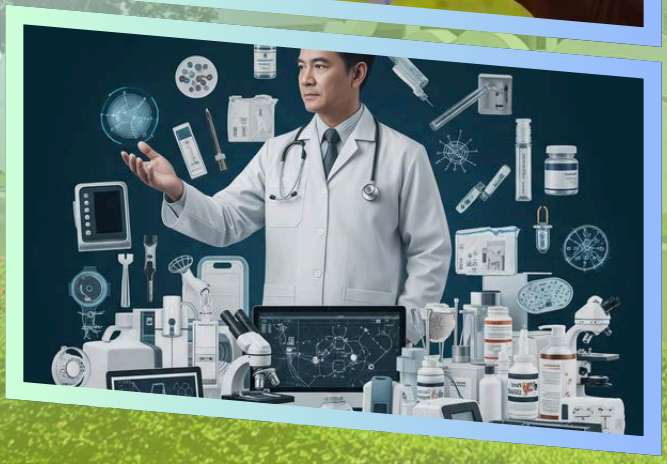
KVAC2025
Trend & Innovation in Veterinary Practice
The 26th Khon Kaen Veterinary Annual International Conference

PROCEEDINGS KVAC2025



The 26th Khon Kaen Veterinary Annual International Conference

Trend & Innovation in Veterinary Practice

July 16-17 July 2025

Pullman Khon Kaen Raja Orchid
Khon Kaen, THAILAND

KVAC 2025

The 26th Khon Kaen Veterinary Annual International Conference 2025
"Trend & Innovation in Veterinary Practice"

16th-17th July 2025
Faculty of Veterinary Medicine, Khon Kaen University, Thailand

PROCEEDINGS

Organized by the Faculty of Veterinary Medicine, Khon Kaen University, Thailand

Edited by
Jatesada Jiwakanon
Peerapol Sukon
Pinsaw Va Kromratanaphorn

Proceedings of The 26th Khon Kaen Veterinary Annual International Conference 2025
"Trend & Innovation in Veterinary Practice"

16th-17th July 2025 Pullman Khon Kaen Raja Orchid, Khon Kaen, Thailand
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Contents

Welcome Messages 6
The 26th KVAC Committee 14
Scientific Program 19
Invited Speakers 21
Speaker's Notes 38

Oral Presentation

Effects of *Crocodylus Siamensis* Liver Extracts on Glutathione S-Transferase Activity in Vital Organs of the Rats 50
Thanyanant Sahabantherngsin, Payu Srisuporn, Phitsanu Tulayakul

Modeling Hookworm Risk in Dogs Using Random Forest: A Machine Learning Toolkit Approach 57
Petcharat Chompo, Veerasak Punyapornwithaya, Sirikachorn Tangkawattana

Impact of Sperm Sexing on Functional Sperm Parameters in Small Ruminants: A Meta-Analysis 65
Naela Wanda Yusria Dalimunthe, Uswatun Muslykhah, Clara Ancilia Pramita Kusumasri, Dian Meididewi Nuraini, Morsid Andityas, Peerapol Sukon, Saksiri Sirisathien, Panisara Kunkitti

Molecular Prevalence of Canine Babesiosis Across Asia: A Systematic Review and Meta-Analysis 75
Clara Ancilia Pramita Kusumasri, Dian Meididewi Nuraini, Naela Wanda Yusria Dalimunthe, Morsid Andityas, Uswatun Musklykhah, Numfa Fungbun, Patchara Puektes

Identification and Pathological Assessment of *Mycobacterium* spp. Infections in Wild Marine Mammals from the Andaman Sea, Thailand 84
Pimwarang Sukkarun, Apinya Arnuphapprasert, Piyarat Khumraksa, Chayanis Daochai, Sasibha Jantrakajorn, Narissara Keawchana, Peerapon Sornying, Watcharapol Suyapoh

The Efficacy of Nonnai (*Tadehagi triquetrum L.*) grass in Preventing, Eliminating Flies and Killing Pathogenic Bacteria 96
Vongpasith Chanthakhoun, Xaykham Onphachanh, Somsay Phovisay, Toum Phonpadith

Poster Presentation

Effect of Idebenone in Extender on Frozen Semen Quality in Thai Native Chickens 107
Kodchakorn Jaroensiri, Chanisara Siriruang, Pimchana Pilunthanadilok, Sarawut Sringam

Efficacy of Endoscope Assisted Transcervical Artificial Insemination (ETCAI) on Pregnancy Rates in Goats 108
Sarawut Sringam, Pongthorn Suwannathada, Peerapat Deesuk, Avirut Wichaiwong, Patchanee Sringam

Preliminary Synchrotron SAXS Analysis of Dog Hair with Diverse Characteristics and Surgical Conditions 109
Kochakorn Direksin, Nusara Suwannachot, Pisit Suwannachot

Retrospective Analysis of Risk Factors and Postoperative Management of Surgical Site Infections in Canine Patients at Chiang Mai University's Small Animal Hospital (2017–2024) 119
Tanapat Ketvaraporn, Kanika Na-Lampang, Worapat Prachasilchai

Reproductive Tract-Derived Extracellular Vesicles Improve Bovine Embryo Development During *In Vitro* Culture 120
Apisit Polrachom, Kamolchanok Tonekam, Worawalan Samruan, Mariena Ketudat-Cairns, Traimat boonthai and Rangsun Parnpai

Impact of Resveratrol in Culture Media on the Development and Cryotolerance of *In Vitro* Produced Bovine Embryos 135
Kamolchanok Tonekam, Apisit Polrachom ,Yanapon Anthakat, Worawalan Samruan, Preeyanan Anwised, Pakpoom Boonchuen, Mariena Ketudat-Cairns, Rangsun Parnpai

OCT-4 Activating Compound 1 (OAC1) Enhanced *In Vitro* Development of SCNT Porcine Embryos 146
Thida Praeksamut, Phattarawadee Noita, Rangsun Parnpai

16S rRNA Metagenomic Analysis of Fecal Microbiota in Dairy Cattle with Different Body Condition Score 157
Bhuripit Saraphol, Woranich Hinthong, Peerut Chienwichai, Natapol Pumipuntu, Onrapak Reamtong, Thassanee Srisook, Jiraphan Premsuriya

Heart Rate and Heart Rate Variability in the First and Second Trimester Pregnant Mares 158
Sutheema Suwannarueang, Wanpitak Pongkan, Theerapong Pontaema, Wootichai Kenchaiwong ,Pongphol Pomgthaisong,Chayanon Chompoosan, Wichaporn Lerdweeraphon

Seasonal Variation and Antimicrobial Resistance of *Salmonella* Isolated from Pork at Slaughterhouses and Markets in Northeast of Thailand 159
Hongmei Liu, Fanan Suksawat, Sunpetch Angkititrakul, Jetesada Jiwakanon, Arunee Ritthipanun

Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Poultry in Thailand 160
Zhihui Zhang, Sunpetch Angkititrakul, Fanan Suksawat, Jetesada Jiwakanon, Arunee Ritthipanun

Development of detection of *Escherichia coli* O157 in Cow Milk using Loop Mediated Isothermal Amplification (LAMP) assay 163
Teerayut Chawut, Pawat Seritrakul, Sirichai Eardmusic

Seroprevalence and Risk Factors of Brucellosis in Goats in Muang District, Nakhon Phanom Province 164
Chatree Chumnandee, Nawarat Pha-obnga, Mattaneeya Sarakul, Warin Wongsamart

Ocular disease of captive pinnipeds in the Zoological Park Organization of Thailand 173
Chayanuch Utara, Anucha Sirimalaisuwan, Wanlaya Tipkantha, Kannika Na-Lampang
L-Asparaginase Monotherapy as a Salvage Treatment for Chemotherapy-Resistant Extragenital Transmissible Venereal Tumor (TVT) in a Mixed-Breed Dog: A Case Report 174
Arthit Sriart, Sarankorn Tipkositkun
Acute Stabilization and Management of MMVD Stage C with Pulmonary Hypertension and Tick-Borne Co-infection in a Geriatric Dog 175
Picha Kittipreeya , Siraprapa Pimthong, Nittaya Boonbal
Chronic Pancreatitis Presenting with Pancreatic Calcification in a Male Cat: A Case Report 176
Nichanan Maneeganondh, Nitaya Boonbal
Comparison of Primary Chicken Embryo Liver and Primary Chicken Embryo Fibroblast Cells for the Efficient Isolation of Fowl Adenovirus Serotype 2, 8 and 11 177
Kingkarn Angkabkingkaew, Natroupsorn Tantiwongvanich, Budsarin Thammasati, Pornpanom Toomtone, Patchareeporn Ninvilai
Effects of Crocodile (*Crocodylus Siamensis*) Liver Extracts on Detoxification Enzyme Activities in the Rat Liver and Kidney 178
Thanyanant Sahabantherngsin, Payu Srisuporn, Phitsanu Tulayakul
Maxillary Osteonecrosis Secondary to Brachycephalic Malocclusion in a Juvenile Pug: Clinical Management and Fungal Isolation Without Disease 179
Sarankorn Tipkositkun, Thanakorn Srirat
A Portable LAMP Assay for Rapid Molecular Detection of *Leishmania* in Resource-Limited Settings 180
Benjawan Saechue, Yasuhiro Soga, Naganori Kamiyama, Takashi Kobayashi
The efficacy of In Straw Dilution Method on Survival Rates of Vitrified Bovine Embryos 181
Chanyada Angchuan, Traimat Boonthai, Rangsun Parnpai
Innovation And Model for Creating Rabies-Free Zones in Kantharawichai Communities 182
Intrakamhaeng M, Duang-onnarm A, Hirunwatthanakul P, Sombattheera C
Immunosuppressive Therapy for the Management of Fistula Tract in a Cat: A Case Report 184
Sakdakorn Tiptawingoon, Karn Yongvanit, Natruethai Thititanasub
ABOUT VM-KKU 185
THANK YOU TO OUR SPONSORS 192

Welcome

Welcome message from the President of Khon Kaen University

Dear distinguished guests, esteemed colleagues, respected speakers, and all participants,

On behalf of Khon Kaen University, it is my great honor and privilege to welcome you all to the 26th Khon Kaen Veterinary Annual International Conference—KVAC 2025. I extend my heartfelt appreciation to the Faculty of Veterinary Medicine for organizing this important event, which has consistently served as a vital platform for knowledge exchange, innovation, and global collaboration.

This year's theme, *"Trend & Innovation on Veterinary Practice,"* truly reflects the dynamic evolution of veterinary medicine in the face of rapid technological advancement. The conference underscores our commitment to fostering new ideas, integrating scientific knowledge with practical applications, and making a meaningful impact on animal health and public well-being.

This international conference is more than just an academic event. It is a significant opportunity to position the Faculty of Veterinary Medicine at Khon Kaen University as a regional and global leader in veterinary education, research, and innovation. Through the collaboration of experts, researchers, and practitioners from around the globe, we aim to advance animal health, strengthen veterinary services, and contribute to better societal outcomes.

We believe KVAC 2025 will serve as a powerful catalyst for change—driving impactful research, fostering sustainable innovation, and creating lasting partnerships that will benefit our profession and our communities far into the future.

Once again, I warmly welcome you all and wish you a productive, inspiring, and memorable conference.

Thank you.

Associate Professor Dr. Charnchai Panthongviriyakul
The President of Khon Kaen University

Welcome Message from the Dean of the Faculty of Veterinary Medicine, KKU

Welcome to the 2025 Khon Kaen Veterinary Annual International Conference (KVAC).

It is a great honor for the Faculty of Veterinary Medicine, Khon Kaen University, to host this distinguished event that brings together veterinarians from across Thailand and around the world. We are pleased to welcome you to Khon Kaen city as we unite to explore the central theme: "Trends & Innovations in Veterinary Practice."

With the rapid changes taking place in many aspects of our world—such as global mobility, disruptive technologies, the trend of pet parenting, international trade, and the One Health approach—these factors are increasingly impacting the veterinary profession. What will the trends and innovations in veterinary practice be in the future?

This conference provides a valuable platform for exchanging knowledge, sharing experience, and developing innovative solutions to the emerging challenges and opportunities in our field.

I would like to express my sincere appreciation to the President of KKU, our distinguished guests, the KVAC committees, VM KKU alumni and staff, as well as our partners for their unwavering commitment and hard work in making this conference possible. I also extend special thanks to our distinguished speakers, whose expertise, insights, and contributions will be invaluable to our discussions.

Thank you for joining us at this conference, and I hope you enjoy your time in Khon Kaen city.

Sincerely,

Associate Professor Dr. Naruepon Kampa
The Dean of the Faculty of Veterinary Medicine, Khon Kaen University

Welcome Message from the Conference Chairperson of KVAC 2025

Dear esteemed colleagues, distinguished guests, and participants,

On behalf of the Faculty of Veterinary Medicine, Khon Kaen University, it is my great pleasure to welcome you to the 26th Khon Kaen Veterinary Annual International Conference (KVAC 2025). Our theme, "Trend and Innovation in Veterinary Practice," reflects our commitment to advancing veterinary science.

This conference is built upon key pillars:

- Driving Innovation: We aim to be a crucial platform for promoting cutting-edge research and innovative solutions in veterinary medicine. This fosters creativity, elevates animal healthcare standards, and enhances global disease prevention.
- Integrating Technology and Knowledge: KVAC 2025 will connect researchers, practitioners, and industry leaders worldwide. This strengthens collaboration, facilitates knowledge exchange, and encourages the adoption of advanced diagnostic and treatment technologies.
- Creating Positive Impact: We aspire to present impactful research and solutions to contemporary animal health challenges. Participants will gain practical knowledge and skills to improve field services, ultimately benefiting animal welfare and society.
- Enhancing Global Leadership: Hosting KVAC 2025 elevates Khon Kaen University's reputation as a leader in veterinary education and research. It attracts top professionals and fosters international academic partnerships.

KVAC 2025 is more than a knowledge exchange forum; it is a testament to our vision of becoming a regional and global hub for veterinary innovation. Through our collective efforts, we aim to drive significant advancements in animal health and societal well-being. Thank you for joining us. We wish you a productive and memorable conference.

Sincerely,

Associate Professor Dr. Weerapol Taweenan
Chairperson of the conference

Welcome Message from the Dean of the Faculty of Veterinary Science, MSU

Dear Esteemed Delegates,

On behalf of the Faculty of Veterinary Science, Mahasarakham University, it is my great honour to extend a warm welcome to all participants of KVAC 2025. We are truly privileged to join the Faculty of Veterinary Medicine, Khon Kaen University, as co-hosts of this prestigious international conference for the second consecutive year.

This continued partnership between our two institutions reflects not only a shared vision for advancing veterinary education and research but also the enduring bond between two leading faculties of veterinary science in the Northeastern region of Thailand. Together, we strive to cultivate academic excellence, strengthen research collaboration, and enhance the quality of veterinary practice for the benefit of animals, people, and society.

As the Dean of the Faculty of Veterinary Science, Mahasarakham University, and an alumna of the Faculty of Veterinary Medicine, Khon Kaen University, I am especially proud to be part of this significant gathering. It is both a personal and professional privilege to contribute to the ongoing growth and progress of our field alongside distinguished colleagues, mentors, and the next generation of veterinary professionals.

Under the theme "Trends and Innovation in Veterinary Practice," KVAC 2025 serves as a vital platform for the exchange of knowledge, the forging of meaningful partnerships, and the exploration of innovative approaches to improving animal health and welfare on a global scale.

Thank you for your participation and contribution. I wish you a productive, inspiring, and memorable conference experience.

Warmest regards,

Assistant Professor Dr. Sukanya Leethongdee
The Dean of the Faculty of Veterinary Science
Maha Sarakham University

Welcome Message from the Dean of the Faculty of Agriculture, National University of Laos

On behalf of the Faculty of Agriculture, National University of Laos, co-host of KVAC2025, I extend a warm and sincere welcome to all participants. As we come together to celebrate the 26th anniversary of this distinguished conference, it is a great honor to acknowledge KVAC's longstanding contribution to advancing animal health and promoting collaboration across our region and beyond.

This year's theme, **"Trend & Innovation in Veterinary Practice,"** reflects our shared commitment to exploring how emerging technologies are transforming the landscape of animal health. The integration of artificial intelligence and innovation is opening new frontiers, improving disease detection, enhancing veterinary services, and supporting the development of sustainable solutions that benefit animals, humans, and ecosystems alike.

KVAC2025 offers a unique platform to exchange ideas, showcase research, and build meaningful partnerships among scientists, professionals, and organizations. Through these discussions, we aim to spark innovations, strengthen networks, and advance our collective efforts toward improving animal health systems.

I wish you all a successful, insightful, and inspiring conference. May the connections you make here, the knowledge you gain, and the ideas you share drive continued progress in research, policy, and practice across the field.

Thank you, and once again, welcome to KVAC2025!

Associate Professor Dr. Vannaphone PUTTHANA
The Dean of the Faculty of Agriculture, National University of Lao

Welcome message from the President of The Veterinary Practitioner Association of Thailand (VPAT)

Distinguished guests, esteemed colleagues, ladies and gentlemen, it is a tremendous honor and a great pleasure to extend a warm welcome to all of you to The 26th Khon Kaen Veterinary Annual International Conference (KVAC 2025). This year, the Veterinary Practitioner Association of Thailand (VPAT) is proud to co-host this significant event under the insightful theme, "Trend & Innovation in Veterinary Practice."

As you may know, VPAT's core missions revolve around elevating the veterinary profession, serving as a central hub for developing professional standards and continuous education in companion animal practice across Asia, establishing a strong network and representative body for our members, and championing animal welfare for both our members and the general public. This conference perfectly aligns with these missions, offering an unparalleled opportunity to delve into cutting-edge trends, exchange invaluable insights, and foster collaborations that will undoubtedly advance our shared profession.

I am confident that the diverse program will provide each of you with profound knowledge and inspiration, and I wish you all a highly successful conference, sincerely hoping that you achieve all your objectives during your participation.

Warm regards,

Assistant Professor Phudit Maneesaay
VPAT president

Welcome Message from the President of The Veterinary Medicine of Khon Kaen university Alumni Association (VMKKUAA)

On behalf of the Veterinary Medicine of Khon Kaen University Alumni Association (VMKKUAA), it is my distinct pleasure to welcome all honored delegates to 26th Khon Kaen Veterinary International Annual Conference (KVAC 2025). This milestone event shines a spotlight on the "Trend & Innovation in Veterinary Practice". As the fast moving technology we gather here today, we find ourselves as part of technological revolution and global transformation especially in the veterinary and animal health practice. The rapid advancements in AI and innovation are reshaping our world and our profession. It is imperative that we, as veterinarians, stay at the forefront of these global trends, integrating cutting-edge technology into our practices to enhance the care we provide.

Our Alumni Association proudly represents more than 3,000 graduates from the Faculty of Veterinary Medicine at Khon Kaen University. We are committed to ensuring that our members are well-equipped, highly skilled, and intellectually prepared to tackle the challenges of the veterinary field. By embracing AI and other innovations, we can set new standards in animal health and welfare.

As the president of VMKKUAA, I am delighted to see such a distinguished gathering of professionals and experts. I have no doubt that this conference will be a platform for excellent and fruitful discussions, fostering collaboration and the exchange of ideas that will drive our profession forward.

I also encourage you to take some time to immerse yourselves in the rich cultural heritage of Northeastern Thailand. Khon Kaen is renowned for its warm hospitality, beautiful landscapes, and vibrant traditions. From the intricate art of silk weaving to the captivating Mor Lam music and dance, there is much to experience and enjoy. Our local culture, often referred to as our "soft power," is an integral part of what makes this region so special.

Thank you for being here and for your commitment to advancing veterinary medicine. I wish you all an enriching and memorable experience at KVAC 2025.

