Under project of the

"CONTROL OF TUBERCULOSIS AND GLANDERS"

/SATREPS project/

BTB: Progress and Future activity implementation in IVM

Duration: Oct, 2020 to Nov, 2022

Speaker: Ts. BATBOLD, DVM, member of BTB Research Group

by Team of BTB Research Group

17 November, 2022

Tables show the Operation plan of the project (Revised)

Outputs 3.1.1

Collected samples from Slaughterhouses in UB /Emeelt and Nalaikh/				
Lung samples	**Cattle/yak**	**Sheep**	**Camel**	**Total**
2021	**62**	**28**	**2**	**91**
2022	**87**	**77**	**21**	**185**

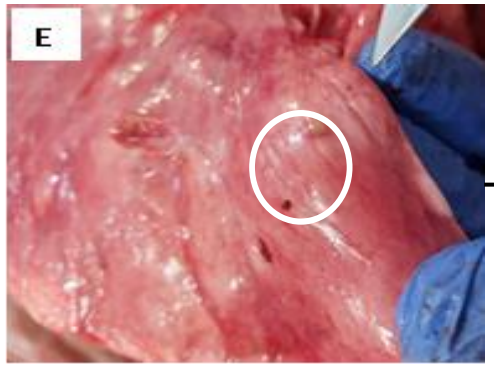
Collected milk from Selenge province	
Milk samples	**Cattle**
2022	**24**

Bacteriology was performed under the "OIE Terrestrial Manual of Standard for Diagnostic Tests and Vaccines" used as a guideline.

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.

Bacteriology results

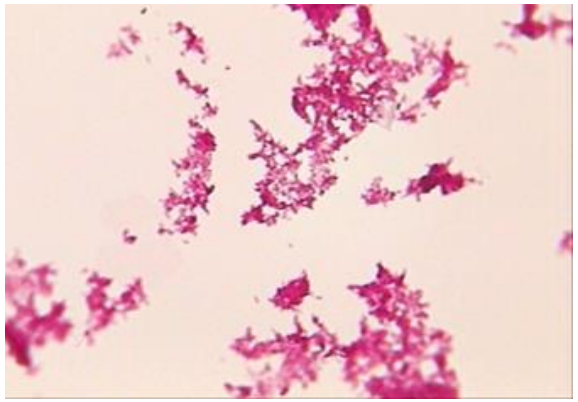
The lung samples were processed then cultured in Lowenstein media incubating at 37°C in for up to 8-10 weeks. Mycobacterium like colony growth was observed in the sample “Y1” and “Sh17”.



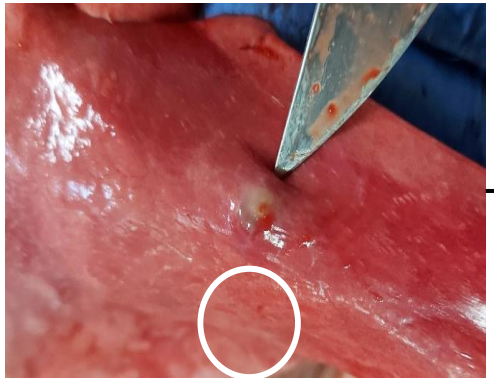
Cattle Lung “Y1”

