

## Under project of the

## "CONTROL OF TUBERCULOSIS AND GLANDERS"

## /SATREPS project/

## Progress report: Glanders/BTB surveillance in IVM

**Between November 2020 to November 2021** 

Ulaanbaatar Mongolia

17 November, 2021

by Research team of IVM

#### **Tables show the Operation plan of the project**

TO STARTED and currently CONTINIUES			TO STARTED and currently CONTINIUES un-performed yet, due to COVID19 fandemic and others	Tent
un-performed yet, due to COVID19 fandemic and others	2020	2021	Inputs	Year         2020         2021         2022           Marriett         Jan-         Apr-         Jul-         Oct-         Jan-         Apr-         Jul-         Jul- <t< th=""></t<>
nputs	Jan- Apr- Jul- Oct- Mar Jun Sep Dec	Jan- Apr- Jul- Oct		Month Jan- Apr- Jul- Oct- Jan- Apr- Jul- Oct- Jan- Apr- Jul- Mar Jun Sep Dec Mar Jun Sep Dec Mar Jun Sep Dec Mar Jun Sep
1.1. Development of a LAMP-based rapid diagnostic method (test kit) for M. infection		Mar Jun Sep De	2-5. Epidemiological evaluation of the epidemics of <i>B. mallei</i> Infection in hum	lan
intection			2.5.1. To collect the biological samples (sputum and/or throat swab) obtained from patients with human infectious pneumonitis for whom no causative agent	Plan
1.1.1. To develop a LAMP-based method for detecting M. bovis-specific genetic			has been identified in the NCCD, followed by transferring them to IVM.	Actual
region at the Hokkaido University (including drying of reagents).			2.5.2. To evaluate the presence of human cases of <i>B. mallel</i> infection in IVM	Plan Plan
			by screening them with the gene detection method developed in Activity 1.2.	Actual
1.1.2. To make the <i>M. bovis</i> gene detection method into a "kit" using an ink-jet printer in the Hokkaido University (trial production of a rapid diagnostic test kit).				Tenta
			TO STARTED and currently CONTINIUES un-performed yet, due to COVID19 fandemic and others	
1.1.3. To evaluate the sensitivity and specificity of the developed kit(s) with			an performed yet, due to CO (101) innocime and others	Year 2020 2021 2022
biological specimens of $M$ . bovis-infected patients and animals in Mongolia .			Inputs	Month Jan- Apr- Jul- Oct- Jan- Apr- Jul- Oct- Jan- Apr- Jul- Mar Jun Sep Dec Mar Jun Sep Dec Mar Jun Sep
				Plan
		Tentative Pl	3-1. Molecular-epidemiological evaluation of the epidemics of <i>M. bovis</i> Infection in livestock	Actual
TO STARTED and currently CONTINIUES un-performed yet, due to COVID19 fandemic and others				
Year 20	20 2021	2022	3.1.1. To isolate tuberculosis complex using the L-J mediua in IVM, from the tubercles-suspected granulomas samples collected from cattle and sheep at	Plan
Inputs Month Jan- Apr- Jan Jun	Jul- Oct- Jan- Apr- Jul- Oct Sep Dec Mar Jun Sep Dec	Jan- Apr- Jul- Oct- Jan Mar Jun Sep Dec Ma	slaughterhouses and meat markets in the project target areas.	Actual
1.3.3. To evaluate the sensitivity and specificity of the developed kit(s) with Plan				
biological specimens of <i>B. mallei</i> -infected animals in Mongolia . Actual			3.1.2. To estimate the prevalence of <i>M. bovis</i> in the tuberculosis complex isolated	d Plan
1.3.4. To prepare SOPs for the genetic diagnosis of <i>B. mallei</i> infections in Plan			from cattle and sheep by determining the presence of <i>M. bovis</i> in the colonies grown on L-J medium using the genetic detection method developed in the	
humans at NCCD following the improvements are made as appropriate based on the aforementioned evaluation results. ( <i>Note: the Activity 1.2.4 should be</i>			Activity 1.1.	Actual
conducted in consideration of the results of epidemiological studies on B.				
mallei infection in human performed in the Activities under the Outcome 2.) Actual			3.1.3. To investigate the endemic status of <i>M. bovis</i> infection including subclinica	Plan
1.3.5. To prepare SOPs for the genetic diagnosis of <i>B. mallei</i> infections in Plan			infection by performing the Interferon-Gamma Release Assay (IGRA) on herds in	
livestock at IVM following the improvements are made as appropriate based on			which <i>M. bovis</i> -detected cattle were kept.	Actual
the aforementioned evaluation results.				