Warm regards,

Dr. Thawatchai Poolsawat (DVM, MBA)

The President of The Veterinary Medicine of Khon Kaen university Alumni Association

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The 26th Khon Kaen Veterinary Annual International Conference (KVAC) 2025
"Trend & Innovation in Veterinary Practice"
16-17 July 2025 Pullman Khon Kaen Raja Orchid, Khon Kaen, Thailand

Faculty of Veterinary Medicine. Khon Kaen University. Muang District. Khon Kaen. Thailand

Day 1 - 16 July 2025

Time	Opening Session [Room: Orchid Ballroom 2-3]
09.00-09.30	**Open ceremony: Welcome remark by the President of Khon Kaen University**
09.30-10.30 (1 hr)	**The Future Trends of Veterinary Practices in Thailand: How to Stay Ahead [English]** **Keynote Speaker: Assoc. Prof. Dr. Theera Rukkwamsuk (รศ.น.สพ.ดร. ธีระ รักความสุข)** Moderator: Dr. Pakpoom Navanukraw
10.30-10.50	Coffee Break – Poster presentation
10.50-11.50 (1 hr)	**From Innovation Lab to Life: Translating Veterinary Research into Real-World Impact [English]** **Keynote Speaker: Prof. Dr. Kaywalee Chatdarong (ศ.สพ.ญ.ดร. เกวลี ฉัตรดรงค์)** Moderator: Dr. Pakpoom Navanukraw
11.50-13.00	Lunch – Poster presentation

Time	Room: Erawan 1-2 Ruminant Moderator: Assoc. Prof. Chaiwat Jarassaeng	Time	Room: Orchid Ballroom 3 Exotic and wildlife Moderator: Assoc. Prof. Sompoth Weerakhun	Time	Room: Orchid Ballroom 2 Companion Pets Moderator: Assoc. Prof. Supranee Jitpean
13.00-13.50 (50 min)	**Main Topic** **Premium Beef Production: The Future and Survival Strategy for Thai Farmers** การผลิตโคเนื้อคุณภาพพรีเมียม: อนาคตและทางรอดของเกษตรกรไทย **Lessons from a Decade: Building the Surin Wagyu Beef Brand** Speaker: Mrs. Kiangduan Sanguanchue (คุณเกียง เดือน สงวนชื่อ)	13.00-13.50 (50 min)	**Case Study: Dolphin Incursion into the Bang Pakong-Prachinburi River Estuary** กรณีศึกษาโลมาเข้าปากแม่น้ำบางปะกง-ปราจีนบุรี Speaker: Dr. Rachawadee Chantra (สพ.ญ.ราชาวดี จันทรา)	13.00-13.50 (50 min)	**New Evidence, Real-World Proof: Advancing Modern Parasite Protection** อัปเดตข้อมูลใหม่ ที่พิสูจน์ได้จริง สู่การป้องกันปรสิตยุคใหม่ที่ดีกว่าเดิม Speaker: Assoc.Prof.Dr.Piyarat Chansiripornchai (รศ.สพ.ญ.ดร.ปิยะรัตน์ จันทร์ศิริพรชัย) Asst.Prof.Rungrote Osathanon (ผศ.น.สพ.รุ่งโรจน์ โอสถานนท์)
13.50-14.40 (50 min)	**Experiences and Husbandry Practices for Premium Marbling in Phu Phan Beef Cattle (Tajima Wagyu Line)** Speaker: Dr. Wisut Auekingpetch (น.สพ.วิศุทธิ์ เอื้อกิ่งเพชร)	13.50-14.40 (50 min)	**Disease and Health Management of Elephants in the Elephant Kingdom Surin** โรคและการจัดการสุขภาพช้างที่คชอาณาจักร Speaker: Dr. Nuttapon Bangkaew (น.สพ. ณัฐพล บางแก้ว)	13.50-14.40 (50 min)	
14.40-15.00	Coffee Break 20 min – Poster presentation	14.40-15.00	Coffee Break 20 min – Poster presentation	14.40-15.00	Coffee Break 20 min – Poster presentation
15.00-15.50 (50 min)	**Genetic Detection of Marbling Genes: A Tool for Enhancing Beef Quality** Speaker: Assoc.Prof.Dr. Mariena Ketudat-Cairns (รศ.ดร.มารินา เกตุทัต-คาร์นส์)	15.00-15.50 (50 min)	**Reptiles: Understanding Biological Differences, Diseases, and Best Practice Management** สัตว์เลื้อยคลาน.ความแตกต่างจากสัตว์ชนิดอื่น โรคและการจัดการอย่างถูกทาง Speaker: Dr. Tulyawat Sutthipat (น.สพ. ตุลยวรรธ สุทธิแพทย์)	15.00-15.50 (50 min)	**New Evidence, Real-World Proof: Advancing Modern Parasite Protection** อัปเดตข้อมูลใหม่ ที่พิสูจน์ได้จริง สู่การป้องกันปรสิตยุคใหม่ที่ดีกว่าเดิม Speaker: Assoc.Prof.Dr.Piyarat Chansiripornchai (รศ.สพ.ญ.ดร.ปิยะรัตน์ จันทร์ศิริพรชัย) Asst.Prof.Rungrote Osathanon (ผศ.น.สพ.รุ่งโรจน์ โอสถานนท์)
15.50-16.40 (50 min)	**Ultrasonography for evaluating marbling score in Beef Cattle** Speaker: Mr. Emmanuel Château	15.50-16.40 (50 min)	**Understanding Common Viral Diseases in Psittacine Birds: What Practitioners Should be Aware of** โรคไวรัสสำคัญที่พบบ่อยในนกแก้ว อะไรคือสิ่งที่สัตวแพทย์ควรตระหนัก Speaker: Dr. Gan Rungpiriyadej (น.สพ. กันต์ รุ่งพิริยะเดช)	15.50-16.40 (50 min)	

The 26th Khon Kaen Veterinary Annual International Conference (KVAC) 2025
"Trend & Innovation in Veterinary Practice"
July 16-17 July 2025 Pullman Khon Kaen Raja Orchid, Khon Kaen, Thailand

Day 2 - 17 July 2025

Time	Room: Erawan 1-2 Equine Moderator: Asst.Suphannika Phutthachalee	Time	Room: Orchid Ballroom 3 Swine Moderator: Assoc.Prof.Dr.Sathorn Porntrakulpipat	Time	Room: Orchid Ballroom 2 Companion Pets Moderator: Dr.Siwayu Rattanakanokchai
08.40-9.40 (1 hr)	**Translating PRP Therapy from Human to Applications for Chronic Wound Healing in Animals** Speaker: Asst.Prof.Dr. Wiyada Punjarauk (ผศ.พญ.ดร.วิยะดา ปัญจรักษ์)	08.40-9.40 (1 hr)	**Managing pig diseases: An outcome-based strategy** การจัดการโรคสุกร: กลยุทธ์ผู้ใช้บริการ Speaker: Asst.Prof.Dr. Pariwat Poolperm (ผศ.น.สพ.ดร.ปริวรรต พูลเพิ่ม)	08.40-10.10 (15 hrs)	**Hematological parameters and their predictive values in diseases** Speaker: Asst.Prof. Phudit Maneesaay (ผศ.น.สพ. ภูดิท มณีสาย)
9.40-10.40 (1 hr)	**The Science of Regeneration and Applications of Mesenchymal stem cells in Horse and other animals** Speaker: Assoc.Prof.Dr. Aree Thayananuphat (รศ.สพญ.ดร.อารีย์ ทยานนุพัทร์)	9.40-10.40 (1 hr)	**Adapting Cost Management and Production Efficiency to the Evolving Global Landscape** การจัดการต้นทุนและประสิทธิภาพการผลิตในยุคแห่งการเปลี่ยนแปลงตลาดการเงินโลก Speaker: Asst.Prof. Nattavut Ratanavanichroj (ผศ.น.สพ.ณัฐวุฒิ รัตนวณิชโรจน์)	10.10-10.30	Coffee Break 20 min – Poster presentation
10.40-11.00	Coffee Break 20 min – Poster presentation	10.40-11.00	Coffee Break 20 min – Poster presentation	10.30-12.00 (15 hrs)	**Biochemical tests for liver and kidney: when and why** Speaker: Asst.Prof. Phudit Maneesaay (ผศ.น.สพ. ภูดิท มณีสาย)
11.00-12.00 (1 hr)	**Effect of cytokines from equine umbilical cord stem cells on chronic wound healing in horses** Speaker: Lt. Col. Kosin Thongsri (พันโท น.สพ.โกสินทร์ ทองศรี)	11.00-12.00 (1 hr)	**From Gut to Growth: Sustainable Nutrition Strategies in Pig Farming** จากลำไส้สู่การเติบโต: กลยุทธ์โภชนการที่ยั่งยืนในฟาร์มสุกร Speaker: Prof.Dr. Chaiyapoom Bunchasak (ศ.ดร.ชัยพูน บัญชาศักดิ์)		
11.00-12.00	Lunch – Poster presentation				
Time	**Room: Erawan 1-2** **Oral Presentation** Moderator: Asst Prof.Dr. Panisara Kunkiti	Time	**Room: Orchid Ballroom 3** **Poultry** Moderator: Dr.Parinya Sroithongkham	Time	**Room: Orchid Ballroom 2** **Companion Pets** Moderator: Dr.Siwayu Rattanakanokchai
13.00-14.40 (1 hr 40 min)	Oral Presentation	13.00-13.50 (50 min)	**Poultry Gut Health** Speaker: Prof.Dr Niwat Chansiripormchai (ศ.น.สพ.ดร.นิวัตร ชันรีภิรัพรชัย)	13.00-13.50 (50 min)	**Advancing Companion Animal Cardiology: Future perspectives on Mitral Valve Repair in Dogs and Emerging Therapies for Hypertrophic Cardiomyopathy in Cats** Speaker: Prof.Dr. Soontaree Petchdee (ศ.สพ.ญ.ดร. สุนทรี เพ็ชรดี)
		13.50-14.40 (50 min)	**Bacteriophage characterization and application in poultry** Speaker: Prof.Dr. Niwat Chansiripormchai (ศ.น.สพ.ดร.นิวัตร ชันรีภิรัพรชัย)	13.50-14.40 (50 min)	**Central versus Peripheral Vestibular Disease: enhance diagnostic skills to pinpoint precise location** Speaker: Dr. Pohonyoot Soraned (น.สพ. พหลยุทธ โสรเนตร)
14.40-15.00	Coffee Break 20 min – Poster presentation	14.40-15.00	Coffee Break 20 min – Poster presentation	14.40-15.00	Coffee Break 20 min – Poster presentation
15.00-16.00 (1 hr)	Oral Presentation	15.00-16.00 (1 hr)	**Antimicrobial Resistance in Poultry Isolates: Current Trends and Emerging Challenges** Speaker: Assoc.Prof.Dr. Thotsapol Thomrongsuwannakij (รศ.น.สพ.ดร.ทรงพล ร้อยอรรถากร)	15.00-15.50 (50 min)	**Veterinary Diagnostics: Emerging Approaches in Medical Image Analysis** Speaker: Dr. Panida Pongvittayanon (อ.สพ.ญ. พิมดา พงศ์วิทยานนท์)
				15.50-16.40 (50 min)	Revolutionizing Veterinary CPR: Key Insights from the RECOVER Guidelines Speaker: Dr Natthada Phukit (สพ.ญ. ณัฐฐดา ภูกิจ)

Invited Speakers

Associate Professor Dr. Theera Rukkwamsuk

Educational Background

- 1991 D.V.M. (First Class Honors) Kasetsart University
- 1998 M.Sc. (Veterinary Epidemiology and Economics) Utrecht University, Netherlands
- 1999 Ph.D. (Large Animal Medicine) Utrecht University, Netherlands
- 2015 V.V.M. (Internal Medicine) College of Veterinary Professionals, Expert of Thailand

Current work situation

- President of the Veterinary Council (2024-2027)
- Deputy Head of the Department of Large Animal and Wildlife Clinical Medicine, Faculty of Veterinary Medicine Kasetsart University

Work Experience

1991 -Present Lecturer, Department of Large Animal and Wildlife Clinical Medicine, Faculty of Veterinary Medicine, Kasetsart University
2021 – 2024 President of the Veterinary Council (2021-2024)
2019 – 2021 Chairman of the Executive Committee of the College of Veterinary Professionals of Thailand
2017 – 2019 Incumbent Chairman of the Executive Committee of the College of Veterinary Professionals of Thailand
2014 – 2017 Chairman of the Subcommittee on Training and Examination of Knowledge and Expertise in Veterinary Profession Department of Internal Medicine
2012 – 2018 Council Member of the Veterinary Council of Thailand
2015 – 2021 Chairman of the Committee on Curriculum and Institution Review, Veterinary Council

Expertise

- Ruminant Internal Medicine
- Veterinary Epidemiology
- Clinical Nutrition in Ruminants
- Herd Health Management in Ruminants

Academic Achievements/Awards

- Published research in more than 90 national and international academic journals

Professor Dr. Kaywalee Chatdarong

Professor of Obstetrics, Gynecology, and Reproductive Sciences
Faculty of Veterinary Science, Chulalongkorn University

Educational Background

Ph.D. 2546 Swedish University of Agricultural Sciences (Veterinary Obstetrics & Gynaecology)
M.Sc. 2544 Swedish University of Agricultural Sciences (Veterinary Obstetrics & Gynaecology)
Bechelor of Veterinary Science 2020 Faculty of Veterinary Science, Chulalongkorn University

Current Position

Currently holds the position of Professor in the Department of Obstetrics, Gynecology, and Reproductive Sciences, Faculty of Veterinary Science, Chulalongkorn University

Achievements in Administration

- President of the Thai Veterinary Practitioners Association (2010 – 2014)
- Associate Dean for Academic Services, Faculty of Veterinary Science (2013 – 2017)
- Director of the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University (2013 – 2016)
- Director of the Continuing Education Center, Veterinary Council of Thailand (2015 – 2018)
- Head of the Obstetrics and Reproductive Biology Research Unit (2014 – 2018)
- Associate Dean for Research and Innovation (2018 – 2021)
- Vice President for Strategic Planning, Innovation, and Global Engagement, Chulalongkorn University (2021 – 2024)

Achievements in Academic

- 92 international publications indexed in Scopus with an H-index of 21
- Supervised 10 Ph.D. dissertations and 5 Master's theses as principal advisor

Achievements in Award

- 2010: Distinguished Alumni Award for Academic Services from the Alumni Association, Faculty of Veterinary Science, Chulalongkorn University
- 2017: Outstanding Veterinarian in Academic Field, The Veterinary Council of Thailand under the Royal Patronage

Dr. Wisut Auekingpetch

Dr. Wisut Auekingpetch

Current Position

Veterinary Officer, Senior Professional Level

- Head, Livestock Study and Development Division, Phu Phan Royal Development Study Center (under the Royal Initiative)
- Head of Animal Health Development Group, Sakon Nakhon Provincial Livestock Office

Educational Background

1996 Doctor of Veterinary Medicine (D.M.V.), Faculty of Veterinary Medicine, Khon Kaen University

Work Experience

1996 – 1997 Veterinarian Level 4, Regional Livestock Office 4, Sakon Nakhon Animal Breeding Station

1998 – 1999 Veterinarian Level 4 – 5, Regional Livestock Office 9, Hat Yai Animal Quarantine Station

1998 – 2001 Veterinarian Level 5, Bureau of Disease Control and Veterinary Services, Disease Prevention and Eradication Planning Division

2003 – Present Veterinarian Level 6 – 7, Senior Professional Level, Sakon Nakhon Provincial Livestock Office, Animal Health Development Group

2001 – Present Head of Livestock Study and Development Section, Phu Phan Royal Development Study Center (under the Royal Initiative)

Short course training

1997 Participated in the international 'Young Professional Scientist' training program organized by the Food and Agriculture Organization of the United Nations (FAO).

Dr. Rachawadee Chantra

Current work

Marine and Coastal Resources Research and Development Center (Upper Gulf of Thailand: Samut Sakhon), Department of Marine and Coastal Resources, Thailand

Educational Background

- Doctor of Veterinary Medicine, Khon Kaen University
- Internship in the International Veterinary Fellowship Program offers marine mammal veterinarians, the Marine Mammal Center, Callifornia, U.S.A.

Work Experience

12 years as a Marine Veterinarian under the Department of Marine and Coastal Resources,
Thailand

- Endangered marine species (Dolphin, Whale, Dugong, Sea turtle and Whale shark) rescue management, rehabilitation and necropsy
- Health assessment of Bryde's Whale (*Balaenoptera edeni*) in Upper Gulf of Thailand
- The impacts of marine debris on endangered marine species

Dr. Nuttapon Bangkaew

Position: Veterinarian Level 5, Division of Conservation, Research and Animal Health
Organization: Elephant Kingdom Project, Surin Province, Zoological Park Organization of Thailand

Work Experience

- Asian Elephant Foundation of Thailand (AEFT), 2015-2017
- National Elephant Health Service and Research Institute, Department of Livestock Development, 2017–2019
- Elephant Kingdom Project, Surin Province, Zoological Park Organization of Thailand, 2020 – Present

Expertise: Eleplant

Educational Background: Bachelor of Veterinary Medicine, Faculty of Veterinary Medicine, Khon Kaen University in 2014

Research and academic presentations

1. Individual identification and collecting genetic sample database as well as sperm bank of captive elephant projects
2. Treatment and repair of left hind leg bone fracture at the tibia fibula in an asian elephant
3. Diagnosis and Treatment of Bacterial and Urinary tract infections in Asian Elephants
4. Artificial Insemination Project Based on the Evaluation of Reproductive Efficiency and Appropriate Estrous Cycle of Captive Elephants in the Elephant Kingdom Project, Surin Province, Starting Research in the Fiscal Year 2026

Collaborative Research

1. *The first report on internal transcribed spacer region-based characterization of microfilaria in Asian elephants (Elephas maximus) in Thailand*
2. *Enhancing genetic management in captive Asian elephants: evaluating mitochondrial single nucleotide polymorphism markers for improved breeding and conservation at Elephant Kingdom, Thailand ract infections in Asian Elephants*

Present academic research/work at international conferences

1. Participate in the Southeast Asian Zoos and Aquariums Association in the topic of Diagnosis and Treatment of Bacterial and Urinary tract infections in Asian Elephants, Malasia
2. Lecture and Practical Workshop on Reproductive System Management in Asian Elephants at the A'Famosa Resort Malacca Malaysia

Assistant Professor Tulyawat Sutthipat

Educational Background

Master's Degree: Master of Science (M.Sc.) in Biology, Chiang Mai University, 2011
Bachelor's Degree: Doctor of Veterinary Medicine (D.V.M.), Faculty of Veterinary Medicine, Kasetsart University, 1996

Work Experience and Professional Roles:

1996 – 2001: Veterinarian, Nakhon Ratchasima Zoo, Zoological Park Organization of Thailand
2001 – 2007: Lecturer, Elephant and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University
2002 – 2004: Head of Elephant and Wildlife Clinic Program, Faculty of Veterinary Medicine, Chiang Mai University
2002 – 2006: Member, Ethics Committee for Animal Experimentation and Use of Animal Housing Facilities, Faculty of Veterinary Medicine, Chiang Mai University
2007 – 2017: Assistant Professor, Elephant and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University
2004 – 2006: Head of Companion Animal Group, Faculty of Veterinary Medicine, Chiang Mai University
2004 – 2005: Committee Member, Avian Influenza Surveillance Unit, Faculty of Veterinary Medicine
2004 – 2008: Member, University Academic Senate, Chiang Mai University
2005 – 2007: Committee Member, Central Laboratory Animal Center Project, Chiang Mai University
2016 – 2017: Member, Chiang Mai University Staff Senate
2016 – 2017: Subcommittee Member, Oversight of Chiang Mai Night Safari Office
2002 – 2005: Co-instructor, Intensive Course in Wildlife Veterinary Medicine and Advisor: Association of Thailand Exotic Pet Veterinarians

Dr. Gan Rungpiriyadej

Pursuing a Ph.D in the Veterinary Science Program (International),
Faculty of Veterinary Medicine, Khon Kaen University

Educational Background

2016 Doctor of Veterinary Medicine (D.V.M.), Faculty of Veterinary Medicine, Khon Kaen University
Currently pursuing a Ph.D in the Veterinary Science Program (International), Faculty of Veterinary Medicine, Khon Kaen University

Work Experience

Veterinarian:

- 2020-present: Petland animal hospital
- 2018-2020: Prasu-Arthorn animal hospital
- 2016-2018: Kwunkum animal hospital

Certification and short courses

- Participant and hand-on workshop in the exotic orthopedics, Faculty of Veterinary Medicine, Khon Kaen University, May 24-26, 2024
- Certificate of knowledge in exotic pets medicine international e-learning veterinary school (EAVS ASBL) European school for advanced veterinary studies (ESAVS) completed: July 29, 2024
- Participant in the "handling avian respiratory emergencies" by the Association of Avian Veterinarians (AAV) May 24, 2023
- Participant in the "Pathological radiology" by the Association of Avian Veterinarians (AAV) May 25, 2023
- Participant and hand-on workshop on rabbit dentistry, Faculty of Veterinary Medicine, Kasetsart University. Nov 21, 2020

Assistant Professor Rungrote Osathanon

Educational Background

2000 Doctor of Veterinary Medicine (D.V.M), Faculty of Veterinary Science, Chulalongkorn University

2019 Master of Veterinary Medicine and a residency training program in small animal internal medicine at the Royal Veterinary College, University of London, UK

2022 American College of Veterinary Internal Medicine (ACVIM) diploma and a Board-Certified in Small Animal Internal Medicine

Current situation

Assistant Professor at the Department of Veterinary Clinical Science and Public Health
A director of the residency training program for Thai Board of Veterinary Internal Medicine, Mahidol University.
An IRIS ambassador of the International Renal Interest Society.

