



Under project of the  
“CONTROL OF TUBERCULOSIS AND GLANDERS”  
/SATREPS project/

# GLANDERS/bTB research: **Progress and Future activity** **Implementation in IVM**

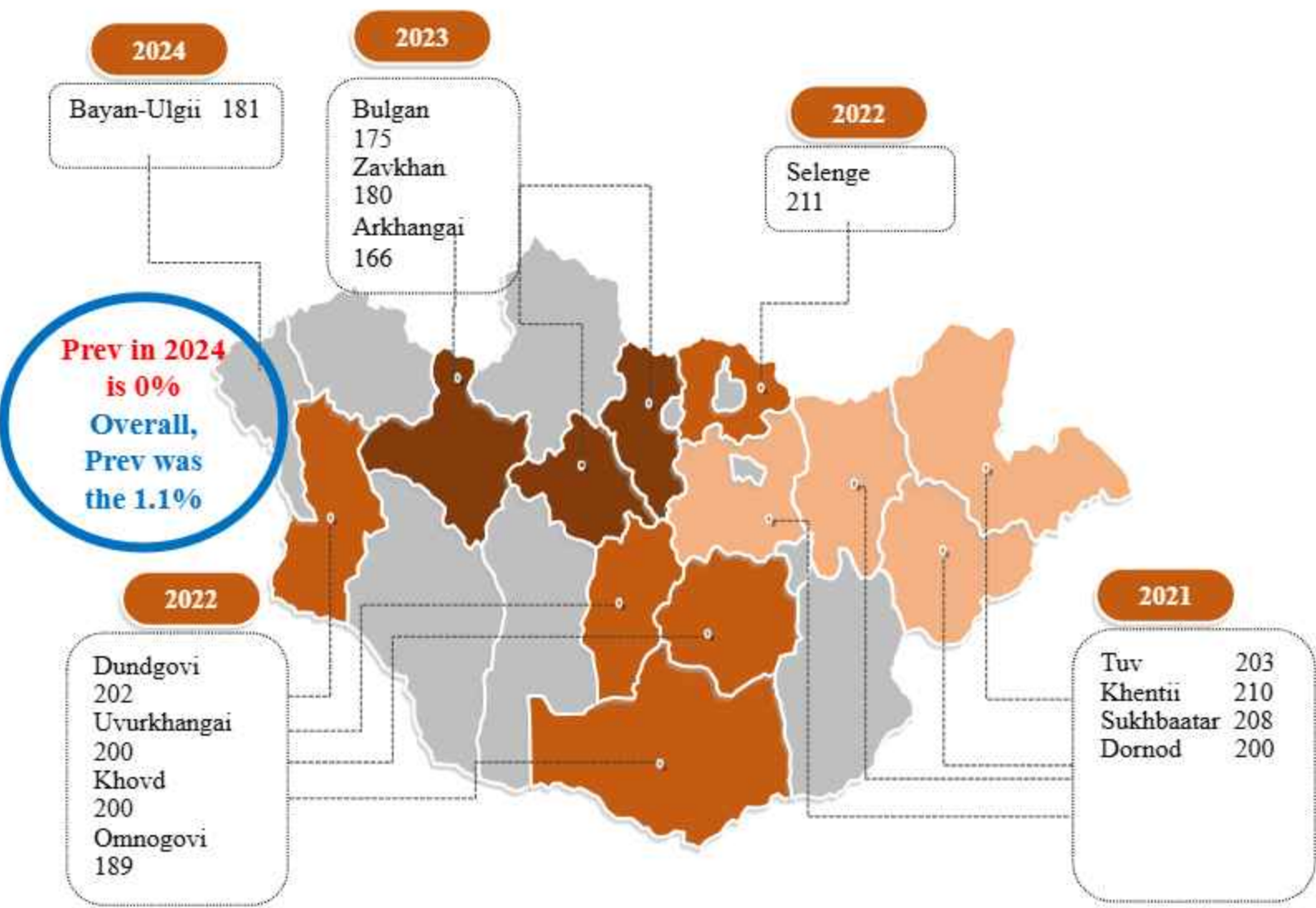
Duration: **Feb 2024 to Dec 2024**

by **Team of Glanders/bTB Research Group**

**5 February 2025**

Inputs	Duration
<b>3-3. Molecular-epidemiological and sero-epidemiological evaluation of the epidemics of <i>B. mallei</i> infection in horses</b>	
3.3.1 To perform a sero-epidemiological survey on <i>B. mallei</i> infection (history) by testing the sera obtained from horse herds in the project area with the conventional methods (complement-fixation test ) in IVM.	10/2020 - 05/2025
3.3.4 To assess the transmission and distribution of <i>B. mallei</i> in horse 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	03/2022 - 05/2025

