BOOK OF PROCEEDINGS

NATIONAL INSTITUTE of ANIMAL HEALTH ANNUAL Conference 2026

JULY 21-23, 2026
FELIX RIVER KWAI RESORT,
KANCHANABURI

BOOK OF PROCEEDINGS

National Institute of Animal Health Annual Conference 2026

NEXT 2026: Elevating Research, Empowering the Future

July 21 – 23, 2026
Felix River Kwai Resort, Kanchanaburi

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WELCOME

Message from the Director of the Veterinary Research and Development Center, Western Region

It is my great pleasure to present the proceedings of the National Institute of Animal Health Annual Conference 2026, held under the theme **"NEXT 2026: Elevating Research, Empowering the Future."** This conference provides an important platform for researchers, veterinarians, scientists, and academic professionals to present, exchange, and disseminate research findings, academic achievements, and innovations in animal health. It also plays a vital role in strengthening professional capacity and promoting academic excellence within the Department of Livestock Development.

As animal health continues to face the challenges of emerging and re-emerging diseases, environmental changes, and rapid advances in science and technology, research and innovation have become essential to enhancing disease diagnosis, surveillance, prevention, and control. These efforts contribute not only to the advancement of veterinary services but also to food security and the sustainable development of Thailand's livestock sector.

This volume of the proceedings reflects the collective efforts and dedication of researchers and academic professionals who have contributed valuable scientific knowledge and research achievements. It serves as an important academic reference for further study, research, and the continued development of veterinary laboratory services and animal health.

On behalf of the Veterinary Research and Development Center, Western Region, and the Veterinary Research and Development Center, Lower Southern Region, the co-hosts of this conference, I would like to express my sincere appreciation to the National Institute of Animal Health for entrusting us with organizing this important event. I also extend my heartfelt gratitude to the organizing committee, reviewers, keynote speakers, authors, participants, and all who have contributed to the success of the conference through their dedication and cooperation.

It is my sincere hope that these proceedings will serve as a valuable academic reference, fostering collaboration, inspiring future research and innovation, and contributing to the continued advancement of animal health in Thailand.

Dr. Trakarnsak Paethaisong
Veterinarian, Senior Professional Level
Acting Director of Veterinary Research and Development Center, Western Region

About NIAHAC 2026

National Institute of Animal Health Annual Conference 2026

The Department of Livestock Development has established a policy to advance Thailand's livestock industry toward sustainable growth and progress. This includes promoting academic development, research activities, and personnel capacity-building to enhance expertise and professional competence in accordance with the Department of Livestock Development Strategic Plan 2023–2027. The strategy aims to develop personnel into **Smart Officers and Smart Researchers** who are capable of becoming internationally recognized researchers. One of the key approaches is to encourage knowledge exchange and dissemination of innovative research and modern technologies under the concept of **"NEXT 2026: Elevating Research, Empowering the Future."** This initiative seeks to strengthen research capabilities, empower future development, and enhance human resources to sustainably drive Thailand's livestock sector and improve its global competitiveness.

The National Institute of Animal Health and its affiliated agencies serve as the principal academic organizations of the Department of Livestock Development. Their missions include conducting studies, analyses, laboratory investigations, and research related to animal and zoonotic diseases; performing diagnostic testing; developing veterinary biologics for disease prevention and control; and ensuring quality standards for livestock products and environmental sanitation in livestock production systems.

Therefore, the National Institute of Animal Health has assigned the Veterinary Research and Development Center (Western Region) to organize the National Institute of Animal Health Annual Conference 2026. The conference aims to provide a platform for personnel to exchange knowledge, share research findings and academic achievements among affiliated agencies, strengthen professional networks, and further develop expertise in their respective fields.

NIAHAC 2026 Committees

National Institute of Animal Health Annual Conference 2026 (NIAHAC 2026) was co-organized by the Veterinary Research and Development Center, Western Region (Ratchaburi province), and the Veterinary Research and Development Center, Lower Southern Region (Songkhla province). Committee work is vital to the success of NIAHAC 2026, and the list of committee members is provided below.

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Conference Chair

Dr. Trakarnsak Paethaisong — Acting Director of Veterinary Research and Development Center, Western Region

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Lists of Reviewers

We sincerely thank all reviewers for their valuable time, effort, and thoughtful contributions in reviewing the conference abstracts.

Dr. Kittichai Unjit	Expert in Poultry Disease Research, National Institute of Animal Health
Mr. Anusorn Yooyen	Expert in Biochemistry and Toxicology Research, National Institute of Animal Health
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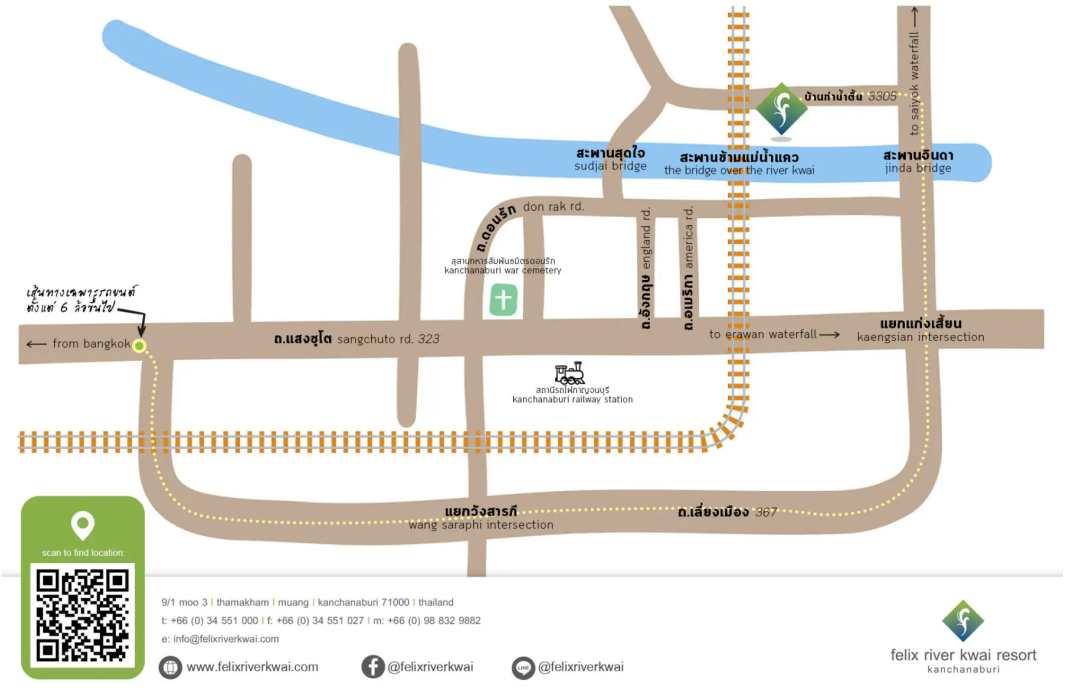
Ms. Supaporn Meeboon	Quality Control of Livestock Products Section, Veterinary Research and Development Center, Western Region

Venue

Felix River Kwai Resort is the legendary riverside garden retreat of Kanchanaburi province. It's a retro contemporary classic Thai-influenced architectural pavilion, built mainly with local hard redwoods, mountainous rocks, and terracotta tiles sealed to create a comfortable homey ambience that is nestled in its own private pristine 133 rai or 53 acres lush tropical garden, which sits on nearly 1 km length riverfront of the south bank of the Kwai River.

Location Map



Sponsors

The Organizing Committee of the National Institute of Animal Health Annual Conference 2026 sincerely acknowledges and appreciates the generous support of our sponsors.

Program at a Glance

National Institute of Animal Health Annual Conference 2026 (NIAHAC 2026)
July 21 – 23, 2026, at Felix River Kwai Resort, Kanchanaburi

	Tuesday 21 July	Wednesday 22 July	Thursday 23 July
09:00 – 10:00		Opening ceremony	Poster presentation session
		Group Photograph	
		Oral presentation session	
10:00 – 11:00		**Break (10:15-10:30)**	**Break (10:15-10:30)**
11:00 – 12:00		Oral presentation session (cont.)	Poster presentation session (cont.)
12:00 – 13:00		**Lunch**	Closing ceremony/ **Lunch**
13:00 – 14:00	**Registrations**	Oral presentation session (cont.)	
14:00 – 15:00	Conference orientation		
	Group assignment and breakout sessions		
15:00 – 16:00	**Break (15:00-15:15)**	**Break (15:00-15:15)**	
	Special topic session	Oral presentation session (cont.)	
16:00 – 17:00			
17:00 – 18:00	**Dinner**	**Dinner**	

*The schedule is subject to change as appropriate.

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Netnapa Sroisena

Position: Product & Marketing Manager
Organization: F.N. Science Co., Ltd.
Topic: Automation Workflow Solution for Animal Disease Detection, Tianlong

Theeraporn Jiratammakun

Position: Field Application Specialist APeC
Organization: Indical Bioscience GmbH
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Phairat Royros

Position: Senior Market Development Manager
Organization: QIAGEN (Thailand) Ltd.
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Akin Hemadhulin

Position: Product Manager
Organization: Molecular Dx Co., Ltd.
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ABSTRACTS OF SPECIAL TOPIC SESSION

Keep up on the Latest Nanopore Sequencing Developments from Oxford Nanopore Technologies

Kanokkarn Pothong

Abstract

Nanopore sequencing, a technology developed by Oxford Nanopore Technologies, is achieving substantial recognition and broad implementation across various sectors, particularly within the domain of infectious diseases. This technology facilitates the analysis of not only bacteria and microbiomes but also enables simultaneous 16S and ITS sequencing, thereby providing comprehensive coverage of pathogenic agents. Metagenomics represents a prominent application employed by researchers for environmental investigations. Furthermore, this approach facilitates the expedited identification of respiratory pathogens in medical field. To overcome the constraints of traditional sequencing methods, plasmid sequencing has been developed, enabling comprehensive characterization of entire plasmids with rapid and complete. Oxford Nanopore Technologies has developed a novel kit for direct RNA sequencing with multiplex samples, specifically designed for RNA pathogen research. Alternatively, RNA virus sequencing, such as for the influenza virus, can be achieved using the Native Barcoding kit. Amplicons can be processed efficiently with the rapid method, and adaptive sampling effectively mitigates host interference.

Keywords: Nanopore sequencing, 16S, ITS, Metagenomics, Plasmid sequencing

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Automation Workflow Solution for Animal Disease Detection, Tianlong

Netnapa Sroisena

Abstract

Effective management of animal disease outbreaks requires rapid, accurate, and high-throughput diagnostic solutions to safeguard livestock industries and public health. This paper presents the Automation Workflow Solution for Animal Disease Detection developed by Xi'an Tianlong Science and Technology. The solution integrates automated nucleic acid extraction, precise liquid handling, and advanced real-time Polymerase Chain Reaction (qPCR) detection into a unified, seamless workflow.

By utilizing state-of-the-art automated extractors (such as the Libex/GeneRotex series) alongside high-performance thermal cyclers (such as the Gentier series), this workflow significantly minimizes manual intervention, reduces human error, and mitigates cross-contamination risks. The system demonstrates exceptional sensitivity and specificity in detecting major veterinary pathogens, including African Swine Fever Virus (ASFV), Avian Influenza Virus (AIV), and Foot-and-Mouth Disease Virus (FMDV). Furthermore, the standardized protocol drastically shortens turnaround times, enabling laboratories to process large volumes of samples efficiently during critical screening windows. Ultimately, Xi'an Tianlong's automated solution provides a robust, reliable, and scalable platform for veterinary laboratories, contributing significantly to global animal biosafety and infectious disease control.

F.N. Science Co., Ltd. is a leading provider of comprehensive scientific solutions, specializing in the distribution of high-quality laboratory instruments, chemical reagents, and specialized analytical equipment. Dedicated to supporting advancements in research, quality control, and industrial testing, the company offers an extensive product portfolio sourced from globally recognized manufacturers. Beyond product supply, F.N. Science delivers end-to-end support, including professional installation, calibration, preventive maintenance, and expert technical consultation. By combining reliable, cutting-edge technology with customer-centric services, F.N. Science Co., Ltd. serves as a trusted partner, empowering laboratories across academic, medical, and industrial sectors to achieve precise, efficient, and compliant analytical results.

Keywords: Animal disease detection, Automation workflow, Nucleic acid extraction, Real-time PCR, High-throughput diagnostics, Xi'an Tianlong

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CTS 2.0: Advancing *Salmonella* Surveillance and Molecular Typing in Veterinary Diagnostics

Theeraporn Jiratammakun

Abstract

In poultry, swine, cattle, and other food-producing animals, *Salmonella* infections threaten animal health, reduce productivity, and contribute to substantial economic losses through decreased production performance, increased treatment costs, and trade restrictions. Effective surveillance and rapid characterization of *Salmonella* are therefore essential for protecting animal health, minimizing economic losses, supporting disease control programs, and reducing the risk of zoonotic transmission along the food production chain.

CTS 2.0 is an advanced multiplex real-time PCR assay developed for the rapid molecular characterization of *Salmonella* isolates. By simultaneously detecting multiple genetic markers in a single reaction, CTS 2.0 delivers sensitive, specific, and reproducible results within hours, providing a standardized and efficient alternative to conventional serotyping. The streamlined workflow reduces hands-on time, laboratory complexity, and turnaround time while supporting high-throughput testing in routine veterinary diagnostic laboratories.

CTS 2.0 enables rapid and reliable characterization of *Salmonella* serotypes and genetic markers, providing valuable information for surveillance programs, outbreak investigations, epidemiological studies, and source attribution. Compared with conventional serotyping, the assay eliminates labor-intensive antigenic testing while improving consistency and reproducibility. Compared with next-generation sequencing (NGS), CTS 2.0 offers a practical, cost-effective solution for routine surveillance without requiring extensive sequencing infrastructure or bioinformatics expertise.

By supporting early detection and timely characterization of *Salmonella*, CTS 2.0 empowers veterinarians, diagnostic laboratories, producers, and animal health authorities to implement targeted interventions, strengthen farm biosecurity, and monitor pathogen dissemination within and between livestock populations. As a rapid and scalable molecular typing platform, CTS 2.0 enhances national and regional *Salmonella* surveillance programs, contributes to more effective disease prevention and control strategies, protects animal health, minimizes economic losses, and supports food safety and One Health initiatives.

INDICAL
BIOSCIENCE

Keywords: *Salmonella* serotyping; Multiplex real-time PCR; Animal health surveillance
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Advancing Veterinary Molecular Research with Digital PCR: From Pathogen Detection to Absolute Quantification

Phairat Royros

Abstract

Digital PCR is emerging as a powerful molecular technology for sensitive, precise, and absolute quantification of viral pathogens in animal health research. This presentation will highlight the application of the QIAcuity Digital PCR System, together with dPCR assays available through QIAGEN GeneGlobe, for the detection and quantification of key veterinary pathogens across poultry, swine, ruminants, horses, and other animal species. Target pathogens include both RNA and DNA viruses, such as avian influenza virus, Newcastle disease virus, African swine fever virus, PRRS virus, foot-and-mouth disease virus, bovine viral diarrhea virus, lumpy skin disease virus, and other economically important infectious agents.

The session will discuss how digital PCR can support veterinary research workflows, including low-copy target detection, pathogen load monitoring, assay validation, multiplex detection, and sample-to-result molecular analysis. By integrating disease biology, nucleic acid type, sample selection, and PCR-based detection strategies, this presentation aims to demonstrate how QIAcuity can strengthen veterinary molecular research, surveillance, and outbreak preparedness through accurate and reproducible absolute quantification.

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Digital Pathology in Animal Field and Cytogenetics Analysis Software: Whole Slide Imaging and Intelligent Software Solutions

Akin Hemadhulin

Abstract

The rapid advancement of digital technologies is transforming veterinary diagnostic laboratories by improving workflow efficiency, data management, and diagnostic capabilities. This presentation introduces two innovative diagnostic solutions: KFbio Digital Pathology and ASI Cytogenetics Analysis Software.

