

*e-ASIA Joint Research Program  
Progress Report*

1. Project Title: 「Evaluation of the pandemic potential of H5N1 highly pathogenic avian influenza viruses circulating in Indonesia」

2. Joint Research Period: April 01, 2015 ~ March 31, 2018

3. Principal Investigators:

Country 1: Yoshihiro Kawaoka, Professor, Univ. of Tokyo, Japan  
Planned Funding Period: April 01, 2015 – March 31, 2018

Country 2: Chairul A. Nidom, Professor, Airlangga Univ., Indonesia  
Planned Funding Period: April 01, 2015 – December 30, 2015

Country 3: Gabriele Neumann, Research Professor, Univ. of Wisconsin-Madison, USA  
Planned Funding Period: None

4. Summary of the Progress of the Joint Research:

Highly pathogenic H5N1 avian influenza viruses have caused outbreaks among poultry in Indonesia every year, leading to human infections. It is imperative that we understand the current situation regarding H5N1 virus circulation in Indonesia and characterize these viruses to assess their pandemic potential and to prepare for a potential H5N1 pandemic.

To this end, the Indonesian research team has strengthened its influenza surveillance systems to collect swab samples from poultry and pigs in outbreak areas. For example, they have established a project to collect samples continually on Madura Island, East Java. Furthermore, they have strengthened their relationship with the local government of West Java province so that H5N1 viruses isolated in West Java can be promptly provided to the Indonesian team. In 2015 and 2016, the Indonesian team conducted surveillance on Java Island and also on Kalimantan Island. They sent 1476 swab samples (496, 120, and 860 samples collected in 2016, 2015, and 2014, respectively) to the Tokyo research team. The Indonesian team also sent 828 swab samples collected in 2014 and 2015 and 70 swab samples collected between 2011 and 2014 to the US research team. They isolated H5N1 viruses in their BSL3 facility and 61 influenza PCR-positive samples were sent to the US team.

The Tokyo research team isolated viruses from the swab samples collected by the Indonesian team by using embryonated chicken eggs in their BSL3 facility. From these samples, 19 H5N1 viruses were isolated (six, one, and 12 H5N1 viruses were isolated from samples collected in 2014, 2015, and 2016, respectively). Phylogenetic analysis of the hemagglutinin (HA) genes showed

that all 19 H5N1 viruses belonged to clade 2.3.2.1. Clade 2.3.2.1 viruses were thought to have been introduced from Vietnam in 2012. Whole genome sequencing showed that all of the other segments of the viruses isolated in 2014 and 2015 also originated from clade 2.3.2.1 viruses. However, some of the viruses isolated in 2016 possessed a PB2 segment from a different source. Blast searches showed that the virus possessing the closest PB2 was a duck H5N2 virus isolated in Malaysia in 2004, suggesting that reassortment occurred between the H5N1 viruses and viruses of a different subtype. Analysis of the HA and neuraminidase (NA) amino acid sequences revealed that none of the isolates have acquired any mutations that are known to confer human-type receptor recognition or resistance to NA-inhibitor drugs, respectively. In 2015 and 2016, the Tokyo research team visited Indonesia and conducted surveillance with the Indonesian team in areas of East Java and West Kalimantan.

The US team has conducted virus isolation on 420 of the 828 samples collected in 2014 and 2015, but no virus has been isolated so far. From the 70 samples collected between 2011 and 2014, 9 viruses were isolated and 6 samples were RT-PCR positive. None of the 61 influenza PCR-positive samples they received contained infectious virus.

In summary, we have strengthened the influenza surveillance systems and collected swab samples in Indonesia. From these samples, we have isolated H5N1 viruses currently circulating in Indonesia and have molecularly characterized them. The information from this study improves our understanding of the current situation regarding H5N1 virus circulation in Indonesia.

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

### 5-1. Training

The Tokyo team provided training and technical support to the Indonesian team to improve their ability to genetically and biologically analyze influenza viruses.

March 7–18, 2016: Shinya Yamada at Airlangga University, Indonesia

March 6–10, 2017: Kiyoko Iwatsuki-Horimoto at Airlangga University, Indonesia

March 21–30, 2017: Shinya Yamada at Airlangga University, Indonesia

### 5-2. Workshop

None

### 5-3. Publication

None

### 5-4. Oral Presentation

- Takeaki Imamura. Analyses of H5N1 highly pathogenic avian influenza viruses in Indonesia. Sixth Negative Strand Virus-Japan. Okinawa, Japan. Jan 2017.
- Chairul A. Nidom. Update of pandemic & H5N1 viruses in Indonesia. Swiss-

Indonesia Vaccine Formulation Symposium. Surabaya, Indonesia. Mar 2017.

- Reviany V. Nidom, Ema Qurnianingsih, Kuncoro P. Santoso, Rahmalia D. Suindari, Ulvie A, Almira F. Lahay, Setyarina Indrasari, Kadek Rachmawati, Chairul A. Nidom. Construction of Indonesian-strain avian flu virus seed vaccine using low pathogenic hemagglutinin gene and neuraminidase PR8 gene through reverse genetic. Swiss-Indonesia Vaccine Formulation Symposium. Surabaya, Indonesia. Mar 2017.
- Ema Qurnianingsih, Kadek Rachmawati, Ira Humairah, Kuncoro P. Santoso, Rahmalia D. Suindarti, Ulvie Apritasari, Reviany V. Nidom, Chairul A. Nidom. Growth optimization of H5N1 influenza vaccine seed strain Indonesia low pathogenic viruses by reverse genetic in MDCK cell. Swiss-Indonesia Vaccine Formulation Symposium. Surabaya, Indonesia. Mar 2017.
- Kadek Rachmawati, Ema Qurnianingsih, Kuncoro P. Santoso, Rahmalia D. Suindari Ulvie Apritasari, Muh Y. Alamudi, Reviany V. Nidom, Chairul A. Nidom. Mutation of H5N1 influenza viruses as a vaccine seed in a variety of hosts by the molecular studies of hemagglutinin and neuraminidase fragment. Swiss-Indonesia Vaccine Formulation Symposium. Surabaya, Indonesia. Mar 2017.

5-5. Patent,  
None

5-6. Award

Yoshihiro Kawaoka

2015	UNESCO Carlos J. Finlay Prize for Microbiology
2016	Fellow of the American Academy of Microbiology
2016	Japan Academy Prize

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

In Indonesia, as of March 2017, the total number of human cases infected with H5N1 viruses reached 199; the second largest number in the world. Although no human cases were reported in 2015 or 2016, H5N1 viruses continue to cause outbreaks among poultry in many parts of Indonesia, suggesting the possibility that circulating H5N1 viruses could infect humans. In this project, we are characterizing the H5N1 viruses currently circulating in Indonesia to assess their pandemic potential and to prepare for a potential H5N1 pandemic. The comprehensive characterization of H5N1 viruses currently circulating in Indonesia is critical to determine whether such viruses pose a pandemic threat. This project will identify novel molecular markers of H5N1 adaptation to humans. Such information is essential for effective preparedness for and prevention of pandemics caused by H5N1 viruses not only in Indonesia but in other parts of the world.

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

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

None