Republic of Indonesia

Bogor Agricultural University (IPB)

地球規模課題対応国際科学技術協力（SATREPS）

# The Project on Ecological Studies on Flying Foxes and Their Involvement in Rabies-related and Other Viral Infectious Diseases

# Project Completion Report

**August 2020**

**JAPAN INTERNATIONAL COOPERATION AGENCY (JICA)**

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# Project Completion Report

**Project Title:** The Project on Ecological Studies on Flying Foxes and Their Involvement in Rabies-related and Other Viral Infectious Diseases

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| **Name** | : Prof. Srihadi Agungpriyono |
| **Title** | : Project Director |
| **Name** | : Prof. Eichi Hondo |
| **Title** | : Chief Advisor |
| **Submission Date** | : September 30th 2020 |

## I. Basic Information of The Project

### 1. Country

Indonesia

### 2. Title of the Project

The Project on Ecological Studies on Flying Foxes and Their Involvement in Rabies-related and Other Viral Infectious Diseases

### 3. Duration of the Project

Planned : 5 years (July 21, 2015 – July 21, 2020)
Actual : 5 years (July 21, 2015 – July 21, 2020)

### 4. Background

Recently the attention has been focused on the role of flying foxes which are carrying several important infectious agents like Lyssavirus causing rabies, Nipah, Hendra, and/or Ebola virus.

The Government of Indonesia (hereinafter referred to as GOI) plans to eliminate rabies by 2020 and has been trying to take appropriate actions in collaboration with all Indonesian Institutions and International organizations such as Food and Agriculture Organization of the United Nations, World Health Organization (hereinafter referred to as "WHO"), and etc.

Flying foxes are able to fly over 100 km a day and migrate over 1,000 km seasonally as average; therefore, they're one of the considerable vectors of the viral infectious diseases across nations. Under the situation, GOI requested JICA to initiate the Indonesia-Japan collaborative project at Agricultural Institute (Institut Pertanian) of the Bogor Agricultural University (hereinafter referred to as IPB) for conducting ecological studies on flying foxes and their involvement in rabies-related and other viral infectious diseases

Indonesian and Japanese researchers are going to conduct research on the relationship of flying foxes and the rabies and other viral infection by technical cooperation scheme. Through the Project, in addition, research capacity of Indonesian counterparts in the field of rabies-related and other viral infectious diseases derived from flying foxes aims to be strengthened.

## 5. Project Purpose

Research capacity of Indonesian researchers in the field of rabies-related and other viral infectious diseases derived from flying foxes is strengthened.

## 6. Implementing Agency

Indonesia side:

- Faculty of Veterinary Medicine (FKH), Institut Pertanian Bogor University (IPB) / Bogor Agricultural University
- (Supporting Institutions)
  Supporting Institutions are designated to assist IPB with available capabilities and expertise in support of implementation of the Project, as coordinated by the Project Director: Research Center for Biology, Indonesian Institute of Science (LIPI) and National Zoonosis Center (NZC-IPB)

Japan side:

- Nagoya University
- Yamaguchi University
- Tokyo University of Agriculture and Technology (TUAT)

## II. Result of the Project

## 1. Result of the Project

### 1-1. Input by the Japanese side

a. Amount of input by the Japanese Side

Planned : JPY 300,000,000.-

Actual : JPY 226,940,352.- (75.6%, as of 30 April, 2020)

Detail expenses are as follows.

Table 1. Detail expenses as input from Japanese side

| Item | Total (JPY) | Note |
|---|---|---|
| Expert dispatch | 44,400,278 | Dispatching Japanese expert to Indonesia |
| Short-term training in Japan | 6,625,191 | Receipt of training participants (1-3 weeks) |
| Equipment provision | 95,703,203 | Equipment |
| Consumables | 4,628,200 | Consumables |
| Overseas activities | 75,583,480 | Business trip, local cost, Regents |
| TOTAL | 226,940,352 | As of April 30th , 2020 |

b. Expert dispatched

Short-term dispatch=119 times (as of 30th, April 2020)

One long-term expert (coordinator) is dispatched from September2015 to July 2020.

Major activities:

- Virus Isolation
- Establishment of Cell line
- Collect and preserve samples of flying foxes
- Set up the biosafety committee in IPB
- Establish the method of serological examination including ELISA
- Run the Next Generation Sequencing and Multiplex PCR
- Determine the site for behavioral observation of flying foxes
- Analyze the movement of flying foxes using the Argos satellite system
- Develop an appropriate method to collect information of

rabies-related and other viral infectious diseases in Indonesia

Various field of experts of Nagoya University, Yamaguchi University, and Tokyo University of Agriculture and Technology (TUAT)) were often dispatched to FKH (Faculty of Veterinary and Medicine), IPB, Indonesia to transfer techniques, technologies and the front line knowledges to be applied and to monitor, as well as to confirm, those techniques were implemented appropriately or not. Not only to the laboratories, but also to the many related Ministries, the advisors were frequently dispatched to improve the IPB's self-ability or it can be said "sustainability" to run the BSL3 Laboratory, which poses the next generation sequencer (NGS).

Timing of dispatching the experts was carefully arranged with the timing of dispatching Indonesian researchers to Japan for training, in order to maintain continuous techniques and knowledge transfer activities and capacity building of Indonesian researchers.

c. Receipt of training participants

Short-term training : 18 persons (all are from Indonesia)

- FKH, IPB : 16 persons
- LIPI : 2 persons

Short-term training was conducted at either the Nagoya University, the Yamaguchi University or TUAT and aimed mainly to learn technology/knowledge from Japanese counterparts to be applied in Indonesian counterparts, including accelerating progress of the research conducted in Indonesian counterparts. Major of the training includes topics as follows.

- Experiment Management of Zoonotic Disease and Education Curriculum
- Improvement of academic dissertation writing ability and acquisition of basic gene manipulation technology
- Virus isolation and Serological study

- Acquisition of monoclonal antibody production technology for newly isolated virus
- Learn cell and virus handling and enable serological epidemiological investigation and virus isolation
- Learn new technologies of gene analysis and detection of pathogens using next generation sequencer and multiplex PCR
- Learn hybridoma preparation technology after the immunization for animals
- Zoonosis Laboratory Management and Education Program

**1-2. Input by the Japanese side**

a. Counterpart assignment: Total 18 persons

- FKH, IPB: 15 persons
- LIPI: 3 persons

List of researchers assigned by the Indonesian counterparts into this project was properly revised every year in order to response the changing of technical strategies and to improve the progress of the project. As of 31 March 2020, Indonesian counterparts assigned 18 persons to be involved in this project.

b. Provision of office and other in-kinds

- FKH, IPB:
  - Office space for project coordinator
  - Four desks for project coordinator, Indonesian assistant staff and Japanese experts
  - One document cabinet
  - One document drawer
  - One air-conditioner for office room
  - Laboratorium space for cell culture, viral isolation and serological examination including ELISA, and to determine genomic sequences of rabies-related and other viruses derived from flying foxes by multiplex PCR and next generation sequencing, BSL2 lab for those experiments.

- Special room space for Next Generation Sequencing, elaborately horizontal desk for installing NGS, and One air-conditioner.
- Special space for BSL3 room and pre-room,
  Assembling the external and internal panels from Japan, installing Bio-safety Level 3 cabinet, put the -80C and -30C Freezers, incubator, autoclave, and one air-conditioner in the decompressed pre-chamber.

c. Other items borne by the Government of Indonesia

Budget allocated by the Government of Indonesia through IPB for this project during the project term FY2016-FY2020 is as follows.

Table 2. Budget allocated by the Government of Indonesia for this project during the project term (FY2016-FY2020)

| Expense Item | Amount (IDR) | Note |
|---|---|---|
| Salaries | 245,000,000.- | Salaries for non-permanent (including Zoonosis IPB center staff, and technicians) |
| Consumables/ Regents | 90,000,000.- | Regents and consumables for virus isolation, culture call, ELISA and PCR |
| Travel | 355,800,000.- | Flying foxes sampling travel to Garut, West Java and Pontianak, West Kalimantan. Telemetry installation for flying foxes in Garut, West Java, and Pontianak, West Kalimantan |
| Meeting | 55,800,500.- | JCC meetings<br>International symposiums |
| Equipment | 85,530,000.- | Generator for Lab,<br>UPS, Electric stabilizers |
| TOTAL | 832,130,500.- | |

## 1-3. Activities (Planned and Actual)

*Output 1: Characteristics of rabies-related and other viruses derived from flying foxes are revealed by viral isolation and genetic analysis.*

The project faced the big problem about importing the equipment from Japan. As we can see in the PO (Plan of Operation), the project was expecting that the whole equipment, which were purchased in Japan were supposed to be imported by the end of December 2015. But shipment had to be divided into two containers according to the import procedure of Indonesia and pre-shipment inspection in Japan, then first cargo was delivered to FKH, IPB on November 2016, and second shipment was eventually delivered to FKH on Mach 2017. It was delayed more than one year. The main reason for this delay will be described later in this paper. The project had to change the timing of the activities such as serological lab work, virus isolation, culture, or running Multiplex / Real-time PCR and next generation sequencing due to the late delivery of the equipment, and behavioral information survey of flying foxes in and surrounding the Garut region, provincial West Java was the focuse of the activities until the equipment was installed.

