

## PROJECT MONITORING SHEET

Project Title: The Project for the Control of Tuberculosis and Glanders

Version of the Sheet: Ver.●● (Term: Month, Year - Month, Year)

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### I. Summary

#### 1 Progress

##### 1-1 Progress of Inputs

JICA HQ will prepare.

##### 1-2 Progress of Activities

Progress of Activities for Output1: The function of laboratory diagnosis for zoonotic diseases is enhanced in Mongolia through the development of LAMP / immunochromatography-based novel rapid diagnostic methods (kits) for detecting *Mycobacterium bovis* and *Burkholderia mallei* as well as updating existing disease diagnostic systems.

1.1. Development of a LAMP-based rapid diagnostic method (test kit) for *M. bovis* infection: **We have developed LAMP-based rapid gene detection method to identify *M. bovis* infection from postmortem tissues by targeting the region of difference 4 (RD4), a 12.7 kbp genomic region that is deleted solely in *M. bovis* and produced on publication (PLOS Neglect Trop Dis 2021;15(1):e0008996).**

1.2. Establishment of the LAMP-based gene detection method for tuberculosis complex in NCCD: **To be performed in later years.**

1.3. Development of a LAMP-based Rapid Diagnostic Method (Test Kit) for *B. mallei* infection: **We have found that *B. mallei* lacks 28.8 kb intergenic DNA that is commonly found in the genome of *B. pseudomallei*. Using this deletion as a target region, we are trying to establish LAMP that can differentiate *B. mallei* from *B. pseudomallei* and other *Burkholderia* species.**

1.4. Development of an immunochromatography-based Rapid Diagnostic Method (Test Kit) for *B. mallei* infection: **大橋先生、記入お願いします**

1.5. To establish production systems for the genetic diagnostic kits described above by introducing Ink-jet printers into NCCD and/or IVM: **Not performed due to the lack of Ink-jet printer in NCCD or IVM. Due to the influence of COVID-19, it is very difficult to import laboratory equipment, including Ink-jet printer, from Japan to Mongolia.**

1.6. Updating of the diagnostic flow for tuberculosis including detection of *M. bovis* as well as of the methods for detecting drug-resistant *M. tuberculosis* in NCCD:

**- Requests for lab. reagents and equipment for the conventional detection method of *M. bovis* were submitted to the Japan International Cooperation Agency in Mongolia.**

- As part of the TB care service, 197 *M. tuberculosis* strains were isolated from a sample of 2004 suspected extra-pulmonary TB in a liquid medium from November 2020 to October 2021. *M.bovis* can be identified in further.

-In framework updating of the diagnostic flow for tuberculosis as well as of the methods for detecting drug-resistant *M. tuberculosis*:

GeneXpert MTB/RIF machine based on real-time PCR for the detection of *M. tuberculosis* and rifampicin-resistant TB are provided to 41 national TB laboratories under the project and GeneXpert MTB/RIF testing is used at the frontline of TB diagnosis. In other words, it has shifted from conventional diagnostic methods to new technologies for early detection of tuberculosis. In generally, this method can detect *M. tuberculosis* but it is not possible to identify *M. bovis*.

The diagnostic algorithm has been updated and reflected in the “TB Care Guidelines” approved by the Order A / 30 of the Minister of Health on January 27, 2021 and is used in care services.

**Progress of Activities for Output2:** The epidemics of tuberculosis and glanders as zoonotic diseases in human are evaluated using molecular epidemiological techniques.

2-1. Molecular-epidemiological evaluation of the epidemics of *M. bovis* infection in human: **To be performed in later years.**

2-2. Molecular-epidemiological evaluation of the emergence of drug-resistant (multidrug-resistant) *M. tuberculosis* in human:

Determining next-generation genome sequencing (NGS), 2 pieces Oxford Nanopore’s MinION Sequencing Device and with MinION mk1b MinION Flow Cell received on August 9, 2021 at the NCCD TB Reference Laboratory.

DST is performed by molecular and conventional methods in direct samples and isolated strains. In the first 9 months of 2021, 110 people were tested DST for MGIT and solid medium, with a susceptible of 72.7% and any resistance of 28.2%.

A total of 1,028 people were tested for DST by molecular methods (LPA and Xpert MTB/RIF), of which 85.0% were susceptible, 9.0% were RIF resistant, and 14.8% were resistant to isoniazid.