The first part highlights the capabilities of KFbio Digital Pathology, including whole slide imaging (WSI), high-resolution slide scanning, digital image management, remote consultation, education, and research applications. The technology enables laboratories to transition from conventional microscopy to a fully digital pathology workflow.

The second part presents ASI Cytogenetics Analysis Software, an advanced platform for chromosome and fluorescence *in situ* hybridization (FISH) analysis. Key features such as automated image acquisition, karyotyping, FISH signal analysis, case documentation, and standardized reporting will be introduced to demonstrate how the software enhances accuracy and laboratory efficiency.

Together, these technologies provide a comprehensive digital solution that supports modern veterinary pathology and cytogenetics, paving the way toward more efficient, collaborative, and data-driven diagnostic laboratories.

Keywords: Digital pathology, Cytogenetics, Fluorescence *in situ* hybridization (FISH), Karyotyping
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ABSTRACTS OF ORAL PRESENTATION SESSION

Evaluation of the Stability of Reconstituted Lumpy Skin Disease Vaccine at 2–8°C for Field Applications

Wilasinee Thaopech[1], Sirintip Khemtong[1,*], Rattanee Thongtha[1], Natekamol Yimpirat[1], Pawatchaya Hansuri[1] and Nilawan Suaykampang[1]

Abstract

Background: Lumpy skin disease (LSD) caused by Lumpy skin disease virus, a member of the genus *Capripoxvirus* affects cattle and buffalo, leading to fever, weight loss, lethargy, lymphadenopathy and nodular skin lesions. Although mortality is low, LSD causes significant economic losses through reduced growth, milk production, reproductive performance and carcass quality. Vaccination with live attenuated lyophilized LSD vaccines is the primary control measure. However, after reconstitution, vaccine potency can be affected by temperature, light and contamination. In field conditions, maintaining recommended storage temperatures and using all doses within the specified timeframe can be challenging, particularly when vial sizes exceed the number of animals and vaccine sharing between farms is limited. This often results in wastage and increased costs. Therefore, evaluating the stability of reconstituted vaccines is essential to ensure their effectiveness under practical conditions.

Objectives: This study evaluated the stability of the reconstituted Lumpy Skin Disease (LSD) vaccine at 2-8°C under practical field-use conditions over various time periods

Methods: Three batches of lyophilized Lumpy Skin Disease vaccine were reconstituted according to the manufacturer's instructions. To simulate field conditions, the reconstituted vaccines were kept in an icebox at 2-8°C for 0-10 hours, then stored in a refrigerator at 2-8°C for testing at 24 hours. MDBK cells were seeded in 96-well plates to form monolayers. The vaccines were serially diluted from 10^{-1} to 10^{-6} and 50 µL of each dilution was inoculated into five wells. Plates were incubated at 37°C with 5% CO_2 and cytopathic effects were monitored for 7 days. Virus titers were calculated using the Spearman-Karber method at 0, 2, 4, 6, 8, 10 and 24 hours post-reconstitution.

Results: Virus titers of three vaccine batches at 0 hours post-reconstitution were 4.2, 4.2 and 3.8 $\log_{10}$ $TCID_{50}$/dose, respectively and remained stable for up to 6 hours. At 8 and 10 hours, Sample 1 remained stable, whereas Samples 2 and 3 showed slight decreases to 4.0 and 3.6 $\log_{10}$ $TCID_{50}$/dose, respectively. All samples remained within the acceptable range (3-4 $\log_{10}$ $TCID_{50}$/dose) according to the guidelines of the World Organization for Animal Health at 24 hours.

Conclusion: The reconstituted LSD vaccine is stable for up to 6 hours at 2-8°C. Although titers remain within the acceptable range at 24 hours, factors such as contamination and light exposure may affect vaccine stability in practice. These findings provide important data for enhancing program efficiency and supporting reliable field use in controlling LSD outbreaks.

Keywords: Lyophilized Lumpy Skin Disease vaccine, Virus titer, Vaccine stability
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Application of Digital PCR for Sensitive Detection of Avian Influenza Viral RNA in Poultry Matrices with Low Viral Loads

Teerapong Prompunya[1,*], Duangrat Wongyai[1], Chainarong Kulchim[1] and Naruepol Promkuntod[1]

Abstract

Background: Avian influenza virus (AIV) is a highly contagious pathogen in poultry that causes substantial economic losses and poses significant risks to animal and public health worldwide. Rapid and accurate detection is essential for early outbreak recognition, effective control, and prevention of virus spread. Although real-time RT-PCR is the current standard for AIV diagnosis, its performance may be limited in specimens with low viral loads and complex poultry-derived matrices. Digital PCR (dPCR), which enables absolute quantification without reliance on standard curves, has emerged as a promising approach to improve sensitivity and precision in challenging diagnostic settings.

Objectives: To evaluate the performance of dPCR for sensitive and precise detection of the AIV genome in diverse poultry sample matrices, with particular emphasis on low viral load specimens.

Methods: A series of 10-fold and 2-fold serial dilutions of quantified synthetic AIV RNA corresponding to the influenza A matrix (*M*) gene were prepared in a no-effect matrix to evaluate analytical performance, including quantification range, limit of detection, linearity, and precision. Specificity was assessed using inclusivity, cross-reactivity, and negative pathogen panels. To assess matrix effects, RNA was spiked into poultry-derived sample matrices, including oropharyngeal swabs, cloacal swabs, and tissue homogenates (n = 20 each), and categorized into four viral load groups (10^4, 10^3, 10^2, and 50 copies/µL, representing high, intermediate, low, and very low viral loads, respectively). Quantification was performed using real-time RT-PCR and dPCR with primers and probes targeting the M1 gene. Analytical performance was evaluated across the dilution series, and agreement between methods was assessed using correlation and Bland–Altman analyses, including estimation of limits of agreement (LoA).

Results: Real-time RT-PCR demonstrated a wider quantification range (50 -10^6 copies/µL), whereas dPCR exhibited a narrower quantification range but a lower limit of detection (1 -10^4 copies/µL). Both methods showed strong linearity across 10-fold serial dilutions ($R^2 > 0.99$). dPCR demonstrated improved precision at low and very low viral loads, as indicated by a lower coefficient of variation. Specificity analysis showed almost perfect agreement, with 100% concordance (11/11) and Cohen's $\kappa = 1.0$. In matrix-derived samples, no significant difference was observed between dPCR and real-time RT-PCR at high, intermediate, and low viral loads in oropharyngeal and cloacal swabs, whereas a significant difference was observed at very low viral loads, with dPCR demonstrating improved detection performance. No significant differences were observed across all viral loads in tissue homogenates. Correlation analysis demonstrated strong agreement between the two methods in oropharyngeal swabs ($R^2 = 0.983$), cloacal swabs ($R^2 = 0.984$), and tissue homogenates ($R^2 = 0.982$). Bland–Altman analysis showed LoA from −0.256 to 0.179 $\log_{10}$ at high viral loads, −0.312 to 0.232 $\log_{10}$ at intermediate viral loads, −0.265 to 0.269 $\log_{10}$ at low viral loads, and −0.398 to 0.350 $\log_{10}$ at very low viral loads, indicating overall good agreement, with increased bias and wider limits of agreement observed at very low viral loads.

Conclusion: dPCR provides a sensitive and precise platform for the detection and absolute quantification of AIV genomes in diverse poultry sample matrices. Its improved performance in very low viral load specimens supports its use as a complementary or confirmatory method to real-time RT-PCR for early diagnosis, surveillance, and outbreak preparedness in avian influenza control programs.

Keywords: Avian influenza virus, Digital PCR, Real time RT-PCR

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Genomic Characterization of a Fowl Adenovirus Isolated from Chickens with Inclusion Body Hepatitis in Thailand Using Whole Genome Sequencing

Nantaporn Wandee[1,*], Tapanut Songkasupa[1], Kultyarat Bhakha[1] and Klairoong Thonsranoi[1]

Abstract

Background: Inclusion body hepatitis (IBH) is a significant viral disease causing substantial economic losses in the global broiler industry due to high mortality rates and characteristic hepatic pathology. The disease is primarily caused by fowl adenovirus (FAdV), a member of the genus *Aviadenovirus*, which comprises five species (FAdV-A to E) and 12 serotypes. Despite recurring outbreaks in Thailand, comprehensive genomic data on circulating strains remain limited.

Objectives: This study aimed to isolate an FAdV strain from an affected poultry farm and utilize whole-genome sequencing (WGS) to analyze the sequences of major structural and virulence genes to identify mutations associated with its biological properties.

Methods: Liver samples were collected in 2022 from broiler chickens in Chonburi Province exhibiting clinical signs and gross lesions pathognomonic for IBH. The virus was isolated and propagated in primary chicken embryo liver (CEL) cells, with viral presence confirmed via polymerase chain reaction (PCR) targeting the *hexon* gene. The extracted viral DNA was subsequently subjected to WGS using Oxford Nanopore technology, leveraging its long-read capabilities to facilitate high-accuracy genome assembly.

Results: Phylogenetic analysis at the whole-genome level confirmed that the isolate belongs to species E, serotype 8b (FAdV-8b). In-depth genomic assessment focused on critical structural genes and virulence determinants, specifically the hypervariable regions (HVRs) of the *hexon* gene and the full-length *fiber* gene. Comparative analysis with global reference strains from GenBank revealed distinct mutation patterns and specific amino acid substitutions within these key genes, highlighting the unique genetic profile of the circulating Thai strain.

Conclusion: The genomic characterization provided by this study establishes a vital molecular epidemiological foundation for disease surveillance and tracking viral evolution. Furthermore, these findings provide a scientific basis for selecting appropriate local candidate vaccine seeds to effectively control IBH within the Thai poultry industry.

Keywords: Fowl adenovirus, Inclusion body hepatitis, Genomic characterization, Whole genome sequencing, Nanopore sequencing

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Genetic Diversity and Evolution of Infectious Bronchitis Virus in Southern Thailand: Dominance of QX-like (GI-19) and Emergence of GI-19/GI-7 Recombinants (2014-2025)

Pattaramon Suanin[1,*], Sasiwimol Thongmee[1], Nusara Pathan[1], Wongkae Watjiranon[1], Khanistar Rattanaburee[1], Khwansanee Suknao[1], Gamonsiri Bhumibhamon[1] and Wandee Kongkaew[1]

Abstract

Background: Avian infectious bronchitis virus (IBV) poses a continuous threat to the poultry industry due to its rapid evolution. High mutation rates, particularly within the S1 gene, drive significant genetic diversity, leading to the emergence of new variants and potential vaccine failure. Previous studies (2008-2016) identified the QX-like lineage (GI-19) as the predominant genotype in Thailand since 2009, co-circulating with 4/91-like (GI-13), Massachusetts (GI-1), and vaccine-related strains.

Objectives: This study aimed to characterize the genetic diversity and phylogenetic relationships of IBV isolates collected from chickens in Southern Thailand between 2014 and 2025 using partial S1 gene analysis.

Methods: Viral RNA was extracted from 12 confirmed IBV clinical cases. Partial S1 gene fragments (980 and 1065 bp) were amplified via reverse transcription polymerase chain reaction (RT-PCR). Phylogenetic trees were constructed using MEGA software (v.12) to determine lineage classification. Potential recombination events were further analyzed and confirmed using RDP software (v.5).

Results: Phylogenetic analysis revealed that the QX-like lineage (GI-19) remained predominant, accounting for 9 out of 12 isolates. Other isolates were identified as 4/91-like (GI-13) and DLD vaccine-like strains. Notably, one isolate showed discordant phylogenetic clustering and was confirmed as a GI-19/GI-7 recombinant. Most genotype assignments were supported by high bootstrap values.

Conclusion: The QX-like (GI-19) genotype remains the predominant IBV strain circulating in Southern Thailand. The identification of a novel GI-19/GI-7 recombinant underscores the high genetic diversity and ongoing evolution of IBV in the region. These findings emphasize the necessity of continuous molecular surveillance. Furthermore, the combined analysis of 980 and 1065 bp S1 fragments proved highly effective for accurate genotyping and recombination detection.

Keywords: Avian infectious bronchitis virus, S1 gene, GI-19 (QX-like), GI-19/GI-7 recombinant, Molecular epidemiology

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Prevalence of *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ Genes in *Escherichia coli* Isolated from Chicken Meat and Pork in Butcher Shop in Eastern Region of Thailand during 2022 – 2024

Phanlert Sakkaew[1,*], Nanticha Rattanamangkalanon[1], Ruchuda Kanjanapen[1] and Supaporn Wongsrichai[2]

Abstract

Background: In eastern region of Thailand, the number of pig and chicken is ranked in the top three of the most population. Then, the antimicrobial drugs may be used in high volume for treatment and ESBL producing - *E. coli* could be seriously concerned in antimicrobial resistance issue in animal, human and environmental problems (One Health). Extended-spectrum beta-lactamase (ESBL) is the hydrolysing enzyme that is produced from *bla*$_{TEM}$, *bla*$_{CTX-M}$ or *bla*$_{SHV}$ gene in plasmid of *Escherichia coli* (*E. coli*). The enzyme can be against the potential of beta-lactam antibiotics such as 3rd and 4th generation cephalosporins and monobactam with beta-lactam ring destruction. Hydrolysis of the carbonyl group of the beta-lactam ring is an important mechanism of drug resistance. As a result, the effectiveness of beta-lactamase drug is significantly decreased. Chicken meat and pork are one of the most main ingredients in daily recipes. Many studies reported that these kinds of meat can be contaminated with ESBL producing - *E. coli* from farms, slaughterhouses and butcher shops. According to the Thailand's Second National Action Plan on Antimicrobial Resistance 2023 – 2027 and the Thailand's integrated antimicrobial resistance surveillance with One Health approach guideline, monitoring of ESBL producing - *E. coli* resistance to 3rd generation cephalosporin in meat is highly recommended.

Objectives: To study the prevalence of *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ genes in *E. coli* isolated from chicken meat and pork from butcher shop in each province in Eastern region of Thailand and the comparison between ESBL producing - *E. coli* in modern and local butcher shops.

Methods: A total of 276 samples of chicken meat (n = 138) and pork (n = 138) were collected from modern and local butcher shops in 9 provinces (Samutprakan, Chachoengsao, Chonburi, Prachinburi, Rayong, Sakaeo, Chanthaburi, Trat and Nakhonnayok) in eastern region of Thailand during August 2022 to July 2024. Those samples were identified with microbiological and biochemical tests. ESBL screening test was done according to the CLSI (Clinical and Laboratory Standards Institute, 2023) for Extended-spectrum beta-lactamase (ESBL) drug sensitivity test. The Polymerase Chain Reaction (PCR) was done for *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ gene detection from ESBL producing- *E. coli*.

Results: The ESBL producing – *E. coli* percentage of chicken meat and pork in 2022 – 2024 was declined from 60.71%, 53.57% and 51.22%, respectively. Pork was detected more ESBL producing - *E. coli, bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{TEM}$ with *bla*$_{CTX-M}$ gene than chicken meat. Almost all provinces have high prevalence (50 – 100%) of *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes except Samutprakarn. Interestingly, none of the provinces were found *bla*$_{SHV}$ gene. The detection of those genes between modern and local butcher shops was not significantly different (p = 0.199).

Conclusion: Chicken meat and pork were most probably contaminated with ESBL producing – *E. coli* during raised on farm, slaughtering and retailing. Good Agricultural Practice (GAP), Antimicrobial-free farm, Good Hygiene Practice (GHP) and Hazzard Analysis and Critical Control Point (HACCP) should be included in each process for minimized the contamination. In addition, ESBL producing – *E. coli* and resistance gene surveillance is still necessary.

Keywords: *E. coli,* ESBL, Chicken meat, Pork, Butcher shop

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Analysis of Phenotypic Antimicrobial Resistance Patterns of *Salmonella* in Meat Using Hierarchical Clustering in Western Thailand, 2022–2024

Supaporn Meeboon[1,*], Kittiya Paiboonkunkon[1] and Trakarnsak Paethaisong[1]

Abstract

Background: Antimicrobial resistance (AMR) in *Salmonella* is a major global concern, especially through contaminated meat. Multidrug-resistant (MDR) strains, defined as resistance to three or more antimicrobial classes, are increasingly detected in livestock and threaten both food safety and treatment success. Although Thailand applies a "One Health" surveillance framework, phenotypic resistance data in food-producing animals remain limited. Strengthening this area is crucial because resistant strains from livestock can enter humans via the food chain. This study used hierarchical clustering to group isolates by similarity in resistance profiles, revealing resistance clusters, MDR patterns, and emerging trends that enhance traditional analyses.

