Report for OIE twinning project on avian influenza between Japan and Mongolia (2016-2018)

Parent laboratory: Hokkaido University Research Center for Zoonosis Control, OIE Reference Laboratory for Highly Pathogenic Avian Influenza and Low Pathogenic Avian Influenza, Japan

Candidate laboratory: State Central Veterinary Laboratory, Ministry of Food, Agriculture and Light Industry, Mongolia

ULAANKHUU Ankhanbaatar, DVM, Msc

August 21, 2018
Ulaanbaatar, Mongolia

The main activities of the OIE twinning project involved 6 objectives, as stated below:

1. Proficiency test for gene detection of avian influenza virus (AIV) by Reverse Transcription Polymerase Chain Reaction (RT-PCR) method

2. Improvement of antigenic characterization of isolated viruses by hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests

3. Improvement of the assessment of pathogenicity of isolated viruses by genetic analyze

4. Establishment of phylogenetic analysis for molecular epidemiology of AIV

5. Experimental infection and pathology of avian influenza

6. Joint surveillance of avian influenza in migratory birds in Mongolia

Objective 1: Proficiency test of gene detection by RT-PCR method:

Purpose: To evaluate gene detection techniques for the recent H5 highly pathogenic avian influenza virus (HPAIV).

Performance: the gene of H5 avian influenza virus was detected from test samples.

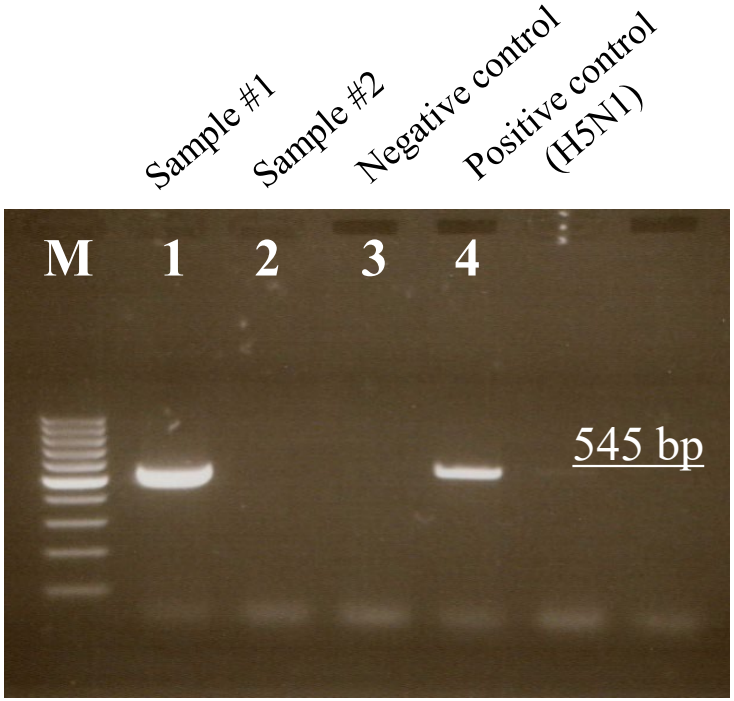


Fig. 1. Result of proficiency test for H5 avian influenza viruses

Status:

- Result of the proficiency test was approved.
- Molecular techniques of candidate laboratory for AIV detection could meet with real situation as emergency diagnosis and detection for recent H5 HPAIV.

Improvement:

- During the test performance, the candidate laboratory recognized that primer set for AIV diagnosis has to update by most recent and specific primer.
- The parent laboratory provided for updating primer sets to subtyping of H5 avian influenza virus with their protocols.

Objective 2: Improvement of antigenic characterization of isolated viruses by HI and NI tests:

Before:

- HI and NI testing skills
- Reagents
- Methods
- Antiserum list
- Virus list were not good.

After:

- After the training, HI and NI tests performed in the candidate laboratory with reference serums which introduced by OIE reference laboratory.
- The candidate laboratory could solve difficulty in preparation of the reagents required in NI test.
- Since re-establishment of the methods, the candidate laboratory can perform HI and NI test of avian influenza viruses.

Objective 3: Improvement of assessment of pathogenicity of isolated viruses by genetic analysis

Activity in Mongolia: To apply knowledge to diagnosis of AIVs.

- We performed proficiency test using learned method and result was HPAI virus indicated by multiple basic amino acid at HA cleavage site.
- Now, we have skill to characterize both HPAI and LPAI by cleavage site analysis.