Indonesian and Japanese joint researchers team walked into the Garut costal preservation area to determine the site for behavioral observation of flying foxes. Moreover, the project worked together with Ministry of environment and Forestry in Indonesia and caught three (3) flying foxes to analyze the movement of flying foxes with using the Agros satellite system.

After the installation of all the equipment, the project worked hard to catch up with the delayed planned operation, but all of them were completely achieved despite of the delay of importing the equipment from Japan.

1.1.1 To obtain researchers' VISA from the Ministry of Research and Technology and Higher Education

- Research permit visa would provide foreign researchers with staying visa called KITAS. For JICA Indonesia office, this was the very first time to get the research permit visa system with KITAS provided by the domiciled immigration office, eventually Prof. Hondo and Ms. Yupadee from Nagoya University obtained the research permit from RISTEK (the Ministry of Research,

Technology and Higher Education) and KITAS (staying permit for one year) from Bogor immigration office in December 2015.

1.1.2. To obtain permission for collecting flying foxes and entering conservation areas from the Ministry of Forest

- Firstly, the project asked the recommendation letter from LIPI (Indonesian Institute of Science) so that the Ministry of Environment and Forestry could provide the project with the permission of catching 100 flying foxes from Leuweung Sancang, Garut region, West Java.
- The project submitted the application letter of permission for collecting flying foxes with recommendation from LIPI to the Ministry of Environment and Forestry, and received the permission on May 2016.
- Also the project needed to get the confirmation for collecting flying foxes and transportation permission of flying foxes from Garut to Bogor from the regional Forestry office in Garut, and received the permission on June 2016.

1.1.3. To have protocol of collecting and transporting flying foxes (and dogs if necessary)

- The project confirmed several protocol of collecting and transporting flying foxes with BKSDA (Nature Resources Conservation Agency) in Garut, on June 2016 and received the permission letter from them.

1.1.4. To determine SOPs for laboratory and field research

- Laboratory SOP (including BSL2 and BSL3) was established with the draft made by Dr. Takemae from Nagoya University.
- Field research SOP, especially for handling of flying foxes was established with the draft made by Dr. Takemae from Nagoya University.

1.2. To collect and preserve samples of flying foxes (and dogs if necessary

- First sampling of flying foxes was conducted on September and

October 2016, in conservation area, Leuweung Sancang, Garut, West Java Province. Total 55 flying foxes were caught and transported to FKH, IPB.

- Second sampling of flying foxes was implemented on March 2017, in Leuweung Sancang conservation area in Garut region, West Java Province. The project caught 36 flying foxes in the rainy reason.
- Next sampling of flying foxes was conducted on September 2017, in Leuweung Sancang conservation area in Garut, West Java Province. 52 flying foxes were caught in the dry season and transported to FKH, IPB.
- The Dog serum sampling was implemented on October 2020, near around the Leuweung Sancang Conservation area and 50 dog's serum were sent to FKH, IPB in the end.
- In 2018, from May to July, sampling was made in Bogor, West Java Province. 33 flying foxes were collected and transported to FKH Laboratorium.
- In March 2019, the project implemented flying foxes sampling for the first time near Pontianak, West Kalimantan. Only 4 flying foxes were caught even though village hunters can get hundreds of them each year.
- In August 2019, the sampling of flying foxes was implemented near Pontianak, West Kalimantan again. This time, 96 flying foxes were caught and transported to IPB, West Java.
- The project was planning the final sampling in March or April 2020 for 100 bats, however it was cancelled due to the COVID-19. The project caught 276 flying foxes totally since 2016 to 2019.

1.3.1 To set biosafety-committee in IPB and to train all people related to laboratory to handle pathogen in BSL-3.

- The biosafety committee in IPB was established in February 2017.
- A biosafety seminar was held by Dr. Shinohara of the National Institute of Infectious Diseases in Japan on February 9$^{th}$2017, and also FKH received some advice to create a suitable regulation for the biosafety committee.
- One of the purposes of this seminar is for the members of the IPB Biosafety Committee to become familiar with the basic precautions

for handling pathogens such as BSL3 levels. As well as it is about developing the ability of the committee to decide many things independently and appropriately. From this point, Dr. Shinohara explained in detail the handling standards for pathogens, the correct usage of equipment such as biosafety cabinets, and the countermeasures in case of contamination.

1.3.2. To train all people related to laboratory to handle pathogens in BSL-3 / To do the lectures for biosafety (BSL-3) to Faculty of Veterinary Medicine, IPB.

- After the afternoon training on 10th February 2017 by Dr. Shinohara from the National Institute of Infectious Diseases in Japan, a test was conducted, and only successful applicants were allowed to enter the BSL3 cabinet and were given permission to work there (all 20 participants passed). From then on, the IPB's Biosafety Committee will implement these permits.
- Chief Advisor Professor Hondo conducted BSL3 training (TOT) for FKH teachers, then the trained teachers actually gave lectures for FKH students. After that, students took the BSL3 tests, and all participants passed. In this way, FKH will independently conduct training and tests for BSL3 users and create a system that provides usage permission.

1.4.1. To isolate rabies-related and other viruses derived from flying foxes using cell lines. / large T antigen expression plasmid/ to isolate the virus.

- Flying Foxes cerebrospinal fluid and swab were inoculated into cultured cell lines, and bat orthoreovirus and mammalian orthoreovirus were successfully isolated.
- As the cell line, BHK cells derived from hamster kidney, HmLu cells derived from hamster lung, and Vero9013 cells derived from African green monkey kidney were used.
- Fifty-five flying fox rectal swabs captured in Garut, West Java from September to October 2016 were inoculated into cell lines, and bat orthoreovirus was isolated from one individual.
- In March 2017, 36 individuals of flying fox rectal swabs captured

in Garut, West Java were inoculated into cell lines, and bat orthoreovirus was isolated from 3 individuals.

- In September 2017, 51 individuals of flying foxes rectal swabs captured in Garut, West Java were inoculated into cell lines, and bat orthoreovirus was isolated from 5 individuals.
- Flying Foxes cerebrospinal fluid from 96 individuals captured in Pontianak, West Kalimantan in August 2019 were inoculated into culture cell lines, and mammalian orthoreovirus was isolated from 5 of 19 pools.
- The Australian bat lyssa virus genome fragment was detected by RT-PCR from the bat cerebrospinal fluid RNA captured in West Kalimantan. The post project, FKH SATREPS team is planning to inoculate this cerebrospinal fluid into mouse neuroblastoma derived Neuro2a cells to attempt virus isolation.

1.4.2. To do counterpart training in immortalization of primary cell from the tissues using SV40

- In order to efficiently isolate the virus from flying foxes, the project attempted to establish the cultured cell-lines derived from flying foxes, and succeeded in immortalizing the kidneys and passage over 100 generations.
- Efficiency is improved by using cultured cells that are the host of the virus for virus isolation.
- Primary cultured cells of the fetal kidney, spleen, and thymus of the flying fox were prepared to establish cultured cells derived from flying foxes.
- The T antigen of monkey virus 40 was transfected to immortalize the cells.
- As a result, only kidney-derived cells were immortalized, and over 100 generations cell lines was successfully established.
- These are the result from the counterpart training in Japan and FKH, IPB is planning to carry out virus isolation using this cultured cell in the future.

1.5.1. To determine genomic sequences of rabies-related and other viruses derived from flying foxes by multiplex PCR and subsequent next

generation sequencing.

- The types of viruses that wild bats carry in their bodies were identified by comprehensive DNA sequence analysis, and the risk of potential infectious diseases was estimated.

