e-ASIA Joint Research Program
Progress Report

1. Project Title: Dengue viral genetic diversity in selected populations in

2. Joint Research Period: : 1st Aug, 2016 ～ 31st March, 2020

3. Principal Investigators:

- Japan: Moi Meng Ling, Associate Professor, Institute of Tropical Medicine, Nagasaki University
  - Planned Funding Period: Aug, 1st, 2016 – March, 31st, 2020

- Myanmar: Hlaing Myat Thu, Department of Medical Research
  - Planned Funding Period: Aug, 1st, 2016 – March, 31st, 2020 (funding in-kind)

- USA: Sujan Shresta, La Jolla Institute of Allergy and Immunology
  - Planned Funding Period: Aug, 1st, 2016 – March, 31st, 2020 (funding in-kind)

4. Summary of the Progress of the Joint Research:

A) Determination of the virus characteristics of epidemic DENV strains

Determination of DENV serotypes, genotypes, genomic sequences and virological characterization were done for virus strains isolated from the Dengue season, 2017. A total of >200 samples were collected and tested for Dengue virus. During the Dengue season of 2016-2017, a large DENV outbreak occurred in Yangon, Myanmar. Samples were collected from suspected-DENV patients, tested for DENV NS1 antigen by rapid ICT kit. Further laboratory tests were performed to test for anti-DENV IgM and IgG. A total of 70 samples were collected from children (ages 4-12, M=38, F=32), 41 cases with warning signs, 3 samples from severe Dengue and 26 samples from Dengue patients without warning signs. A further 19 samples were collected from adults, 7 with warning signs and 12 without warning signs. Out of the 89 clinical cases, 72 were positive for IgM antibodies and 17 were positive for IgG antibodies. Using the IgM/IgG antibody results, 24 cases were of primary infection and 21 cases were determined to be secondary infection whereas 44 cases was underterminable due to high levels of IgM and IgG antibody. A total of 28 virus was isolated by using C6/36 mosquito cell lines, DENV-1 was isolated from 9 cases, DENV-3 from 10 cases and DENV-4 from 9 cases. DENV-2 was not isolated from any of the cases. Sequence analyses showed that the isolate was of DENV-1 genotype I and DENV-4 genotype I. While reports on DENV-4 as a dominant serotype are limited, our data suggest changing patterns in serotypes during DENV epidemic in Myanmar and Southeast Asia. All samples collected were tested positive for DENV Ag and none were positive for concurrent infection of other arboviruses such as Chikungunya and Zika virus. We have found recent evidence that Zika virus causes sporadic outbreaks in Myanmar (Tun Ngwe et al., 2018).

Severe Dengue cases were found in both primary and secondary DENV infections, currently we are analyzing trends of primary and secondary DENV infection in severe vs non-severe cases. On-going sample collection and DENV sequence study using NGS would determine the virological factors that are associated with disease spread and pathogenesis. Data has been shared with Myanmar counterpart, and the results from this study will be used to develop better control measures against Dengue in Myanmar.

(B) Analysis of DENV quasispecies and host factors associated with DENV infection

Using DENV-1 strains isolated from clinical samples, 2 clinical isolates that displays differential growth characteristics were isolated from a single sample by using mammalian and a mosquito cell line. Analyses of the full genome by using NGS suggest that 2 variants co-exist in a single patient. In the analyses of quasispecies in DENV, we have previously used an NGS system that is based on the IonProton system. Improvements of an NGS system based on Miseq protocol is currently being developed to better analyze quasispecies in clinical specimens. A digital-PCR system to detect DENV quasi-species was also developed to determine the percentages of variants in a single virus population.

Next, using the isolated variants, a variant with single amino acid alteration at the non-structural protein 4B (NS4B) of DENV was found to result in enhanced growth in a human cell line (dendritic iPS-cell). Infection with a variant carrying a mutation of the NS4B region (NS4B-116M/A) also resulted in differential expression of interferon-b and interferon stimulated genes (Bui et al., 2018). These results suggest that variants in a DENV population plays an important role in virus replication and adaptation between hosts (mosquito vs human host). The results from this study will be applied for further studies of differential gene expression in human monocytes and a better understanding on the pathological mechanism of DENV infection.

