e-ASIA Joint Research Program
Final Report

1. Project title: **"Comprehensive study on virus quasi-species and vascular permeability factors in severe dengue infection in humans for innovative epidemic and clinical managements"**

2. Joint Research period :

Japan Team January 1, 2014 – March 31, 2017
Philippine Team January 1, 2015 – December 31, 2017
Vietnam Team January 1, 2014 – December 31, 2016

3. Research Team :

Japan Team
Funding period: April 1, 2014 – March 31, 2017
Total Funded Amount (JPY): 46,260,000

Japan Team	Name	Position	Affiliation	Role in the project
PI	Futoshi Hasebe	Professor	Institute of Tropical Medicine, Nagasaki University	Conception of the project, project implementation and, report writing
Collaborator	Kouichi Morita	Professor	Institute of Tropical Medicine, Nagasaki University	Conception of the project, virological analysis
Collaborator	Meng Ling Moi	Associate Professor	Institute of Tropical Medicine, Nagasaki University	Serologic and molecular analysis
Collaborator	Corazon C Buerano	Visiting Professor	Institute of Tropical Medicine, Nagasaki University	Virus isolation and detection, gene sequence and analysis
Collaborator	Takeshi Nabeshima	Assistant Professor	Institute of Tropical Medicine, Nagasaki University	Viral genome analysis using next generation sequencer
Collaborator	Shigeru Tajima	Chief Scientist	Institute of Tropical Medicine, Nagasaki University	Design and construction of recombinant dengue viruses.
Total number of participants including students: 14				

Philippine Team
Funding period: January 1, 2015 – December 31, 2017
Total Funded Amount (PhP): 14,762,100.10

Philippine Team	Name	Position	Affiliation	Role in the project
Program Coordinator	Filipinas F. Natividad, PhD/ Mark Pierre S. Dimamay, PhD	Retired Head/ Associate Director	St. Luke's Medical Center (SLMC)	International and national Linkages
Project leader	Maria Luisa G. Daroy, RCh, MS, DPAM	Scientist	SLMC	Project implementation and overall supervision; report-writing
Project consultant	Maria Terrese A. Dimamay, PhD	Scientist	SLMC	Supervision of laboratory experiments, technical training and vascular permeability experiments; report writing
Project consultant	Ronald R. Matias, PhD	Sr. Res. Scientist and Director	SLMC & United Laboratories, Inc.	Protocol development and laboratory supervision
Epidemiologist	Cynthia A. Mapua, MSPH	Associate Director	SLMC	Database development and data analysis
Consultant doctor	Edith S. Tria, MD	Head	External Affairs, San Lazaro Hospital	Coordination of patient recruitment, sample and data collection
Total number of participants:14-16				

Vietnam Team
Funding period: January 1, 2014 – December 31, 2016
Total Funded Amount (VND): 2,780,000,000

	Name	Position	Affiliation	Role in the project
PI	Le Thi Quynh Mai	Vice Director	National Institute of Hygiene and Epidemiology (NIHE)	Project implementation and overall supervision
Collaborator	Nguyen Thi Thu Thuy	Chief	Arbovirus Laboratory, NIHE	Serologic analysis, virus isolation
Collaborator	Nguyen Thi Nam Lien	Head	Department of Microbiology, Hue Central Hospital	Coordination of patient recruitment,, sample and data collection
Collaborator	Nguyen Kim Phuong	Head	Department of Microbiology, 108 Military Hospital	Coordination of patient recruitment, sample and data collection
Collaborator	Le Thanh Nhan	Head	Department of International Research Collaboration and Education, Nhidong Hospital No.1	Coordination of patient recruitment, sample and data collection
Collaborator	Nguyen Nhat Cam	Director	Hanoi Preventive Medicine Center	Coordination of patient recruitment, sample and data collection
Total number of participants including students: 20				

4. Summary of the joint research (*up to 4 pages for section 4. to 6. including figures. Please note that information described in this report should only be disclosable.*)

The research outputs are summarized as follows:

A. Determination of the virus serotype, genotype and genomic sequences of epidemic DEN strains in the Philippines and Vietnam

In Vietnam, determination of DENV serotypes, genotypes and genomic sequences and virological characterization were done for the virus strains isolated during the 3 year period of research (Table 1) and also for DENV from serum samples collected as far back as 2008.

Table 1. Detection of DENV infection, identification of serotypes and virus isolation in samples from DEN suspected patients in central provinces in Vietnam.

	No. of serum samples	NS1 (+)	Virus detection Results of Real time PCR / Virus isolation			
			DENV-1	DENV-2	DENV-3	DENV-4
2014	953	ND*	ND/ C-57**, V-33***	ND/ C-16, V-5	ND/ C-16, V-17	ND/ C-83, V-4
2015	911	207	36 / C-4, B-3****	1 / C-1, B-1	27 / C-16, B-13	21 / C-10, B-2
2016	1736	418	107 / C-9, B-21	6 / C-2, B-1	0 / 0	68 / C-19, B-6
Total	3600	625	143/ C-70, V-33, B-24	7/ C-19, V-5, B-2	27/ C-32, V-17, B-13	89/C-112, V-4, B-8

*ND: not done, **C: number of virus isolates in C6/36 cell line, ***V: no. of virus isolates in Vero cell line, ****B: no. of virus isolates in BHK Fcγ cell line

The outputs of the work were published as listed in Annex 1. We would like to mention that in 2013, a large DENV outbreak occurred with 204,661 clinical cases in central Vietnam. During the dengue season, September–December 2013, a total of 1532 collected blood samples were screened by dengue NS1 Ag ELISA in Hue central hospital. Out of the 702 samples positive by NS1 Ag ELISA, 501 samples were positive by serotype specific real time – reverse transcription (RT)-PCR method. As a result, DENV-4 was the dominant serotype (245 cases, 48.9%), followed by DENV-1 (141 cases, 28.1%), DENV-3 (63 cases, 12.6%) and the last DENV-2 (52 cases, 10.4%) (Takamatsu Y, et al. J. Clin Virol 66; May 2015,24-26). None of dengue negative specimens showed Chikungunya and other flavivirus such as Zika virus positive by RT-PCR (data not shown).

