PhD Thesis (abridged)

博士論文（要約）

Understanding the vectors of Japanese encephalitis virus:

their viromes and dynamics of the virus infection to transmission

（日本脳炎ウイルスのベクターの研究：蚊のウイロームおよび

感染から媒介までのウイルス動態）

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As a neglected tropical disease, Japanese encephalitis (JE) remains a public health concern due to the possible disease severity of disease presentation. Active vaccination, complemented by periodic surveillances, has resulted in a significant decrease in clinical cases of JE in some endemic countries. However, the global control of JE is hampered by limitations including: the absence of a national JE vaccination program in some endemic countries, the reemergence of genotypes, after decades of absence, and the reported spread of the causative virus (i.e. JEV) into new countries outside the endemic areas, necessitating continuous studies. JEV is a mosquito-borne virus whose continuous presence is dependent on the availability of an amplifier hosts (e.g. swine) and specific mosquito species as vectors. Humans, and other vertebrates, on the other hand are dead-end hosts. Mosquitoes play a significant role in maintaining JEV circulation by transmitting the virus from one amplifier host to another. Due to their importance, this thesis focused primarily on the JEV mosquito vector. The objectives of this study were:

1. To establish a high throughput RNA virus detection and isolation method from mosquitoes to facilitate JEV surveillance
2. To evaluate the competence of different mosquito species in transmitting various JEV genotypes.

In chapter I, a system for RNA virome profile elucidation and arbovirus isolation were established. *Culex vishnui* subgroup mosquitoes were processed for RNA virome analysis, with another sympatric mosquito species for comparison. The obtained virome was made up of 27 viruses, including JEV. The virome profile, diversity and abundance, was dependent on the mosquito taxon.

Successful isolation of JEV genotype I from a *Cx. tritaeniorhynchus* pool confirmed its continuous presence in Japan, indicating that continuous monitoring is needed. This study went beyond elucidating the virome of an important disease vector to provide considerable insights into ecology and virus evolution of both arboviruses and insect specific viruses. This study is the first in Japan to describe the virome of the major JE vectors, *Cx. vishnui* subgroup mosquitoes.

In the chapter II, a combination method of the virus isolation and next-generation sequencing (NGS) previously established was used in identifying JEV. Collected mosquitoes in various locations in two different countries: Ishikawa Prefecture in Japan and Bali Province in Indonesia were processed. Results showed the different compositions of mosquito captured from the two countries. There was no JEV sequence detected from 2,147 *Culex* mosquitoes collected in Ishikawa Prefecture in 2018, whereas in Tabanan Regency in Bali Province, a JEV sequence was detected and isolated from one *Cx. vishnui* mosquito pool collected in 2019. The sequence was subsequently identified by genetic and phylogenetic analyses as JEV genotype (G) IV and its nucleotide identity was 99% with other JEV GIV isolates reportedly obtained from swine sera and a human patient's sample in 2017 and 2019, respectively. These results may suggest the difference of circulating JEV genotype in the two countries. Furthermore, this study may indicate that JEV GIV is actively circulating in certain areas in Indonesia, hence, robust entomological surveillance will be required to assess the risk of virus infection.

This study also evaluated vector competences of different mosquito species in transmitting JEV. In the chapter III, in vitro growth on various mosquito

cells were observed and subsequent in vivo study in two mosquito species (a *Culex sp.* and an *Aedes sp*.) revealed their vector competence in transmitting three JEV genotypes (I, III, and V). Results showed significant differences between the two species, with infection rate of 95% (261/274) and 9% (16/177) in *Cx. tritaeniorhynchus (Cx. tritaeniorhynchus)* and *Aedes japonicus japonicus* (*Ae. j. japonicus)*, respectively. However, the two species were susceptible to three JEV genotypes tested and showed comparable mean viral titer. These results confirmed the *Cx. tritaeniorhynchus'* competence as JEV primary vector, but the fact that JEV was able to establish in *Ae. j. japonicus*, which is not a common JEV vector, is also of public health significance. With the range of 2 to 16% of transmission rate, *Ae. j. japonicus* may have the potential to successfully transmit JEV. The result of this study may explain the human cases and infrequent detection in primary vector-free areas. Importantly, as an invasive species, *Ae. j. japonicus* could be a relevant vector spreading the disease into new areas, indicating the need for security measures in areas where the mosquito is distributed or where it may be introduced.