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THE PROJECT FOR CONTROL OF TUBERCULOSIS AND GLANDERS

SEEKING WAYS TO ERADICATE TUBERCULOSIS AND GLANDERS INTERNATIONAL SCIENTIFIC CONFERENCE 2025

Ulaanbaatar, Mongolia
2025

The pictures of the SEEKING WAYS TO ERADICATE TUBERCULOSIS AND GLANDERS INTERNATIONAL SCIENTIFIC CONFERENCE in 2024

WELCOME MASSAGE

Dear Participants,

I am delighted to welcome you to the Seeking Ways to Eradicate Tuberculosis and Glanders International Scientific Conference 2025. This conference was planned as part of “The Project for The Control of Tuberculosis and Glanders”, implemented under the flamework of Science and Technology Research Partnership for Sustainable Development (SATREPS), which is supported by the Japan International Cooperation Agency (JICA) in collaboration with the Japan Agency for Medical Research and Development (AMED). I am deeply honored to have leading researchers from China, the UK and France delivering lectures at this conference. Additionally, Mongolian and Japanese researchers involved in the project will present their latest findings.

I hope that this conference will be a valuable experience for all participants, fostering new discoveries and collaborations.

Thank you for your participation, and we look forward to engaging discussions and fruitful exchanges.

Sincerely,

Takashi Kimura

Chief Advisor of the SATREPS project Professor

Laboratory of Comparative Pathology

Faculty of Veterinary Medicine

Hokkaido University, Japan

THE PROJECT **FOR** CONTROL **OF** TUBERCULOSIS **AND** GLANDERS

SEEKING WAYS TO ERADICATETUBERCULOSIS AND GLANDERSINTERNATIONAL SCIENTIFIC CONFERENCE-2025

Date: Thursday, May 15, 2025

Time of the day： 8:50～17:15

Location of the event: Bayangol Hotel, **Ulaanbaatar**
Zoom

Link：https://us02web.zoom.us/j/88262860766?pwd=qptIcy8BW4ldFZUh3aP46pzfaAp3Ac.1

Meeting ID: 882 6286 0766 Passcode: 881188

Organizer:
The SATREPS Project for Control of tuberculosis and glanders, JICA, JAPAN
The National Center for Communicable Diseases (NCCD), MOH, MONGOLIA
The Institute of Veterinary Medicine (IVM), MED, MONGOLIA

Chairman: Takashi KIMURA, JICA expert, Chief Advisor (CA) of the SATREPS project, Professor at Laboratory of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University

Time	Agenda	Name and Affiliation of the presenter
8:50 ～8.55	Opening Remarks	**Lkhagva BATTUR** MD, PhD, associate professor, Project Director, Director of the Public Health Policy Department, Ministry of Health
8.55～9.00	Opening Remarks	**Kensuke MIYAGI** Chief Representative (s) of the JICA Mongolia Office
	Session - 1	
	Moderators: **Lkhagva BATTUR,** MD, PhD, associate professor, Project Director, Director of the Public Health Policy Department, Ministry of Health **Badgar BATTSETSEG**, DVM, PhD, Professor, Manager of SATREPS project, Director of Institute of Veterinary Medicine, MED, Mongolia	
9:00 ～9:35	**Epidemiology, Diagnosis, and Integrated Control of Bovine Tuberculosis: Insights from Clinical Trials and Novel Interventions** （30 minutes for presentation + 5 minutes for Q&A）	**Prof. XIANG MEI ZHOU** Professor of Veterinary Pathology, Deputy Director of Department of Basic Veterinary Medicine, College of Veterinary Medicine, China Agricultural University. Chairman of Standing Council, Experimental Animal Pathology Board of Chinese Society of Experiment Animal. Vice-

Time	Agenda	Name and Affiliation of the presenter
		Chairman of Standing Council of Chinese Association of Veterinary Pathology. Member of the Expert Committee of the National Animal Tuberculosis Reference Laboratory. Member of the Standing Committee of Tuberculosis Basic Research Branch of China National Tuberculosis Association. Member of the Standing Committee of Zoonotic Tuberculosis Branch of China National Tuberculosis Association
9:35 ～10:10	**Development and evaluation of an ELISA and a Rapid Strip Test for Glanders** （30 minutes for presentation + 5 minutes for Q&A）	**Prof. XIAOJUN WANG** Deputy Director, Harbin Veterinary Research Institute, China. Chinese Academy of Agriculture Sciences (CAAS) Head of Equine Diseases and Lentiviral Infection Study. WOAH expert, WOAH Reference Laboratory for Equine Infectious Anemia and the National Glanders Reference Laboratory of China
10:10 ～10:45	**Antimicrobial susceptibility testing for second-line anti-TB agents** (30 minutes for presentation + 5 minutes for Q&A）	**Dr. CLAUDIO KÖSER** Department of Genetics, University of Cambridge Cambridge, UK
	10:45-11:00	**Coffee break**
11:00 ～11:20	**Molecular typing of *B. mallei* and *B. pseudomallei*** (15 minutes for presentation + 5 minutes for Q&A）	**Dr. KARINE LAROUCAU** French Agency for Food, Environmental and Occupational Health and Safety, Maisons-Alfort Laboratory for Animal Health
11:20 ～11:40	**Development of a *Burkholderia mallei*-specific real-time PCR method** (15 minutes for presentation + 5 minutes for Q&A）	**Prof. Takashi KIMURA,** JICA expert, Chief Advisor (CA) of the SATREPS project, Professor at Laboratory of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University
11:40 ～12:00	**Improvement of sputum pre-treatment method for mycobacterial culture** (15 minutes for presentation + 5	**Prof. Satoshi MITARAI** JICA expert, Head of Mycobacterium Reference and Research Department, Research Institute of Tuberculosis, Japan