We reported a case of dengue encephalitis caused by DENV3 genotype III in a male patient with atypical symptoms of DENV infection in Hai Phong, Vietnam in 2013. The virus isolated from the cerebrospinal fluid of this case-patient was closely related

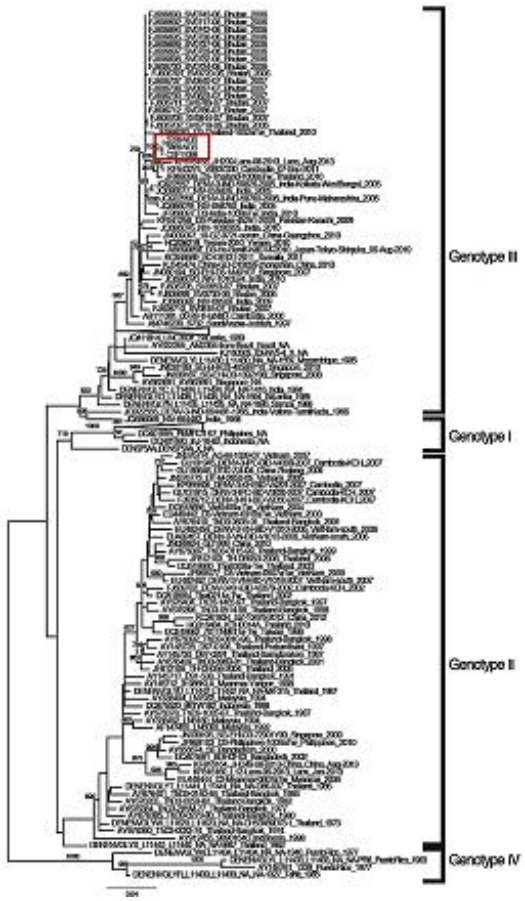

Fig.1 Phylogenetic tree of DENV-3

to DENV3 genotype III strains isolated from serum of two other patients, who manifested classical dengue in the same year and residing in the same area as the case-patient. It is noteworthy to mention that in 2013, DENV3 genotype III was detected for the first time in Vietnam (Minh Huong Phu Ly et. al. J. Clin Virol 2015 Sep;70:93-96) (Fig.1). Currently, the virological and biological characteristics of the CSF and serum isolates are being determined by using nucleotide sequence and virological analyses.

The Philippine team counterpart received the allocated grants starting from fiscal year (FY) 2015. Thereafter, 1,745 sera were collected from 1,290 dengue-suspected patients between 2015 -2017 in Manila, Philippines. 893 were enrolled as outpatients and 397 as inpatients. Virus isolation and virological characterization of these samples was performed. Below are some outputs (Tables 2 and 3):

Table-2. Laboratory test results of samples for Filipino outpatients enrolled in e-ASIA JRP

Year	Number of samples collected	Anti-DENV IgM (ELISA)	Realtime RT-PCR (Alm et al. 2014, serum &/or ICF)
2015	437	82/435	178/431
2016	256	23/256	86/255
2017	200	1/155	56/200
Total	893	106/846	320/886

379 samples were DENV(+) by realtime RT-PCR, with the most prevalent serotype being DENV-1 (34%) in 2015, DENV-3 (40% and 64.3%) in 2016 and 2017. Four specimens from 2017 showed co-infection with DENV-1&3. 98 (25.9%) specimens were unserotypeable using both conventional and realtime RT-PCR methods.

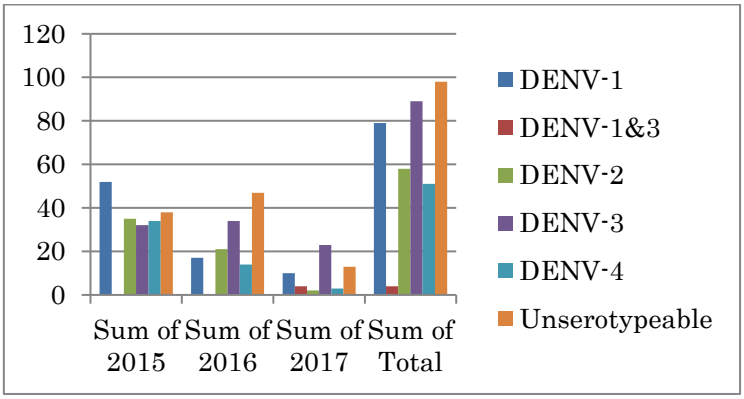

Fig. 2 Distribution of DENV serotypes in 2015-2017 in two Metro Manila hospitals.

Table-3. Distribution of dengue serotypes in the Philippines by using realtime RT-PCR.

Serotype	**2015**	**2016**	**2017**	**Total (%)**
DENV-1	52	17	10	79 (20.8)
DENV-2	35	21	2	58 (15.3)
DENV-3	32	34	23	89 (23.5)
DENV-4	34	14	3	51 (13.5)
DENV-1&3	0	0	4	4 (1.06)
Unserotypeable	38	47	13	98 (25.9)
Total	191	133	55	379

As of December 2016, a total of 678 serum and plasma samples were sent from the Philippines as part of the e-Asia JRP project in NEKKEN. These samples included outpatient and inpatient samples from 537 individual patients. From IgM and IgG capture ELISA experiments, 62.3% of outpatient samples and 45.7% of inpatient samples were found to be of secondary infection. The serotype distribution of DENV in these samples was 20.7% (n=29) DENV1, 20.0% (n=28) DENV2, 18.6% (n=26) DENV3, and 18.6% (n=26) DENV4 with one sample having coinfection from DENV3 and DENV4. Thirty (30) of the samples were unserotypeable. Interestingly, there have been an increase in the percentage of DENV4 patients. Phylogenetic analyses of the envelope region of the DENV4 demonstrated that the isolated DENV4 belongs to genotype II, and is closely related to those DENV4 strains circulating in South East Asia. DENV4 isolated from the Philippines demonstrated a mixture of 2 different strains with mutations in the E-protein hinge region Thr-58-Ile. Further studies would be needed to determine the role of the mutations in the changing epidemic DENV patterns in the Philippines.

