voutilainen L., Vene S., Vaheri A., Vapalahti O. & Lundkvist A. (2013). – The three subtypes of tick-borne encephalitis virus induce encephalitis in a natural host, the bank vole (*Myodes glareolus*). *PLoS ONE*, **8** (12), e81214.
38. Tonteri E., Jaaskelainen A.E., Tikkakoski T., Voutilainen L., Niemimaa J., Henttonen H., Vaheri A. & Vapalahti O. (2011). – Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008–2009. *Emerg. Infect. Dis.*, **17** (1), 72–75.
39. Venturi G., Martelli P., Mazzolini E., Fiorentini C., Benedetti E., Todone D., Villalta D., Fortuna C., Marchi A., Minelli G. & Ciufolini M.G. (2009). – Humoral immunity in natural infection by tick-borne encephalitis virus. *J. Med. Virol.*, **81** (4), 665–671.
40. Hubalek Z., Rudolf I. & Nowotny N. (2014). – Arboviruses pathogenic for domestic and wild animals. *Adv. Virus Res.*, **89**, 201–275.
41. Klaus C., Horugel U., Hoffmann B. & Beer M. (2013). – Tick-borne encephalitis virus (TBEV) infection in horses: clinical and laboratory findings and epidemiological investigations. *Vet. Microbiol.*, **163** (3–4), 368–372.
42. Muller K., König M. & Thiel H.J. (2006). – Tick-borne encephalitis (TBE) with special emphasis on infection in horses. *Dtsch. Tierärztl. Wochenschr.*, **113** (4), 147–151.
43. Rushton J.O., Lecollinet S., Hubálek Z., Svobodová P., Lussy H. & Nowotny N. (2013). – Tick-borne encephalitis virus in horses, Austria, 2011. *Emerg. Infect. Dis.*, **19** (4), 635–637.
44. Pfeffer M. & Dobler G. (2011). – Tick-borne encephalitis virus in dogs: is this an issue? *Parasit. Vectors*, **4**, 59.
45. Chambouris R., Sixl W., Stunzner D. & Kock M. (1989). – Antibodies in dogs to the virus of tick-borne encephalitis (early summer encephalomyelitis/tick-borne encephalitis) in Greece [in German]. *Geogr. Med.*, **3**, 11–14.
46. Lindhe K.E., Meldgaard D.S., Jensen P.M., Houser G.A. & Berendt M. (2009). – Prevalence of tick-borne encephalitis virus antibodies in dogs from Denmark. *Acta Vet. Scand.*, **51**, 56.
47. Keane D.P., Parent J. & Little P.B. (1987). – California serogroup and Powassan virus infection of cats. *Can. J. Microbiol.*, **33** (8), 693–697.
48. Ernek E., Kozuch O., Nosek J., Teplan J. & Folk C. (1977). – Arboviruses in birds captured in Slovakia. *J. Hyg. Epidemiol. Microbiol. Immunol.*, **21** (3), 353–359.
49. Mikryukova T.P., Moskvitina N.S., Kononova Y.V., Korobitsyn I.G., Kartashov M.Y., Tyuten Kov O.Y., Protopopova E.V., Romanenko V.N., Chausov E.V., Gashkov S.I., Konovalova S.N., Moskvitin S.S., Tupota N.L., Sementsova A.O., Ternovoi V.A. & Loktev V.B. (2014). – Surveillance of tick-borne encephalitis virus in wild birds and ticks in Tomsk city and its suburbs (Western Siberia). *Ticks Tick Borne Dis.*, **5** (2), 145–151.
50. Waldenström J., Lundkvist A., Falk K.I., Garpmo U., Bergström S., Lindegren G., Sjöstedt A., Mejlon H., Fransson T., Haemig P.D. & Olsen B. (2007). – Migrating birds and tickborne encephalitis virus. *Emerg. Infect. Dis.*, **13** (8), 1215–1218.

51. Geller J., Nazarova L., Katargina O., Leivits A., Jarvekul L. & Golovljova I. (2013). – Tick-borne pathogens in ticks feeding on migratory passerines in western part of Estonia. *Vector Borne Zoonotic Dis.*, **13** (7), 443–448.
52. Pogodina V.V. (1958). – Resistance of tick-borne encephalitis virus to gastric juice. *Vopr. Virusol.*, **3** (5), 271–275.
53. Kerbo N., Donchenko I., Kutsar K. & Vasilenko V. (2005). – Tickborne encephalitis outbreak in Estonia linked to raw goat milk, May–June 2005. *Eurosurveillance*, **10** (25), pii: 2730.
54. Balogh Z., Ferenczi E., Szeles K., Stefanoff P., Gut W., Szomor K.N., Takacs M. & Berencsi G. (2010). – Tick-borne encephalitis outbreak in Hungary due to consumption of raw goat milk. *J. Virol. Meth.*, **163** (2), 481–485.