Time	Agenda	Name and Affiliation of the presenter
	minutes for Q&A）	
12:00 ～12:20	**MGIT-seq for the Identification of Nontuberculous Mycobacteria and Drug Resistance** (15 minutes for presentation + 5 minutes for Q&A）	**Dr. Shiomi YOSHIDA** Department of Mycobacterium Reference and Research, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association Department of Infection Metagenomics, Bioinformatics center, Research Institute for Microbial Diseases, Osaka University
12:20 ～12:40	**Comparative analysis of drug resistance prediction using targeted NGS with MDR-TB isolates in Japan** (15 minutes for presentation + 5 minutes for Q&A）	**Dr. Akiko TAKAKI** JICA expert, Center for Japan Pre-Entry TB Screening Quality Assessment (cJPQA) Department of Mycobacterium Reference and Research (DMRR), Research Institute of Tuberculosis (RIT), JATA, Japan
12:40 ～13:00	**Development of an equine IFNγ release assay (IGRA) for glanders** (15 minutes for presentation + 5 minutes for Q&A）	**Dr. Borjigin LIUSHIQI** JICA expert, Lecturer at Faculty of Veterinary Medicine, Okayama University of Science, Japan
	13:00 - 14:00 Lunch break	
	Session - 2	
Moderators: **Ochirbat BATBAYAR,** MD, MPH**,** Deputy director in charge of Research, Training and Communication, National Center for Communicable Diseases, Mongolia **Vanaabaatar BATBAATAR**, DVM, PhD, Head, Laboratory of Infectious Diseases and Immunology, Institute of Veterinary Medicine, Mongolia		
14:00 ～14:20	**Evaluation of Nanopore sequencing for drug resistance of Mycobacterium tuberculosis strains in Mongolia** (15 minutes for presentation + 5 minutes for Q&A）	**Dr. Tumenbayar OYUNTUYA** PhD candidate at Mongolian National University of Medical Sciences**,** Head of National TB reference laboratory, NCCD
14:20 ～14:40	**Status of tuberculosis infection among different age groups of healthy people in Mongolia** (15 minutes for presentation + 5 minutes for Q&A）	**Dr. Narmandakh ERDENEGEREL** PhD candidate at Mongolian National University of Medical Science, Doctor of National TB reference laboratory, NCCD
14:40 ～15:00	**Serological survey of the bovine paratuberculosis**	**Ms. Nyamdorj ENKHTSETSEG** Biotechnologist, PhD student at Mongolian

Time	Agenda	Name and Affiliation of the presenter
	(Johne's disease) in the Mongolia (15 minutes for presentation + 5 minutes for Q&A)	University of Life Science, Associate Researcher of Laboratory of Infectious Disease and Immunology, Institute of Veterinary Medicine, Ministry of Economy and Development, Mongolia
15.00 ～15:20	**First survey of tuberculosis in Mongolian sheep using IGRA assay** (15 minutes for presentation + 5 minutes for Q&A)	**Dr. Gombosuren ULZIISAIKHAN,** DVM, PhD student at Mongolian University of Life Science, Associate Researcher of Laboratory of Infectious Disease and Immunology, Institute of Veterinary Medicine, Ministry of Economy and Development, Mongolia
	15:20 - 15:35 Coffee break	
	Session - 3	
	Moderators: **Enkhbold ANKHBAYAR**, MD, MSc, Manager of SATREPS project, General Director, National Center of Communicable Diseases, MOH Mongolia **Adilbish ALTANCHIMEG**, Ass. professor, DVM, Scientific Secretory of Institute of Veterinary Medicine, MULS, Mongolia	
15:35 ～15:55	**Sero epidemiological situation of equine glanders in risk-based flocks** （15 minutes for presentation + 5 minutes for Q&A）	**Ms. Baasansuren LKHAM,** Nuclear technologist, MSc, Associate Researcher of Laboratory of Infectious Disease and Immunology, Institute of Veterinary Medicine, Ministry of Economy and Development, Mongolia
15:55 ～16:15	**Equine glanders is re-emerging in Mongolia:** *Results of region wide surveillance.* （15 minutes for presentation + 5 minutes for Q&A）	**Dr. Batchuluun ENKHTUUL,** DVM, PhD student at Mongolian University of Life Science, Associate Researcher of Laboratory of Infectious Disease and Immunology, Institute of Veterinary Medicine, Ministry of Economy and Development, Mongolia
16:15 ～16:35	**Antibiotics susceptibility of the *Burkholderia mallei* field strains**. （15 minutes for presentation + 5 minutes for Q&A）	**Dr. Ochirbat KHURSTBAATAR,** DVM, PhD student at Mongolian University of Life Science, Associate Researcher of Laboratory of Infectious Disease and Immunology, Institute of Veterinary Medicine, Ministry of Economy and Development, Mongolia
16:35 ～16:55	**Result of the surveillance of glanders among herders** （15 minutes for presentation + 5 minutes for Q&A）	**Dr. Durved ALTMAA,** MD, MPH, Epidemiologist, Department of Infectious Disease Surveillance and Research, National Center for Communicable Diseases, Mongolia
16:55	**Closing Remarks**	**Prof. Takashi KIMURA**

Time	Agenda	Name and Affiliation of the presenter
～17:15		JICA expert, CA of SATREPS project, Professor at Laboratory of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University

Epidemiology, Diagnosis, and Integrated Control of Bovine Tuberculosis: Insights from Clinical Trials and Novel Interventions

Yuanzhi Wang[1], Puxiu Shen[1], Yuhui Dong[1], Xin Ge[1], Ruichao Yue[1],

Xiangmei Zhou[1]

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Xiangmei Zhou: zhouxm@cau.edu.cn

Keywords: *bovine tuberculosis, epidemiology, new detection assay, control strategies*

Abstract:

The report presented a comprehensive analysis of the epidemiology, detection, and control strategies for bovine tuberculosis (bTB). Caused by Mycobacterium bovis (M. bovis), bTB is a zoonotic disease with significant global public health and economic impacts. In 2021, M. bovis caused approximately 140,000 human infections and 11,400 deaths worldwide, while the incidence and mortality rates fell short of the WHO's Sustainable Development Goals. In China, the overall prevalence of bTB in dairy cattle was 2.4% (2021), with higher risks in northern and eastern regions, young heifers, large-scale farms, and humid climates.

Detection: Immunological methods (e.g., gamma-interferon release assay) and molecular techniques (e.g., PCR) showed high sensitivity, but cost-effective skin tests remain critical for field applications. A novel diagnostic tool developed by the

research team achieved 94.12% sensitivity and 85.54% specificity in differentiating latent and active infections, enabling targeted interventions.

