Under project of the

“CONTROL OF TUBERCULOSIS AND GLANDERS”

/SATREPS project/

Progress report: Glanders/BTB surveillance in IVM

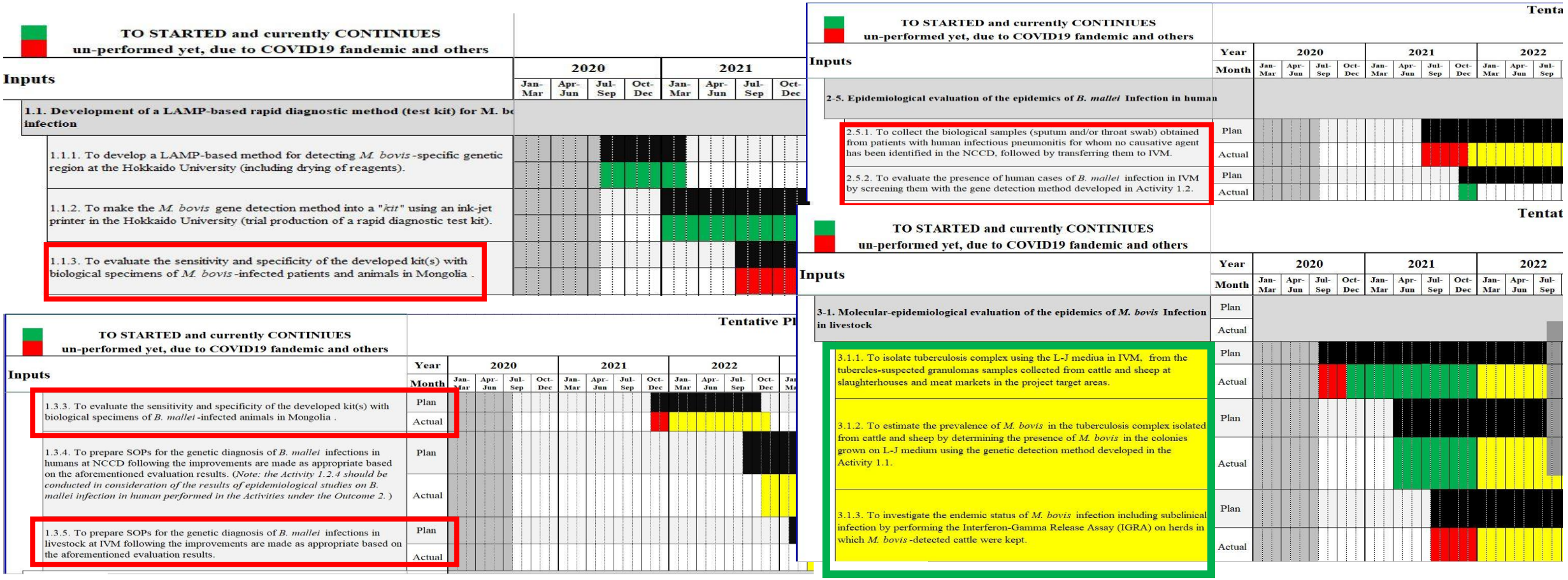
Between November 2020 to November 2021

Ulaanbaatar Mongolia

17 November, 2021

by Research team of IVM

Tables show the Operation plan of the project



Outputs 1.1.3/1.3.3/1.3.5 and 2.5.1/2.5.2

UNACCOMPLISHED, DUE TO COVID-19 PANDEMICS/OTHERS

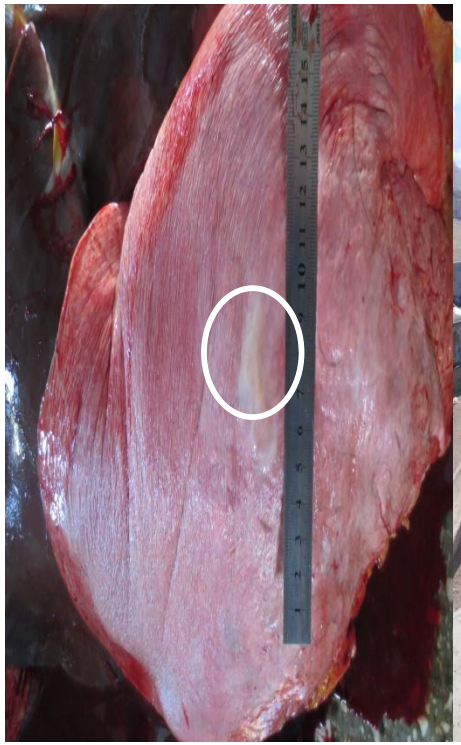
FUTURE ACTIVITIES

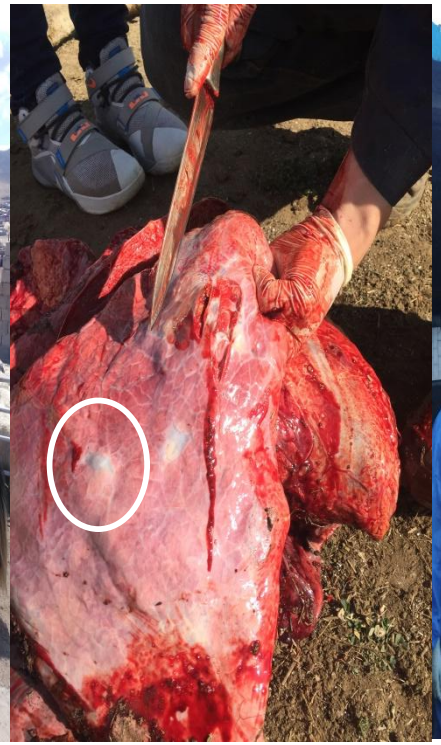