Map of Collection Area under the Equine Glanders Surveillance in 2024



RESULT OF GLANDERS SURVEY OF RISK-BASED FLOCKS IN THE 2024

Date	Province	n_suspected horse	n_CFT pos (%)
2024	Dornod	38	0 (0)
	Tuv	15	0 (0)
	Selenge	56	1 (1.8)
	Khentii	8	0 (0)
	Dundgovi	5	1 (20)
	Uvurkhangai	18	2 (11.1)
	Sukhbaatar	22	0 (0)
Total		162	4 (2.5)



This surveillance shown that disease prevalence was 1.1%. However, equine glanders detected in 3/4 of the provinces, in 2/3 of soums, and in 7.9% of horse flocks, and it was indicating that the equine glanders distribution is increased and re-emerging in Mongolia

Inputs	Duration
<b>3-3. Molecular-epidemiological and sero-epidemiological evaluation of the epidemics of <i>B. mallei</i> infection in horses</b>	
<p>3.3.1 To perform a sero-epidemiological survey on <i>B. mallei</i> infection (history) by testing the sera obtained from horse herds in the project area with the conventional methods (complement-fixation and plate agglutination) in IVM.</p>	<p>10/2020 - 05/2025</p>
<p><b>3.3.4 To assess the transmission and distribution of <i>B. mallei</i> in horse 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</b></p>	<p><b>03/2022 - 05/2025</b></p>





## First molecular characterization of *Burkholderia mallei* strains isolated from horses in Mongolia

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### ARTICLE INFO

**Keywords:**  
Glanders  
Mongolia  
WGS  
HRMA  
SNP  
cgSNP

### ABSTRACT

Glanders, a highly contagious and often fatal disease affecting equids, is caused by *Burkholderia mallei*. Although sporadic cases of equine glanders have recently been documented in Mongolia, genome sequencing and molecular studies of the bacteria within this region are lacking. This study provided the first molecular characterization of *B. mallei* isolated from four native Mongolian horses from two different provinces in 2019 and 2022 by applying whole-genome sequencing with two SNP types (previously developed genotyping with 15 SNP markers that provide global coverage of the *B. mallei* population and the core genome coding SNP typing developed in this study). The Mongolian isolates were located within the L3B1 cluster, which was previously associated with the V-120 strain from Russia. Within the L3B1 cluster shared by neighboring countries, they were in a unique sub-branch. In this study, specific SNP markers unique to the Mongolian strains were identified to track these strains using a high-resolution melting analysis (HRMA). This study revealed the unique phylogenetic background of Mongolian strains isolated from the eastern part of Mongolia. HRMA specific to the Mongolian subbranch may contribute to the molecular epidemiological monitoring of glanders in Mongolia and surrounding countries.

## Conclusion

First, whole-genome sequencing of Mongolian *B. mallei* strains bridged the gap in the molecular epidemiological information on glanders in Mongolia. The Mongolian isolates were positioned within a unique subbranch of the L3B1 cluster. This finding implies evolutionary divergence specific to the Far East region following geographical isolation. High genetic variation was observed among the Mongolian strains isolated from horses in the same herd. HRMA based on these genomic data provides high resolution and simplifies the identification of Mongolian strains in the Far East region. The future direction of this research will focus on expanding the genomic database for *B. mallei* in Mongolia as well as in the Far East region, enhancing the precision of molecular typing, assessing the entire endemic situation in Mongolia and promoting glanders eradication.

Inputs	Duration
<b>1-3. Development of a LAMP- based Rapid Method (Test kit) for <i>B.mallei</i> infection</b>	
1.3.1 To develop a LAMP-based method for detecting <i>B.mallei</i> -specific genetic region at the Hokkaido University (including drying of reagents).	07/2020 - 07/2021
1.3.2 To make the <i>B.mallei</i> gene detection method into a “kit” using an ink-jet printer in the Hokkaido University (trial production of a rapid diagnostic test kit).	04/2021 - 04/2022
<b>1.3.3 To evaluate the sensitivity and specificity of the developed kit (s) with biological specimens of <i>B. mallei</i> infected animals in Mongolia.</b>	10/2021 - 10/2022

No	Livestock species	Samples (nasal swabs)	LAMP Positive (%)
1	Horse	520	62 (11.9)

## ***B. mallei infection surveillance in human***

***ACTIVE SURVEILLANCE AMONG HERDERS:*** Collect blood samples from the herder's families who was closely conducted with horse such as reported suspected & affected of equine glanders (EG)