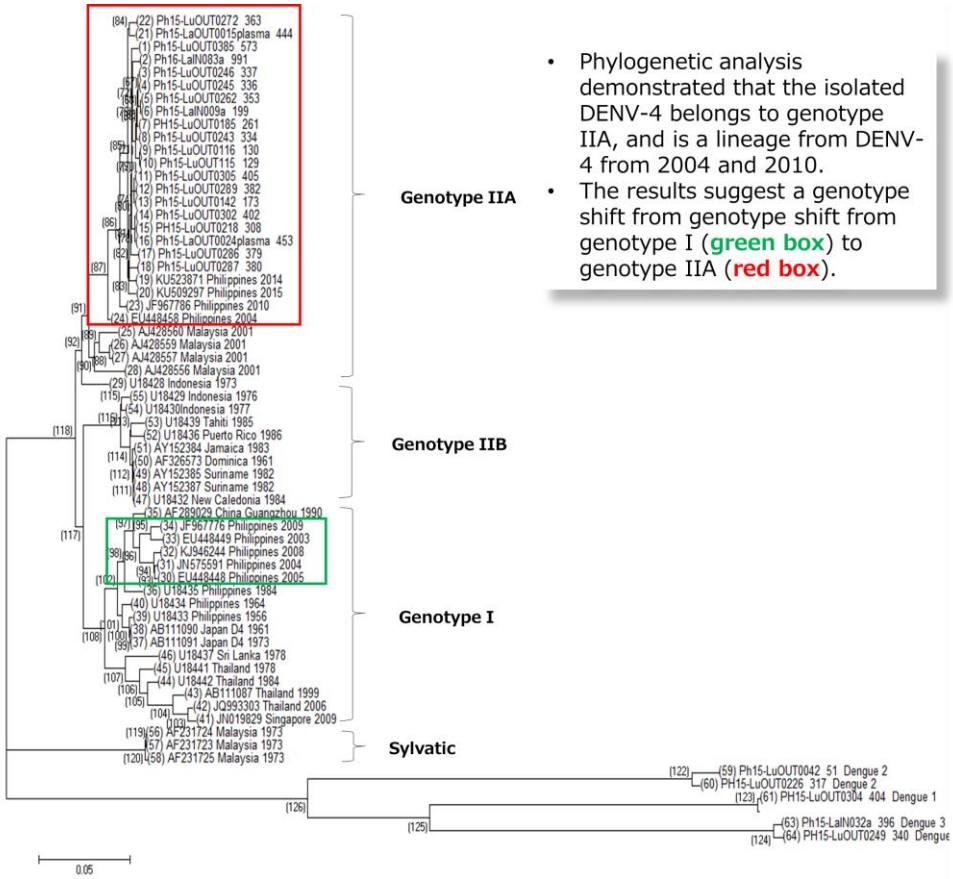

Fig. 3 Phylogenetic tree based on the envelope gene sequence of selected DENV-4 isolates

B. **Analysis of DENV quasispecies and factors associated with vascular permeability to determine the mechanism of severe dengue**

In FY2014, amino acid mutations of the NS4B region, which are hypothesized to be associated with host adaptability in mosquito (C6/36) and mammalian cells (Vero) in DENV-1 was determined. Using the limited dilution method, 3 phenotypes of DENV quasispecies were successfully isolated from a single patient. Additionally, by using a DENV-1 infectious plasmid clone with same mutations introduced into the NS4B region, studies to determine the biological functions of the virus protein are currently being performed.

Clinical samples (blood and or ascites or pleural effusions) were obtained starting August 2015 from patients at Children Hospital No. 1 (Ho Chi Minh, Southern Vietnam) where annually, a high number of severe dengue patients are treated. Clinical samples were

obtained from 154 patients with different degrees of disease severity and the samples were analyzed for correlation with disease severity. All specimens were serologically analyzed to differentiate primary from secondary infection and tested by real time RT-PCR for dengue virus serotyping. Seventy three out of 154 samples were found positive by real time RT-PCR (DENV-1: 35, DENV-2: 15, DENV-3: 2, DENV-4: 20, DENV-1&2 mix infection: 1). Correlation between disease severity and infection status (primary or secondary infection) and dengue virus serotype were determined (Fig.2, Fig.3). Unexpectedly, many severe dengue cases were found in primary infection (34%). DENV-2 might be more virulent compare with other serotypes.

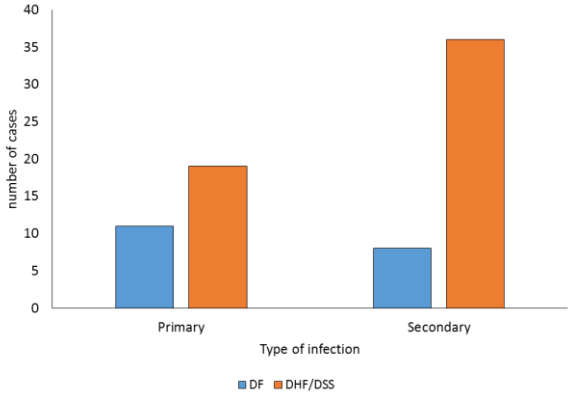

Fig.2 Disease severity and infection status.

