Form No.5

Abstract of Thesis/Dissertation

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Title： Epidemiological survey of tick-borne encephalitis virus in wild animals and livestock, 1998–2018, Japan. (日本において、1998 年～2018 年に行われた野生動物と家畜におけるダニ媒介脳炎ウイルスの疫学的調査)

Abstract

Tick-borne encephalitis (TBE) is one of the severe viral infections of the human central nervous system. TBE virus (TBEV) infections tend to be asymptomatic that outnumbers the symptomatic disease with characteristic neurological abnormalities including meningitis and encephalitis. In TBEV-endemic Eurasian countries, over 10,000 cases are annually estimated, of which about 70% leave the disease sequelae.

Usually, humans become infected by tick bites, or rarely via contaminated milk of mothers or domestic animals infected with TBEV. Although TBE is an efficiently vaccinated disease, people living in the TBEV endemic countries remain at risk.

TBEV is maintained in nature alternating between ticks and vertebrate hosts. The most identified vector ticks are Ixodid ticks, and their feeding hosts are forest and domestic animals which serve as the hosts for TBEV transmission. Among the vertebrate hosts, rodents act as both maintenance and amplifying hosts and also as a reservoir host due to persistent and high-level viremia. Other large animals cannot transmit the virus to ticks, because of a short and low-level viremia. However, in masses they provide the place that ticks can feed and multiply on their bodies, resulting in

support for virus circulation. In TBEV-endemic countries, moreover, both large wild and domestic animals such as deer, wild boar, sheep, goat, and cattle are known to be valuable indicator hosts (sentinels) to monitor the distribution and abundance of vector ticks to avoid TBE risk in humans. However, very little known regarding TBEV infection in these sentinel species in Japan. The distribution of TBEV has been reported in 32 Eurasian countries including Japan with an estimated approximately 30,000 TBEV foci.

TBE is a notifiable disease in Japan. The first human case of TBE was recorded in the southern part of Hokkaido in 1993, and it was subsequently revealed that this region was a TBEV-endemic area based on the isolation of TBEV from ticks, rodents, and dogs, as well as serological evidence in rodents, dogs, and horses. Although there were no further records of TBE for a long period, four confirmed cases were reported in the southern, central and northern part of Hokkaido during 2016−2018, suggesting the re-emergence of TBE and the widespread distribution of TBEV in Hokkaido. Seropositive Hokkaido sika deer were detected in the northern part of Hokkaido before the occurrence of TBE in 2018. However, TBEV foci are unknown in other parts of Hokkaido such as the eastern part. Outside Hokkaido, Shimane Prefecture was the only area where seropositive rodents were found in 2001. Thus, it is an urgent issue to examine and clarify the distribution of TBEV foci in Japan to avoid the further occurrence of TBE. Therefore, the thesis aimed to uncover and identify the previously unknown TBEV risk areas by testing wild animals, such as raccoons, sika deer and wild boars, and livestock using ELISA and a 50% plaque reduction neutralization test (PRNT50).

The first chapter describes the optimization of ELISA in order to detect TBEV antibodies in sera from large animals. Several parameters such as the Strep-SPs concentration, blocking and antigen coating buffers, and diluents, were evaluated using a rabbit anti-TBEV antiserum and normal rabbit serum. As ELISA positive antigens, TBEV subviral particles (SPs) with Strep-tag (WSHPQFEK) that were secreted into the supernatants of HEK293T cells transfected with the plasmid expressing Strep-SPs were used (Strep-SP ELISA). Negative antigens were similarly prepared from the supernatants of normal HEK293T cells. As a result of parameter evaluation, the carbonate-bicarbonate buffer was chosen as coating buffer of alkaline phosphatase labeled Strep-Tactin instead of PBS due to a good signal. The supernatant containing