Objectives: To characterize phenotypic AMR patterns of *Salmonella* isolates from pork and poultry meat in western Thailand (2022–2024) using hierarchical clustering, and to assess epidemiological differences in MDR contamination.

Methods: A total of 446 *Salmonella* isolates were cultured and identified from pork and poultry meat samples collected in western Thailand between 2022 and 2024. The dataset used in this study was secondary data, derived from routine surveillance activities and subsequently analyzed for research purposes. Antimicrobial susceptibility testing was performed against 11 agents following Clinical and Laboratory Standards Institute (CLSI) guidelines. Minimum inhibitory concentration (MIC) values were converted into ordinal scales and then normalized to place all variables on a comparable 0–1 range, ensuring equal contribution to distance calculations. The processed data were analyzed using agglomerative hierarchical clustering, employing Euclidean distance and Ward's linkage in Orange Data Mining (version 3.36). Epidemiological differences in multidrug resistance (MDR) contamination were assessed using odds ratios (OR) with 95% confidence intervals and the chi square test.

Results: Five distinct resistance clusters were identified (average Silhouette coefficient = 0.16, consistent with the expected heterogeneity of phenotypic AMR data). Cluster 1 (n = 183) exhibited minimal resistance (<6%). Cluster 2 (n = 64) showed extensive MDR, with AMP (100%), CHL, GEN, and TET (>95%), and CTX (>92%). Cluster 3 (n = 82) displayed moderate resistance to AMP (78%) and TET (76%) but remained susceptible to cephalosporins. Cluster 4 (n = 37) demonstrated high resistance to older antimicrobials (AMP 100%, CHL 95%, TET 89%, and SXT 86%) but full susceptibility to CTX, CAZ, CST, GEN, and MEM. Cluster 5 (n = 80) showed a resistance profile similar to Cluster 3 but with markedly higher resistance to AMP (95%) and TET (96%). Epidemiological analysis revealed that only pork had a significantly higher risk of MDR contamination than poultry meat (OR 2.16, 95% CI 1.47–3.19, $p < 0.001$), while no difference was observed between slaughterhouses and retail markets.

Conclusion: This study revealed substantial heterogeneity in phenotypic antimicrobial resistance among *Salmonella* isolates from pork and poultry meat in western Thailand, resulting in five distinct resistance clusters ranging from fully susceptible to extensively multidrug-resistant profiles. The identification of clusters with high resistance to older antimicrobials (AMP, CHL, TET, SXT) and the presence of a strongly MDR group underscore the ongoing selective pressure within the food production system. Pork was identified as a significantly higher-risk source of MDR contamination than poultry meat, highlighting a critical control point for targeted interventions. Overall, the findings emphasize the need to strengthen AMR surveillance within the One Health framework and to implement evidence-based strategies to mitigate the spread of resistant *Salmonella* through the food chain.

Keywords: *Salmonella,* Antimicrobial resistance, Multidrug resistance, Hierarchical clustering, Western Thailand

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Molecular Characterization of Rabies Virus in Thailand, 2022-2025

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Abstract

Background: Rabies remains a fatal zoonotic disease and a significant public health concern in Thailand. Understanding the epidemiological patterns and genetic diversity of circulating rabies virus strains is essential for effective surveillance and control. Analysis of the nucleoprotein (N) and glycoprotein (G) genes, together with whole genome sequencing (WGS) of selected isolates, enables detailed characterization of circulating viruses and supports improved rabies monitoring and control strategies in Thailand.

Objectives: To characterize the genetic features of rabies virus in Thailand and generate updated molecular data, including information related to antigenicity (G gene) and molecular characteristics of key viral genes (N, P, M, and L), to support rabies surveillance and contribute to effective prevention and control strategies.

Methods: Rabies virus–positive brain samples from animals collected in Thailand during 2022–2025 were confirmed by the Direct Fluorescent Antibody (DFA) test and real-time RT-PCR. Viral RNA was extracted, and the N and G genes were amplified by RT-PCR and sequenced using Oxford Nanopore technology in 100 samples. In addition, WGS was performed on eight representative samples using the same platform. The obtained sequences were compared with reference and vaccine strains from GenBank and analyzed using standard bioinformatics tools to identify genetic variations and construct phylogenetic trees.

Results: Phylogenetic analysis of the N and G genes showed that rabies virus strains circulating in Thailand belong to the Asian clade. Viruses in the northern and upper northeastern regions (n = 17) were mainly classified into the SEA1 subclade, while the SEA3 subclade (n = 83) was distributed across the country. WGS of representative samples showed consistent clustering with the N and G gene analyses. The Thai strains were genetically related to viruses reported from neighboring countries in the GenBank. In addition, the virus detected in a golden jackal was highly similar to strains circulating in domestic dogs in Loei Province. Comparative analysis suggested that current rabies vaccines remain effective against the circulating strains.

Conclusion: This study provides updated information on the genetic characteristics of rabies viruses circulating in Thailand. The findings demonstrate that the circulating strains belong to the Asian clade and are genetically related to viruses reported in neighboring countries. The detection of genetically similar rabies viruses in wildlife and domestic animals also highlights the potential role of wildlife in rabies transmission. These results support ongoing rabies surveillance and indicate that current vaccines remain effective for rabies prevention and control.

Keywords: Rabies, Whole genome sequencing, N gene, G gene, Vaccine

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Rapid Diagnosis of Surra in Water Buffaloes Using Plasma for CATT/*T. evansi* Method: A Field Laboratory Troubleshooting and Case Report

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Abstract

Background: Surra, caused by *Trypanosoma evansi*, is an economically significant haemoparasitic disease affecting livestock in Thailand. Standard parasitological diagnosis via direct microscopic examination often faces sensitivity limitations, particularly during periods of low parasitemia or when blood samples deteriorate during transport. This report focuses on utilizing plasma to overcome sample constraints in urgent diagnostic situations.

Objectives: To demonstrate the practical use of EDTA plasma as an alternative to serum for the Card Agglutination Test for *T. evansi* (CATT/*T. evansi*) to provide a rapid and accurate serological diagnosis under field laboratory conditions when serum samples are unavailable.

Methods: One sudden death (3-year-old male) and one clinically ill buffalo (7-year-old female) were found in a herd of 23 water buffaloes (9 males and 14 females) in an upper northeastern province of Thailand. EDTA-anticoagulated blood samples from both animals were submitted to the laboratory following overnight transport. Initial diagnostic tests included determination of packed cell volume (PCV), Woo's technique, and microscopic examination of thin blood and buffy coat smears. Due to the lack of serum samples, EDTA plasma was obtained by centrifugation and used for CATT/*T. evansi* testing.

Results: Initial laboratory results for the deceased buffalo showed positive results (+4) in both blood smears and buffy coat smears, while Woo's technique was negative. In contrast, the sick buffalo exhibited severe anemia (PCV 14%), but all parasitological examinations yielded negative results, possibly due to low parasitemia or parasite lysis during transit. However, CATT/*T. evansi* testing using EDTA plasma showed strongly positive results in both animals. This serological finding confirmed the infection in the sick buffalo, highlighting the high sensitivity of CATT even when parasites were not microscopically detectable.

Conclusion: This report demonstrates the potential utility of EDTA plasma as a practical substitute for serum in CATT/*T. evansi* method when rapid diagnostic results are required, and serum samples are unavailable. The detection of specific antibodies in plasma ensures diagnosis accuracy, enabling veterinarians to administer timely treatment with Diminazene aceturate. In this case, prompt intervention successfully cured the sick animal and facilitated effective disease control within the herd.

Keywords: *Trypanosoma evansi*, Surra, CATT, Plasma, Water buffalo

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Unexpected Strangles in Imported Horses: Molecular Identification of *Streptococcus equi* subsp. *equi*

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Abstract

Background: Strangles is a highly contagious equine respiratory disease caused by *Streptococcus equi* subsp. *equi* (SEE) and is characterized by fever, mucopurulent nasal discharge, and abscess formation in the submandibular and retropharyngeal lymph nodes. In contrast, *Streptococcus equi* subsp. *zooepidemicus* (SEZ) is a commensal and opportunistic pathogen. Young horses aged 1–5 years are most susceptible, particularly under stress such as transportation or overcrowding. Strangles is currently included among the diseases that must be certified negative prior to importation into Thailand. However, the disease has previously been reported in association with imported horses. In 2014, a SEE outbreak was documented in horses imported from Europe.

In September 2025, several horses were imported into Thailand for a competition scheduled for December. During quarantine, a yearling horse showed clinical respiratory signs with nasal discharge and a submandibular abscess. After a week in the same location, ten additional horses developed similar symptoms. Then the horse's owner notified DLD to investigate the situation.

Objectives: To investigate a suspected outbreak in a horse herd and confirm SEE as the causative agent of strangles in imported horses using bacteriological and molecular diagnostic methods.

Methods: Thirty-five sera were collected from the horse herd showing clinical respiratory signs. Then, the sera were subjected to serological testing by ELISA. Further sampling included 52 whole bloods, 5 pus swabs, 3 wound swabs, and 23 nasal swabs from seropositive herds, which were subjected to bacterial isolation and direct real-time PCR targeting the *SIR2* gene. Subsequently, suspected colonies were confirmed by conventional PCR targeting both the *SeM* and *SIR2* genes. Then, Sanger sequencing of universal *16S rRNA*, *SeM,* and *SIR2* genes was performed for confirmation. Furthermore, antimicrobial susceptibility of confirmed isolates was tested by disc diffusion. Epidemiological investigation included two additional farms with a history of horse import from the same batch.

Results: Seropositivity to SEE was 48.6% (17/35 samples). Bacterial isolation was obtained primarily from pus swabs, whereas most nasal swabs were culture-negative despite positive molecular detection. Isolates produced small, beta-hemolytic colonies on blood agar, and microscopic examination revealed Gram-positive cocci consistent with *Streptococcus* spp.

Direct real-time PCR targeting the *SIR2* gene detected SEE in clinical samples, while conventional PCR targeting both *SeM* and *SIR2* genes confirmed the identity of cultured isolates, with results confirmed by Sanger sequencing (100% sequence identity). These findings indicate that pus swabs with 80% positive (4/5 samples) are more suitable for bacterial isolation, whereas nasal swabs with 4.3% positive (1/23 samples) are reliable for molecular detection. For the horse with a positive nasal swab, the corresponding serum sample was also positive by ELISA. No additional samples were collected from this animal as the clinical condition did not improve, and the horse was subsequently euthanized. The 52 blood samples and 3 wound swabs were all negative. Additionally, SEEs were most susceptible to antibiotics, and horses recovered after treatment. Furthermore, one of two additional farms that had imported horses from the same source was investigated, and clinical samples from these farms were also positive for SEE.

Conclusion: This study confirms SEE infection in imported horses in Thailand and highlights the risk of post-import disease introduction. Pus swabs were more suitable for bacterial isolation, whereas nasal swabs were reliable for molecular detection. The use of *SIR2*-targeted real-time PCR enabled specific detection, while dual-target PCR improved diagnostic confidence. Molecular methods demonstrated higher sensitivity than culture, and epidemiological findings suggest that horse movement contributed to disease transmission between farms. These findings emphasize the importance of strict post-arrival quarantine protocols, active surveillance, and integrated diagnostic approaches for effective control of transboundary equine diseases.

Keywords: Equine, PCR, Strangles, *Streptococcus equi* subsp. *equi*

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Evaluation of the Efficacy of Disinfectants Against Foot-and-Mouth Disease Virus in Laboratory Conditions

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Abstract

Background: Foot-and-Mouth Disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals, causing substantial economic losses to the livestock industry and significant disruptions to international trade. Effective disinfection is a fundamental component of biosecurity measures for the prevention and control of outbreaks caused by the Foot-and-Mouth Disease Virus (FMDV). However, the virucidal efficacy of disinfectants against FMDV varies considerably depending on their chemical composition and environmental conditions.

Objective: This study aimed to evaluate the efficacy of selected commercially available disinfectants and laboratory-developed formulations for the inactivation of FMDV under controlled laboratory conditions.

Methods: Disinfectants evaluated included quaternary ammonium compounds (QACs), chlorine-based disinfectants, and other candidate formulations. Viral inactivation was assessed against FMDV serotypes O, A, and Asia1 using standard virological methods, including plaque reduction assays and median tissue culture infectious dose ($TCID_{50}$) assays. Key parameters including disinfectant concentration, contact time, temperature, and the presence of organic matter were systematically investigated.

Results: Comparative analysis was conducted to determine the relative efficacy of each disinfectant against highly transmissible FMDV strains. The results provide quantitative evidence on the conditions required to achieve effective viral inactivation and identify disinfectant formulations exhibiting strong virucidal activity under specific conditions. Chlorine-based disinfectants demonstrated the highest virucidal efficacy, achieving a reduction of >4 log_{10} in viral titer within 5 minutes at a concentration of 0.5%. In contrast, QACs showed moderate efficacy, requiring higher concentrations (≥1.0%) and longer contact times (≥10 minutes) to achieve comparable viral reduction. The presence of organic matter significantly reduced the efficacy of QACs, whereas chlorine-based disinfectants retained relatively strong activity under the same conditions.

Conclusion: These findings contribute to the optimization of disinfection protocols and the strengthening of biosecurity measures for the effective prevention and control of FMD outbreaks.

Keywords: Foot-and-mouth disease, Efficacy, Disinfectants

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Hidden Zoonotic Risk in Cardiac Sarcocystosis of Cattle: Integrative Histopathological and Molecular Evidence from Upper Northern Thailand

Phawut Nueangphuet[1,*], Pornchanan Srikam[1], Natdhan Saenbuaphan[1], Natthawoot Inkamchuea[1]
Natthinee Kittiwan[1] and Wichittra Anukool[1]

Abstract

Background: *Sarcocystis* spp. are globally important protozoan parasites of livestock with recognized veterinary and public health significance, particularly due to the zoonotic potential of certain species such as *Sarcocystis hominis*. Infection in food animals is often subclinical but may have implications for meat safety and public health. However, information on their pathological characteristics, species distribution, and zoonotic implications in upper northern Thailand remains limited.

Objectives: This study aimed to investigate *Sarcocystis* spp. infection in cardiac tissues from livestock, with emphasis on histopathological characterization and molecular species identification.

Methods: A total of 298 fresh heart samples from cattle (227 samples) and buffalo (71 samples) collected from eight provinces in upper northern Thailand between October 2023 and March 2026, were examined using a parasitological stamp smear method. Histopathological analysis was performed to characterize cyst morphology. For molecular confirmation, 17 representative samples were analyzed by PCR targeting 18S rRNA and mitochondrial cox1 genes, followed by phylogenetic analysis.

Results: Sarcocysts were detected in 82/298 samples (27.5%), with occurrences 31.7% in cattle (72/227) and 14.1% in buffalo (10/71). Histopathological examination revealed numerous intramyocytic sarcocysts embedded within cardiomyocytes without associated inflammatory response. Two distinct morphological groups were identified from 17 randomly selected samples based on cyst wall measurements: thick-walled cysts (6.45 ± 1.11 μm; n = 16 cysts) and thin-walled cysts (1.99 ± 0.40 μm; n = 30 cysts). Wall thickness was significantly greater in the thick-wall group ($p < 0.001$). Within these selected samples, molecular analysis identified mixed infections consistent with *Sarcocystis hominis* and *Sarcocystis cruzi* in four cattle samples, which related exclusively with the presence of thick-walled cysts (*Sarcocystis hominis*) in histopathology; in contrast, the remaining samples were associated only with thin-walled cysts. Phylogenetic analysis based on both markers clustered predominantly with *S. cruzi*, with 18S sequences showing close relatedness to isolates from Thailand and neighboring countries. The consistent association between thick-walled cyst morphology and molecular detection of *S. hominis* suggests that zoonotic species may be present but underrepresented in phylogenetic analysis.