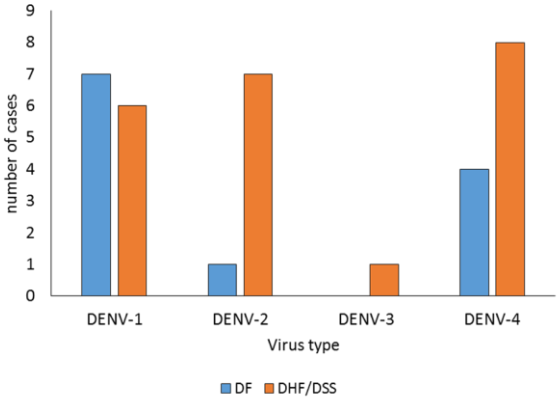

Fig.3 Disease severity and dengue virus serotype.

All real time RT-PCR positive specimens were directly analyzed by next generation sequencer, Ion Proton, and subjected to virus isolation using different cell lines. Only 1 virus strain (No.69) was isolated from a DSS patient. Four mixed infection cases were found by analysis using Ion Proton (No.49 & 69: DENV-1 &4, No.176: DENV-2 & 4, No.204: DENV-1 & 2) (Fig.4). Several sequence variants were found in DENV-1 and DENV-2 (Fig.5). For the determination of correlation between disease severity and sequence variants there is a need to analyze samples from more severe dengue cases.

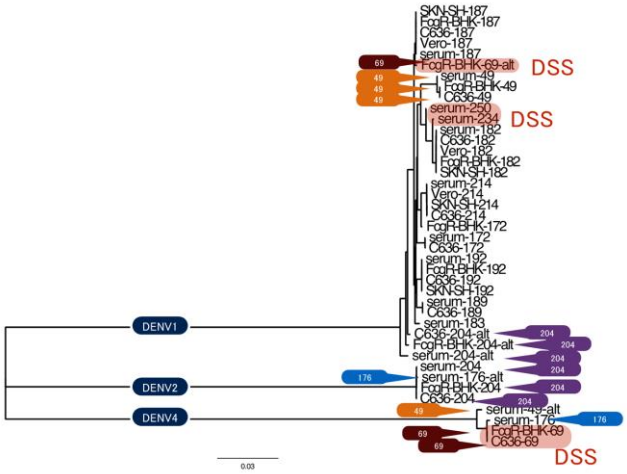

Fig.4 Phylogenetic tree of DENV strains from serum samples and from the viruses isolated after each serum was inoculated in arthropod (C6/36 cells) and mammalian (FcgR BHK cells, Vero and SKN-SH) cell lines. DSS= dengue shock syndrome, the rest of the samples came from patients with dengue fever.

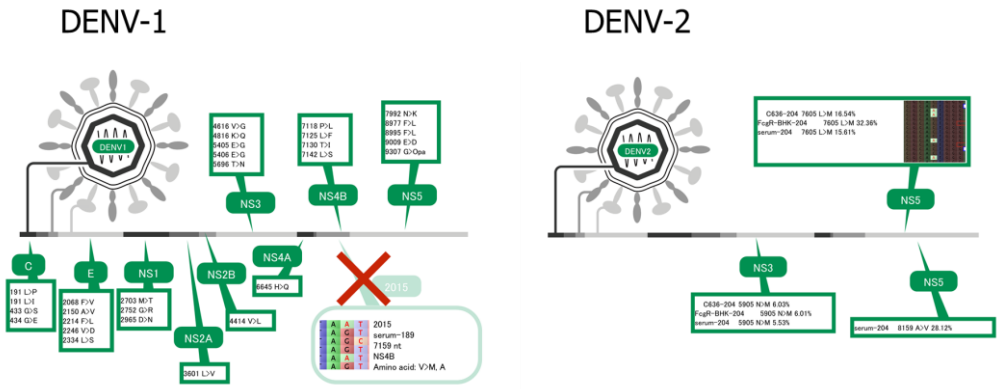

Fig.5 Quasivirus variations found in DENV-1 and DENV-2.

On the Philippine side, inpatient samples were used to study the mechanism of vascular permeability and molecular factors associated with it. Current results showed a significant increase in the in vitro vascular permeability ($P \leq 0.05$) during the acute phase of DV infection, which gradually decreased to baseline levels during the critical and convalescence phases (Fig. 6A and B) A delay in onset of vascular permeability was seen among patients with warning signs versus patients without warning signs. Interestingly, the kinetics of transforming growth factor beta (TGF-beta) showed a significantly decreased level during the acute phase and gradually increased to normal baseline levels at the critical and convalescence phases ($P \leq 0.0001$). Significantly lower concentrations were found among the patients with warning signs versus those without warning signs during the acute and critical phases of DV infection (data not shown). In this study, we believe that TGF-beta and possibly other molecular host factors play an important role in the immune mechanism of increased vascular permeability in severe DV infection, as indicated by our inhibition assay *in vitro* (data not shown).

