



Epidemiology and pathological mechanisms of tick-borne encephalitis

Kentaro YOSHII^{1)*}¹⁾Laboratory of Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido 060-0818, Japan

ABSTRACT. Tick-borne encephalitis virus (TBEV), a member of the genus *Flavivirus* within the family *Flaviviridae*, causes fatal encephalitis with severe sequelae in humans. TBEV is prevalent over a wide area of the Eurasian continent including Europe, Russia, Far-Eastern Asia, and Japan. While it was previously thought that TBEV was not endemic in Japan, the first confirmed case of serologically diagnosed TBE was reported in 1993 in the southern area of Hokkaido Prefecture, Japan. In addition, TBEV has been isolated from dogs, wild rodents and ticks in the area. Our epizootiological survey indicated that endemic foci of TBEV were maintained in Hokkaido and other areas of Honshu. TBEV can be divided into three subtypes based on phylogenetic analyses. The Japanese isolates were classified as the Far Eastern subtype, which causes severe neural disorders with a higher mortality rate up to 30%. However, how viral replication and pathogenicity contribute to the neurological manifestations remains unclear. Recent studies have revealed distinctive mechanisms of TBEV pathogenicity and viral genetic factors associated with virulence. This review discusses the recent findings regarding the epidemiology and pathogenesis of TBEV.

KEY WORDS: epidemiology, flavivirus, pathogenicity, tick-borne encephalitis virus

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Tick-borne encephalitis virus (TBEV), belonging to the *Flavivirus* genus of the *Flaviviridae* family, causes mild or moderate febrile illness and fatal encephalitis with sequelae in humans. TBEV is divided into European, Siberian, and Far-Eastern subtypes. The Far-Eastern subtype is the most lethal. The virus is endemic in many parts of Europe and Asia [27, 39], and >10,000 cases of the disease are reported annually. TBEV is maintained in transmission cycles between ixodid ticks and wild mammalian hosts, particularly rodents. Furthermore, transovarial and transstadial transmission have been reported in tick vectors [6, 35]. TBEV infects a wide range of animal species via bites from infected ticks. Alimentary transmission has been also reported via the consumption of unpasteurized milk and milk products from infected livestock [4].

Tick bites often remain unnoticed, and the incubation period of TBE is usually 7–14 days (range, 2–28 days) [19, 28]. The symptoms of TBEV infection can be categorized in two phases. The European subtype produces biphasic febrile illness, whereas the other two subtypes display a monophasic course. Flu-like symptoms are observed during the initial viremic phase of the illness, including fever, fatigue, general malaise, and headache and muscular pains; these symptoms last 2–7 days (range, 1–10 days) [12, 19]. No signs or symptoms of meningoencephalitis are usually seen during this phase. The asymptomatic interval before the second phase lasts up to 7 days (range 1–21 days). Illness caused by the Far-Eastern and Siberian subtypes usually progress without this asymptomatic phase. The second phase presents with symptoms and signs ranging from mild meningitis to severe encephalitis. The main clinical neurological syndromes associated with TBE are febrile headache, aseptic meningitis, meningoencephalitis, meningomyeloencephalitis, and postencephalitic syndrome. The chronic form of TBE is often associated with the Siberian subtype [33]. Chronic disease begins with or without the typical acute-phase symptoms. In many cases, it can take years to develop neurological symptoms after the tick bite.

As no specific antiviral treatments are available for TBE, prophylaxis is important for its control. Active immunization with a vaccine is effective for the prevention of TBE. Immunogenicity is mostly associated with virions, particularly with the viral envelope protein. Formaldehyde-inactivated and purified whole virus is used as a TBE vaccine, which is currently produced by five manufacturers. The European TBE vaccines have a well-established safety record [2]. In Austria, the FSME-immune vaccine is used extensively, and mass vaccination has reduced the number of reported cases [14]. However, no TBE vaccines are currently licensed in Japan.

In Japan, patients infected with a virus within the TBE serocomplex had encephalitis during an epidemic of Japanese encephalitis (JE) in 1948 in the Tokyo area. The isolated virus, named Negishi virus, was retrospectively identified as a member of the louping ill virus through antigenic and phylogenetic analyses conducted decades later [32, 42]. No subsequent cases of TBE