Conclusion: These findings highlight the presence of thick-walled sarcocysts suggestive of zoonotic *Sarcocystis hominis* in the study area. From a One Health perspective, this raises potential public health concerns associated with the consumption of raw or undercooked meat, emphasizing the need for increased awareness, surveillance, meat inspection, and appropriate food hygiene practices in endemic regions of northern Thailand.

Keywords: Cardiac sarcocystosis, Histopathology, Zoonotic risk, Molecular evidence, Upper northern Thailand
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ABSTRACTS OF POSTER PRESENTATION SESSION

Development and Validation of an Analytical Method for Tetracycline Antibiotic Residues in Honey Using OPT-SPE and LC-MS/MS Detection

Saranya Wutthiosot[1,*] and Jamras Lerdsri[1]

Abstract

Background: The use of tetracycline antibiotics to control bee diseases can lead to residues in honey, posing risks to consumer safety and creating trade barriers, particularly with the European Union market, which enforces a zero-tolerance policy for such residues. Therefore, it is necessary to develop and validate an analytical method for detecting residues of oxytetracycline, tetracycline, chlortetracycline, and doxycycline in honey using liquid chromatography–tandem mass spectrometry (LC–MS/MS), a technique known for its high sensitivity, specificity, and reliability.

Objectives: To develop and validate an analytical method for determination of tetracycline residues in honey for routine implementation at the Veterinary Research and Development Center, Upper Northern Region.

Methods: The sample preparation method was developed by comparing the extraction efficiency of three solid-phase extraction (SPE) techniques, namely C18, OPT, and C8/SCX cartridges, using sulfaphenazole as the internal standard. Method validation was conducted in accordance with EURACHEM guidelines, covering the following parameters: matrix effects, analytical range and linearity, accuracy, precision, measurement uncertainty, decision limit (CCα), specificity, and method robustness.

Results: The OPT cartridge exhibited the highest extraction efficiency with percentage recoveries ranging from 92.5 to 103.2%. The developed method showed no significant matrix effects and demonstrated a linear analytical range of 2.0–40.0 ng/g with a coefficient of determination (R^2) ≥ 0.9978. The method exhibited good accuracy, with recoveries between 94.00 and 102.95%, and satisfactory precision, with %CV values ranging from 1.42 to 6.50. The measurement uncertainty was estimated in the range of ±6.4% to ±19.3%, and the decision limits (CCα) were between 2.10 and 5.17 ng/g, which are below the minimum required performance limit (10 ng/g) established by the European Union. The analytical method complied with the criteria of European Commission Implementing Regulation (EU) 2021/808. Furthermore, the developed method demonstrated adequate specificity and robustness against variations in sample preparation conditions.

Conclusion: The developed LC-MS/MS method coupled with OPT-SPE is suitable, sensitive, accurate, and reliable for routine determination of tetracycline residues in honey.

Keywords: Method development, Honey, Antibiotic residue, LC-MS/MS, Tetracycline
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Determination of Selenium in Goat and Sheep Sera by ICP-OES with Continuous Flow Hydride Generation Using a Modified Pre-reduction and Digestion Method

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Abstract

Background: Selenium deficiency leads to muscle weakness and poor reproductive performance in goats and sheep. Determination of selenium (Se) by ICP-OES is challenging because this trace element is a weak emitter, which makes it difficult to measure low levels in serum samples. One technique to enhance the sensitivity of Se is gas-phase hydride conversion. In addition, the combination procedures of pre-reduction and digestion are used to improve the limit of detection. Our recent in-house Se analytical method is complicated, time-consuming, and suffers from excessive hazardous acids. Therefore, a simplified and effective modified method is necessary to improve diagnostic readiness and strengthen laboratory capacity.

Objectives: This study aimed to determine selenium concentrations in goat and sheep sera, assess method accuracy using percent recoveries of fortified method blanks (FMB), fortified analytical solutions (FAS), and fortified analytical portions (FAP), and evaluate method precision using relative percent difference (RPD) and relative standard deviation (RSD) of replicate samples.

Methods: Nineteen goat sera and nineteen sheep sera were collected from the same origin in Lopburi province in October, 2025. All samples, calibration standards, blanks, and fortification solutions were prepared in 1% nitric acid. In the pre-reduction step, samples were mixed with a small volume of concentrated hydrochloric acid and heated in a dry block heater at 100 °C for 20 minutes prior to digestion. Then, a micro-Kjeldahl digester was used for 30 minutes. After that, the samples were mixed in the chemifold assembly with the Sodium Borohydride/Sodium Hydroxide solution to form the hydrides. The hydride gases were separated from the liquid and continuously flowed into the ICP-OES plasma. All quantitative Se measurements were made against linear through-zero external calibration curves from 10, 25, 50, 75, 100 µg/L. The limit of detection (LOD), limit of quantitation (LOQ), recoveries, RPD, and RSD were calculated using Microsoft Excel®. Demonstrated Se levels in goat and sheep sera compared to the reference range values (goat 62-158 µg/L, sheep 55-170 µg/L). Likewise, the method performance parameters were evaluated by method criteria acceptance.

Results: LOD of this modified method was 2 µg/L, and LOQ was 9 µg/L. Linearity was confirmed across the concentration range of 10 to 100 µg/L with a correlation coefficient (r) of 0.9987. Percent recoveries of FMB, FAS, and FAP for mid-level fortified solutions in goat matrix serum were 96%, 91%, and 81%, respectively. Percent recoveries of FMB, FAS, and FAP for mid-level fortified solutions in sheep matrix serum were 96%, 92%, and 83%, respectively. The average RPDs of representative duplicate goat and sheep sera were 4.9% and 9.5%, respectively. The average RSDs of ICP-OES triplicate measurements of goat and sheep pooled sera were 3.5% and 2.8%, respectively. All accuracy and precision parameters met the predefined acceptance criteria. The method demonstrated consistent robustness across different animal matrices, remaining stable for both goat and sheep sera.

The concentration ranges of Se in goat and sheep sera were <9 – 67.73 µg/L and 26.64 – 58.16 µg/L, respectively. For goat sera, only 16% (3/19) of the samples were within the normal range, leaving 84% (16/19) interpreted as selenium deficiency. Furthermore, 95% (18/19) of sheep were determined to have selenium deficiency, while only 5% (1/19) reached a normal level. These findings revealed a significant health concern for this studied group. The practical application of this modified method could detect a high prevalence of selenium deficiency in a field sample of goats and sheep.

Conclusion: This study successfully established a modified pre-reduction and digestion procedure for Se determination by ICP-OES assembled with hydride generation chemifold. This method not only increases analytical sensitivity but also overcomes the limitations of the hazardous traditional method. The new modified method proved to be a reliable and efficient tool for Se diagnostic preparedness and was crucial for identifying livestock health status. As evidenced by this investigation in these small ruminants, there is an urgent need for nutritional intervention and improved selenium supplementation strategies.

Keywords: Hydride generation, ICP-OES, Selenium, Serum, Small ruminants

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PRRSV-2 Detection and Phylogenetic Classification from Swine in Northern Thailand Using ORF5 Gene

Nuttun Pengpetch[1] and Kultyarat Bhakha[1]

Abstract

Background: Porcine reproductive and respiratory syndrome virus (PRRSV) causes severe reproductive failures and respiratory illness in pigs worldwide. The type 2 genotype (PRRSV-2) is prevalent in North American and Asia, and ORF5 gene sequencing has been used to classify PRRSV-2 into multiple genetic lineages. In Thailand, PRRSV-2 has circulated for decades, and recent studies found Thai isolates belonging to at least five lineages (L1, L5, L8, L9, L10). Notably, lineage 10 appears to be Thai-specific, as global sequence data show L10 sequences exclusively from Thailand.

Objectives: This study aimed to detect PRRSV-2 in pig blood samples from northern Thailand (2025) and to determine the genetic lineage of any detected virus. Specifically, we sought to amplify and sequence the PRRSV ORF5 gene from positive samples and classify the viruses by phylogenetic analysis.

Methods: Two porcine blood samples were screened for PRRSV using a real-time reverse-transcription PCR assay. For each PRRSV-positive sample, the ORF5 gene was amplified by RT-PCR; PCR products were purified and subjected to Sanger sequencing. The resulting ORF5 sequences were aligned with global reference sequences and phylogenetic analysis was performed using the maximum-likelihood method in IQ-TREE with the GTR+G model. Branch support was assessed using ultrafast bootstrap and SH-aLRT with 1,000 replicates each.

Results: Both samples tested positive for PRRSV-2, and their ORF5 sequences clustered firmly within lineage 10. This Thai-specific lineage has low intra-lineage diversity. Each sequence showed >99% nucleotide identity to a reference lineage-10 strain (GenBank AF494042.1), indicating very high similarity. These findings are consistent with prior reports that circulating Thai PRRSV-2 strains group in lineage 10.

Conclusion: We report detection of PRRSV-2 lineage 10 in pigs from northern Thailand, highlighting the persistence of this Thai-specific lineage. ORF5-based phylogenetic analysis proved effective for classifying the virus. This integrated molecular approach improves understanding of PRRSV-2 diversity and underscores the importance of ongoing surveillance to guide control measures.

Keywords: PRRSV-2, ORF5 gene, Phylogenetic classification, Lineage 10, Thailand

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Evaluation of Recombinant Foot-and-Mouth Disease 2C3AB Protein for Use as an Antigen

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Abstract

Background: Foot-and-mouth disease (FMD) represents a major economic threat to global cattle production systems. While vaccination programs effectively control this highly contagious viral disease, endemic countries require robust surveillance systems to distinguish naturally infected animals from vaccinated populations. Nonstructural proteins (NSPs) are essential for viral replication within infected cells and serve as valuable diagnostic antigens since they are absent from inactivated vaccines but produced during natural infection.

Among these NSPs, the 3ABC polyprotein has emerged as serological diagnosis in vaccinated populations, offering high sensitivity and specificity for DIVA (Differentiating Infected from Vaccinated Animals) testing. However, recent evidence suggests that the 2C3AB polyprotein may provide comparable diagnostic performance while potentially offering additional advantages, such as avoiding the autocatalytic processing issues associated with 3C protease and incorporating broader epitope coverage through the inclusion of 2C domain sequences alongside 3AB components.

Objectives: Clone and express the 2C3AB gene from FMDV O/Mya-98 strain using the baculovirus expression system under the control of AcNPV promoters; develop and optimize an indirect ELISA protocol using the recombinant 2C3AB protein as coating antigen, and establish diagnostic performance parameters including optimal cutoff values, sensitivity, and specificity through ROC curve analysis for potential implementation in DIVA-based serological surveillance programs

Methods: A total of 96 bovine serum samples were utilized, comprising 64 positive and 32 negative samples as determined by commercial blocking ELISA. The gene encoding 2C3AB from FMDV serotype O/Mya-98 was amplified by RT-PCR and initially cloned into pGEM-T Easy vector. Subsequently, the 2C3AB gene was subcloned into the pFastBac1 donor plasmid and transformed into DH10Bac competent *E. coli* to generate recombinant bacmid through site-specific transposition. Following purification, the recombinant bacmid was transfected into Sf9 insect cells, and passage 3 (P3) recombinant baculovirus was harvested for protein expression in High Five cells. The recombinant 2C3AB protein, containing a C-terminal 6×His tag, was purified using Ni-NTA affinity chromatography, and protein concentration was quantified. SDS-PAGE and Western blot analyses were performed to confirm specific protein expression and molecular weight. ROC curve analysis was conducted using MedCalc software to determine optimal cutoff values, sensitivity, and specificity parameters.

Results: RT-PCR amplification successfully generated a 1.6 kb fragment corresponding to the 2C3AB gene. Positive clones were confirmed by double restriction enzyme digestion analysis. The recombinant r2C3AB protein was expressed with an apparent molecular weight of approximately 61 kDa without Histag, consistent with theoretical calculations. Western blot analysis using rabbit anti-6×His antibody demonstrated specific recognition of the recombinant protein, confirming successful expression and purification. Following optimization studies, the indirect ELISA protocol was established using 1 μg/mL of r2C3AB as coating antigen and bovine serum diluted at 1:20. ROC curve analysis revealed optimal diagnostic performance with a recommended cutoff value of OD $\geq$ 0.24. At this threshold, the assay demonstrated high sensitivity (96.87%) and specificity (93.73%) with 95% confidence intervals of 91.1% to 99.4%. The area under the curve (AUC) value of 0.969 indicates excellent diagnostic accuracy, confirming the potential of r2C3AB-based indirect ELISA for DIVA applications in FMD surveillance.

Conclusion: The 2C3AB gene from FMDV O/Mya-98 was successfully cloned and expressed using the baculovirus expression system, yielding a functionally active recombinant protein. The r2C3AB demonstrated strong potential as a diagnostic antigen, as evidenced by its specific recognition by sera from naturally FMDV-infected cattle. The developed indirect ELISA achieved excellent diagnostic performance (96.87% sensitivity, 93.73% specificity, AUC = 0.969), comparable to commercial blocking ELISA standards, confirming its capability for serological detection of FMDV infection in DIVA applications. These findings support the use of r2C3AB as an alternative diagnostic antigen that may offer advantages over conventional 3ABC-based assays, including avoidance of autocatalytic processing issues and broader epitope coverage. However, further validation studies using larger, more diverse sample populations across different geographical regions and serotypes are essential to establish the assay's reliability for routine diagnostic implementation in FMD surveillance programs.

Keywords: Foot-and-mouth disease, 2C3AB protein

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A Comparative Study on the Diagnostic Performance of a Locally Produced iELISA Kit Versus a Commercially Available Foreign Kit for Bovine Brucellosis

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Abstract

Background: Bovine brucellosis, caused by *Brucella abortus* (*B. abortus*), is a significant zoonotic disease that leads to substantial economic losses in the livestock industry. Although indirect ELISA (iELISA) is highly effective for diagnosis, current imported commercial kits are expensive, involve long delivery times, and lack of geographical specificity of *B. abortus* antigens.

Objectives: This study aimed to develop a highly efficient in-house iELISA for bovine brucellosis diagnosis using highly purified lipopolysaccharide (LPS) derived from local *B. abortus*, as the antigen, and to comparatively evaluate its diagnostic performance against commercially available ELISA kits in terms of sensitivity, specificity, accuracy, and agreement (Cohen's kappa) using field serum samples.

Methods: The LPS was extracted and purified using the cold butanol method, enzymatic treatments and size exclusion chromatographic (SEC). Then, it was characterized by monitoring of the obtained weight, the size, the morphological structure, the purity, the physiochemical properties, the specificity using of SDS-PAGE, Western blot, fluorescent-based Qubit test, the limulus amoebocyte lysate (LAL) including HPLC. To determine the optimal condition of in-house iELISA and a cut off value of the test, a checkerboard titration method with reference positive bovine serum and fetal bovine serum (FBS) were used to perform for obtaining the proper LPS, serum and conjugate concentration in the reaction. The efficiency of developed in-house iELISA such as sensitivity, specificity and accuracy, were evaluated using 215 positive and 94 negative bovine composite reference serum (CRS) samples obtained from commercial iELISA and Rose Bengal test (RBT). The agreement of the in-house and commercial iELISA was tested using randomized field bovine serum (n = 200) and reported in Cohen's kappa coefficient value.

Results: The highly, purified LPS of multiplex PCR-confirmed *B. abortus* was obtained from the 1st peak of SEC chromatogram contained 1.46% (w/w) of dried weight (DW) of *B. abortus*. Under the SDS-PAGE stained by silver nitrate, LPS showed the ladder pattern with the molecular weights ranged from >25-100 kDa similar to *E. coli* LPS. Low nucleic acid and protein contamination as 0.023% (w/w) and 1.04% (w/w) were observed. Western blot method illustrated the immunological specific reaction between monoclonal antibody (mAb) against *B. abortus* LPS and obtained purified LPS at a band position of 75-100 kDa. Retention time (Rt) of purified *B. abortus* LPS was 1.63 minutes closely to 1.59 minutes of purified *E. coli* LPS after examining by HPLC. LAL test displayed that the obtained LPS (1 mg/ml) had low endotoxin biological activity equal to 9.45 EU/ml. The checkerboard titration test indicated the optimal concentration of purified LPS, serum dilution and conjugate concentration used in in-house iELISA were 0.5 µg/100 µL, 1:80 and 1:8,000 with substrate incubation time as 1.20 minutes. The cut off value of the test was 0.191. The in-house iELISA had the sensitivity, specificity as 87% of 98.94%, respectively with an area under the curve (AUC) of 0.930. Compared with a commercial iELISA test, the in-house version demonstrated a strong agreement, with a Cohen's kappa coefficient value as 0.868.