Lowenstein Jensen media

multiple colony observed

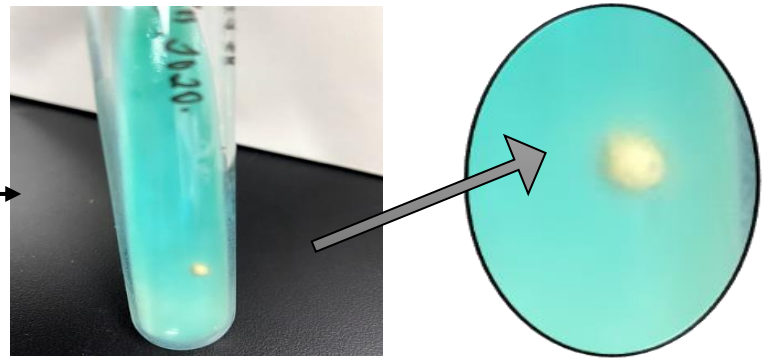


Zeihl-neelsen staining

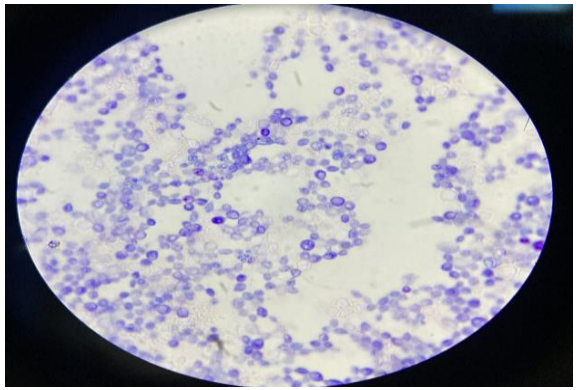


Sheep Lung

Lowenstein Jensen media

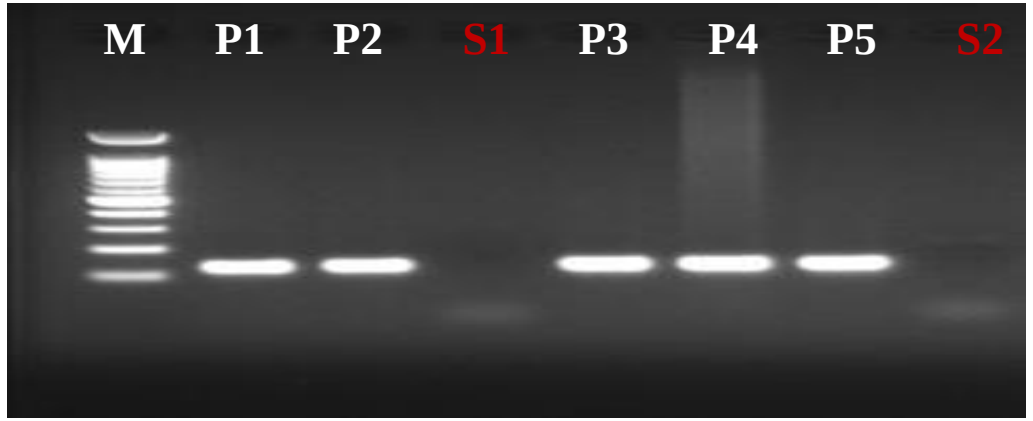


Single colony observed



Zeihl-neelsen staining

Result (Molecular biology)



M - 100 bp molecular weight marker
P1 - positive control M.tuberculosis
P2 - positive control M. bovis
S1- lung sample Y1
P3 - positive control BCG
P4 - positive control from NCCD
P5 - positive control from NCCD
S2- lung sample SH17

Protocol:

- ✓ DNA was isolated from a suspected colony by a purification kit and measured its concentration at 260/280 nm
- ✓ PCR mixer mix was prepared using MTBC specific primer (IS6110) PCR conditions were as below

  94°C for 2 min

  94°C for 30 sec

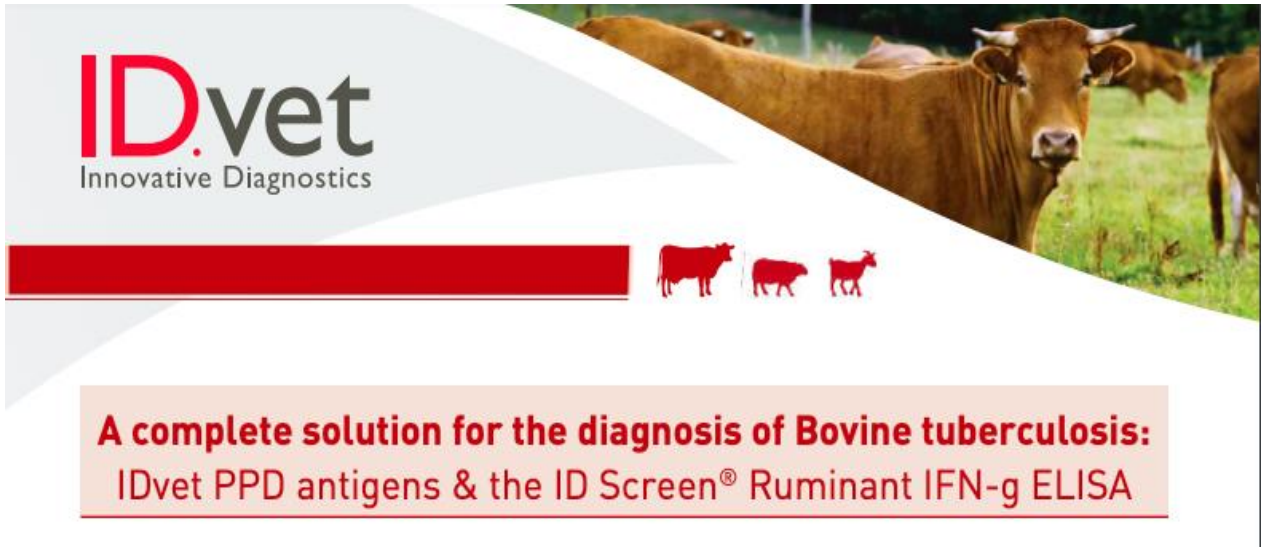
  63°C for 30 sec

  68°C for 1 min

  4°C with total of 35 cycles
- ✓ PCR products were run at 100V for 25 mins in 1.5% agarose gel