Technical training for collection and analyses of Dengue patient samples (including PBMC samples) for the staff of Department of Medical Research (Myanmar) are being carried out by USA and Japan counterparts. PBMC collection and analyses are currently ongoing for current Dengue season (2018-2019).

4B. Outputs and Anticipated Outcomes of Joint Research

4B-1 Scientific achievements and implemented activities of the joint research

1. Establishment and improvements of electronic dengue database that includes clinical and virological data
2. Dengue biobank of serum/plasma, virus cultures and viral nucleic acid
3. Analyses of dengue virus factors – DENV cultures, RNA/cDNA, serotype/genotype
4. Analyses of human host factors – anti-DENV antibody and immunological factors
5. Genome sequence analyses of dengue isolates by NGS
6. Better understanding of the epidemiological features of DENV outbreak in the region

4B-2 Synergistic effects of the international joint research

1. Training in laboratory methods and technologies developed in Nagasaki University (Japan counterpart) and in La Jolla Institute of Allergy and Immunology (USA counterpart)
2. Exchange of information and ideas on Dengue disease control in the region
3. Collaboration in preparation of result dissemination to develop better strategies for disease control

4-B3 Broader impacts including contribution to society

1. Data and results from this study will be used as a baseline for implementation on control strategies, including dengue vaccination program, by the relevant ministries in Myanmar
2. Integration of research efforts in both hospital, academic and governmental institutes for Dengue and arboviral disease control

4-B4 Development and sustainability of the cooperation

1. Current exchanges (student and technical staff) and training in Japan and USA will lead to future global leaders that are responsible for further cooperation in Myanmar and Dengue endemic region. Currently, 2 students from Myanmar are enrolled in Nagasaki University for PhD program and will undergo training overseas

2. Japan-Myanmar-USA have expressed willingness to contribute to further research collaboration in the future
3. Strengthening of ties between 3 countries to develop measures against Dengue and other diseases. This will be useful, particularly during emergency outbreaks, because the foundation for exchange has been developed through this eASIA program

5. Scientific Achievements and Implemented Activities (Research Exchange, Workshop, Publication, etc. if any):

For this item, please fill in the attached Excel file.

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

1. Continuation of Dengue virus genome sequencing and analyses to determine the epidemiological characteristics in the region
2. Continuation in the development of a database for Dengue virus sequence and patient data of Myanmar and the region
3. Develop sustainable relationship between research groups in Myanmar and USA to further research work on Dengue and other arboviruses of the region

7. Recommendations and Comments to the Program (if any):
(ex. Any support to request from the Program in order to achieve item 6.)

The eASIA project has offered an excellent platform for exchange between the 3 groups –Japan, Myanmar and USA. Using this platform has brought the 3 groups together to develop a network for information exchange and collaborate on strategies to control infectious diseases of importance in Myanmar and Southeast Asia. Through the eASIA program, we were given the opportunity to train 2 students from Myanmar for their PhD program in Nagasaki University. It will be better that some form of funding could be extended/provided to support the research of these students during their study period in Nagasaki University (up to Sept 2022). This will support both research and their program, and further enhance the research network between the 3 counterparts.

Currently, both research institutions in Myanmar and USA are supporting this research program “in-kind” and partner countries would have to use resources from other funding for both man-power and financial support. In these cases, it would be better if Japan-side could offer flexibility in funding, particularly in lower-middle income countries that would benefit greatly with materials and research equipment.

Lists of Achievements and Implemented Activities

1. Original Publication of Articles etc.

【Notes】
Please fill in **only the achievements of this project** by country in order of publication date. Only "published" is targeted, but please write "in press" too only for Final Report.
Please count Proceedings with peer review as original paper.
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1. 1 Original Publications (Articles co-authored among Research Teams)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)
Taro Kagaku and Jiro Kagaku、Distinct roles of MLCK and ROCK in the regulation of membrane	doi: 10.1083/jcb.201506	in press	
Ngwe Tun MM, Kyaw AK, Hmone SW, Inoue S, Buerano CC, Soe AM, Moi ML, Hayasaka D, Thu HM, Hasebe F, Thant KZ, Morita K. Detection of Zika Virus Infection in Myanmar. Am J Trop Med Hyg. 2018 Mar;98(3):868-871.	10.4269/ajtmh.17-0708	published	Impact factor 2.2
Kyaw AK, Ngwe Tun MM, Moi ML, Nabeshima T, Soe KT, Thwe SM, Myint AA, Maung KTT, Aung W, Hayasaka D, Buerano CC, Thant KZ, Morita K. Clinical, virological and epidemiological characterization of dengue outbreak in Myanmar, 2015. Epidemiol Infect. 2017 Jul;145(9):1886-1897	doi: 10.1017/S0950268817000735	published	Impact factor 2.1