Conclusion: The developed in-house iELISA for bovine brucellosis diagnosis test contributed the high diagnostic efficiency and strong agreement result to the commercial iELISA test that it has the promisingly potential for further development and use as a low-cost field-scale testing in the future.

Keywords: Bovine brucellosis, *Brucella abortus*, Indirect ELISA, Lipopolysaccharide antigen, Diagnostic performance

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Genomic Characterization of *Staphylococcus aureus* with Methicillin-resistant Phenotypes from Chicken and Pork in Upper Northern Thailand Using Long-Read Whole Genome Sequencing

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Abstract

Background: *Staphylococcus aureus* is a significant member of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*.), posing major concerns for public health and food safety. In particular, strains resistant to β-lactam antibiotics, such as methicillin, referred to as methicillin-resistant *Staphylococcus aureus* (MRSA), are of critical importance. Whole-genome analysis of MRSA provides in-depth insights into genetic diversity, transmission dynamics, and antimicrobial resistance with high accuracy.

Objectives: To comprehensively characterize the genomic features of *Staphylococcus aureus* isolates exhibiting methicillin-resistant phenotypes from chicken and pork in Upper Northern Thailand using long-read whole genome sequencing (WGS).

Methods: A total of 341 meat samples were tested for *S. aureus*. All detected *S. aureus* isolates were examined for phenotypic resistance to cefoxitin and oxacillin using the broth microdilution method for minimum inhibitory concentration (MIC) determination. Isolates exhibiting methicillin-resistant phenotypes were analyzed using long-read WGS on the Oxford Nanopore MinION Mk1C platform. The genomes were de novo assembled using Flye and subsequently annotated using Prokka.

Results: *S. aureus* was isolated from 9.7% (33/341) of meat samples, with a markedly higher prevalence in pork (11.4%; 32/280) compared to chicken (1.6%; 1/61). Antimicrobial susceptibility testing was performed on all 33 isolates, revealing that 6.1% (2/33) of the isolates exhibited methicillin-resistant phenotypes. One isolate showed high-level resistance to both cefoxitin and oxacillin (MIC >64 μg/mL), while the other was resistant to cefoxitin (32 μg/mL) but remained susceptible to oxacillin (<1 μg/mL). These phenotypically resistant isolates were subsequently selected for whole genome sequencing. Long-read WGS generated high-quality genome assemblies (~2.7–2.9 Mb) comprising two circular contigs. All isolates were confirmed as *S. aureus* based on average nucleotide identity (ANI) values (~0.996), with sequence types ST6 and ST672 identified by multilocus sequence typing (MLST). Genomic analysis detected the β-lactamase gene *blaZ* in all isolates and *tet*(K) in selected isolates. However, neither *mecA* nor *mecC* genes were identified. The observed discordance between phenotypic and genotypic resistance suggests the involvement of non-*mec*-mediated mechanisms

Conclusion: This study demonstrates the genetic diversity and antimicrobial resistance profiles of *S. aureus* isolated from chicken and pork in Northern Thailand. Although multiple resistance genes, particularly *blaZ* and *tet*(K), were identified, no classical methicillin resistance genes (*mecA* or *mecC*) were detected despite the presence of phenotypic resistance to cefoxitin and oxacillin in some isolates. This discrepancy between phenotypic and genotypic resistance suggests the involvement of non-*mec*-mediated mechanisms. The application of Long-read WGS provides enhanced resolution for genomic characterization and highlights its value in antimicrobial resistance surveillance within a One Health framework.

Keywords: *Staphylococcus aureus*, Methicillin-resistant phenotype, Long-read whole genome sequencing, Antimicrobial resistance

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Validation of a Real-time RT PCR (qRT-PCR) Assay for the Detection of Foot-and-Mouth Disease Virus in Thailand Using a Portable Real-time PCR Machine

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Abstract

Background: Foot and mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals. Molecular diagnostic techniques, especially qRT-PCR, show high sensitivity and specificity and save time compared to traditional diagnostic methods. However, in an FMD diagnostics, a qRT-PCR assay by the portable real-time PCR machine (Picogene®) needs to be validated and evaluated to ensure test performance.

Objectives: This study aims to validate a qRT-PCR assay by the portable real-time PCR machine (Picogene®) to ensure its accuracy under laboratory conditions.

Methods: FMDV serotype Asia 1 was used to evaluate the limit of detection (LOD) and PCR efficiency, while 10 infectious samples from cloven-hoof animals were used to evaluate analytical specificity (Asp). 45 Positive and 15 negative reference samples from viral fluids were used to determine the diagnostic sensitivity and specificity (DSe and DSp), and accuracy. All samples were extracted, and qRT-PCR was performed using primers and probes specific to the 3D region of FMD virus using a portable real-time PCR machine. Then, compared the DSe results with the qRT-PCR assay using a conventional real-time PCR machine.

Results: The qRT-PCR assay by the portable real-time PCR machine for FMD detection showed 100% of DSe, DSp, and accuracy. The LOD of the portable real-time PCR machine was 10^3 $TCID_{50}$/mL. For linearity results, the portable real-time PCR machine exhibited R^2 greater than 0.99 ($R^2 = 0.9947$), confirming that the test is consistent and reliable across different concentrations of FMD virus. Moreover, this experiment showed that PCR efficiency was 90%, confirming the suitability of the protocol, including primer/probe concentrations and PCR temperature. In part of Asp, the results presented 100% Asp, which showed no cross-reactivity to other viruses, including Bluetongue virus (BTV), Vesicular Stomatitis Virus (VSV), Bovine Viral Diarrhea Virus (BVDV), Bovine respiratory syncytial virus (BRSV), Infectious Bovine Rhinotracheitis/Bovine Herpesvirus (IBR/BHV), and Parapoxvirus. Lastly, the qRT-PCR assay by the portable PCR machine presented a Ct value (mean Ct = 24.28) greater than the conventional qRT-PCR machine (mean Ct = 20.83) ($p < 0.01$).

Conclusion: This study validated the qRT-PCR assay by the portable real-time PCR machine as an effective, reliable, and reproducible diagnostic tool for FMD virus detection, which is suitable for use in FMD virus diagnostics, contributing to improving FMD surveillance and control of FMD outbreaks in Thailand.

Keywords: Foot and mouth disease, qRT-PCR, Validation

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Genomic Analysis of *Salmonella* Isolates from Broilers and Swine in Upper Northern Thailand Using Long-Read Whole Genome Sequencing

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Abstract

Background: *Salmonella spp.* is an important foodborne pathogen affecting public health and livestock production. The increasing prevalence of antimicrobial resistance (AMR), particularly multidrug resistance (MDR), poses a critical challenge to effective treatment and control. Recent advances in whole genome sequencing (WGS), especially long-read technologies, enable high-resolution and comprehensive genome analysis, facilitating precise characterization of genetic diversity, transmission dynamics, and resistance profiles.

Objectives: To comprehensively characterize and analyze the genomic features, antimicrobial resistance profiles, and phylogenetic relationships of *Salmonella* isolates from broilers and swine in Upper Northern Thailand using long-read WGS, and to evaluate the concordance between phenotypic and genotypic antimicrobial resistance.

Methods: A total of 20 *Salmonella* isolates obtained from cecal and meat samples of broilers and swine collected from slaughterhouses and retail meat shops across six provinces in Upper Northern Thailand in 2023 were analyzed using long-read WGS. The assembled genomes were subjected to bioinformatic analyses for comprehensive genomic characterization, including serotype determination and sequence type (ST) assignment based on multilocus sequence typing (MLST). Pangenome and phylogenetic analyses were conducted to characterize gene diversity and assess genetic relationships among isolates. Antimicrobial resistance genes (ARGs) and plasmid profiles were identified to characterize resistance determinants. Phenotypic susceptibility to nine antimicrobial agents was determined by broth microdilution test, and concordance between phenotypic and genotypic resistance was assessed using Cohen's kappa.

Results: All 20 isolates were successfully sequenced and assembled into high-quality genomes. Nine serotypes and nine sequence types (STs) were identified, with ST29 (Stanley) and ST469 (Rissen) predominating. Several STs (ST29, ST462, ST469, and ST1543) were distributed across multiple provinces and hosts, suggesting potential cross-host transmission and regional dissemination. Pangenome analysis identified 8,956 genes, including 41.4% core genes, which exhibited phylogenomic clustering that strictly aligned with ST classifications. A total of 25 ARGs across 10 antimicrobial classes were detected, including clinically important *blaCTX-M-55, qnrS1* and *mcr-1.1*. MDR was observed in most STs (7/9 STs). Plasmids were detected in 60% of isolates, predominantly Col and Inc types. Phenotypic–genotypic AMR concordance ranged from moderate to substantial agreement ($\kappa = 0.53–0.70$).

Conclusion: *Salmonella* isolates from Upper Northern Thailand exhibited substantial genetic diversity, widespread MDR, and clinically important resistance determinants. These findings, enabled by long-read WGS, highlight the need for strengthened genomic surveillance in livestock production and the food chain to inform targeted control strategies and mitigate antimicrobial resistance risks within a One Health framework.

Keywords: *Salmonella*, Whole genome sequencing, Antimicrobial resistance, Livestock, Northern Thailand
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Application of Digital PCR for Evaluating the Performance of Nucleic Acid Extraction Kits in the Detection of Avian Paramyxovirus Serotype-1

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Abstract

Background: Newcastle Disease (ND), remains one of the most devastating infectious diseases affecting the global poultry industry. The disease is caused by the Newcastle Disease Virus, taxonomically as *Avian Paramyxovirus serotype-1* (APMV-1), a negative-sense, single-stranded RNA genome. Currently, real-time reverse transcription PCR (qRT-PCR) is the standard technique for routine diagnostics. Digital PCR (dPCR) represents a novel nucleic acid detection technology that enables direct absolute quantification of target molecules without the need for a standard curve. In this workflow, nucleic acid extraction is a critical step, as the quality and integrity of the extracted genetic material directly determine the efficiency and accuracy of molecular diagnostic outcomes.

Objectives: This study aimed to apply qRT-PCR in conjunction with dPCR to evaluate the efficiency of various commercial nucleic acid extraction kits for the detection of APMV-1.

Methods: Three commercial kits, two magnetic bead-based (A and B) and one spin column-based (C) were evaluated. The reference virus used in this study was APMV-1/Queensland-V4 (lentogenic strain). By utilizing primers and probes specific to the *Matrix (M)* gene. Cloacal swab samples from poultry, pre-confirmed as negative for ND via qRT-PCR, were spiked the reference virus at concentrations 1.325 to 53,000 copies/µL of sample. Following extraction with each respective kit, diagnostic performance was evaluated using both qRT-PCR and dPCR. The evaluation criteria included the detection range, limit of detection (LOD), linear regression analysis (R^2), repeatability and recovery rates.

Results: Using qRT-PCR, extraction kits A, B, and C demonstrated a detection range of 2.65–53,000 copies/µL, with 100% limits of detection of 2.65, 1.325, and 2.65 copies/µL, respectively. All kits showed excellent linearity, with $R^2 > 0.996$, and good repeatability, with overall coefficients of variation (%CV) between 1.6% and 7.9%. In parallel with qRT-PCR, all samples were also evaluated using dPCR. By dPCR, all kits exhibited a detection range of 5.3–53,000 copies/µL, with 100% LOD values of 5.3, 1.325, and 2.65 copies/µL for extraction kits A, B, and C, respectively, and All kits showed excellent linearity, with $R^2 > 0.996$. In recovery testing, extraction kit B demonstrated significantly higher recovery rates than extraction kits A and C ($p < 0.05$).

Conclusion: The magnetic bead-based extraction kit B demonstrated outstanding performance for APMV-1 nucleic acid extraction, particularly when combined with dPCR, providing the highest sensitivity and recovery rates. These results support its use for developing highly accurate and reliable molecular diagnostic standards for Newcastle disease.

Keywords: Newcastle disease virus, Nucleic acid extraction kits, Digital PCR, Real-time RT-PCR

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Evaluation of Immune Responses in Beef Cattle Following Vaccination with Live Attenuated and Inactivated Lumpy Skin Disease Vaccines

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Roipim Mapongpeng[2] and Supunsa Mahongchai[3]

Abstract

Background: Lumpy Skin Disease (LSD) is a transboundary viral disease causing substantial economic losses in beef cattle production. Vaccination is the primary control measure; however, comparative field evidence on the immunogenicity and durability of live attenuated vaccines (LAV) versus inactivated vaccines (IV) remains limited.

Objectives: To compare humoral immune responses and antibody persistence following LAV and IV vaccination against LSD in beef cattle under field conditions.

Methods: A longitudinal field study was conducted on 177 beef cattle were vaccinated with either LAV (n = 93) or IV (n = 84). Serum samples were collected on days 0, 30, 60, 90, and 120 post-vaccination. LSD virus–specific antibody levels and seropositivity rates were compared between vaccine groups over time. In addition, the association between age and antibody persistence was evaluated. Statistical significance was set at $p < 0.05$.

Results: Seropositivity rates did not differ significantly between the groups at days 30 and 60 ($p > 0.05$). However, from day 90 onward, the IV group showed significantly higher seropositivity. By day 120, the seropositivity rate in the IV group was 82.1%, compared to 60.2% in the LAV group ($p < 0.05$). Antibody responses induced by IV were more stable over time, particularly in cattle aged 6–9 years.

Conclusion: Inactivated LSD vaccines induce more durable humoral immunity than live attenuated vaccines under field conditions, supporting their use in endemic or high-risk areas. This information is essential for effective disease control and vaccination optimization at the farm level in beef cattle operations. It is particularly important in areas of Thailand with recurrent outbreaks that require sustained herd immunity.

Keywords: Lumpy skin disease, Beef cattle, Inactivated vaccine, Live attenuated vaccine, Antibody persistence
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Effect of Storage Conditions on Salbutamol Stability in Spiked Swine Urine Samples: ELISA Analysis with Linear Mixed Models

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Abstract

Background: Beta-agonists, specifically salbutamol, are pharmacologically essential for treating respiratory disorders in humans. However, their illicit use in the swine industry as repartitioning agents to enhance leanness and reduce fat poses significant risks to food safety and public health. The Department of Livestock Development utilizes LC-MS/MS as the gold standard for confirmatory residue testing in urine and animal products. While, field test and regional laboratory centers use ELISA as it remains a critical high-throughput screening tool due to its cost-effectiveness and speed. Positive samples from screening test are stored and then sent for confirmatory testing. Whether these conditions affect sample stability have not been clarified. Therefore, this study focuses on the effect of storage conditions on sample stability, a critical factor for the accuracy of analytical results prior to confirmatory testing.

Objectives: To evaluate the effect of storage temperature, duration, and initial concentration on the stability of salbutamol in swine urine.

Methods: Urine samples from swine, previously confirmed to be free of salbutamol, were spiked with salbutamol at three concentrations (2.5, 5, and 10 ng/mL; n=3 per level) and stored under three conditions: room temperature (RT, 25–30 °C), refrigeration (RE, 2–8 °C), and freezing (FR, below −20 °C). Salbutamol concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) with a commercial test kit (Kwinbon Biotech, China) at 0, 7, 14, 21, and 28 days. Data were analyzed using a Linear Mixed Model (LMM) to evaluate main effects (temperature, time and initial concentrations) and their interactions on the stability and concentration of salbutamol. Sample ID was specified as a random effect to account for the correlation within repeated measurements (within-subject variance), with statistical significance set at $p \leq 0.05$.