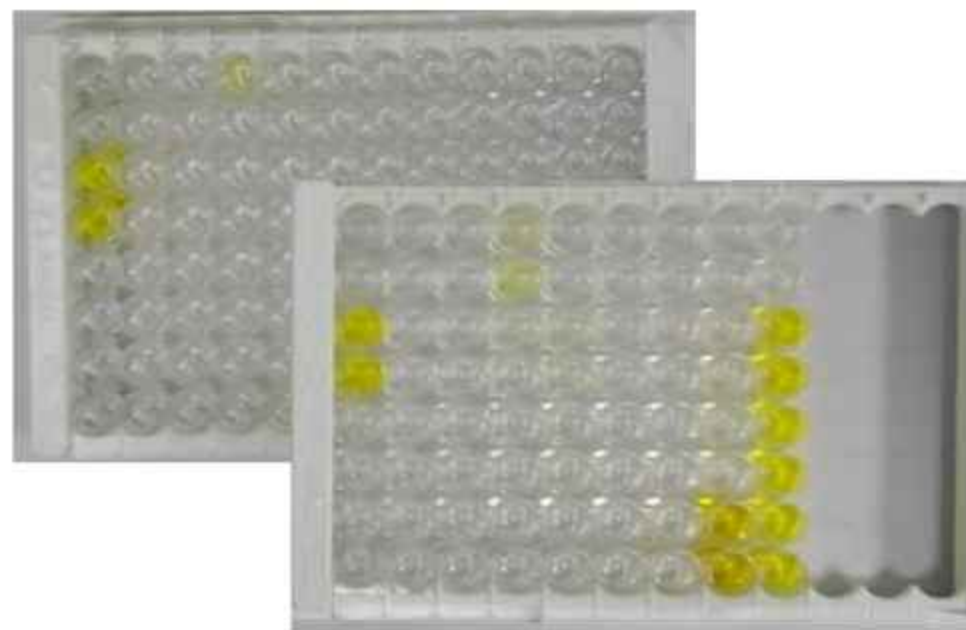
**Scope:** 75 face-to-face families of 18 soums of Khentii, Uvurkhangai, Selenge, Bayan-Ulgii, Sukhbaatar, Dornod and Dundgobi provinces

### **Data collection:**

- ✓ Questionnaire focused on risk factors (25 open and closed questions) by face-to-face interview
- ✓ Blood samples collected from 75 herders



№	Sampling Date	Provinces	n_soum	n_herders	ID Screen Glanders Double Antigens Multi-species		%
					n_tested	n_result	
1	2024.05	Arkhangai	1	4	4	0	0
2	2024.06	Khentii	3	8	8	0	0
3	2024.06	Uvurkhanga i	3	15	15	0	0
4	2024.06	Selenge	2	4	4	0	0
5	2024.07-08	Bayan- Ulgii	3	22	22	0	0
6	2024.1	Uvs	2	6	6	0	0
7	2024.09	Dornod	2	9	9	0	0
8	2024.1	Sukhbaatar	2	8	8	0	0
Total			18	76	76	0	0



	1	2	3	4	5	6	7	8	9	10	11	12
A	neg	Ar-3	Khe- 3	Khe- 7	Uv- 3	Uv- 7	Uv- 11	Uv- 15	Se-4	B-U1-4	B-U1-8	B-U1-12
C	neg	Ar-3	Khe- 3	Khe- 7	Uv- 3	Uv- 7	Uv- 11	Uv- 15	Se-4	B-U1-4	B-U1-8	B-U1-12
E	pos	Ar- 4	Khe- 4	Khe- 8	Uv- 4	Uv- 8	Uv- 12	Se-1	B-U1-1	B-U1-5	B-U1-9	B-U1-13
G	pos	Ar- 4	Khe- 4	Khe- 8	Uv- 4	Uv- 8	Uv- 12	Se-1	B-U1-1	B-U1-5	B-U1-9	B-U1-13
A	Ar- 1	Khe- 1	Khe- 5	Uv- 1	Uv- 5	Uv- 9	Uv- 13	Se-2	B-U1-2	B-U1-6	B-U1-10	B-U1-14
C	Ar- 1	Khe- 1	Khe- 5	Uv- 1	Uv- 5	Uv- 9	Uv- 13	Se-2	B-U1-2	B-U1-6	B-U1-10	B-U1-14
E	Ar-2	Khe- 2	Khe- 6	Uv- 2	Uv- 6	Uv- 10	Uv- 14	Se-3	B-U1-3	B-U1-7	B-U1-11	B-U1-15
G	Ar-2	Khe- 2	Khe- 6	Uv- 2	Uv- 6	Uv- 10	Uv- 14	Se-3	B-U1-3	B-U1-7	B-U1-11	B-U1-15