1.5.2. To design the degenerate primers for multiplex PCRs for Rhabdovirus, Filovirus, Paramyxovirus, and Coronavirus.

- Primer design that can comprehensively detect Lyssavirus by the CODEHOPE method.
- The L gene, which has a storage region for amino acids, was used as the amplification site.
- The L gene sequences of 17 Lyssavirus species registered in GenBank were aligned with amino acids, and one primer was designed from each of the four more conserved regions to form a nested PCR system.
- Synthetic DNA was purchased from each of the 17 Lyssavirus species and used in order to evaluate the sensitivity of nested PCR.
- The nested PCR system was then changed to a system containing four primers at high concentrations.
- The final sensitivity was 10 squared / reaction.
- When the cDNA synthesized from the bat sample extracted RNA stored at our laboratory was spiked into the synthetic DNA and the sensitivity was evaluated again, non-specific bands frequently occurred regardless of the type of synthetic DNA.
- Currently, we are studying the conditions so that non-specific bands do not appear in the spike test by improving some primer sequences.

- Establishment of a method for efficiently detecting genomic sequences derived from pathogenic viruses by the RDA method [In the RDA method, RNA extracted from healthy individuals and RNA extracted from pathogen-bearing individuals are compared and subtracted in a PCR tube, and only RNA (in this case, assuming an RNA virus genome) that exists only in pathogen-bearing individuals is extracted and will be analyzed with NGS].
- First, RNA extracted from cultured cells was mixed with known virus-derived RNA and verified by real-time PCR.

- Unfortunately, cell-derived RNA was not significantly reduced in the post-RDA sample.
- The cause was expected to be the inefficiency of hybridization, which is important during "subtraction".
- Currently, the FKH, IPB team and TUAT(Tokyo University of Agriculture and Technology) are studying the buffer used for hybridization.

1.5.3. To setup multiplex PCR, to detect virus in flying foxes by multiplex PCR, to analyze viral genomes by NGS, to detect viruses from dogs, if necessary, by multiplex PCR, to analyze viral genomes by NGS.

- It is considered that there are unknown types of viruses that wild bats carry in their bodies.
- With the permission of Indonesian related Ministries, the project captured wild flying foxes in Garut, Java.
- DNA samples were extracted from the livers of 6 individuals for verification including individual differences.
- It is predicted that this DNA sample will contain the intracellularly infected viral genome sequence in addition to the genome sequence of the flying fox itself.
- By next-generation sequencer analysis, DNA sequence information of 15-23 Gb (equivalent to 7-11 times the genome of flying foxes) was obtained per individual.
- The virus homologous sequences contained in the acquired sequences were listed, and the virus species contained in wild flying foxes were determined.
- The results are being submitted to some of the science journals. After it is accepted, the project will try to share the results as open access.

*Output 2: Method of serological examination including ELISA in flying foxes for the viruses analyzed by the Project is established.*

2.1. To do counterpart training in serological surveillance methods including ELISA for the viruses.

- In Yamaguchi University, the project conducted the Cell / virus

handling and serum epidemiology survey and implementation training for several young counterparts from FKH, IPB.

2.2. To prepare for recombinant antigens and corresponding antibodies against rabies-related and other viruses derived from flying foxes based on their genomic sequences.

- Expression plasmids for N protein (8 types) and G protein (7 types) of lyssavirus including rabies virus were prepared.

2.3. To establish the method of serological examination for rabies-related and other viral infectious diseases derived from flying foxes.

- When ELISA was performed using N protein, G protein expressing cells and infected cells of rabies virus, ELISA using N protein had the highest correlation with the results of the virus neutralization test and showed high specificity and sensitivity.
- Based on the above results, ELISA using N protein-expressing cells was performed on Indonesian flying foxes with 8 types of lyssavirus.
- As a result, antibodies against Australian bat lyssavirus and Lagos bat lyssavirus, which have not been confirmed in Indonesia, were detected.
- Based on the above results and observations, the certain method of serological examination for rabies-related and other viral infectious diseases derived from flying foxes was established.

2.4. To examine the efficiency of the method of serological examination to the flying foxes (and dogs if necessary).

- As described in 2.3, when ELISA was performed using N protein, G protein expressing cells and infected cells of rabies virus, ELISA using N protein had the highest correlation with the results of the virus neutralization test and showed high specificity and sensitivity. This shows that using the appropriate protein will bring the efficient result eventually.

2.5. To write papers and publish in academic journals

- The results are being submitted to some of the science journals. After it is accepted, the project will try to share the results as open access.

*Output 3: Behavioral information of flying foxes in and surrounding the province of West Java and West Kalimantan are reported.*

3.1. To determine the site for behavioral observation of flying foxes, conduct administrative procedures for research permission, and to create SOPs for safety of researchers engaged in the field work.

- FKH, IPB and the project determine "Leuweung Sancang" conservation area near Garut, West Java as the site for behavioral observation of flying foxes.
- The project applied research permission in Leuweung Sancang(PADIA) to the Directorate of Biodiversity Conservation, Directorate General of National Resources and Ecosystem Conservation, the Ministry of Environment and Forestry.
- The research permission(PADIA) was approved by the Directorate of Biodiversity Conservation, Directorate General of National Resources and Ecosystem Conservation.
- Besides the research permission(PADIA), both Indonesian and Japanese researchers are required "SIMAKSI" (Surat Izin Masuk Kawasan Konservasi – Entry permission letter to Conservation Area) and the project applied it to the Regional office of Environment and Forestry in Bandung, capital city of West Java province.
- The Entry permission(SIMAKSI) was approved by the Regional office of Environment and Forestry in Bandung, West Java province.
- The Entry permission(SIMAKSI) was only provided to the foreign researchers who have research permit from RISTEK-DIKTI.
- Standard Operating Procedures(SOP) for the field work was made on the basis of the IPB's field work regulation in accordance with the Indonesian regulation for the Conservation area.

3.2. To investigate the behavior of the flying foxes in the roosting site.

- Day time and night time behavior of the flying foxes were investigated by the both Indonesian and Japanese researchers in Leuweung Sancang and outskirts of the conservation area on May 2016.
- The project researchers team found the several roosting site of the flying foxes in the Leuweung Sancang Conservation Area and

observed their behavior for about 20 days.

- The "Daytime behavior of *Pteropus vampyrus* in a natural transmission" by **Ms. Yupadee Hengian / Nagoya University** was accepted in The Journal of Veterinary Medical Science in 2017.
- Night time behavior of the flying foxes were investigated by the both Indonesian and Japanese researchers in Leuweung Sancang and outskirts of the conservation area on October 2017.
- Night time behavior of the flying foxes were observed near the fruit trees which were located close to the Leuweung Sancang Conservation Area.
- The project researchers team had to change observation locations each time flying foxes changed their food-securing trees.
- "Night time behavior of *Pteropus Vampyrus* in a natural transmission" by **Ms. Yupadee Hengian / Nagoya University** was accepted by the Journal of Veterinary Medical Science in 2018.

3.3. To interview with residents near the site about behavior of flying foxes.

- The interview with residents near the Leuweung Sancang Conservation Area was conducted in seven (7) villages by the FKH, IPB researchers team with the instruction of Dr. Chaerul Basri, FKH, IPB.
- The questionnaire was divided into three groups: flying fox habitat within 1 km, within 1 km to 5 km, and over 5 km.
- The interview survey took 40 minutes per person and was conducted on 10 to 15 people a day.
- In this way, the interview survey was conducted on about 150 villagers.
- "Potential risk of viral transmission from flying foxes to domestic animals and humans on the southern coast of West Java, Indonesia" by **Dr. Chaerul Basri /FKH IPB** was accepted in The Journal of Veterinary Medical Science in 2017.

3.4. To analyze the movement of flying foxes using the Argos satellite system.

- The first challenge to install the three (3) telemetries to flying foxes were conducted when the project researchers team observed the night time behavior of flying foxes on October 2017.

