

## Results report

1. Title of Research and Development: An integrated research for the development of a scheme to control emerging vector-borne viral diseases in Asia
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4. Results of Research and Development:

### Collection and identification of ticks

Manual for collection and identification of ticks were prepared and the methods were shared among each counterparts. In Philippines, Indonesia and Thailand, ticks were collected and genetically identified by sequencing of mitochondrial COI gene. In Indonesia and Thailand, most of collected ticks were *Rhipicephalus microplus* and the other was *Haemaphysalis wellington*. On the other hand, the major specie of ticks was collected in Japan. Especially, in Wakayama and Yamaguchi prefectures, ticks were collected monthly and the number of collected species and stage of ticks were compared by months and regions.

### Detection of viral genes from ticks

Detection of genes of flaviviruses and phleboviruses from ticks in Thailand and Indonesia were performed. Genes of Langkat virus were detected from *Rhipicephalus microplus* in Thailand and the sequence was consisted with those in the previous report. In Japan, a novel flavivirus (Yamaguchi virus), seven novel phleboviruses including severe fever with thrombocytopenia syndrome (SFTS) virus and Kabuto Mountain (KAM) virus, a novel rhabdovirus (NMR virus), and a novel thogoto virus (OZ) were successfully detected from ticks.

### Collection and identification of mosquitoes

Manual for collection and identification of mosquitoes were prepared and the methods were shared among each counterparts. In Philippines, Indonesia and Thailand, mosquitoes were collected and genetically identified by sequencing of mitochondrial COI gene. Many species of mosquitoes including *Culex tritaeniorhynchus*, the vector for Japanese encephalitis virus, and *Aedes albopictus*, the vector for dengue, Zika and Chikungunya viruses, were identified. In Japan, mosquitoes were collected weekly around cowshed. Interestingly, it was confirmed that *Culex tritaeniorhynchus*, the vector for Japanese encephalitis virus, suck blood from cows in early spring. In addition, molecular classification of mosquitoes in each country were performed by sequencing of mitochondrial COI gene.

### Serological survey of arboviruses in animals

We established serological method to detect antibody against SFTS virus, JE virus, tick-borne encephalitis (TBE) virus, OZ virus, KAM virus and NMR virus and examined seroprevalence of these viruses among dogs and cats in Thailand dogs. The results showed that 20% of dogs were infected with JEV and some dogs had antibodies against TBE and SFTS viruses. However, seroprevalence of JEV infection in cats was lower than that in dogs.

### Serological survey of phleboviruses

Expression plasmid of envelope and nucleocapsid proteins of 12 phleboviruses including SFTS virus and Heartland virus are under construction. Serological survey of SFTS virus and KAM virus in wild boars, deer, wild animals and hunters in Japan were performed by ELISA using extracts from virus-infected cells, indicating that SFTS and KAM viruses are spreading at eastern part of Japan and almost entire region of Japan, respectively.

### Virus isolation

For isolation of virus from mosquitoes, *Aedes aegypti* originated cell line, YU-AeAe-1265 cell, was kindly provided by Dr. Kobayashi and the growth of dengue virus-1 to -4 and JE virus were compared. Now, we prepared three cell lines originated from *Aedes aegypti*, *Aedes albopictus* and *Culex tritaeniorhynchus* for virus isolation in Asian country.

### Kick-off meeting and exchange of information

On 25 March, all participants of this project got together from 6 institutes of five countries to Yamaguchi University and Kick-off meeting was held and each information was exchanged. Methods and periods for collection of ticks, mosquitoes and sera from animals were confirmed and plan for further three years was discussed.