Field of Interest

Canine and feline internal medicine, particularly cardiology, nephrology and endocrinology.

Assistant Professor Dr Wiyada Punjaruk

Work address:
The Department of Physiology and the Cellular therapy center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, 40002

Educational Background

- 2004 Medical Doctor (Honor class) from the Faculty of Medicine, Khon Kaen University, Thailand
- 2010 Doctor of Philosophy (Ph.D.) (Medical genetics: Cancer stem cells) from the University of Nottingham, UK
- 2011 Short course training in Clinical Epidemiology Research from the Faculty of Medicine, Khon Kaen University
- 2014 Mini MBA Executive from MBA, Khon Kaen University
- 2019 Training in Cellular immunotherapy from the Department of Cell therapy, the Oslo University Hospital, Norway

Work Experience and Award

- The Director of The Cellular Therapy Center, The Faculty of Medicine, Khon Kaen University since 2023-present
- Lecturer at the Department of Physiology, Faculty of Medicine, Khon Kaen University for medical students and paramedical students since 2004-present
- The Deputy Director of The Blood Transfusion Center, Srinagarind Hospital, the Faculty of Medicine, Khon Kaen University since 2019-2024
- The Assistant Dean for the Academic affairs, the Faculty of Medicine, Khon Kaen University sine 2017-2018
- The Assistant Dean for the Student development, the Faculty of Medicine, Khon Kaen University since 2014-2017
- Promotion for the Assistant Professor in Physiology, the Faculty of Medicine, Khon Kaen University in 2015

Publication

Over 30 published articles in national and international peer-reviewed journals

CURRICULUM VITAE: AREE THAYANANUPHAT, D.V.M., Ph.D., DTBVS

EDUCATION

D.V.M. Veterinary Medicine, Kasetsart University, Thailand
Ph.D. Animal Science University of Minnesota, USA

TRAINING

Ophthalmology Clinic, University of California, Davis, US
Ophthalmology Clinic, University of Minnesota, St Paul, US
Ophthalmology chapter lecture and workshop: Retina and Vision, Australian college of veterinary scientists, Gold Coast, Australia
Intraocular surgery for advanced surgeon, Berlin, Germany

PREVIOUS STATUS

Associate Professor: Department of Small Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University
Director of Kasetsart University Veterinary Teaching Hospital Bangkhen

CURRENT STATUS

CEO: Precision Vet Co., Ltd.
Special Instructor, Faculty of Veterinary Medicine, Kasetsart University
Diplomate, Thai Board of Veterinary Surgery
Committee of The Thai Society of Veterinary Ophthalmology Practitioners

Lieutenant Colonel Kosin Thongsri

Lieutenant Colonel Kosin Thongsri currently holds the position of Director of the veterinary hospital within the Veterinary Corps. His working experience includes serving as a Veterinarian judge at the SOUTH EAST ASIA ENDURANCE CUP CEI 1* TIEP in Terengganu, Malaysia in 2016, and as a Team Veterinarian in Equestrian Terengganu State, Malaysia in 2017. He also served as Veterinarian and Coordinator for RTA. USAPAC. USASOC. Joint Exercises in HI., NC. USA. in 2022, and in the same role for the Special Forces Multipurpose Canine Handler Basic Course, 1st SFG (Airborne), 1st SFC (Airborne), JBLM UASASOC WA. USA. in 2023.

His education background includes graduating from the Armed Forces Academies Preparatory School (Class 41) in 1999 and earning a Doctor of Veterinary Medicine from Mahanakorn University of Technology in 2007. He also obtained a Bachelor of Laws from Sukhothai Thammathirat Open University in 2007, and a Certificate in Pet Reproductive unit and Exotic pet unit from Kasetsart University in the same year. He further received a Master's degree in Agricultural Resource Management (Animal Production Resource Management) from Sukhothai Thammathirat Open University in 2013, and two Bachelor's degrees in Management (Agribusiness) (2012-2013) and Management (Animal Production Management) (2010) from the same university. Additionally, he completed The Postgraduate Diploma in Strategic and Defence Studies (PASS WITH CREDIT) from the National Defence University Malaysia in 2024.

His military education includes the Royal Thai Army Airborne Course (Class 241 RTA), the Military Dog Handler Observation Course (Class 96 (1/2012) RTA), a Diploma in Royal Thai Army Military Research and Development (Class 6 RTA), the Veterinary Officer basic course (Class 22 RTA), and the Veterinary Officer advanced course (Class 24 RTA). He also completed the COMMAND AND GENERAL STAFF OFFICER COURSE (CGSC) Class 96 RTA, the USASOC Airborne Friendship Jump at Fort Bragg (Fort Liberty) NC. USA in 2022, the USASOC parachute Friendship Jump at Fort Bragg (Fort Liberty) NC. USA in 2022, and the Special Forces Multipurpose Canine Handler Basic Course, 1st SFG (Airborne), 1st SFC (Airborne), JBLM UASASOC WA. USA. in 2023. He also attended The Malaysian Armed Forces Staff College National Defence Education Centre Putrajaya Malaysia the Malaysian Command and Staff Course 53/2024. His foreign education includes the FEI Veterinarian Course in TIEP Kuala Terengganu, Malaysia in 2018, the UASASOC. Airborne Friendship Jump, fort bragg (Fort Liberty) NC. USA. in 2022, the UASASOC. parachute Friendship Jump, fort bragg (Fort Liberty) NC. USA. in 2022, and the Special Forces Multipurpose Canine Handler Basic Course, 1st SFG (Airborne), 1st SFC (Airborne), JBLM UASASOC WA. USA. in 2023.

Professor Dr. Chaiyapoom Bunchasak, PhD

Professor in the Department of Animal Science
Kasetsart University in Bangkok, Thailand.

Prof. Dr. Chaiyapoom Bunchasak is a distinguished professor in the Department of Animal Science at Kasetsart University in Bangkok, Thailand. His research focuses on animal nutrition, with a particular emphasis on poultry and pigs. Prof. Bunchasak has made significant contributions to understanding dietary protein and amino acids, especially methionine, and their impact on growth performance, carcass characteristics, metabolic responses and microbiome in poultry and pigs. He also explores the utilization of alternative protein/energy sources and feed additives to enhance livestock health and productivity.

Prof. Bunchasak earned his Science (Agriculture; Honor), KhonKaen University, Thailand and completed his PhD in Animal Science at Gifu University, Japan. He has published extensively, contributing to the advancement of poultry nutrition and management. To date, he has authored or co-authored 70 peer-reviewed publications in scientific journals, 89 proceedings of international meetings, 128 abstracts of presentations at national and international scientific meetings, 34 popular press articles, 8 book chapters, and 3 books. His notable studies include research on the impact of dietary energy and methionine sources on broiler performance and carcass yield.

Professor Dr. Niwat Chansiripornchai

Educational Background

1993 DVM, Chulalongkorn University, Bangkok, Thailand
2000 M.Sc. (Molecular Microbiology and Epidemiology), Swedish University of Agricultural Sciences, Sweden
2004 Ph.D. (Infectious Diseases and Immunology), Utrecht University, The Netherlands
2025 Diploma Thai Board of Veterinary Medicine

Office

Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University
39 Henri Dunant Rd., Bangkok, Thailand 10330; e-mail: niwat.c@chula.ac.th

Experience and Awards

- Teaching, research and poultry extension services for more than 30 years local and international. Published more than 100 publications
- WOAH Charge De Mission, World Organisation for Animal Health (WOAH), Paris, France 2013
- President of Thai Association of Veterinary Laboratory Diagnosis (TAVLD) 2019- 2023
- Master Science Scholarship at Swedish University of Agricultural Science
- PhD Scholarship at Utrecht University
- Prince Mahidol Foundation Award to work in World Organisation for Animal Health (WOAH, Paris, France)
- Thai Veterinary Exemplary in Academics 2023 Awarded by Thai Veterinary Medical Association under the Royal Patronage

Current position:

- Professor at Chulalongkorn University
- Executive board: World Association of Veterinary Laboratory Diagnosticians (WAVLD)
- Administration board: Thai Veterinary Medical Association under the Royal Patronage
- Administration board: Thai Society of Mycotoxicosis
- Experts:
    * Egg Board, Ministry of Agriculture and Cooperative
    * Meat Board, Department of Livestock Development
    * National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperative
    * Food and Drug Administration, Ministry of Public Health

Dr. Thotsapol Thomrongsuwannakij

Office:
Walailak University, Akkhraratchakumari Veterinary College, 222 Thaiburi, Thasala District Nakhonsithammarat 80160 Thailand, Thotsapol.th@wu.ac.th

Educational Background

- Veterinary Medicine in Poultry, Faculty of Veterinary Science, Chulalongkorn University, Thailand, 2017
- Doctor of Veterinary Medicine (DVM), 1st class honours, Faculty of Veterinary Science,

Chulalongkorn University, Thailand, 2008

Work Experience

2018 Postdoctoral researcher fellow – Endeavour fellowship at The University of Queensland
2017 Postdoctoral research fellow at Chulalongkorn University

Expertise

1) Avian diseases
2) Antimicrobial resistance
3) Foodborne pathogens from poultry

Academic Achievement for 5 years ago

Research article

Thomrongsuwannakij, T., Charoenvisal, N. and Chansiripornchai, N. (2021). Comparison of two attenuated infectious bursal disease vaccine strains focused on safety and antibody response in commercial broilers. Veterinary World, 14(1): 70-77.
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Thomrongsuwannakij T., Blackall P.J. and Chansiripornchai. N. (2017). A Study on Campylobacter jejuni and Campylobacter coli through Commercial Broiler Production Chains in Thailand: Antimicrobial Resistance, the Characterization of gyrA Mutation, and Genetic Diversity by flaA-RFLP. Avian Diseases. 61(2): 186-197.
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Award and honor Award and honor Year

Endeavour Research Fellowships – Australian government scholarships, 2018

Professor Dr. Soontaree Petchdee

Educational Background

2021 Undergraduate: Diplomate of Asian College of Veterinary Internal Medicine (Cardiology)
2009 Ph.D.: Cardiac Electrophysiology
2006 M.Sc.: Physiology, Mahidol University
1998 Bachelor's Degree: Veterinary Medicine, Khon Kaen University

Expertise: Veterinary Cardiology

Work Experience

- 2009 - Present: Department of Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University

Research Interests:

My research interests in cardiac research incorporate basic biology, biochemistry, cardiac electrophysiology, translational, and clinical research.

Achievements in Award and Patents

- 2023 Petty Patent: "Process for extracting germinated brown rice extract," Agricultural Research Development Agency (ARDA) and Kasetsart University, 2023
- Outstanding Personnel Award in Research at the Kasetsart University Kamphaeng Saen Campus Foundation Day in 2013 and 2016

Excellent Poster Award, National Rice Conference, 2012, on "Effects of Germinated Brown Rice in Preventing Cardiovascular Diseases"

Dr. Pohonyoot Soraned

Position: Veterinary Practitioner

2014 Bachelor of Veterinary Medicine from the Faculty of Veterinary Medicine, Khon Kaen University

2014 Clinical Experience in Small Animal Internal Medicine and General Surgery, Small Animal Hospital, Faculty of Veterinary Medicine, Khon Kaen University

2020 – Present Neurology Specialty Clinic, Small Animal Hospital, Faculty of Veterinary Medicine, Khon Kaen University. Primary responsibilities in neurology cases and clinical practice.

Speaker's Notes

Experiences and Husbandry Practices for Premium Marbling in PhuPhan Beef Cattle

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Abstract

This abstract outlines the comprehensive development of PhuPhan Beef Cattle, a Wagyu breeding initiative in Thailand established under royal patronage. The program began in 1988 with the donation of purebred Tajima Wagyu cattle from Japan to the Thai Department of Livestock Development. Since then, the project has focused on crossbreeding and genetic enhancement to adapt Wagyu cattle to Thailand's tropical climate while preserving their superior marbling characteristics.

Key achievements include the establishment of the PhuPhan Beef Cattle breed, with up to 96.09% Wagyu genetic composition (F5 generation), and the development of optimized feeding and management practices suitable for small-scale farmers. Research within the program highlights the significance of the role of customized nutritional strategies. Challenges including heat stress and parasite resistance have been mitigated through adaptive husbandry techniques.

The program also places a strong emphasis on knowledge transfer through farmer training and international collaborations with Wagyu experts. Remarkably, PhuPhan Beef Cattle now achieves beef marbling scores (BMS) comparable to imported Wagyu, offering a sustainable and high-quality beef alternative within Thailand. This initiative aligns with the philosophy of His Majesty King Bhumibol Adulyadej, promoting low-cost, high-value agriculture as a pathway to rural development.

Keywords: PhuPhan Beef Cattle, Wagyu, Tajima genetics, Beef marbling score (BMS), F1 Wagyu crossbreeding, Thailand

Genetic Detection of Marbling Genes: A Tool for Enhancing Beef Quality

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Abstract

Marbling, defined as the intramuscular deposition of fat, is a key determinant of beef quality, contributing significantly to attributes such as tenderness, juiciness, and flavor. Recent advancements in genomics have facilitated the identification of major genes and genetic markers associated with marbling traits, including **Thyroglobulin (TG)**, **Diacylglycerol O-Acyltransferase 1 (DGAT1)**, **Fatty Acid Binding Protein 4 (FABP4)**, as well as **Calpain 1 (CAPN1)** and **Calpastatin (CAST)**, which are also critical for meat tenderness. In our laboratory, a tetra-primer amplification refractory mutation system polymerase chain reactions (ARMS-PCRs) methods were developed for the efficient detection of single nucleotide polymorphisms (SNPs) within these genes. The implementation of this cost-effective ARMS-PCR approach substantially reduces the expense of SNP genotyping, thereby enhancing accessibility to molecular breeding technologies for Thai cattle farmers. This advancement enables more precise selection for breeding and fattening purposes. The technology has been licensed to a student startup company, **Gen-A-Tech**, for further development and dissemination.

Keywords: Marbling, Beef quality, genetic markers, ARMs-PCR

Case Study: Dolphin Incursion into the Bang Pakong - Prachinburi River Estuary

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Abstract

The Bang Pakong River mouth, Chachoengsao Province has been an important habitat for Irrawaddy dolphins (*Orcaella brevirastris*), a species currently at high risk of extinction. Occasionally, Irrawaddy dolphins have been reported entering the Prachinburi River, located approximately 150 kilometers from the river mouth. The most recent case occurred on January 19, 2025, when an Irrawaddy dolphin was observed entering the Prachinburi River. The animal was found alive in Ban Sang District, Prachinburi Province, and the carcass was later recovered on January 22, 2025. Necropsy findings revealed that the dolphin was an elderly female in good body condition. Frothy exudate was observed emerging from the blowhole, and the cause of death was determined to be asphyxiation due to water aspiration. Histopathological examination of the lungs identified focal emphysema pulmonary edema and interstitial edema. No evidence of pathology related to prolonged freshwater exposure was observed. Other findings, such as mineralization in the renal medulla, were consistent with age-related changes and not considered contributory to the cause of death. This case provides important evidence of the Irrawaddy dolphin's ability to inhabit and thrive in freshwater environments, including river mouths with low salinity. Additionally, this study highlights patterns and causes of dolphin stranding in the Bang Pakong Estuary and Prachinburi River, offering valuable insights for improving stranding response plans and conservation strategies within the Bang Pakong River Basin.

Keywords: Irrawaddy dolphin, Stranding, Bang Pakong estuaries, Prachinburi River

Understanding the 3 key molecules in modern internal and external parasite protection

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Abstract

Parasite infection, such as those caused by helminths and arthropod ectoparasites, continue to cause significant morbidity and mortality in cats and dogs. These infections occur frequently despite the available and use of several very potent and selective antiparasitic drugs. In 2025, the FDA approved a new indication for a parasiticide combination of sarolaner, moxidectin and pyrantel chewable tablets for dogs. This approval makes the combination drug the only canine parasiticide indicated for the treatment of flea tapeworm (*Dipyridium caninum*) infections by killing the vector fleas (*Ctenocephalides felis*) before transmission occurs. Additionally, this newly approved drug is indicated for use against roundworms, including *Toxocara canis* (L5 and adults), *Ancylostoma caninum* (L4, L5, and adults), *Toxascaris leonina* (adults), *Uncinaria stenocephala* (adults), microfilariae of *Dirofilaria immitis, Angiostrongylus vasorum* (L5), and *Thelazia callipaeda* (adult eyeworm). For ectoparasite control, the combination drug can be used for the treatment and control of tick infestations with *Rhipicephalus sanguineus* (brown dog tick), *Amblyomma americanum* (lone star tick), *A. maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *D. reticulatus*, *Ixodes scapularis* (black-legged tick),*I. ricinus, I. hexagonus*, *Haemaphysalis longicornis* (Asian longhorned tick), *C. felis*, and *C. canis* for one month in dogs and puppies 8 weeks of age and older, weighing 1.25 kg or more. It is also indicated for the treatment of demodicosis caused by *Demodex canis* and scabies caused by *Sarcoptes scabiei* var *canis*. Furthermore, the way in which these drugs are used and integrated with other approaches to parasite control is critical for successful and sustained management of parasitic infections. Guidelines for optimizing the use of antiparasitic drugs include: 1. use of an effective drug class; 2. rotation among drug groups; 3. combination of drugs with different mechanism of action, and 4. combination of drugs with management strategies.

Keywords: Antiparasitic drugs, Moxidectin, Parasiticide, Pyrantel, Sarolaner

From Gut to Growth: Sustainable Nutrition Strategies in Pig Farming

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Abstract

The success of pig production is intricately tied to the principles of One Health and sustainability. Of all operational costs, feed constitutes 60–70% of total expenses on a pig farm. Specifically, dietary energy contributes approximately 60% of feed costs, while protein accounts for around 25%. Nonetheless, a considerable portion of dietary energy is lost, typically through feces (10–25%), urine (1–5%), and heat production (20–30%). Enhancing gut function is a pivotal strategy to mitigate these energy and protein losses. A healthier and more efficient gut improves digestion and nutrient absorption and supports the immune system, microbiome balance, and the gut-brain axis. These enhancements directly contribute to elevating the farm's Production Index (PI). Meeting the animal's nutritional requirements, especially by minimizing the Feed Conversion Ratio (FCR), supports both carcass leanness and overall animal health. Therefore, focusing on reducing FCR can significantly positively impact PI. Additionally, minimizing variation and identifying sources of variation within the farm are crucial for better management and performance optimization. Integrating data from various areas such as feed quality control, on-farm production metrics, carcass traits, and economic indicators is essential to formulate the most effective and cost-efficient diets for pigs.

Keywords: Gut function, Pig Farm, Feed Utilization, Energy&Protein Utilization, Production Index

Poultry Gut Health

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Abstract

Poultry gut health is a critical determinant of overall productivity, disease resistance, and growth performance in both commercial and backyard poultry systems. The gastrointestinal tract (GIT) serves not only as the site of nutrient digestion and absorption but also as a key immunological organ interfacing with the external environment. A healthy gut is characterized by a balanced and diverse microbiota, intact mucosal barriers, and regulated immune responses. Disruptions to this delicate ecosystem—whether from pathogens, dietary imbalances, antibiotics, or environmental stressors—can lead to dysbiosis, inflammation, and decreased performance. Current research emphasizes the role of gut microbiota in modulating host metabolism, enhancing immune function, and outcompeting pathogenic organisms. Feed additives such as prebiotics, probiotics, organic acids, and phytogenics are increasingly used as alternatives to antibiotics to support gut health and sustainable poultry production. Additionally, early-life programming, biosecurity measures, and nutritional strategies are being employed to optimize gut integrity from hatch to market. Understanding and managing poultry gut health is therefore fundamental not only for improving animal welfare and productivity but also for addressing global concerns related to food safety, antibiotic resistance, and environmental sustainability.