/COVID 19 Quarantine with full lockdowns (4 times) and Government agencies have been operating under the restricted working hours/other issues/

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℃ in 5-10% CO_2 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).

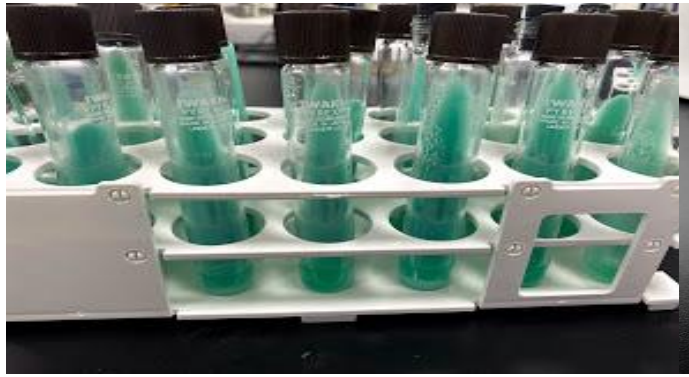


Fig 2. 20 lung tissue samples growth on Lowenstein Jensen solid medium

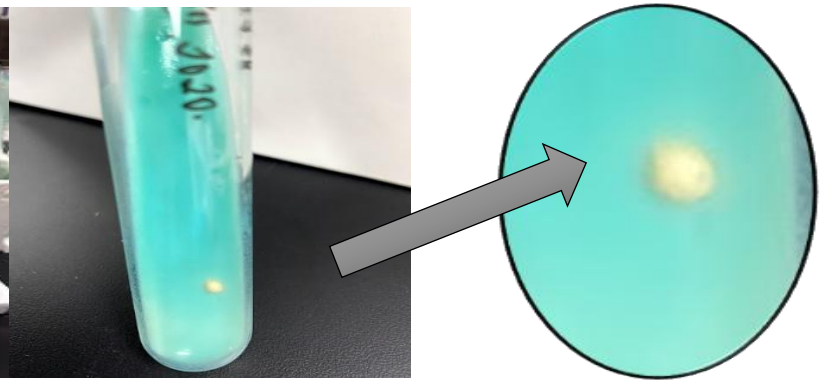


Fig 3. Bacteria colonies observed like *Mycobacteria* spp in LJ medium (isolated ID_20EU36)