#### Outputs 1.1.3/1.3.3/1.3.5 and 2.5.1/2.5.2

**UNACCOMPLISHED, DUE TO COVID-19 PANDEMICS/OTHERS** 

#### **FUTURE ACTIVITIES**

/COVID 19 Quarantine with full lockdowns (4 times) and Government agencies have been operating under the restricted working hours/other issues/

## Outputs 3.1.1-3.1.3

- Bacteriology was performed under the "OIE Terrestrial Manual of Standard for Diagnostic Tests and Vaccines" used as a guideline.
- In September and November of 2020, approximately 1320 cattle lungs were examined at slaughterhouses/abattoirs near of UB (Emeelt and Nalaikh). As a result, 91 lung samples with microscopical changes were collected and examined by the method of bacteriology for the purpose of detecting agents of Bovine Tuberculosis in this surveillance.



Molecule biology: In reference to Detection of Mycobacterium tuberculosis by PCR amplification with pan-Mycobacterium primers and hybridization to an M. tuberculosis-specific probe

## **Bacteriology:**

- 20 out of 91 lung samples had a formation of nonvascular nodular granulomas of tubercles observed.
- The 20 lung samples then cultured in Lowenstein Jensen media incubating at 35-37°C in 5-10% CO<sub>2</sub> for up to 8 weeks.
- After 5-8 weeks, only one lung tissue (sample ID: 20EU36, collected from slaughterhouse of Emeelt) had a *Mycobacterium* like colony growth observed.

(The sampled cattle belongs to a herder household from Battsengel soum, Arkhangai province).



**Fig 2.** 20 lung tissue samples growth on Lowenstein Jensen solid medium

**Fig 3.** Bacteria colonies observed like *Mycobacteria* spp in LJ medium (isolated ID\_20EU36)

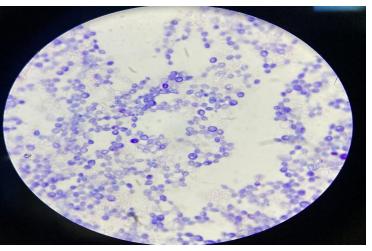


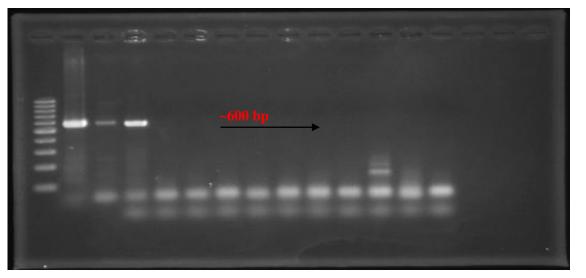
Fig 4. Zeihl-Neelsen Staining results

## PCR result of isolates (sample ID\_20EU36)

PCR result of the lung sample 20EU36 from Emeelt,

resulted a positive reaction (around 600 bps).

M P P 1 2 3 4 5 6 7 8 9 10 11 N



#### Figure 6

M – 100 bps marker

- P positive control /*M. tuberculosis\_NCCD*/
- P positive control /*M. bovis\_NCCD*/

#### 1–Sample of 20EU362

2-11 other lung samples out of the 20 nodular samples

N – negative control

**Reference to article:** Detection of Mycobacterium tuberculosis by PCR amplification with pan-Mycobacterium primers and hybridization to an M. tuberculosis-specific probe

## **Conclusion**

20 lungs had a formation of nodular granulomas of Tuberculosis observed. Bacteriology and molecular diagnosis show that sample ID 20EU36 resulted in a positive reaction, which suggests Mycobacterium. by spp the colony formation and PCR