- The one telemetry became undetectable shortly after being attached to the flying fox.
- One flying fox, which was equipped with the device on October 4, continued flight measurements until January 24, 2018, and it was recorded that it was moving within 20 to 30 km around the nesting site.
- Another flying fox, which was fitted with the device, telemetry on October 4, continued flight measurements until April 9, 2018. This flying fox was recorded flying to the suburbs of Subang, West Java, about 100 kilometers away from the nesting site.
- The next flying fox flight survey was conducted in July 2019 near Pontianak, West Kalimantan, and three flying foxes were released with telemetry attached.
- One of the flying foxes, telemetry-mounted on July 17, 2019, was tracked by the Argos satellite until September 16, 2019, and was found to have flown a total of about 800 kilometers.
- One flying fox, also telemetrically fitted on July 17, 2019, was tracked by the Argos satellite until November 11, 2019, and was found to have flown a total of approximately 2,272 kilometers.
- One flying fox, also telemetrically fitted on July 17, 2019, was tracked by the Argos satellite until September 30, 2019. The bat flew about 160 kilometers north in a day, approaching the Sabah province border of Malaysia to 65 kilometers.
- As a final Argos survey, telemetry was attached to four (4) male flying foxes on August 21, 2019, near Pontianak, West Kalimantan, and released for observation.
- Seven flying foxes were able to be tracked by Argos Satellite system in flight from July to up to January 2020.
- One of the flying foxes was tracked until September 20 and flew about 29 kilometers near the nesting site.
- One of the flying foxes was tracked until December 30, 2019 and flew about 1,282 kilometers near the nesting site.
- The last flying fox was tracked by the Argos satellite until January 9, 2020, and was recorded to have flown a total of about 1,213 kilometers near the nesting site.

3.4.1. To analyze annual patterns of behavior of flying foxes using the Argos satellite system.

- Even in Leuweung Sancang Conservation Area near Garut, West Java and Pontianak, West Kalimantan, it was extremely difficult to carry out year-round observations due to the weather and the time of migration to the nesting sites of flying foxes.
- Observations that consider when the fruits that serve as food for flying foxes will remain an issue for IPB researchers.

3.5. To analyze the data of climate, meteorology, topography and changes of land use in and around the roost of flying foxes from the satellite.

- The project held the seminar in IPB Convention Center in Bogor for about the climate, meteorology, topography and changes of atmosphere by using the land in Indonesia on August 2016.
- Dr. Ardhasena Sopaheluwakan (Indonesia Meteorology, Climate and Geophysics Agency: BMKG) gave a lecture on the Climate of the Indonesian region and the recent ENSO episode. In particular, Dr. Ardhasena explained interesting data on climate characteristics, precipitation fluctuations, wind changes, and climate changes associated with the 2015 El Nino-Southern Oscillation (ENSO) in the Indonesian region.
- Dr. Taisei Kanamori gave a lecture on Climate variability over the Maritime Continent (Institute for Space-Earth Environment, Nagoya University).
- Professor. Yonny Koesmaryono (Bogor Agricultural University) gave a lecture entitled "Climate Change and Its Impacts on Livestock" on the warming effect associated with oxygen dioxide emissions, the risks associated with it, and the impact on livestock.
- Dr. I Wayan Teguh Wibawan (Bogor Agricultural University) gave a lecture on the outbreak of zoonotic diseases in Indonesia with the content of "Important Zoonotic Diseases in Indonesia and Their Occurrence".
- Dr. Shimoda (Yamaguchi University) gave a lecture on Bat-derived infectious diseases.
- Dr. Takemae (Nagoya University) gave a lecture entitled "The protozoan research and its application to SATREPS project".

- Dr. Ryosuke Kobayashi (Nagoya University) gave a presentation on Immunology of bats.
- Dr. Yupadee Hengjan (Nagoya University) gave a lecture "Daytime behavior of Pteropus vampyrus and habitat sharing with Trachipterus auratus: the possibility of disease transmission among them".
- Dr. Tsutomu Omatsu (Tokyo University of Agriculture and Technology) gave a lecture entitled "How to detect the virus genome with high-throughput sequencing".
- In particular, young Indonesian researchers were able to learn a lot about the environmental impacts of wild animals, e.g. flying foxes, such as habitats, through meteorology and topography.

3.5.1. To do counterpart training in satellite image analysis (Alos 2 image)

3.5.1. To correspond the places of the individual flying foxes to the real-time land image of ALOS-2, JAXA

- The images on Google map have been updated, and we decided that there was no merit in using the expensive images issued by JAXA, so the project used Google map images that can be used online for free.

3.5.2. To examine changes of atmospheric condition and their effect on patterns of behavior of flying foxes including a change of roost.

- Using the Argos satellite system, we conducted a training to investigate the flight area, distance, and altitude of flying foxes on a Google map.
- The work of observing the route, altitude, speed, etc. of the flying foxes actually flying in Argos Satellite system for each individual and incorporating those data into the Google map has been completed.

3.6. To integrate all data and write papers in academic journals.

- Currently, a young IPB researcher is planning to submit to a specialized academic journal.

*Output 4: Capacity of Indonesian researchers in collecting and analyzing viral information derived from flying foxes is developed.*

4.1. To develop an appropriate method to collect information of rabies-related and other viral infectious disease in Indonesia.

- Ministry of Agriculture, Directorate of Animal Health, Directorate General of Livestock and Animal Health, department collects livestock and wildlife infectious disease data throughout Indonesia every year.

4.2. To collect and analyze data of rabies-related and other viral infectious diseases from Indonesia.

- Access to the Agricultural Department's database has made it easier to access state data, especially on rabies, over the past five years to get information on rabies-derived infectious diseases.

## 2. Achievement of the Project

### 2-1. Outputs and indicators

*Output 1 : Characteristic of rabies-related and other viruses derived from flying foxes are revealed by viral isolation and genetic analysis.*

1.1. Biosafety committee is immediately set in IPB once the Project starts and biosafety guidelines concerning the Project are prepared.
The output was achieved in the 2nd year.

- The biosafety committee in IPB was established in February 2017.
- Initially, the committee was supposed to be established soon after the project started, but the arrival of the equipment provided from Japan was delayed by a year and a half, so the establishment of the committee was significantly delayed.
- The biosafety guidelines were drafted at the same time as the committee was established, based on the guidelines of Nagoya University and the rules of the IPB Animal Ethics Committee.

1.2. Methods of cell culture and viral isolation are established by Indonesian researchers.

The output was achieved in the 4th year.

- Virus isolation studies began in October 2016 after the flying foxes were sampled.
- Fifty-five flying fox rectal swabs captured in Garut, West Java from September to October 2016 were inoculated into cell lines, and bat orthoreovirus was isolated from one individual.
- In March 2017, 36 individuals of flying fox rectal swabs captured in Garut, West Java were inoculated into cell lines, and bat orthoreovirus was isolated from 3 individuals.
- In September 2017, 51 individuals of flying foxes rectal swabs captured in Garut, West Java were inoculated into cell lines, and bat orthoreovirus was isolated from 5 individuals.
- Flying Foxes cerebrospinal fluid from 96 individuals captured in Pontianak, West Kalimantan in August 2019 were inoculated into culture cell lines, and mammalian orthoreovirus was isolated from 5 of 19 pools.
- Through numerous cell cultures and virus isolation experiments, a virus isolation method was established by young Indonesian researchers.

1.3. Rabies-related and other viruses derived from flying foxes are distinguished by genetic analysis.

The output was achieved in the 5th year.

- The Australian bat lyssa virus genome fragment was detected by RT-PCR from the bat cerebrospinal fluid RNA captured in West Kalimantan.
- The post project, FKH SATREPS team is planning to inoculate this cerebrospinal fluid into mouse neuroblastoma derived Neuro2a cells to attempt virus isolation.
- In this way, through many experiments, the path to find out the rabies-related virus derived from flying foxes using cell culture, virus isolation, PCR and NGS will be acquired as a skill of Indonesian researchers.

*Output 2: Method of serological examination including ELISA in flying foxes for the viruses analyzed by the Project is established and epidemiological study is performed.*

2.1. Recombinant antigens and their antibodies responding to the viruses analyzed are generated by Indonesian researchers.

The output was achieved in the 3rd year.

- Expression plasmids for N protein (8 types) and G protein (7 types) of lyssavirus including rabies virus were prepared.
- In particular, recombinant antigens against genetically analyzed viruses and their antibody preparation were appropriately instructed to Indonesian researchers in 2016, 2017, and 2018 in the counterpart training at Yamaguchi University.

2.2. Efficiency of the method of serological examination to the flying foxes and /or dogs.

The output was achieved in the 5th year.

- When ELISA was performed using N protein, G protein expressing cells and infected cells of rabies virus, ELISA using N protein had the highest correlation with the results of the virus neutralization test and showed high specificity and sensitivity.
- This shows that using the appropriate protein will bring the efficient result eventually.

*Output 3. Behavioral information of flying foxes in and surrounding the province of West Java and West Kalimantan are reported.*

3.1. Protocol for the research of the behavior of flying foxes is prepared.

Output was achieved in the 2nd year.