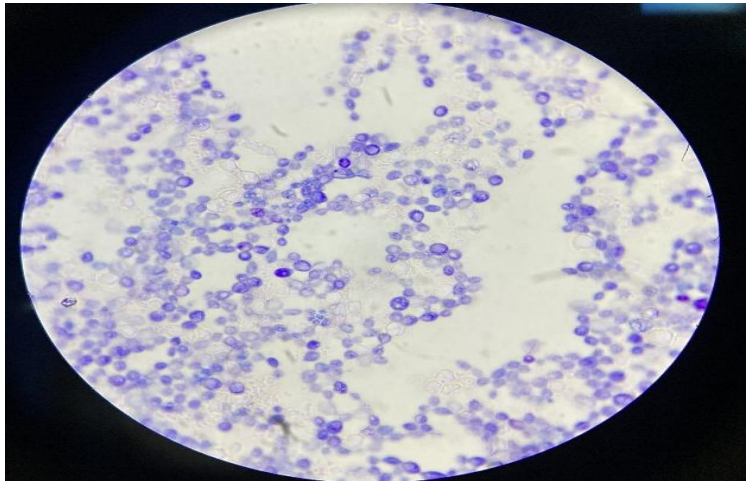


Fig 4. Zeihl-Neelsen Staining results

PCR result of isolates (sample ID_20EU36)

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PCR result of the lung sample 20EU36 from Emeelt,

resulted a positive reaction (around 600 bps).

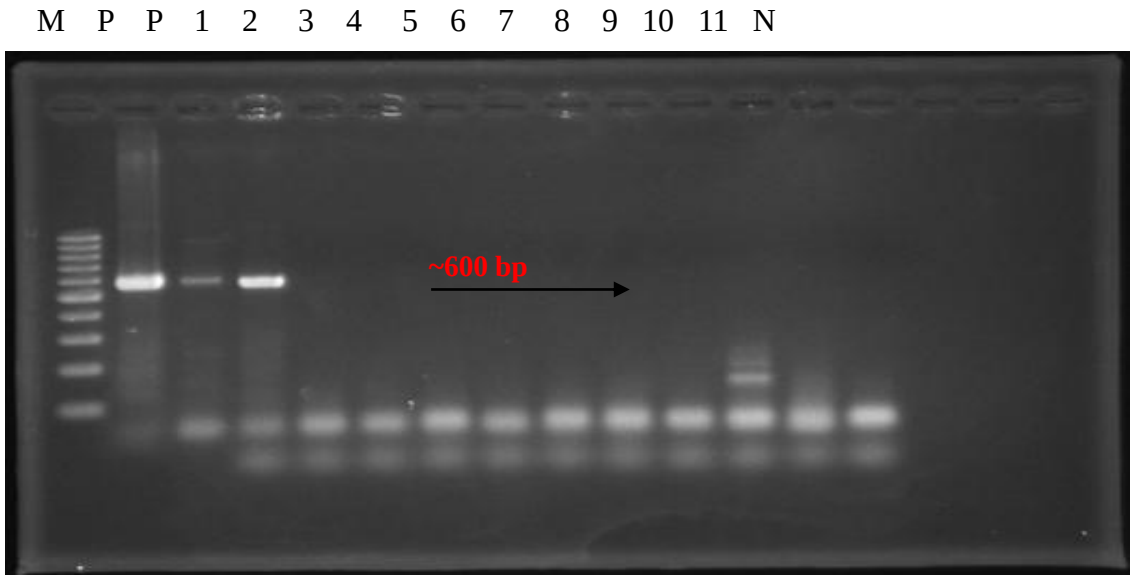


Figure 6
M – 100 bps marker
P – positive control /*M. tuberculosis_NCCD/*
P – positive control /*M. bovis_NCCD/*
1– **Sample of 20EU362**
2-11 other lung samples out of the 20 nodular samples
N – negative control