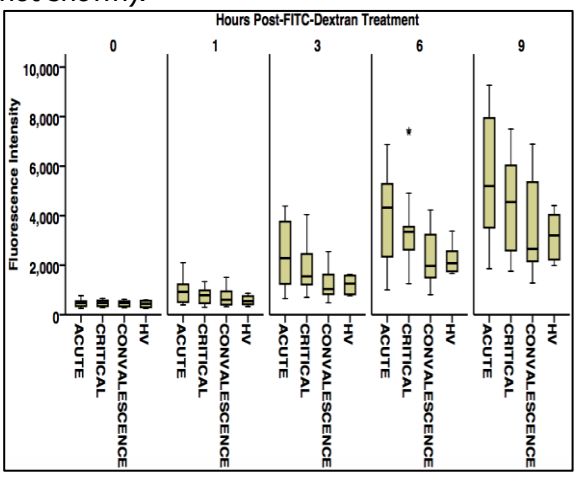

Fig. 6A. In vitro vascular permeability result of dengue patients with warning signs (n=13). HV V – healthy voluntee

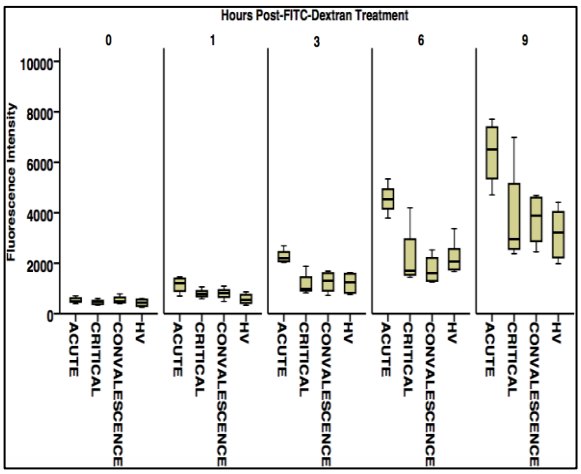

Fig. 6B. In vitro vascular permeability result of dengue patients without warning signs (n=4). HV – healthy volunteers

C. Determination of DENV antibody neutralizing titer to predict DENV epidemics

In collaboration with the Preventive Medicine Center, Hanoi, samples from 100 residents (healthy individuals) were collected before and after the 2015 outbreak in Hanoi, and the levels of anti-DENV IgM and IgG antibodies were determined by using an in-house ELISA assay. The antibody seroconversion rates, and data on primary and secondary infection among the residents of Hanoi have been obtained. The serum samples were sent to the Institute of Tropical Medicine, Nagasaki University for further analyses on the levels of neutralizing antibodies by using the Fc gamma R-

expressing BHK cells. We were able to establish PRNT for all serotypes of dengue viruses and also for Zika virus. We faced Zika virus outbreak in Vietnam and the Philippines in 2016. Dengue viruses and Zika virus belong to the same family Flaviviridae, therefore it is difficult to differentiate by using ordinary serologic tests such as IF test, IgG-ELISA and IgM-capture ELISA. However we can detect and titrate each virus specific antibody by PRNT method. The first Zika virus infection case and microencephaly cases caused by Zika virus infection in Vietnam were identified by our study members. The introduction of PRNT method was very significant for research on flavivirus infections, especially dengue or Japanese encephalitis in endemic countries.

5. Outputs and Anticipated Outcomes of Joint Research

5-1 Scientific achievements and implemented activities of the joint research

1. Establishment of electronic dengue database
2. Dengue biobank of serum/plasma, virus cultures, and viral nucleic acids
3. Analyses of dengue virus factors – DENV cultures, RNA/cDNA, serotype/genotype
4. Analyses of human host factors – anti-DENV IgM, vascular permeability, cytokines
5. Complete genome sequences of dengue virus isolates by NGS
6. Development of molecular protocols: real-time RT-PCR, vascular permeability assay, modified PRNT, NGS & bioinformatics

5-2 Synergistic effects of the international joint research

1. Training in advanced technologies and protocols
2. Exchange of information and ideas on dengue epidemiology of national and regional significance
3. Close collaboration in preparation of results for publication and presentation.

5-3 Broader impacts including contribution to society

1. Data gathered provide baseline information for implementation of dengue vaccination program by Philippine government
2. Integration of research efforts with other dengue projects within the group and with other groups.

5-4 Development and sustainability of the cooperation

1. Japan (Nagasaki University), Philippines (SLMC, PCHRD) and Vietnam have expressed willingness to continue collaborative research on dengue.

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

1. Continuation of research focus on viral and host factors of dengue severity
2. Whole RNA genome sequencing of dengue virus isolates (virome?)
3. Development of rapid diagnostics for dengue
4. Molecular epidemiology and biostatistics for *in silico* modelling

7. Recommendations and Comments to the Program

It is indeed a very good opportunity to study dengue virus infection among the 3 the countries–Japan, Philippines and Vietnam. We could make a network for the exchange of information and specimens and collaborate research work on infectious diseases. The study period is not long enough, however we got 3 government-financed foreign students–1 student from Philippines and 2 students from Vietnam–to help us in the project. Therefore, we are still able to continue to work on the study subjects. We are aware that at least for one recent e-ASIA JRP project between Japan and two other foreign countries, financial funding is from Japan side only and the other partner countries do not provide cash but only manpower and support in kind. So some budget in Japan side should be flexible for use especially for the arrangement of sample collection.

Annex: List of Scientific Achievements and Implemented Activities of the Joint Research

(*Please lists only achievements that are relevant to e-ASIA JRP.*)

1 Original Publications (All Authors' Names, Title, Journal Name, Volume, Page, Year, DOI)

1.1-1 Ngwe Tun MM, Muthugala RV, Thuy NT, Ly PH, Thu LT, Dinh DT, Hoang NV, Mai LT, Moi ML, Buerano CC, Morita K, Hasebe F. “Dengue-associated acute encephalitis syndrome cases in Son La Province, Vietnam.” Jpn J Infect Dis. 2016 Oct 31. [Epub ahead of print]

1.1-2 Minh Huong Phu Ly, Yuki Takamatsu, Takeshi Nabeshima, Linh Ly Pham Hoai, Hang Pham Thi, Dinh Dang Thi, Ngoc Linh Nguyen, Thu Thuy Nguyen Thi, Quynh Mai Le Thi, Corazon C. Buerano, Kouichi Morita,Futoshi Hasebe. “Isolation of dengue serotype 3 virus from the cerebrospinal fluid of an encephalitis patient in Hai Phong, Vietnam in 2013.” J. Clin Virol 2015 Sep;70:93-96. doi: 10.1016/j.jcv.2015.07.295. Epub 2015 Jul 17

1.1-3 Kwoon Yong Pok, Raynal C Squires, Li Kiang Tan, Tomohiko Takasaki, Sazaly Abubakar, Futoshi Hasebe, Jeffrey Partridge, Chin Kei Lee, Janice Lo, John Askov, Lee Ching Ng and Frank Konings. “First round of external quality assessment of dengue diagnostics in the WHO Western Pacific Region, 2013.” Western Pac Surveil Response J. 2015 Jun 30;6(2):73-81. doi:10.5365/ WPSAR.2015.6.1.017. eCollection 2015 Apr-Jun.