	1	2	3	4	5	6	7	8	9
A	neg	B-U1-18	B-U1-22	Uvs-4	Do- 2	Do- 6	Su- 2	Su- 6	Do- 9
C	neg	B-U1-18	B-U1-22	Uvs-4	Do- 2	Do- 6	Su- 2	Su- 6	Do- 9
E	pos	B-U1-19	Uvs-1	Uvs-5	Do- 3	Do- 7	Su- 3	Su- 7	2303
G	pos	B-U1-19	Uvs-1	Uvs-5	Do- 3	Do- 7	Su- 3	Su- 7	2303
A	B-U1-16	B-U1-20	Uvs-2	Uvs-6	Do- 4	Do- 8	Su- 4	Su- 8	240117
C	B-U1-16	B-U1-20	Uvs-2	Uvs-6	Do- 4	Do- 8	Su- 4	Su- 8	240117
E	B-U1-17	B-U1-21	Uvs-3	Do- 1	Do- 5	Su- 1	Su- 5	220315	241009
G	B-U1-17	B-U1-21	Uvs-3	Do- 1	Do- 5	Su- 1	Su- 5	220315	241009

# INTER-SECTORAL RISK ASSESSMENT

**Participants:** 20 medical doctors & vets

**Participated organizations:** NCCD, GAVS, IVM, Capital Veterinary Service, some of private Veterinary Units & some of slaughterhouses near UB

**Organized time:** 5<sup>th</sup> of Jul, 2024

**Venue:** EOP of NCCD



**DISCUSSED & ASSESSED:** A TOTAL OF 31 QUESTIONS

Магадлалын түвшин	маш өндөр (80-100%)					
	өндөр (60-79%)					
	дунд (40-59%)					
	бага (20-39%)					
	маш бага (0-19%)					
		маш бага (0-19%)	бага (20-39%)	дунд (40-59%)	өндөр (60-79%)	маш өндөр (80-100%)
Үр дагаврын түвшин						

**ASSESSED:** Probability 37.5% & impact 40%

**RESULT:** Have transmission risks of EG among high-risk people in Mongolia? - **MODERATE**

# **CONCLUSION**

- **Among the conducted horses included in the surveillance (survey) were diagnosed equine glanders & It means, peoples with high-risk are may have to exposed by *B. mallei* and it is one of the risk factors in Mongolia**
- **Risks of EG to transmit among high-risk people were evaluated as a moderate in Mongolia**



**bTB research**

## Outputs 3.1.1 and 3.1.2

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

3.1.1. To isolate tuberculosis complex using the L-J media in from the tubercles-suspected granulomas samples collected from cattle and sheep at slaughterhouses and meat markets in the project target areas.

3.1.2. To estimate the prevalence of *M. bovis* in the tuberculosis complex isolated from cattle and sheep by determining the presence of *M. bovis* in the colonies grown on L-J medium using the genetic detection method developed in the Activity 1.1.

3.1.3. To investigate the endemic status of *M. bovis* infection including subclinical infection by performing the Interferon-Gamma Release Assay (IGRA) on herds in which *M. bovis*-detected cattle were kept.

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

3.1.1. To isolate tuberculosis complex using the L-J media in IVM, from the tubercles-suspected granulomas samples collected from cattle and sheep at slaughterhouses and meat markets in the project target areas.

3.1.2. To estimate the prevalence of *M. bovis* in the tuberculosis complex isolated from cattle and sheep by determining the presence of *M. bovis* in the colonies grown on L-J medium using the genetic detection method developed in the Activity 1.1.

3.1.3. To investigate the endemic status of *M. bovis* infection including subclinical infection by performing the Interferon-Gamma Release Assay (IGRA) on herds in which *M. bovis*-detected cattle were kept.

### Under 3.1.1

Previously isolated 10 *Mycobacterium*-like cultures in 2023 were re-inoculated and conducted for PCR



Collected milk samples were from 6 cows out of 19 that tested positive by IGRA ELISA in 2023 and conducted bacteriological analysis.

Additionally, bacteriology of *Paratuberculosis* was conducted on fecal samples from 12 cows.

### Under 3.1.2

- ✓ DNA was isolated from 10 mycobacterium-like cultures and its concentration was measured at 260/280 nm using a nanodroplet purification device.
- ✓ *M. tuberculosis* complex (IS6110R, IS6110F)
- ✓ *M. bovis* (pncAMB, pncAMT, pncATB)
- ✓ PCR products were run at 100V for 25 mins in 1.5% agarose gel



### under.... outputs 3.1.1

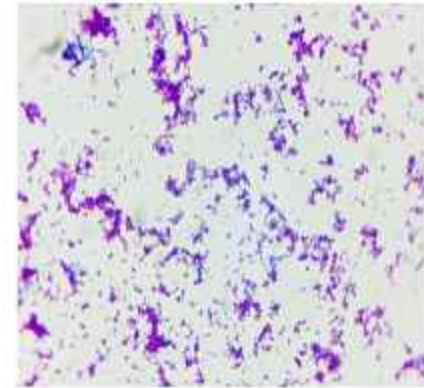
### *Bacteriological results of M. tuberculosis complex and M. bovis*



Mycobacterium-like colonies cultured in LJ medium



Mycobacterium-like colonies cultured in LJ medium



Zeihli-neelsen staining

Through bacteriological analysis, 10 isolates obtained in 2023 were fully cultured within 14–21 days. In 7H9 liquid medium, they produced uniform turbidity, while on LJ (Lowenstein-Jensen) solid medium, they formed small, rough, yellow colonies.