2-3. To develop a draft revision of the current guidelines or equivalent document(s) for the diagnosis of tuberculosis in human on the basis of the prevalence of *M. bovis* human infections as well as the results of epidemiological evaluations of drug-resistant *M tuberculosis*, with consultation from relevant authorities such as the Ministry of Health: **To be performed in later years.**

2-4. To conduct specific consultations with the authorities concerned such as the Ministry of Health for the revision of the guidelines or equivalent document(s) on the basis of the epidemiological evidences: **To be performed in later years.**

2-5. Epidemiological evaluation of the epidemics of *B. mallei* infection in human:

Project meetings are being held between the institutes. An agreement is being drafted to exchange information, samples, and to share the laboratory in the study. Currently, researchers are working to share experiences.

Progress of Activities for Output3: The epidemics of tuberculosis and glanders as zoonotic diseases in livestock are evaluated using seroepidemiological and molecular epidemiological / seroepidemiological techniques, respectively.

3-1. Molecular-epidemiological evaluation of the epidemics of *M. bovis* Infection in livestock:

Totally 91 lung tissues of slaughtered cattle were collected in September to October, 2020 and to performed bacteriology of *Mycobacteria* spp of BTB for those samples. One of *Mycobacteria*-like isolate was obtained when growth in L-J medium. And then to performed the general PCR with *Mycobacteria* specific primers.

Also, we collected 668 serum samples of cattle, from Khentii Tuv and Sukhbaatar province in September to November 2021. To be perform the Ab detection of against BTB and diagnostic kits purchasing is currently ongoing.

We have agreed to working on NCCD's collection/stocks of *Mycobacteria complex* spp isolates to detect/check relevant the *M. bovis* with of Assistant professor Dr. Lishiqu Borjigin from Hokkaido University, Japan. This work will be start on 15<sup>th</sup> of November, 2021.

3-2. To evaluate the *M. bovis* contamination status in milk, which are sold in markets such as milk stands, by testing them with the gene detection method developed in the Activity 1.1:

Milk sample have not been collected yet. Because, main terms of milking season in the county are already done due to seasonally. And we cannot performe to collected of milk samples because, due to both reason the COVID 19 quarantine in UB and no vehile.

3-3. Molecular-epidemiological and seroepidemiological evaluation of the epidemics of *B. mallei* infection in horses:

The risk-based surveillance of Equine Glanders was performed by serology and intradermal testing of horses in Ulaanbaatar, Khentii, Dornod, Sukhbaatar, Dornod, Dundgovi, and Tuv aimags and some soums of those aimags.

Between Nov 2020 to July 2021, a total of 1,271 equines were tested for serology and intradermal test (Mallein test). Sero-prevalence was a 5.4% and Mallein test was a 5.2% and bacteriological samples (from 12 horses) was obtained and performed.

All isolated isolations were '*Burkholderia* spp' like cultures by colone/cell morphology and staining test etc. In these 12 isolates were tested 'positive' for '*Burkholderia* spp' detection general PCR.

Further, we tested 12 positive samples by detection PCR for the *B. cepacia* complex, all of which were negative.

We collected 696 samples of horse from Khentii Tuv and Sukhbaatar province in September to November, 2021 and those samples are have exanimated by CFT and RBT to diagnosis for the Equine Glanders.

Progress of Activities for Output4: A platform for One-Health approach-based infectious disease control is functioning for the practical application of research outcomes including risk analyses of tuberculosis and glanders as zoonotic diseases.

4.1. To establish a platform, such as regular liaison and coordination meeting, regular technical working group, etc., in Mongolia for comprehensive evidence-based zoonotic disease control, consisting of Mongolian and Japanese as well as medical and veterinary research, educational and administrative institutions: **To be performed in later years.**

#### 4.2. Risk assessment of *M. bovis* infection as a zoonotic disease:

Due to Covid-19 pandemic, the Mongolian government enforced a complete lockdown from November, 2020 to July, 2021. The quarantine was implemented four times with full lockdowns in UB (approximately 4~5 months consumed). The government agencies have been operating under restricted working hours (approximately ~20 hours per week) with half of the work force. Result of frequent lockdown we could not able to perform this activity.

