SATREPS "The Project for the control of tuberculosis and glanders"

Development of immunochromatography as a simple serodiagnostic method for glanders

Kazuki Mizuta¹, Yoshiki Ichikawa¹, Tomohiro Okagawa², **Kazuhiko Ohashi²**, **Takashi Kimura**¹

Laboratory of Comparative Pathology¹ and Infectious Diseases² Faculty of Veterinary Medicine (FVM) Hokkaid University, Japan

The 3rd JCC. February 21' 2024. Ulaanbaatar

Development of simple diagnostic methods for glanders by the FVM, Hokkaido University

Dry-LAMP method

- 1.3. Development of a LAMP-based Rapid diagnostic Method (Test Kit) for *B. mallei* infection
- 1.5. To establish production systems for the genetic diagnostic kits described above by introducing Ink-jet printers into NCCD

Finished

Immunochromatography

• 1.4. Development of an immunochromatography-based Rapid Diagnostic Method (Test kit) for *B. mallei* infection

	Inputs			20	020			20	021			20	22			20	023			20	0 2 4		202	5
			Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec	Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec	Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec	Jan- Mar	Apr- Jun	Jul- Sep	Oct- Dec	an- far	Apr- Jun	Jul- Sep	Oet- Dec	Jan- Mar	Apr- Jun
PO 1.4. Immunochromatography development plan																								
	1.4.1. To search <i>B. mallei</i> -specific antigen by investigating the reactivity of protein,																							
	expressed in <i>E. coli</i> , etc. from selected specific genetic region of a <i>B. mallei</i> standard strain, with the serum collected from <i>B. mallei</i> -infected horses, in the Hokkaido University.	Revised plan																						
		Actual																						
	142 To devote an immunication results based without for detection P wallsi	Plan																						
	 1.4.2. To develop an immunochromatography-based method for detecting <i>B. mallei</i>-specific antibodies using the specific antigen protein selected in the Activity 1.3.1 and the positive serum of <i>B. mallei</i>-infected horses. 1.4.3. To make the <i>B. mallei</i>-specific antibody detection method into a "kit" in collaboration with private enterprises in Japan or Mongolia (trial production of a rapid diagnostic test kit). 	Revised plan																						
		Actual																						
		Plan Revised																						
		plan																						
		Actual		 ; ;																				
		Plan																						
	1.4.4. To assess the sensitivity and specificity of the kit by performing non-inferiority or comparative superiority test with conventional methods such as complement fixation test and plate agglutination test using biological samples from infected horses in Mongolia.	Revised plan																						
	1.4.5. To prepare SOPs for the serological diagnosis of <i>B. mallei</i> infections in livestock at IVM following the improvements are made as appropriate based on the aforementioned evaluation results.	Pian																						
		Revised plan																						
		Actual																						

Diagnosis of Glanders

Conventional methods

		Recommended by WOAH, Highly sensitive						
Complement fixation test (CFT)		High false positive rate, Takes time to make diagnosis Requires rigorous quality control of kit components						
	Molloin reaction	Once widely used, May give inconclusive results						
	Mallein reaction	Against animal welfare, Inducing false positive for CFT						
	Destarial isolation	Gold standard for disease identification						
	Bacterial isolation	Requires several days and biosafety laboratory						
Rose Bengal test (Plate agglutination test)		Low sensitivity and specificity						

Recently developed methods

ELISA, Western-blot:

Test (Kits) are expensive Requires equipment

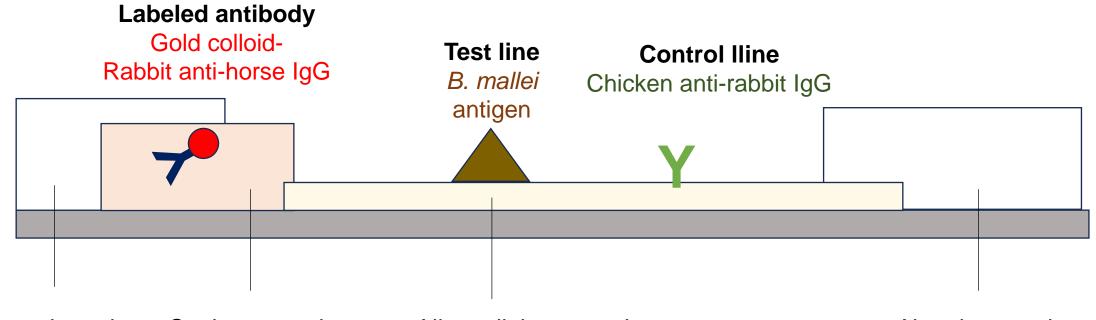
Recommended by MOAH Highly consitive

Need for a highly accurate, rapid and inexpensive diagnostic method

Immonochromatographic test (ICT)