#### TO STARTED and currently CONTINIUES

un-performed yet, due to COVID19 fandemic and others

	Year		20	20			20	21			20	)2
ts	Month	Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec	Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec	Jan- Mar	Apr- Jun	
3.3.1. To perform a seroepidemiological survey on <i>B. mallei</i> infection (history) by testing the sera obtained from horse herds in the project area with	Plan											
the conventional methods (complement-fixation and plate agglutination) in IVM.	Actual											×
3.3.2. To evaluate the epidemics of <i>B. mallei</i> infection in Mongolia seroepidemiological, by testing the horse sera collected in the Activity 3.2.1	Plan											
with the immunochromatography-based <i>B. mallei</i> -specific antibody detection method developed in the Activity 1.3.	Actual										Apr-	
3.3.3. To analyse <i>B. mallei</i> infection in horses histopathologically and	Plan											
immunohistrogically by dissecting infected horses.	Actual											2
3.3.4. To assess the transmission and distribution of <i>B. mallei</i> in horses by performing the comprehensive gene screening using a next-generation												
sequencer on the isolated strains, which are obtained by culturing specimens of lesioned part of the infected horses.	Actual											

## **Outputs 3.3.1**

 Table 1. Results of serology and intradermal test in 2020/2021

#### (RISK-BASED SURVEILLANCE)

 Table 2. Results of serology in 2021

#### (RANDOM SURVEILLANCE)

Date	Province	Number of samples	<b>CFT</b> (%)	Mallein test (%)	Date	Province	Number of samples	<b>CFT (%)</b>
	Tuv	185	8 (4.3)	8 (4.3)		Tuv	202	1 (0 40)
	Khentii	382	18 (4.7)	18 (4.7)			203	1 (0.49)
2020	Sukhbaatar	220	-	-	2021	Khentii	210	1 (0.48)
	Dornod	86	3 (3.5)	1 (1.2)		Sukhbaatar	208	9 (4.3)
	Dundgobi	44	24 (54.5)	24 (54.5)				
	Dornod	5	1 (20)	-		Dornod	200	2 (1.0)
2021	Tuv	363	13 (3.6)	14 (3.9)		Total	821	13 (1.58)
	Sukhbaatar	1	1 (100)	1 (100)				
	Total	1271	<b>68</b> ( <b>5.4</b> )	66 (5.2)				

#### Outputs 3.3.3

## **CLINICAL SYMPTOMS OF GLANDERS CASE in 2020/2021**



Fig 2. Skin rash with pustules horse



Fig 3. Signs of a punctate stroke in the spleen



Fig 4. Skin test positive horse

#### Continuous

#### Table 3: Results of bacteriology and molecular biology tested samples in 2020/2021

	Uandan		Clinic			Bacteriol -		PCR det	ection	
Date	Herder 's ID	Location	sympto ms	CFT	Mallein	ogy	All Burkholderia	B.mallei	B.pseudo mallei	<i>B.cepacia</i> complex
2020	1	Khui 7, Tuv	+	+	+	+	+	-	-	-
2020	1	Khui 7, Tuv	+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-
2020	2	Khentii	+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-

#### Continues Table 3

								PCR dete	ction	
Date	Herder's ID	Location	Clinic symptoms	CFT	Mallein	Bacteriolo gy	All Burkholderia	B. mallei	B. pseudoma Ilei	<i>B. cepacia</i> complex
2021	3	Tuv	+	+	+	+	+	-	-	-
2021	4	Tuv	+	+	+	+	+	-	-	-
2021	5	Khentii	+	+	+	+	+	-	-	-
2021	6	Tuv	+	+	+	+	+	-	-	-
2021	6	Tuv	+	+	+	+	+	-	-	-

#### **Continuous**

## **Definition of species-specific oligonucleotide primers**

Table 4: Primers used for species specific PCR

Target species	Primer <sup>a</sup>	23S rDNA helices containing target position	Sequence	Size of PCR product (bp)
B. vietnamiensis, B. mallei, and B. pseudomallei	VMP 23-1	9ab/10a	5'-CTT TTG GGT CAT CCT RGA-3'	1,051
B. mallei and B. pseudomallei All Burkholderia spp. B. mallei All Burkholderia spp. excluding B. mallei	MP 23-2 CVMP 23-1 M 23-2 CVP 23-2 <sup>b</sup>	45ab/36b 5b/8ab 78ab 78ab	5'-TCC TAC CAT GCG AGA CT-3' 5'-AAA CCG ACA CAG GTG G-3' 5'-CAC CGA AAC TAG CA-3' 5'-CAC CGA AAC TAG CG-3'	526

<sup>a</sup> V, B. vietnamiensis; M, B. mallei; P, B. pseudomallei; C, B. cepacia. A suffix of 1 indicates a sense primer; a suffix of 2 indicates an antisense primer. <sup>b</sup> Contains an NH<sub>2</sub> modification at the 3' end to supress amplification of Burkholderia species other than B. mallei.