- Standard Operating Procedures(SOP) for the field work was made on the basis of the IPB's field work regulation in accordance with the Indonesian regulation for the Conservation area.
- The protocol also made up for the shortfall in light of the rules of the Animal Ethics Committee of Nagoya University.
- The protocol was all translated into Indonesian and completed.

3.2. Satellite tracking data of flying foxes are collected and analyzed by Indonesian researchers.

Output was achieved in the 5th year.

- The first flying fox flight survey was conducted on October 4, 2017 in Garut, West Java, with telemetry attached to three male flying foxes.
- The one telemetry became undetectable shortly after being attached to the

flying fox.

- One flying fox, which was equipped with the telemetry device on October 4, continued to fly around until January 24, 2018, and it was recorded that it was moving within 20 to 30 km around the roosting site.
- Another flying fox, which was fitted with the device, telemetry on October 4, continued to fly around until April 9, 2018. This flying fox was recorded flying to the suburbs of Subang, West Java, about 100 kilometers away from the roosting site.
- October is already the beginning of the rainy season around the Leuweung Sancang Conservation Area, near Garut, West Java, and it is also the time when the fruits that flying foxes like to eat grow. From this reason, it is presumed that the individuals wearing telemetries did not move long distances because food could be secured without large-scale movement from the roosting site.
- The next flying fox flight survey was conducted in July and August 2019, targeting flying foxes roosting around Pontianak, West Kalimantan, and released to the natural world with telemetries attached to a total of seven males.
- Some flying foxes flew 160 kilometers in a day and approached 65 kilometers to Sabah Province, the border with Malaysia.
- However, most flying foxes traveled to and from the feeding site not far from the roosting site, and the daily flight distance was only within 5 to 8 km.
- Since the observation period is the dry season and many fruits are eaten by flying foxes around Pontianak during this period, it is considered that they do not move far from the roosting site. However, in the event of a full-scale rainy season or when food is not available near the roosting site, it is necessary to continue to investigate whether the herd itself will move somewhere.
- Flying surveys of flying foxes near Garut, West Java and Pontianak, West Kalimantan show that their flight distance and direction are closely related to the time and duration of ripening of the fruits that feed primarily.

*Output 4. Capacity of Indonesian researchers in collecting and analyzing viral information derived from flying foxes is developed.*

4. Data on viral information derived from flying foxes is analyzed.
Output was achieved in the 5th year.

- The project's activities began by investigating which ministries and agencies are collecting and managing information on viruses derived from wild animals and livestock in Indonesia.
- As a result of the investigation, it was found that the Directorate of Animal Health, Directorate General of Livestock and Animal Health, Ministry of Agriculture, statistically manages viral data derived from wildlife animals in Indonesia.
- The project was approved by requesting access to this database maintained by the Directorate General of Livestock and Animal Health, Ministry of Agriculture, with the cooperation of one of our senior counterparts, Dr. Etih. In response, young IPB researchers have begun investigating statistical records of rabies and other rabies-related viral infectious diseases in 34 provinces in Indonesia.
- In response, one of the young IPB researchers has begun to investigate statistical records of rabies and other rabies-related viral infectious diseases throughout 34 provinces in Indonesia.
- The results will be submitted to some of the science journals. After it is accepted, the project will try to share the results as open access.

## 2-2. Project Purpose and indicators

*Project purpose: Research capacity of Indonesian researchers in the field of rabies-related and other viral infectious diseases derived from flying foxes is strengthened.*

1. Characteristic of rabies-related and other viral infectious diseases derived from flying foxes are able to be clarified through genetic analysis by Indonesian researchers.
The indicator is achieved in the 5th year of the project.

- More than 100 individuals of flying fox rectal swabs captured in Garut,

West Java since 2016 until 2017 were inoculated into cell lines, and bat orthoreovirus was isolated from 9 individuals.

- Flying Foxes cerebrospinal fluid from 96 individuals captured in Pontianak, West Kalimantan in August 2019 were inoculated into culture cell lines, and mammalian orthoreovirus was isolated from 5 of 19 pools by the collaboration work with Indonesian and Japanese researchers.
- The Australian bat lyssa virus genome fragment was detected by RT-PCR from the bat cerebrospinal fluid RNA captured in West Kalimantan. The post project, FKH SATREPS team is planning to inoculate this cerebrospinal fluid into mouse neuroblastoma derived Neuro2a cells to attempt virus isolation.
- Thus, in the fifth year of the project, Indonesian researchers have been able to clarified genetic markers for rabies-related and other infectious disease viruses derived from flying foxes.

2. Behavioral information of flying foxes is able to be collected and analyzed by Indonesian researchers.

The indicator is achieved in the 5$^{th}$ year of the project.

- Telemetry ID number 164317 was attached to a male flying fox captured in Leuweung Sancang Conservation Area in Garut, West Java province on 4$^{th}$ October 2017, and was released for the tracking observation by the Argos Satellite system. The ID 164317 was tracked by the Argos satellite until 24 January 2018 and was found to have flown a total of approximately 800 kilometers. The ID 164317 once flew to Subang city, approximately 100 km far from his roosting site, and came back to his roosting site soon.
- The flying fox with telemetry ID number 164318 could not be tracked for some reason, such as a mechanical failure, shortly after the telemetry was installed(4$^{th}$ October 2017, Leuweung Sancang Conservation Area).
- Telemetry ID number 164319 was attached to a male flying fox captured in Leuweung Sancang Conservation Area in Garut, West Java province on 4$^{th}$ October 2017, and was released for the tracking observation by the Argos Satellite system. The ID 164319 was tracked by the Argos satellite until 4 April 2018 and this male bat was flying over 15 kilometers around the Leuweung Sancang Conservation Area almost every day.
- The male flying fox with ID 164320 was released with telemetry on July

17, 2019, near Pontianak, West Kalimantan, and its flight was tracked by the Argos satellite system until September 30, 2019. ID164320 flew up to 520m above sea level, flew until 165km north, and even reached 65km to Sabah province on the Malaysian border. ID164320 flew a total of 1,189km in about two and a half months.

- The male flying fox with ID 164322 was telemetry-equipped on July 17, 2019, released to nature near Pontianak, West Kalimantan, and was tracked by the Argos Satellite system until November 11, 2019. ID164322 flew a total of 2,272km in about 4 months.
- The male flying fox with ID 164323 was telemetrically fitted on August 21, 2019, released to the natural world near Pontianak, West Kalimantan, and was tracked by the Argos system until September 20, 2019. ID164323 flew a total of 29km in about a month. This flying fox spent a month with almost no movement, probably because there were many fruits to eat near the roosting site or because of illness.
- The male flying fox with ID 164324 was telemetry-equipped on August 21, 2019, released to nature near Pontianak, West Kalimantan, and followed by the Argos system until January 9, 2020. ID164324 flew a total of 1,213km in about four and a half months. Data confirmed that it had traveled about 150 kilometers northwest and southeast and spent several days there, perhaps to secure food.
- The male flying fox with ID 164325, fitted with telemetry on August 21, 2019, was released to nature near Pontianak, West Kalimantan, but was captured again by a hunter on August 24.
- The male flying fox with ID 164326 was attached telemetry on 21 August 2019, released to nature near Pontianak, West Kalimantan, and tracked by the Argos Satellite system until 30 December. ID164326 flew a total of 1,282km in about 4 months. The data confirmed that it traveled about 200 kilometers northwest and southeast and spent several days there, perhaps to secure food.
- The results will be submitted to some of the science journals. After it is accepted, the project will try to share the results as open access.

3. More than 5 research articles related to the Project, in which first author is Indonesian researchers or comparative responsibility with first author, are

published in academic journals.
This indicator is achieved in the 5th year of the project.