Immunoassay using capillary phenomenon

Procedure	Add a few drops of sample solution and leave it	Simple
Time required	15 to 20 minutes	Rapid
Judgment	Visual inspection (no equipment required)	Low cost

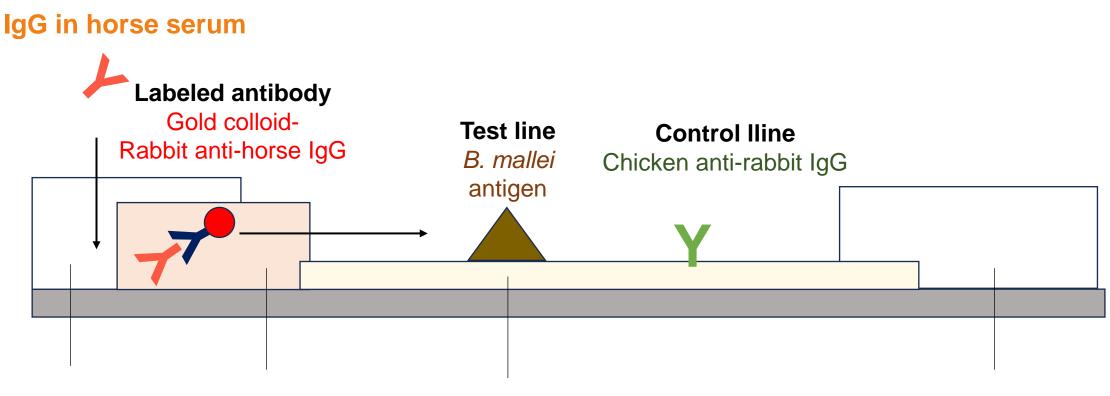


Sample pad Co

Conjugate pad

Nitrocellulose membrane

Absorbent pad

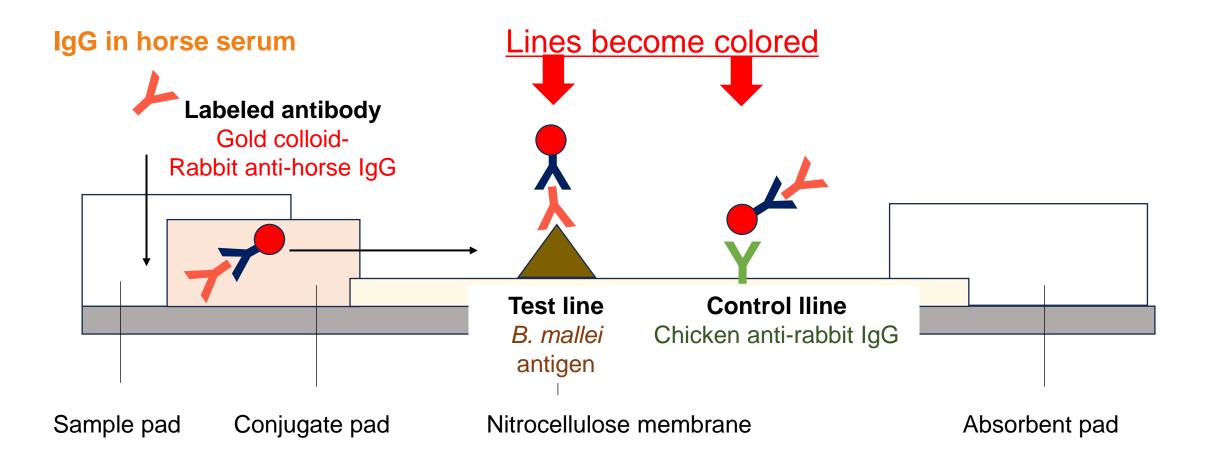


Sample pad Conjugate pad

