Under project of the

"CONTROL OF TUBERCULOSIS AND GLANDERS"

/SATREPS project/

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

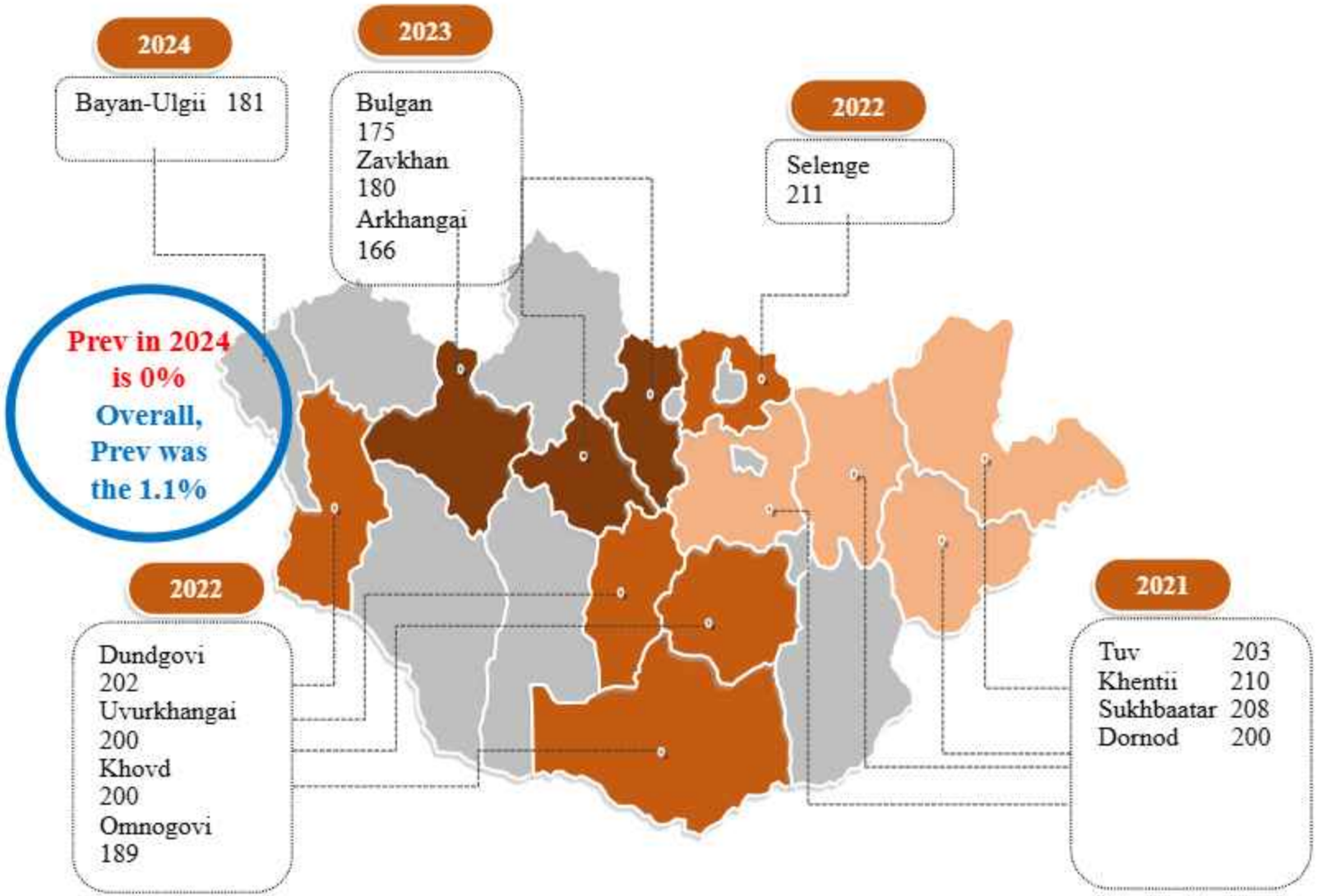
Duration: Feb 2024 to Dec 2024

by Team of Glanders/bTB Research Group

5 February 2025

Inputs	Duration
3-3. Molecular-epidemiological and sero-epidemiological evaluation of the epidemics of *B. mallei* infection in horses	
3.3.1 To perform a sero-epidemiological survey on *B. mallei* 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 *B. mallei* 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)**

SEEKING WAYS TO ERADICATE TUBERCULOSIS AND GLANDERS

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Sero-epidemiological situation of the equine glanders in Mongolia

Aim of this study

To determine of equine glanders prevalence and distribution in the country

Objective

- To collect samples through randomly selected horse flocks and horses.
- To perform serology for the glanders.