Control Strategies: China’s "test-and-cull" policy, aligned with national goals to achieve bTB-free status in 50% of large-scale dairy farms by 2030, emphasizes intensified monitoring, biosecurity, and culling of infected cattle. Promisingly, the mannose-modified lipoarabinomannan (ManLAM) adjuvant therapy demonstrated efficacy in clinical trials: Phase I achieved a 90% skin test conversion rate, while Phase II showed a 1.42 L/day increase in milk yield and a 24% reduction in dry milk rate, offering innovative solutions for disease management.

Challenges and Prospects: Persistent hurdles include wildlife reservoirs, implementation gaps in grassroots diagnostics. The report advocates interdisciplinary collaboration, policy optimization, and adoption of advanced diagnostic/therapeutic technologies to safeguard livestock productivity and public health. This work provides a scientific foundation and practical roadmap for integrated bTB control.

Development and Evaluation of an ELISA and a Rapid Strip Test for Glanders

Zenan ZHANG[1], Zhe Hu[1], **Xiaojun WANG[1,2]**

[1] National Glanders Reference Laboratory, State Key Laboratory for Animal Disease Control and Prevention, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, Harbin, China

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Corresponding author: Prof. Xiaojun Wang, E-mail: wangxiaojun@caas.cn

Keywords: *Glanders, Burkholderia mallei, ELISA, Rapid Test Strip, Antibody Detection*

Background: Glanders, caused by *Burkholderia mallei*, is a highly contagious disease in equids and a zoonosis listed as a notifiable disease by the World Organization for Animal Health (WOAH). Given its significant impact on animal health and public safety, timely and accurate diagnosis is crucial for epidemic monitoring and control.

Objectives: This study aimed to develop and evaluate an indirect ELISA and a colloidal gold immunochromatographic (GICG) strip test for antibody detection for glanders.

Scope: In this study, the Hemolysin-coregulated proteins 1 (Hcp1) were purified from bacteria transformed with a recombinant plasmid pET-28a- Hcp1 encoding the *hcp1* gene of *Burkholderia* mallei. The purified Hcp protein is used as coating antigen for ELISA method. The test strip method, based on a double-antigen sandwich format, used the same Hcp protein, with the T-line coated with Hcp and the C-line coated with rabbit polyclonal anti-Hcp antibodies. Sensitivity, specificity, and stability were evaluated through serum dilution, testing of other disease-positive

sera, and accelerated experiments. The ELISA and test strip were compared with IDvet and BIOSTON ELISA kits, as well as the complement fixation test. Finally, the methods were applied to clinical sample detection.

Results: The recombinant Hcp protein showed good expression in a prokaryotic system and verified by Western blot. The ELISA cut-off value was set at S/P > 0.2. Both methods exhibited high specificity and specificity. For the sensitivity, the strip test shows higher sensitivity than ELISA, but lower than IDvet ELISA. In a comparison test with 40 reference sera, the accuracy of the developed methods exceeded that of the complement fixation test, with 100% consistency with the IDVET kit. Testing of 630 clinical samples yielded all-negative results with no false positives.

Conclusion: In this study, we developed two diagnostic methods for glanders: an indirect ELISA and a colloidal gold strip test. The ELISA is ideal for high-throughput lab testing, while the colloidal gold test strip is perfect for rapid on-site detection. Both methods are highly specific, sensitive, and repeatable, providing strong support for glanders control and prevention.

Antimicrobial susceptibility testing for second-line anti-TB agents

Claudio Köser

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Keywords: *Mycobacterium tuberculosis complex, antimicrobial susceptibility testing, bedaquiline, delamanid, pretomanid*

Abstract:

Thanks to the introduction of bedaquiline and the nitroimidazoles (i.e. delamanid and pretomanid), the World Health Organization was able to recommend several all-oral 6- and 9-month regimens for treating rifampicin-resistant TB. I will discuss five related factors that may affect the longevity of these regimens. First, it is not clear whether BPaL(M) is as effective against lineage 1 compared with lineages 2, 3, and 4. Second, there is limited evidence about how well these regimens prevent acquired resistance. Third, even though bedaquiline and the nitroimidazoles are novel drugs, relatively high rates of pre-existing have been reported in some settings (e.g. in Eswatini and South Korea). Fourth, there is no or insufficient capacity for genotypic and phenotypic antimicrobial susceptibility testing (AST) in many countries. Finally, AST for these agents is more technically challenging than for most traditional drugs.

PCR-HRM typing schemes for *Burkholderia* spp.: Application to Glanders and first use in Melioidosis

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Keywords: *glanders, melioidosis, epidemiology, genotyping*

Abstract:

Glanders and melioidosis are two infectious diseases caused by closely related pathogens, *Burkholderia mallei* and *Burkholderia pseudomallei*, respectively. Both are of animal and public health concern due to their high virulence, environmental persistence (for *B. pseudomallei*) and potential for emergence or re-emergence in new areas. Despite their importance, tools for rapid and informative molecular typing of strains remain limited, particularly in contexts requiring timely outbreak investigation or risk assessment in non-endemic areas.

To address this gap, a flexible and scalable molecular typing method has been developed based on the use of informative genetic markers selected from whole genome data. This approach provides a rapid alternative to whole-genome sequencing for strain differentiation and epidemiological investigations.

1. Application to glanders: An initial panel of 15 markers has been established to differentiate *B. mallei* strains and predict their geographical origin. This tool has proved useful in tracing sources of infection in horses and in clarifying transmission chains, especially in cases where movement history is unclear. The method is designed to be extensible by incorporating newly sequenced genomes from circulating strains, allowing markers to be refined or new ones to be added. This evolving strategy will improve the accuracy of source attribution and facilitate a

better understanding of disease transmission pathways - key components of effective surveillance and control.

2. Application to melioidosis: In newly identified endemic areas, such as the French overseas territories in the Americas and parts of Africa, rapid strain typing is essential to increase clinical awareness and diagnostic capacity. The method allows differentiation between cases associated with travel to known endemic regions (e.g., Southeast Asia, northern Australia) and locally acquired infections that might otherwise go unrecognized. Timely characterization of circulating strains supports the identification of autochthonous transmission and informs public health responses.