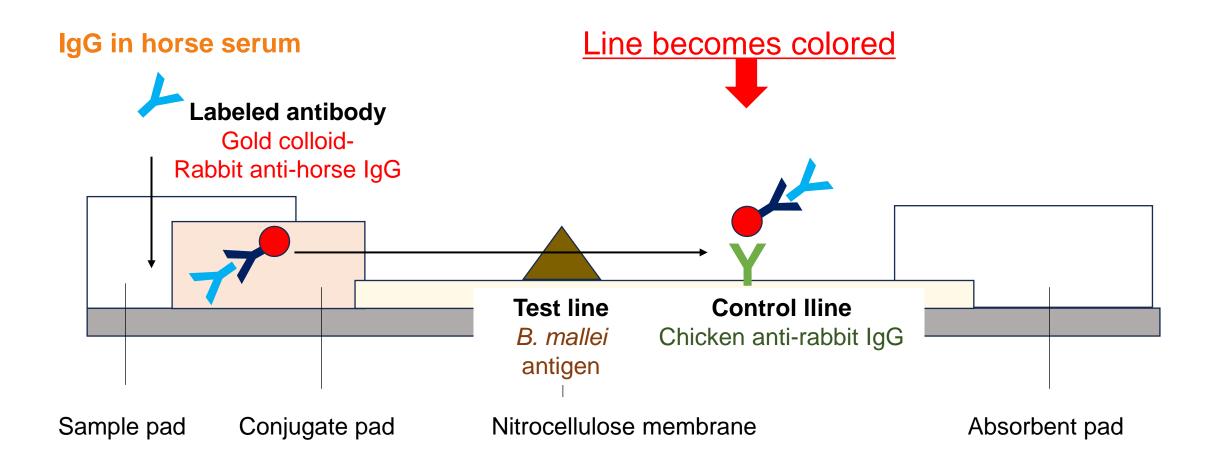
Nitrocellulose membrane

Absorbent pad

Infected horse serum (with anti-B. mallei IgG)



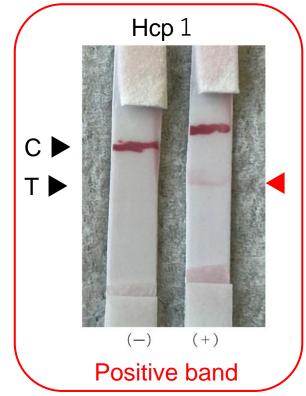
Uninfected horse serum (without anti-B. mallei IgG)



Comparison of reactivity of B. mallei antigens

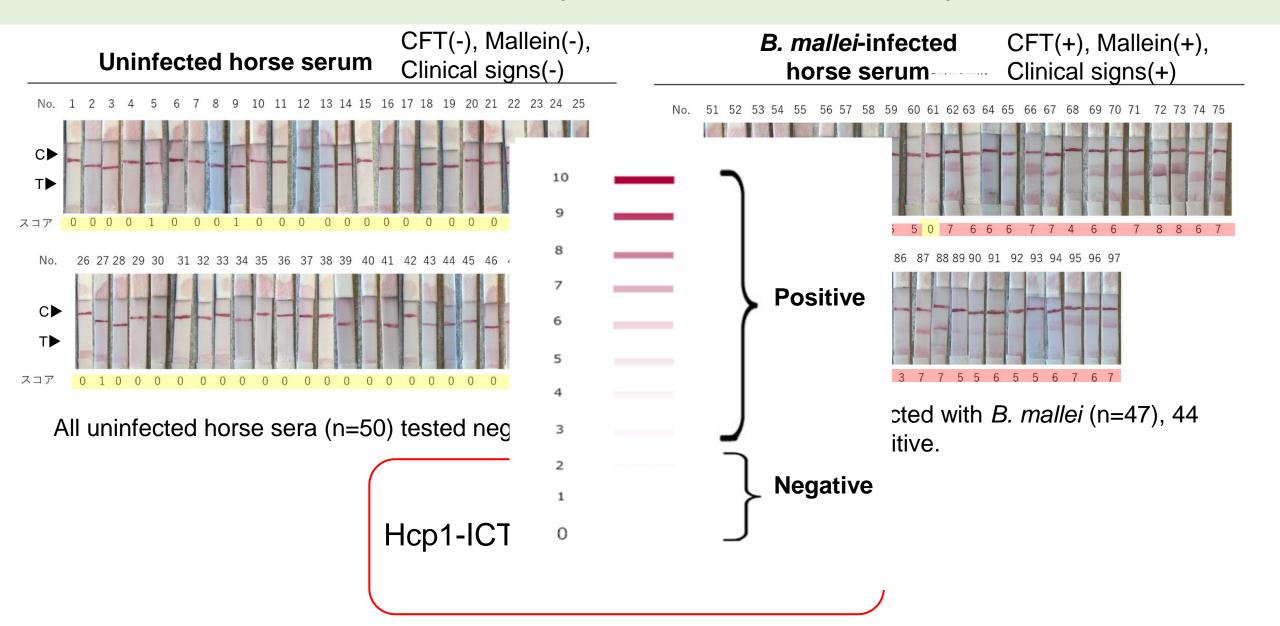
Candidate of B. mallei antigen applied to the test line