Tables show the Operation plan of the project

Outputs 3.1.3



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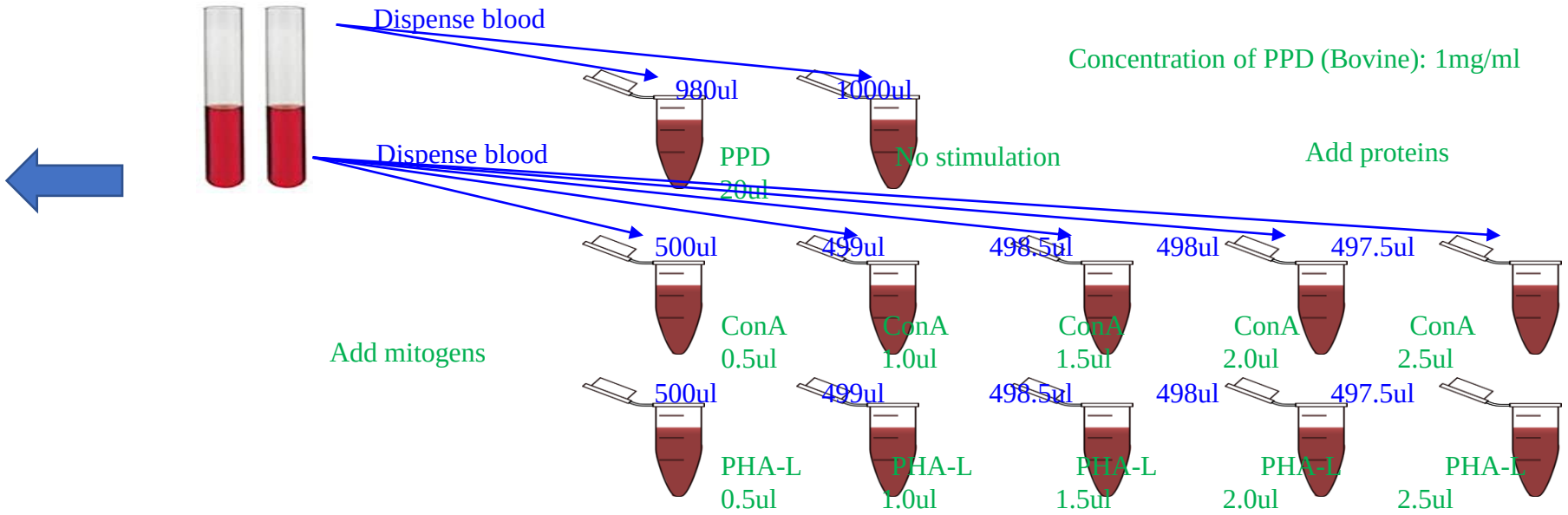
Slide illustrates different approaches that was taken for collecting plasma samples induced by PPD from *M. bovis* and *M. avium* and several mitogens conducted for the IGRA assay

This approach was taken in **Uvurkhangai, Dundgovi** and **Selenge provinces**



2, Dispense blood

980ul

980ul

Bovine-PPD
20ul

Avian-PPD
20 ul

3, Add proteins

Final concentration of proteins: 20 ug/ml

1, Collect heparin blood 5 ml

Less than 16 hrs at room temperature (17 – 27℃).