Materials and methods

Sampling → **Randomly and multi stage sampling** *(WHO, 2015)*

- *1st stage: Pilot* area: Provinces and soums are pilot in the project
- *2nd stage:* Randomly selected horse flocks (or herds)
- *3th stage:* Randomly selected horses

Serology → **Complement Fixation Test** *(all components manufactured in Biocombinat, Ulaanbaatar, Mongolia) Performing under the standard as a "MNS 5200: 2002"*

→ Confirmatory test: iELISA with whole cell lysate antigen *(cut-off point -0.343)*; Western blotting with lipopolysaccharide antigen *(recommended by WOAH)*

analysis → **(EXCEL & ArcGIS Ver. 10.4)**

Diag 2. Level of equine glanders detected in provinces, in soums and in flocks (for the %)

Fig 2. Map of sampling distribution

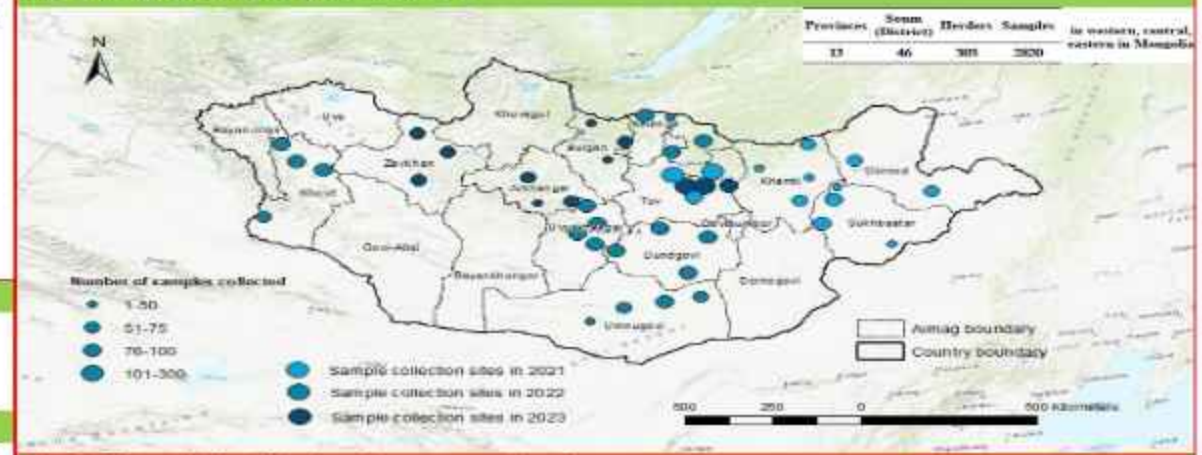
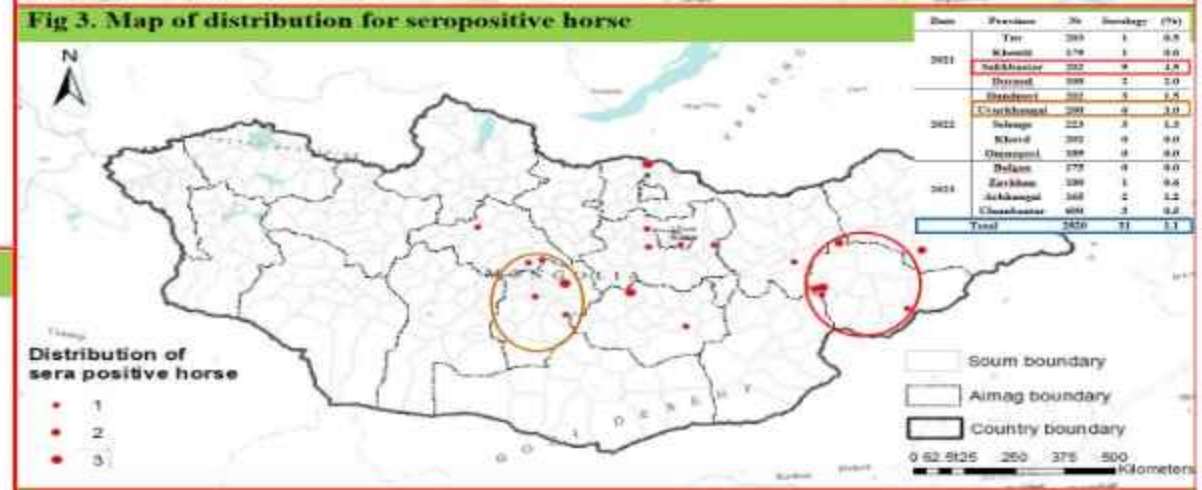