Table 1. Determination of HA cleavage site and estimation of the pathogenicity.

№	Name	Length	Cleavage site	Pathogenicity	Subtype
1	Sample #1	907	PQREKR/GLF	LP	H5
2	Sample #2	412	RERRRKR/GLF*	HP	H5
3	Sample #3	973	RERRRKR/GLF	HP	H5
4	Sample #4	1625	PEEPKGR/GLF	LP	H7

*HPAIV contain multiple basic amino acids at the HA cleavage site.

Objective 4: Establishment of the methods of phylogenetic analysis for the molecular epidemiology

Activity in Mongolia: To apply the learned knowledge to AIV characterization.

- The trainee performed phylogenetic analyses on TADs such as FMD, Goat and sheep Pox, and PPR.

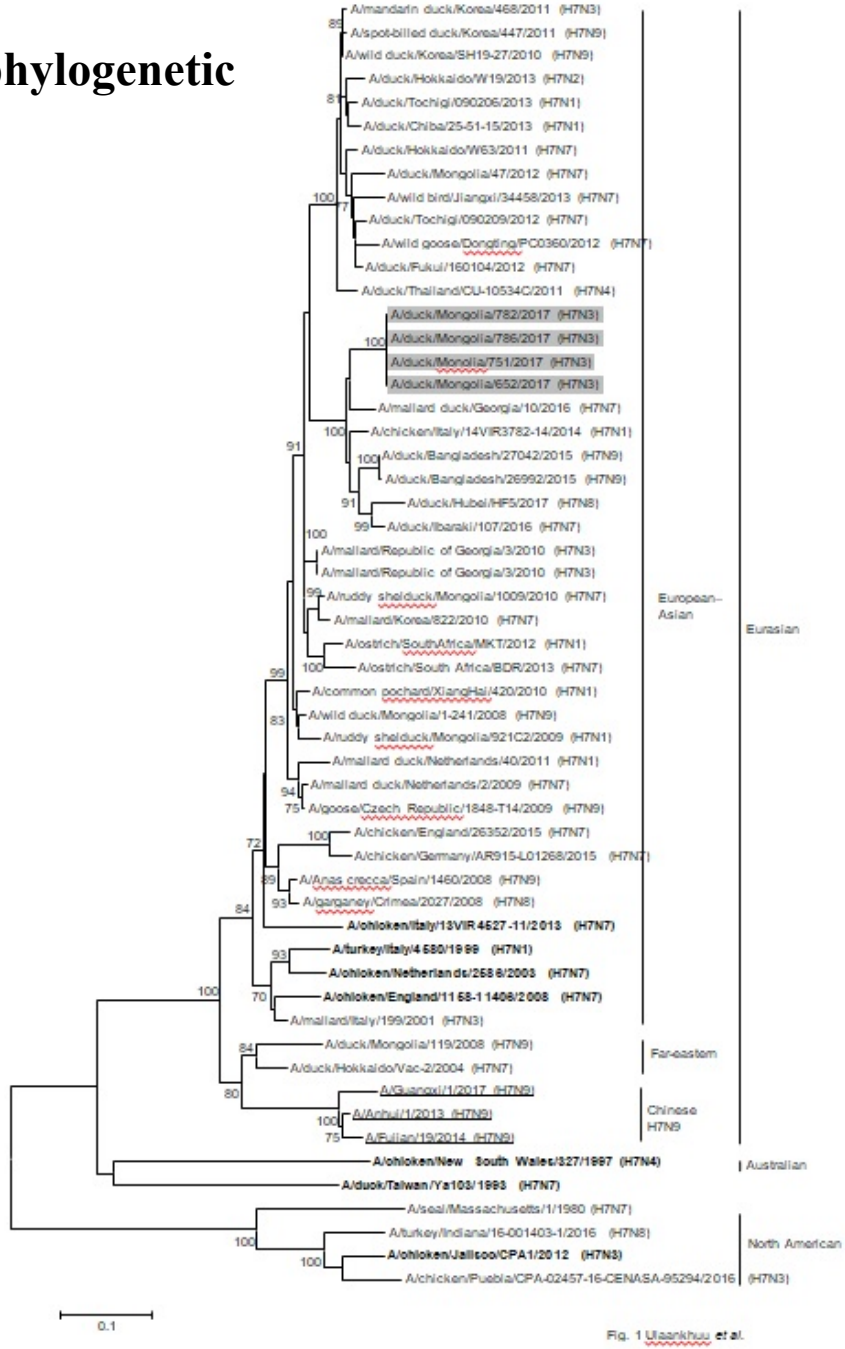


Fig 2. Phylogenetic tree of HA gene

Objective 5: Experimental infection and pathology of avian influenza

Activity in Japan: To perform experimental infection of H5N6 HPAIV in chickens and ducks and to diagnosis AIV.