Keywords: Gut Health, Microbiome, Performance, Poultry

Bacteriophage characterization and application in poultry

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Abstract

The present study explores alternatives to antibiotics for poultry farms. The aims of this study were to isolate and characterize bacteriophages for selection of the appropriate phage, to reduce *Salmonella* in the gastrointestinal tract of broiler chickens and observe gut microbiota alterations after bacteriophage treatments. In this study, bacteriophages were isolated from 2 broiler chicken farms, 2 poultry processing plants, a goat farm and a pig farm in the central region of Thailand. Out of the 33 samples analyzed, 25 (75.5%) tested positive for the presence of *Salmonella* bacteriophages. Among the 63 isolates examined, SEpBS-1 was selected for its ability to infect five *Salmonella* serovars: *S.* Enteritidis, *S.* Hadar, *S.* Typhimurium, *S.* Dublin, and *S.* Poona.Thermal stability test of phages showed that phages were stable at -6.5 to 50°C for 30 minutes, and significantly decreased ($p < 0.05$) at 60°C, and drastically decreased at 70°C. Furthermore, pH stability test of phages showed that phages were stable at pH 5-9. Phage SEpBS-1 was stable in acidic condition. Phage titers decreased with increased salinity. The morphological characterization of the phage using transmission electron microscope (TEM) revealed icosahedral heads and thin, long, non-contractile, flexible tails. The phage SEpBS-1 was classified as a member of the *Siphoviridae* family. The growth curve of the bacteriophage revealed that phage SEpBS-1 for SE had a latent period of 2 h, burst time of 2-3.5 h and burst size of 166 pfu/infected cell. Phage SEpBS-1 for *S.* Typhimurium had a latent period of 2.5 h, burst time of 2.5-4 h and burst size of 973 pfu/infected cell. Studying the effects of phage SEpBS-1 against *Salmonella* infection in broiler chickens found that *Salmonella* counts were slightly increased at 7 and 14 days after phage treatment. However, there was no statistically significant difference between groups ($p > 0.05$). *Salmonella* counts decreased by 40% at 14 days, while the positive control found the highest number of *Salmonella* in ceca. The application of lytic bacteriophages in the biocontrol of foodborne pathogens presents a promising approach for targeting *Salmonella*. Bacteriophage therapy offers an effective alternative to antibiotics for pathogen control.

Keywords: bacteriophages, *Salmonella*, broiler chickens, livestock industry

Advancing Companion Animal Cardiology: Future Perspectives on Mitral Valve Repair in Dogs and Emerging Therapies for Hypertrophic Cardiomyopathy in Cats

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Part 1: Mitral Valve Disease (MVD) in Dogs

1. Mitral Valve Anatomy and Degenerative Disease: The mitral valve comprises the annulus, leaflets, chordae tendineae, papillary muscles, LA, and LV.; Degenerative mitral valve disease (MMVD) involves disorganization of these components, accumulation of proteoglycans, and collagen changes, and is prevalent in breeds like CKCS and Dachshunds and some small breeds.; MMVD grading ranges from Whitney type 0–4, with increasing structural distortion.
2. Diagnosis and Medical Treatment: Diagnosis via transthoracic echocardiography (TTE), ACVIM staging (B1–D) guides treatment, Medications: diuretics, vasodilators, pimobendan, spironolactone, ACE inhibitors.
3. Surgical and Minimally Invasive Interventions: TEER (Transcatheter Edge-to-Edge Repair): A minimally invasive alternative to open surgery, adapted from human MitraClip®.; Indications for M-TEER: Symptomatic/asymptomatic MMVD B2–D, LA dilation, or pulmonary hypertension with favorable anatomy.; Hybrid Approaches: Use of V-clamp devices via thoracotomy and transapical access.
4. Surgical Techniques: Posterior and anterior leaflet prolapse correction via triangular resection and edge-to-edge repair.; Open-heart surgery with cardiopulmonary bypass (CPB) and hypothermia (sHT) has been explored.

Part 2: Hypertrophic Cardiomyopathy (HCM) in Cats

1. Overview and Clinical Presentation: Affects 15–34% of cats; often subclinical.; Risks: CHF, sudden death, thromboembolism (ATE).; Diagnosis includes murmurs, SAM, LVOTO, and LA enlargement.
2. Diagnostic Tools: Cardiac Biomarkers: NT-proBNP, Troponin I.: Genetic Testing: MYBPC3 mutations (e.g., A31P in Maine Coons).: LAMP-LFD testing: Promising for on-site mutation detection.
3. Advanced Echocardiography: Use of 2D, TTE, tissue Doppler, strain imaging (longitudinal, radial, circumferential).; Strain echocardiography reveals early myocardial dysfunction.; LA remodeling assessed for stroke/AF risk.

4. Treatment Approaches
- CHF: oxygen, diuretics, thoracocentesis.
- ATE: clopidogrel, rivaroxaban, enoxaparin.
- Clopidogrel resistance is linked to genetic polymorphisms.
- Rivaroxaban/enoxaparin + clopidogrel showed effectiveness in reducing thrombosis and cardiac symptoms.

5. Emerging Therapies
- Myosin inhibitors: Aficamten, Mavacamten — reduce LVOT obstruction, improve relaxation.
- Rapamycin: Potential to delay hypertrophy progression.
- LAA Occlusion Devices: Under investigation for thrombus prevention in HCM.

Concluding Remarks
- For Dogs: M-TEER and hybrid surgeries are closing treatment gaps in advanced MMVD.
- For Cats: Genetics, biomarkers, advanced imaging, and targeted drugs are paving new directions for HCM care.
- Further research is essential to refine therapies and enhance survival and quality of life in companion animals.

Veterinary Diagnostics: Emerging Approaches in Medical Image Analysis

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Abstract

At present, Medical Imaging analysis has been playing an important role to be the non-invasive tools for enhancing ability of medical diagnosis and prediction in both human medicine and veterinary medicine e.g. pathological classification, grading abnormality, early disease detection, imaging biomarker-based prediction (prediction of disease progression, treatment outcome, or body composition). Radiomics, a subset of medical imaging analysis, is a task focusing on image processing, segmentation, feature extraction, feature selection, and statistical analysis/machine learning. In the last decade, the use of machine learning in both human medicine and veterinary medicine has been growing exponentially. Radiomics provides meaningful and quantitative features from medical images and serves as valuable input for these machine learning models. This presentation will inform about radiomics which would help veterinarians understand and gain more insight into the perception on medical images and raise awareness of limitations of human visualization to diagnosis tasks. Furthermore, this presentation also aims to inspire new research ideas; and to bridge the gap between traditional image interpretation and image biomarker-based diagnosis.

Keywords: Radiomics, Medical imaging analysis

Oral Presentation

Effects of *Crocodylus Siamensis* Liver Extracts on Glutathione S-Transferase Activity in vital organs of the Rats

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Background: *Crocodylus Siamensis*, a freshwater crocodile native to Southeast Asia, possesses an exceptional detoxification capacity, particularly through high hepatic glutathione S-transferase (GST) activity. Previous studies have shown that crocodile liver extracts could modulate detoxification enzymes and inhibit apoptosis in hepatocellular carcinoma cells.

Objective: This study aimed to investigate the effects of oral *C. Siamensis* liver extracts on GST activity and enzyme kinetics in the liver and kidney of the rats.

Methods: Male Wistar rats were divided into six groups (n= 5) and received oral administration of liver extract (50–800 mg/kg) for seven days. GST activity was assessed using CDNB as the substrate. Enzyme kinetics (V_{max}, K_m) were calculated by Michaelis-Menten modeling. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test.

Results: In the liver, GST activity increased significantly at 200–400 mg/kg, with V_{max} values of 0.67–0.90 µmol/min/mg protein and enhanced catalytic efficiency (V_{max}/K_m) observed, despite stable K_m values (around 1.3–1.7 mM). In the kidney, GST activity significantly increased only at 800 mg/kg, with a V_{max} of 0.50 µmol/min/mg protein and K_m of 0.38 mM. Enzyme kinetics revealed a biphasic response decreased efficiency at mid-doses (e.g., efficiency dropped to 0.18 at 100 mg/kg) and recovery at higher doses. These shifts in V_{max} and K_m suggest tissue-specific modulation of catalytic function and substrate affinity.

Conclusion: Oral administration of *C. Siamensis* liver extracts modulated GST activity in both liver and kidney with a dose-dependent and tissue-specific manner. These findings highlight the extract's potential role in enhancing xenobiotic detoxification pathways and provide a basis for further investigation into its use in other mammals.

Keywords: Glutathione S-transferase (GST), *Crocodylus Siamensis*, Liver extracts, Detoxification enzymes, Rat liver and kidney

Introduction

Crocodylus Siamensis, commonly known as the Siamese crocodile, is a freshwater crocodile species native to Southeast Asia and holds significant economic and ecological value in South East Asia. The species is known for its robust physiology and resilience to environmental toxins, which may be partly attributed to its efficient detoxification systems, including high activity of GST enzymes in the liver. Previous studies have indicated that the liver of *C. Siamensis* exhibits high GST levels compared to other organs, particularly GST isoforms pi and mu, which demonstrate high catalytic rates. Moreover, crocodile liver extracts have shown significant inhibition of apoptosis gene expression in AFB1-treated human liver cancer cells (HepG2). Glutathione S-transferase (GST) is a crucial enzyme involved in the detoxification process, especially in the liver, which serves as the primary organ for xenobiotic clearance. GST catalyzes the Phase II detoxification reaction by conjugating glutathione (GSH) to toxic compounds or reactive oxygen species, thereby reducing their toxicity and increasing their solubility for safe excretion from the body. Therefore, this study aimed to investigate the effects of orally administered of *C. siamensis* liver extract on GST enzyme activity in the liver and kidney of Wistar rats. Enzyme kinetic parameters (K_m and V_{max}) were analyzed to evaluate the functional changes in GST activity in these organs following treatment.

2. Materials and Methods

2.1. Animals and Treatment

Male Wistar rats 24 weeks were randomly divided into six groups (n = 5/group): control (PBS) and treatment groups receiving *C. Siamensis* liver extract at 50, 100, 200, 400, and 800 mg/kg BW via oral gavage. At the end of the treatment period, liver and kidney tissues were collected for analysis of detoxification enzyme activities. All procedures were approved by the Kasetsart University Research and Development Institute (ACKU61-VET-088). The protocol was adapted from Thiendedsakul et al. (2020).

2.2. Protein Determination

Protein concentrations of samples were determined by the Bradford assay to normalize GST activity, which was expressed as µmol/min/mg protein.

2.3. Preparation of Cytosolic Fraction

At the end of the treatment period, rats were anesthetized, and liver samples were collected. Liver tissues were homogenized in 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 10,000 × g for 15 minutes. The supernatant was further centrifuged at 105,000 × g for 60 minutes to obtain the cytosolic fraction. The cytosolic supernatant was stored at –80°C until enzyme analysis.

2.4. Determination of GST Activity

GST activity was measured spectrophotometrically using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. The change in absorbance at 340 nm was monitored in a buffer system containing GSH and CDNB at varying concentrations to determine kinetic parameters Km and Vmax by Michaelis-Menten kinetics. Data analysis was performed using GraphPad Prism software.

2.5. Statistical Analysis

All data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 10.3 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. GST Enzyme Activity in Rat Liver and Kidney

The enzymatic activity of glutathione S-transferase (GST) was measured in the liver and kidney tissues of Wistar rats following oral administration of *C. Siamensis* liver extract at varying doses. In liver, the groups receiving 200 and 400 mg/kg BW of the extract showed a significant increase in V_{max} compared to the control group ($p < 0.05$), whereas K_m values remained relatively unchanged. This suggests enhanced catalytic efficiency of GST without altering substrate affinity. In kidney, GST activity also increased, with significant elevation of V_{max} observed in the 400 and 800 mg/kg BW groups ($p < 0.05$). However, a slight increase in K_m values was noted, indicating a possible requirement for higher substrate concentration to achieve optimal enzyme activity in renal tissue.

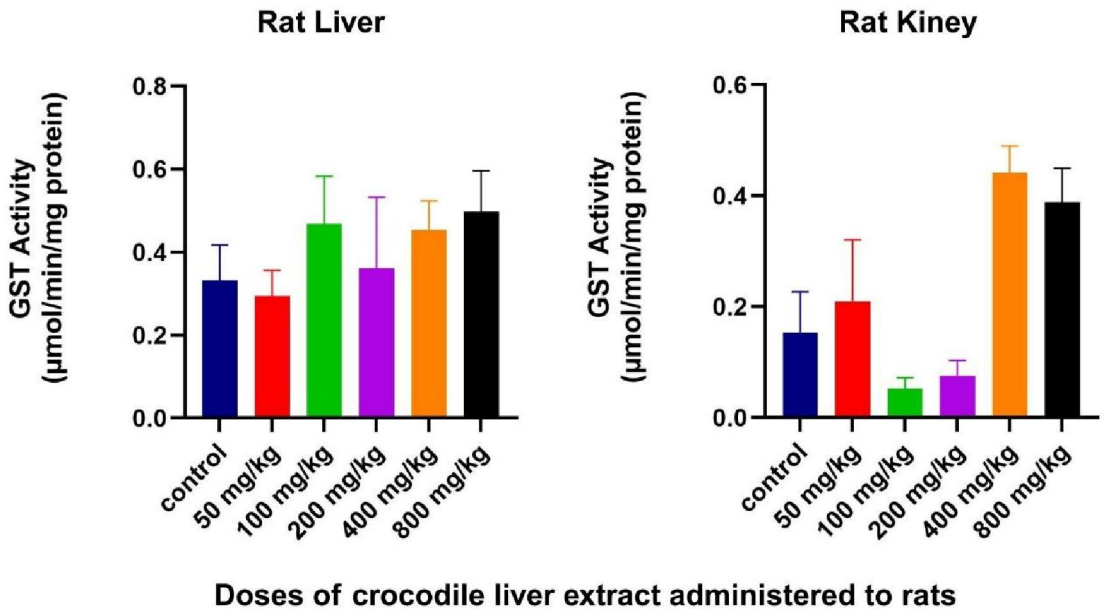

Figure 1 *Enzyme activitiy of glutathione S-transferase (GST universal) in liver and kidney tissues of rats following oral administration of C. Siamensis liver extract at different doses (control, 200, 400, and 800 mg/kg BW). Data are expressed as mean ± SD (n = 5 per).* Indicating of significant differences compared to the control group ($p < 0.05$) Although one-way ANOVA indicated significant overall differences among treatment groups in both organs ($p < 0.05$), Tukey's post hoc test did not identify significant pairwise differences between the groups in the liver but not for kidney.

3.2. Kinetics of GST Enzyme Activity

GST kinetic analysis revealed tissue- and dose-dependent effects of crocodile liver extract. In the liver, V_{max} decreased at 50 mg/kg but increased at higher doses, while K_m fluctuated slightly. Catalytic efficiency (Vmax/Km) improved in all treated groups, peaking at 800 mg/kg (0.53 mL/min/mg protein) versus control (0.39), suggesting enhanced GST function. In the kidney, V_{max} decreased at 100–200 mg/kg, with Km undetermined at 200 mg/kg. Catalytic efficiency dropped in mid-dose groups but recovered at 400–800 mg/kg. The control showed the highest efficiency (1.46 mL/min/mg protein). These results indicate a biphasic response in renal GST activity, contrasting the more consistent hepatic activation.

Table 1 Kinetic parameters of GST activity in rat liver and kidney tissues (n=5)

Organ	Treatment (mg/kg)	V_{max} (µmol/min/mg protein)	K_m (mM)	V_{max}/K_m ratio (ml/min/mgprotein)
Liver	Control	0.92	2.34	0.39
	50	0.46	1.23	0.37
	100	0.80	1.59	0.50
	200	0.67	1.29	0.52
	400	0.90	1.73	0.52
	800	0.73	1.39	0.53
Kidney	Control	0.16	0.11	1.46
	50	0.40	1.17	0.34
	100	0.06	0.35	0.18
	200	0.07	nd	nd
	400	0.59	0.54	1.10
	800	0.50	0.38	1.32

Remark; The kinetic parameters, V_{max} (maximum reaction velocity) and K_m (Michaelis constant), were determined from Michaelis-Menten kinetics using various concentrations of GST substrate (1, 2, 4, and 8 mM) for each treatment group (Control, 50, 100, 200, 400, 800 mg/kg) in rat liver and kidney tissues. The V_{max}/K_m ratio represents the catalytic efficiency. Data presented are derived from single Michaelis-Menten curve fits for each group. "nd" indicates that the parameter could not be determined due to a non-physiological value obtained from the curve fitting.

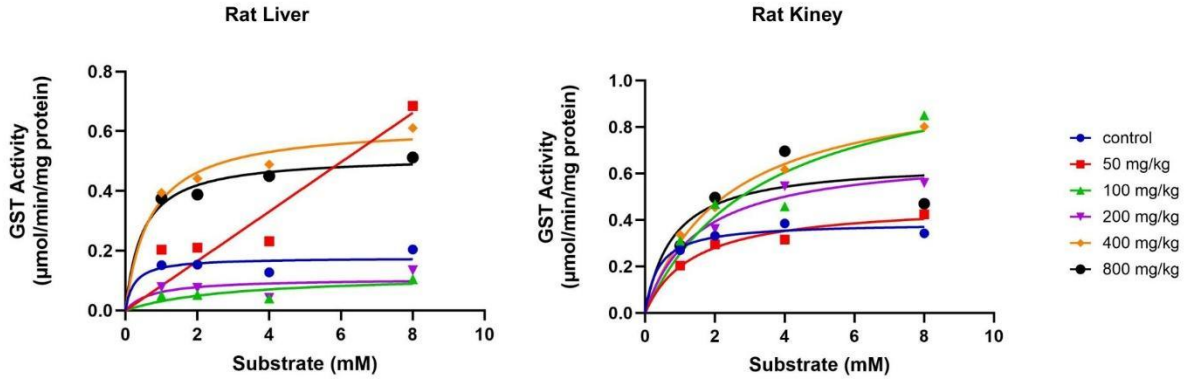

Figure 2 Lineweaver-Burk plots of GST activity in rat liver and kidney tissues. GST activity was determined at various concentrations of GST substrate (1, 2, 4, and 8 mM) for different treatment groups: control (0 mg/kg), 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg, and 800 mg/kg. The reciprocal of reaction velocity (1/v) was plotted against the reciprocal of substrate concentration (1/[S]). Kinetic parameters, V_{max} and K_m, were derived from the y-intercept ($1/V_{max}$) and x-intercept ($-1/K_m$) of the linear regression lines, respectively. Data points represent single measurements for each substrate concentration and treatment group.

4. Discussion

The present study investigated the effects of orally administered of *crocodile* liver extract on GST enzyme activity and its kinetic parameters in the rat liver and kidney. GST is a crucial detoxification enzyme which this finding could indicate that *C. Siamensis* liver contains remarkably high hepatic GST activity. **In the rat liver tissues**, the universal GST activity showed no statistically significant differences compared to the control group ($p > 0.05$). However, analysis of kinetic parameters (Table 1) revealed that while Vmax initially decreased at 50 mg/kg,however, it turned with an increasing trend at higher doses of200 and 400 mg/kg), suggesting a potential of high speed activation of hepatic GST function. Km values remained largely unchanged, indicating stable substrate affinity. The catalytic efficiency (Vmax/Km) generally improved across most treated groups, aligning with the inherent high detoxification capacity observed in crocodiles themselves. **In rat kidney tissues**, the effects were more pronounced. Overall GST activity for the dose of 400 mg/kg and 800 mg/kg groups showed a **statistically significant increase** compared to the control group ($p < 0.05$). The kinetic analysis (Table 1) indicated a complex, biphasic response. Lower to mid-doses (50-200 mg/kg) generally led to decreased Vmax and catalytic efficiency, with the Km for 200 mg/kg group being problematic. Conversely, at higher doses of 400 and 800 mg/kg, Vmax significantly increased, and catalytic efficiency recovered to levels comparable to the control. This suggested that while some extract concentrations might transiently affect kidney GST, higher doses may induce a compensatory or beneficial activation.