Overall, this molecular typing approach is designed to be user-friendly and adaptable, providing a cost-effective means of generating actionable knowledge for clinicians, veterinarians and public health stakeholders. It contributes to early detection and understanding of the transmission dynamics of two high impact, yet often neglected diseases, and supports preparedness in the context of changing epidemic patterns.

Development of a *Burkholderia mallei*-specific real-time PCR method

Ai Koshikawa[1], Yoshiki Ichikawa[1], Mitsuru Nakase[1],
Vanaabaatar Batbaatar[2], **Takashi Kimura[1]**

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Keywords: *Burkholderia mallei, glanders, real-time PCR*

Abstract:

Glanders is a bacterial zoonotic disease caused by *Burkholderia mallei*. While polymerase chain reaction (PCR) has been increasingly used for glanders diagnosis through *B. mallei* DNA detection, differentiating it from *B. pseudomallei* remains challenging due to their high genomic similarity, with *B. mallei* postulated to have evolved from *B. pseudomallei* via genome reduction and large-scale rearrangements.

In this study, we identified a novel 494 bp sequence exclusive to *B. mallei* chromosome 1 through comparative analysis of *Burkholderia* spp. genome in database. Initial attempts to develop an intercalation-based real-time PCR using 14 primer sets revealed one primer pair specific to *B. mallei*, although primer dimer formation limited its utility.

Subsequently, we attempted to develop a more specific probe-based method. Among 11 primer-probe combinations we designed, 2 sets demonstrated high specificity for *B. mallei* in two-step real-time PCR assays, with no cross-reactivity with *B. pseudomallei*, *B. thailandensis*, *B. cepacia*, or *B. vietnamiensis*. Furthermore, these two sets exhibited lower detection limits (32 femtogram of

bacterial genome) than existing methods. Current efforts focus on optimizing amplification parameters and preparing for clinical validation studies.

Improvement of sputum pre-treatment method for mycobacterial culture

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Keywords: *Mycobacterium tuberculosis, NALC-NaOH, dithiothreitol, benzalkonium chloride*

Abstract:

Objective: NALC-NaOH is the current standard sputum pre-treatment method including digestion/liquefaction and inactivation of general bacteria. Although it works effectively as pre-treatment process of mycobacterial culture, 1% of NaOH (final concentration) is still harmful even to *Mycobacterium tuberculosis* (MTB), resulting approximately 40% inactivation of MTB before inoculating to culture medium. It is an inefficient process but no other alternative is actually available. Then, we tried to develop a new pre-treatment method which is not harmful to MTB.

Methods: Sputum specimen was liquefied with semi-alkaline protease (SAP), NALC and 5% dithiothreitol (DTT). After liquefaction, the homogeneity was assessed by naked eye and rheometer. Separately, benzalkonium chloride (0.1-0.2%), benzethonium chloride (0.1-0.2%) and isopropylmethylphenol (0.05-0.1%) was tested to inactivate several microorganisms commonly contaminate the culture even after NaOH treatment: *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Aspergillus fumigatus*.

Results: SAP showed sufficient liquefaction but required high volume. NALC treatment showed suboptimal liquefaction without NaOH. DTT showed a complete

liquefying effect but some mucus remained. As to the inactivation/decontamination of general bacteria and fungi, the benzalkonium chloride showed the most effective result at 0.2%. There was no clear combination synergy between these disinfectants. Conclusion: DTT seemed to be a potential sputum liquefying method but its chemical instability by oxidation will be a problem in practical use. The use of benzalkonium chloride at 0.2% after complete sputum liquefaction will more viable MTB in the specimen and will increase the culture efficiency. Further studies evaluating culture effciency is now on-going.

MGIT-seq for the Identification of Nontuberculous Mycobacteria and Drug Resistance

Shiomi Yoshida[1], Yuki Matsumoto[2], Shota Nakamura[2], Satoshi Mitarai[1]

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Keywords: *Non-tuberculosis Mycobacteria, direct sequencing, MGIT, species-level identification, drug susceptible test*

Abstract:

Objective: Because nontuberculous mycobacterial pulmonary disease (NTM-PD) is a considerable health burden, a simple and clinically applicable analytical protocol enabling the identification of subspecies and drug-resistant NTM-PD is required to determine the treatment strategy. We aimed to develop a simplified workflow consisting only of direct sequencing of mycobacterial growth indicator tube cultures (MGIT-seq).

Methods: In total, each sputum sample from 138 patients was prospectively submitted for the mycobacterial culture between April 2021 and May 2022. Sequence analysis was conducted to identify species using core genome multilocus sequence typing (cgMLST) and to predict macrolide (CAM) and amikacin (AMK) resistance based on previously reported mutations in *rrl*, *rrs*, and *erm*(41) directly from MGIT culture-positive broths using the MinION sequencer, a portable next-generation sequencer. The results were compared to clinical routine tests for species identification and drug susceptible testing (DST).

Results: A total of 116 patients with positive MGIT cultures were included in the analysis. MGIT-seq yielded 99.1% accuracy in species-level identification and identified 98 isolates (84.5%) at the subspecies level. CAM and AMK resistance were detected in 19.4% and 1.9% of *Mycobacterium avium* complex (MAC) and *M. abscessus* isolates. The predicted CAM and AMK resistance were consistent with the results of phenotypical DST, with specificities of 97.6% and 100.0%, respectively.

Conclusion: Direct MGIT-seq has achieved comprehensive identification and drug resistance prediction of NTM, which could be applicable to determine the treatment strategy for NTM-PD by a single test in clinical practice.