- The parent laboratory organized experimental infection and it was first time conducted to virus inoculation into poultry using BSL-3 animal facility as research purpose following IVPI index calculation.
- We learned knowledge and skills listed below.
    - Working on BSL-3 animal facility
    - Perform animal experiment by HPAIV
    - Monitoring the AIV infection by clinical sign
    - Collection of the swab and tissue samples from infected poultry
    - Performing virological and pathological examination on dead poultry

Fig. 3. Chicken experiment with H5N6 highly pathogenic virus in the BSL 3 animal facility

Objective 6: Joint surveillance of avian influenza in migratory birds and poultry in Mongolia.

Since 2009, the research team of Hokkaido University collaborated with our laboratory to organize joint surveillance in Mongolia for avian influenza in migratory birds.

In 2016 and 2017, our laboratory shared samples collected for joint surveillance. By skill of virus isolation, subtyping of HA and NA and molecular genetic analysis, our laboratory isolated 2 (H3N8 and H4N6) subtype in 2016, isolated 3 (H2N2 and H4N6) viruses from the joint surveillance in 2017.

	2016 (100 samples)	**2017 (200 samples)**
Japan	2 (H3N8, H4N6)	3 (H2N2, H4N6)
Mongolia	2 (H3N8, H4N6)	3 (H2N2, H4N6)

Fig. 4. The joint surveillance of avian influenza in August 2015 and 2017 in Mongolia

Summary

		Before /Candidate laboratory/	After /Candidate laboratory/	Problems
1	Proficiency test of gene detection by RT-PCR method	**Primer preparation : △** Primer list : ○	○	-
2	Improvement of antigenic characterization of isolated viruses by HI and NI tests	HI : ○ **NI : ×** **Virus list : ×** Antiserum list : △	○	-
3	Improvement of the assessment of pathogenicity of isolated viruses by genetic analysis	BLAST: ○ **Alignment : ×**	○ Alignment: △	Animal facility laboratory
4	Establishment of the methods of phylogenetic analysis for the molecular epidemiology	**Not performed**	○	Learn more
5	Experimental infection and pathology of avian influenza	-	△ *But the candidate laboratory does not have animal facility laboratory.	Animal facility laboratory
6	Joint surveillance of avian influenza in migratory birds and poultry in Mongolia	Joined	Joined	-

○ : good, △ : not bad, × : learn more

Acknowledgement:

We would like to express our special thanks to the parent laboratory (Hokkaido University Research Center for Zoonosis Control, OIE Reference Laboratory for HPAI and LPAI), as well as our principal (the OIE), which gave us the golden opportunity to implement this fruitful project facilitating our research.

Joint Closing Meeting of OIE Laboratory Twinning Projects on FMD and HPAI between Japan and State Central Veterinary Laboratory of Mongolia
August 21, 2018, Ulaanbaatar, Mongolia

北海道大学

How we transferred our research mind, knowledge and skills to the staffs of SCVL

Yoshihiro Sakoda, DVM, PhD

OIE Reference Laboratory for HPAI and LPAI

Faculty of Veterinary Medicine
Hokkaido University, Sapporo, Japan

Background (1)

2001- present :

Joint surveillance of avian influenza for wild migratory birds in Mongolia

Table 4
Isolation of avian influenza viruses from fecal samples of migratory waterfowl in Mongolia.