#### 4.3. Risk assessment of *B. mallei* infection as a zoonotic disease:

Due to Covid-19, the Mongolian government enforced a complete lockdown from December, 2020. Since then, quarantine was implemented frequently with full lockdowns in UB (approximately 4-5 months consumed). The government agencies have been operating under restricted working hours (approximately ~20 hours per week) with half of the

4.4. To conduct discussions with medical and/or veterinary authorities as well as other eligible stakeholders concerned of medical and veterinary services as well as zoonotic disease control with regard to practical application of preventive measures of its epidemics: **To be performed in later years.**

4.5. To hold symposiums and/or joint seminars on project collaborative research at least once a year: **We held online Project Technical Committee (TC) twice, on October 21' 2020 and on August 29' 2021, using Zoom.**

### 1-3 Achievement of Output

**Progress of Activities for Output1:** The function of laboratory diagnosis for zoonotic diseases is enhanced in Mongolia through the development of LAMP / immunochromatography-based novel rapid diagnostic methods (kits) for detecting *Mycobacterium bovis* and *Burkholderia mallei* as well as updating existing disease diagnostic systems.

**Papers presented at scientific meetings:**

1. **Jimuna Y, Okagawa T, Ichikawa Y, Liushiqi B, Maekawa N, Murata S, Konnai S, Kimura T, Ohashi K.** Investigation of recombinant *Burkholderia mallei* antigens for the establishment of novel serodiagnostic method for glanders. 164<sup>th</sup> Annual Meeting of the Japanese Society of Veterinary Science, September 7-13' 2021, online (in Japanese)←大橋先生、修正お願いします
2. **Mitarai S.** DNA based TB Diagnostics for Precision Treatment. 2<sup>nd</sup> Indonesian Tuberculosis International Meeting. Virtual. August 28-29, 2020.←御手洗先生、掲載場所はここで OK ですか？結構かと思います（御手洗）。

**Published papers:**

1. **Kapalamula TF, Thapa J, Akapelwa ML, Hayashida K, Gordon SV, Hang' Ombe BM, Munyeme M, Solo ES, Bwalya P, Nyenje ME, Tamaru A, Suzuki Y, Nakajima C.** Development of a loop-mediated isothermal amplification (LAMP) method for specific detection of *Mycobacterium bovis*. 2021, PLoS neglected tropical diseases, 15(1), e0008996, doi: 10.1371/journal.pntd.0008996.

**Other products (i.e. SOP, protocol, guideline):**

**Progress of Activities for Output2:** The epidemics of tuberculosis and glanders as

zoonotic diseases in human are evaluated using molecular epidemiological techniques.

Papers presented at scientific meetings:

Published papers:

1. Narmandakh E, Tumenbayar O, Borolzoi T, Erkhembayar B, Bold T, Dambaa N, Burneebaatar B, Nyamdavaa N, Mitarai S, Jav S, Chiang CY. Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis* in Mongolia. Antimicrobial Agent Chemother 2020 Apr 20. pii: AAC.00537-20. doi: 10.1128/AAC.00537-20.

Other products (i.e. SOP, protocol, guideline): SOP for Culture examination in solid and MGIT has been prepared by English and Mongolian version (NCCD).

**Progress of Activities for Output3:** The epidemics of tuberculosis and glanders as zoonotic diseases in livestock are evaluated using seroepidemiological and molecular epidemiological / seroepidemiological techniques, respectively.

Papers presented at scientific meetings:

Published papers:

1. Erdemsurakh O, Ochirbat K, Gombosuren U, Tserendorj B, Purevdorj B, Vanaabaatar B, Aoshima K, Kobayashi A, Kimura T. Seroprevalence of equine glanders in horses in the central and eastern parts of Mongolia. Journal of Veterinary Medical Science. 2020, 82(9), 1247-1252, doi: 10.1292/jvms.20-0219.
2. Erdemsurakh O, Purevdorj B, Ochirbat K, MGIT Adilbish A, Vanaabaatar B, Aoshima K, Kobayashi A, Kimura T. Pathological and Immunohistochemical Analyses of Naturally Occurring Equine Glanders Using an Anti-BpaB Antibody. Veterinary Pathology. 2020, 57(6), 807-811, doi: 10.1177/0300985820953422.
3. Baatarjargal P, Ochbayar E, Mungu-Ochir B, Nyamdolgor U, Soyolmaa G, Batbaatar V, Khurtsbaatar O, Ulziisaikhan G, Batbold TS, Altanchimeg A and T. Kimura. "Result of study of macro and micro textures of Equine Glanders". This paper has been submitted and accepted to the Journal of Veterinary Science and Technology, IVM, Mongolia and the Journal will be published mid of the November, 2021.
4. Khurtsbaatar O, Ulziisaikhan G, Batbold TS, Baatarjargal TS, Ochibayar E, Batbaatar V and T. Kimura. "Results on risk-based surveillance for Equine Glanders between 2020 to 2021" This paper has been submitted and accepted to the Journal of Veterinary Science and Technology, IVM, Mongolia and the Journal will be published mid of the November, 2021

**Progress of Activities for Output4:** A platform for One-Health approach-based infectious disease control is functioning for the practical application of research outcomes including risk analyses of tuberculosis and glanders as zoonotic diseases.