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.*** spp by the colony formation and PCR

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

TO STARTED and currently CONTINIUES
un-performed yet, due to COVID19 fandemic and others

Ter

Inputs

	Year	2020				2021				2022		
	Month	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Ju Se
3.3.1. To perform a seroepidemiological survey on *B. mallei* 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.	Plan											
	Actual											
3.3.2. To evaluate the epidemics of *B. mallei* infection in Mongolia seroepidemiological, by testing the horse sera collected in the Activity 3.2.1 with the immunochromatography-based *B. mallei* -specific antibody detection method developed in the Activity 1.3.	Plan											
	Actual											
3.3.3. To analyse *B. mallei* infection in horses histopathologically and immunohistrogically by dissecting infected horses.	Plan											
	Actual											
3.3.4. To assess the transmission and distribution of *B. mallei* 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.	Plan											
	Actual											

Plan of Operation | Sheet1

Outputs 3.3.1

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

(RISK-BASED SURVEILLANCE)

Date	Province	Number of samples	CFT (%)	Mallein test (%)
2020	Tuv	185	8 (4.3)	8 (4.3)
	Khentii	382	18 (4.7)	18 (4.7)
	Sukhbaatar	220	-	-
	Dornod	86	3 (3.5)	1 (1.2)
	Dundgobi	44	24 (54.5)	24 (54.5)
2021	Dornod	5	1 (20)	-
	Tuv	363	13 (3.6)	14 (3.9)
	Sukhbaatar	1	1 (100)	1 (100)
	Total	**1271**	**68 (5.4)**	**66 (5.2)**

Table 2. Results of serology in 2021

(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)
	Total	**821**	**13 (1.58)**