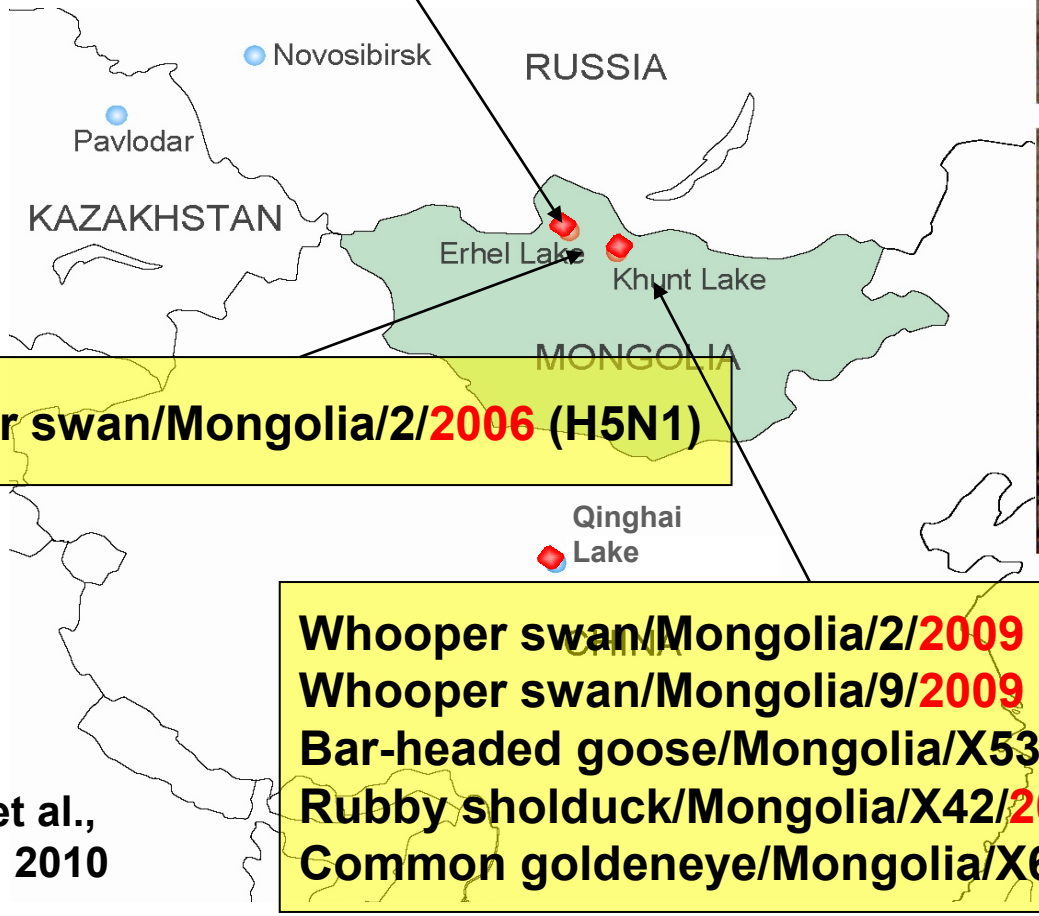
Sampling date	Name of lakes	Isolated viruses/Total samples	Subtypes of viruses[a] (No. of isolates)
Sep., 2001	Ugii, Doityn tsagaan,	37/725	H1N1 (1), H3N2 (1), H3N6 (3), H3N8 (11), H4N2 (1), H4N6 (12), H5N2 (1), H5N3 (2), H7N1 (1), H10N3 (4)
Sep., 2002	Erkhel, Ugii	109/959	H1N1 (3), H3N3 (2), H3N6 (20), H3N8 (53), H4N6 (12), H4N7 (1), H4N8 (1), H7N1 (1), H7N7 (9), H8N4 (5), H10N7 (1), H12N5 (1)
Sep., 2003	Ugii,	68/750	H1N1 (1), H2N3 (1), H3N6 (6), H3N8 (28), H4N2 (1), H4N6 (25), H9N2 (1), H10N5 (5)
Sep., 2005	Ugii,	32/476	H3N2 (1), H3N6 (2), H3N8 (10), H4N6 (6), H8N4 (1), H10N3 (11), H10N7 (5)
Aug., 2006	Khunt, Ugii, Borgin, Shorvog, Baga Tsaisam, Duut, Ikh Tsaidam, Doityn tsagaan	18/545	H2N2 (1), H3N8 (8), H4N6 (9)
Aug., 2007	Khunt, Ugii, Dunt, Ikh Tsaidam, Doityn tsagaan	20/943	H3N8 (14), H4N3(1), H7N6 (1), H7N7 (4)
Aug., 2008	Khunt, Ugii, Dunt, Ikh Tsaidam, Doityn tsagaan	40/792	H3N6 (3), H3N8 (23), H4N6 (8), H4N8 (3), H7N9 (3)
Aug., 2009	Ugii, Doityn tsagaan, Khunt Doroo, Sharga	9/1021	H1N8 (1), H3N8 (2), H4N6 (3), H8N4 (3)

Sakoda et al., Virology, 2010

Background (2)