### *Paratuberculosis*

*Paratuberculosis*-like colonies were not observed in the 12 fecal samples.

Additionally, *Mycobacterium*-like colonies were not detected in the six milk samples.

## under.... outputs 3.1.2

## Result (Molecular biology)

PCR (using IS6110R and IS6110F primers) for detecting the *Mycobacterium tuberculosis* complex in 10 *Mycobacterium*-like isolates.

PCR (using pncAMB, pncAMT and pncATB primers) for detecting the *M. bovis* in 10 *Mycobacterium*-like isolates.



M – Marker (100bp), PC – Positive control, PC – Positive control, NC – Negative control, 1 – SEBR191, 2 – 373062, 3 – Y-1, 4 – Arg7, 5 – BN3, 6 – Arg2, 7 – 5, 8 – BN7, 9 – Y-1(1-1)10 – 373403



M – Marker (100bp), PC – Positive control, NC – Negative control, 1 – SEBR191, 2 – 373062, 3 – Y-1, 4 – Arg7, 5 – Arg2, 6 – BN3, 7 – BN7, 8 – 5, 9 – 373403, 10 – Y-1(1-1)

### Purification and concentration of DNA.

	Sample Name	Conce (ng/ μL)	A260/280 (Purity)
№			
1	Arg2	231.9	1.74
2	5	483.2	1.69
3	BN7	401.8	1.69
4	Y-1(1-1)	344.8	1.63
5	373403	432.7	1.67
6	SEBR191	762.9	1.82
7	373062	417.0	1.66
8	Y-1	317.6	1.60
9	Arg7	506.6	1.66
10	BN3	610.3	1.68

### Outputs 3.1.3

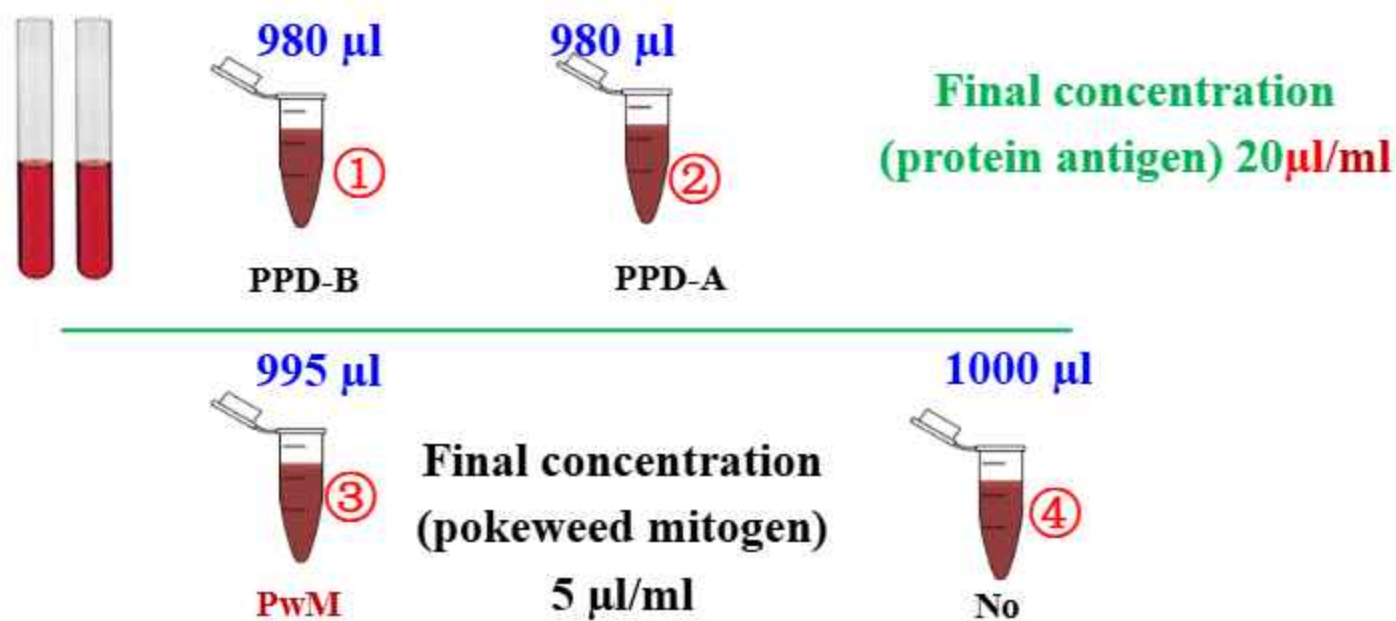
#### 3-1. Molecular-epidemiological evaluation of the epidemics of *M. bovis* Infection in livestock

3.1.1. To isolate tuberculosis complex using the L-J media in IVM, from the tubercles-suspected granulomas samples collected from cattle and sheep at slaughterhouses and meat markets in the project target areas.