Outputs 3.3.3

CLINICAL SYMPTOMS OF GLANDERS CASE in 2020/2021

Fig 2. Skin rash with pustules horse

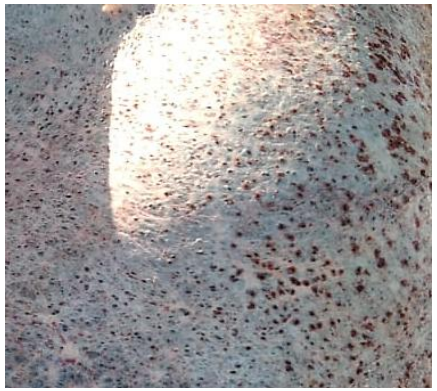


Fig 3. Signs of a punctate stroke in the spleen

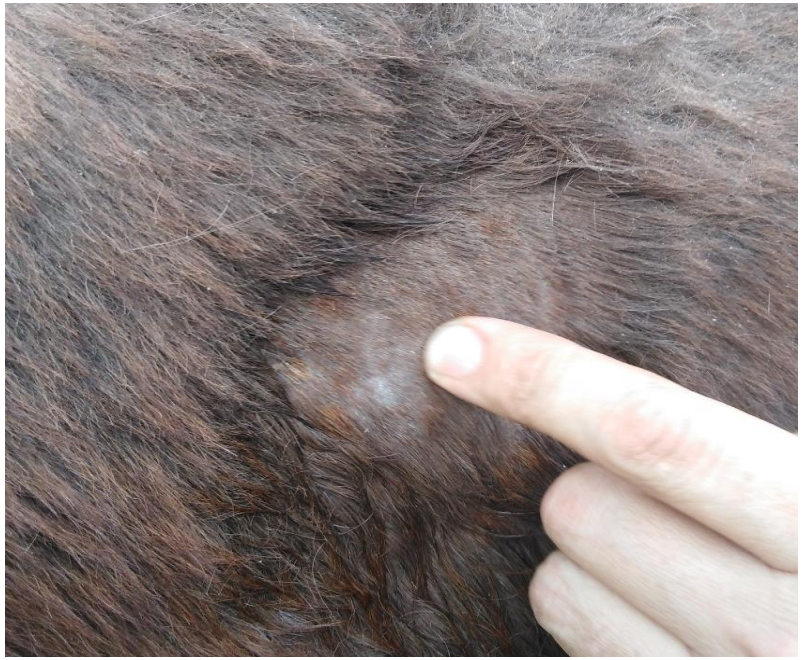


Fig 4. Skin test positive horse

Continuous

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

Date	Herder's ID	Location	Clinic symptoms	CFT	Mallein	Bacteriology	PCR detection			
							All *Burkholderia*	*B.mallei*	*B.pseudo mallei*	*B.cepacia* complex
2020	1	Khui 7, Tuv	+	+	+	+	+	-	-	-
2020	1	Khui 7, Tuv	+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-
2020	2	Khentii	+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-
			+	+	+	+	+	-	-	-

Continues Table 3

Date	Herder's ID	Location	Clinic symptoms	CFT	Mallein	Bacteriology	PCR detection			
							All *Burkholderia*	*B. mallei*	*B. pseudomallei*	*B. cepacia* complex
2021	3	Tuv	+	+	+	+	+	-	-	-
2021	4	Tuv	+	+	+	+	+	-	-	-
2021	5	Khentii	+	+	+	+	+	-	-	-
2021	6	Tuv	+	+	+	+	+	-	-	-
2021	6	Tuv	+	+	+	+	+	-	-	-

Definition of species-specific oligonucleotide primers

Table 4: Primers used for species specific PCR

Target species	Primer[a]	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*	MP 23-2	45ab/36b	5′-TCC TAC CAT GCG AGA CT-3′	
All *Burkholderia* spp.	CVMP 23-1	5b/8ab	5′-AAA CCG ACA CAG GTG G-3′	
B. mallei	M 23-2	78ab	5′-CAC CGA AAC TAG CA-3′	526
All *Burkholderia* spp. excluding *B. mallei*	CVP 23-2[b]	78ab	5′-CAC CGA AAC TAG CG-3′	

[a] 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.
[b] Contains an NH_2 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

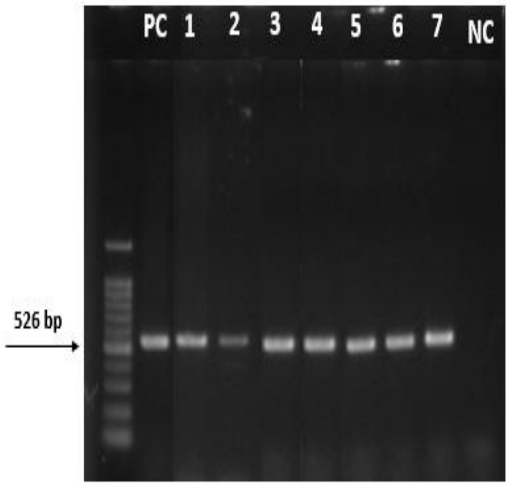


Fig 5. PCR amplification of all *Bukholderia* spp using primer pairs CVMP23-1, M23-2, CVP23-2 (in