2 Total

1. 2 Original Publications (Articles by Single Team only)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)	Country name of the team
Taro Kagaku and Jiro Kagaku、Distinct roles of MLCK and ROCK in the regulation of membrane	doi: 10.1083/jcb.201506	in press		Thailand
Bui TT, Moi ML, Nabeshima T, Takemura T, Nguyen TT, Nguyen LN, Pham HTT, Nguyen TTT, Manh DH, Dumre SP, Mizukami S, Hirayama K, Tajima S, Le MTQ, Aoyagi K, Hasebe F, Morita K. A single amino acid substitution in the NS4B protein of Dengue virus confers enhanced virus growth and fitness in human cells in vitro through IFN-dependent host response. J Gen	10.1099/jgv.0.001092	in press	Impact factor 2.9	Japan
Moi ML, Takasaki T, Kurane I. Detection of virus-antibody immune complexes in secondary dengue infection. in "Hemorrhagic Fever Viruses: Methods and Protocols", Editor, Maria S Salvato, Springer 2018;1604:331-337. (book chapter)	10.1007/978-1-4939-6981-4_25	published	-	Japan
Ly MHP, Moi ML, Vu TBH, Tun MMN, Saunders T, Nguyen CN, Nguyen AKT, Nguyen HM, Dao TH, Pham DQ, Nguyen TTT, Le TQM, Hasebe F, Morita K.Dengue virus infection-enhancement activity in neutralizing antibodies of healthy adults before dengue season as determined by using Fc γ R-expressing cells. BMC Infect Dis. 2018 Jan 10;18(1):31	10.1186/s12879-017-2894-7	published	Impact factor 2.8	Japan

3 Total

Lists of Achievements and Implemented Activities

2. presentations at Academic Conferences etc. (Seminars, Workshops, Symposia)

【Notes】
Please fill in **only the achievements of this project** by country in order of presentation date.
The information on this form is only disclosable. Please submit Non-disclosable information in a separate file.

2. 1 Conference Presentations (Joint Presentations among Research Teams)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location, etc.
March 4, 2018	Guest/Invited Speaker	Taro Kagaku, "xxx", yyy, Tokyo,
Sept 2017	Oral Presentation	Ngwe Tun MM, Aung KK, Hmone SQ, Inoue S, Buerano CC, Aung MS, Moi ML, Hayasaka D, Hlaing MT, Thant KZ, Hasebe F, Morita K. Detection of Zika virus infection in Myanmar. Toga, pesti, flavivirus Research Meeting, Osaka
Nov 2016	Poster Session	Ngwe Tun Mya Myat, ムタガラ ロヒタ, キョウ アウンキョウ, フェヂナ アヅンゴ, モイ メンリン, 早坂 大輔, 吾郷 昌信, ブエラノ コロゾン, テャン キョウジン, 森田 公一. Differential type I interferon response mediated by RIG-I and MDA-5 in human glioblastoma cells (T98G) following with clinical Chikungunya virus isolate and prototype strain, Japanese Society of Tropical Medicine, Tokyo
Nov 2016	Poster Session	Kyaw Aung Kyaw, Ngwe Tun Mya Myat, Moi Meng Ling, Takeshi Nabeshima, Soe Kyaw Thu, Myint Aye Aye, Daisuke Hayasaka, Buerano Corazon, Thant Kyaw Zin, Kouichi Morita. Clinical, virologic and epidemiologic characterization of Dengue outbreak in Myanmar, 2015. Japanese Society of Tropical Medicine, Tokyo