55. Caini S., Szomor K., Ferenczi E., Szekeleyne Gaspar A., Csohan A., Krisztalovics K., Molnar Z. & Horvath J. (2012). – Tick-borne encephalitis transmitted by unpasteurised cow milk in western Hungary, September to October 2011. *Eurosurveillance*, **17** (12), 20128.
56. Cisak E., Wojcik-Fatla A., Zajac V., Sroka J., Buczek A. & Dutkiewicz J. (2010). – Prevalence of tick-borne encephalitis virus (TBEV) in samples of raw milk taken randomly from cows, goats and sheep in eastern Poland. *Ann. Agric. Environ. Med.*, **17** (2), 283–286.
57. Holzmann H., Aberle S.W., Stiasny K., Werner P., Mischak A., Zainer B., Netzer M., Koppi S., Bechter E. & Heinz F.X. (2009). – Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerg. Infect. Dis.*, **15** (10), 1671–1673.
58. Klaus C., Ziegler U., Kalthoff D., Hoffmann B. & Beer M. (2014). – Tick-borne encephalitis virus (TBEV): findings on cross reactivity and longevity of TBEV antibodies in animal sera. *BMC Vet. Res.*, **10** (1), 78.
59. Balogh Z., Egyed L., Ferenczi E., Ban E., Szomor K.N., Takacs M. & Berencsi G. (2012). – Experimental infection of goats with tick-borne encephalitis virus and the possibilities to prevent virus transmission by raw goat milk. *Intervirology*, **55** (3), 194–200.
60. Klaus C., Hoffmann B., Moog U., Schau U., Beer M. & Suss J. (2010). – Can goats be used as sentinels for tick-borne encephalitis (TBE) in nonendemic areas? Experimental studies and epizootiological observations. *Berl. Münch. Tierärztl. Wochenschr.*, **123** (11–12), 441–445.
61. Studahl M., Lindquist L., Eriksson B.M., Gunther G., Bengner M., Franzen-Rohl E., Fohlman J., Bergstrom T. & Aurelius E. (2013). – Acute viral infections of the central nervous system in immunocompetent adults: diagnosis and management. *Drugs*, **73** (2), 131–158.
62. Suss J., Gelpi E., Klaus C., Bagon A., Liebler-Tenorio E.M., Budka H., Stark B., Müller W. & Hotzel H. (2007). – Tickborne encephalitis in naturally exposed monkey (*Macaca sylvanus*). *Emerg. Infect. Dis.*, **13** (6), 905–907.
63. Leschnik M.W., Kirtz G.C. & Thalhammer J.G. (2002). – Tick-borne encephalitis (TBE) in dogs. *Int. J. Med. Microbiol.*, **291** (Suppl. 33), 66–69.
64. Robertson S.J., Mitzel D.N., Taylor R.T., Best S.M. & Bloom M.E. (2009). – Tick-borne flaviviruses: dissecting host immune responses and virus countermeasures. *Immunol. Res.*, **43** (1–3), 172–186.
65. Best S.M., Morris K.L., Shannon J.G., Robertson S.J., Mitzel D.N., Park G.S., Boer E., Wolfinbarger J.B. & Bloom M.E. (2005). – Inhibition of interferon-stimulated JAK-STAT signaling by a tick-borne flavivirus and identification of NS5 as an interferon antagonist. *J. Virol.*, **79** (20), 12828–12839.
66. Werme K., Wigerius M. & Johansson M. (2008). – Tick-borne encephalitis virus NS5 associates with membrane protein scribble and impairs interferon-stimulated JAK-STAT signalling. *Cell. Microbiol.*, **10** (3), 696–712.
67. Atrasheuskaya A.V., Fredeking T.M. & Ignatyev G.M. (2003). – Changes in immune parameters and their correction in human cases of tick-borne encephalitis. *Clin. Experim. Immunol.*, **131** (1), 148–154.
68. Gunther G., Haglund M., Lindquist L., Forsgren M., Andersson J., Andersson B. & Skoldenberg B. (2011). – Tick-borne encephalitis is associated with low levels of interleukin-10 in cerebrospinal fluid. *Infect. Ecol. Epidemiol.*, **1**, 6029.
69. Lepej S.Z., Mistic-Majerus L., Jeren T., Rode O.D., Remenar A., Sporec V. & Vince A. (2007). – Chemokines CXCL10 and CXCL11 in the cerebrospinal fluid of patients with tick-borne encephalitis. *Acta Neurol. Scand.*, **115** (2), 109–114.