*Correspondence to: Yoshii, K.: kyoshii@vetmed.hokudai.ac.jp

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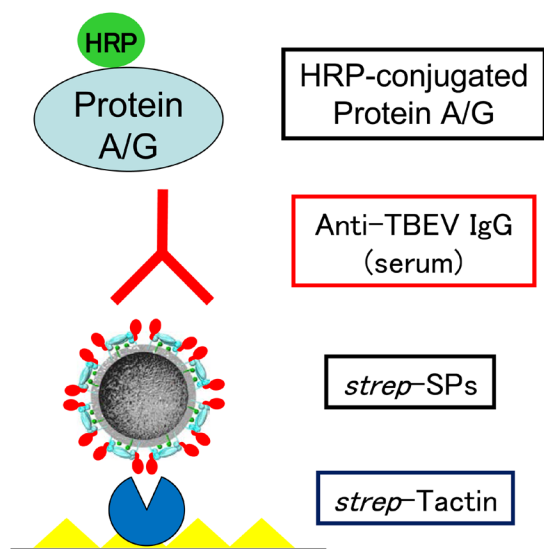


Fig. 1. IgG-ELISA using *strep*-tagged subviral particles of TBEV. Strep-tagged subviral particles (strep-SPs) of TBEV are captured by strep-tactin coated onto a plate, and anti-TBEV IgG antibodies in serum samples are reacted. The IgG antibodies are detected by enzyme-conjugated Protein A/G.

Table 1. Comparison of the results obtained by neutralization and IgG-ELISA using *strep*-SPs (SPs ELISA) in human serum (From Inagaki *et al.* [18])

Neutralization test	SPs ELISA		Total
	Positive	Negative	
Positive	78	4	82
Negative	1	24	25

in Japan were reported until 1993, when a case of viral encephalitis in southern Hokkaido was diagnosed as TBE [29]. TBEV was isolated from dogs and *Ixodes ovatus* in the area where the patient lived, and the virus strains were identified as the Far-Eastern subtype [40, 41]. In 2016 and 2017, three more cases of TBE were identified in Hokkaido [43, 45]. To control TBEV infection and to design an effective prevention and vaccination plan, requisite specific targeting was performed to define TBEV-endemic areas and to identify the characteristics of endemic TBEV.

DEVELOPMENT OF NEW SEROLOGICAL DIAGNOSTIC METHOD AND ITS APPLICATION TO EPIDEMIOLOGICAL RESEARCH FOR TBE IN JAPAN

In the Prevention of Infectious Diseases and Medical Care for Infectious Patients Act that became legislation in Japan in 1999, TBE was classified as a reportable infectious disease and TBEV was classified as a Class 3 select agent handled under bio-safety level (BSL) 3 conditions. Therefore, laboratory examinations are available only in limited facilities due to restrictions in handling TBEV as a BSL 3 agent, leading to the difficulties in conducting epidemiological survey of TBE in Japan. Several diagnostic tests are available to detect TBEV infection. The neutralization test (NT) is useful for areas where two or more flaviviruses are endemic, as it has high specificity for each virus. However, performing the NT is time-consuming, and high-biosafety-level facilities are required to handle the live virus. The enzyme-linked immunosorbent assay (ELISA) is a relatively safe diagnostic method for detecting antibodies in infected individuals because it utilizes inactivated TBE virions. However, the commercially available TBE-ELISA kit shows cross-reactivity against antibodies to other flaviviruses [7, 16, 30], and these kits are applicable only to infection in humans, not in other animals. Therefore, it is critical to develop a safe and simple serological diagnostic method that can be applied to a wide range of animal species.

The entire pre-membrane (prM) and envelope (E) gene sequences are expressed and the products are secreted as membrane-bound subviral particles (SPs) [1]. SPs consist of a viral envelope without a nucleocapsid or genomic RNA. Because SPs maintain antigenicity similar to that of authentic virions, they have been applied as substitutes for infectious virions in serological diagnoses [8, 17, 31]. In a previous study, we constructed SPs of TBEV by fusing a small peptide tag (*strep*-tag), which has a high binding affinity for strep-tactin, to the N-terminus of the E protein. The SPs with strep-tag (strep-SPs) were used in an IgG-ELISA to detect reactive antibodies in a broad range of mammal species (Fig. 1) [18]. Compared to NT results, the ELISA showed high sensitivity and specificity (>95%) (Table 1), showing no cross-reactivity with antibodies to Japanese encephalitis virus.

Our IgG-ELISA using *strep*-SPs was introduced to prefectural institute of public health for serological diagnoses of TBE-suspected human cases, which led to the detection of patients with TBE in 2017 [43]. In addition, the IgG-ELISA was employed in sero-epizootiological surveys in wild animals, which revealed that the endemic foci of TBEV are widely distributed in Hokkaido, and that TBEV or TBE-serocomplex viruses may be broadly distributed in western Japan.

