

e-ASIA Joint Research Program Progress Report

1. Project Title: 「An integrated research for the development of a scheme to control emerging vector-borne viral diseases in Asia」

2. Joint Research Period: June 1, 2015 ~ May 31, 2019

3. Principal Investigators:

JAPAN: Ken Maeda, Professor, Yamaguchi University

Planned Funding Period: June 1, 2015 - May 31, 2019

Philippines: Emmanuel T. Baltazar, Professor, Central Mindanao University

Planned Funding Period: January 1, 2017 – December 31, 2019

U.S.A.: Hideki Ebihara, Principal Investigator/Unit Chief, NIAID (August, 2016-: Mayo Clinic)

Planned Funding Period: June 1, 2015 –

Indonesia: Srihadi Agungpriyono, Professor, Bogor Agricultural University

In kind

Thailand: Worawut Rerkamnuaychoke, Associate Professor, Kasetsart University

In kind

4. Summary of the Progress of the Joint Research:

In this project, we propose to conduct a comprehensive surveillance of emerging vector-borne diseases in wild animals, arthropods, and livestock in South Asian countries and work towards developing risk assessments of the outbreak potential of vector-borne viruses. Our results obtained in this project are described below.

Mosquito-borne virus surveillance in Asian countries

Mosquito collection were conducted in Japan (Yamaguchi and Oki Island), Thailand (around Bangkok), Philippine (Central Mindanao University), and Indonesia (Java Island, Bogor) from 2014 to 2016 by using mainly sweeping nets or aspirator, and partially by using CDC trap (Japan). *Aedes* mosquitoes were collected in residential area in daytime, and *Culex* mosquitoes were collected in cowshed in night time. Until today, a total of **17,344** female mosquitoes, comprising seven genera and at least **30** species, were collected from four Asian countries. The most abundant genus of collected mosquitoes was *Culex*. In Thailand and Philippine, more than 80% of collected mosquitoes were *Culex*. *Culex* mosquitoes were collected mainly in the cowsheds, and *Aedes* mosquitoes were collected mainly in residential area.

We performed virus isolation from collected mosquitoes. In brief, these collected mosquitoes were sorted into 595 pools with a maximum of 50 mosquitoes per pool, and the pools of mosquitoes were homogenized to prepare the inocula. The mosquito homogenates were inoculated onto monolayers of mosquito C6/36 cell, mammalian Vero and BHK-21 cells, respectively. After at least three blind passages, culture supernatants were collected and checked for viral genomes by RT-PCR or high-throughput sequencing analysis. We successfully obtained several positive for flavivirus from Japan and Thailand, and one positive for reovirus from Indonesia. We determined the sequences of viral genome and conducted phylogenetic analyses. As the results, all of positive samples for flavivirus were belonging to the member of an insect-specific flavivirus group and were divided into three species. Among three flaviviruses, one is a novel insect flavivirus (named Oki flavivirus), and the others are *Culex* flavivirus and Quang Bing virus. We found that one positive sample for reovirus from Indonesia is Banna virus.

Surveillance of Tick-borne virus in Asian countries

- One novel Thogotovirus was isolated from serum of a deer in Japan and was similar to

those isolated from ticks in Kyoto.

- One novel Coltivirus was isolated from brain of a dead raccoon dog in Japan and was similar to Colorado tick fever virus.
- 94 *Rhipicephalus microplus* ticks were collected from the surface of cattle in Thailand. A total of 85 *Rhipicephalus microplus* and 10 *Haemaphysalis welingtoni* ticks were collected from the surface of cattle in Indonesia. As the result of detection of viral RNA of flaviviruses and tick-borne phleboviruses, 3 pools out of 7 pools of *R. microplus* ticks were positive for flaviviruses. Sequence analysis revealed that detected viruses were LGTV.

Surveillance of Tick-borne bacteria from ticks infested lizard in Indonesia

23 *Aponomma lucasi* (21 male, a Female and Nymph) were collected from a Lizard (*Varanus* sp.). In these 23 ticks, 18 live ticks were dissected and prepared for DNA extraction and cultivation using BSK medium.

	Prevalence by PCR	Characterization	Cultivation Salivary gland	Cultivation Midgut
<i>Borrelia</i>	22/23 (96%)	<i>Borrelia</i> sp.	15/18 (83%)	15/15 (100%) ³
<i>Rickettsia</i>	5/23 (22%) ²	<i>R. tamurae</i> like	NT ¹	NT
<i>Ehrlichia/ Anaplasma</i>	4/23 (17%)	<i>Anaplasma</i> spp.	NT	NT

¹Not tested

²Both 17kDa and gltA detected samples were defined as positive for *Rickettsia*.

³Not included contaminated samples.

We also analyzed tick mitochondrial 12S rRNA and 16A rRNA genes for future references of tick molecular identification (fig. 5 and 6).