70. Grygorczuk S., Zajkowska J., Swierzbinska R., Pancewicz S., Kondrusik M. & Hermanowska-Szpakowicz T. (2006). – Concentration of the beta-chemokine CCL5 (RANTES) in cerebrospinal fluid in patients with tick-borne encephalitis [in Polish]. *Acta Neurol. Scand.*, **40** (2), 106–111.
71. Ruzek D., Salat J., Palus M., Gritsun T.S., Gould E.A., Dykova I., Skallova A., Jelinek J., Kopecky J. & Grubhoffer L. (2009). – CD8+ T-cells mediate immunopathology in tick-borne encephalitis. *Virology*, **384** (1), 1–6.
72. Heinz F.X. (2003). – Molecular aspects of TBE virus research. *Vaccine*, **21** (Suppl. 1), S3–S10.
73. Gomez I., Marx F., Saurwein-Teissl M., Gould E.A. & Grubeck-Loebenstien B. (2003). – Characterization of tick-borne encephalitis virus-specific human T lymphocyte responses by stimulation with structural TBEV proteins expressed in a recombinant baculovirus. *Viral Immunol.*, **16** (3), 407–414.

74. Gelpi E., Preusser M., Garzuly F., Holzmann H., Heinz FX. & Budka H. (2005). – Visualization of Central European tick-borne encephalitis infection in fatal human cases. *J. Neuropathol. Experim. Neurol.*, **64** (6), 506–512.
75. Gelpi E., Preusser M., Laggner U., Garzuly F., Holzmann H., Heinz FX. & Budka H. (2006). – Inflammatory response in human tick-borne encephalitis: analysis of postmortem brain tissue. *J. Neurovirol.*, **12** (4), 322–327.
76. Dörrbecker B., Dobler G., Spiegel M. & Hufert FT. (2010). – Tick-borne encephalitis virus and the immune response of the mammalian host. *Travel Med. Infect. Dis.*, **8** (4), 213–222.
77. Leonova G.N. & Maistrovskaia O.S. (1996). – Viremia in patients with tick-borne encephalitis and in persons with attached ixodes ticks. *Vopr. Virusol.*, **41** (5), 224–228.
78. Haglund M. & Gunther G. (2003). – Tick-borne encephalitis: pathogenesis, clinical course and long-term follow-up. *Vaccine*, **21** (Suppl. 1), S11–S18.
79. Ruzek D., Dobler G. & Donoso Mantke O. (2010). – Tick-borne encephalitis: pathogenesis and clinical implications. *Travel Med. Infect. Dis.*, **8** (4), 223–232.
80. Holzmann H. (2003). – Diagnosis of tick-borne encephalitis. *Vaccine*, **21** (Suppl. 1), S36–S40.
81. Schwaiger M. & Cassinotti P. (2003). – Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. *J. Clin. Virol.*, **27** (2), 136–145.
82. Schultze D., Dollenmaier G., Rohner A., Guidi T. & Cassinotti P. (2007). – Benefit of detecting tick-borne encephalitis viremia in the first phase of illness. *J. Clin. Virol.*, **38** (2), 172–175.
83. Achazi K., Nitsche A., Patel P., Radonic A., Donoso Mantke O. & Niedrig M. (2011). – Detection and differentiation of tick-borne encephalitis virus subtypes by a reverse transcription quantitative real-time PCR and pyrosequencing. *J. Virol. Meth.*, **171** (1), 34–39.
84. Ruzek D., Stastna H., Kopecky J., Golovljova I. & Grubhoffer L. (2007). – Rapid subtyping of tick-borne encephalitis virus isolates using multiplex RT-PCR. *J. Virol. Meth.*, **144** (1–2), 133–137.
85. Saksida A., Duh D., Lotric-Furlan S., Strle F., Petrovec M. & Avsic-Zupanc T. (2005). – The importance of tick-borne encephalitis virus RNA detection for early differential diagnosis of tick-borne encephalitis. *J. Clin. Virol.*, **33** (4), 331–335.
86. Jaaskelainen A., Han X., Niedrig M., Vaheri A. & Vapalahti O. (2003). – Diagnosis of tick-borne encephalitis by a mu-capture immunoglobulin M-enzyme immunoassay based on secreted recombinant antigen produced in insect cells. *J. Clin. Microbiol.*, **41** (9), 4336–4342.
87. Gunther G., Haglund M., Lindquist L., Skoldenberg B. & Forsgren M. (1997). – Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis. Long-term follow-up related to clinical course and outcome. *Clin. Diagn. Virol.*, **8** (1), 17–29.
88. Klaus C., Beer M., Saier R., Schubert H., Bischoff S. & Suss J. (2011). – Evaluation of serological tests for detecting tick-borne encephalitis virus (TBEV) antibodies in animals. *Berl. Münch. Tierärztl. Wochenschr.*, **124** (11–12), 443–449.
