



HOKKAIDO UNIVERSITY

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学位論文内容の要旨
Abstract of the dissertation

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The title of the doctoral dissertation

Studies on Isolation and Characterization of Alphaviruses Sindbis
Virus and Eilat Virus
identified from mosquitoes in Zambia

（ザンビアの蚊から単離されたアルファウイルス属シンドビスウイルス
スおよびエイラットウイルスの性状解析）

Alphaviruses are RNA viruses transmitted by mosquito vectors which infect a variety of hosts including humans, and cause a wide variety of clinical symptoms in patients. While they are associated with human disease, alphaviruses remain poorly understood in terms of their host specificity mechanisms, evolution and pathogenesis. Sindbis virus (SINV) belongs to an alphavirus species within the Western Equine Encephalitis (WEE) complex and are found in the Old World. Eilat virus (EILV) is an insect-specific alphavirus recently identified in mosquitoes and forms a distinct group from other known alphavirus complexes. In this study, a strain of SINV and a strain of EILV were isolated for the first time from mosquitoes captured in Zambia. These viruses were characterized in terms of their phylogenetic relatedness to previously reported viruses as well as their growth properties. The purpose of this study was to compare the fundamental biological properties of these newly isolated alphavirus strains with previously reported isolates.

In Chapter I, a new strain of SINV designated strain zmq_17M115 (SINV M115) was isolated during alphavirus screening in Zambia and characterized. Phylogenetic analysis revealed that SINV M115 belongs to Clade D of SINV Genotype 1, which is also found in Europe, the Middle East and Africa. From a global epidemiological perspective, this finding suggests that SINV may have been introduced to Zambia via birds serving as vectors or amplifying

hosts. The growth efficiency of SINV M115 in mammalian cells, such as BHK-21 cells was similar as that of the prototype SINV AR339; however SINV M115 proliferated more rapidly than SINV AR339 in mosquito-derived C6/36 cells. The pathogenicity of the Zambian SINV was assessed in immunodeficient as well as immunocompetent mouse models in comparison with reference SINV strain AR339. *In vivo* experiments revealed that both SINV strains caused lethal infection of AG129 mice within 3 days post infection (dpi) inoculated by subcutaneous (SC) route using 10⁴-1,000 plaque forming units (PFU) of the viruses. These results demonstrate that SINV M115 is highly pathogenic in AG129 mice. This study indicates the possible circulation of SINV in Zambia and highlights the need for surveillance of potential epidemics in humans.

In Chapter II, a newly isolated EILV strain from *Culex* mosquitoes in Zambia was characterized in terms of its genetic relation in alphaviruses and replication in mosquito and mammalian cells. The genetic characterization showed that Zambian EILV (strain zmq19_M44) exhibited a close phylogenetic relationship with other insect-specific alphaviruses and shared a close nucleotide identity to those of EILV isolate (90.4%) and Mwinilunga alphavirus (MWAV) (75.5%), which is a novel alphavirus previously identified in mosquitoes collected in Zambia. The EILV zmq19_M44 replicated in C6/36 cells and attained a saturating titer at 6 to 8 dpi but was unable to replicate in mammalian cell lines, Vero and BHK-21 cells. This study revealed that, in addition to MWAV and SINV, EILV is also present in mosquitoes inhabiting Zambia, indicating the existence of diverse alphaviruses in the region. Thus it is an important work for understanding the dissemination of alphaviruses across major geographic regions.