- Six scientific papers in which first author are Indonesian researcher had been published in academic journals.
  - ➢ Chaerul BASRI, Eko Muhammad Zainal ARIFIN, Hitoshi TAKEMAE, Yupadee HENGJAN, Keisuke IIDA, Etih SUDARNIKA, Abdul ZAHID, Retno Damayanti SOEJOEDONO, Heru SUSETYA, Bambang SUMIARTO, Ryosuke KOBAYASHI, Srihadi AGUNGPRIYONO and Eiichi HONDO (2017). Potential risk of viral transmission from flying foxes to domestic animals and humans on the southern coast of West Java, Indonesia. The Journal of Veterinary Medical Science. doi: 10.1292/jvms.17-0222.
  - ➢ Desrayni Hanadhita, Anisa Rahma, Andhika Yudha Prawira, Ni Luh Putu Ika Mayasari, Aryani Sismin Satyaningtijas, Eiichi Hondo, Srihadi Agungpriyono (2019). The spleen morphophysiology of fruit bats. Anatomia Histologia Embryologia, Journal of Veterinary Medicine. doi: 10.1111/ahe.12442.
  - ➢ Desrayni Hanadhita, Anisa Rahma, Muhammad R. Wahid, Ni Luh Putu Ika Mayasari, Aryani Sismin Satyaningtijas, Eiichi Hondo, Srihadi Agungpriyono (2019). Extracellular matrix composition of different spleen compartments of fruit bats. Anatomia Histologia Embryologia, Journal of Veterinary Medicine. doi: 10.1111/ahe.12526.
  - ➢ Supriyono, Ai Takano, Ryusei Kuwata, Hiroshi Shimoda, Upik K. Hadi, Agus Setiyono, Srihadi Agungpriyono, Ken Maeda (2019). Detection and isolation of tick-borne bacteria (Anaplasma spp., Rickettsia spp.,and Borrelia spp.) in Amblyomma varanense ticks on lizard (Varanus salvator ). Microbiology and Immunology, WILEY. doi: 10.1111/1348-0421.12721.
  - ➢ SUPRIYONO, Ryusei KUWATA, Shun TORII, Hiroshi SHIMODA, Keita ISHIJIMA, Kenzo YONEMITSU, Shohei MINAMI, Yudai KURODA, Kango TATEMOTO, Ngo Thuy Bao TRAN, Ai TAKANO, Tsutomu OMATSU, Tetsuya MIZUTANI, Kentaro ITOKAWA, Haruhiko ISAWA, Kyoko SAWABE, Tomohiko TAKASAKI, Dewi Maria YULIANI, Dimas ABIYOGA, Upik Kesumawati HADI, Agus SETIYONO, Eiichi HONDO, Srihadi AGUNGPRIYONO, Ken MAEDA (2020). Mosquito-borne viruses, insect-specific flaviviruses

(family Flaviviridae, genus Flavivirus), Banna virus (family Reoviridae, genus Seadornavirus), Bogor virus (unassigned member of family Permutotetraviridae), and alphamesoniviruses 2 and 3 (family Mesoniviridae, genus Alphamesonivirus) isolated from Indonesian mosquitoes. The Journal of Veterinary Medical Science. Advance Publication.

➢ Ronald Tarigan, Hiroshi Shimoda, Karla Cristine C. Doysabas, Maeda Ken, Atsuo Iida, Eiichi Hondo (2020). Role of pattern recognition receptors and interferon-beta in protecting bat call lines from encephalomyocarditis virus and Japanese encephalitis virus infection. Biochemical and Biophysical Research Communications ELSEVIER. https://doi.org/10.1016/j.bbrc.2020.04.060

## 3. History of PDM Modification

Project Design Matrix (PDM) was modified twice during the project term.

### 3-1. Determine the number of the articles.

(a) When the RD was tied up and the project started, one of the indicators to measure the degree of achievement of the project purpose was to set the number of papers written by Indonesian researchers. After the project started and the skills of Indonesian researchers and the progress of research were observed, it was discussed within the member of the project that the number of the articles.

(b) At the 2nd JCC meeting held in November 2016, one of the indicators of the project purpose, the number of papers to be written, was decided to be "5". This new number was recorded in Minutes of Meeting of JCC Meeting.

(c) New “Objectively Verifiable Indicators” is as follows:
More than 5 research articles related to the Project, in which first author is Indonesian researchers or comparative responsibility with first author, are published in academic journals.

### 3-2. Additional sampling location, West Kalimantan.

(a) As the result of the three years’ sampling from the Leuweung Sancang

Conservation Area in Garut, West Java, only the isolation of orthoreovirus was achieved. Under these circumstances, the project wanted to sample flying foxes in West Kalimantan, where the incidence of rabies was higher than in West Java and wanted to conduct a comparative survey.

(b) Both Indonesian and Japanese researchers agreed and decided to add this area to the sample location since the high catch of edible flying foxes by local residents around Pontianak, West Kalimantan was observed.

(c) The project first received consent from the director of Research Center for Biology, Indonesian Institute of Science, LIPI on this matter and asked them to prepare a recommendation letter to the Directorate of Biodiversity Conservation, Directorate General of National Resources and Ecosystem Conservation, Ministry of Forestry. The project brought this letter and met with the Director, Dr. Indra Exploitasia in person, Directorate of Biodiversity Conservation, Directorate General of National Resources and Ecosystem Conservation, Ministry of Forestry to explain the need for additional flying fox capture sites.

(d) The project was formally approved by the Director, and West Kalimantan was added to the sampling site.

(e) As a result, West Kalimantan has been added to the PDM Project site as follows:

"Project site: West Java (Garut and Bogor) and West Kalimantan.

(f) Also, Output 3 was changed as follows:

3. Behavioral information of flying foxes in and surrounding the province of West Java and West Kalimantan are reported.

### 3-3. Project member list

There were several changes in each year. All changes were reported in JCC Meeting and approved. They were all recorded in the Minutes of Meeting of JCC Meeting and the project member list were revised each time.

## 4. Others

4.1. Results of Environmental and Social Considerations

(Not applicable)

4.2 Results of Considerations on Gender/Peace Building/Poverty Reduction

(Not applicable)

## III. Results of Joint Review

### 1. Results of Review based on DAC Evaluation Criteria

1-1. **Relevance** (Consistency of this project with development policies, high-level plans and needs etc. to the partner country)

Review result: **HIGH**

Currently, people are still dying in Indonesia due to rabies and zoonotic diseases derived from wild animals. As can be seen from the statistics of the Directorate of Animal Health, Directorate General of Livestock and Animal Health, Ministry of Agriculture for the past five years, it cannot be said that zoonotic diseases such as rabies have decreased in the 34 provinces of Indonesia.

Although flying foxes are already known as a host for rabies, the SATREPS project is based on the idea that flying foxes living in Indonesia, which has a vast land area, may also be a host for rabies and some infectious disease viruses, then it started in 2015. At the same time, the Indonesian government is focusing on eradication of rabies and other zoonotic diseases in 34 states (mainly by the Ministry of Agriculture, Ministry of Environment and Forests, and Ministry of Health). It can be said that the project has the same purpose in a sense, in line with the policy of the Indonesian government.

Strengthening the ability of Indonesian researchers to grasp the actual situation of infectious diseases, which is also the project purpose, means that Indonesian researchers can perform virus culture, isolation, and genome analysis by correctly identifying infectious diseases derived from wild animals including flying foxes. It is expected that this will be possible through this project, which will lead to the prediction and prevention of infectious diseases not only in Indonesia but also in Asia.

In fact, while the new coronavirus (COVID-19) is sweeping the world, it can be said that the start of research on viral infectious diseases

derived from wild animals in this SATREPS Project is completely in line with Indonesia's national policy, and it is an indispensable field and must be studied more and more in the future.

1-2. **Effectiveness** (Achievement level of the project purpose, influence of impediments, relations between outputs and project purposes, etc.)

Review result: **HIGH**

Project purpose of this project is "Research capacity of Indonesian researchers in the field of rabies-related and other viral infectious diseases derived from flying foxes is strengthened." Indicators for evaluating the level of achievement of the project purpose were also defined at the starting point of this project. As already described in Section II of this Report (Result of the Project), all these indicators have been achieved successfully by the Project.

Input from Government of Japan (GOJ) is described in Section II.1 (Result of the Project). Although the import of equipment was delayed, all of them are installed in IPB and their use is amazingly effective. Anyway, after the equipment arrived safely at Faculty of Veterinarian Medicine, (FKH), IPB, the properties of rabies-related and other infectious disease viruses derived from Flying Foxes were clarified by virus isolation and genetic analysis, and many ELISA experiments conducted by Indonesian researchers. It can be evaluated that the effectiveness of implementing the project was high because the serological test method was established by the Project.