Emergency diagnosis for wild birds in Mongolia

Whooper swan/Mongolia/3/2005 (H5N1)
Bar-headed goose/Mongolia/1/2005 (H5N1)
Common goldeneye/Mongolia/12/2006 (H5N1)



Whooper swan/Mongolia/2/2006 (H5N1)

Whooper swan/Mongolia/2/2009 (H5N1)
Whooper swan/Mongolia/9/2009 (H5N1)
Bar-headed goose/Mongolia/X53/2009 (H5N1)
Rubby sholduck/Mongolia/X42/2009 (H5N1)
Common goldeneye/Mongolia/X60/2009 (H5N1)

Sakoda et al.,
Virology, 2010

Background (3)

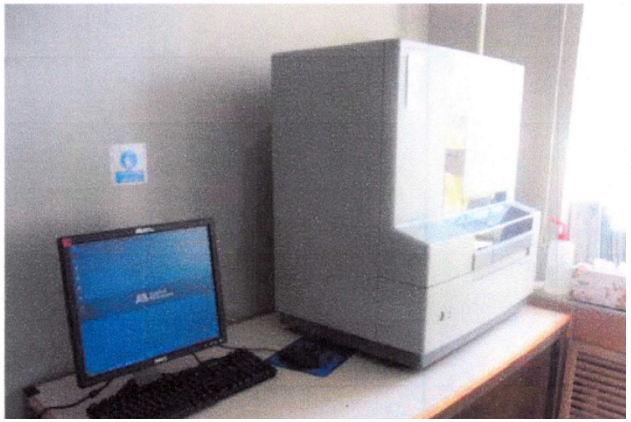
Organisation Mondiale de la Santé Animale

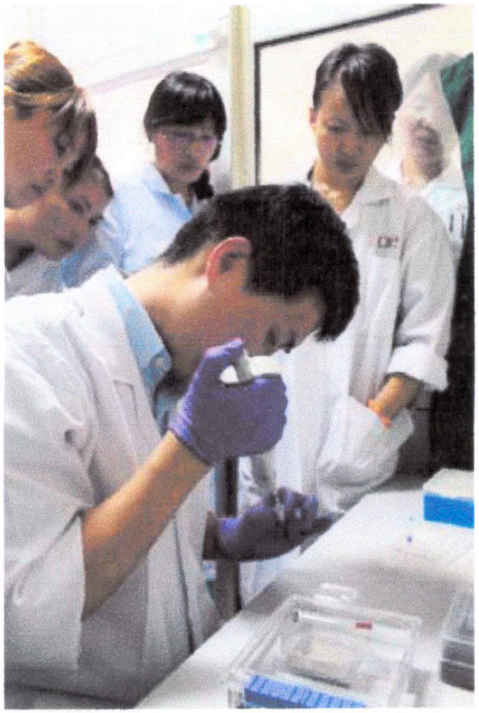
World Organisation for Animal Health

2009- 2012 :

OIE/Japan Trust Fund Project for Strengthening HPAI Control in Asia

2009





August, 2011

Background (4)

2014- present : Strengthening the capacity for human resource development in the field of Veterinary and Animal husbandry

WHAT WE DO

To support the advancement and strenghtening the capacity of SVM of MULS and MOFALI, this project consists of three main activities: Providing necessary equipment for the project, dispatching high level experts from Japan, and granting the opportunity for Mongolian professionals to be trained in Japan.

PROVIDE EQUIPMENT

JICA grants research equipment equal to the value of 4,076 million MNT

SEND EXPERTS

2014 ~ 2019
JICA dispatches approximately 100 high level experts on the field of veterinary medicine

TRAIN MONGOLIAN PROFESSIONALS IN JAPAN

JICA sends more than 50 Mongolian veterinary experts for training in JAPAN

PROJECT ACTIVITIES

Project for Strenghtening the Capacity for Human Resource Development in the Field of Veterinary and Animal Husbandry (VEP)

OIE Twinning Project for avian influenza

Period: September 2016-August 2018 (2 years)
Budget: Self-fund

1st term (Sep. 2016- Feb. 2017)

- Kick-off meeting for Twinning project
- Evaluation and improvement of the RT-PCR skills
- Field sampling (first round, in 2016) as joint work
- Training of invited trainee to improve laboratory skills

2nd term (Mar. 2017- Aug. 2017)

- Characterization of avian influenza viruses isolated from the field sampling
- Evaluation of first surveillance activities and solve a problem
- Training of invited trainee to improve laboratory skills