Fig 3. Map of distribution for seropositive horse



Date	Province	Jn	Serology	(%)
2021	Tov	203	1	0.5
	Khentii	179	1	0.6
	Sukhbaatar	202	9	4.5
	Dornod	199	2	1.0
	Dundgovi	202	3	1.5
2022	Uvurkhangai	200	6	3.0
	Selenge	223	3	1.3
	Khovd	202	0	0.0
	Omnogovi	189	0	0.0
2023	Bulgan	175	0	0.0
	Zavkhan	180	1	0.6
	Arkhangai	160	2	1.2
	Govi-Altai	400	3	0.8
Total		2820	31	1.1

Conclusion

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

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

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

№	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

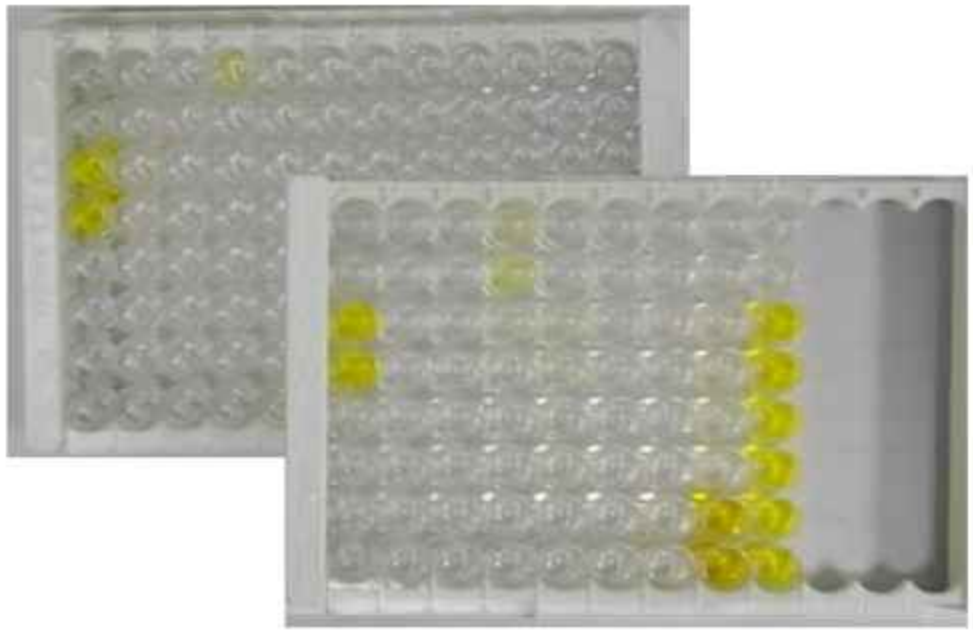
Data collection:

✓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	Uvurkhangai	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	202409	Dornod	2	9	9	0	0
8	2024.1	Sukhbaatar	2	8	8	0	0
Total			18	76	76	0	0