Results: Salbutamol concentrations decreased significantly with increasing storage duration compared to baseline (day 0) under all temperature conditions ($p < 0.001$). The most degradation occurred at RT, where concentrations fell to 89.70% by day 28—significantly lower than the 93.98% retention observed under FR conditions ($p = 0.02$, 95% CI = -7.89, -0.66). Samples stored under RE conditions retained 91.49% of the initial concentration. These findings indicate that salbutamol exhibited the highest stability under FR conditions. Notably, the initial spike concentration did not significantly influence the degradation rate ($p > 0.05$), indicating a consistent percentage-based decline.

Conclusion: Storage temperature is a critical determinant of long-term salbutamol stability in swine urine. For samples requiring storage exceeding three weeks, freezing is recommended to ensure analytical accuracy. Prolonged exposure to room temperature must be avoided to prevent erroneous results that could compromise the efficacy of food safety surveillance and subsequent confirmatory processes. These findings support the development of standardized protocols for sample handling prior to confirmatory analysis.

Keywords: Salbutamol stability, Swine urine, Sample storage conditions, ELISA, Linear mixed models
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A SISPA-Nanopore Sequencing Approach for Genomic Characterization of Viruses from Clinical Samples

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Abstract

Background: Genomic characterization of viruses directly from clinical samples remains technically challenging because of low viral load, high host nucleic acid background, and incomplete recovery of viral genetic material. These limitations can affect nucleic acid quality and viral nucleic acid recovery, thereby reducing library quality and sequencing performance. Although Oxford Nanopore sequencing provides rapid and flexible sequencing capability, successful characterization from clinical samples still depends on sufficient recovery of viral nucleic acids. While direct RNA sequencing is now available, its routine application in diagnostic and surveillance settings may still be limited by relatively high cost and practical workflow considerations. Sequence-Independent Single-Primer Amplification (SISPA) is therefore a useful amplification-based approach for enhancing viral sequence recovery prior to sequencing and supporting direct genome-based characterization of dsRNA viruses from clinical samples.

Objectives: This study aimed to evaluate a SISPA-based Nanopore sequencing workflow for genomic characterization of dsRNA viruses directly from clinical samples and compare sequencing outcomes generated from three sample preparation workflows—cDNA synthesis, double-stranded cDNA synthesis, and SISPA—Representative dsRNA viruses, including Bluetongue virus (BTV) and African horse sickness virus (AHSV), were used as target viruses in this study.

Methods: Viral RNA was extracted from clinical samples using the QIAamp Viral RNA Mini Kit. The extracted RNA was processed using three separate preparation workflows for comparative analysis: cDNA synthesis using random hexamers, double-stranded cDNA synthesis, and sequence-independent single-primer amplification (SISPA). Libraries generated from each workflow were prepared using a Native Barcoding Kit and sequenced on an Oxford Nanopore MinION Mk1C platform with an R10 flow cell. Basecalling was performed using Dorado v7.6.7 integrated in MinKNOW v24.11.8. Reads were adapter-trimmed using Porechop v0.2.4, quality-assessed using NanoPlot v1.43.0, and length-filtered using Chopper v0.8.0. Reference-based analysis was conducted using minimap2 v2.28, SAMtools and BCFtools v1.21 for read mapping, sorted alignments and consensus sequences. Viral detection was defined as the presence of mapped viral reads against the reference genome, while successful sequence recovery was determined based on ≥50% genome coverage with a mean sequencing depth of ≥30× in reference-based analysis.

Results: Comparative analysis showed that the SISPA workflow yielded the most informative sequencing output among the three preparation methods. Relative to cDNA and double-stranded cDNA, the SISPA workflow markedly improved viral read recovery, with approximately 518.5-fold (1,037 vs 2 reads) and 148.1-fold (1,037 vs 7 reads) higher viral read counts, respectively. Although limited numbers of mapped viral reads were identified in the cDNA and double-stranded cDNA datasets, these workflows did not meet the predefined criteria for successful sequence recovery. In contrast, the SISPA-derived dataset successfully recovered the target viral sequence, achieving the defined genome coverage and sequencing depth thresholds in reference-based analysis.

Conclusion: These findings indicate that SISPA can enhance Nanopore-based recovery of dsRNA viral sequence data from a clinical sample and enable downstream reference-based analysis. This workflow provides a useful approach for the genomic characterization of dsRNA viruses directly from clinical samples.

Keywords: Sequence-Independent Single-Primer Amplification (SISPA), Oxford nanopore sequencing, Clinical samples, RNA viruses, Viral sequencing

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Association Rule Mining of Bacterial Species and Antimicrobial Susceptibility in Milk from Cows with Mastitis: A Case Study of Livestock Region 7, Thailand, 2023–2025

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Abstract

Background: Bovine mastitis is a major problem in the dairy industry and is one of the primary causes of antimicrobial use in dairy production systems, which may contribute to the development of antimicrobial resistance. However, conventional data analysis methods have limitations in detecting complex relationships, particularly co-occurring resistance patterns among multiple antimicrobial agents in bacterial isolates.

Objectives: This study aimed to apply association rule mining techniques to analyze the relationships between bacterial species and antimicrobial susceptibility, as well as to identify co-resistance patterns among antimicrobial agents from raw milk samples of mastitic dairy cows within the jurisdiction of Regional Livestock Office 7, Department of Livestock Development, Thailand.

Methods: A retrospective analytical study was conducted using data on bacterial isolation and antimicrobial susceptibility testing from raw milk samples of mastitic cows submitted to the Veterinary Research and Development Center (Western Region), Ratchaburi Province, Thailand, between 2023 and 2025. A total of 945 milk samples were analyzed, yielding 26 bacterial species and 22 antimicrobial agents for testing. Antimicrobial susceptibility testing was performed using the disk diffusion method and categorized as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The dataset was structured into a long format for analyzing the relationships between bacterial species and antimicrobial responses (16,330 records) and into a wide format for identifying co-resistance patterns (1,246 records). Association rule mining was performed using the Apriori algorithm in WEKA version 3.8.6, and the generated rules were selected based on predefined minimum thresholds of support, confidence, and lift.

Results: A total of 9 strong association rules were identified between bacterial species and antimicrobial susceptibility test results (support 0.01–0.02, confidence 0.86–1.00, lift 1.53–2.90). *Escherichia coli* showed a strong association with resistance to penicillin G, whereas *Streptococcus uberis* was associated with susceptibility to β-lactam antibiotics. Resistance to sulfamethoxazole/ trimethoprim was also observed in some rules. In addition, ten co-resistance patterns were identified (support 0.11–0.32, confidence 0.90–1.00, lift 1.72–3.08), indicating frequent co-occurrence of resistance to multiple antimicrobial agents, particularly within the β-lactam group. Notably, clear co-resistance patterns were observed within the tetracycline group.

Conclusion: Association rule mining enabled the identification of complex, multi-dimensional relationships among bacterial species, antimicrobial susceptibility, and co-resistance patterns that are difficult to capture using conventional analytical methods. This approach provides added value for antimicrobial resistance surveillance and offers a promising tool for supporting evidence-based and rational antimicrobial use in dairy production systems.

Keywords: Association rule mining, Antimicrobial resistance, Bovine mastitis, Raw milk, Apriori algorithm
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Forensic Characterization of Electrical Contact Lesions in Asian Elephants Using Histopathology and Histochemistry

Phurida Sripipattanakul[1,*], Wachareeporn Starrat[1], Kirana Noradechanon[2], Chananya Kanchanasaka[2] and Dusita Chincharoendee[2]

Abstract

Background: Suspected electrocution is a recurring cause of mortality in wild Asian elephants (*Elephas maximus*) inhabiting agricultural areas adjacent to forest habitats in Thailand, particularly in regions affected by ongoing human–elephant conflict. Confirmation of electrocution can be challenging, as gross lesions may resemble other types of cutaneous injury. Electrical contact lesions may exhibit characteristic histopathological features, and metal transfer from electrical conductors to the skin may occur during contact. Histochemical techniques targeting collagen alteration and iron deposition may provide additional diagnostic evidence in suspected electrical contact lesions.

Objectives: To characterize the histopathological features of suspected electrical contact lesions in elephants and to assess collagen alterations and iron deposition as supportive forensic evidence of electrocution.

Methods: During 2020–2025, skin samples from burn-like lesions in Asian elephants suspected of electrocution were submitted by field officers from the Department of National Parks, Wildlife and Plant Conservation to the National Institute of Animal Health for histopathological examination to confirm electrical contact injury. Tissue samples from suspected electrical contact sites were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histopathological examination. Masson's trichrome and Prussian blue histochemical stains were performed to assess collagen alterations and detect ferric iron (Fe^{3+}) deposits, respectively.

Results: Gross examination revealed focal burn-like skin lesions at suspected electrical contact sites in all cases (n = 4). Histopathological examination revealed consistent epidermal and dermal alterations, including coagulative necrosis of the epidermis, intraepidermal separation, and nuclear streaming. Dermal changes included collagen homogenization and hemorrhage. Histochemical staining with Masson's trichrome staining highlighted collagen alterations characterized by homogenization and loss of normal collagen architecture in the dermis. Prussian blue staining demonstrated ferric iron-positive particulate material within the stratum corneum in all cases.

Conclusion: The combined histopathological and histochemical findings support the diagnosis of electrical contact injury in elephants. The persistence of ferric iron-positive particulate material within the stratum corneum following routine histologic processing is suggestive of metallization associated with electrical contact, rather than superficial contamination. These findings highlight the potential value of histopathology and histochemistry as complementary diagnostic tools in the forensic investigation of suspected elephant electrocution.

Keywords: Asian elephant, Electrocution, Forensic pathology, Electrical contact lesion, Histochemistry, Metallization

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Verification of Commercial AGID Kits for Equine Infectious Anemia Antibody Detection

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Abstract

Background: The Agar gel immunodiffusion (AGID) test is the gold standard for the serological detection of Equine Infectious Anemia (EIA). However, the discontinuation of the previous kit requires a switch to new commercial alternatives. These alternatives must be verified to ensure that diagnostic standards are maintained for consistent and accurate disease surveillance.

Objectives: This study aimed to verify and compare the diagnostic performance of two commercial AGID test kits to determine their suitability for routine laboratory diagnosis.

Methods: The verification process evaluated the diagnostic sensitivity (DSe), diagnostic specificity (DSp), analytical specificity (ASp), and repeatability of the two kits. A total of 118 serum samples were used, consisting of 81 known positive samples (including reference materials from the National Veterinary Services Laboratories, USA) and 37 known negative samples. Analytical specificity was assessed by testing for cross-reactivity with antibodies against other pathogens, such as African Horse Sickness (AHS), Equine Influenza (EI), and Foot-and-Mouth Disease (FMD). Repeatability was determined through 20 testing replicates of samples.

Results: The results indicated that kit A achieved a DSe of 92.59%, while kit B demonstrated a higher DSe of 96.30%. Both kits exhibited a DSp of 100% and an ASp of 100%, showing no cross-reactivity with the other tested pathogens. Additionally, repeatability testing for both commercial kits showed 100% agreement across all replicates and sample concentrations.

Conclusion: The verification study confirmed that both commercial AGID test kits exceed the established performance criteria (DSe $\geq$ 90% and DSp = 100%). These findings support the implementation of these kits as reliable replacements for discontinued diagnostic tools, ensuring the continued accuracy and continuity of EIA surveillance programs.

Keywords: Equine infectious anemia (EIA), Agar gel immunodiffusion (AGID), Verification, Diagnostic sensitivity, Diagnostic specificity

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Development, Optimization, and Validation of a Multiplex Real-time RT-PCR Assay for the Simultaneous Detection of BVDV, BTV, and BLV in Cattle

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Abstract

Background: The National Institute of Animal Health (NIAH) is responsible for the disease diagnostic screening of imported live cattle to prevent transboundary and emerging diseases into Thailand. Bovine Viral Diarrhea Virus (BVDV), Bluetongue Virus (BTV), and Bovine Leukemia Virus (BLV) are classified under the Animal Epidemics Act B.E. 2558 (2015). They are considered high-risk diseases that require mandatory health screening and certification prior to international movement into the country. Currently, the NIAH utilizes singleplex real-time PCR, which requires separate testing for each pathogen. This process is resource-intensive, extends operational timelines and increases costs which increases the turnaround time necessary for rapid biosecurity screening.

Objectives: This study aimed to develop, optimization and validation a multiplex Real-time RT-PCR assay for the simultaneous detection of BVDV, BTV, and BLV in a single reaction to enhance the efficiency and speed of diagnostic services for imported cattle.

Methods: Specific primers and probes were designed targeting the 5'-UTR (BVDV), NS3 (BTV), and env (BLV) regions in accordance with WOAH Terrestrial Manual guidelines. Probes were labeled with reporter dye (FAM, VIC, and Cy5) to ensure non-overlapping signals. Synthetic plasmids containing these target sequences were used as positive controls. The one-step RT-qPCR master mix was used for the simultaneous detection of RNA and DNA targets. This assay optimization focused on primer/probe concentrations and thermal cycling conditions to maximize fluorescence signals. The Limit of Detection (LOD) was determined using serial dilutions of synthetic plasmids. Performance was evaluated using 60 reference samples (20 positive samples for each virus). Diagnostic sensitivity (D-Sn), diagnostic specificity (D-Sp), and Cohen's kappa statistics were calculated following WOAH validation standards.

Results: The optimized multiplex real-time RT-PCR demonstrated high performance in the simultaneous detection of BVDV, BLV, and BTV with the ability to distinctly identify each target through unique fluorescence signals and non-overlapping amplification plots. Assay optimization revealed that a primer and probe concentration of 400 nM provided the most optimal performance. The thermal cycling conditions were established with a reverse transcription step at 50°C for 10 minutes, followed by pre-amplification at 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds and 60°C for 45 seconds. The LOD was 3.21 x 10^5 copies/reaction for BVDV, BTV, and BLV. Consequently, the analytical sensitivity was consistently high, and both D-Sn and D-Sp reached 100% when validated against 60 reference clinical samples. Statistical analysis yielded a Cohen's kappa coefficient of 1.0, confirming perfect agreement with reference singleplex assay.

Conclusion: The preliminarily validated Multiplex Real-time RT-PCR provides a rapid, cost-effective, and highly reliable diagnostic tool. It significantly strengthens the biosecurity measures of NIAH by accelerating the health certification process for imported live cattle and ensuring the effective prevention of transboundary disease outbreaks into Thailand.

Keywords: Multiplex real-time RT-PCR, Optimization, Imported cattle, BVDV, BTV, BLV

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A Color Absorption Strategy for Chromatic Analysis by Using a Portable 3D-printed Tube Holder Coupled with a Notebook Screen and a Smartphone

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Abstract

Background: A colorimetric assay is the simplest detection method to interpret results through discoloration in a solution that is visible to the naked eye. However, visual interpretation has some limitations regarding the variability in individual color perception caused by several factors such as color blindness and experience, which needs trained and experienced personnel to determine between positive and negative results.

Objectives: This study aimed to develop a color readout method for differentiating between positive and negative colors based on color absorption strategy by using a portable 3D-printed tube holder device, a notebook screen, and then readout by a smartphone. After that, our strategy was used to analyze the colorimetric loop-mediated isothermal amplification coupled with malachite green dye (cLAMP-MG) assay for detecting cestodes belonging to the genus *Raillietina* as a colorimetric assay model.

Methods: A portable 3D-printed tube holder was designed using 123D design software and printed by using a 3D printer with a black polylactic acid filament. Color absorption can be easily analyzed using a notebook screen as a chromatic light source and a smartphone camera coupled with the ColorAssist application as a color detector. Moreover, a convex lens (15× magnification) was placed in front of the back camera's own lens of iPhone 13 (f = 1.6 mm) in order to increase the image magnification and focus on the reaction tube in close range. To conduct the experiment, the cLAMP-MG solution in a 0.2 mL tube was inserted into the tube holder hanging on a red notebook screen. The red light from the notebook screen passed through the solution in the tube, and the transmitted light was measured by a ColorAssist on smartphone and were calculated according to the following formula:

$$\text{Absorption}_{\text{sample}-\text{negative control}} = -\log\frac{R_{\text{p}}}{R_{\text{p0}}} + \log\frac{R_{\text{n}}}{R_{\text{n0}}}$$

where R_{p} and R_{n} are the color values of sample and negative control, respectively. R_{p0} and R_{n0} are the red color values of the background (light source) before measuring the sample and negative control, respectively. For a cut-off value setup, the empirical rule was applied to compute a cut-off value from negative controls according to the following: the three-fold standard deviation of the negative controls (3SD) were calculated to confirm with 99.7% confidence that a reaction higher than the cut-off is considered a positive result. This method was used to analyze the results of cLAMP-MG validation, including analytical and clinical evaluations, using a total of 180 reaction tubes for testing.