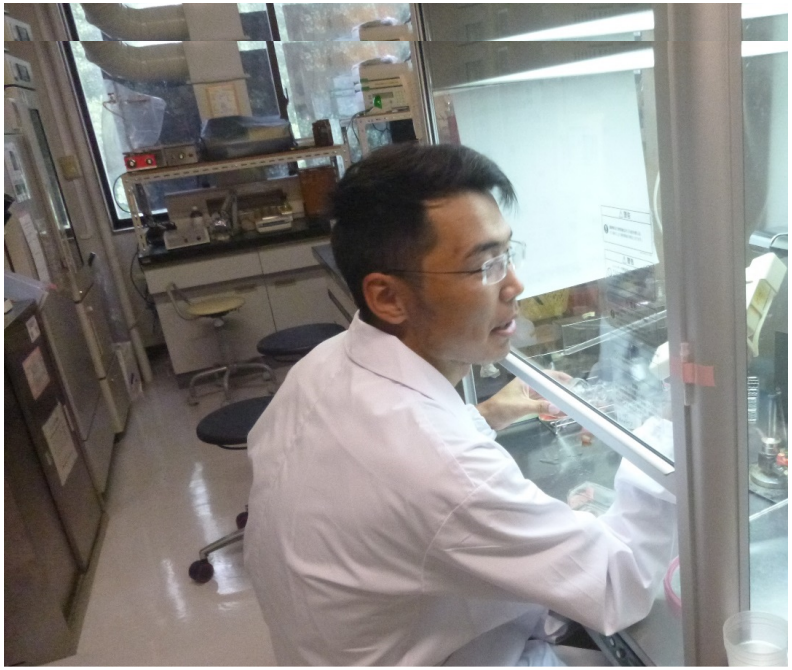
3rd term (Sep. 2017- Feb. 2018)

- Second round field sampling in 2017 as joint work
- Characterization of avian influenza viruses isolated from the field sampling
- Invitation and training for top management and senior researcher

4th term (Mar. 2018- Aug. 2018)

- Joint paper by Mongolian researchers
- Evaluation of diagnosis and research skills of candidate laboratory
- Wrap-up discussion to close twinning project and new proposal of future collaboration.

Training in Sapporo



Ulaankhuu A.

August 19, 2016 – October 14, 2016
(2 months)

July 1, 2017 – August 31, 2017
(2 months)

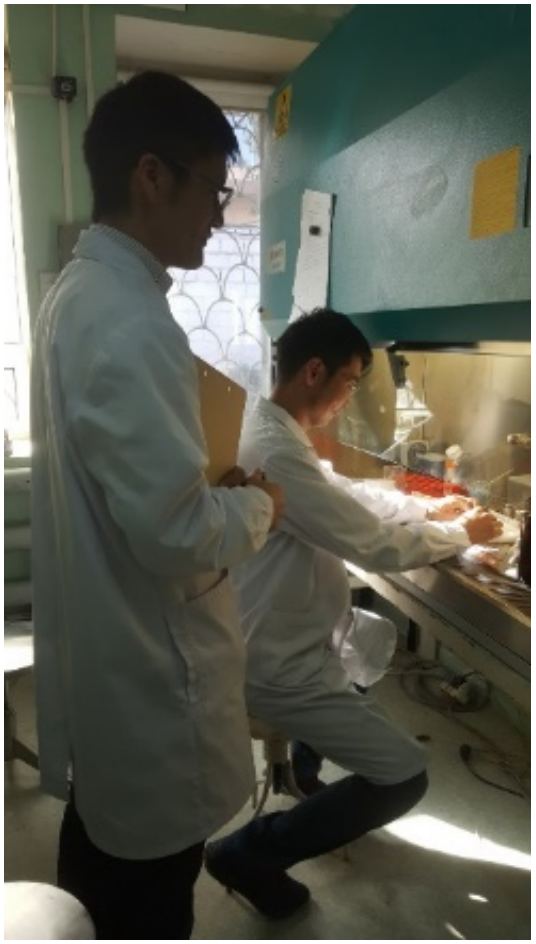
Bazarragchaa E.