5. Conclusion

This study demonstrates that oral administration of *crocodile* liver extract modulates GST enzyme activity in the liver and kidney of the rat, as a dose-dependent and tissue-specific manner. In the liver, the extract generally led to an enhancement of GST catalytic efficiency, consistent with the known high detoxification capacity of the crocodiles offal. In contrary, the GST in the rats showed a biphasic response, with lower

to mid-doses potentially impacting enzyme function, while higher doses resulted in a statistically significant increase in overall activity and restored to enhanced of catalytic efficiency.

These findings provide evidence that the extract influences xenobiotic detoxification pathways, highlighting its potential physiological effects from reptile like crocodile into the mammal species like the rat. Further research into the underlying molecular mechanisms responsible for these observed changes is warranted and the innovative use of this beneficial finding in other companion animals would also be planned.

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Modeling Hookworm Risk in Dogs Using Random Forest: A Machine Learning Toolkit Approach

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Abstract

Objective This study aimed to identify environmental and host-related risk factors associated with hookworm infection in owned dogs in Chaiyaphum Province, Thailand, using a hybrid spatial random forest model.

Methods A cross-sectional survey was conducted during August–September 2023 in 12 villages of Khon Sawan District. Fecal samples were collected from 221 dogs across 143 households. Environmental and demographic predictors were evaluated using the spatialRF package in R. Feature selection was performed with the mRMR algorithm, and model residuals were tested for spatial autocorrelation using Moran's I.

Results The individual-level prevalence of hookworm infection was 23.53%. Normalized Difference Vegetation Index (NDVI) was identified as the top global predictor, followed by land surface temperature and proximity to open water. No spatial autocorrelation was found in model residuals. Risk mapping revealed distinct high-risk clusters, especially in the Si Samran sub-district.

Conclusion Hookworm infections in dogs were primarily driven by environmental factors in this study area. The absence of spatial confounding supports the potential of the non-spatial model. Findings highlight the need for targeted interventions in high-risk areas, including environmental sanitation and regular deworming.

Keywords: dogs, environmental risk, hookworms, spatial random forest

1. Introduction

Hookworms are parasitic nematodes that inhabit the small intestines of mammalian hosts, including humans, dogs, and cats. They cause helminthiasis, one of the most prevalent soil-transmitted infections and a major neglected tropical disease (Hossain and Bhuiyan, 2016; Tenorio et al., 2024). In Thailand, hookworm infection is the most commonly reported parasitic disease in dogs and cats, which serve as reservoirs. Dogs, as definitive hosts, acquire infection through ingestion or skin penetration of third-stage larvae. Clinical signs include chronic intestinal blood loss, iron-deficiency anemia, and cutaneous larva migrants (Wongwigkan and Inpankaew, 2020).

Machine learning (ML) models such as the random forest can capture complex, non-linear associations without strict data assumptions (Wiemken and Kelley, 2020). The recent development of hybrid spatial random forest offers a novel approach for integrating spatial regression with the random forest algorithm. (Benito, 2021).

Despite advances in ML-based modeling, few studies have applied these techniques to investigate environmental and host-level risk factors for hookworm infection in companion animals within endemic regions. There remains a need to understand how spatial and environmental factors influence dynamic infection, particularly in rural areas. This study aimed to explore environmental and host-related risk factors for hookworm infections in owned dogs in Chaiyaphum Province, Thailand, using random forest and its spatial extension as a machine learning framework for veterinary epidemiological analysis.

2. Materials and Methods

2.1 Study Area

Dog fecal samples were collected from August to September 2023 across 12 villages in Khon Sawan District, Chaiyaphum Province, northeastern Thailand.

2.2 Animal Ethics and Sample Size

The study protocol was approved by the Khon Kaen University Animal Ethics Committee IACUC-KKU(C)- 1/67. A total of 221 dogs from 143 households were randomly selected for the study. Eligible dogs included all breeds and both sexes, conditional upon owner consent. Exclusion criteria included dogs under three months of age, pregnant or lactating females, and those that were visibly ill or clinically unstable.

2.3 Animal Fecal Sample Collection

After signing of owner's consent, each dog was clinically examined, and a saline enema was administered to collect the feces. Approximately 2 grams of feces were analyzed using the modified formalin-ethyl acetate concentration technique (M-FECT). Microscopic examination focused on detecting *Ancylostoma* spp. eggs based on morphological characteristics.

2.4 Data Collection and Processing

Owner and animal demographic data were recorded via a structured interview. Environmental variables were retrieved from publicly available geospatial datasets (**Table 1**). To enhance model performance and minimize redundancy, feature selection was conducted using the Minimum Redundancy Maximum Relevance (mRMR) algorithm. Additionally, exploratory data analysis (EDA) was performed to examine variable distributions and relationships. Predictor variables were further evaluated for multicollinearity, spatial autocorrelation, and potential interaction effects. These screening steps were implemented using built-in functions provided by the spatialRF package,

ensuring that only relevant, non-redundant, and spatially unbiased variables were retained for model development.

2.5 Model Fitting and Spatial Diagnostics

Random Forest (RF) models were implemented using the spatialRF package in R version 4.3.2 (R Core Team, 2023). Model development followed the framework described by Breiman (2001). Initially, a non-spatial RF model was fitted. Spatial autocorrelation in model residuals was assessed using Moran's I statistic across multiple distance thresholds. If significant spatial autocorrelation had been detected, a spatial RF model would have been implemented.

2.6 Variable Importance and Spatial Interpretation

Global permutation importance was computed on out-of-bag (OOB) data to identify overall predictor contributions. In addition, local variable importance was mapped to examine spatial heterogeneity in predictor influence across the study areas.

2.7 Risk Surface Mapping

Model predictions were visualized as continuous surface risk maps using Inverse Distance Weighting (IDW) interpolation, allowing identification of high-risk zones for hookworm infection in dogs.

3. Results

3.1 Prevalence of hookworm infections in dogs

Hookworm infection was detected in 52 of 221 dogs (23.53%; mean EPG: 125.93 ± 274.75). At the household level, 43 of 143 households were infected (30.07%; 43/143).

3.2 Spatial autocorrelation and modeling

Figure 1 presents the spatial autocorrelation (Moran's I) of the response and predictor variables across distance thresholds (0–3 km). All variables exhibited low Moran's I values with p-values ≥ 0.05, indicating no significant spatial autocorrelation. Following model fitting, residuals were not normally distributed, as shown by the Shapiro-Wilk test ($W = 0.854$, $p < 0.001$). Nonetheless, Moran's I values calculated for model residuals at multiple distance thresholds (0–3 km) remained low and statistically non-significant ($p \geq 0.05$), suggesting that spatial dependence was not a concern in the final model. Therefore, residuals showed no spatial structure, the non-spatial model was retained.

3.3 Global and Local Variable Importance

Global permutation importance identified NDVI as the top predictor (**Figure 2**), with its strongest local influence observed in northeastern clusters, highlighting spatial heterogeneity (**Figure 3**). Land surface temperature ranked second, showing consistent positive effects, particularly in the southern and northeastern areas. Proximity to open water ranked third, with higher importance concentrated in the same regions, indicating localized relevance. Soil wetness showed uniformly positive contributions, especially in the southern cluster, suggesting a stable influence. Minimum land surface temperature demonstrated moderate but consistent local importance across the study area. Conversely, the 40–60-year-old owner group exhibited negative global importance, indicating minimal predictive contribution in most locations.

3.4 Surface risk map

The spatial prediction map shows heterogeneity in hookworm infection risk across Khon Sawan District, Chaiyaphum Province. High-risk clusters were concentrated in Si

Samran sub-district, while low to moderate values appeared in both Si Samran and Khok Mang Ngoi. Lower risk was mainly found in southwestern Khok Mang Ngoi (**Figure 4**).

4. Discussion

Our study revealed a 23.53% individual-level prevalence of hookworm infection in dogs, consistent with global patterns indicating high burdens in tropical regions (Hossain and Bhuiyan, 2016). The results of the present study identified NDVI as the top global predictor, highlighting its significant association with infection risk. Similar findings have been reported in human helminth studies, where elevated NDVI was strongly linked to increased hookworm prevalence (OR = 32.5) (Cociancic et al., 2021). This relationship may be attributed to the environmental conditions in forested areas, where shaded and humid microclimates help retain soil moisture, thereby promoting the survival of soil-transmitted helminth larvae (Ngui et al., 2014). Mean and minimum land surface temperature also showed a positive influence, suggesting that suitable temperature ranges facilitate larval development. A previous study reported the highest hookworm prevalence at a mean temperature of 25.1°C and relative humidity of 68%, with infection rates declining at more extreme values (De et al., 2017).

Furthermore, proximity to open water bodies emerged as a locally important factor, particularly within the northern and southern clusters. Wet environments near rivers and ponds help sustain soil moisture, which is essential for the survival and longevity of hookworm larvae (Forrer et al., 2015). Similarly, soil wetness highlights the role of humidity in sustaining transmission in riverine areas. Statistical tests (Shapiro-Wilk, Moran's I) revealed no spatial autocorrelation in residuals, indicating that the non-spatial regression captured key drivers without bias from spatial clustering.

Our findings indicate that hookworm infections in dogs are predominantly driven by environmental conditions. This aligns with similar ecological patterns in human and animal studies, emphasizing the need to monitor cross-species transmission risks (Arcenillas-Hernández et al., 2024). Prevention efforts should prioritize environmental management in wet, vegetated areas, including fecal hygiene and strategic deworming. The hybrid spatial random forest model effectively identified high-risk zones, supporting targeted surveillance and control interventions.

5. Conclusion

This study identified key environmental risk factors associated with hookworm infections in owned dogs in Chaiyaphum Province, Thailand. A high individual-level prevalence of 23.53% was observed, highlighting the public and veterinary health importance of this parasite. Random forest modeling with spatial diagnostics, revealed that NDVI, land surface temperature, proximity to water, and soil wetness were the most influential predictors of infection. These findings emphasize the role of humid, vegetated environments in sustaining transmission. The integration of ML with spatial epidemiology offers a powerful approach for identifying high-risk areas and guiding targeted interventions. The modeling framework demonstrated here may be applicable to other parasitic diseases in similar settings, supporting future One Health surveillance and control programs.

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7. Table and figures

Table 1 Environmental and spatial variables used in the analysis.

	Variables	Data Sources	Resolution	Time frame	Processing Tools
1	Precipitation	CHIRPS (UCSB-CHG/CHIRPS/DAILY)	~5 km (daily)	2019 – 2023	Google Earth Engine (GEE)
2	Land Surface Temperature – Min	MODIS Night-time LST (MODIS/061/MYD11A2)	1,000 m	2019 – 2023	GEE
3	Land Surface Temperature – Max/Mean	Landsat 8 (LANDSAT/LC08/C02/T1_L2)	30 m	2019 – 2023	GEE
4	NDVI	MODIS/061/MCD12Q1(LC_Prop1)	500 m	2019 – 2023	GEE
5	Soil wetness	NASA/SMAP/SPL4SMGP/007 (sm_surface_wetness)	~9 km	2019 – 2023	GEE
6	Elevation	DIVA-GIS (https://diva-gis.org)	Variable (~90 m SRTM)	Static	GEE + QGIS
7	Proximity to Water Bodies	Derived from surface water layers or hydrological data (not specified)	Variable	Static	GEE + QGIS
8	Administrative Boundary	'USDOS/LSIB_SIMPLE/2017' (for clipping)	N/A	Static	GEE

Note. GEE; Google Earth Engine, QGIS; Quantum Geographic Information System

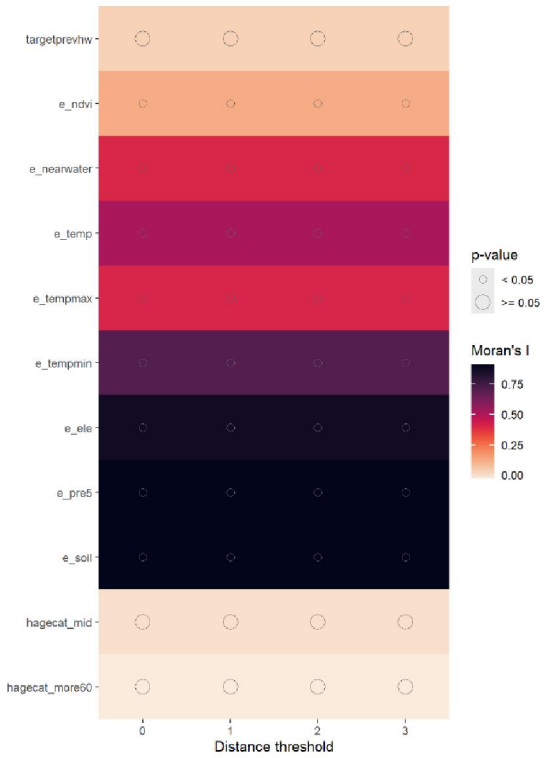

Figure 1 Spatial autocorrelation of hookworm infection and predictor variables across distance thresholds.

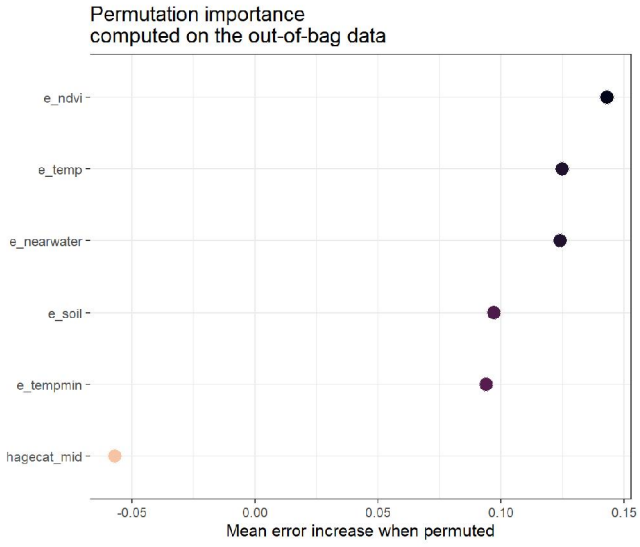

Figure 2 Global variable importance ranked by mean error increase.

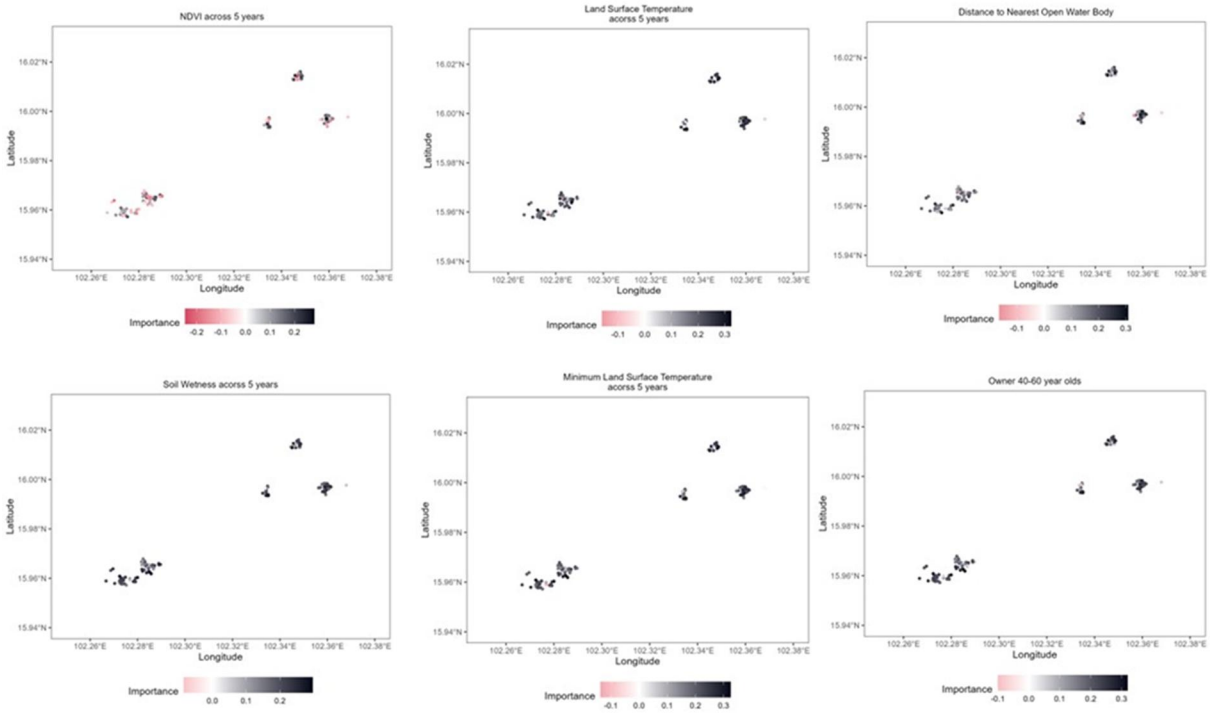

Figure 3 Mapped local variable importance shows spatial heterogeneity in predictor influence across the study area.

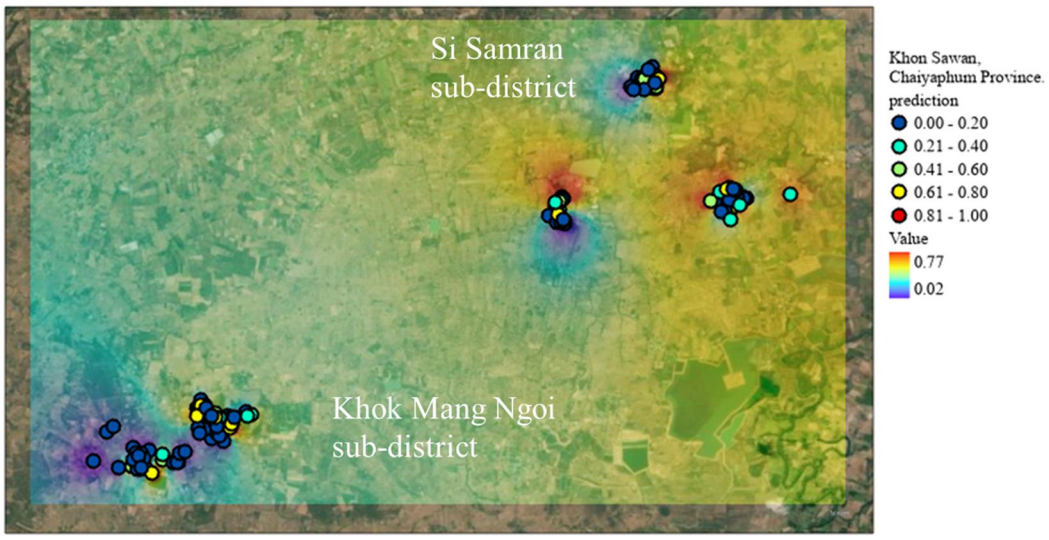

Figure 4 Localized risk distribution of canine hookworm Infection in two sub-districts of Chaiyaphum Province.

Impact of Sperm Sexing on Functional Sperm Parameters in Small Ruminants: A Meta-Analysis

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Abstract:

Background: Sperm sexing is increasingly used in small ruminant breeding to control offspring sex, but its impact on sperm quality remains unclear. This meta-analysis evaluates the effects of sexing techniques on key sperm quality parameters in small ruminants.

Methods: A systematic review was conducted in April 2024 following PRISMA-P guidelines, using PubMed, Scopus, EBSCO, ProQuest, and Google Scholar. Thirteen observational studies (2003–2024) on sheep and goat sperm sexing were included. Screening and data extraction were independently performed by two reviewers using the Rayyan platform. Meta-analyses were performed in R (v4.3.1) using a random-effects model to calculate standardized mean differences (SMDs) with 95% confidence intervals (CIs). Heterogeneity (I^2) and publication bias were assessed using standard methods.