3 Total

2. 2 Conference Presentations (by Single Team)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location etc.	Country name of the team
March 4, 2018	Guest/Invited Speaker	Taro Kagaku, "xxx", yyy, Tokyo,	Thailand
Oct 2017	Poster Session	Moi ML, Nguyen CT, Wijesooriya SL, Nguyen TTT, Vu TBH, Le TQM, Morita K, Dang DA, Hasebe F. Seroepidemiological surveillance of Zika virus in Vietnam, 2014-2016, Japanese Society of Virology, Osaka	Japan
Oct 2017	Poster Session	Bui TT, Moi ML, Nabeshima T, Pham Hoai LL, Pham Thi H, Dang Thi D, Nguyen Thi TT, Le Thi QM, Morita K, Hasebe F. Single amino acid substitution on NS4B protein of dengue virus increases virus fitness in mammalian cells, Japanese Society of Virology, Osaka	Japan
Oct 2017	Oral Presentation	Bui TT, Moi ML, Hasebe F, Morita K. One-step RT-LAMP for detection of Zika virus. Toga, pesti, flavivirus Research Meeting, Osaka	Japan
Sept 2017	Oral Presentation	Phu Ly MH, Moi ML, Hau VTB, Ngwe Tun MM, Saunders T, Nguyen NC, Nguyen ATK, Nguyne MH, Dao TH, Pha, QD, Nguyen TTT, Le MTQ, Hasebe F, Morita K.Absence of dengue virus infection-enhancement activity in neutralizing antibodies of healthy adults before dengue season as determined by using Fc γ R-expressing cells. Kyushu Society of Virology (Okinawa)	Japan
Sept 2017	Poster Session	Moi ML, Wijesooriya SL, Nguyen CT, Nguyen TTT, Vu TBH, Tun Ngwe MM, Pha, TD, Pham T, Tran T, Le TQM, Dang DA, Hasebe F, Morita K. Zika virus infection and microencephaly in Vietnam, 2014-2016. The 16th Awaji International Forum on Infection and Immunity, Hyogo	Japan
Sept 2017	Guest/Invited Speaker	Moi ML. 古くて新しい～節足動物媒介性感染症～ジカウイルスの世界的な流行と最近の知見(招待講演). 第160回日本獣医学会学術集会.(鹿児島)	Japan
May 2017	Poster Session	Luz MA, Moi ML, Dimamay MT, Nabeshima T, Pangilinan LA, Dimamay MP, Matias R, Buerano C, Tria E, Natividad F, Daroy ML, Hasebe F, Morita K. Virological characterization of DENV circulating in Metro Manila, 2015-2016. The 13th Nagasaki-Singapore Medical Symposium.	Japan
May 2017	Poster Session	Nguyen CT, Moi ML, Wijesooriya SL, Tun Ngwe MM, Nguyen TTT, Hau VTB, Pha, TTH, Le TQM, Hasebe F, Morita K. Serological surveillance of Zika virus in Central Vietnam, 2016-2017. The 13th Nagasaki-Singapore Medical	Japan
May 2017	Poster Session	Wijesooriya SL, Moi ML, Nguyen CT, Inoue S, Nguyen TTT, Hau VTB, Pham TTH, Le TQM, Morita K, Hasebe F. Seroepidemiological survey for Zika virus antibodies in febrile patients, Central and North Vietnam, 2014-2015. The 13th Nagasaki-Singapore Medical Symposium.	Japan
May 2017	Poster Session	Phu Ly MH, Moi ML, Takamatsu Y, Nabeshima T, Pham HLL, Pham TH, Dang TD, Nguyen NL, Nguyen TTT, Le TQM, Buerano CC, Morita K, Hasebe F. Neurotropic characteristics of dengue serotype 3 virus isolated from a dengue encephalitis patient in Viet Nam. The 13th Nagasaki-Singapore Medical Symposium.	Japan
May 2017	Poster Session	Bui TT, Moi ML, Nabeshima T, Pham HLL, Pham TH, Dang TD, Nguyen NL, Nguyen TTT, Le TQM, Morita K, Hasebe F. Dengue viral genetic diversity in selected dengue patients. The 13th Nagasaki-Singapore Medical Symposium.	Japan