Results: Negative controls were calculated to define the cut-off value in color absorption difference between positive and negative results, which was found to be 0.0286. All positive results showed absorption values higher than the cut-off with an initial 0.0341 up to 0.2001, which is consistent with the results that were analyzed by gel electrophoresis. The results of colorimetric analysis based on the color absorption did not show false positive or negative results in analytical sensitivity, analytical specificity, and clinical testing. Meaning that our method could assist in differentiating between positive and negative colors of the cLAMP-MG assay

Conclusion: Our color readout method is rapid, simple, and easy to interpret after the ending of the reaction, indicating the possibility to be applied in other colorimetric assays of a wide range of pathogens for supporting farm management and solve animal health problems.

Keywords: Colorimetric assay**,** Chromatic absorption, Colorimetric LAMP, *Raillietina* spp., Malachite green

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Method Validation for Moisture Content in Combined Live Newcastle Disease and Infectious Bronchitis Vaccines by Vacuum Oven Method

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Abstract

Background: The appropriate moisture content is critical for the viability of the vaccine, which affects to vaccine efficacy. The ASEAN standard for animal vaccines, the moisture content in freeze dried vaccines must not exceed 4%. Moisture content in freeze-dried vaccines is commonly determined using Karl Fischer titration and vacuum drying. Currently, the Veterinary Biologics Assay and research Center uses a vacuum oven method to determine moisture content, under conditions of 60±3°C, vacuum < 25 millibar, at least 3 hours drying time and room relative humidity (RH) < 45%. Therefore, this study conducted a validation of the vacuum oven method for moisture content by reducing the drying time from 3 to 2 hours to time-saving and cost-effective method for determining the moisture content in freeze dried live vaccines.

Objectives: To validate the vacuum oven method for moisture content determination in freeze dried live vaccines at Veterinary Biologics Assay and research Center.

Methods: Ten batches of combined live Newcastle disease and Infectious Bronchitis vaccine were tested using vacuum oven at 60°C, 24 millibar, RH < 45% for duration time 2 and 3 hours. For precision, 100 µL of DW was dispensed in to weighed container and weighed. Samples were dried in vacuum oven at 60°C, 24 millibar, RH < 45% and reweighed. Weight difference before and after drying was used to calculate % moisture and the acceptance was determined using the F-test. Accuracy was tested by adding 100 µL of DW to moisture-free combined live Newcastle disease and infectious bronchitis vaccine and determine the acceptance of the method using %Recovery. Method validation was performed using ten batches of combined live Newcastle disease and Infectious Bronchitis vaccine, acceptance of the method variability was assessed using the F-test and t-test.

Results: At 2 and 3 hours, the precision average moisture content was showed 101.23% and 101.39% with Fcal of 0.79. Accuracy average moisture content was 100.04±1.05% and 100.05±1.34% and %R were 101.23 and 101.39. The method validation average moisture content was 3.09±0.68% and 3.00±0.44% with Fcal=1.54 and tcal=0.26 (n=10, Fcrit=3.18, tcrit=2.10, p=0.05). This results within the acceptable range according to AOAC (1993) and method met the validation of Fcal<Fcrit and tcal<tcrit at 95% confidence level.

Conclusion: Validation results of the moisture content in combined live Newcastle disease and Infectious Bronchitis vaccines by vacuum oven method at temperature of 60°C, vacuum 24 millibar and drying time of 2 hours can be used as a reliable method for determining moisture content in combined live Newcastle disease and infectious bronchitis vaccines.

Keywords: Method validation, Moisture content determination, Combined live Newcastle disease and infectious bronchitis vaccines, Vacuum oven method

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Development and Validation a Method for Determining the Total Bacterial Count in Raw Cow Milk by an Automate Instrument Using Flow Cytometry Technique

Sunantha Rungmee[1,*], Chonlada Thongdee[1] and Siriprapha KhamKhote[1]

Abstract

Background: Total bacterial count contamination is recognized as an indicator of raw milk quality, hygienic conditions during production, packaging and storage. According to Thai agricultural standard TAS 6003-2010, the regulation required for microbiological criteria of standard plate count in raw cow milk shall not exceed 5×10^5 cfu/mL. Currently, Upper Northeastern Veterinary Research and Development Center (VRDNE) laboratory analyze the total bacterial count in raw cow milk using the standard AOAC 986.33 method with Petrifilm™, which requires an incubation period of 48±3 hours. In contrast, automated instruments for testing total bacterial count by the flow cytometry technique can rapidly analyze at least fifty samples per hour. Therefore, an automated raw milk bacterial count analyzer utilizing flow cytometry has been developed to optimize both the accuracy and efficiency of microbial analysis, particularly in high-volume testing operations, where rapid results are required.

Objectives: The study aimed to develop and validate a method using an automated instrument using flow cytometry technique to determine the total bacterial count in raw cow milk.

Methods: A total of 237 raw cow milk samples with natural microbial contamination were collected from twenty-five milk collection centers and submitted to the VRDNE laboratory for analysis of milk quality. All samples are subject to testing for bacterial enumeration by using an automated total bacterial count instrument (BactoCount IBCm3.0) compared with the Petrifilm™ method. After that, all the obtained datasets were then used to establish a scatter plot graph and an equation relationship between the two methods. Subsequently, An adjusted equation was used to replace the original equation in the BactoCount machine to convert the test results from IBC/mL to cfu/mL in accordance with ISO 21187/IDF 196:2021. Then, the validity of BactoCount instrument method is verified according to ISO 16140-2:2016/Amd 1:2024.

Results: The analysis of 237 raw cow's milk samples to determine the correlation between the BactoCount IBCm3.0 method and the AOAC 986.33 standard method revealed a standard error (Sy,x) of 0.2245, which complies with the requirement of ISO 16297/IDF 161:2020, which specifies a maximum error of 0.4. When the relationship was analyzed using a linear regression test, the equation correlation obtained from the BactoCount instrument and the standard AOAC 986.33 Petrifilm™ method was Log_{10} cfu/mL = 1.1343 x Log_{10}IBC/mL - 1.2862, with an R^2 value of 0.9887, which showed no statistically significant difference between the two analytical methods ($p \geq 0.05$). For the relative trueness test according to the ISO 16140-2:2016/Amd 1:2024, the results of the BactoCount instrument compared to the Petrifilm™ method showed a consistency of 95% confidence. This demonstrates that both methods are consistent. In addition, it was found that the accuracy profile of the total bacterial count from all samples ranged from 3.86 Log_{10} cfu/mL to 7.2 Log_{10} cfu/mL which are smaller than the acceptability limits level. Therefore, the accuracy profile of this method is acceptable.

Conclusion: The BactoCount instrument, using the flow cytometry technique, was developed and validated to determine the total bacterial count in raw cow milk. It indicated reliability and equivalence to the Petrifilm™ method.

Keywords: Raw cow milk, Total bacterial count, Validation method, Flow cytometry technique

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Molecular Identification of Microfilariae in Native Cattle in Thepha District, Songkhla Province

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Abstract

Background: Microfilariae are blood-borne nematode larvae transmitted by mosquito vectors and are widely distributed in tropical and subtropical regions. In cattle, adult worms typically reside in the peritoneal cavity. Infections are generally asymptomatic; however, aberrant larval migration to the central nervous system can result in paralysis. Morphological diagnosis has limited capacity to differentiate species, posing a challenge for identifying the specific species circulating within a region. Therefore, molecular techniques employing highly specific primers are required for accurate species identification, which is essential for the development of effective prevention and control strategies.

Objectives: To identify microfilarial species in native cattle in Thepha District, Songkhla Province, based on the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene.

Methods: Blood samples were collected from three asymptomatic native cattle at a farm in Thepha District, Songkhla Province. All cattle had previously tested positive for microfilariae using Woo's method and Giemsa-stained blood smear examination. Genomic DNA was extracted from blood samples, and polymerase chain reaction (PCR) amplification targeting the *cox1* gene was performed, followed by DNA sequencing. The obtained sequences were analyzed using BLASTn and compared with reference sequences in the NCBI GenBank database to confirm species identity.

Results: An approximately 682 bp amplicon of the *cox1* gene was successfully obtained from all three microfilaria-positive samples. All sequences showed high similarity to GenBank entries assigned to *Setaria* spp. Two sequences exhibited 100% identity with *Setaria digitata* (GenBank accession no. KY284626). The third sequence showed high similarity to both *Setaria marshalli* (LC719467) and *Setaria labiatopapillosa* (NC044071), with sequence identities of 99.66% and 99.38%, respectively.

Conclusion: Molecular techniques based on the *cox1* gene effectively identified microfilarial species in asymptomatic cattle; however, closely related species, *Setaria marshalli* and *Setaria labiatopapillosa*, remain difficult to distinguish. Integrating morphological analysis of adult worms would improve diagnostic accuracy. Despite the limited sample size, this study provides preliminary molecular evidence of *Setaria* diversity in Thepha District, Songkhla Province, and provides baseline data for future studies and surveillance.

Keywords: Molecular identification, Microfilariae, Native cattle, Thepha District

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Performance Evaluation of DNA Amplification Reagent Kits for the Molecular Detection of Lumpy Skin Disease Virus Using Real-time PCR

Krongkan Srikhawkhum[1,*] and Aroonpan Prerdprao[2]

Abstract

Background: Lumpy skin disease (LSD) is an economically important viral disease affecting cattle and buffaloes and is caused by the Lumpy skin disease virus (LSDV), a member of the genus *Capripoxvirus* within the family *Poxviridae*. The disease has a substantial impact on animal health and livestock production. Laboratory diagnosis using real-time polymerase chain reaction (real-time PCR) is widely accepted as a standard diagnostic method because of its high sensitivity, specificity, and ability to provide rapid and reliable results. In addition, this technique plays an important role in disease surveillance, animal movement control, and the certification of disease-free farms. However, the accuracy and reliability of molecular detection may be affected by several factors, particularly the selection of an appropriate and high-quality DNA amplification reagent kit (PCR Master Mix) for routine laboratory use.

Objectives: This study aimed to evaluate and compare the analytical performance of three commercial DNA amplification reagent kits (Kit F, Kit L, and Kit Q), currently used in the virology laboratory of the Western Veterinary Research and Development Center for the molecular detection of LSDV by real-time PCR.

Methods: The analytical performance of the three reagent kits was assessed using plasmid samples serially diluted to concentrations ranging from 10^6 to 10 copies/5 µL. Each dilution level was tested in triplicate. The reagent kits were evaluated based on their ability to detect LSDV genetic material across the dilution series. Cycle threshold (Ct) values obtained from each kit were statistically compared using analysis of variance (ANOVA).

Results: All three reagent kits were able to reliably detect LSDV genetic material at concentrations as low as 10^2 copies/5 µL. At the lower concentration of 10 copies/5 µL, Kit Q successfully detected all replicates (3/3), whereas Kit F detected only one (1/3), and Kit L failed to detect any replicates (0/3). Statistical analysis using ANOVA revealed no statistically significant differences in Ct values among the three reagent kits across all detectable dilution levels ($p > 0.05$).

Conclusion: The findings of this study indicate that all three reagent kits are suitable for the molecular detection of LSDV by real-time PCR. However, Kit Q demonstrated superior analytical sensitivity for the detection of low-copy-number samples, while Kit F and Kit L offered practical advantages in terms of lower cost and shorter assay time. Therefore, the selection of an appropriate reagent kit should take into account not only analytical performance but also cost-effectiveness, operational efficiency, and procurement feasibility, in accordance with the specific requirements and operational context of the laboratory.

Keywords: Lumpy skin disease, Lumpy skin disease virus, DNA amplification reagent kit, Real-time PCR

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An Integrated Morphological and Molecular Approach for Detection of Enteric Parasites in Captive Reptiles

Tanapon Sukkankah[1] and Chanakan Chotiphutthikul[1,*]

Abstract

Background: The rising popularity of exotic pet cafés and animal markets has created high-density, mixed-species environments for captive reptiles. These settings can act as epidemiological hotspots for gastrointestinal parasites, increasing the risk of cross-contamination and impacting animal welfare. Due to limited baseline surveillance in these settings, a targeted pilot study is necessary to determine the diversity of existing endoparasites.

Objectives: This pilot study aimed to detect and identify gastrointestinal parasites in captive reptiles from exotic pet cafés and animal markets using an integrated morphological and molecular approach.

Methods: Twelve fecal samples (10 tortoises and 2 iguanas) were submitted to the parasitology laboratory from zoo collections and exotic pet cafés in Chonburi, Saraburi, and Phra Nakhon Si Ayutthaya provinces between January and February 2026. Initial morphological screening was conducted using direct smears and simple flotation to detect ova, cysts, and trophozoites. In addition, modified Ziehl–Neelsen (MZN) staining was performed for specific screening of *Cryptosporidium* spp. For molecular identification, DNA was extracted from fecal samples and subjected to polymerase chain reaction (PCR) amplification targeting protozoa (EukA/B primers targeting 18S rRNA), nematodes (NEM18F/R primers targeting 18S rRNA), and *Cryptosporidium* (nested PCR: SSU2F/R and SSU3F/R targeting SSU rRNA). Positive PCR amplicons were subsequently sequenced for species-level identification. Sequence identification was performed using BLAST against the NCBI database and selecting the result with the highest percent identity.

Results: Microscopic examination detected gastrointestinal nematode eggs (4/12), ciliate protozoan cysts consistent with *Nyctotherus*-like organisms (7/12), and coccidian oocysts (3/12), while *Cryptosporidium* spp. were not detected. PCR amplification using NEM18F/R primers detected nematodes in 6/12 samples, whereas EukA/B primers detected protozoa in 10/12 samples. No amplification of *Cryptosporidium* was observed using nested PCR. All visible PCR bands were excised and sequenced. High-quality sequences were obtained from six samples, based on clear chromatogram peaks without overlapping signals. For nematodes (NEM18F/R primers), two samples yielded identifiable sequences, corresponding to *Thelandros tinerfensis* (97.50% identity) and *Ozolaimus linstowi* (99.41% identity). For protozoa (EukA/B primers), four samples were successfully identified, including *Nyctotherus ovalis* (92% identity) and *Nyctotheroides* spp. (99.12%, 98.35%, and 92.08% identity). Low sequence quality limited identification in some samples. Overall, molecular findings were generally consistent with microscopic observations, particularly for ciliate protozoa. However, PCR detected additional positive samples that were not identified by microscopy.

Conclusion: This study demonstrates that an integrated morphological–molecular approach enhances the detection and identification of gastrointestinal parasites in captive reptiles. While microscopic examination identified broad parasite groups, molecular analysis enabled species-level identification and detected a greater number of positive samples. These findings contribute to improving diagnostic approaches for parasite surveillance in captive environments.

Keywords: Reptiles, Gastrointestinal parasites, Molecular diagnosis, Protozoa, Nematodes

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Development of In-house DNA Extraction Method for *Trypanosoma evansi* from Dried Blood Samples on FTA Cards

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Abstract

Background: Surra, caused by *Trypanosoma evansi*, is an important parasitic disease that affects animal health and livestock production worldwide, including in Thailand. PCR-based diagnostics offer high sensitivity, capable of detecting the parasite even at low concentrations. However, field application remains limited due to the need for transporting fresh blood samples under controlled temperature conditions and the high cost of commercial extraction kits. Dried blood spots (DBS) on storage cards offer a practical alternative; nevertheless, efficient and cost-effective DNA extraction methods from this matrix remain limited. Therefore, the development of an in-house extraction method suitable for DBS is essential to enhance accessibility and support large-scale surveillance.