There are two other items that have led to the success of the project purpose. One is that in the ecological study of flying foxes. Japanese researchers and Indonesian researchers have teamed up to investigate both daytime and nighttime activities over a long period of time. It can be said that it was a great achievement to be able to observe the Flying Fox's behavior in detail even to the fact that they are contacting with humans. Using the Argos Satellite system gave the Project a lot of new findings. Especially, the flight paths and distances of flying foxes were observed and one of the results was closely related to food in their roosting site. It can also be said that the effectiveness of the observations was extremely high. Second, Indonesian researchers have gained access to a wildlife infectious disease database, primarily

owned by the Ministry of Agriculture. It is the result of this project's frequent approach to the Ministry of Agriculture. Gaining the access makes possible to connect and analyze not only the data of the laboratory of IPB but also the data of the whole country stored in Ministry's data base system. It was remarkably effective for Indonesian researchers.

1-3. **Efficiency** (Relations with the achievement level of inputs and outputs, etc.)

Review result: **HIGH**

Both GOI and GOJ contributed inputs to the Project, including budget, experts, equipment such as PCR (Polymerase Chain Reaction) and NGS (Next Generation Sequencer), Laboratorium, and office space. These inputs need to be secured at the right time because the sampling time is limited, and the timing of virus isolation, ELISA, and genome analysis with PCR or NGS should be scheduled by considering the timing of dispatching the Japanese researchers who convey technology and knowledge. Because of the cooperation period is five years, researchers on the Indonesian side and the Japanese side had to always consider its timeline and the timing of the inputs. Most of these inputs were implemented based on the planned schedule and utilized by the Project to achieve all indicators for project outputs and project purpose as described in Section II (Results of the Project). Thus, efficiency of this project is regarded as high.

1-4. **Impact** (Contribution to the achievement level of the overall goal, level of contribution to policies and communities, contribution to other projects, etc.)

Review result: HIGH

The major impacts from this project are given to the local government such as the Department of Health, Department of Environment and Forestry, and Department of Agriculture, where the Project captured flying foxes, i.e. Garut regional office, West Java Province and Pontianak city office, West Kalimantan Province. Also, the reports from the Project on the results of ELISA or virus isolation, etc. gave the remarkable impact to the Ministry of Agriculture, Ministry

of Environment and Forests, and Ministry of Health.

Although it is an ELISA antibody reaction, the agricultural departments of both provinces where Australian bat lyssavirus and Nipah virus were detected (neutralization tests and virus isolation must be done though) assume that a very dangerous infectious diseases are lurking in the wild animals, very close to their villages.

One of the central Government, Directorate of Animal Health, Directorate General of Livestock and Animal Health, Ministry of Agriculture is also incredibly surprised to these results. Since Nipah virus is biosafety level 4, the Directorate of Animal Health has decided that it is quite necessary to start with a degree of enlightenment to provincial government, regional office, residents of villages informing like "wild animals often have rabies-like diseases, so avoid contact as much as possible" to avoid any panic.

In response to this situation, IPB Indonesian researchers have gathered residents of villages near the Leuweung Sancang Conservation Area, near Garut, West Java, and staff of Agricultural and Livestock Department, staff of the Health Department, and the staff of the Environmental Forest Department in West Java provinces and held the seminar to inform the risks of the rabies and other related infectious diseases derived from the wild animals.

In this way, the data obtained in the field can have a strong impact not only on researchers but also on government officials, not only achieving project purpose, but also supporting the activities of local residents and various central ministries in and local agencies.

1-5. **Sustainability** (Likely continuation from the aspects of policy, technology, organization, finance, etc.)

Review result: **HIGH**

Sustainability is also a particularly important point in this SATREPS project. A lot of equipment imported from Japan, including Real-time PCR, NGS (Next Generation Sequencer), and BSL3 Laboratory constantly require high running and maintenance costs. Immediately after the project started, the Dean of Faculty of Veterinarian and Medicine, IPB, Prof. Srihadi Agungpriyono and Chief advisor of the Project, Prof. Eichi Hondo and the Project coordinator

discussed these issues. Initially, the project sought a grant by the Indonesian government and applied for it in an attempt to obtain ample operational funding after the Project. However, there are very few public offering programs offered by the Indonesian government, it seemed difficult to get the grantse.

The project team had to consider another way for securing the maintenance and operation cost. Professor Mizutani of Tokyo University of Agriculture and Technology considered possibilities of collaboration between FKH and IPB and one of Japanese private companies. In order to promote research on the effects of sterilizer that the private Japanese company is going to sell in Indonesia, the company is setting up its branch at FKH and conduct research with experiments at FKH's SATREPS lab by the Indonesian researchers.The company will be asked to bear the cost of reagents for operating NGS, IPB's BSL3 lab to use the equipment for their research. In this way, the program that draws Japanese private companies to the IPB University side will enable to secure part of the operation cost of SATREPS laboratories regardless of the limited FKH budget.

In addition, in Indonesia, there are few researchers who can analyze the results of NGS even if they own NGS. On the other hand, the ability of IPB Indonesian researchers who have achieved results in the SATREPS lab is exceptional. The Project team went to see the Director of Directorate of Animal Health, Directorate General of Livestock and Animal Health, the Ministry of Agriculture many times to explain the importance of NGS operation method and data analysis method, and succeeded in analyzing the viruses stored by their Laboratories at the SATREPS Lab. Since the director has increased the reliability of IPB's SATREPS lab, it should be inquired in the future for analysis of unknown viruses.

## 2. Key Factors Affecting Implementation and Outputs

1) Sampling of Flying Foxes

In carrying out this project, flying foxes must first be captured in Garut, West Java and Pontianak, West Kalimantan. It is necessary to

ensure that flying foxes are captured during the scheduled experiment period, taking into consideration the rainy season, the dry season, whether the fruits as their food are abundant harvests or poor crops, and smoke damage caused by shifting cultivation.

In addition, it is necessary to receive an annual recommendation letter for the capture of flying foxes from LIPI, obtain approval from the Directorate of Biodiversity Conservation, Directorate General of National Resources and Ecosystem Conservation, the Ministry of Environment and Forestry, and also receive a final permit from BKSDA in West Java and West Kalimantan.

Moreover, permission from the Quarantine Department of the Directorate of Animal Health, Directorate General of Livestock and Animal Health, Ministry of Agriculture is required to transport flying foxes' organs which were dissected from Pontianak, West Kalimantan, to Bogor, West Java, where IPB University is located.

2) Biosafety system

A biosafety level 3 facility was set up at the IPB's Faculty of Veterinary Medicine, despite a year and a half delay in importing the equipment from Japan. In order to catch up with the schedule, the IPB Biosafety Committee was immediately set up, the rules / regulations and SOPs were quickly created, and the foundation for safely handling and experimenting with pathogens was established. The important point is that the IPB's Biosafety Committee has created a system in which users are given training and tests, and permission to enter the room and research is granted.

3) Importing the equipment from Japan properly

Since 2015, the Indonesian government has changed the import system for project equipment from public institutions such as JICA without detailed notification. From the perspective of protecting the industry of the country, the Indonesian government insisted that even publicly imported goods should follow the same procedures as commercial cargo. The problem was that, not only JICA, but also the Indonesian government's SETNEG did not know that such changes would be implemented, which initially caused great confusion for all

parties. Eventually, the Project obtained a license for import permission from the Ministry of Commerce, Ministry of Health, and Ministry of Energy and Mineral Resources, and especially for the freezer whose import was restricted by the Indonesian government, the JICA Indonesia office wrote a letter to the Minister of Commerce of Indonesia and finally the Project were able to receive all equipment. Although the import of equipment was delayed for a year and a half from the original plan, we tried to minimize the impact of the delay by conducting an ecological survey and study of flying foxes first, and to prevent any problems in the subsequent project research. Such sudden policy changes of the partner government can occur from time to time, but once the facts are understood, it is strongly required that the Project and related organizations ought to work together quickly to respond to the problem.

4) Communications and capacity building

One of the most important points in the implementation and outcome of the project is communication between the researchers of the both countries involved. In particular, the Project asked Japanese researchers who stay in Indonesia for a long time to participate in the mutual communication network using WhatsAPP as well as online mail. On top of that, the Project requested both Indonesian and Japanese researchers always send and share information.

## 3. Evaluation on the results of the Project Risk Management

### 3-1. Risk Management Results

a. Sampling of Flying Foxes

- The capture of Flying Foxes

Prior to the commencement of the project, the Indonesian government (LIPI and the Ministry of Environment and Forestry) promised the Project to capture 100 flying foxes each year for research. However, it has gradually become clear that the flying foxes are migrating to roosting sites differently from those of the previous year and a few years ago due to delays in

the rainy and dry seasons, smoke damage from shifting cultivation, and abundant and poor crops of flying foxes.