Papers presented at scientific meetings: Takaki A, Aono A, Chikamatsu K, Igarashi Y, Shimomura Y, Hosoya M, Murase Y, Mitarai S. Evaluation of Deeplex-MycTB with MDR-TB isolates in Japan. International Union against Tuberculosis and Lung Diseases TBScience Late breaker session. 19-22 Nov., 2021, On-line.

Other products (i.e. SOP, protocol, guideline): SOP for whole genome sequence has been prepared (NCCD).

1-4 Achievement of the Project Purpose

To be filled in later years

## 1-5 Changes of Risks and Actions for Mitigation

- COVID-19 pandemic is causing restrictions and delays in research activities (for details, please see “2 Delay of Work Schedule and/or Problems”), which could severely affect the project if it continues for long.

## 1-6 Progress of Actions undertaken by JICA

JICA HQ will prepare.

## 1-7 Progress of Actions undertaken by Gov. of Mongolia

## 1-8 Progress of Environmental and Social Considerations (if applicable)

Inapplicable

## 1-9 Progress of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

Inapplicable

## 1-10 Lessons learned from the Project for applying to other crops seed multiplication

None as of now

## 1-11 PR activities in this term

We made short introductory movie of this SATREPS project with the support of AMED. [https://www.youtube.com/watch?v=EJO9OYE\\_zzU](https://www.youtube.com/watch?v=EJO9OYE_zzU)

## 1-12 Other remarkable/considerable issues related/affect to the project (such as other JICA's projects, activities of counterparts, other donors, private sectors, NGOs etc.)

**2 Delay of Work Schedule and/or Problems (if any)**

## 2-1 Detail

## - Implementation of the project plan

1.5. To establish production systems for the genetic diagnostic kits described above by introducing Ink-jet printers into NCCD and/or IVM:

4.2 and 4.3 have not been done due to long term of Covid-19 lockdown in Mongolia. And most of the requested reagents and equipments has not been arrived on time. because of delay further activities postponed.

## - Expert (Project coordinator, external expert and teachers)

- Equipment supply: Necessary experimental instruments and equipment for research activities in the Project

- Training (Bacteriology, Immunology, Epidemiology, Pathology, Molecular Biology, Bioinformatics and other necessary specialized area

## 2-2 Cause and Problems

Due to Covid-19 pandemic, the Mongolian government enforced a complete lockdown from November, 2020 to July, 2021. The quarantine was implemented four times with full lockdowns in UB (approximately 4~5 months consumed). The

government agencies have been operating under restricted working hours (approximately ~20 hours per week) with half of the work force. Result of frequent lockdown we could not able to perform some activity.

Due to the influence of COVID 19:

- Logistics between country and intercountry
- Delays:
  - Project coordinator appointment and placement
  - External expert visit
  - Training coverage of researchers
  - Laboratory supplies and placement
- Human resource mobilization and overwork

## 2-3 Action to be taken

-Replaning of the Project plan implementation with related COVID-19 situation

-Resolve the issue of crossing the Mongolian border and 14 days of isolation  
COVID-19 situation

-Placement of appointed project coordinator

- Conduct training (Bacteriology, Immunology, Epidemiology, Pathology, Molecular Biology, Bioinformatics and other necessary specialized area

- Procurement lab. supplies Training (Bacteriology, Immunology, Epidemiology, Pathology, Molecular Biology, Bioinformatics and other necessary specialized area

- Modification of the Project Implementation plan

## 3 Modification of the Project Implementation Plan

### 3-1 PO

Plan of operation was reviewed and modified as Attachement-1

### 3-2 Other modifications on detailed implementation plan

*(Remarks: The amendment of R/D and PDM (title of the project, duration, project site(s), target group(s), implementation structure, overall goal, project purpose, outputs, activities, and input) should be authorized by JICA HQs. If the project team deems it necessary to modify any part of R/D and PDM, the team may propose the draft.)*

## 4 Preparation of Gov. of Mongolia toward after completion of the Project

To be filled in later years

END

Attachment-1 Revised Plan of Operation

Attachment-2 Project Design Matrix

project activities are could not implemented on planned time  
Implementation of the project action plan