Table 2. Viral genetic determinant of TBEV pathogenicity identified by using reverse genetics

Viral gene	Functions in TBEV pathogenicity	Reference
5' UTR	Genomic RNA cyclization and replication	[9, 20]
	Viral RNA transport in dendrites	[15]
E	Receptor-binding and neurovirulence	[23]
	Glycosaminoglycan-binding affinity in neuroinvasiveness	[26]
	Maturation of the E protein in mammalian cells	[46]
NS5	Interferon antagonism	[22]
	Attenuation of neurite outgrowth	[44]
3' UTR	Genomic RNA cyclization and replication	[9, 20]
	Adaptation or selection in mammalian cells	[37, 38]

VIRAL GENETIC DETERMINANT OF TBEV PATHOGENICITY

The flavivirus genome consists of a positive-polarity, single-stranded RNA of approximately 11 kb, which encodes three structural proteins: (the core [C], prM, and E proteins) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) within a single open reading frame [5]. The 5' and 3' UTRs predict secondary structures that are implicated in viral replication, translation, and packaging of the genome [11, 34].

Reverse genetics of viruses allows the generation and manipulation of viral genomes to investigate the direct effects of changes on virus biology and pathogenesis. Infectious cDNA clones of flaviviruses, including TBEV, generated using reverse genetics systems [10, 13, 24] have provided useful platforms on which to investigate the genetic determinants of flavivirus virulence.

Several viral determinants of TBEV pathogenicity have been identified using reverse genetics (Table 2). For example, the important role of the E protein in pathogenicity has been well characterized. Mutations of specific amino acid residues in the upper lateral surface of domain III in the E protein affect neurovirulence in mice [23]. Mutations that increase glycosaminoglycan (GAG)-binding affinity, located on the outer surface of the E protein, usually occur near existing positive clusters [26]. Increased GAG-binding affinity appears to be a major mechanism of attenuation of neuroinvasiveness via the E proteins. In the majority of TBEV strains, as in other flaviviruses, the E protein contains a conserved N-linked glycosylation site. In mammalian cells, loss of glycosylation affects the conformation of protein E during secretion, reducing the infectivity of secreted virions and affecting TBEV pathogenicity in mice [46].

The viral NS5 protein consists of two principle domains: the methyltransferase (MTase) domain located on the N terminal side of the protein and the RNA-dependent RNA polymerase (RdRp) domain on the C terminal side [21, 36]. NS5 in several flaviviruses has interferon (IFN) antagonist activity [3]. Tick-borne flavivirus infection downregulates the cellular expression of IFN receptor subunit, IFNAR1, through an interaction between NS5 and the host protein prolidase [22]. A TBEV variant with a NS5 mutation lacking IFN-I antagonism shows reduced virulence in mice. While the majority of TBE complex viruses cause encephalitis, three tick-borne flaviviruses cause hemorrhagic disease, including Omsk hemorrhagic fever virus (OHFV). A recent study that compared TBEV and OHFV revealed that a 4 amino acid region near the C-terminus of NS5 is critical for the neuropathogenesis of TBEV [44]. This region is involved in the attenuation of neurite outgrowth, resulting in the neurological disease phenotype in mice.

UTRs are important for many functions in flavivirus multiplication. The complementary sequences in the 5' and 3' UTRs cyclize the viral genome, which is essential for viral genome replication [9, 20]. A recent study showed that the 5' UTR of TBEV functions in RNA transport in neuron dendrites. Using this function, TBEV hijacks the transport system of host mRNA in dendrites and affects neuronal functions, such as neurogenesis and the plasticity of synaptic communication [15].

The 3' UTR can be divided into two regions: the variable region, which varies among TBEV strains, and the core element, which has a highly conserved sequence [11]. The core element shows a high degree of sequence conservation among TBEV strains and contains sequences necessary for viral genome replication, such as those involved in cyclization [20]; deletions in the core element cause virus attenuation in mice [25]. Recent studies have identified deletions and insertions of poly (A) sequences in the variable region, which cause severe pathological changes associated with the Far Eastern subtype of TBEV [37, 38]. A deletion in the 3' UTR occurs during passage in mammalian cell cultures or in mice; however, strains freshly isolated from ticks and wild rodents have no deletion in the variable region. Therefore, this region is considered essential for the natural transmission cycle of TBEV. These data suggest that the deletion caused by adaptation or selection in mammalian cells may be associated with the increased virulence in mammals.

Important advances in TBEV research have been made in recent years. Modern diagnostic methods are being used to investigate TBEV in an increasing number of laboratories, which will lead to further understanding of TBEV and raise greater awareness of TBE. Understanding the pathogenic mechanism of TBEV will contribute to the future development of vaccines and therapies to treat TBE.

CONFLICTS OF INTEREST. The authors declare no conflicts of interest.

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