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3.1.3. To investigate the endemic status of *M. bovis* infection including subclinical infection by performing the Interferon-Gamma Release Assay (IGRA) on herds in which *M. bovis*-detected cattle were kept.

### bTB surveillance in cows in UB



#### Methodology for stimulating 500 cows collected from 3 districts of Ulaanbaatar city in 2023/2024

- The ID Screen *Paratuberculosis* indirect ELISA (IDVet, France) and *John* disease kit (Japan) were compared (According to the methodology)
- ID Screen® Ruminant IFN-  $\gamma$  and BOVIGAM TM 2G TB ELISA were compared. (According to the methodology)



## ID Screen Ruminant INF-g ELISA results in 2023

№	District	Number of cows	Total positive	Type 1 antigen positive		Type 2 antigen positive (stiPPDB <sup>+</sup> ; stiPPDA <sup>+</sup> )
				stiPPDB <sup>+</sup>	stiPPDA <sup>+</sup>	
1	Songinokhairkhan	270	55	20	35	17
2	Bayanzurkh	40	6	4	1	0
3	Khan-Uul	190	8	3	5	2
Total		500	68	27	41	19

# Comparison of BOVIGAM™ 2G TB ELISA and ID Screen® Ruminant IFN- $\gamma$ results in 2024

ID_Sample	ID Screen Ruminants IFN-g ELISA						BOVIGAM™ 2G TB ELISA					
	Gamma interferon (IFN-γ) was stimulated by											
	None	PPDB	PPDA	PwM	PC-EC	PC-HP	PPDB	PPDA	PwM	PC-EC	PC-HP	Nil
	S/P	S/P	S/P	S/P	S/P	S/P	S/P	S/P	S/P	S/P	S/P	S/P
SHB-40	0.00	5.50	8.60	455.60	0.30	1.00	0.07	0.06	0.98	0.05	0.06	0.05
SHB- 98	0.00	1.70	4.30	179.60	-0.20	0.30	0.06	0.05	0.39	0.05	0.05	0.05
SHB-117	0.00	-1.60	-2.30	118.90	-2.30	-1.30	0.05	0.06	0.30	0.06	0.05	0.06
SHB- 158	0.00	3.30	7.70	474.00	3.50	1.30	0.06	0.05	0.26	0.06	0.06	0.07
SHB- 160	0.00	0.50	-0.20	146.40	2.40	0.50	0.06	0.06	0.31	0.05	0.07	0.05
SHB-233	0.00	3.70	11.40	402.20	-4.80	-4.50	0.06	0.08	0.96	0.06	0.06	0.06
SHB-240	0.00	0.80	1.70	382.70	0.20	0.00	0.05	0.05	0.67	0.06	0.05	0.05
KUB-21-4	0.00	-0.50	3.00	190.00	0.30	-1.50	0.05	0.06	0.11	0.05	0.05	0.06
ID Screen Ruminants IFN-g ELISA:						BOVIGAM™ 2G TB ELISA:						
S/P% <15% negative, S/P ≥ 15% positive						OD утга <0.130 negative OD утга 1.2 -2.0 positive						

All samples were negative.

## SEEKING WAYS TO ERADICATE TUBERCULOSIS AND GLANDERS INTERNATIONAL SCIENTIFIC CONFERENCE

### RESULTS OF BOVINE TUBERCULOSIS SURVEILLANCE IN THE ULAANBAATAR OF MONGOLIA

#### Result

Table 1. Skin test result

No	name of district	n_cows	positive only in		positives in both tuberculin
			tstPPDB	tstPPDA	
1	Songinokhairkhan	270	105	55	55
2	Bayanzurkh	40	2	0	0
3	Khan-Uul	190	17	2	2
<b>Total</b>		<b>500</b>	<b>124</b>	<b>57</b>	<b>57</b>

Figure 1. Skin test performing procedure



#### Material and Methods



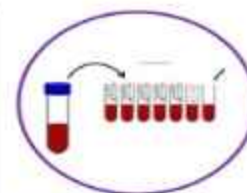
##### Randomly and multi-stage sampling:

1. Randomly select farms
2. Randomly select cows



##### Tuberculin skin test (TST)

- ✦ Injected on the left side by Bovine tuberculin (Bioconbinat, Mongolia)
- ✦ Injected on the right side by Avian tuberculin (Bioconbinat, Mongolia)