1.1-4 Takamatsu Y, Nabeshima T, Nguyen Thi Thu Thuy, Dang Thi Dinh, Pham Hoai Linh Ly, Pham Thi Hang, Nguyen Thi Nam Lien, Phu Ly Minh Huong, Bui Thu Thuy, Le Thi Quynh Mai, Morita K, Hasebe F. “A dengue virus serotype 4-dominated outbreak in central Vietnam, 2013.” J. Clin Virol 66; May 2015,24-26.

1.1-5 Tsuzuki A, Duoc VT, Sunahara T, Suzuki M, Le NH, Higa Y, Yoshida LM, Hasebe F, Phong TV, Minakawa N. “Possible association between recent migration and hospitalization for dengue in an urban population: a prospective case-control study in northern Vietnam.” Trop Biomed. 2014 Dec;31(4):698-708.

1.1-6 Nguyen Thi Thu Thuy, Le Thi Quynh Mai, Dang Thi Dinh, Pham Hoai Linh Ly, Nguyen Ngoc Linh, Vu Trong Duoc, Futoshi Hasebe. “CHARACTERIZATION OF DENGUE 1 EPIDEMIC STRAINS PROLIFERATED IN HANOI, VIET NAM, BETWEEN 2008 AND 2009.” Vietnam Journal of Preventive Medicine, Vol. XXV, Issue 8(168) 2015.

1.1-7 Nguyen Thi Thu Thuy, Nguyen Ngoc Linh, Dang Thi Dinh, Le Thi Hien Thu, Le Thi Quynh Mai, Nguyen Nhat Cam, Futoshi Hasebe. “DETECTION CAPACITY OF DENGUE ACUTE INFECTION WITH METHODS BY NIHE, 2014.” Vietnam Journal of Preventive Medicine, Vol. XXV, Issue 8(168) 2015.

1.2-1 長谷部太「蚊媒介性ウイルス感染　ーデング熱・日本脳炎・チクングニア熱ー」「科学療法の領域」2015 年 5 月号（2015 年 4 月 25 日発行）特集「野外活動と感染症」
1.2-2 長谷部太、西條政幸、高田礼人、沢辺京子「感染症は一国の問題ではない。～エボラ出血熱、デング熱を例として～」　2015 年 2 月 16 日　公益社団法人国際校正事業団　初版発行　監修
1.2-3 長谷部太 「デング熱、日本脳炎、ウエストナイル熱」　臨床と微生物　話題の新興・再興感染症　Vol. 41, No.1, 2014: 309-315.
1.2-4 Nguyen Thi Thu Thuy, Nguyen Ngoc Linh, Pham Do Quyen, Pham Thi Thu Hang, Le Thi Hien Thu, Le Thi Quynh Mai. “DENGUE SITUATION IN HANOI, VIETNA, BETWEEN 2000 AND 2015.” Vietnam Journal of Preventive Medicine, Issue 10 (183) 2016.

2 Presentations at conferences (Speaker, Title, Conference Name, Location, Date, Type of Presentation, etc.)

2.1-1 Le Thi Quynh Mai, Report on the dengue situation in Vietnam, e-ASIA JRP Dengue Project Kickoff Meeting, Ryoujyun Hall (Nagasaki University), 09 May 2014, Oral presentation.
2.1-2 Maria Luisa G. Daroy and Filipinas F. Natividad, Report on the dengue situation in the Philippines, Ryoujyun Hall (Nagasaki University), 09 May 2014, Oral presentation.
2.1-3 Takeshi Nabeshima, Application of the next generation sequencer, e-ASIA JRP Dengue Project Kickoff Meeting, Ryoujyun Hall (Nagasaki University), 09 May 2014, Oral presentation.
2.1-4 Corazon C. Buerano, Vascular permeability assay, e-ASIA JRP Dengue Project Kickoff Meeting, Ryoujyun Hall (Nagasaki University), 09 May 2014, Oral presentation.
2.1-5 Futoshi Hasebe. Dengue and Dengue Research in Vietnam. Scientific Workshop to Explore e-ASIA Research Collaboration Opportunities Focused on Emerging Infectious Disease and Cancer priorities in South East Asia and Pacific Rim (Convened in Conjunction with the e-ASIA JRP Board Meeting) Inya Lake Hotel, Yangon, Myanmar, 13-14 August 2015, Oral presentation.
2.1-6 Phu Ly Minh Huong, Yuki Takamatsu, Takeshi Nabeshima, Pham Hoai Linh Ly, Pham Thi Hang, Dang Thi Dinh, Nguyen Ngoc Linh, Nguyen Thi Thu Thuy, Le Thi Quynh Mai, Corazon C. Buerano, Kouichi Morita and, Futoshi Hasebe. In vitro growth and pathogenicity characterization of a dengue serotype 3 virus from the cerebrospinal fluid of a dengue encephalitis patient. The 52nd Kyushu Branch Meeting of the Japanese Society for Virology. Beppu International Convention Center, B-ConPlaza. 4-5 September 2015, Oral presentation.
2.1-7 Le Thi Quynh Mai, Country report–Vietnam, e-ASIA JRP Dengue Project Annual Meeting, St. Luke’s Medical Center, (Quezon City Philippines), 16 November 2015, Oral presentation.
2.1-8 Eugene Lopez, Country report–Philippines, e-ASIA JRP Dengue Project Annual Meeting, St. Luke’s Medical Center, (Quezon City Philippines), 16 November 2015, Oral presentation.
2.1-9 Meng Ling Moi, Overview of e-Asia research project in Japan, , e-ASIA JRP Dengue Project Annual Meeting, St. Luke’s Medical Center, (Quezon City Philippines), 16 November 2015, Oral presentation by Skype.
2.1-10 Takeshi, Nabeshima, Srategies for the investigation of viral diseases with next generation sequencer, e-ASIA JRP Dengue Project Annual Meeting, St. Luke’s Medical Center, (Quezon City Philippines), 16 November 2015, Oral presentation by Skype.
2.1-11 Satoshi Shimada, Clinical experience in Ho Chi Minh City, Vietnam 2015, e-ASIA JRP Dengue Project Annual Meeting, St. Luke’s Medical Center (Quezon City Philippines), 16 November 2015, Oral presentation by Skype.
2.1-12 Nguyen Thi Thu Thuy, Yuki Takamatsu, Dang Thi Dinh, Nguyen Thi Nam Lien, Doang Hoai Linh Ly, Pham Thi Hang, Bui Thu Thuy, Takeshi Nabeshima, Le Thi Quynh Mai, Kouichi Morita, Futoshi Hasebe. A dengue virus serotype 4 dominant outbreak in central Vietnam, 2013. The 63rd Annual Meeting of the Japanese Society for Virology. Fukuoka International Congress Center. 22-24 November 2015. Poster presentation.