Strep-SP that were concentrated 100-fold showed a good signal as antigen. As a blocking buffer, 1% BlockAce highly reduced background reaction compared with 4% bovine serum albumin (BSA), 5% skim milk or 1% casein. As serum and conjugate diluents,1% BSA was superior to 3% BSA, because of lower background. In the optimized ELISA, the results were recorded as the positive/negative (P/N) ratio (i.e., the ratio of the OD value at 450 nm with Strep-SPs to that with the negative antigens). A total of 411 sera including 368 raccoons and 43 sika deer were first tested to judge whether the assay can detect antibodies to TBEV. In this study, I regarded Strep-SP ELISA positive when serum samples indicated a P/N ratio of ≥1.5. PRNT50 confirmed Strep-SP ELISA performance. Neutralizing titers ($PRNT_{50}$) of ≥1:50 were determined to be seropositive against TBEV. PRNT50 found that Strep-SP ELISA could detect the presence of TBEV-specific antibodies in raccoons and sika deer.

Chapter II describes an expanded, serological survey of TBEV on Hokkaido and Honshu islands by testing a total of 1,649 animal sera sampled in 2003−2018, including 1,072 raccoons, 519 sika deer, and 58 wild boars using Strep-SP ELISA and PRNT50. In addition, 507 total Ixodid ticks and 22 raccoon blood were examined by real-time reverse transcription polymerase chain reaction and virus isolation tests. As a result of the serological survey, the most identified TBEV seropositive species was raccoons (3.9%; 42/1,072), followed by sika deer (0.6%; 3/519). The central part of Hokkaido had the highest seroprevalence at 5.7% (39/685), followed by the Kenhoku part of Tochigi Prefecture in Honshu at 2.4% (1/42), and the eastern part of Hokkaido at 0.6% (5/819). Significant differences in TBEV seroprevalence among raccoons were observed between Sorachi District (9.5%) in the central part of Hokkaido and Iburi (2.1%; $p < 0.05$), Ishikari (2.1%; $p < 0.01$), and Tokachi District in (0.8%; $p < 0.01$). Neither raccoons and wild boars in Fukushima and Tochigi prefectures had TBEV antibodies. All Ixodid ticks and raccoon blood were tested negative for TBEV.

In the study, TBEV foci were newly discovered in Tokachi District in the eastern part of Hokkaido with TBEV seroprevalence in raccoons (0.8%; 3/368) and Hokkaido sika deer (0.5%; 2/401), and in Tochigi Prefecture with TBEV prevalence in Honshu sika deer (2.4%; 1/42). From the data of these seropositive sera collected in 2016–2018, both areas may have been recently exposed to TBEV, which may suggest a possibility that

TBEV is distributed in a wide range not only throughout Hokkaido but also other islands.

Chapter III includes a serological survey on cattle and sheep sera collected across the country using Strep-SP ELISA. A total of 728 cattle sampled in 1998–1999 from 13 prefectures on all islands of Japan, and 177 sheep sera in the central part of Hokkaido were tested. Of these, 70 cattle and three sheep sera showed P/N ratios of ≥1.5. Unexpectedly, however, TBEV specific neutralizing antibodies were not detected in cattle sera. Only one cattle serum showed a low-level titer (1:20) against TBEV in PRNT50. In this study, Strep-SP ELISA was first applied to the examination of cattle sera. Although the reason of high nonspecific reaction in cattle sera is unknown, as a possible explanation for this unexpected result, non-specific IgG in cattle sera might have associated with the reaction of Strep-tag of TBEV-SPs or Strep-Tactin AP. However, it may not deny the possibility that TBEV Strep-SPs might have reacted to antibodies against unknown flaviviruses that are antigenically related to TBEV.

In conclusion, this thesis study revealed the presence of new TBEV foci in the eastern part of Hokkaido and Tochigi Prefectures (Honshu island) through serological surveys on large wild animals. The present study may suggest that TBEV is widely distributed in Japan more than expected, especially in Hokkaido. For continuous large-scale monitoring of TBEV foci using large wild animals such as raccoons, sika deer or wild boars as sentinels, a combination of Strep-SP ELISA with PRNT50 seems to be useful. To avoid emerging TBE, it is required to find out the exact locations of TBEV foci in the areas where the serological evidence of TBEV infection was given.

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2. Abstract should be between 1,800 and 2,200 characters in Japanese or be between 1,000 and 1,400 words in English.
3. Do not include figures and tables.
4. Abstract can be longer than one page.