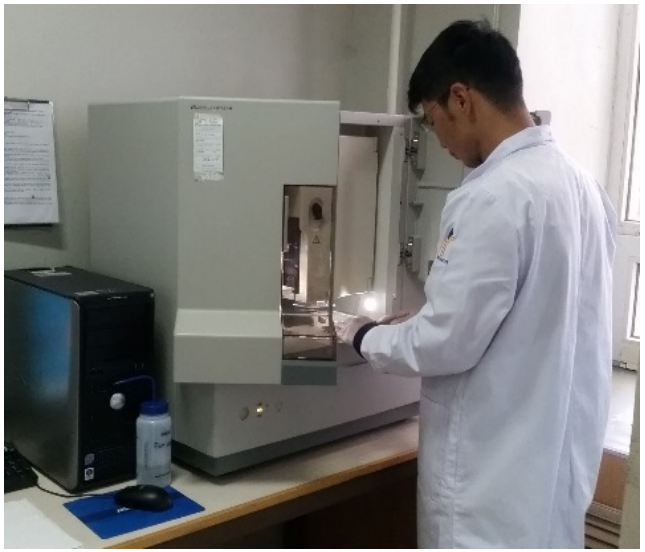
July 1, 2017 – August 31, 2017
(2 months)

October 1, 2017 – September 30, 2021
(4 years)

Training in Sapporo in 2017

Training in Mongolia





Prof. Sakoda Y.

Nov. 2016, Feb. 2017, May 2017, Oct. 2017, May 2018

Prof. Okamatsu M.

(Mar. 2016), Oct. 2017

Support by Skype discussion

November 28, 2016

February 21, 2017
March 22, 2017
May 2, 2017
June 23, 2017

February 6, 2018
April 2, 2018
June 29, 2018
August 10, 2018

Objective 1: Proficiency test of gene detection by RT-PCR method

Before  After 

Some primer sets,

but no primer list for RT-PCR detection
(H5, H7, M gene,,)

Primer list available!!
for RT-PCR detection
(H1-H16, HPAIV H5, H7, M gene,,)

<Future challenge>
Evaluate new primers and update their list

Objective 2: Improvement of antigenic characterization of isolated viruses by HI and NI tests

Before  After 

No list of antiserum for the tests

No preparation of reagents for NI test

Reagents are well prepared by yourselves

Antiserum list available!!

Antiserum well equipped from OIE ref. Lab., Italy

Objective 3: Improvement of the assessment of pathogenicity of isolated viruses by genetic analysis

Before  After 

- Just sequencing

- No skill to handle genetic software (BioEdit)

- No knowledge about cleavage site of HA

Sequencing
>> Genetic analysis
>> Identification of cleavage site of recent H5 and H7 viruses

<Future challenge>
Evaluate new primers for new HPAIVs

Objective 4: Establishment of the phylogenetic analyses for molecular epidemiolog

Before  After 

- Just sequencing

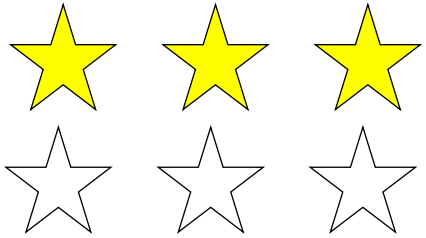
- No skill to handle genetic software (MEGA) for phylogenetic tree

Phylogenetic tree by themselves!!

<Future challenge>
How to select reference strains for the tree

Objective 5: Experimental infection and pathology of avian influenza

Before 

After 

No skill and experience of animal experiments

Skill and knowledge about the pathogenicity in chickens

<Future challenge>
Facility of animal experiments in Mongolia

Objective 6: Combined surveillance of avian influenza in migratory birds and poultry in Mongolia