2.1-13 Futoshi Hasebe, Nguyen Thi ThuThuy, Nguyen Co Thach, Dang Thi Dinh, Pham Hoai Linh Ly, Pham Thi Hang, Takeshi Nabeshima, Le Thi Quynh Mai, and Kouichi Morita. Severe dengue cases in Vietnam in 2015. Symposium Researches on infectious diseases in Vietnam and their application to the improvement of diagnosis, prevention and treatment, Seminar Room L (Global Health General Research Bldg, Nagasaki University), 16 May 2016.

2.1-14 Mya Myat Ngwe Tun , Rohitha V. Muthugala , Nguyen Thi Thu Thuy , Pham Hoai Linh Ly , Le Thi Hien Thu , Dang Thi Dinh , Nguyen Viet Hoang , Le Thi Quynh Mai , Meng Ling Moi, Corazon C. Buerano , Kouichi Morita , Futoshi Hasebe. Dengue associated acute encephalitis syndrome cases in Son La Province, Vietnam in 2014, The 15th Awaji International Forum on Infection and Immunity, Awaji Island, 6-9 September 2016, Poster presentation.

2.1-15 Futoshi Hasebe Dengue situation in Vietnam and quasispecies of dengue viruses. Laboratory Medicine Congress & Exhibition & Korean Society Laboratory Medicine 57th Annual Meeting. The K-Hotel, Seoul, Korea 26-28 October 2016. Symposium Oral presentation, Invited speaker.

2.1-16 Futoshi Hasebe, Recent dengue situation in Vietnam and dengue virus quasispecies. e-ASIA JRP Seminar and Final Meeting in Vietnam, 2016. Library, National Institute of Hygiene and Epidemiology, November 22, 2016, Oral presentation.

2.1-17 Phu Ly Minh Huong, Neurotropic characterization of dengue virus serotype-3 isolated from a dengue encephalitis patient in Vietnam. e-ASIA JRP Seminar and Final Meeting in Vietnam, 2016, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation by Skype.

2.1-18 Bui Thu Thuy, Isolation and characterization of dengue virus serotype-1 with single mutation substitution in the non-structural protein NS4B in dengue patients, Vietnam 2013, e-ASIA JRP Seminar and Final Meeting in Vietnam, 2016, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation.

2.1-19 Corazon C. Buerano, Molecular epidemiology of dengue viruses in the Philippines, e-ASIA JRP Seminar and Final Meeting in Vietnam, 2016, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation.

2.1-20 Maria Luisa G. Daroy, Laboratory profiling of dengue virus infections in two major hospitals in Metro Manila, e-ASIA JRP Seminar and Final Meeting in Vietnam, 2016, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation.

2.1-21 Maria Terrese A. Dimamay, Increased vascular permeability in Filipino patients during the course of dengue virus infection, e-ASIA JRP Seminar and Final Meeting in Vietnam, 2016, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation.

2.1-22 Satoshi Shimada, Clinical observation on dengue virus infection in Ho Chi Minh City, Vietnam 2016, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation by Skype.

2.1-23 Takeshi Nabeshima, Transcriptome analysis of severe dengue clinical samples, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation by Skype.

2.1-24 Meng Ling Moi, Implications of dengue cross-reactive antibodies in flavivirus endemic areas: the past, the present and the future, Library, National Institute of Hygiene and Epidemiology, 22 November 2016, Oral presentation by Skype.

2.1-25 Mark Anthony Luz, Meng Ling Moi, Maria Terrese A. Dimamay, Takeshi Nabeshima, Mark Pierre Dimamay, Ronald R. Matias, Corazon C. Buerano, Edith Tria, Filipinas F. Natividad, Maria Luisa G. Daroy, Futoshi Hasebe, Kouichi Morita. Virological characterization of Dengue Virus circulating in Metro Manila, 2015-2016. The 13th Nagasaki-Singapore Medical Symposium, Nagasaki, May 2017.