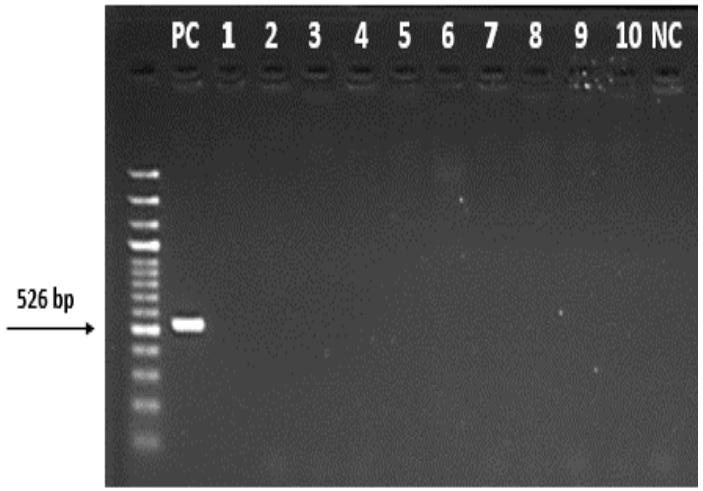


Fig 7. PCR amplification of *B. mallei* and *B. pseudomallei* using primer pairs CVMP23-1, MP23-2, CVP23-2

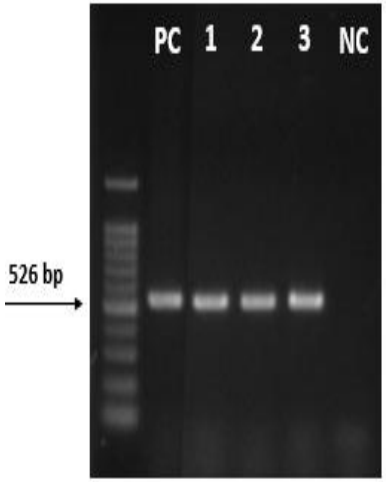
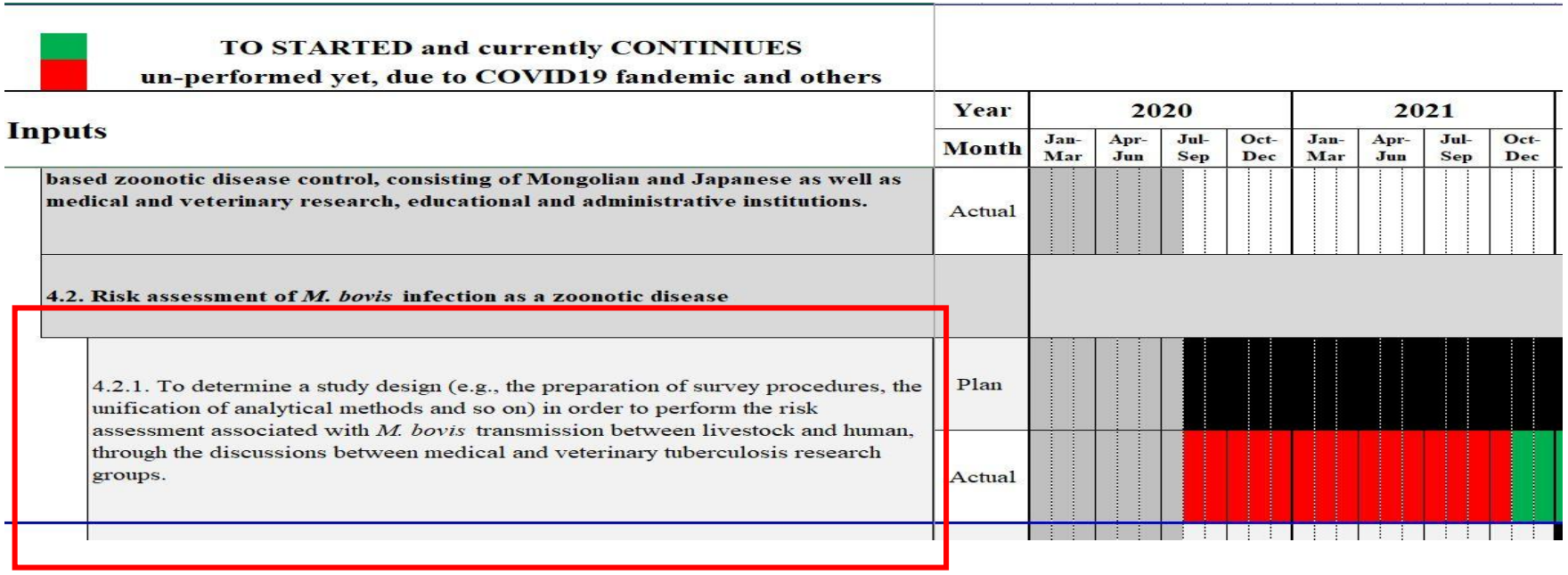