Before 

After 

Just skill for sampling, no activity of virus isolation and characterization

Simultaneous characterization of samples in Mongolia and Japan in 2016 and 2017

<Future challenge>
Continue this activities in 2018 and future

INTRODUCTION

~~A surveillance~~Surveillance of avian influenza in wild birds has increased substantially worldwide in recent years ~~due to~~because of the spread of ~~the~~ H5N1 highly pathogenic avian influenza viruses (HPAIVs) among domestic poultry and wild birds in Asia, Europe, and Africa~~.~~ (Machalaba, et al., EID, 21, 2015 on line, CEIRS, www.niaidceirs.org~~.~~). Since the emergence of H5N1 ~~HPAIV~~HPAIVs in Asia (Xu et al., 1999, Smith et al., 2006), numerous global efforts ~~worldwide~~ have focused on elucidating the relative roles of wild ~~birds~~bird and poultry movement in virus dissemination. In ~~order~~an effort to better understand the ecology of avian influenza virus (~~AIV~~AIVs) in wild birds, ~~a~~ data ~~of~~from wild bird surveillance ~~study~~studies can be used to identify ~~a factor that correlate~~factors correlated with AIV detection ~~from~~in wild birds, such as: reservoir species, bird health status, age, season, and location (Spackman & Suarez, 20XX). ~~The each~~Each of the known subtype viruses (H1~~-~~H16 and N1~~-~~N9) of influenza A virus has been isolated from ~~waterfowls~~waterfowl, especially ~~from~~ migratory ducks ~~which~~that were orally infected with the viruses ~~by~~via waterborne transmission at their nesting lakes close to the Arctic Circle in Siberia, Alaska, and Canada~~,~~ during their breeding season in ~~the~~ summer (Fouchier, Munster, Keawcharoen, Osterhaus, & Kuiken, 2006). These viruses re
crypts in the colon, and they are excreted in feces (Kida et a
diversity of birds does not show clinical signs of illness. Howev
Asia, Europe, and Africa ~~has been~~and found to have caused ~~mar~~
(Rose et al., 2006).~~–~~

Mongolia is located on three flyways ~~such as~~, namely the
Asian, and East ~~Africa~~ African West ~~Asia of~~ Asian flyways,
their northern territory in Siberia to ~~the south.~~southern region
~~influenza~~AIVs in Mongolia ~~was~~has been conducted since aut
Manzoor, et al., Virus Genes, 2008, Sakoda et al., Virology 20
Genes, 2015). ~~–~~Accordingly, the surveillance of ~~AIV for~~ mig
Mongolia ~~was~~is essential ~~to monitor~~for monitoring AIVs that w

コメントの追加 [Editor2]: Tip: Serial comma: In American English, a comma (called serial or Oxford comma) is inserted before "and" in a series of three or more items.

Journal of Virological Methods

VIRMET_2018_286 | Research Paper

Evaluation of a rapid isothermal nucleic acid amplification kit, Alere™ i influenza A&B, for the detection of avian influenza viruses

Yoshihiro Sakoda | Hokkaido University, Laboratory of Microbiology, Graduate School of Veterinary Medicine, Kita18, Nishi9, Japan.

Status: **Ready for Decision (4 days)** | Submitted: **02/Jul/2018**

Zip File

Overview

Other Authors Show Details

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Abstract

Rapid and accurate diagnosis of influenza virus infection is essential for quick responses for both human and animal health. The Alere™ i influenza A&B is a novel isothermal nucleic acid amplification kit capable to detect and differentiate between influenza A and B viruses in human specimens in approximately 15 min. In this study, the Alere™ i influenza A&B kit was evaluated on its sensitivity and specificity for the rapid diagnosis of avian influenza in chickens. The kit detected all representative viruses of hemagglutinin subtypes (H1–H16), and the detection limits for the virus varied between 10¬-1.4–102.1 50% egg-infective dose per test which is higher than that of antigen detection immunochromatography kit, ESPLINE® A INFLUENZA. In addition, the kit could detect recent H5 and H7 highly pathogenic avian influenza viruses. In the study of experimental infection, viral RNA was detected in tracheal and cloacal swabs of chickens inoculated with a highly pathogenic avian influenza virus strain, A/chicken/Hokkaido/002/2016 (H5N6). The kit was found to be sensitive and specific enough for the rapid screening test of influenza A virus infection in chickens.

Keywords

avian influenza, nucleic acid, amplification, rapid detection, chicken

Highlights

• Highly sensitive screening test for LPAIV and HPAIV infections in chickens • Simple and rapid detection of avian influenza virus genes • Application of the kit at a field without laboratory facility

Additional Information View

Future plans

Scientific paper

Contribution to neighbor countries

SOP

Future tasks

Researcher's Mind!!

Strong mind to master the skills and knowledge necessary for the diagnosis of avian influenza on our own

Improvement of environment that will contribute to ongoing knowledge and technology improvement for veterinary medicine and virology

Training report in Hokkaido University

Training and evaluations

		Before the Project	August 2018	
			Hokkaido training (July3-Aug31)	SCVL
1	gene detection by RT-PCR method	△	○	△
2	HI and NI	△	○	○
3	the assessment of pathogenicity by genetic analysis	×	○	○
4	the phylogenetic analyses for molecular epidemiology	×	○	○
5	Experimental infection and pathology of avian influenza	NA	○	NA
6	Combined surveillance of avian influenza	△	○	△

○: good, △: not bad, ×: learn more

6項目について最終判定を入れてください