(LOT-O06)
Exp: 2026/02



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

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

INTER-SECTORAL RISK ASSESSMENT

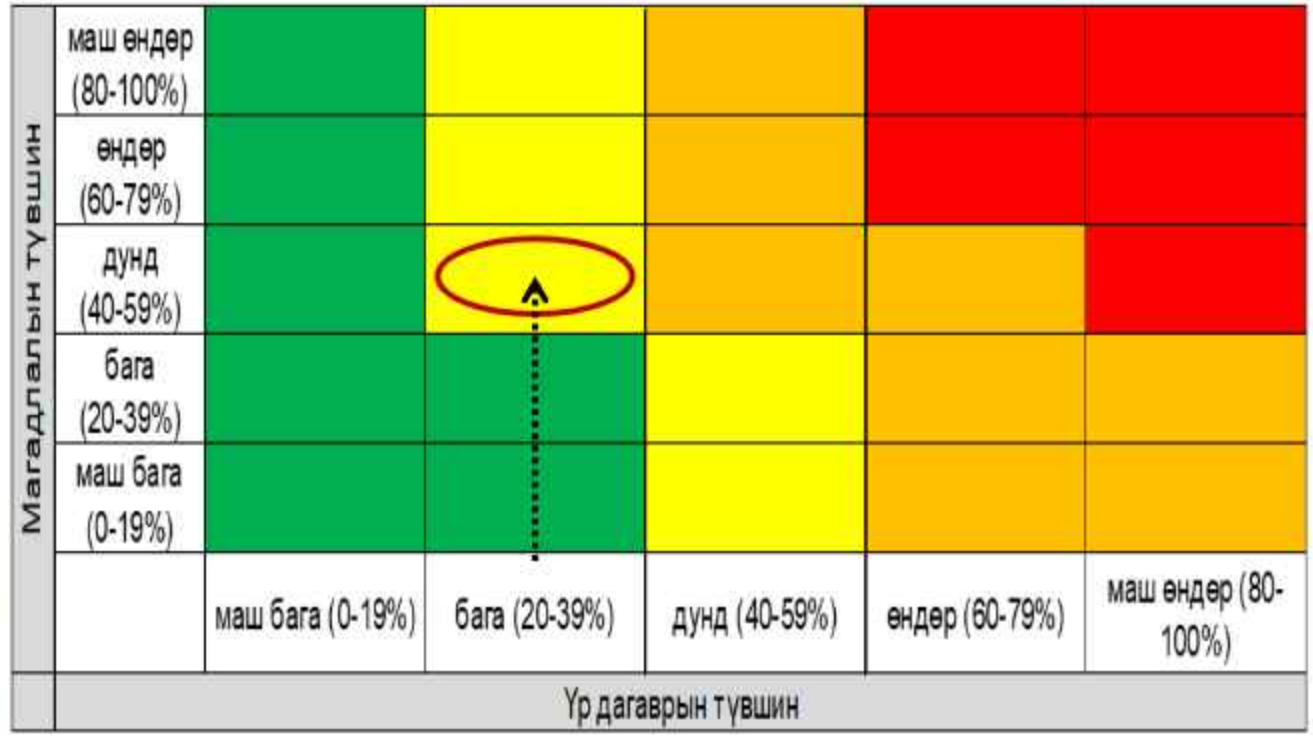
Participants: 20 medical doctors & vets

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

Organized time: 5th of Jul, 2024

Venue: EOP of NCCD

DISCUSSED & ASSESSED: A TOTAL OF 31 QUESTIONS



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

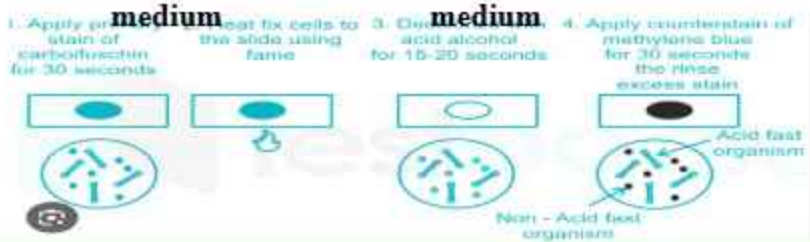
3-1. Molecular-epidemiological evaluation of the epidemics of *M. bovis* Infection in livestock
3.1.1. To isolate tuberculosis complex using the L-J mediua in from the tubercles-suspected granulomas samples collected fro cattle and sheep at slaughterhouses and meat markets in the pr target areas.
3.1.2. To estimate the prevalence of *M. bovis* in the tuberculos complex isolated from cattle and sheep by determining the pre of *M. bovis* in the colonies grown on L-J medium using the ge 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-G Release Assay (IGRA) on herds in which *M. bovis*-detected cat 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 mediua 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

7H9 OADS medium → **LJ medium**



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

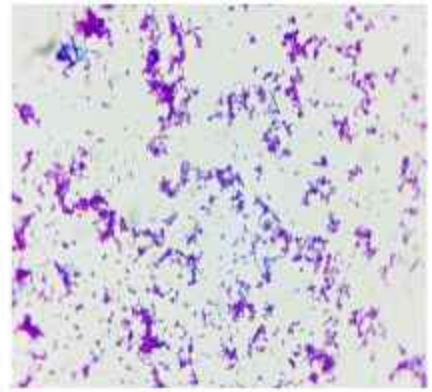
- ✓ **DNA was isolated from 10 mycobacterium-like cultures and its concentration was measured at 260/280 nm using a nanodroplet purification device.**
- ✓ ***M. tuberculosis* complex (IS6110R, IS6110F)**
- ✓ ***M. bovis* (pncAMB, pncAMT, pncATB)**
- ✓ **PCR products were run at 100V for 25 mins in 1.5%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

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

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

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 mediua 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.

bTB surveillance in cows in UB

980 µl

PPD-B

980 µl

PPD-A

Final concentration (protein antigen) 20µl/ml

995 µl

PwM

Final concentration (pokeweed mitogen) 5 µl/ml

1000 µl

No

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- γ 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$^+$; stiPPDA$^+$)
				stiPPDB$^+$	stiPPDA$^+$	
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 BOVIGAMTM 2G TB ELISA and ID Screen® Ruminant IFN- γ 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	Nill
	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: S/P% <15% negative, S/P ≥ 15% positive							BOVIGAM™ 2G TB ELISA: OD ytra <0.130 negative OD ytra 1.2 -2.0 positive					

All samples were negative.