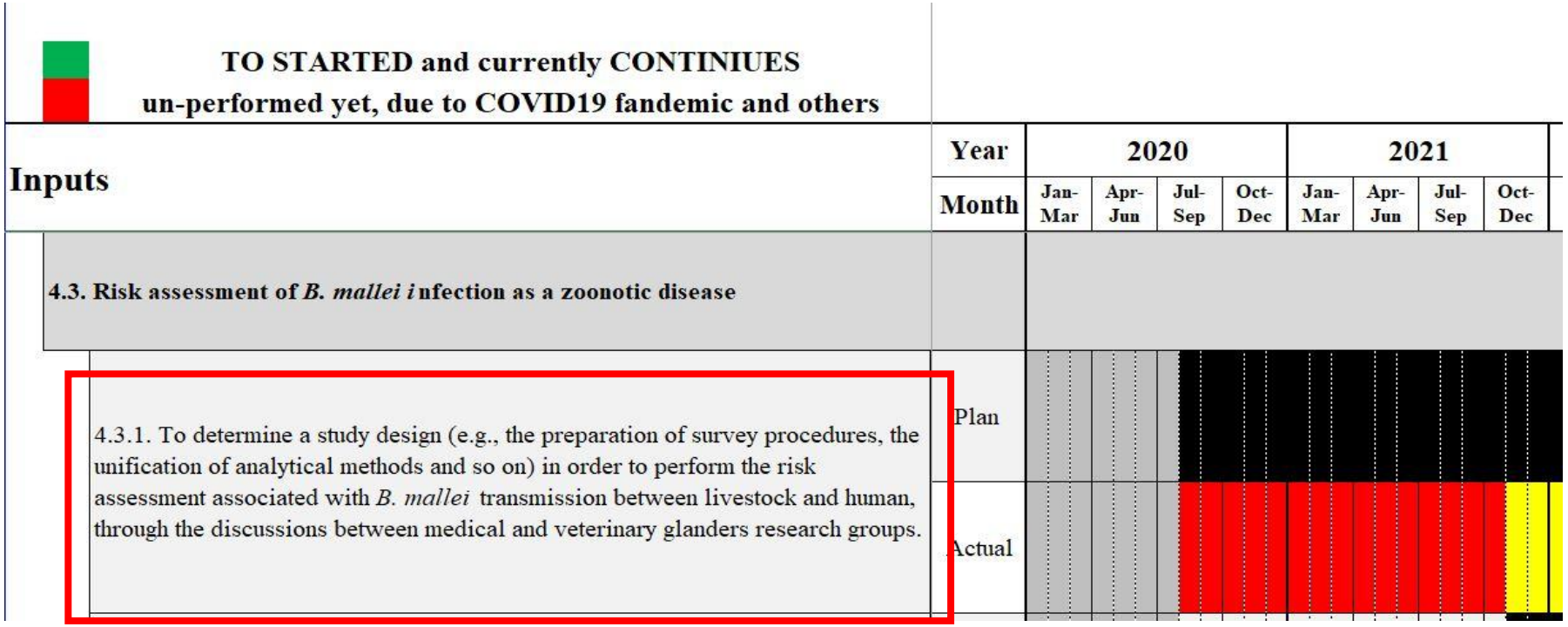
Fig 6. PCR amplification of all *Bukholderia spp* using primer pairs CVMP23-1, M23-2, CVP23-2 (in 2021)

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 symptoms and were confirmed by the serology/intradermal tests were **SLAUGHTERED in agreement with owner (THE 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).





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/

THANK YOU FOR YOUR KIND ATTENTION