995ul

1000ul

4, Add mitogen

PHA-L
5.0ul

No stimulation

Final concentration of mitogen: 5 ug/ml

This approach was taken in **Khovd, Umnugovi and Selenge** (Risk-based) **provinces**

Total number of collected plasma samples and results of IGRA assay in 2022

n	Province	Soum	Plasma sample no stimulation	Plasma sample induced with PPD_B			Plasma sample induced with PPD_A			Plasma sample induced with ConA			Plasma sample induced with PHA-L		
				n	IGRA assay		n	IGRA assay		n	IGRA assay		n	IGRA assay	
					tested	positive		tested	positive		tested	positive		tested	positive
1	Dundgovi	4	40	40	5	1	0	n/a	0	40	5	0	40	5	2
2	Uvurkhangai	4	60	60	7	1	0	n/a	0	60	7	0	60	7	3
3	Selenge	4	53	53	38	8	0	n/a	0	53	38	0	53	38	6
4	Khovd	4	56	56	53	6	56	53	1	0	0	0	56	n/a	0
5	Umnugobi	4	195	195	53	7	195	53	0	0	0	0	n/a	n/a	0
	Total	20	**404**	404	156	**23**	251	106	**1**	153	50	0	209	50	**11***

IFN-g ELISA was conducted on plasma samples induced by mitogens and PPDs in 156 cows.
Collected plasma samples from 404 cows but didn't test yet on 248 cows. **Because:** Due to the finish of the plates in the kit.
However, we ordered IFNg-ELISA kits and waiting for them.
Each blood sample was induced by various mitogens (5 different concentrations in each mitogen) and PPDs (PPD from *M. bovis* and *M. avium*).

***The positive reaction of the assay was not strong, the value of ODs was a little bit higher or similar to plasma without stimulation**

Risk-based surveillance results in Selenge on Sept, 2022

Positive samples from IFN-g ELISA results were further examined by skin tuberculin test and blood plasmas were collected once again for comparison

n	ID of sample	IGRA assay		Skin thickness before inoculation (mm)	After inoculation at 48 hrs (mm)	
		PPD-B	PHA-L		PPA/differ	PPB/differ
1	SeB 95	+	-	9	11/2	12/23
2	SeB 97	+	+	9	11/2	16/7?
3	SeB 111	-	-	9	12/3	12/3
4	SeB 116	-	-	-	-	-
5	SeB 117	-	-	-	-	-
6	SeB 118	-	-	-	-	-
7	SeB 126	-	-	8	-	9/1
8	SeB 127	-	-	9	-	13/4
9	SeB 128	-	+	-	-	-
10	SeB 138	-	-	-	-	-
11	SeB 141	+	-	-	-	-
12	SeB 148	+	+	-	-	-
13	SeB 162	+	+	8	13/5?	-
14	SeB 166	+	+	-	-	-
15	SeB 169	+	+	-	-	-
16	SeB 190	-	-	11	11/0	12/1
17	SeB 191	-	-	9	11/2	12/2
18	SeB 200	+	-	11	13/2	13/2
19	SeB 201	-	-	9	11/2	12/2
20	SeB 204	-	-	14	12/2	10/2
21	SeB 210	-	-	11	11/0	10/1
22	SeB 214	-	-	12	11/-1	13/1
23	SeB 244	-	-	8	11/3	13/5?
24	SeB 245	-	-	8	12/4?	13/5?
25	SeB 256	-	-	9	13/4?	14/5?
26	SeB 257	-	-	8	11/3	12/4?