Serosurveillance of arbovirus infections in Asian countries

Japan

- Seroprevalence of Japanese encephalitis virus and Langat virus infection among wild boars in Japan by ELISA is 39% (262/666) and 26% (172/666), respectively.
- Seroprevalence of Banna virus and Getah virus infections among wild boar in Japan are 0% (0/37) and 15.6% (166/1060), respectively.
- Seroprevalence of Banna virus and Getah virus infections among sika deer in Japan is 0% (0/53) and 0% (0/35), respectively.

Philippines

- Seroprevalence of Japanese encephalitis virus, Langat virus, Zika virus and Dengue virus infections among cattle in Philippine are 26.8% (40/149), 0% (0/148), 2.2% (3/136), 0.7% (1/147), respectively.
- Seroprevalence of Japanese encephalitis virus, Langat virus, Zika virus and Dengue virus infections among dogs in Philippine are 45.2% (19/42), 0% (0/41), 2.4% (1/41) and 0% (0/41), respectively.
- Seroprevalence of Japanese encephalitis virus, Langat virus, Zika virus and Dengue virus infections among water buffalo in Philippine are 33.3% (10/30), 0% (0/31), 3.7% (1/27) and 0% (0/29), respectively.
- Seroprevalence of Japanese encephalitis virus, Langat virus, Zika virus and Dengue virus infections among goats in Philippine are 36.7% (11/30), 0% (0/32), 0% (0/34) and 12.1% (4/33), respectively.
- Seroprevalence of Japanese encephalitis virus, Langat virus, Zika virus and Dengue virus infections among pig in Philippine are 92.3% (12/14), 7.7% (1/13), 0% (0/10) and 0% (0/12), respectively.

Thailand

- Seroprevalence of Japanese encephalitis (JEV) virus and Langat virus (LGTV) infection among cattle in Thailand in 2016 by ELISA is 68% (170/250) and 15% (38/250), respectively.
- Seroprevalence of JEV and DENV-2 among dogs in Thailand in 2016 by 80% plaque reduction neutralization test is 47.2% (109/231) and 18.6% (43/231), respectively. 10

dogs were only seropositive against DENV-2.

- Seroprevalence of JEV and LGTV among cats in Thailand in 2016 by ELISA is 11.3% (18/160) and 5.0% (8/160), respectively.
- Total 113 dogs and 207 cats from 2015 to 2016 in Thailand were examined. One dog was seropositive for SFTSV (0.9%) and no cat (0%) was seropositive.

Development and characterization of mouse model of Severe fever with thrombocytopenia syndrome (U.S.A.)

We have developed the IFNAR^{-/-} mouse model recapitulating fatal diseases with hematologic manifestations similar to human SFTS cases. Using this model, we have also identified the target cell(s) of virus infection, as well as lesions that are potentially associated with hematological changes.

5. Outstanding Results and Achievements (Training, Workshop, Publication, etc, if any):

5-1. Training

- Two and one seminars in Thailand and Indonesia, respectively
- One student from Philippines to Yamaguchi University
- Two researchers from Indonesia to Yamaguchi University
- One researcher and four students from Thailand to Yamaguchi University
- Six, four, and seven researchers from Japan to Philippines, Thailand and Indonesia, respectively

5-2. Publication

Sakai K, Hagiwara K, Omatsu T, Hamasaki C, Kuwata R, Shimoda H, Suzuki K, Endoh D, Nagata N, Nagai M, Katayama Y, Oba M, Kurane I, Saijo M, Morikawa S, Mizutani T*, Maeda K*. Isolation and characterization of a novel rhabdovirus from a wild boar (*Sus scrofa*) in Japan. *Vet Microbiol* 2015. 179(3-4):197-203.

Kuwata R, Sugiyama H, Yonemitsu K, Dung NV, Terada Y, Taniguchi M, Shimoda H, Takano A, Maeda K* Isolation of Japanese encephalitis virus and a novel insect-specific flavivirus from mosquitoes collected in a cowshed in Japan. *Archives of Virology* 2015. 160(9): 2151-2159.

Matsuno K, Orba Y, Maede-White K, Scott D, Feldmann F, Liang M, Ebihara H. Animal Models of Emerging Tick-Borne Phleboviruses: Determining Target Cells in a Lethal Model of SFTSV Infection. *Front Microbiol*. 2017. 8:104.

5-3. Oral Presentation

Four presentations in international symposium and three presentations in conferences

6. Future Goals and Plan of Activities within and after the project period:

Within the project period

- This project will improve our understanding on the mechanisms of evolution, ecological cycle, and emergence/re-emergence of vector-borne viruses in South Asia.

After the project period

- This project will also facilitate the prevention of vector-borne emerging infectious disease in humans and animals in Asia, and it will undoubtedly aid in the development of improved differential diagnostics for these neglected emerging pathogens.
- This collaboration should expand throughout Asia and will provide the foundation for the establishment of strong diagnostic and epidemiological capabilities in Asian countries.

7. Recommendations and Comments to the Program (if any):

(ex. Any support to request from the Program in order to achieve item 6.)

Financial support from all countries will further improve this project.