2.1-26 Mark Anthony Luz. フィリピンにおいて流行するデングウイルスの血清型、遺伝子型及びウイルス遺伝子の特性解析. サイエンスアンゴラ.東京.2017 年 11 月

2.2-1 長谷部太 「デングウイルスとデング熱」平成 26 年度新型インフルエンザ等新興・

再興感染症研究推進事業シンポジウムプログラム「感染症は一国の問題ではない　～エボラ出血熱、デング熱を例として～」ホテル椿山荘東京ジュピターホール シンポジスト　平成 27 年 2 月 16 日

2.2-2 長谷部太　「デング熱/デング出血熱とは」Dengue Fever/Dengue Hemorrhagic Fever (DF/DHF)　「緊急開催!!デング熱の脅威」　大下財団主催　ANA クラウンプラザホテル広島【広島市中区中町】　平成 26 年 11 月 13 日　招待講演

3 Organization of workshops, seminars, symposia, etc. (Organizer, Title of Event, Date, Location, Number of Participants, etc.)

a. e-ASIA JRP Japan team, e-ASIA JRP Dengue Project Kickoff Meeting, 09 May 2014, Ryojun Hall (School of Medicine, Nagasaki University), 25 participants.
b. e-ASIA JRP Philippine team, e-ASIA JRP Dengue Project Annual Meeting, 16 November 2015, St. Luke's Medical Center, (Quezon City Philippines), 19 participants.
c. e-ASIA JRP Vietnam team, e-ASIA JRP Seminar and Dengue Project Final Meeting, National Institute of Hygiene and Epidemiology (Hanoi, Vietnam), 22 November 2016, 24 participants.

4 Researcher exchanges including students (Description of Exchange, Destination, Duration, etc.)

[Japan Team]

a. Maria Terrese Alonzo, Analysis of dengue patient samples, St Luke's Medical Center (Quezon City, Philippines), 17 February-10 March 2014.
b. Kouichi Morita, Meeting for sample collection from dengue patients in the Philippines, St Luke's Medical Center (Quezon City, Philippines), 12-15 November 2014.
c. Moi Meng Ling, Meeting for the analysis of dengue patient serum samples by modified plaque reduction neutralization assays and for other laboratory experiments, National Institute of Hygiene and Epidemiology (Hanoi, Vietnam), 23-26 May 2015.
d. Futoshi Hasebe, Meeting for sample collection from dengue patients and sample analysis, St Luke's Medical Center (Quezon City, Philippines), 31 May-3 June 2015.
e. Satoshi Shimada, Observation of clinical treatment and medical care method for dengue patients at St Luke's Medical Center and San Lazaro Hospital (Quezon, City and Manila, Philippines), 15-21 November 2015.
f. Moi Meng Ling, Analysis of dengue patient samples. St Luke's Medical Center (Quezon City, Philippines), 19 June-01 July 2016.
g. Moi Meng Ling, Conduct of a training course for modified plaque reduction neutralization assays and other laboratory experiments for differentiation of dengue and Zika virus infection in Vietnam in collaboration with WHO Vietnam office. National Institute of Hygiene and Epidemiology,　08-19 August 2016.
h. Satoshi Shimada, Observation of clinical treatment and medical care method for severe dengue patients in Children Hospital No.1 (Ho Chi Minh City, Vietnam), 04 September-17 November 2016.
i. Phu Ly Minh Huong, Clinical data analysis and collection of samples from severe dengue patients in Children Hospital No.1 (Ho Chi Minh City, Vietnam), 02-27 October 2016.

[Philippine Team]

a. Mark Anthony Luz – Monbusho scholarship under the PhD programme, Dept. of Virology (Institute of Tropical Medicine, Nagasaki University), from Oct 2015.
b. Maria Terresse S. Dimamay, PhD – conduct of training and research on vascular permeability assays, Dept. of Virology (Institute of Tropical Medicine, Nagasaki University), 07-19 June 2016, 06-31 March 2017.
c. Lady-Anne S. Pangilinan – training on modified plaque reduction neutralization assays and other laboratory experiments Department of Virology (Institute of

Tropical Medicine, Nagasaki University), 05-25 July 2015.

d. John Paul Llido – training on modified plaque reduction neutralization assays and other laboratory experiments, Department of Virology (Institute of Tropical Medicine, Nagasaki University), 05-25 July 2015.

[Vietnam Team]

a. Bui Thu Thuy – Monbusho scholarship under the PhD programme, Department of Virology (Institute of Tropical Medicine, Nagasaki University), from April 2014.
b. Nguyen Co Thach – Monbusho scholarship under the PhD programme, Department of Virology (Institute of Tropical Medicine, Nagasaki University), from Oct 2016,
c. Tran Thi Nha, Meeting for sample collection, Vietnam, 30 September to 04 October 2014.
d. Nguyen Thi Kim Phuong, Meeting for sample collection, Vietnam, 30 September-04 October 2014.
e. Do Phuong Loan, Analysis of dengue patient samples, Department of Virology (Institute of Tropical Medicine, Nagasaki University), 04 November to 01 December 2014.
f. Nguyen Ngoc Linh, Analysis of dengue patient samples, Department of Virology (Institute of Tropical Medicine, Nagasaki University), 08 November-05 December 2014.

5 Number of patent applications : None

6 Awards: None

7 Others (Including agenda of workshop, photos of research teams, meetings, and etc.)

e-ASIA JRP DENGUE PROJECT KICKOFF MEETING
8 May 2014, Ryojun Hall, Nagasaki University, Nagasaki City, Japan

e-ASIA JRP DENGUE PROJECT ANNUAL MEETING
16 November 2015, St. Luke's Medical Center, Quezon City, Philippines

e-ASIA JRP DENGUE PROJECT ANNUAL/FINAL MEETING
22 November 2016, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

e-ASIA JRP DENGUE PROJECT ANNUAL/FINAL MEETING
22 November 2016, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam