Under project of the

“CONTROL OF TUBERCULOSIS AND GLANDERS”

/SATREPS project/

GLANDERS: Progress and Future activity Implementation in IVM

Duration: Oct, 2020 to Nov, 2022

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by Team of Glanders Research Group

17 November, 2022

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

Outputs 3.3.1

Table 1. Results of serology in 2021/2022

(RANDOM SURVEILLANCE)

Date	Province	Number of samples	CFT (%)
2021	Tuv	203	1 (0.49)
	Khentii	210	1 (0.48)
	Sukhbaatar	208	9 (4.3)
	Dornod	200	2 (1.0)
2022	Dundgovi	202	3 (1.4)
	Uvurkhangai	200	6 (3)
	Selenge	211	55 (26)
	Khovd	200	0
	Omnogovi	189	0
Total		**1823**	**77 (4.22)**

Table 2. Results of serology test in 2020-2022

(RISK-BASED SURVEILLANCE)

Date	Province	Number of samples	CFT (%)
2020	Tuv	185	8 (4.3)
	Khentii	382	18 (4.7)
	Sukhbaatar	220	0
	Dornod	86	3 (3.5)
	Dundgovi	44	24 (54.5)
2021	Dornod	5	1 (20)
	Tuv	363	13 (3.6)
	Sukhbaatar	1	1 (100)
2022	Ulaanbaatar	143	10 (6.9)
	Tuv	20	4 (20)
	Dornod	3	0
	Sukhbaatar	4	0
	Bulgan	19	0
	Dornogovi	2	0
Total		**1477**	**82 (5.5)**

Table 3. Results of serology test for Camel in 2022

Date	Province	County	Number of samples	CFT (%)
2022	Umnugovi	**Tsogttsetsii**	22	0
		Sevrei	19	0
		Dalanzadgad	17	0
	Bayankhongor	Bogd	31	0
		Bayanlig	24	0
		Bayangovi	29	0
	Dundgovi	Ulziit	85	0
	Total	**7**	**227**	**0**

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Outputs 3.3.4

Table 4. Collected samples from slaughterhouses and stakeholder in 2020-2022

№	Livestock species	Samples (lung. spleen, testis, kidney, liver and skin)	Bacteriology	PCR detection	
				All Burkholderia	*B. mallei*
1	Horse[1]	33	33	2	-
	Camel[1]	7	7	2	-
2	Horse[2]	38	38	21	7
TOTAL		**78**	**78**	**25**	**7**

Bacteriology was performed under the "**OIE Terrestrial Manual" used as a guideline.**

[1]Samples collected from slaughtered horses in Slaughterhouses

[2]Samples collected from Glanderous horse that were Euthanized

Clinical Symptoms of Glanders Case

1

Fig 1. Glanders diagnosed horse (Respiratory phenotype)

2

Fig 2. Glanders diagnosed horse (Skin phenotype)

3

Fig 3. Positive reaction at 24 hrs after skin test was performed

Bacteriology Result

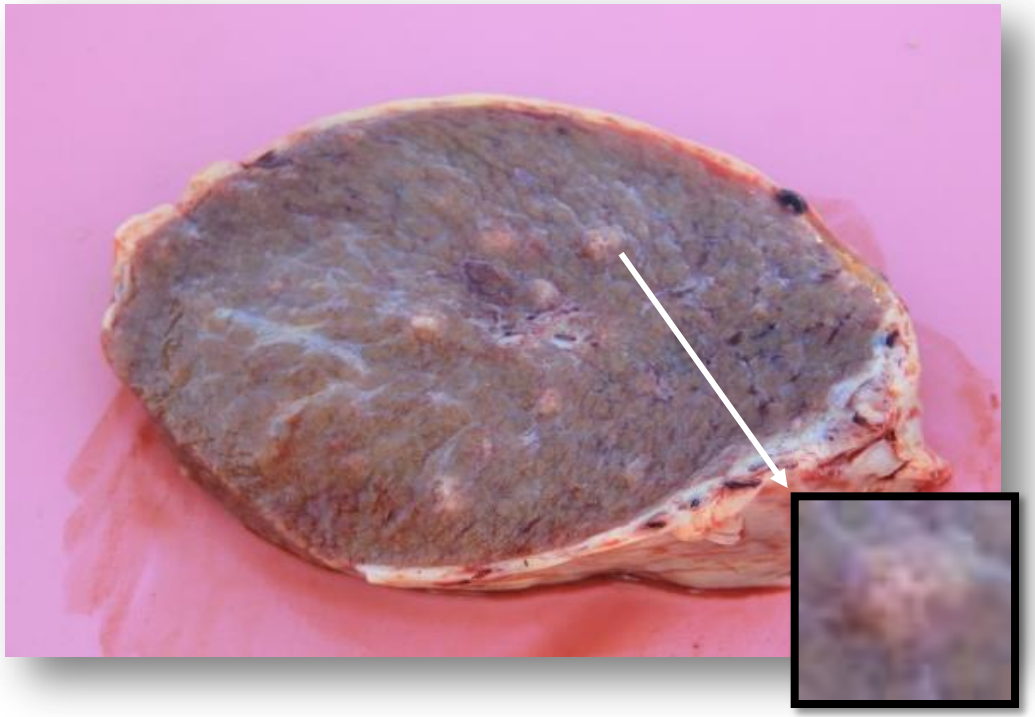


Fig 4. Granular nodule formation in the testis of glanders diagnosed horse

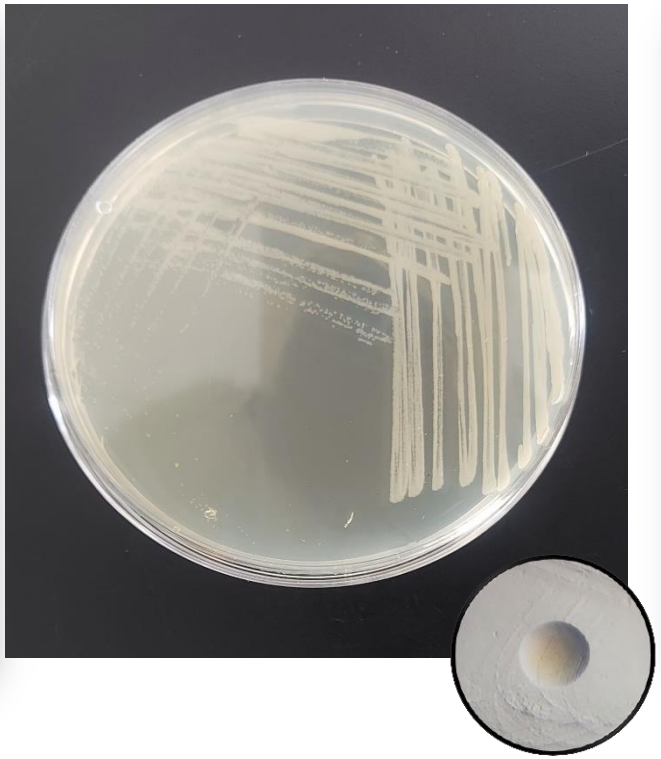


Fig 5. The morphology of *B. mallei* like colony on selective agar containing 4% glycerin.

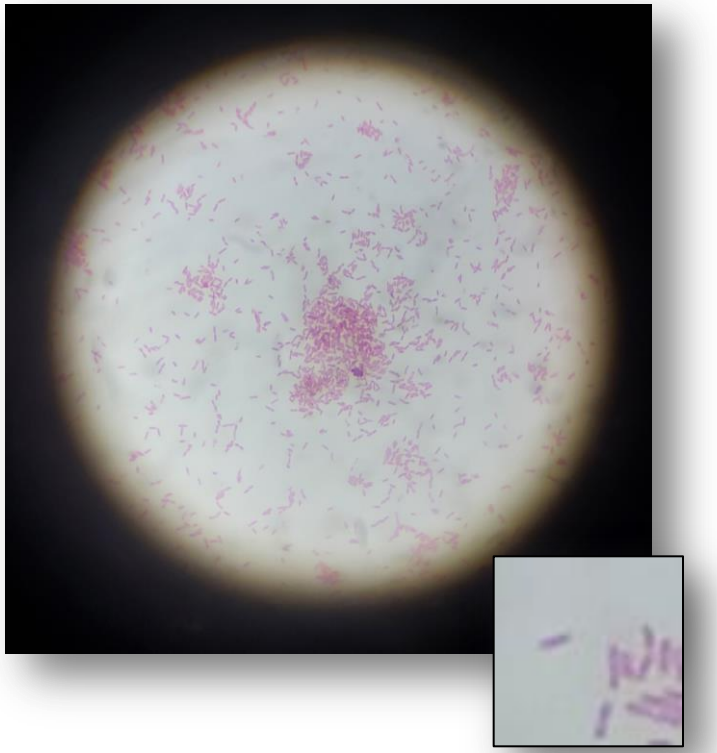


Fig 6. Gram staining of *B. mallei* showing Gram-negative rod-shaped with bipolar staining. (100x)

Molecular Biology Result

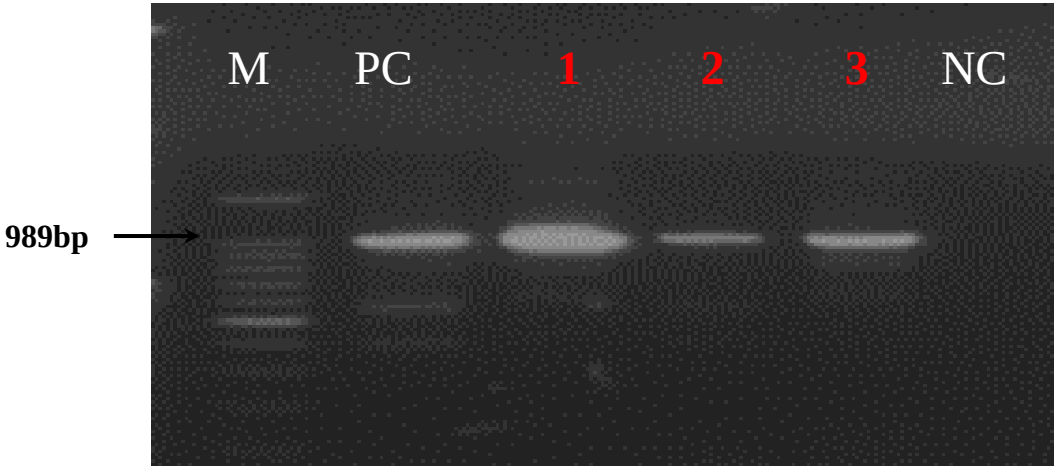


Fig 7. Result of standard PCR with species specific primers of *B. mallei* in **2020**

M - 100bp DNA ladder
PC - positive control
1 -3 new isolates
NC - negative control

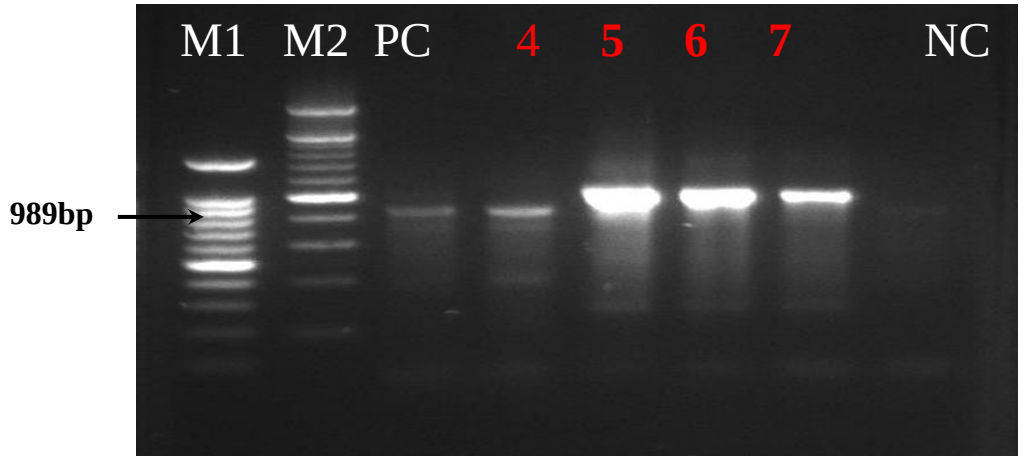


Fig 8. Result of standard PCR with species specific primers of *B. mallei* in **2022**

M1 - 100bp DNA ladder
M2 - 200bp DNA ladder
PC - positive control
4 - 7 new isolates
NC - negative control

PCR detection of *B. mallei* using primer pairs
Bma-ISO407-flipF (5"TCA-GGT-TTG-TAT-GTC-GCT-CGG-3")
Bma-ISO407-flipR (5"CTA-GGT-GAA-GCT-CTG-CGC-GAG-3")

Reference Article: ***OIE manual. Chapter 3.6.11 GLANDERS AND MELIOIDOSIS***

CONCLUSION

Between 2020/2021 to Oct, 2022, a total of 3300 horses subjected to the serological testing. As a result,

Prevalence was at 4.8%.

The 3 isolates in 2020/2021 and 4 isolates in 2022, (a total 7) were confirmed as *B. mallei* by bacteriology and PCR, respectively.

Except for those confirmed 7 isolates, all 18 isolates tested positive for PCR of *Burkholderia* spp.

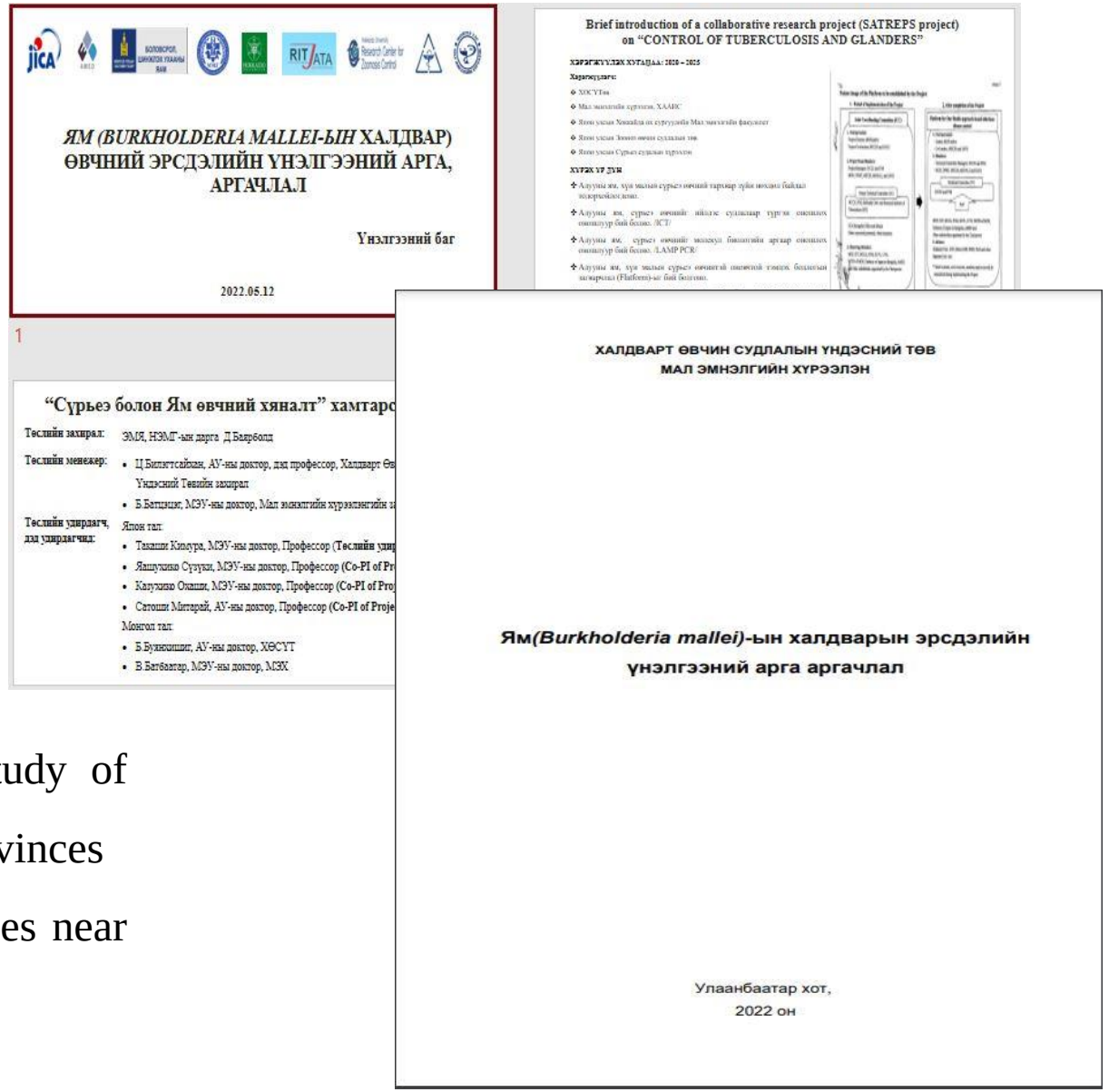
However, none of the 18 isolates were confirmed to be *B. mallei*.

In the future, we would like to identify which species of the *Burkholderia* spp of the 18 isolates belong.

Inputs

4.3. Risk assessment of *B. mallei 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 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 **97** herders of **20** soums in 5 provinces
2. The collected questionnaires 14 slaughterhouses near UB (located in Emeelt and Nalaikh)

Next Goal

- To perform random and Glanders risk-based surveillance until end of project
- To isolate more *B. mallei* culture
- To conduct evaluation of the rapid serological and molecular assays
- To conduct risk assessment of *B. mallei* infection as a zoonotic disease
- To send trainees to HU, Japan

THANK YOU FOR YOUR

KIND ATTENTION