Results: The analysis revealed significant reductions in motility (SMD = −1.03 [−1.66, −0.40], progressive motility (SMD = −0.67 [−0.84, −0.49]), ATP levels (SMD = −1.14 [−2.01, −0.27]), viability (SMD = −1.49 [−3.02, 0.03]), VSL (−0.71 [−0.98, −0.44], and linearity (SMD = −0.08 [−2.03, 1.87]). Plasma membrane integrity (SMD = −0.53 [−0.90, −0.15]) and mitochondrial activity showed a significant decline (SMD = −1.24 [−2.80, 0.32]). In contrast, acrosome integrity (SMD = −0.17 [−0.36, 0.03]) and VAP (−0.85 [−1.12, −0.58]) were not significantly affected.

Conclusion: Sperm sexing in small ruminants disrupts motility, viability, progressive motility, VSL, LIN, plasma membrane integrity, mitochondrial activity, and ATP levels, potentially reducing fertilization capacity. Refinement of gentler and precise protocols is needed to minimize these side effects.

Keywords: sperm sexing, small ruminants, and sperm quality.

1. Introduction

Selective control of offspring sex through sperm sexing has significantly advanced genetic improvement strategies in livestock. In small ruminants such as sheep and goats, sexed semen enhances breeding efficiency, accelerates genetic gain, and improves economic outcomes. For example, male biased progeny is preferred in meat production (Hossein-Zadeh et al., 2010), while in dairy goats, female offsprings are economically favorable due to lower rearing costs and higher returns (Meijer et al., 2021). Sheep contribute to the livestock sector through wool, meat, and milk production (Moradi et al., 2022).

Researchers have long sought effective methods to separate X- and Y-chromosome bearing spermatozoa. Among sexing methods, flow cytometric sorting remains the most widely used due to its high accuracy (~90%), relying on differential DNA content between X- and Y-chromosome bearing spermatozoa (Magata et al., 2021; Ortega Ferrusola et al., 2017). Alternative techniques include Percoll and albumin gradient centrifugation (Suprayogi et al., 2022; Wolf et al., 2018), swim-up (Azizeddin et al., 2014), immunological separation (Liu et al., 2021; Sringarm et al., 2022), and TLR 7/8 (Huang et al., 2022; Ren et al., 2021; Setiawan et al., 2024). However, the sex-sorting process exposes bovine sperm to physical and chemical stresses, including centrifugation, incubation, DNA staining, and laser exposure, leading to molecular alterations that impair motility, viability, acrosome integrity, membrane stability, mitochondrial function, and sperm-oviduct interactions (Bermejo Alvarez et al., 2008; Carvalho et al., 2018; Pirez et al., 2020).

A good sperm sexing process is important to maintain its fertilization potential. Fertilization by sperm involves a series of physiological processes, including capacitation, hyperactivation, and the acrosome reaction (Rotimi et al., 2024). Thus, yet, findings on the impact of sexing procedures on these traits in small ruminants remain inconsistent. This meta-analysis synthesizes available evidence to evaluate the effects of sperm sexing on key sperm quality parameters in small ruminants.

2. Materials and Methods

2.1. Ethical approval

This particular kind of study has no requirement for ethical approval.

2.2. Search strategies

This research was conducted in April 2024 through a comprehensive literature search using databases including PubMed, Scopus, EBSCO, ProQuest, and Google Scholar. The search employed the keywords: 'ruminant,' 'sheep,' 'goat,' 'sexing,' and 'spermatozoa.' Article screening and selection followed the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P)(Page et al., 2021).

2.3. Screening and Data extraction

All retrieved records were imported into the Rayyan Intelligent Systematic Review platform for initial screening, which was conducted independently by three reviewers. The studies included in the analysis were observational in design and contained data on ruminant (sheep and goat) sperm sexing and quality parameters of sperm. Disagreements were resolved through discussion or by a fourth reviewer. The chosen papers comprised 13 studies on sheep and goat that were published from November 2003 to September

2024. Data was extracted to Excel by the main reviewer and verified by a second reviewer. The data includes the author, year of publication, type of animals, study location, language, sample size, quality parameter of sperm sexing, and methods of sperm sexing.

2.4. Statistical Analysis

Statistical analyses were performed using R software (v4.3.1), employing the 'meta' and 'metafor' packages to conduct random-effects meta-analyses. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were calculated to assess effect sizes across studies. The parameters analyzed included motility, progressive motility, straight line velocity (VSL), average path velocity (VAP), linearity (LIN), plasma membrane integrity (PMI), viability, mitochondrial activity, acrosome integrity, and adenosine triphosphate (ATP) levels. Heterogeneity was evaluated using the I^2 statistic, p-value, and Q score. Publication bias was assessed via funnel plots and Egger's test to detect potential asymmetry in effect size distributions.

3. Results

A meta-analysis was conducted on 10 sperm quality parameters across multiple studies assessing the impact of sperm sexing techniques on small ruminants. In total, 1,632 studies were screened, resulting in 13 included studies. The SMDs, CIs, heterogeneity indices (I^2), and statistical significance for each parameter are summarized in Table 1.

Sperm sexing significantly reduced motility, with an SMD of –1.03 (95% CI: –1.66 to –0.40; $p < 0.0001$). This reflects consistent declines in overall motile sperm count post-sexing across the 13 included studies. Progressive motility also declined significantly (SMD = –0.67; 95% CI: –0.84 to –0.49; $p = 0.0001$).

Table 1. Summary of meta-analysis results assessing the impact of sperm sexing on functional sperm parameters in small ruminants

Parameter	No of study	SMD	95% CI	I^2	p-value
Motility	13	−1.03	[−1.66; −0.40]	83.0%	p < 0.0001
Viability	4	−1.49	[−3.02; 0.03]	86.4%	p <0.0001
Progressive motility	7	−0.67	[−0.84; −0.49	49.1%	p = 0.0001
Straight line velocity (VSL)	7	−0.71	[−0.98; −0.44]	34.2%	p = 0.0363
Average path velocity (VAP)	7	−0.85	[−1.12; −0.58]	10.1%	p = 0.3089
Linearity (LIN)	3	−0.08	[−2.03; 1.87]	82.2%	p < 0.0001
Plasma membrane integrity (PMI)	7	−0.53	[−0.90; −0.15]	55.4%	p = 0.0003
Acrosome integrity	9	−0.17	[−0.36; 0.03]	17.5%,	p = 0.1636
Mitochondria activity	3	−1.24	[−2.80; 0.32]	80.6%	p < 0.0001
ATP level	3	−1.14	[−2.01; −0.27]	81.2%	p < 0.0001

A reduction in viability was observed (SMD = –1.49; 95% CI: –3.02 to 0.03; p < 0.0001). Similarly, a statistically significant decline in PMI was reported (SMD = –0.53; 95% CI: –0.90 to –0.15; p = 0.0003).

A statistically significant decrease in VSL was found (SMD = –0.71; 95% CI: –0.98 to –0.44; p = 0.0363). contrary, VAP, no significant, with an SMD of –0.85 (95% CI: –1.12 to –0.58; p = 0.3089).

Linearity significantly decreased (SMD = –0.08; 95% CI: –2.03 to 1.87, p < 0.0001). whereas no significant effect for acrosome integrity (SMD = –0.17; 95% CI: –0.36 to 0.03; p = 0.1636).

Mitochondria activity showed a significant decrease (SMD = –1.24; 95% CI: –2.80 to 0.32; p < 0.0001). A statistically significant decrease in ATP levels was noted (SMD = –1.14; 95% CI: –2.01 to –0.27; p < 0.0001).

4. Discussion

This meta-analysis highlights the detrimental effects of sperm sex determination procedures on key functional sperm parameters in small ruminants, with important implications for offspring sex control of significant economic and genetic importance, as well as assisted reproductive technologies (ARTs) such as artificial insemination (AI) and in vitro fertilization (IVF).

Motility, a key kinematic parameter, is widely regarded as a primary indicator of male fertility, as it underpins sperm migration through the female reproductive tract and successful gamete interaction at fertilization (Robayo et al., 2008). In this study, motility was significantly reduced in sexed sperm (SMD = –1.03), indicating compromised sperm movement and fertilization capacity. The observed heterogeneity (I^2 = 83.0%) may stem from differences in species, sexing protocols, the environment of spermatozoa, and semen extenders used across studies (He et al., 2023; O'Brien et al., 2003; Yotov et al., 2021). Comparable losses have been reported for flow-sorted porcine, approximately 15% ((Winters et al., 2018) and bovine semen (Carvalho et al., 2018) underscoring the motility compromising impact of the sorting process.

Progressive motility refers to the directed, straight movement, which is also decreased (SMD = –0.67), reflecting impaired directional movement essential for oocyte penetration and a critical trait for successful navigation through the female reproductive tract (Hollinshead et al., 2003;Li et al., 2016; Moscatelli et al., 2017; Tanga et al., 2021). Similar declines have been documented in bovine (Carvalho et al., 2018; Palma et al., 2008) and stallion sperm (Balao da Silva et al., 2013), suggesting that sorting procedures negatively affect directional movement. These reductions are likely attributable to sorting induced stressors such as flagellar damage, mitochondrial dysfunction, from laser and fluorescent dye exposure during flow cytometry (De Graaf et al., 2006; Garner & Seidel, 2008; O'Brien et al., 2003).

Our findings confirm that sperm sexing significantly compromises membrane related sperm function in small ruminants. While the overall significantly decreased in viability (SMD = –1.49). Similarly, PMI showed a significant decline (SMD = –0.53), confirming vulnerability to mechanical and oxidative stress during flow cytometry, centrifugation, and incubation (Mocé et al., 2006; Quan et al., 2015; Suprayogi et al., 2022). Flow cytometric sex sorting exerts detrimental effects on sperm quality in small ruminants, leading to reduced DNA integrity, compromised plasma membrane structure, and increased apoptotic activity, all of which negatively impact fertilization (Holden et al., 2017), Similar effects are reported in bovine sex sorted (Carvalho et al., 2018). These processes generate reactive oxygen species (ROS) that disrupt membrane permeability and reduce the longevity of sperm (Mishra et al., 2018; Steele et al., 2020). As membrane integrity is critical for sperm metabolism, capacitation, ova binding, and acrosome reaction, and consequently fertilization and subsequently embryo development, these findings support the development of protective protocols to enhance post sorting sperm function (Allai et al., 2018; He et al., 2023; Li et al., 2018; Tanga et al., 2021).

In the present study, sperm sexing significantly reduced VSL, while VAP remained unchanged. Similar results were reported in stallions, where both VSL and VCL declined post sorting, with no effect on VAP (Balao da Silva et al., 2016). Conversely, Steele et al., (2020) reported a significant VAP reduction in bovine sexed sperm. These velocity parameters, VSL, VAP, and VCL, are key indicators of fertilization potential (De Graaf et al., 2006; Tanga et al., 2021). The decrease in VSL (SMD = –0.71) likely reflects the influence of flagella and mitochondrial activity during sorting or altered sperm velocity (Blondin et al., 2009; Rotimi et al., 2024). Despite variability in protocols, studies incorporating antioxidant supplements showed better outcomes, underscoring the potential to mitigate motility loss through optimized methodologies (Qin et al., 2018; Suprayogi et al., 2022).

Likewise, LIN showed a significant decrease (SMD = –0.08), possibly due to increased hyperactivation or oxidative stress in ram sperm (De Graaf et al., 2006). This decline, driven by reduced VSL relative to VCL, may reflect hyperactivated motility, as seen in bull sperm (Missio et al., 2018). This effect reflects the possible sensitivity of LIN in sperm to sorting conditions and extender composition.

Acrosome integrity, in contrast, remained stable (SMD = –0.17), indicating that the acrosomal membrane is relatively resistant to sorting-induced stress. This aligns with findings in goat, bovine, and mouse sperm sorted with resiquimod (R848), which reported negligible acrosomal damage (Huang et al., 2022; Ren et al., 2021; Umehara et al., 2019; Wen et al., 2023) and by flowcytometry on sorted boar semen, likewise retains motility, plasma membrane, and acrosome integrity (del Olmo et al., 2013). On the other hand,

several studies have shown that X/Y-chromosomes bearing spermatozoa for in vitro fertilization exhibit reduced fertilization rates, largely due to damage incurred during the sorting process. Factors such as centrifugation, incubation, exposure to DNA stains, and physical stress from lasers and electrical charges compromise sperm integrity (Bermejo-Álvarez et al., 2008). In stallions, sex sorting increased acrosome-reacted sperm, likely due to the loss of loosely bound stabilizing proteins from high dilution and hydrostatic pressure, which destabilizes the plasma membrane and promotes acrosome reaction (Balao da Silva et al., 2013). Moreover, post-thaw analysis showed increased numbers of acrosome reactive sperm in sorted bull and female sperm compared to unsorted sperm (Hollinshead et al., 2003; Mocé et al., 2006). Additional impairments include altered motility, reduced lifespan, premature capacitation, and accelerated acrosome reactions, all of which disrupt the acrosomal function essential for successful fertilization (Aitken, 2017; Mocé et al., 2006). Several studies even reported preserved or improved acrosomal integrity, supporting continued use of sexed semen in IVF programs (Rath et al., 2009; Ren et al., 2021).

Sperm sexing was associated with marked impairments in mitochondrial activity (SMD = –1.24) and ATP levels (SMD = –1.14), reflecting significant energy metabolism disruption (Balao da Silva et al., 2016; He et al., 2023). Comparable ATP reductions have been reported in stallion sperm (Balao da Silva et al., 2013) and X-chromosome bearing sperm of cattle and mice (Umehara et al., 2019; Umehara et al., 2020; Wen et al., 2023). These effects likely result from reduced mitochondrial activity, increased ROS, mechanical or phototoxic stress during the sperm sorting process (Balao da Silva et al., 2016; Madeja et al., 2021; Ren et al., 2021; Setiawan et al., 2024). The resulting ATP depletion undermines flagellar motility, membrane stability, and fertilization potential (Balao da Silva et al., 2013; Carvalho et al., 2018).

5. Conclusion

This meta-analysis revealed that sperm sexing in small ruminants adversely affects motility, viability, progressive motility, VSL, LIN, plasma membrane integrity, mitochondrial activity, and ATP levels, which may affect the ability of sperm to fertilize oocytes (fertilization ability). Although acrosome integrity and VAP were less affected, the considerable variability across studies underscores the need for gentler and precise protocols.

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Molecular Prevalence of Canine Babesiosis Across Asia: A Systematic Review and Meta-Analysis

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Abstract

Background: Canine babesiosis is a globally recognized tick-borne disease caused by intraerythrocytic parasites of the *Babesia* genus. It poses a serious threat to dog populations, especially in tropical and subtropical regions such as Asia. This study aimed to provide a comprehensive systematic review and meta-analysis of the molecular prevalence and evaluate potential factors contributing to prevalence variation.

Methods: A systematic search was conducted using PubMed, Scopus, EBSCOhost, and Google Scholar to identify relevant studies published from 2001 to April 2024. Only molecular-based, cross-sectional, or survey studies on canine babesiosis in Asia were included. Statistical analyses, including subgroup and meta-regression, were performed using the R software to examine variations in prevalence based on factors such as co-infection status, age, sex, breed, ownership status, target gene, and identified *Babesia* species.

Results: From a total of 4,859 publications, 114 studies were included to the study. The overall pooled molecular prevalence of canine babesiosis in the Asia region was estimated at 11.89% (95% CI: 9.58–14.68). Subgroup analysis revealed significant variation by co-infection status and *Babesia* species. Single infection was most common, with prevalence rates of 9.69%. Among the identified species, *Babesia gibsoni* was the most detected, with prevalence rates of 13.27%. The *18S rRNA* gene was the most frequently used molecular target. The funnel plot asymmetry and Egger's test ($p<0.01$) suggested potential publication bias.

Conclusion: The molecular prevalence of canine babesiosis across Asia is estimated at 11.89%. Regular monitoring and control strategies of babesiosis in dogs, especially in endemic regions, remain important to prevent the transmission of the tick and protect canine health.

Keywords: Asia, canine babesiosis, meta-analysis, molecular prevalence

1. Introduction

Babesiosis is a hemoprotozoan parasite transmitted by ticks that may infect several animals, including dogs. Canine babesiosis is an intraerythrocytic parasitic infection caused by the genus *Babesia*, posing a global danger to canines and is primarily transmitted through tick bites (Mandal et al., 2014; Wang et al., 2019). Among the Asian region, canine babesiosis cases were reported first time in India in 1910, with the finding of small *Babesia* piroplasm (Jain et al., 2017). The distribution of ticks, especially in the Asian region, is expected to expand because of tropical and subtropical areas that give seasonal activity to the tick, so it can enhance the pathogen transmission (Tan et al., 2021).

Babesia apicomplexan can be divided into two categories based on the morphology diameter size: large forms (3-5µm) and small forms (1-3 µm). Large babesia species such as *Babesia canis*, *Babesia vogeli*, and *Babesia rossi*. Small babesia species such as *Babesia gibsoni*, *Babesia vulpes,* and *Babesia conradae* (Karasová et al., 2022; Muguiro et al., 2023). Canine babesiosis clinical manifestation can range from subclinical to severe disease based on the host immunity, age of the host, and protozoan parasite species. Clinical signs of babesiosis include fever, jaundice, anorexia, lethargy, pale mucous membrane, splenomegaly, anaemia, and thrombocytopenia (Koster et al., 2015; Solano-Gallego et al., 2016).

Although many individual studies have reported the presence of canine babesiosis across Asian countries, the overall molecular prevalence has not yet been comprehensively synthesized. Molecular diagnostic tools, particularly PCR-based methods, offer higher sensitivity and specificity compared to traditional methods, making them a preferred approach in recent years (Khanmohammadi et al., 2021; Kivrane et al., 2021).

This study aims to systematically review and analyze the molecular prevalence of babesiosis in dogs across the Asian continent and evaluate potential factors contributing to prevalence variation.

Materials and Methods

2.1. Ethical statement

This study utilized data from previous published reports and didn't involve animals or animal products; therefore, ethical approval was unnecessary.

2.2. Search strategy

This study was carried out in April 2024 to search for the database. This included studies covered publications on canine babesiosis in Asia from 2001 to April 2024. Scientific papers were identified using a total of four databases, including PubMed, Scopus, EBSCOhost, and Google Scholar. The search technique included combinations of the keywords of ((“Canis” OR “dog*” OR “canine”)) AND ((“Babesia” OR “babesiosis”)) NOT (“review*” OR “systematic review*” OR “meta-analysis" or "scoping review”)). Articles obtained from the database are subsequently collated and extracted for further analysis.

2.3. Study selection and data extraction

Two independent reviewers conducted an initial screening of titles and abstracts using Rayyan-Intelligent Systematic Review. Studies were eligible for inclusion if they were peer-reviewed, observational (cross-sectional or survey), and focused on canine babesiosis within Asia, employed molecular diagnostic techniques, and were published in English. Studies were excluded if they relied solely on serological or blood smear methods, had fewer than 30 samples, focused on non-canine hosts or humans, presented duplicated data, or lacked sufficient detail. Studies that used both serological and molecular diagnostic methods were still considered eligible, but only the molecular data were included in the analysis. Any disagreements during this process were resolved through discussion.

Relevant data from the selected articles were extracted by one author and cross-checked by another. The collected information included publication details (author and year), study location, sample size and types, diagnostic methods and target genes, *Babesia* species identified, estimated prevalence, and potential variables such as age, gender, breed, ownership status, and infection type (single or mixed). All retrieved data were organized in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA).

2.4. Quality assessment of individual studies

Study quality was assessed using a basic scoring checklist adapted from Ding et al, (2017), focusing on five key points: whether the study clearly stated its objective, described its sampling method, mentioned the study period, explained the molecular diagnostic method used, and grouped subjects into relevant subcategories. Each point was rated as "yes", "unclear", or "no", with scores 2, 1, or 0, respectively. The total score for each study could range from 0 to 10.

2.5. Statistical analysis

A random-effects model was used to perform the meta-analysis in the R software (v.4.3.0; Comprehensive R Archive Network, Vienna, Austria) using the "Meta" R package. The data were transformed into logit to calculate the overall pooled molecular prevalence of babesiosis in dogs along with the 95% confidence interval (CI), heterogeneity, and subgroup meta-analysis. Heterogeneity was evaluated using I^2, Cochrane's Q, and the p-value. A forest plot was generated in R software to visualize the overall pooled prevalence, CI, heterogeneity level, and 95% prediction interval (PI).