89. Kollaritsch H., Paulke-Korinek M., Holzmann H., Hombach J., Bjorvatn B. & Barrett A. (2012). – Vaccines and vaccination against tick-borne encephalitis. *Expert Rev. Vaccines*, **11** (9), 1103–1119.
90. Ludolfs D., Reinholz M. & Schmitz H. (2009). – Highly specific detection of antibodies to tick-borne encephalitis (TBE) virus in humans using a domain III antigen and a sensitive immune complex (IC) ELISA. *J. Clin. Virol.*, **45** (2), 125–128.
91. Kaiser R. (2008). – Tick-borne encephalitis. *Infect. Dis. Clin. N. Am.*, **22** (3), 561–575.
92. Waldvogel K., Bossart W., Huisman T., Boltshauser E. & Nadal D. (1996). – Severe tick-borne encephalitis following passive immunization. *Eur. J. Pediatr.*, **155** (9), 775–779.
93. Broker M. & Kollaritsch H. (2008). – After a tick bite in a tick-borne encephalitis virus endemic area: current positions about post-exposure treatment. *Vaccine*, **26** (7), 863–868.
94. Klaus C., Beer M., Saier R., Schau U., Moog U., Hoffmann B., Diller R. & Suss J. (2012). – Goats and sheep as sentinels for tick-borne encephalitis (TBE) virus: epidemiological studies in areas endemic and non-endemic for TBE virus in Germany. *Ticks Tick Borne Dis.*, **3** (1), 27–37.
95. Klaus C., Hoffmann B., Beer M., Muller W., Stark B., Bader W., Stiasny K., Heinz FX. & Suss J. (2010). – Seroprevalence of tick-borne encephalitis (TBE) in naturally exposed monkeys (*Macaca sylvanus*) and sheep and prevalence of TBE virus in ticks in a TBE endemic area in Germany. *Ticks Tick Borne Dis.*, **1** (3), 141–144.
96. Csango P.A., Blakstad E., Kirtz G.C., Pedersen J.E. & Czettel B. (2004). – Tick-borne encephalitis in southern Norway. *Emerg. Infect. Dis.*, **10** (3), 533–534.
97. Ytrehus B., Vainio K., Dudman S.G., Gilray J. & Willoughby K. (2013). – Tick-borne encephalitis virus and louping-ill virus may co-circulate in Southern Norway. *Vector Borne Zoonotic Dis.*, **13** (10), 762–768.
98. Piesman J. & Eisen L. (2008). – Prevention of tick-borne diseases. *Annu. Rev. Entomol.*, **53**, 323–343.
99. Cisak E., Wojcik-Fatla A., Zajac V. & Dutkiewicz J. (2012). – Repellents and acaricides as personal protection measures in the prevention of tick-borne diseases. *Ann. Agric. Environ. Med.*, **19** (4), 625–630.

100. Morozova O.V., Bakhvalova V.N., Potapova O.F., Grishechkin A.E., Isaeva E.I., Aldarov K.V., Klinov D.V. & Vorovich M.F. (2014). – Evaluation of immune response and protective effect of four vaccines against the tick-borne encephalitis virus. *Vaccine*, **32** (25), 3101–3106.
101. Marth E. & Kleinhappl B. (2001). – Albumin is a necessary stabilizer of TBE-vaccine to avoid fever in children after vaccination. *Vaccine*, **20** (3–4), 532–537.
102. Girgsdies O.E. & Rosenkranz G. (1996). – Tick-borne encephalitis: development of a paediatric vaccine. A controlled, randomized, double-blind and multicentre study. *Vaccine*, **14** (15), 1421–1428.
103. Fritz R., Orlinger K.K., Hofmeister Y., Janecki K., Traweger A., Perez-Burgos L., Barrett P.N. & Kreil T.R. (2012). – Quantitative comparison of the cross-protection induced by tick-borne encephalitis virus vaccines based on European and Far Eastern virus subtypes. *Vaccine*, **30** (6), 1165–1169.
104. Orlinger K.K., Hofmeister Y., Fritz R., Holzer G.W., Falkner F.G., Unger B., Loew-Baselli A., Poellabauer E.M., Ehrlich H.J., Barrett P.N. & Kreil T.R. (2011). – A tick-borne encephalitis virus vaccine based on the European prototype strain induces broadly reactive cross-neutralizing antibodies in humans. *J. Infect. Dis.*, **203** (11), 1556–1564.
105. Kollaritsch H., Chmelik V., Dontsenko I., Grzeszczuk A., Kondrusik M., Usonis V. & Lakos A. (2011). – The current perspective on tick-borne encephalitis awareness and prevention in six Central and Eastern European countries: report from a meeting of experts convened to discuss TBE in their region. *Vaccine*, **29** (28), 4556–4564.
-