11 Total

Lists of Achievements and Implemented Activities

3. Workshops, Seminars, Symposia and Other Events （Organized by the Project）

【Notes】
Please fill in **only the achievements of this project** in order of event date.
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Event duration	Name of Organizer	Title of the Event	Location (Country, City, Venue)	Number of Participants (Including Team Members)	Overview
Mar 4-16, 2018	Taro Yamada	○○○○	Germany、Hamburg、○○○○	10	
Jan, 22-23 2017	Moi Meng Ling	eASIA JRP Kickoff Meeting	Nagasaki, Japan, Nagasaki University	20	
Mar 13-14 2017	Hlaing Myat Thu	Analyses of Genetic Diversity of Dengue Virus, Myanmar: Research Discussion	Yangon, Myanmar, Department of Medical R	15	
June 6, 2017	Hlaing Myat Thu	Analyses of Genetic Diversity of Dengue Virus, Myanmar: Research Discussion	Yangon, Myanmar, Department of Medical R	8	

3 Total

Lists of Achievements and Implemented Activities

4. Record of Research Exchanges

【Notes】
Please fill in the record of **resaerch exchange only of this project**.
"Duration of exchange" is not the number of days stayed on the site, but the number of days from departure to return home.
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Date of Departure	Date of Return	Last Name & First Name	Country of Affiliation	Affiliation	Position	Exchange Destination (Country, City, Research Organization etc)	Description of Exchange Content/Purpose	Duration of Exchange (autocompleted)
January 6, 2016	January 16, 2016	Taro Yamada	Japan	Yamada University	Professor	NANOTEC,NECTEC, Bangkok	○○	11
H29.1.21	H29.1.25	Sujan Shresta	USA	La Jolla Institute of Allergy	Associate Professor	Nagasaki, Japan	eASIA JRP Kick-off Meeting	5
H29.1.21	H29.1.25	Kyaw Zin Thant	Myanmar	Department of Medical	Director General	Nagasaki, Japan	eASIA JRP Kick-off Meeting	5
H29.1.21	H29.1.25	Hlaing Myat Thu	Myanmar	Department of Medical	Deputy Director	Nagasaki, Japan	eASIA JRP Kick-off Meeting	5
H29.3.12	H29.3.14	Moi Meng Ling	Japan	Nagasaki University	Associate Professor	Yangon, Myanmar	Research discussion, IRB meeting	3
H29.6.4	H29.6.9	Moi Meng Ling	Japan	Nagasaki University	Associate Professor	Yangon, Myanmar	Research discussion, technical exchange	6
H29.7.10	H29.7.14	Aung Min Soe	Myanmar	Department of Medical	Researcher	Nagasaki, Japan	Research discussion, technical exchange	5
H29.11.6	H29.11.10	Moi Meng Ling	Japan	Nagasaki University	Associate Professor	Yangon, Myanmar	Research discussion, technical exchange	5
H30.6.9	H30.6.14	Khine Saw Nwe	Myanmar	Department of Medical	Researcher	Nagasaki, Japan	Research discussion, technical exchange	6
								0
					Total (Person)	8	Total (Persond-day)	40

Lists of Achievements and Implemented Activities

5. Patent Applications

【Notes】
Please fill in **only the achievements of this project** by country in order of presentation date.
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5. 1 Independent Applications by Single Team

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)	Country Name of the Team
WO20xx-xxxxxx		January 21, 2016	○○ Univ、Univ.of xx	WO/2016/xxxxxx	○○○○、○○・○○	PCT	WO20xx-xxxxxx (20xx.xx.xx)	Thailand

0 Total (Number of Application)

0 Total (Number of Registration)

5. 2 Joint Applications

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)
WO20xx-xxxxxx		January 21, 2016	○○ Univ、Univ.of xx	WO/2016/xxxxxx	○○○○、○○・○○	PCT	WO20xx-xxxxxx (20xx.xx.xx)

0 Total (Number of Application)

0 Total (Number of Registration)

Lists of Achievements and Implemented Activities

6. Awards

【Notes】
Please fill in **only the achievements of this project** by country in order of date of Award.
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Date of Award	Name of Award	Recipient	Remarks	Country Name of the Team
December 24, 2015	○○ Prize	Taro Yamada		Thailand
Feb 2018	Nagasaki University Doumonkai, Best Paper Award	Moi Meng Ling		Japan
Feb 2018	Nagasaki University Habataku Female Researcher Award	Moi Meng Ling		Japan

2 Total