2.6. Publication bias, subgroup, and meta-regression analyses

Subgroup analyses were conducted based on co-infection status, age, ownership status, sex, breed, target gene, and *Babesia* species to enhance the robustness of the meta-analysis. Co-infection status was classified into single infection, 2 mixed infections, and more than two mixed infections with other hemaprotozoan species. Age was grouped into ≤ 2 years and > 2 years, while ownership status was classified as owned, stray, shelter, or kennel. Breed was divided into purebred or mixed breed. The target gene was classified into *18S rRNA* gene, *TRAP*, *hsp70*, and others. And for the subgroup, *Babesia* species was divided into *Babesia* spp., *Babesia gibsoni*, *Babesia vogeli*, *Babesia canis,* and the other *Babesia* species with a low amount to be one group, as others.

Meta-regression was performed to assess the trend in prevalence over the years. Results were reported using a regression equation and moderator p-value, and illustrated with a trend plot.

Publication bias was evaluated using Egger's and Begg's tests, with a p-value <0.01 indicating potential bias. Funnel plots were used to visualize asymmetry. If bias was suspected, a trim-and-fill method was applied to estimate missing studies and to adjust the prevalence accordingly.

3. Results

3.1. Literature search

A total of 4,859 articles were acquired from the search results utilizing keywords from 4 databases. The number of articles that needed to be screened for titles and abstracts was 3,107 after the removal of similar articles. Numerous papers were excluded for the following reasons: solely described the serology method, were not observational studies, utilized tick samples, contained inadequate data, were not available in English, were not on the Asian continent, or had a sample size of less than 30. The total number of papers that fulfilled the qualifying criteria and were included in the analysis was 114, from 22 countries, as illustrated in Figure 1.

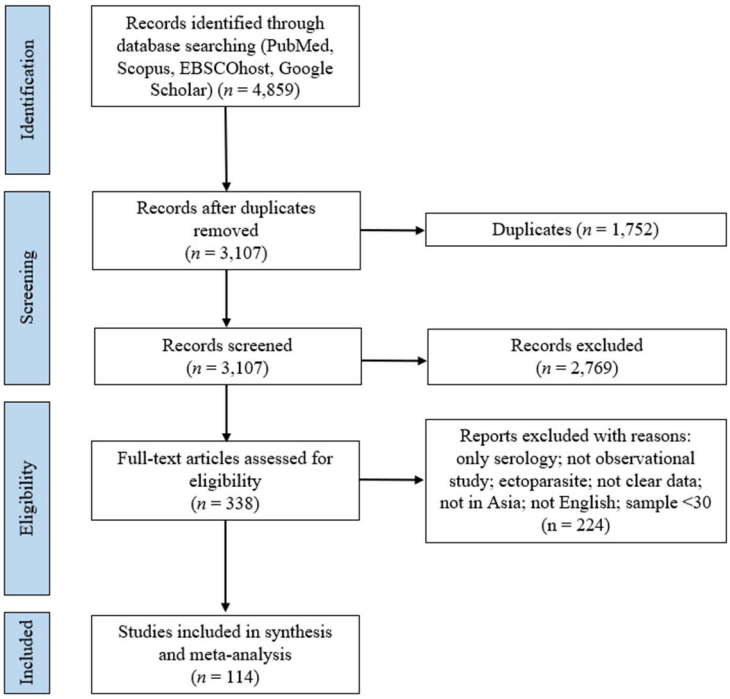

Figure 1 PRISMA flow chart of the selected eligible studies

3.2. Pooled estimates prevalence and quality assessment

Based on a systematic review and meta-analysis, the overall pooled molecular prevalence of dog babesiosis in the Asia continent was estimated at 11.89% (95% CI: 9.58–14.68), with a significant heterogeneity result (Q = 3824.19, I^2 = 97%, p-value <0.05) (Table 1). The quality assessment indicated moderate quality across the study, within the range of a total score of 2-10, with a mean score was 7.28 ± 1.82.

3.3. Subgroup meta-analysis

The subgroup meta-analysis was performed on the subgroup's co-infection status, age, ownership status, sex, breed, target gene, and *Babesia* species (Table 1). Based on the analysis results, among all subgroups, only co-infection status and identified *Babesia* species resulted in significant differences among the parameters in the subgroups ($p<0.01$). For the co-infection status subgroups, single infection showed the highest prevalence (9.69% [95% CI: 7.67–12.17]), followed by mixed infection between 2 species (5.49%, [95% CI: 3.82–7.82]), while the lowest prevalence was found in the >2 species mixed infection (3.36%, [95% CI: 2.32–4.84]) ($p<0.01$). Among the identified *Babesia* species, *Babesia* gibsoni was significantly highest with prevalence rates of 13.27% [95% CI: 9.61–18.04]. Regarding the dog ages, molecular prevalence between dogs with age ≤ 2 years and > 2 years (11.83, [95% CI: 7.85–17.44] and 13.68%, [95% CI: 9.21–19.85], respectively), female and male (12.02%, [95% CI: 9.01–15.86] and 11.76%, [95% CI: 8.27–16.47], respectively), and pure breed and mixed breed (9.27, [95% CI: 5.60–14.98] and 16.14%, [95% CI: 9.53–26.03], respectively) were also showed no significant difference ($p>0.05$). Based on ownership status, the molecular prevalence of dog babesiosis was in the range of 13.93% (95% CI: 8.74–21.48) in shelter dogs to 18.21% (95% CI: 2.80–63.26) in kennel dogs ($p=0.99$). Based on the target gene used for molecular methods, the prevalence was in the range from 18.21% [95% CI: 5.32–46.87] using TRAP to 7.09% [95% CI: 3.81–12.80] using other target genes.

Categories	No. of studies	Prevalence (%)		Heterogeneity			*p*-value for subgroup
		Estimates	(95 % CI)	Q	*p*-value	I^2	
Overall	114	11.89	[9.58; 14.68]	3824.19	0	97%	
Subgroup analysis							
Co-infection status							
Single infection	109	9.69	[7.67; 12.17]	3591.50	0	97%	<0.01
2 mixed infections	40	5.49	[3.82; 7.82]	903.68	<0.01	96%	
> 2 mixed infections	23	3.36	[2.32; 4.84]	108.40	<0.01	80%	
Age							
≤ 2 years	25	11.83	[7.85; 17.44]	509.99	<0.01	94%	0.61
> 2 years	30	13.68	[9.21; 19.85]	415.03	<0.01	94%	
Ownership status							
Owned	33	14.81	[9.67; 22.01]	1089.92	<0.01	97%	0.99
Stray	20	14.64	[10.61; 19.86]	182.91	<0.01	90%	
Shelter	9	13.93	[8.74; 21.48]	82.56	<0.01	90%	
Kennel	4	18.21	[2.80; 63.26]	47.86	<0.01	94%	
Sex							
Male	37	11.76	[8.27; 16.47]	670.37	<0.01	95%	0.92
Female	37	12.02	[9.01; 15.86]	435.70	<0.01	92%	
Breed							
Pure breed	14	9.27	[5.60; 14.98]	151.46	<0.01	91%	0.13
Mixed breed	12	16.14	[9.53; 26.03]	71.22	<0.01	85%	
Target gene							
18S rRNA	103	11.66	[9.23; 14.64]	3271.26	0	97%	0.39
hsp70	3	11.48	[4.47; 26.46]	37.50	<0.01	95%	
TRAP	4	18.21	[5.32; 46.87]	51.24	<0.01	94%	
Others[a]	6	7.09	[3.81; 12.80]	39.79	<0.01	87%	
Babesia species							
Babesia spp.	10	4.33	[1.59; 11.28]	73.24	<0.01	88%	<0.001
Babesia gibsoni	65	13.27	[9.61; 18.04]	2849.33	0	98%	
Babesia canis	20	4.77	[2.45; 9.10]	455.05	<0.01	96%	
Babesia vogeli	56	6.79	[5.17; 8.88]	739.60	<0.01	93%	
Others[b]	6	1.52	[0.52; 4.32]	21.34	0.007	77%	

Table 1 Overall pooled prevalence and subgroup analyses of babesiosis in dogs in Asia region
[a]Others included *ssrRNA, ITS1, Bc28.1, cytb, P18,* and *5.8S rRNA* target gene.
[b]Others included *Babesia caballi, Babesia odocoilei-like species, Babesia vulpes, Babesia microti, Babesia bigemina* and *Babesia venatorum*.

3.4. Meta-regression

Based on meta-regression analysis, the molecular prevalence of babesiosis in dogs showed no significant changes over time (p = 0.15). The regression equation for molecular prevalence in canine babesiosis is: logit event rate = -78.9067 + 0.0381*year (95% CI, -0.01– 0.09).

3.5. Risk of publication bias

Based on the result of the risk of publication bias assessment showed the potency of publication bias as indicated by p<0.01 in Egger's and Begg's test, along with an asymmetry funnel plot (Figure 2). The trim and fill test showed 39 missing studies to create a symmetrical plot which resulted an increasing pooled molecular prevalence of 22.85% (95% CI, 17.99; 28.55) if the additional studies were included.

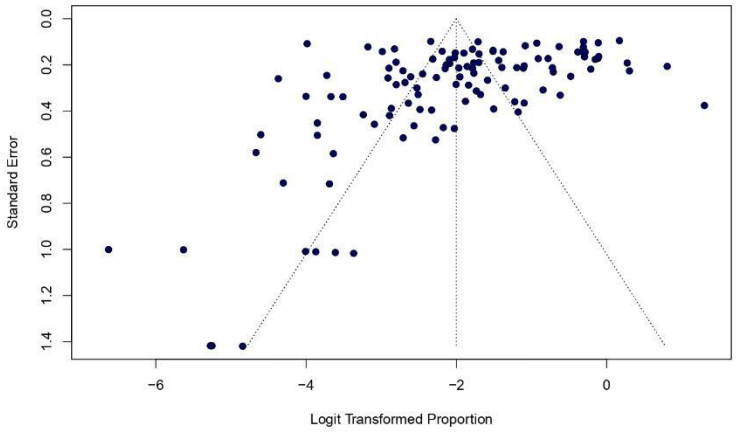

Figure 2 Asymmetrical funnel plot for molecular detection studies of canine babesiosis in Asia

4. Discussion

The relatively pooled prevalence in this study was 11.89% (95% CI: 9.58–14.68), with the highest estimated pooled prevalence found in single infection was 9.69% (95% CI: 7.67– 12.17). This result was close to the global finding average (12%) reported by Abdoli et al (2024). The molecular prevalence of *Babesia* in dogs in the Asian region has been documented in 22 countries, with the highest number of publications were related to India (n = 34). The subgroup of single infection of *Babesia* was found to be the highest prevalence. This can be cause of diagnostic limitations and tick vector. Most of molecular assay just only test for single agent that can't detect for the co-infection (Sanchez-vicente & Tokarz, 2023).

Babesia gibsoni was the most commonly found species in the Asia region, with the highest prevalence is 13.17%. This species has been found endemic in Asia, primally transmitted by *Haemaphysalis* spp. and *Rhipicephalus sanguineus,* which are distributed in countries in the Asian region such as Japan, China, and Taiwan, and can cause moderate to severe clinical signs. And also commonly found to be infected by horizontal transmission (Irwin, 2009; Jongejan et al., 2018; Tan & Xu, 2022). Despite being less commonly reported, zoonotic species, including *Babesia venatorum* (n=1) and *Babesia microti* (n=1), were also found in the dataset and should be taken into consideration due to their potential implications in public health (Young et al., 2019).

Target genes used for the detection of canine babesiosis for the subgroup analysis were *18S rRNA* gene, *hsp70*, TRAP, and other group. *18S rRNA* gene was found to be most useful to be the target gene for detection of babesiosis in dogs, as we found in our study. It is because of the *18S rRNA* gene be a useful genetic marker to study the genetic divergence, evolution and relationship within *Babesia* species isolates. Because this gene contains highly conserved and variable regions of *Babesia* (Mandal et al., 2014; Ulucesme et al., 2024; Wang et al., 2023).

5. Conclusion

This study shows that canine babesiosis remains an important tick-borne disease across Asia, with the overall molecular prevalence estimated at 11.89%. Most subgroup differences were not statistically significant; however, differences were observed across co-infection status and *Babesia* identified species. Although molecular tools offer improved diagnostic accuracy, the wide heterogeneity across studies suggests a need for more standardized surveillance and reporting. Regular monitoring and control strategies of babesiosis in dogs, especially in endemic regions, remain important to prevent the transmission of the tick and protect canine health.

Acknowledgement

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Identification and Pathological Assessment of *Mycobacterium* spp. Infections in Wild Marine Mammals from the Andaman Sea, Thailand

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Abstract

Background: *Mycobacterium* spp. are significant pathogens in marine mammals, capable of causing systemic infections such as dermatitis, pneumonia, and tuberculosis-like disease, with potential for zoonotic transmission. While *Mycobacterium* infections have been reported in marine mammals globally, data from Thai waters, particularly the Andaman Sea, remain scarce. This study aimed to investigate the presence of *Mycobacterium* spp. and associated pathology in stranded dugongs and cetaceans recovered from the Andaman Sea, Thailand.
Methods: Twenty-three marine mammal carcasses, comprising 18 dugongs (*Dugong dugon*) and five cetaceans (including *Stenella attenuata*, *Lagenodelphis hosei*, and *Stenella coeruleoalba*), were submitted for histopathological evaluation. Tissue samples—including liver, lung, spleen, lymph nodes, gastrointestinal tract, skin, and muscle—were examined microscopically to assess pathological changes. Detection of *Mycobacterium* spp. DNA was Performed using conventional PCR targeting the 16S rRNA gene. Selected PCR-positive and PCR-negative tissues were further evaluated using immunofluorescence (IF) staining with a rabbit polyclonal anti-*Mycobacterium* antibody to confirm antigen localization.
Results: *Mycobacterium* spp. DNA was detected in 13 out of 23 animals (56.5%), including 10 of 18 dugongs (55.6%) and 3 of 5 cetaceans (60%). Suppose staining confirmed the presence of *Mycobacterium* antigens within necrotic and inflammatory lesions in PCR-positive tissues, while PCR-negative samples showed no fluorescent signals. Histopathological analysis revealed multisystemic lesions in affected organs, particularly the lungs, liver, spleen, and gastrointestinal tract, consistent with chronic infection.
Conclusion: These preliminary findings indicate the presence of Mycobacterium in Thai marine mammals, suggesting a possible role in mortality. The results underscore the need for further investigation into its epidemiology and raise awareness of potential zoonotic risks for field veterinarians and researchers handling marine mammal carcasses.

Keywords: *Mycobacterium*, Marine mammals, Dugong, Marine mammal, Pathology

1. Introduction

Marine mammals comprise a highly diverse group of approximately 137 species, including cetaceans (such as whales, dolphins, and porpoises), pinnipeds (seals and sea lions), sirenians (manatees and dugongs), as well as certain otters and bears [1]. These animals fulfill essential ecological functions within marine environments, playing key roles in nutrient recycling, regulating predator-prey relationships, and supporting the stability of oceanic food webs [2, 3]. Despite their ecological importance, marine mammals are increasingly exposed to a wide range of threats stemming from both human activities and natural environmental changes. This situation highlights an urgent need for targeted conservation efforts, as continued population declines could result in long-term and potentially irreversible disruptions to marine ecosystem processes and the services they provide [4, 5].

Thailand's coastal waters, including the Andaman Sea and the Gulf of Thailand, host a rich diversity of marine mammals, including whales, dolphins, and dugongs. Many are protected under the Wildlife Conservation and Protection Act, B.E. 2562, and are listed as threatened on the IUCN Red List [6]. Twenty-five cetacean species and one sirenian species have been recorded, with notable examples, including the Indo-Pacific bottlenose dolphin and pantropical spotted dolphin [7, 8]. Marine mammal strandings are a multifactorial phenomenon influenced by both natural and human-related factors. These include climate and oceanographic changes, magnetic anomalies leading to navigational disorientation, and various anthropogenic disturbances [9, 10]. In Thai waters, recent investigations have identified natural causes—particularly infectious diseases such as parasitic, viral, and bacterial infections—a significant contributors to marine mammal strandings [11]. Mycobacteriosis represents an important infectious disease in marine mammals, caused by a diverse group of bacteria within the genus Mycobacterium [12]. These bacteria are aerobic, non-motile, acid-fast bacilli belonging to the family Mycobacteriaceae within the phylum Actinobacteria, and are commonly found as intracellular pathogens [13]. Several *Mycobacterium* species with zoonotic potential have been documented in marine mammals, including *M. marinum* [14], *M. pinnipedii* [15, 16], and *M. abscessus* [17]. Infections in marine mammals may present as dermatitis, panniculitis, pneumonia (Clayton et al., 2012), or a tuberculosis-like disease, all of which carry the potential for zoonotic transmission to humans [12].

Given the significance of this disease, the present study aims to detect *Mycobacterium* spp. in stranded dugongs and cetaceans from the Andaman Sea using conventional PCR and immunofluorescence staining, in conjunction with histopathological examination. The results of this investigation are expected to enhance awareness of *Mycobacterium* infections in marine mammals and improve our understanding of the associated pathological changes. These findings may contribute valuable insights for future health monitoring, disease management, and conservation strategies for affected marine species.

2. Materials and Methods

2.1. Ethics approval and consent to participate

All procedures involving the use of leftover animal tissues were conducted in accordance with institutional guidelines and regulations. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Prince of Songkla University (Approval No. MHESI 68014/1236). The committee

determined that the submitted protocol qualified for exemption under the criteria for Exempt Determination Research. These tissue samples were collected under permission granted by the Department of Fisheries of Thailand (Permit No. 0510.5/12652), issued in accordance with Section 73(1) of the Wildlife Preservation and Protection Act, B.E. 2562 (2019).

2.2. Tissue sample collection and distribution

A total of 22 marine mammal carcasses, consisting of 17 dugongs (*Dugong dugon*) and 5 cetaceans (including *Stenella attenuata* and *Tursiops aduncus*), were necropsied by veterinary pathologists at the Marine and Coastal Resources Research Center (Lower Andaman Sea), Trang Province, Thailand. Postmortem tissue samples were collected from each individual and submitted to the Pathology Unit, Department of Veterinary Science, Faculty of Veterinary Science, Prince of Songkla University, for histopathological and molecular investigations. In dugongs, the most commonly sampled organs included the heart (n = 15), lungs (n = 14), liver (n = 13), intestine (n = 13), spleen (n = 10), and kidneys (n = 10). In cetaceans, fewer tissue samples were obtained, with the heart, lungs, liver, brain, and lymph nodes each collected from five individuals. The gall bladder and urinary bladder were infrequently available in dugongs (n = 1 for each) and were absent in cetacean specimens. All tissues underwent standard histopathological processing and were examined microscopically. Additionally, approximately 25 g of each tissue was preserved in sterile centrifuge tubes for subsequent genomic DNA extraction to support molecular analyses.

2.3. Histopathological Examination

Histopathological evaluation was performed on all submitted tissue samples. Specimens were processed using standard histological procedures [18]. Tissues were initially fixed in 10% neutral buffered formalin to preserve cellular morphology, then dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax to maintain tissue architecture. Paraffin blocks were sectioned at a thickness of 4 µm, and the resulting sections were stained with hematoxylin and eosin (H&E) for routine microscopic assessment of morphological alterations. The stained slides were then mounted with coverslips for detailed histopathological examination.

2.4. Molecular identification of Mycobacterium spp.

To detect *Mycobacterium* spp. in tissue samples, approximately 25 mg of pooled tissue from the same individual was used for genomic DNA extraction, performed using the GeneJET Genomic DNA Purification Kit (Thermo Scientific™, USA) according to the manufacturer's instructions. The pan-mycobacterial 16S rRNA gene was selected as the genetic marker for *Mycobacterium* spp. identification due to its high specificity and reliability in differentiating among *Mycobacterium* species [19, 20]. Nested PCR was employed to amplify the 16S rRNA gene. For the first round of amplification, external primers 16SmF (5′-TGCACACAGGCCACAAGGGA-3′) and 16Sr (5′-GAGAGTTTGATCCTGGCTCAG-3′) were used at a concentration of 10 pmol/µL. The 25 µL PCR reaction mixture contained 25–40 ng of DNA template, 0.5 µL of each primer, 12.5 µL of OnePCR Ultra Master Mix (Bio-Helix, New Taipei City, Taiwan), and 5.5 µL of nuclease-free water. Amplification was performed using a thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation at 94°C for 5

minutes; 30 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. For the second round, internal primers MnF (5′-CTTAACACATGCAAGTCGAAC-3′) and MnR (5′-TTTCACGAACAACGCGACAA-3′) were used under the same reaction conditions and thermal cycling parameters as the first round. PCR amplification products were subsequently analyzed by agarose gel electrophoresis. After mixing with loading dye, PCR products were resolved on an agarose gel, and the presence of a 550 bp amplicon was used to confirm the detection of *Mycobacterium* spp.