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Evaluation of targeted NGS Using Nanopore technology for drug resistance prediction in Mycobacterium tuberculosis isolates from Japan

Akiko Takaki[1], Kinuyo Chikamatsu[1], Yuriko Igarashi[1], Akio Aono[1], Yoshiko Shimomura[1], Makiko Hosoya[1], Miori Nagai[1], Yoshiro Murase[1], Satoshi Mitarai[1,2]

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Keywords: *Mycobacterium tuberculosis, Drug-Resistant tuberculosis, targeted NGS*

Abstract:

Background: Rapid and comprehensive drug susceptibility testing (DST) using clinical specimens is essential for early diagnosis and appropriate treatment of drug-resistant tuberculosis (DR-TB). Recently, targeted next-generation sequencing (tNGS) methods have emerged to predict genotypic resistance to a broader range of drugs, including bedaquiline (BDQ) and linezolid (LZD). The World Health Organization (WHO) has endorsed three tNGS kits for DR-TB. In this study, we evaluated the performance of AMpore-TB (Oxford Nanopore Diagnostics), a soon-to-be-released tNGS assay, using *Mycobacterium tuberculosis* (MTB) isolates from Japan, including a comparison with Deeplex Myc-TB (GenoScreen) and assessment of the updated V14 nanopore chemistry.

Method: Genomic DNA was extracted from 110 MTB isolates, including 101 multidrug-resistant (MDR) strains collected in Japan between 2011 and 2014. Multiplex PCR was performed using AMpore-TB prototype primers, and sequencing was conducted on the GridION platform with both R9 and V14

chemistries. Resistance to 16 anti-TB drugs was predicted using the EPI2ME platform (Metrichor Ltd.). Phenotypic DST (including MIC and MGIT AST), Deeplex, and Illumina-based whole-genome sequencing were used as reference methods.

Results: The sequencing run time required for nanopore analysis was 4 hours, twice the recommended duration. The sensitivity of AMpore-TB for the key anti-TB drugs isoniazid (INH), rifampicin, pyrazinamide, and fluoroquinolones was 86.7%, 99.0%, 100%, and 92.6%, respectively. Resistance prediction for BDQ, LZD, and delamanid remains limited. Of the 2,750 total targets, resistance prediction was undetermined for 15 due to insufficient read coverage. The V14 chemistry demonstrated comparable performance to R9 chemistry in terms of both sequencing run time and analytical accuracy.

Conclusions: AMpore-TB is a tNGS method that enables rapid and reliable prediction of drug resistance within 1–2 days from specimen collection, using a simple and streamlined workflow. In this study, we demonstrated that AMpore-TB achieved high accuracy in MTB isolates from Japan, comparable to that of Deeplex. We also showed that the updated nanopore chemistry, V14, supported equivalent diagnostic performance. However, further refinement of mutation catalogs is essential to improve the accuracy of resistance prediction for INH and second-line anti-TB drugs.

DEVELOPMENT OF AN EQUINE IFNΓ RELEASE ASSAY (IGRA) FOR GLANDERS

Liushiqi Borjigin[1], Thapa Jeewan[3], Yoshiki Ichikawa[2], Vanaabaatar Batbaatar[5], Ochirbat Khurtsbaatar[5], Batchuluun Enkhtuul[5], Jugderkhorloo Unenbat[5], Baasansuren Lkham[5], Takashi Kimura[2], Yasuhiko Suzuki[3, 4]

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Keywords: *Burkholderia mallei, glanders, equine IFNγ, ELISA, IGRA*

Abstract:

Burkholderia mallei (B. mallei) is the causative agent of glanders, a contagious zoonotic disease that affects not only horses, donkeys, and mules but also humans. Although sporadic cases of equine glanders have recently been reported in Mongolia, Pakistan, India, and other countries. Currently, the complement fixation test (CFT), Mallein test, and Rose Bengal test (RBT) are used to diagnose glanders, but these methods present various issues, such as low sensitivity, false positives, false negatives, and being time- and labor-intensive. Previous research found that B.

mallei infection activates cellular immunity, increasing cytokines like IFNγ. In IFN-γ knockout mice, bacterial replication was significantly higher than in wild-type mice. Therefore, *B. mallei* specific IFNγ is considered a potential candidate as a diagnostic marker for glanders. Our study aims to develop an IGRA with high sensitivity and specificity. Additionally, we will develop an immunochromatographic method that is user-friendly for Mongolian nomadic people. To begin, we synthesized eqIFNγ cDNA with a His-Trx tag by referring to the NCBI sequence, inserted it into the pET29a+ plasmid, and expressed the protein (His-Trx-eqIFNγ protein) using Rosetta-gami 2 (DE3) pLysS competent cells. Then, we injected the His-Trx-eqIFNγ protein into rabbits and collected the serum containing eqIFNγ-specific and His-Trx-specific antibodies. Then, eqIFNγ cDNA with a His-Nus tag and an HRV3C cleavage site was synthesized and expressed in Rosetta-gami 2 (DE3) pLysS cells, and the eqIFNγ protein was purified after cleavage by HRV3C protease. The purified eqIFNγ protein was immobilized on HiTrap NHS-Activated HP Columns, and eqIFNγ-specific antibodies were purified from rabbit serum by filtration. Sandwich ELISA confirmed the specific interaction between the antibody and its target antigen, indicating that the antibody was successfully purified with high specificity. In the future, we will biotinylate the purified eqIFNγ-specific antibodies using the Biotin Labeling Kit-NH_2. Subsequently, an ELISA assay will be developed using eqIFNγ and His-Trx proteins as antigens, with non-biotinylated eqIFNγ-specific antibodies coating as the capture antibody and biotinylated antibodies as the detection antibody. Finally, field samples will be employed to identify suitable blood-stimulating antigens, evaluate the sensitivity and specificity of the assay, and ultimately establish a reliable diagnostic assay. This assay is expected to contribute to improved immunological diagnostics for glanders in equine populations.

