Serologic Evidence of Tick-Borne Encephalitis Virus Infection in a Patient with Suspected Lyme Disease in Japan

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Abstract. Tick-borne encephalitis (TBE) is widely prevalent on the Eurasian continent, including Japan, but four cases of TBE have been reported in Japan. To inspect unconfirmed TBE cases in Japan, we conducted a retrospective seroepidemiological study of a total of 158 samples from 81 meningoencephalitis patients suspected as Lyme disease. Two serum samples from one patient showed neutralizing antibodies against TBE virus. The patient with severe and progressive encephalitis had a history of tick bite in Hokkaido in 2012. These results demonstrated that tick-borne encephalitis virus (TBEV) case was actually unconfirmed in Japan. Further seroepidemiological surveys are required to identify unconfirmed TBEV infections to consider the pros and cons of introducing specific countermeasures including vaccination in Japan.

Tick-borne encephalitis (TBE) virus (TBEV) belongs to the Flaviviridae family and *Flavivirus* genus, and may causes fatal encephalitis in humans. In nature, TBEV is transmitted between ticks and wild vertebrate hosts, especially rodents.¹ Tick-borne encephalitis is widely prevalent on the Eurasian continent, including Japan. In Japan, the first confirmed case of TBE was reported in 1993 in Hokkaido, the northernmost prefecture.² Since that time, three other cases of TBE have been reported in Japan in 2016 and 2017,^{3,4} although endemic foci of TBEV have been detected in various parts of Japan, especially in Hokkaido.⁵ Therefore, the possibility exists that TBE patients are being unconfirmed in Japan.

Tick-borne encephalitis virus is transmitted by *Ixodes* ticks, which are also the primary vector for Lyme disease-causing bacteria, *Borrelia*.⁶ In Japan, almost 5–20 cases of Lyme disease patients are reported annually. Through the dissemination of the bacteria, Lyme disease often presents with neurological symptoms, similar to those of TBE. In a previous study, confirmed infection of Powassan virus, one of the TBE serocomplex viruses, was detected in patients with suspected Lyme disease in a Lyme disease-endemic area, indicating that Powassan virus infection had been unconfirmed in northern Wisconsin.⁷ In Japan, many meningitis or meningoencephalitis cases associated with tick bites are subjected to the diagnosis of Lyme disease, but not to that of TBE. This is because of the low awareness of TBE and the difficulty in performing laboratory examinations for the diagnosis of TBEV infection. In this study, we conducted a retrospective seroepidemiological study of meningoencephalitis patients suspected as Lyme disease patients to inspect unconfirmed TBE cases in Japan.

We selected 158 serum or spinal fluid samples from 81 meningoencephalitis patients with suspected Lyme disease collected by the National Institute of Infectious Disease (NIID) between 2010 and 2017. The samples were from patients in their first to eighth decade of life living throughout Japan, including Hokkaido. A neutralizing antibody test was conducted using the TBEV isolate from Hokkaido in 1993.² To differentiate TBEV infections from Japanese encephalitis virus

(JEV) infection, which is seen in southwestern Japan, a neutralization test for JEV was conducted on all TBEV-positive samples. A sample was determined positive if a plaque reduction of 50% or greater compared with the virus control was observed. A neutralizing titer was defined as the reciprocal of the highest dilution. Approval for this study was obtained from the Medical Ethics Committee of the Faculty of Veterinary Medicine, Hokkaido University and the NIID.

Of 158 total samples, two serum samples from one patient showed neutralizing antibodies against TBEV but were both negative for JEV (antibody titers < 20). Significant increases in the neutralizing antibody titers against TBEV (100–800) were detected in the paired serum samples. The patient was a male in his 80's who was reported ill in Hokkaido in 2012. His symptoms included fever, nausea, and vomiting 21 days after a tick bite. After hospitalization, his condition deteriorated over several days. He experienced progressive and severe paralysis as well as difficulty in breathing. A physician initially diagnosed the patient with neuroborreliosis, because he had a history of tick bite in an endemic area of Lyme disease, and he had a fever (ranging from 37.6 to 39.7 degrees) with elevation of C-reactive protein level (4.4–7.5 mg/dL). Polymerase chain reaction and serological tests for Lyme disease were negative.

In this study, infection with TBEV in a Lyme disease-suspected patient was diagnosed serologically, demonstrating that TBEV cases are actually unconfirmed in Japan. Although TBEV or TBEV-infected animals have been detected, four severe human encephalitis cases have been reported in humans in Japan; two of these patients died, and the other two recovered, but severe sequelae such as paralysis remained. Furthermore, two patients infected with a virus within the TBE serocomplex developed encephalitis during an epidemic of Japanese encephalitis in 1948 in the Tokyo area. The isolated virus was retrospectively identified as a member of the louping ill virus through antigenic and phylogenetic analyses conducted decades later.^{8,9} It is likely that other human TBEV infections existed during these years, including not only subclinical and mild cases but also unconfirmed severe cases, as shown in this study. For example, we recently reported unrecognized subclinical infections with TBEV in Japan Self Defense Force members from the Northern Army.¹⁰ Further seroepidemiological surveys with larger sample sizes and a broader scope of subjects are required

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to identify unconfirmed TBEV infections to consider the pros and cons of introducing specific countermeasures including vaccination. Such a survey is also desirable to measure the virulence of TBEV of the so-called Far Eastern subtype, because the detection of subclinical or mild cases may lead to an overall decrease in its alleged high risk of fatality.¹¹

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