Reference Article: Molecular Procedure for Rapid Detection of Burkholderia mallei and Burkholderia pseudomallei., Adolf Baueernfeind et all., Publishded Sept 1998, Journal of Clinical Microbiology, Recommended OIE.

#### **Conclusion**

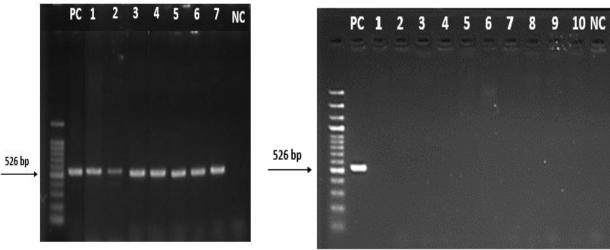


Fig 5. PCR amplification of all *Bukholderia* spp using primer pairs CVMP23-1, M23-2, CVP23-2 (in

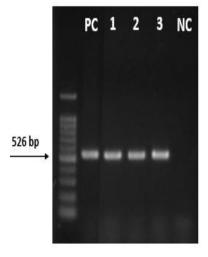


Fig 7. PCR amplification of *B. mallei* and *B. pseudomallei* using primer pairs CVMP23-1, MP23-2, CVP23-2

Fig 6. PCR amplification of all *Bukholderia spp* using primer pairs CVMP23-1, M23-2, CVP23-2 (in 2021) Between Nov 2020 to July 2021, a total of 1,271 equines were tested for serology and the intradermal test. Sero-prevalence was a 5.4% and Mallein prev. was 5.2% (Table1).

/SAMPLE COLLECTION; collaboration with owners of horses and private of veterinary units/

Thus, horses that exhibited Glanders like clinical confirmed symptoms and were by the serology/intradermal tests were **SLAUGHTERED** in with (THE agreement owner **EXTERMINATING PROCESS** CAUSED DIFFICULTIES DUE TO **OWNERS DEMANDING COMPENSATION FOR THE LOST ANIMAL**). And bacteriological samples (8) horses from 6 owners) were obtained and performed (Table3).

#### TO STARTED and currently CONTINIUES

un-performed yet, due to COVID19 fandemic and others

	Year		20	20	ll- Oct- Jan- Apr- Jul- Oc			
aputs	Month	Jan- Mar	Apr- Jun	Jul- Sep			200	Oct- Dec
based zoonotic disease control, consisting of Mongolian and Japanese as well as medical and veterinary research, educational and administrative institutions.	Actual							
4.2. Risk assessment of <i>M. bovis</i> infection as a zoonotic disease	7							
4.2.1. To determine a study design (e.g., the preparation of survey procedures, the unification of analytical methods and so on) in order to perform the risk	Plan							
4.2.1. To determine a study design (e.g., the preparation of survey procedures, the	Actual							

# Outputs 4.2.1 and 4.3.1

#### UNACCOMPLISHED, DUE TO COVID-19 PANDEMICS/OTHERS

#### AND

#### FUTURE ACTIVITIES /WILL BE DONE BEFORE END OF 2021/

#### TO STARTED and currently CONTINIUES

un-performed yet, due to COVID19 fandemic and others

4.3. Risk assessment of <i>B. mallei i</i> nfection as a zoonotic disease			20	20			20	21	
		Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec	Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec
4.3. Risk assessment of <i>B. mallei i</i> nfection as a zoonotic disease									
4.3.1. To determine a study design (e.g., the preparation of survey procedures, the unification of analytical methods and so on) in order to perform the risk	Plan								
4.3. Risk assessment of <i>B. mallei i</i> nfection as a zoonotic disease	Actual								

# THANK YOU FOR YOUR KIND ATTENTION