##### Whole blood stimulation (or induce) and plasma collection

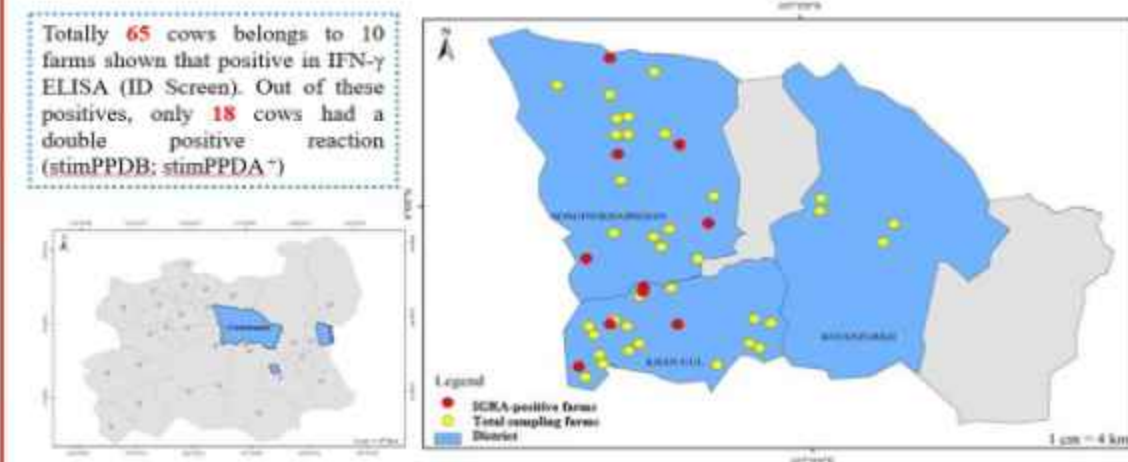
- ✦ PPDH (BOVIGAM™)
- ✦ PPDA (BOVIGAM™)
- ✦ Polysorbate monoglycidyl ether (BOVIGAM™)
- ✦ PC-EC peptide (BOVIGAM™)
- ✦ PC-EP peptide (BOVIGAM™)
- ✦ none stimulation



##### ID Screen Ruminant IFN-gamma ELISA (batch: 08140222)

BOVIGAM 2G TB  
ELISA KIT (lot: BG220907Z)

Figure 2. Geographically location of the sampling area and farms with IGRA positive cows in the Ulaanbaatar



**In conclusion:** Based on this surveillance, result indicating that 500 cows from 50 farms were not detecting infection with *M. bovis* infection

In such cases, conducting to differential diagnosis of John's disease (MAP)

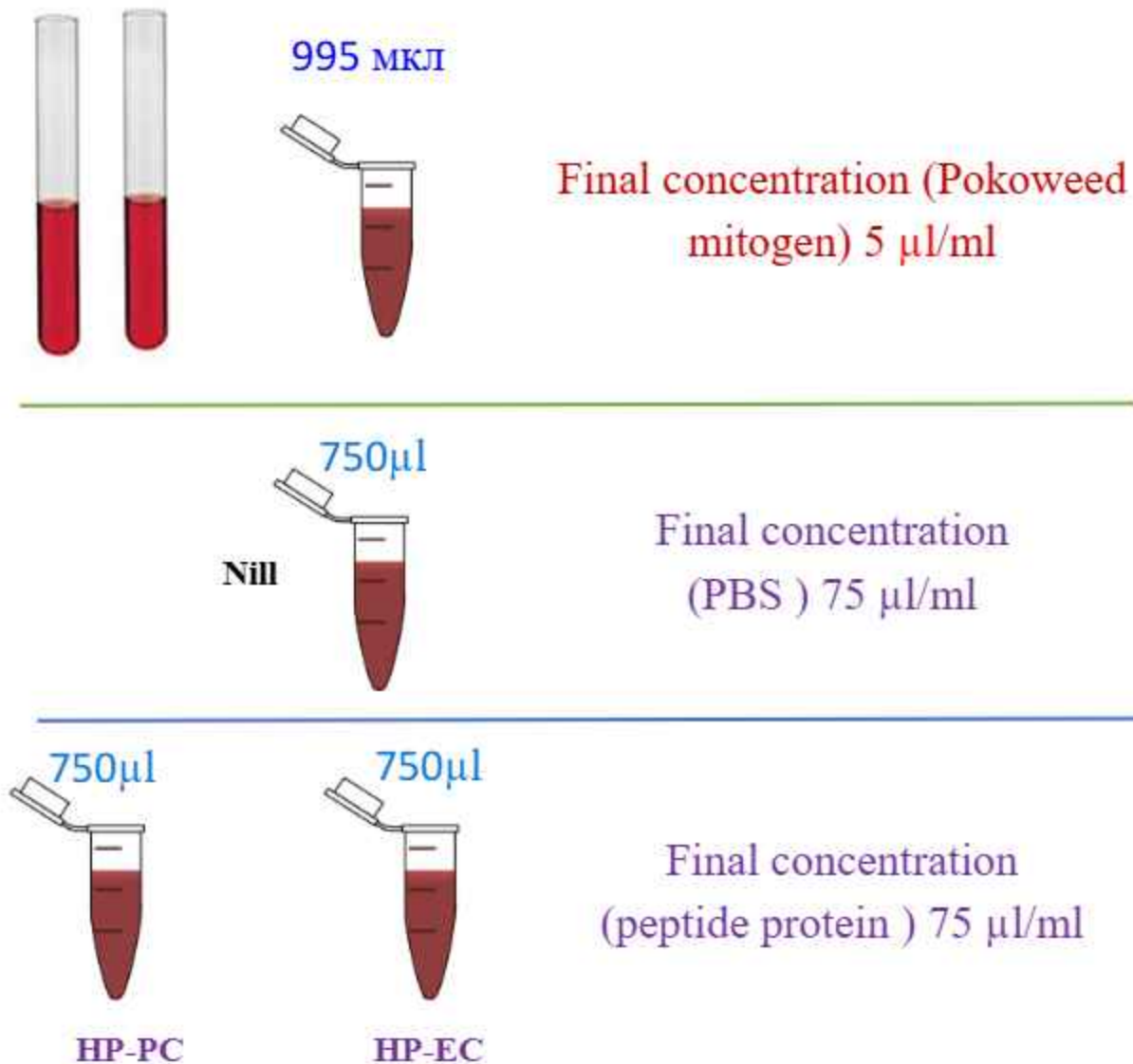
or *M. avium* infection is should be become necessary