Objectives: This study aimed to develop and evaluate methods for extracting *T. evansi* nucleic acids from dried blood on FTA cards.

Methods: An isolate of *T. evansi* (archived at NIAH, Thailand) was serially diluted in confirmed negative bovine blood to obtain concentrations ranging from 10^1 to 10^6 parasites/mL. Each dilution was spotted onto FTA cards. DNA was extracted from three 3-mm punches of each DBS using seven methods, including a commercial kit and six modified protocols: (1) SDS–Proteinase K; (2) Proteinase K-Chelex; (3) SDS-TE (95°C); (4) SDS-TE (room temperature); (5) TE-Chelex; and (6) TED10(TE+10% DMSO)-Chelex. PCR targeting the *T. evansi*-specific repetitive sequence (TBR1/TBR2 primers; 164 bp) was performed under standard conditions. Testing with positive control samples was performed to determine the limit of detection (LOD), which all three replicate samples were consistently detected.

Results: The TE–Chelex method achieved a limit of detection of 10^3 parasites/mL, which was comparable to that of the commercial extraction kit. The TED10-Chelex method showed similar sensitivity but required more complex processing steps. The SDS–Proteinase K method and the Proteinase K–Chelex method showed lower sensitivity, with LOD values of 10^6 parasites/mL. The SDS–TE methods at both temperatures did not yield detectable amplification under the conditions tested.

Conclusion: The developed in-house TE-Chelex extraction method is a reliable and cost-effective alternative for the molecular diagnosis of *T. evansi*. It provides comparable sensitivity to a commercial kit while offering a simpler workflow and reduced reagent requirements.

Keywords: *Trypanosoma evansi*, PCR, DNA extraction, Dried blood spot, FTA card

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Evaluation of DNA Extraction Kits for the Detection and Differentiation of *Salmonella* Enteritidis and Typhimurium in Chicken Feces by Multiplex PCR

Ruchuda Kanjanapen[1,*] and Nanticha Rattanamangkalanon[1]

Abstract

Background: *Salmonella* spp. are important causative agents of human salmonellosis, particularly *S.* Enteritidis and *S.* Typhimurium. Human can become infected through consumption the contaminated food, especially chicken meat, where contamination may originate from environmental sources. Currently, the multiplex PCR technique has been applied for the detection of *Salmonella* spp. However, it may yield false-negative results due to the presence of PCR inhibitor commonly found in chicken feces, such as bilirubin, phytic acid, and bile salts. Therefore, selecting an appropriate DNA extraction method is crucial to reduce false-negative results.

Objectives: This study aim to evaluate three DNA extraction methods from chicken fecal samples for the detection of *Salmonella* spp. and differentiation of the serovars Enteritidis and Typhimurium using the multiplex PCR technique.

Methods: Chicken fecal samples confirmed negative for *Salmonella* spp. by the standard culture method (ISO 6579-1: 2017/Amd 1: 2020) were spiked with reference strains *S.* Enteritidis ATCC 13076 and *S.* Typhimurium ATCC 14028 at concentrations ranging from 10^1 to 10^7 cfu/mL, with one sample prepared for each concentration. DNA extraction was performed using three methods: Taco™, MagMax™, and QIAamp® Fast DNA Stool Mini Kit. The extracted DNA was subsequently analyzed by multiplex PCR. The most suitable extraction method was selected based on DNA yield and purity, and differences among extraction methods were statistically analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). The selected method was then applied to field chicken fecal samples submitted to the laboratory, and multiplex PCR results were compared with those obtained using the standard culture method.

Results: Significant differences in DNA yield and purity were observed among the three extraction methods. The MagMax™ method produced the highest DNA yield and purity (average DNA concentration: 110.27 ng/µL; DNA purity: 2.19), followed by the QIAamp® Fast DNA Stool Mini Kit, whereas the Taco™ method yielded the lowest DNA concentration. Consequently, MagMax™ was identified as the most appropriate method for this study. Multiplex PCR detection of *Salmonella* spp. detection and differentiation of *S.* Enteritidis and *S.* Typhimurium in field chicken fecal samples demonstrated complete agreement with the standard culture method. Among the evaluated methods, MagMax™ and Taco™ exhibited advantages in terms of reduced processing time, lower labor requirements, simplified procedures, and lower reagent costs; however, both methods require an automated nucleic acid extraction system. In contrast, the QIAamp® Fast DNA Stool Mini Kit was more suitable for laboratories without automated extraction instruments, although it required more labor-intensive, time-consuming procedures and incurred higher costs.

Conclusion: DNA extraction using the MagMax™ method demonstrated high efficiency and suitability for extracting DNA from chicken fecal samples for the detection of *Salmonella* spp. and differentiation of *S.* Enteritidis and *S.* Typhimurium using multiplex PCR. Therefore, the three evaluated DNA extraction methods may serve as alternative approaches for researchers to adapt according to laboratory conditions.

Keywords: DNA extraction method, chicken feces, multiplex PCR, *S.* Enteritidis, *S.* Typhimurium

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Development of a Loop-mediated Isothermal Amplification Assay for Screening *Mycoplasma* Contamination to Reduce Contamination Problems in Cell Culture

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Abstract

Background: *Mycoplasma* contamination is a major issue in cell culture, leading to abnormal cell growth and often necessitating the disposal of contaminated cultures, resulting in significant loss of time and resources. It also poses a risk of contaminating vaccine products derived from cultured cells. Common contaminating species in cell culture include *Mycoplasma orale*, *Mycoplasma arginini*, *Mycoplasma hyorhinis*, *Mycoplasma fermentans*, and *Acholeplasma laidlawii*. Standard detection methods such as culture and PCR provide high accuracy but are time-consuming or require costly equipment, while imported commercial test kits, although convenient, are expensive.

Objectives: To develop a Loop-mediated Isothermal Amplification (LAMP) assay for rapid screening of *Mycoplasma* contamination in cell culture and to evaluate its diagnostic performance.

Methods: A LAMP assay was developed for detecting *Mycoplasma* species commonly found in contaminated cell cultures including *Mycoplasma orale*, *Mycoplasma arginini*, *Mycoplasma hyorhinis*, *Mycoplasma fermentans*, and *Acholeplasma laidlawii*. The assay performance was evaluated in terms of analytical sensitivity, specificity, diagnostic sensitivity, diagnostic specificity, and overall accuracy. The limit of detection (LOD) was determined using serial dilutions of target DNA. Positive reactions were interpreted by turbidity.

Results: The developed assay demonstrated high specificity for common *Mycoplasma* species found in cell culture, with a limit of detection (LOD) of 10^3 copies/µL, diagnostic sensitivity of 97.14%, diagnostic specificity of 100%, and overall accuracy of 99.36%. In addition, the assay provided rapid results, was simple to perform, did not require sophisticated instruments, and was four times more cost-effective than imported commercial kits.

Conclusion: The developed LAMP assay is a reliable, rapid, cost-effective, and user-friendly method for screening *Mycoplasma* contamination in cell culture systems. This assay has strong potential as an effective surveillance tool to reduce contamination problems in cell culture processes and improve biosafety in vaccine and biological product production by enabling rapid early detection and prompt management of contaminated cell cultures. However, the high amplification efficiency of LAMP increases the risk of carryover contamination and false-positive results if strict laboratory precautions are not followed. In addition, visual interpretation based on turbidity may be subjective, particularly in samples with low target concentrations or weak positive reactions. Therefore, further validation and standardized interpretation criteria are recommended.

Keywords: *Mycoplasma* contamination, Cell culture, LAMP assay, Screening test

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Differentiation of *Streptococcus suis* between Serotypes 1 and 14, and Serotypes 2 and 1/2, Using PCR-RFLP

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Abstract

Background: *Streptococcus suis* is an important zoonotic pathogen causing streptococcosis in pigs and severe invasive disease in humans, including meningitis and septicemia. Among the 29 recognized serotypes, serotype 2 is considered the most virulent. Two-step multiplex PCR has been used for routine serotyping. However, this method cannot distinguish between serotypes 1 and 14 or between serotypes 2 and 1/2. Capillary precipitation is therefore required for definitive differentiation, but this technique is costly and requires antisera produced by laboratory animals. A single nucleotide polymorphism (SNP) at position 483 (G→C/T) in the *cpsK* gene has been reported to differentiate these serotypes, providing a potential molecular alternative.

Objectives: To differentiate *S. suis* serotypes 1 and 14, and serotypes 2 and 1/2, using a PCR-restriction fragment length polymorphism (PCR-RFLP) assay targeting the *cpsK* gene at position 483.

Methods: A total of 76 *S. suis* isolates obtained from diseased pigs, together with reference strains representing the four serotypes, were included. Isolates were initially classified as serotype 1 or 14 (n = 6) and serotype 2 or 1/2 (n = 70). A DNA fragment covering position 483 of the *cpsK* gene was amplified and subjected to digestion with the restriction enzyme *Bst*NI. Serotypes were confirmed by Sanger sequencing of the same locus and verified by capillary precipitation using serotype-specific antisera (serotypes 1, 2, and 14).

Results: PCR-RFLP results were fully concordant with both Sanger sequencing and capillary precipitation. Among 6 isolates initially classified as serotype 1 or 14, while 2 isolates were identified as serotype 1 and 4 as serotype 14. Among the 70 isolates classified as serotype 2 or 1/2, 64 isolates were confirmed as serotype 2 and 6 isolates as serotype 1/2. In addition, Sanger sequences show that all serotypes 1 and 1/2 contain allele T at the SNP while all serotypes 2 and 14 contain allele G at the same loci.

Conclusion: The PCR-RFLP assay targeting the *cpsK* SNP at position 483 (G→C/T) accurately differentiates *S. suis* serotypes 1 and 14, as well as serotypes 2 and 1/2. This method provides a reliable, cost-effective alternative to capillary precipitation and reduces the need for laboratory animals.

Keywords: *Streptococcus suis*, Serotyping, PCR-RFLP, Pig, SNP

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Prevalence, Antimicrobial Resistance Genes, and Antibiotic Susceptibility of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Isolated from Chicken and Pork Meat in the Lower Northeastern Region of Thailand, 2022–2024

Yaowarat Sawasdee[1,*] and Kloyjai Phonsena[2]

Abstract

Background: *Escherichia coli* is a Gram-negative bacterium commonly found in the intestinal tract of humans and animals and is widely used as an indicator of food contamination. Although most strains are harmless, some can cause severe infections, including urinary tract infections, diarrhea, and foodborne illnesses. The emergence of extended-spectrum β-lactamase (ESBL)-producing *E. coli* has become a major public health concern due to its resistance to β-lactam antibiotics and its potential for dissemination through the food chain and environment.

Objectives: This study aimed to determine the prevalence of ESBL-producing *E. coli* in chicken and pork meat, detect ESBL-associated resistance genes (*bla*TEM, *bla*SHV, and *bla*CTX-M), evaluate antimicrobial susceptibility, and assess the association between resistance genes and phenotypic resistance patterns.

Methods: A total of 1,440 meat samples (681 chicken and 759 pork) were collected from slaughterhouses and retail markets in the lower northeastern region of Thailand from 2022 to 2024. Isolation and identification of *E. coli* were performed using standard microbiological methods. ESBL production was confirmed by the combination disk method. Antimicrobial susceptibility testing was conducted using the broth microdilution method according to CLSI (2025) guidelines. Resistance genes were detected by PCR, Statistical analysis was performed using Microsoft Excel and the Chi-square test, with a confidence level set at 95%.

Results: The prevalence of *E. coli* in pork samples was 46.93% (176/375) from slaughterhouses and 39.84% (153/384) from retail markets, while in chicken samples it was 57.58% (171/297) and 32.29% (124/384), respectively. A statistically significant difference ($p \leq 0.05$) in prevalence was observed in chicken samples between sources. A total of 532 isolates contaminated with ESBL-producing *E. coli* were detected, of which 329 isolates were obtained from pork meat and 203 isolates were obtained from chicken meat. High resistance to ampicillin was observed in both pork (88.75%) and chicken (70.94%) isolates. No resistance to ceftazidime and meropenem was detected. The most prevalent ESBL gene was *bla*CTX-M (64.13% in pork and 61.58% in chicken), followed by *bla*TEM (56.84% in pork and 51.23% in chicken), while *bla*SHV was not detected in any samples. The analysis of the relationship between genotype and phenotype revealed that *bla*CTX-M and *bla*TEM were not significantly associated with resistance to ampicillin ($p > 0.05$). However, a significant association was observed between *bla*CTX-M and resistance to cefotaxime ($p < 0.05$). In contrast, no significant association was found between ESBL genes and resistance to non-β-lactam antibiotics, including tetracycline, ciprofloxacin, gentamicin, and sulfamethoxazole/trimethoprim.

Conclusion: This study revealed a high prevalence of *E. coli* contamination in chicken and pork from the lower northeastern region of Thailand, with slaughterhouse samples showing higher positivity than retail markets. A substantial proportion of isolates were ESBL-producing, particularly those carrying *bla*CTX-M, which was significantly associated with cefotaxime resistance. In contrast, ESBL genes showed no association with resistance to ampicillin or non β-lactam antibiotics, indicating the involvement of additional resistance mechanisms. Overall, these findings highlight ongoing food safety risks and underscore the need for strengthened surveillance, improved hygiene practices, and prudent antimicrobial use to limit the spread of antimicrobial resistant bacteria to consumers.

Keywords: ESBL-producing *Escherichia coli*, Antimicrobial resistance, Chicken and pork, *bla*CTX-M, Food safety

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Diagnosis of *Burkholderia pseudomallei* in Soil Samples from Goat Farms in the Lower Northeastern Region

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Abstract

Background: Melioidosis is a severe infectious disease caused by the environmental bacterium *Burkholderia pseudomallei*. Livestock, particularly goats, have been identified as the most susceptible species to *B. pseudomallei* infection, showing the highest morbidity rate and causing significant economic losses due to high mortality and abortion rates. This disease is prevalent in the lower northeastern region of Thailand. Soil serves as a critical reservoir for the pathogen, posing a health risk to both animals and humans.

Objectives: This study aimed to investigate the contamination of *B. pseudomallei* in soil at three different depths (surface soil, 15 cm, and 30 cm) and to evaluate the antimicrobial susceptibility of the isolated strains from goat farms in the lower northeastern region.

Methods: A total of 69 soil samples were collected from 23 sampling points at three depths: surface soil, 15 cm, and 30 cm. The samples were analyzed using culture techniques with Threonine Basalt Salt Solution (TBSS) and Modified Ashdown's agar (ASH). Microbial identification was confirmed by polymerase chain reaction (PCR) using specific primers targeting the genomic region TTS1-orf2 (BpTT4176F: 5'–CGTCTCTATACTGTCGAGCAATCG–3', BpTT4290R: 5'–GTGCACACCGGTCAGTATC–3'), yielding a 115 bp PCR product. Antimicrobial susceptibility testing was performed using the disk diffusion method against 10 antibiotics: amoxicillin, colistin, gentamicin, cefotaxime, ceftazidime, ciprofloxacin, doxycycline, norfloxacin, ofloxacin, and tetracycline.

Results: *B. pseudomallei* was isolated from 27.54% (19/69) of the soil samples. The detection rates were 8.70% (6/69) at the surface level, 10.14% (7/69) at a depth of 15 cm, and 8.70% (6/69) at a depth of 30 cm. Antimicrobial susceptibility testing revealed that 100% (19/19) of the isolates were susceptible to cefotaxime, ceftazidime, ciprofloxacin, doxycycline, norfloxacin, ofloxacin, and tetracycline. Conversely, all isolates exhibited resistance to amoxicillin, gentamicin, and colistin.

Conclusion: The findings demonstrate that *B. pseudomallei* persists at various soil depths within goat farm environments. Soil contamination, especially during high-moisture periods such as the rainy season, indicates an increased risk of melioidosis transmission to both livestock and humans in this region. Antimicrobial susceptibility testing of the soil isolates demonstrated 100% susceptibility to ceftazidime and doxycycline. Conversely, complete resistance was observed against amoxicillin, gentamicin, and colistin.

Keywords: *Burkholderia pseudomallei*, Melioidosis, Goat

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