A. Skin thickness before inoculation

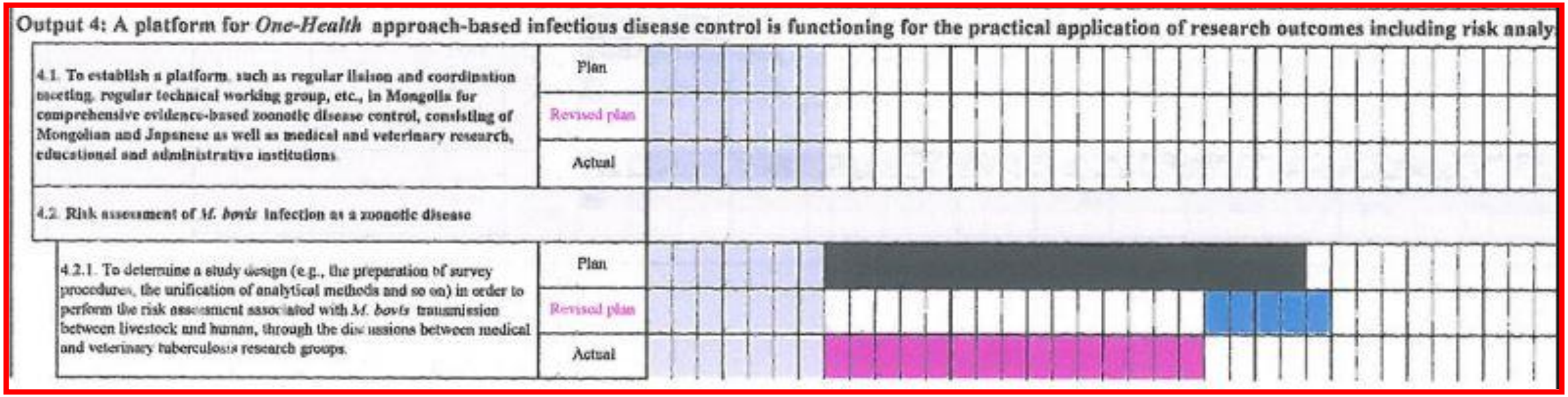
B. Skin thickness after 48 hours

CONCLUSION

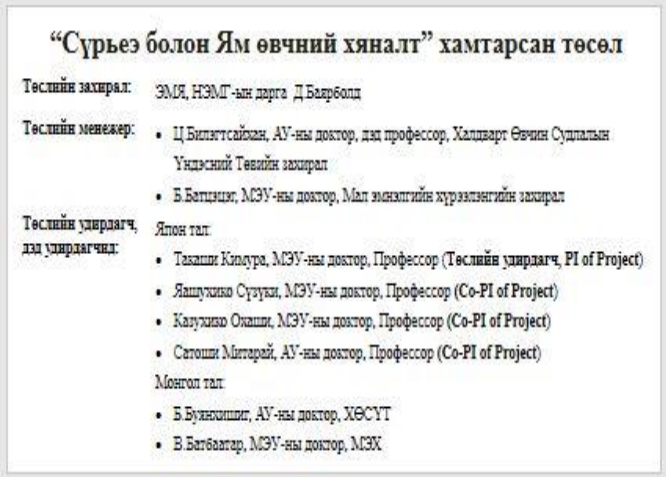
Total of 1495 cattle lungs were examined at the slaughterhouses in the 2021/2022, Out of those, 149 lungs with microscopically changed. We collected **65 samples observed with formation of nodular granulomas of tuberculosis** and further examined for BTB research.

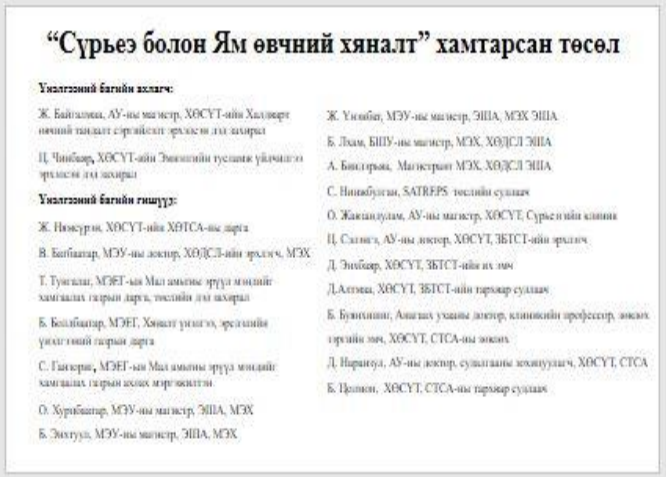
For the bacteriology result, 2 isolates (Y1 and Sh-17) showed ***Mycobacteria* spp** like growth on LJ agar **but showed the negative results in PCR.**

The IGRA assay suggested, **23 samples with PPD-B** and **1 sample with PPD-A were positive,** respectively.


Output 4: A platform for One-Health approach-based infectious disease control is functioning for the practical application of research outcomes including risk analy
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.
Plan
Revised plan
Actual
4.2. Risk assessment of M. bovis infection as a zoonotic disease
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 assessment associated with M. bovis transmission between livestock and human, through the discussions between medical and veterinary tuberculosis research groups.
Plan
Revised plan
Actual


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.
Plan
Revised plan
Actual
4.3.2. To perform the risk assessment of B. mallei transmission between livestock and human as well as its pathogenicity by analysing the results of the epidemiological analyses of B. mallei infection in human and livestock obtained in the Output 2 and the Output 3 in an integrated manner.
Plan
Revised plan
Actual





1. **The collected the questionnaires (for the RA study of BTB) from 98 herders of 20 soums in pilot 5 provinces in 2022**
2. **The collected the questionnaires from 14 workers of slaughterhouses near UB (located in Emeelt and Nalaikh) in 2022**

Next Goal

- To perform random and BTB risk-based surveillance until end of project
- To obtain causative agents of BTB
- To conduct evaluation of developed serological and molecular assays for BTB
- To conduct risk assessment *of M. bovis* infection as a zoonotic disease
- To send trainees to HU, Japan

THANK YOU FOR YOUR

KIND ATTENTION