# **Comparison of positive results with ID Screen Paratuberculosis indirect ELISA (IDVet, France) and Johne disease kit (Japan) in 2024**

№	District	Number of cows tested	ID Screen Paratuberculosis kit	Johne disease kit (Japan)
			Number of positive cows	
1	Songinohairkhan	270	4	2
2	Bayanzurkh	40	1	1
3	Khan-Uul	190	2	2
Total		500	7	5

## TB surveillance in sheep in 2024



1. PwM (Pokoweed mitogen)
2. PC-EC (peptide cocktails stimulating Ag)
3. PC-HP (peptide cocktails stimulating Ag)
4. Null: Dilution 1:10, by PBS

<i>Dornod province</i>	<i>40 sheep</i>
<i>Sukhbaatar province</i>	<i>45 sheep</i>
<i>Tuv province</i>	<i>13 sheep</i>
<i>UB</i>	<i>29 sheep</i>
<i>Total</i>	<i>127 sheep</i>

# Inputs

## 4.3. Risk assessment of *B. mallei* infection 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 assessment associated with *B. mallei* transmission between livestock and human, through the discussions between medical and veterinary glanders research groups.

1. The collected questionnaires (for the RA study of Glanders from 76 herders of 20 soums in 8 provinces



**ЯМ (BURKHOLDERIA MALLEI)-ЫН ХАЛДВАР**  
**ӨВЧНИЙ ЭРСДЭЛИЙН ҮНЭЛГЭЭНИЙ АРГА,**  
**АРГАЧЛАЛ**

Үнэлгээний баг

2022.05.12

**Brief introduction of a collaborative research project (SATREPS project) on "CONTROL OF TUBERCULOSIS AND GLANDERS"**

ХАМГАЙГАЛЫН ХАТГАЛ: 2018-2021

Харгалууд:

- 2018-2019
- 2019-2020
- 2020-2021
- 2021-2022
- 2022-2023
- 2023-2024
- 2024-2025
- 2025-2026
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# International Scientific Conference was organized



## Installation and opened BSL3 laboratory







## Training at HU

An assessment of the social impact and transmission risk of **bovine Tuberculosis/Glanders** was conducted in collaboration with experts from the National Center for Disease Control and Prevention (NCDC).



## Goal in 2025

- To continue conduct evaluation of the newly developed rapid serological (ICT) and molecular (dry-LAMP) assays
- To write at least 3 research manuscripts:

about .....

“Report of the Equine Glanders Surveillance”

“Isolation and Characterization of the *B. mallei* isolates”

“Report of the bTB surveillance in UB”

want to more .... **“Report of the John Disease Surveillance in UB”**

- To conduct of the John disease Ab surveillance in cattle serum (from bull) collected from 21 province (including 330 soums), ..... **about more than 2200 cattle serum collected in 2024/**
- To cooperate to establish “OH Zoonosis **Platform**”

**Example:** “Online platform on control of Glanders/bTB”

- An international conference will be organized
- To be send trainees to Kimura sensei

**/training on to produce of ICT/**