Evaluation of Nanopore sequencing for geneticdrug susceptibility testing of *Mycobacterium tuberculosis* isolates in Mongolia

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Keywords: *M.tuberculosis, drug resistance, Oxford Nanopore technology, Next-generation sequencing*

Background: Antibiotic resistance presents a critical obstacle to global tuberculosis (TB) control efforts, posing an ongoing public health crisis. Timely and comprehensive drug susceptibility testing (DST) is essential, particularly through methods that can be applied directly to sputum or decontaminated sputum samples. In Mongolia, The drug resistance problem is significant: the recent DRS results provided an estimated incidence of TB patients with rifampicin resistance and multidrug resistance (RR/MDR)-TB of 1100 for 2022. Effective implementation of the National TB Control Program requires an in-depth understanding of transmission dynamics and the spread of drug-resistant TB in high-risk populations. Next-generation sequencing (NGS), notably Oxford Nanopore Technologies (ONT),, offers a rapid, portable, and real-time platform for detecting mutations in resistance-associated genes. To facilitate this advancement, the World Health Organization (WHO) has developed a standardized mutation catalog and

implementation guidelines for sequencing-based DST. In response to the growing burden of drug-resistant TB in Mongolia, ONT sequencing has been introduced at the National Tuberculosis Reference Laboratory (NTRL) under the “Control of Tuberculosis and Glanders” SATREPS project, jointly supported by the Japan International Cooperation Agency (JICA) and the Japan Agency for Medical Research and Development (AMED). Running from 2020 to 2025, this initiative also fosters collaboration with the Research Institute of Tuberculosis (RIT), aiming to strengthen local capacity for advanced TB diagnostics and surveillance.

Methods: Genomic DNA from *M. tuberculosis* isolates was analyzed using a third-generation long-read sequencing approach. The DNA was fractionated and subjected to library preparation using a Ligation Sequencing Kit, Barcoding kit according the manufacturer’s protocol. The barcoded library was captured, washed, and eluted from magnetic beads (AMPure XP). A total of 700 ng of pooled library was loaded onto flow cells and sequenced. Sequencing was performed using the GridION platform, and the resulting reads were processed and analyzed via the EPI2ME Desktop Agent, with quality and coverage metrics assessed on the EPI2ME platform (https://epi2me.nanoporetech.com).

Results: This study utilized *M.tuberculosis* isolates from the existing culture bank at the NTRL. Subculturing was performed prior to DNA extraction and sequencing. As of 2024, a total of 1,000 isolates have been sequenced. These efforts enabled the successful establishment of a functional and sustainable ONT-based sequencing workflow at the NTRL.

Conclusion: The implementation of ONT sequencing at the NTRL marks a significant advancement in the capacity to understand transmission dynamics and detect drug-resistant *M. tuberculosis* by single method. Ongoing capacity building and integration of sequencing data into routine surveillance systems will be essential to fully realize the public health impact of this technology in Mongolia..

Tuberculosis infection by relatively healthy age group in UB city of Mongolia

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Keywords: *tuberculosis infection, QFT Plus, age group*

Background: Mongolia is one of the 30 countries with a high TB burden an estimated incidence rate of 491 per 100,000 population. The country has a population of approximately 3.5 million, with nearly 1.7 million residing in the capital, Ulaanbaatar. This study aimed to determine the prevalence of tuberculosis infection among relatively healthy individuals residing in crowded metropolitan settings.

Materials and methods: A cross-sectional study was conducted between June 2024 and March 2025 at the National Center for Communicable Diseases in Ulaanbaatar, Mongolia. Eligibility criteria included being relatively healthy, asymptomatic for TB, with no history of TB treatment or known contact with TB cases. Participants completed questionnaires, and tested QuantiFERON-TB Gold Plus (QFT-Plus) assay in whole blood. Python (3.8.8) and other libraries were utilized to analyze the dataset. Statistical models were used to examine the relationship between demographic factors and QFT-Plus test results.

Results: A total of 766 individuals were included in the analysis. The mean age was 38.2 years (SD=15.7), and the median age was 36 years, with an age range from 10

to 87 year. Of the participants, 47.1% (361/766) were female and 52.9% (405/766) were male. The weighted prevalence of TB infection was estimated at 33.4% (95% CI: 30.1%–36.8%). The QFT-plus positivity rate was 10-19 years (7.9%, 95%CI: 2.3-13.6%), 20-29 (27.5%, 95%CI: 20.8-34.3%), 30-39 (37.9%, 95%CI: 31.3%-44.6%), 40-49 (42.1%, 95%CI: 33.0-51.2%), 50-59 (36.7%, 95%CI: 26.9-45.6%), 60+ (46.1%, 95%CI: 35.7-56.4%), respectively. The QFT-plus positivity rates increase with age across both genders, peaking in the 60< group (p-0.000). Females in age groups 40–59 show slightly higher positive rates than males, whereas the 30–39 male group has the highest observed rate (41.6%, 95% CI: 30.6 to 52.6%) among males. However, gender was not significantly associated with positivity ($p = 0.652$).

Conclusions: This study identified a TB infection prevalence of 33.4%, indicating that nearly one-third of the study population tested positive for TB infection. Age was significantly associated with higher TB infection rates, whereas gender was not a determining factor, indicating the decrease of active transmission in young ages and shift to a situation where TB from latent TB infection is predominant.

SEROLOGICAL SURVEY OF THE BOVINE PARATUBERCULOSIS (JOHNE'S DISEASE) IN THE MONGOLIA

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Keywords: *Mycobacterium avium subs paratuberculosis, Johne's disease, Bovine paratuberculosis, i-ELISA*

Abstract:

Paratuberculosis (causative agent is *Mycobacterium avium* subspecies *paratuberculosis*) is a chronic, infectious, epizootic disease that primarily affects ruminants such as cattle, camels, sheep, goats, and deer. The pathogen causes inflammation in the gastrointestinal tract, leading to thickening of the intestinal wall and mucosa, chronic or bloody diarrhea, weight loss, reduced milk production, and

ultimately death due to progressive debilitation. This disease poses a significant economic burden, particularly on the intensive livestock sector.

In this survey study, based on density of cattle and the presence of numerous cattle farming operations, and a representative subset of 2,100 cattle samples (all bulls) (ranging 4 to 50 samples per soum) from 115 soums in the 21 provinces was selected for testing.

The ID Screen Paratuberculosis Indirect Screening ELISA kit (PARAS ver 0516 EN) by ID.vet, France was used to detect of paratuberculosis. Optical density was read at 450 nm and results were calculated as S/P% that according to the manufacturer.

Of the 2,100 samples, totally 16 samples (0.76%) were shown in positive reaction, and repeat testing was to confirm the initial results in these all positives.

These positive cattle were belongs in 14 soums across 11 provinces. Geographical distribution of the positive cattle showed that 3 soums (out of 19) in Arkhangai province, 2 soums (out of 24) in Khuvsgul province and both soums in Orkhon province has been detected.