There once was the worst case that even if the schedules of Japanese researchers and Indonesian researchers were adjusted and sampling was planned (Japanese researchers flew from Japan to Pontianak, West Kalimantan, Indonesia), only four (4) flying foxes were caught. Through this experience, for research on these natural phenomena, the Project often asked the local hunters and BKSDA (Department of Natural Resources and Ecosystem Conservation) staff about the migration condition of flying foxes in the local location. If the number of flying foxes is small, we will request them to capture flying foxes from a month or two in advance, and have them bred at BKSD facilities, etc., aiming to secure the number of flying foxes according to the sampling time.

- The many complicated administrative work for capturing Flying Foxes.

  At the stage of RD preparation, the Ministry of Environment and Forestry promised to catch 100 flying foxes a year for the project, but the Project had to face the many administrative work such as the permit application includes the acquisition of the recommendation letter from LIPI, and the acquisition of the approval letter from the Directorate of Biodiversity Conservation, Directorate General of National Resources and Ecosystem Conservation, Ministry of Environment and Forestry. In addition to central approval, the next step required permission from the provincial government to capture flying foxes. Once this was done, permission from the prefectural office (BKSDA / Department of Natural Resources and Ecosystem Conservation) at the capture site was also required. Since it takes about half a year to complete all the procedures from the recommendation letter from LIPI to the final approval at BKSDA, it will not be possible to make the sampling in time unless the paperwork is done early, so the Project has to pay close attention.

- The Quarantine and 48 hours

  Sampling in Pontianak, West Kalimantan will require an application to the Ministry of Agriculture's quarantine station to transport flying fox organs by air. Normally, organs need to be stored at 4 ° C and placed in a freezer at -80 ° C within 48 hours, so project staff will be assigned to the quarantine station in advance to prevent problems at airport quarantine stations in Pontianak and Jakarta.

b. Biosafety system

- Establishment of BSL2 and BSL3 Laboratories

  In addition to the BSL2 lab, the BSL3 lab was set up to deal with rabies virus, related viruses, and unknown infectious disease viruses derived from flying foxes. To protect the safety of researchers, everyone should change shoes, jackets in the BSL2 Lab, wear appropriate gloves, and use autoclaves for waste, and the Project held the safety seminars for staff handling waste.

  In the BSL3 lab, the entire room itself is assembled including the doors, the ceiling, and air-conditioner, and the whole parts were imported from Japan. Six staff members of Nihon Airtech, the contractor of the BSL3 Lab, came all the way from Japan to IPB, Indonesia to carry out the assembly work.

  The IPB immediately set up a biosafety committee, and Dr. Shinohara from the National Institute of Infectious Diseases in Japan, to hold a seminar for BSL3 to deepen the understanding of Indonesian researchers so that they can conduct research safely. The most important point for the risk management for the Biosafety Level 3 is the independent operation by the IPB Biosafety Committee, which shall conduct seminars and examinations for new researchers and only researchers who pass the exam can use the BSL3 facility.

- Establishment of Standard Operating Procedure (SOPs)

  In order to maintain safety in the laboratories, as well as

to obtain a reliable data without any contamination during the experiment, solid and appropriate SOPs are crucial. While referring to the BSL2 SOP and the regulations of the Animal Ethics Committee that have been used in IPB so far, the Project created an SOP suitable for Indonesia based on the BSL3 SOPs of Nagoya University.

c. GOI Regulation related to the Equipment Import

- Change of Import Regulations

As described in 1-3. Activities (Planned and actual), Indonesian Government changed its import system since 2015. Before 2015, JICA Indonesia office got the permission of importing various kinds of goods and equipment from Japan in accordance with the letter from SETNEG (Ministry of State Secretariat of the Republic of Indonesia) called PP-19. However, Indonesia has reviewed the mechanisms, taxation, exports, imports, and other national policies, and has made it transparent.

The Indonesian government, led by the Ministry of Finance and the Ministry of Commerce, started revising the method of importing goods in 2015. Government agencies such as JICA were also requested the acquisition of import licenses for the import of equipment even for ODA projects, as same as commercial cargo. In this way, the Project was requested to acquire several licenses from the Ministry of Commerce, the Ministry of Mineral Resources, and the Ministry of Health for customs clearance of equipment from Japan.

It was extremely difficult for both JICA and the Project to import the equipment from Japan by acquiring several licenses from those Ministries as this was the very first time. The Project collected several licenses but must ask JICA Indonesia office to prepare the special letter to the Minister of Commerce, GOI in order to obtain the import permission especially for the freezers and air-conditioner for BSL3 room. And these freezers needed the pre-inspection in Japan to get the special letter for the import custom clearances in Indonesia.

Finally, every equipment was delivered after those

complicated procedures. It took for about one year and half. The project carried out field ecology surveys of flying foxes, responded to delays in the arrival of equipment from Japan, and did not pose a major problem as a five-year project. Nevertheless, the enormous amount of work had increased, and there was no doubt that it had become a major obstacle to the original work execution of the project. It will be very important to explain these experiences to other projects in advance in Indonesia so that they do not face the same problems.

d. The Blackout

- The Frequent Blackout and IPB's response

It can be said that a blackout occurs once a month in Bogor. Power outage can be caused by rolling blackouts from the central electric power company (PLN) and equipment failures or malfunctions that occur on campus or in Bogor city. Although rolling blackouts are notified in advance, there are more power outages caused by sudden electrical component failures. The power outage caused by the failure of this electric component is very troublesome because it is unknown how many hours it will last. In the Project, the organs of flying foxes are stored in three -80 ° C freezer, so how to back up the power supply of these freezers in the event of a power outage becomes a big and important issue.

FKH had a large generator that covered the entire faculty, but it had been out of order for a long time. In December 2018, FKH's large generator was repaired and put into operation. This has made it possible to secure a safer power source for the freezers even if a power outage occurs.

- The Countermeasure for the Blackout

In response to the repeated power outages, the Project installed stabilizers between the freezers and the power supply to prevent the damage to the freezers caused by the unstable power transmission .

Another issue is the manpower which can be

necessary in time of the Blackout. Even if the stabilizer was turned on, if there was a power outage, a technician was needed to connect these freezers to a small generator, return to power transmission via the stabilizer when the main power supply was restored.

Even if there was a blackout on Saturdays, Sundays, and holidays, FKH's two engineers could visit FKH's SATREPS lab to secure power for the freezer.

The project was able to respond to frequent blackout by having two engineers pay a power outage fee to carry out the mission. The Project would like IPB to sustain the follow-up system by these engineers so that the SATREPS Lab is maintained and operated appropriately even at the time of the power outage.

3-2. Results of the Use of Lessons Learnt

a. Sampling of the Flying Foxes

The capture of flying foxes in the Project is agreed by the time of signing the R/D, but it took some time to grasp the actual procedure flow. First, obtain a recommendation letter from LIPI (specify the number and location of capturing flying foxes), and submit the application letter of catching flying foxes to Directorate of Biodiversity Conservation, Directorate General of National Resources and Ecosystem Conservation, Ministry of Environment and Forestry. If permission is given, submit the application for confirmation of capturing flying foxes to the Provincial office of Environment and Forestry in the location where the Project would like to conduct the sampling of Flying Foxes. In addition, procedures such as permission to carry the sampled flying foxes to the IPB and permission from the quarantine office to carry the organs of flying foxes were also required.

In the first year, the Project was overwhelmed by these complicated procedures, but from the following year, the Project was able to know when it was appropriate to carry out such

paperwork, and able to proceed smoothly according to the sampling schedule. In Indonesia, it often takes a few months to get a permit, which seems easy to obtain, so it was very efficient to be able to apply the experience of the first year to the next year.

b. Import Regulations

As mentioned earlier, Indonesia's import procedure has undergone a major system review, and it is necessary to take necessary action of the import considering the duration of the procedure.

## IV. For the Achievement of Project Purpose after the Project Completion

### 1. Prospects to Achieve Project Purpose

Project Purpose:

Research capacity of Indonesian researchers is enhanced through the genetic analysis based on the characteristics of rabies-related and other viral infectious diseases derived from flying foxes.

Prospects

There are several prospects for achieving project purpose as below.

a. Capacity on isolation and identification of infectious diseases derived from flying foxes is improved.
   This prospect will be realized through frequent implementation of genetic analysis by Indonesian researchers.
b. Behavioral information of flying foxes is able to be collected and analyzed by Indonesian researchers.
   This prospect will be realized through research collaboration with local official office such as Forestry or Livestock offices.