- GroEL
- Hcp 1
- Whole cell lysate (WCL)



Indire	ct ELIS	A Ichikaw	Ichikawa <i>et al.</i> , submitted				
		Sensitivity	Specifi	city (%)			
Gr	oEL	100	92				
Ho	p 1	97.3	100)			
W	/CL	100	100)			
C ► T ►	Gro	DEL	WCI				
	(-)	(+)	(-)	(+)			
No positive band							

Hcp1-ICT: sensitivity and specificity



Discussion

Hcp1-ICT prepared in this study has high diagnostic accuracy, suggesting that it may be useful as a rapid and simple serological diagnosis comparable to the conventional diagnosic method for glanders

However,

Three of the 47 infected horse serum samples tested negative
 GroEL-ICT and WCL-ICT did not show positive band against infected horse serum

What we need to work on;

- Increasing the sensitivity of Hcp1-ICT so that a band appears even with a small amount of anti-B. mallei IgG in the blood
- Making GroEL-ICT and WCL-ICT that can be used for diagnosis

Opinion about the project as a whole

- It is necessary to proceed with risk assessment of animal-tohuman transmission of tuberculosis and glanders.
- In particular, little analysis has been conducted regarding the risk of *B. mallei* transmission from animals to humans.
- In order to proceed with this matter efficiently, I would like to propose a revision to PDM2.5. "Epidemiological evaluation of the epidemics of B. mallei Infection in human" (please refer to the next slide)
- We have developed novel diagnostic methods; however, some reagents appear to be difficult to obtain in Ulaanbaatar. We would appreciate your cooperation in this regard.

	Current PDM	Revision proposal	Reasons
2.5.1	NCCDにおいて原因病原体が特定されていないヒト感染性肺炎患者から得られた生体試料(喀痰ないし咽頭拭い液)を収集し、IVMに移送する。To collect the biological samples (sputum and/or throat swab) obtained from patients with human infectious pneumonitis for whom no causative agent has been identified in the NCCD, followed by transferring them to IVM.	GAVS等の機関が所有している鼻疽 罹患馬を飼育している牧夫の情報 をNCCDに供与し、該当する牧夫 と必要であればその関係者より血 液を採取する。To collect blood from the herdsmen and their related persons who raise B. mallei-infected horses by providing information on the herdsmen who raise B. mallei- infected horses owned by organizations such as GAVS to NCCD.	現在の PDM の手法を使用した場合、 鼻疽馬と接触した人々を選択的に分 析することは困難であるため Because using current PDM method, it is difficult to selectively analyze people who have had contact with glandrous horses.
2.5.2	収集した生体試料をIVMにおいて活動1.2で開発した遺伝子検出法で スクリーニングし、B. malleiヒト 感染例の有無を評価する。To evaluate the presence of human cases of B. mallei infection in IVM by screening them with the gene detection method developed in Activity 1.2.	収集した血液をIVMにおいてスク リーニングし、B. malleiヒト感染 例の有無を評価する。To evaluate the presence of human infection with B. mallei by screening the collected human blood in IVM.	