But one positive sample each was found in Dornod, Dundgovi, Selenge, Bayan-Ulgii, Bulgan, Govisumber, and Darkhan-Uul provinces. In addional, S/P% values of iELISA were above 70% in all positive samples.

In conclusion, the results of this nationwide survey indicate that the seroprevalence of paratuberculosis in Mongolia is relatively low. Positive cases were sporadically distributed across 14 soums in 11 provinces, suggesting a limited and scattered presence of the disease rather than widespread endem. The high S/P values (>70%) in positive samples support the reliability of these findings. Despite the low prevalence, the detection of positive cases in multiple regions highlights the importance of continuous monitoring, especially in areas with intensive livestock farming.

Overall, these survey findings indicate that paratuberculosis currently has a low national prevalence in Mongolia.

FIRST SURVEY OF TUBERCULOSIS IN MONGOLIAN SHEEPUSING IGRA ASSAY

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Key words: *Ovinus, sheep tuberculosis, IGRA assay,*

Abstract:

Tuberculosis (TB) is a chronic infectious disease caused by bacteria belonging to the Mycobacterium genus, which can affect both humans and animals. Bovine tuberculosis (bTB) is primarily caused by *Mycobacterium bovis* and negatively impacts animal health, product yields, and poses a risk of zoonotic transmission. Although sheep are considered less susceptible to tuberculosis compared to cattle, the role they play in the spread of the disease remains unclear. Therefore, conducting a tuberculosis survey in sheep is to be important for assessing the disease status, and developing preventive measures. Immunological tests,

particularly those involving interferon-gamma (IFN-γ) detection assay are beneficial in identifying the initial immune response.

In this study, we conducted in 127 sheep in Mongolia. Of these, 29 samples were collected from Songinokhairkhan district in Ulaanbaatar, 13 in Jargalant soum of Tuv province, 45 in Munkhaan soum of Sukhbaatar province, and 40 in Dashbalbar soum of Dornod province, respectively.

Ech blood samples were collected and stimulated by Pokeweed mitogen (PwM) and HP-PC and HP-EC (peptide cocktail stimulating antigens) and including a control sample in each sheep taken with no stimulated. Harvest plasma after stimulated in each blood samples, and all samples were tested by the BOVIGAM 2G TB ELISA kits.

Seeing results of the IFN-γ release assay, all samples were shown in negative results. These results indicate that no immune response against *Mycobacetria* spp infection was detected among these sampled sheep.

Finally, these findings provide one of the baseline information on the sheep tuberculosis situation in Mongolia. Further large-scale studies are necessary, alongside the use of molecular biology and bacteriological diagnostic methods to be include using large scale of IFN-y release assay.

SERO EPIDEMIOLOGICAL SITUATION OF EQUINE GLANDERS IN RISK-BASED FLOCKS

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Kyewords: *Equine gladers, risk-based surveillance, seroprevalence*

Abstract:

Glanders is a highly contagious and often fatal disease of equines caused by *Burkholderia mallei*, posing significant health threats to both animals and humans. As a zoonotic disease, it also presents occupational risks to veterinarians and herdsmen. Although many countries have eliminated glanders, it remains endemic in parts of Asia, including Mongolia, where millions of horses are part of daily livelihood and culture. Despite this, recent data on the prevalence of the disease has been limited.This study aimed to evaluate the seroepidemiological status of equine glanders in risk-based horse populations across several Mongolian provinces. From

2020 to 2024, a total of 982 horse serum samples were collected from herds considered to be at higher risk—based on geographic proximity to previously affected areas, frequent inter-herd movement, and limited implementation of biosecurity measures. All samples were tested by the Complement Fixation Test (CFT), which revealed an overall seroprevalence of 9.2%, indicating ongoing exposure and potential transmission within the studied population.

Our results are consistent with previous findings. Ochbayar et al. (2020) conducted a serological investigation in central and eastern Mongolia and found seroprevalence rates of 7.7% (RBT) and 8.3% (CFT) among 337 tested horses, with higher rates observed in crossbred horses. In another risk-based study, Khurtsbaatar et al. (2021) reported that 3.7% of 1,694 horses tested positive for equine glanders by both RBT and CFT, with 93.6% of those also testing positive via the intradermal mallein test. These consistent findings further support the conclusion that glanders continues to circulate in the region and may be re-emerging after a period of apparent decline.

Our findings emphasize the importance of ongoing surveillance, especially in high-risked areas. Serological testing using validated tools like a CFT, combined with field diagnostics such as the intradermal test (mallein test), remains essential for early detection and control. Strengthening preventive strategies, raising awareness among equine owners, and investigating genetic or breed-related susceptibilities may further aid in reducing disease burden.

This study contributes to the growing body of evidence underscoring the need for coordinated national strategies to monitor, to control, and eventually to eliminate for equine glanders in Mongolia.

EQUINE GLANDERS IS RE-EMERGING IN MONGOLIA:

Results of region wide surveillance.

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Keywords: *Equidae*, equine glanders, re-emerging, disease prevalence, disease distribution

Abstract:

Equine glanders is a zoonotic bacterial infectious disease caused by the gram-negative bacterium *Burkholderia mallei (B. mallei).* Glanders is transmissible to humans by direct contact with diseased/ or infected animals or with infected or contaminated materials. Known since antiquity, glanders was eradicated in the early 20th century in Australia, Europe, Japan, North America, and some other countries. The disease has never been reported in New Zealand and remains endemic in India, Iraq, Pakistan, Mongolia, and some regions in Brazil. Recent outbreaks or cases

have occurred sporadically in parts of Asia, the Middle East, Africa, and South America. Cases were reported in Russia and Republic of Armenia on 2023, and most recently in in Iraq in 2024, as notified to the World Organization of Animal Health (WOAH).

In Mongolia, the first large-scale surveillance conducted in 1943 and 1944 showed an infection rate of 32-36.5 percent, which then decreased to 0.05 percent in 1988 due to measures taken to control the disease. Last nationwide surveillance implemented in 2011, the prevalence was increased to 0.19 percent.

Here, we present the outcome of glanders serological surveillance carried out between 2021-2024 to know the status of equine glanders among different regions in Mongolia.