SEEKING WAYS TO ERADICATE TUBERCULOSIS AND GLANDERS
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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
Total		**500**	**124**	**57**	**57**

Figure 1. Skin test performing procedure

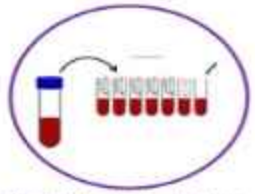
Material and Methods

Randomly and multistage 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

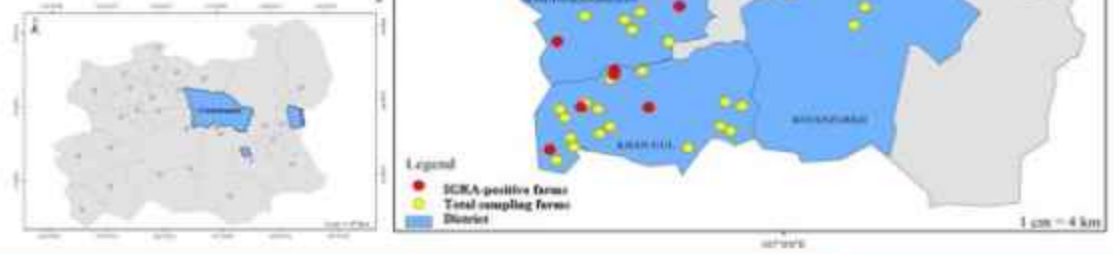
- PPDB (**BOVIGAM™**)
- PPDA (**BOVIGAM™**)
- Pokeweed mitogen-PrtM (**BOVIGAM™**)
- PC-EC peptide (**BOVIGAM™**)
- PC-HP peptide (**BOVIGAM™**)
- none stimulation

ID Screen Ruminant IFN-g 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

Totally **65** cows belongs to 10 farms shown that positive in IFN-γ ELISA (ID Screen). Out of these positives, only **18** cows had a double positive reaction (stimPPDB: stimPPDA⁻)



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

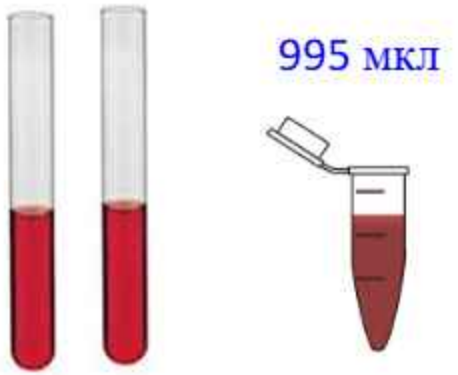
In such cases, conducting to differential diagnosis of John's disease (MAP)

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

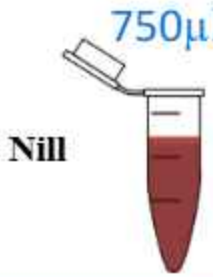
№	District	Number of cows tested	ID Screen Paratuberculosis kit	Johne disease kit (Japan)
			Number of positive cows	
1	Songinohairkhan	270	4	2
2	Bayanzurkh	40	1	1
3	Khan-Uul	190	2	2
	Total	500	7	5

TB surveillance in sheep in 2024

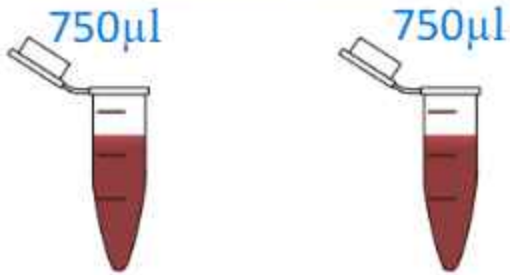


995 мкл

Final concentration (Pokoweed mitogen) 5 µl/ml



Final concentration (PBS) 75 µl/ml



Final concentration (peptide protein) 75 µl/ml

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

Dornod province	***40 sheep***
Sukhbaatar province	***45 sheep***
Tuv province	***13 sheep***
UB	***29 sheep***
Total	***127 sheep***

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

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/