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Name

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Surveillance of arbovirus infection in Zambia and characterization of the untranslated regions
of insect-specific flaviviruses

(ザンビアにおける蚊媒介性ウイルス感染症の調査、
および蚊特異的フラビウイルス非翻訳領域の解析)

Surveillance of arthropod-borne virus (arbovirus) infection was conducted in Zambia to obtain information for arbovirus disease prevention and control and to make a precaution for upcoming emerging diseases. Zambia is located at southern Africa with tropical climate that favourable for mosquito breeding and arthropod-borne disease spreading. To monitor arbovirus dissemination, serosurveillance in mammalian host and mosquitoes are need to be conducted. In addition, virus isolation and characterization will provide further data related to virus transmission and their ability to infect various hosts.

Serosurveillance of arbovirus infection was conducted by examination of serum-specific antibodies against Zika virus (ZIKV) using plaque reduction neutralization test (PRNT) from sera collected from three species of non-human primates (NHPs) in the Livingstone (southern) and Mfuwe (eastern) districts between 2009 and 2010. Species of NHPs were confirmed by sequencing of mitochondrial *cytochrome b* gene. As the results, 34% (33/97) of NHP sera had neutralizing antibodies against ZIKV. Seroprevalence of anti-ZIKV antibodies of samples from Livingstone (43%: 21/49) was higher than that of Mfuwe (25%: 12/48). Cross-neutralization activity of one serum NHPs with yellow fever virus was detected, but not with tick-borne encephalitis virus. Despite the relatively high sero prevalence of ZIKV neutralizing antibodies, we could not detect ZIKV genomes in splenic tissues. These serological findings suggest the possibility of ZIKV circulation in NHPs in Zambia.

Further investigation of arbovirus infection was conducted by collecting 3,304 mosquitoes. A pan-flavivirus RT-PCR analysis was performed to identify flavivirus genome in total RNA extracted from the pooled mosquito lysates. Moreover, virus isolation was performed from the filtered lysates followed by next-generation sequencing and rapid-amplification cDNA ends analysis to determine the whole genome sequence of the isolated viruses. Flavivirus RNA was detected in 4/217 pools, and two flaviviruses, a new strain of Barkedji virus (BJV Zambia; 10,899 nt) and a novel flavivirus tentatively named Barkedji-like virus (BJLV; 10,885 nt) were

isolated from *Culex* spp. mosquitoes. Both viruses could replicate effectively in mosquito-derived cell lines. Phylogenetic tree analysis revealed that those viruses belong to the dual-host affiliated insect-specific flavivirus (dISFVs) group.

The secondary structure the of 5'- and 3'-untranslated regions (UTRs) of BJV Zambia and BJLV were analyzed *in silico* to investigate their evolutionary conserved structural RNA. The sequence folding in the secondary structure and consensus structural prediction revealed that the 5'-UTRs structure of both viruses were similar to those of mosquito-borne flaviviruses (MBFVs). The 3'-UTRs of BJV Zambia and BJLV were structurally homolog in the stem loop (SL), dumbbell (DB) and terminal 3'SL of MBFVs. The function of exoribonuclease resistant-RNA (xrRNA) structure which found in BJV Zambia and BJLV 3'-UTRs was investigated using exoribonuclease-1 (Xrn1) degradation assay, and the stop point of Xrn1 degradation was then determined. As the results, the xrRNA structure of BJV Zambia and BJLV blocked RNA degradation by Xrn 1 at the upstream of those structures. These results indicated that BJV Zambia and BJLV may produce subgenomic flavivirus RNA (sfRNA) which can modulate host's immune response.