A total of 3001 equine serum collected from 49 soum of 14 provinces in Western, Eastern, Central, Khangai regions of Mongolia. All samples were conducted to CFT and followed confirmatory test by Immunoblotting and GLANDA-ELISA.

During this four-year surveillance, a total of 31 samples positive were detected from 19 soums (including district) of 10 provinces (including Ulaanbaatar capital). Overall seroprevalence ranged between 1.03% (95% CI: 0.7–1.5). The study revealed increasing of prevalence from 2011 occurrence in Mongolia.

The present surveillance unveils territorial ingression of equine glanders to ten provinces like Tuv, Dundgobi, Selenge, Dornod, Arkhangai, Khentii, Ulaanbaatar, Sukhbaatar, Zavkhan, Uvurkhangai. In addition, re-emerging equine glanders have been reported by serological test in Tuv, Dundgobi, Selenge, Dornod, Arkhangai, Khentii, Ulaanbaatar, Zavkhan after a gap of 10 years. In this may, Lack of awareness but are due to the movement of horse trainer without any veterinary control for the purpose of participating in horse racing and breeding, knowledge about glanders. Therefore, we believe that information from this study will provide a latest baseline data on equine glanders for devising surveillance and control strategies in Mongolia.

ANTIBIOTICS SUSCEPTIBILITY OF THE BURKHOLDERIA MALLEI FIELD STRAINS

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Keywords: *Equine glanders, Burkholderia mallei, antimicrobial susceptibility*

Abstract:

Glanders is a zoonotic infectious disease caused by *Burkholderia mallei*, a highly virulent bacterium primarily affecting solipeds such as horses, mules, and donkeys. Due to its potential to infect humans and its high virulence, the Centers for Disease Control and Prevention (CDC) has classified B. mallei as a Category B biological threat agent.

In Mongolia, studies on the disease prevalence, diagnosis, control of disease, and especially the antibiotic susceptibility of causative agents of equine glanders are limited.

This study aimed to evaluate the antibiotic susceptibility of B. mallei field strains isolated from horses in Mongolia.

A total of 8 isolates were included in the study, and their antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method and the broth microdilution method to determine the ‘Minimum Inhibitory Concentration (MIC)’.

According to the disk diffusion results, all isolates were 100% resistant to ‘Fosfomycin (FF-30, FF-200)’ and ‘Amoxicillin (AX-10)’.

Additionally, 80% of all examined isolates were resistant to ‘Cefoxitin (FOX-30)’ and ‘Penicillin-G (P-2)’. On the other hand, most isolates showed sensitivity to ‘Gentamicin’, ‘Doxycycline’ and ‘Erythromycin’.

MIC values were determined based on optical density at 600 nm (OD600). The broth dilution assay revealed that Novobiocin and Tetracycline were effective against B. mallei, while the isolates exhibited resistance to ‘Sulfanilamide’, ‘Tetracycline disodium salt’, ‘Streptomycin sulfate’ and ‘Amoxicillin’.

This study provides essential first research report for the diagnosis for disease, treatment, and control of equine glanders in Mongolia, especially in guiding appropriate antibiotic selection.

RESULT OF THE SURVEILLANCE OF GLANDERS AMONG HERDERS

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Keywords*: Glanders, surveillance, herders*

Abstract:

Equine glanders is a zoonotic disease caused by *Burkholderia mallei*, primarily affecting solipeds such as horses, mules, and donkeys, as well as other predators, including cats, and humans through contact with infected animals. Herders, horse caretakers, and equine butchers and bacteriologist and bacteriologist laboratory workers have a risk of being affected by glanders. In 2020, 5.6% of 901 horses are tested positive. According to research by IVM, in 2021-2023, 1.1% of all horses tested positive. Between 1966 and 1977, three cases of glanders were unofficially reported in our country and reported two mortality. Glanders has a few studies. Until now there were no official guideline on glanders diagnosis, treatment and surveillance. Equine glanders is reported among horses in our country, but glanders in humans is rarely reported. Therefore, there is a need to study glanders among risk groups.

Goal: The aim of the study was to conduct surveillance on glanders among herders

Material and Methods: Among herders' family for investigation, the herders were interviewed face-to-face using a questionnaire containing 25 open and closed questions. Blood samples were collected from herders and were tested using the enzyme-linked immunosorbent assay to detect antibody against *Burkholderia mallei*

in the Laboratory of Infectious Diseases and Immunology of the IVM. The study data were entered into Microsoft Excel 2010, and the analysis was conduct.

Result: In 2024, 75 herder families of 18 soums from 7 provinces were interviewed face-to-face using a questionnaire and 70 herder families were tested to detect antibody against Burkholderia mallei. Of all the herders included in the study, 14.7% were exposed to risk factors for glanders by consuming raw equine testicles, 2.7% of them had consumed fresh mares milk, 22.7% of all the herders included in the study consumed raw water to deep well, and spring-well and 45.3% of them were cleaned muckheap. 44% [95 CI 32.8-55.5] had purchased horses from other soums, provinces and countries and 22.7% [95 CI 13.2-32.2] had sold horses to other soums, provinces and countries without any glander testing. Herders purchase and sell horses from other soums, provinces and countries for breeding, racing and consuming a food source. 16% of all herder families were answered to confirmation equine glanders on horse. 32% of all herders included in the study were answered to knowing about glanders and gather information related to glanders from veterinarian books, newspapers, television, and radio. Between 1.3% and 16% of all interviewed herders reported that their horses exhibited suspected clinical signs of equine glanders, including cough, pus-forming lesions, skin nodules, nasal discharge, and bloody nose and enlarged submandibular lymph nodes. However, 10.7% of these herders report these signs to the local veterinarian and 5.3% of all these herders were their horses for equine glanders. All herders included in the study did not manifest suspected signs of glanders, including fever of unknown origin, cough, chest pain, myalgia, and cutaneous nodular, lesions, and abscesses. 70 herders, tested using enzyme-linked immunosorbent did not detect an antibody against *Burkholderia mallei.*

Conclusion: Among the herders included in the study, there were risk factors for glanders and were not detected an antibody against *Burkholderia mallei***.**

Field survey photos in 2023: цithin SATREPS project for Control of Tuberculosis and Glander


JICA