2.5. Immunofluorescence identification of Mycobacterium spp.

To confirm the presence of *Mycobacterium* spp. antigen in tissue samples, immunofluorescence (IF) staining was performed using a rabbit polyclonal anti-*Mycobacterium* antibody. Formalin-fixed, paraffin-embedded intestinal tissue sections from both positive and negative samples were processed according to standard IF protocols. Briefly, 4 µm sections were incubated at 60 °C for 15 minutes, followed by deparaffinization in xylene and rehydration through a graded ethanol series. Antigen retrieval was conducted in citrate buffer (pH 6.0) using a high-pressure cooker for 10 minutes. To block non-specific binding, the sections were incubated with 1% normal horse serum in PBS for 45 minutes at room temperature [21, 22]. The primary antibody (rabbit polyclonal anti-*Mycobacterium*, ab214721, Abcam) was applied at a 1:100 dilution and incubated overnight at 4 °C. After washing with PBS, sections were incubated for 1 hour at room temperature in the dark with the secondary antibody (CF™ 555-conjugated Rabbit Anti-Goat IgG [H+L], Sigma-Aldrich) diluted in 1% bovine serum albumin (BSA). Nuclei were counterstained with ProLong™ Gold Antifade Mountant containing DAPI (Thermo Fisher Scientific, USA). Fluorescence signals were visualized using a fluorescence microscope (ECLIPSE Ni-U, Nikon, Tokyo, Japan).

3. Results

3.1. Major necropsy findings in marine mammals

A total of 23 marine mammal carcasses were included in this study based on suspicion of infectious disease at necropsy. These comprised 18 dugongs (*Dugong dugon*), 2 pantropical spotted dolphins (*Stenella attenuata*), 1 Fraser's dolphin (*Lagenodelphis hosei*), and 1 striped dolphin (*Stenella coeruleoalba*). All animals were submitted for postmortem examination. According to the necropsy reports, the majority of dugong deaths were attributed to natural causes (15/18), with common findings including severe cachexia, pneumonia, septicemia, intestinal obstruction, and chronic hepatitis. A few cases also presented with liver masses or abscesses, and one with suspected chronic infectious disease. Three dugong cases were linked to anthropogenic trauma, including traumatic bite wounds, incision wounds, and distal vertebral fractures with associated suppurative inflammation. All cetacean cases (5/5) were classified as natural deaths, with pneumonia being the predominant lesion noted across species. These findings support the inclusion of all 23 animals in the present study, which investigates marine mammal mortality potentially associated with Mycobacterium spp. infection in the Andaman Sea, Thailand.

Figure 1. Suspected causes of death in stranded marine mammals submitted for necropsy. (a) Pie charts illustrating the proportion of natural versus anthropogenic causes of death in dugongs (left) and cetaceans (right). The majority of cases were suspected to have died from natural causes. (b) Representative image of a cachectic dugong carcass. (c) Representative image of a cetacean carcass with a slightly poor body condition score.

3.2. Histopathology findings in marine mammals

Histopathological evaluation of tissues from 23 stranded marine mammals—comprising 18 dugongs and 5 cetaceans—revealed a wide range of lesions affecting multiple organ systems, with more than 80 distinct pathological findings documented.

The liver was one of the most commonly affected organs, with lipofuscinosis and ferritin pigmentation identified in 5/23 animals (21.7%) (Figure 2a), frequently accompanied by periductal or mild hepatic fibrosis in 3/23 cases (13.0%). Additional hepatic changes included vacuolar degeneration, sinusoidal congestion, and cytoplasmic pigment accumulation in Kupffer cells, suggesting chronic hepatic stress. Portal inflammation and cholangiocellular hyperplasia were observed in a smaller subset. Autolytic changes and pseudo-lesions such as periportal connective tissue disruption, vessel lysis, and sinusoidal widening were present in all liver samples, though the severity varied by case. The gastrointestinal tract displayed lesions in 8/23 animals (34.8%), including granulomatous and eosinophilic enteritis, verminous granulomas, and villous atrophy (Figure 2b). Mucosal erosion, ulceration, and abscesses were also identified, with inflammation extending into deeper layers in many cases. Pseudo-lesions, including connective tissue disruption and cellular lysis, were universally noted, though their expression ranged from mild to extensive.

Splenic lesions were observed in 9/23 animals (39.1%), with the most common findings being moderate to severe vascular congestion, splenic follicular hyperplasia, and extramedullary hematopoiesis (Figure 2c). In 3/23 cases (13.0%), chronic changes, such as capsular fibrosis and hyalinized germinal centers, were evident. Autolytic changes and pseudo-lesions—including connective tissue disruption, lymphoid cell lysis, and nuclear degeneration—were also present in all spleen samples, with varying degrees of severity. Lymph node lesions were recorded in 3/23 animals (13.0%), showing lobular disorganization, reactive follicular hyperplasia, and macrophages and eosinophilic infiltration, accompanied by autolysis and formalin pigment accumulation (Figure 2d). These degenerative features were present in all lymph node specimens, albeit with varying intensities.

The lungs were significantly affected, with bronchopneumonia diagnosed in 8/23 animals (34.8%), including all five cetaceans. Additional pulmonary lesions included eosinophilic bronchitis, granulomatous pneumonia, and bronchointerstitial pneumonia (2/23, 8.7%) and mild interstitial pneumonia (1/23, 4.3%) (Figure 2e). Widespread findings included mononuclear and eosinophilic infiltration, alveolar collapse, tracheal epithelial degeneration, and inflammatory involvement of bronchial walls. Pseudo-lesions and autolytic changes, such as alveolar epithelial lysis, pyknosis, and cytological debris accumulation, were noted in all lung specimens.

Cutaneous and musculoskeletal involvement was found in 2/23 cases (8.7%), including chronic skin erosion, myositis, and vasculitis (Figure 2g, h). Muscle samples revealed myofiber degeneration, loss of striation, and pseudo-lesions, along with perivascular inflammatory infiltration and occasional hemosiderin-laden macrophages. Again, autolytic artifacts were evident in all examined sections. In cetaceans, 5/5 individuals (100%) exhibited bronchopneumonia, and 2/5 (40%) showed multiorgan helminthiasis. Other findings included mild myocarditis, meningitis, villous atrophy, eosinophilic enteritis, and granulomatous pneumonitis (Figure 2f), all of which point toward chronic systemic infection or parasitism. Chronic verminous cholangitis with granuloma formation was noted in 1/5 cetaceans (20%).

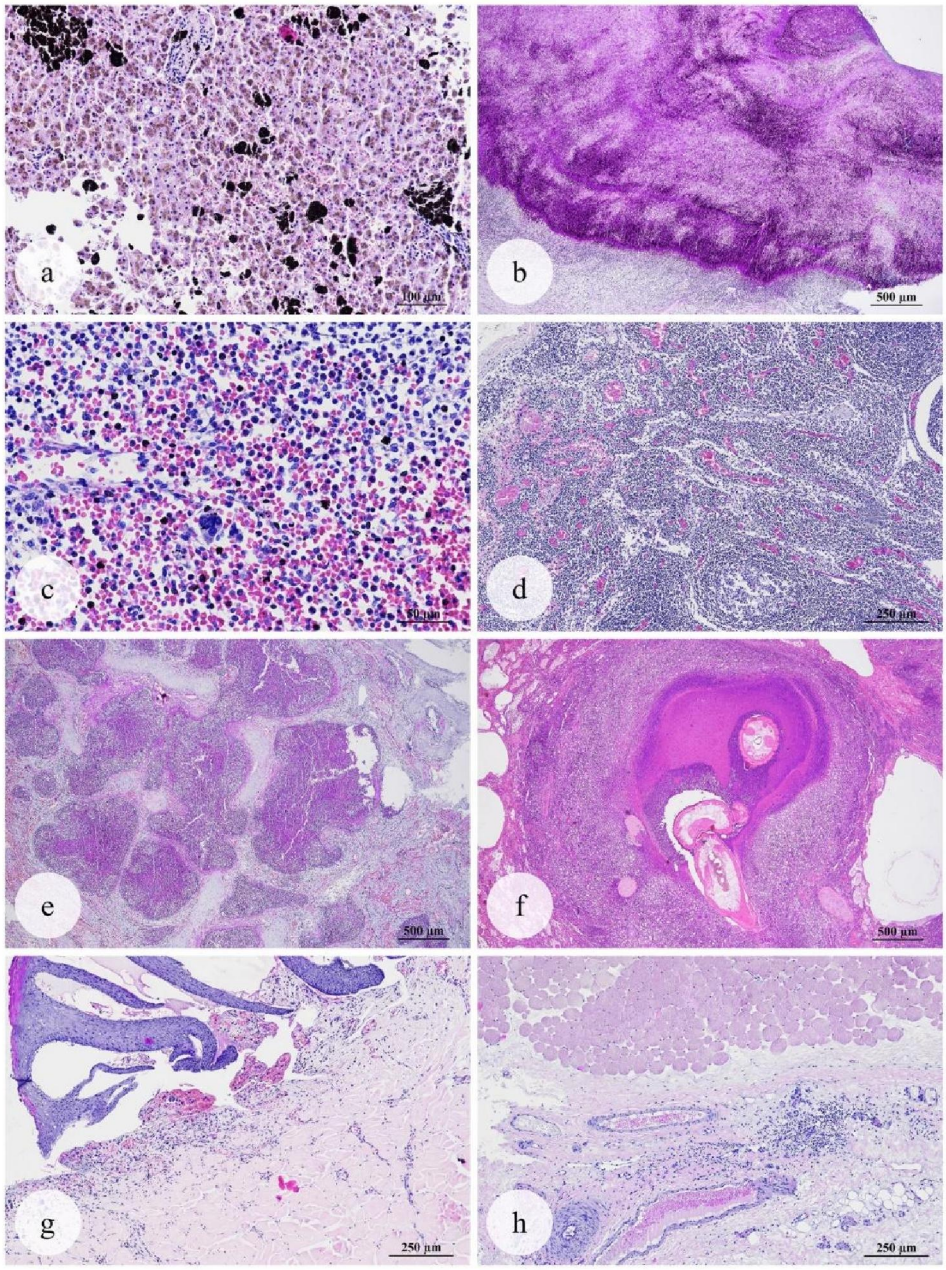

Figure 2. Histopathological lesions observed in the organs of dugongs and cetaceans.

(a) Hepatic hemosiderosis, lipofuscinosis, and ferritin pigmentation are characterized by the accumulation of lipofuscin, hemosiderin, and ferritin pigments within the hepatic parenchyma, accompanied by hepatocellular degeneration. (b) Granulomatous enteritis showing eosinophilic mucosal thickening with villous atrophy. (c) Splenic extramedullary hematopoiesis is indicated by the presence of megakaryocytes within the splenic parenchyma and vascular spaces. (d) Reactive lymph node displaying macrophage infiltration at the mantle zone, perifollicular regions, and some germinal centers. (e) Bronchointerstitial pneumonia with inflammation involving the alveoli, interstitium, and lower respiratory tract. (f) Granulomatous pneumonitis associated with helminth infection. (g) Chronic inflammatory response at an erosive skin wound site. (h) Vasculitis of the deep dermis with inflammation surrounding and infiltrating vascular structures. (Hematoxylin and Eosin [H&E] staining; original magnifications: b, e, f = ×4, scale bar = 500 µm; d, g, h = ×10, scale bar = 250 µm; a = ×20, scale bar = 100 µm; c = ×40, scale bar = 50 µm.)

3.3. Molecular identification of Mycobacterium spp. and inter-species patterns

Among the 23 stranded marine mammals tested for *Mycobacterium* spp. using a 16S rRNA gene-targeted PCR assay, 13 individuals (56.5%) tested positive. In comparison, 10 individuals (43.5%) tested negative, indicating that over half of the sampled animals harbored detectable mycobacterial DNA in at least one tissue. When results were categorized by species, dugongs accounted for 18 of the total cases, of which 10 (55.6%) were PCR-positive and 8 (44.4%) were negative, suggesting a considerable presence of mycobacterial infection within this species, which may be associated with underlying systemic or organ-specific pathology as described histologically.

Among the five cetaceans examined, one of the two pantropical spotted dolphins (50%) tested positive, while the other yielded a negative result. Notably, the single Fraser's dolphin tested positive, reflecting 100% detection in that species, whereas the striped dolphin tested negative, providing a more limited data point due to the small sample size. These findings demonstrate that *Mycobacterium* spp. DNA was detected across multiple species of marine mammals, with the highest number of positive cases occurring in dugongs. The relatively high proportion of PCR-positive results supports the hypothesis that mycobacterial infections may play a significant role in the morbidity and mortality of marine mammals inhabiting the Andaman Sea, warranting further investigation into potential environmental sources, transmission dynamics, and species susceptibility.

3.4. Immunofluorescence (IF) staining for Mycobacterium spp. antigen detection

To confirm the presence of *Mycobacterium* spp. antigens in tissue samples, immunofluorescence (IF) staining was conducted on selected formalin-fixed, paraffin-embedded (FFPE) sections previously categorized as PCR-positive and PCR-negative. A rabbit polyclonal anti-*Mycobacterium* antibody was employed as the primary marker to detect antigenic expression. Fluorescent signals indicative of *Mycobacterium* spp. antigen localization were detected within inflammatory and/or necrotic lesions in PCR-positive samples (Figure 3), suggesting a correlation between antigen expression and histopathological damage. In contrast, PCR-negative tissue sections did not exhibit any detectable fluorescent labeling, supporting the specificity of the immunolabeling for *Mycobacterium* spp.

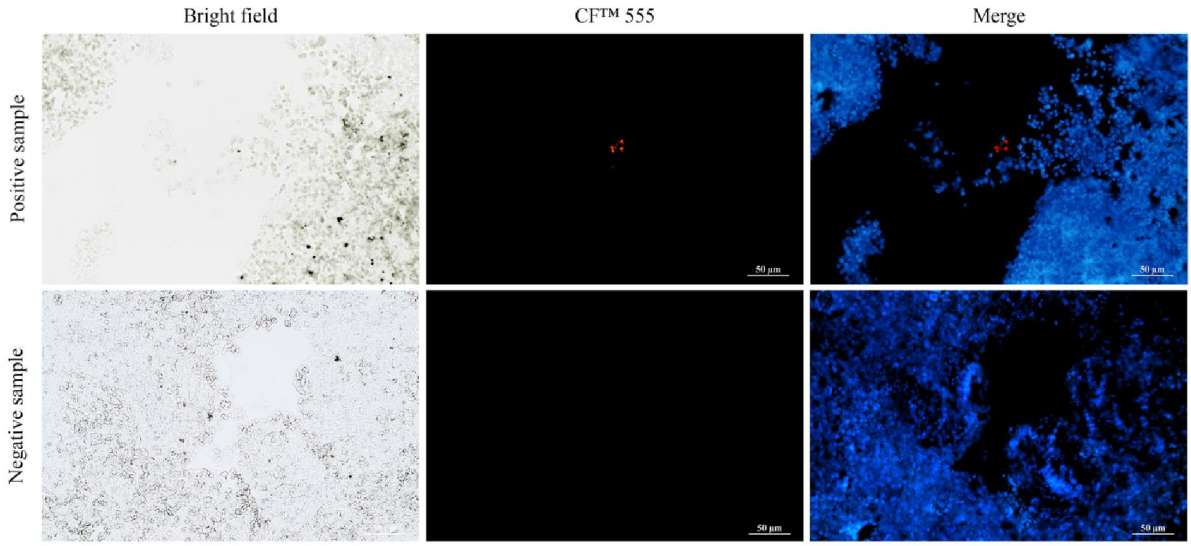

Figure 3. Representative photomicrographs of *Mycobacterium*-positive and -negative immunofluorescence staining. Upper panel: A PCR-positive spleen sample showing *Mycobacterium* spp. antigen localized within the tissue parenchyma. Rod-shaped bacteria are visualized as bright red fluorescent signals, indicating positive immunoreactivity.
Lower panel: A PCR-negative spleen section showing no detectable fluorescent signal, confirming the absence of *Mycobacterium* antigen. (Bright field, CFTM555, and DAPI channels; original magnification ×40, scale bar = 50 µm for all images.)

4. Discussion

This study represents the first systematic investigation of *Mycobacterium* spp. infections in stranded dugongs and cetaceans along the Andaman Sea coast of Thailand. The detection of *Mycobacterium* DNA in 56.5% of examined individuals, corroborated by immunofluorescent and histopathological findings, suggests a considerable prevalence of mycobacterial infection in this population. The detection rate observed in this study is notably higher than that reported in previous surveys of marine mammal tuberculosis, which indicated a relatively low prevalence of approximately 1.6% [16]. Nonetheless, mycobacterial infections, particularly those caused by *Mycobacterium pinnipedii*, are well-documented in pinnipeds, with significantly higher incidence rates reported. For example, tuberculosis prevalence reached 46.4% in captive sea lions housed in Dutch zoological institutions [15], and up to 74% in free-ranging New Zealand fur seals and sea lions [16, 23]. Although there have been reports of *Mycobacterium* infection in Amazonian manatees (*Trichechus inunguis*) [24, 25], comprehensive epidemiological studies in wild sirenian populations remain lacking. To our knowledge, this study is the first to document *Mycobacterium* spp. infections in dugongs (*Dugong dugon*) and wild marine mammals from the Andaman Sea region of Thailand, thereby contributing novel insights into the epidemiology of mycobacterial diseases in tropical marine ecosystems. However, further molecular characterization through sequencing and phylogenetic analysis is necessary to confirm the specific *Mycobacterium* species involved, assess genetic relatedness to known strains, and better understand the transmission dynamics, potential reservoirs, and zoonotic implications within marine environments.

Previous studies have identified *Mycobacterium* spp. as causative agents of chronic diseases in marine mammals worldwide, frequently manifesting as tuberculosis-like conditions, pneumonia, and systemic granulomatous inflammation [12, 17]. Consistent with these findings, the present study observed similar pathological changes, including granulomatous enteritis and granulomatous pneumonia. Infections with *M. pinnipedii* have been previously associated with widespread systemic pathology affecting multiple organs, including the lungs, kidneys, spleen, liver, and lymph nodes [15, 26, 27]. Our findings are in concordance with these reports, as evidenced by the presence of splenic follicular hyperplasia and vascular congestion, reactive lymphadenopathy, hepatic inflammation, and iron-associated lesions such as hepatic hemosiderosis and ferritin pigmentation. Additionally, some carcasses in our study exhibited cutaneous and subcutaneous lesions, including dermatitis and panniculitis, which are consistent with non-tuberculosis-like manifestations reported in earlier studies [28-30].

Given that many mycobacterial species are zoonotic, these findings also raise important public health considerations. Individuals involved in marine mammal rescue, necropsy, or research—especially in field settings—should be aware of the potential risk for transmission and adopt appropriate biosafety protocols.

5. Conclusion

The findings of this study indicate that *Mycobacterium* spp. may play a substantial role in the morbidity and mortality of marine mammals in the Andaman Sea. The presence of multisystemic pathological lesions, along with consistent localization of bacterial antigens within affected tissues, supports the pathogenic potential of these organisms. Further investigations are warranted to accurately identify the *Mycobacterium* species involved, determine possible environmental sources of infection, and assess the long-term health impacts on wild marine mammal populations in Thailand.

Acknowledgement

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The Efficacy of Nonnai (*Tadehagi triquetrum L.*) grass in Preventing, Eliminating Flies and Killing Pathogenic Bacteria

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Abstract

Background: This study investigates the efficacy of Nonnai grass (*Tadehagi triquetrum* L.), a traditional Lao plant, as a natural agent for fly repellency and antibacterial activity. In response to growing concerns over resistance to synthetic pesticides and antibiotics, the research explores sustainable alternatives for use in veterinary and agricultural contexts.

Methods: Various preparations of Nonnai grass ethanol extracts, water extracts, and fermented forms (T1: Control, T2: Ethanol extraction, T3: Water extraction, T4: Fermentation with alcohol, T5: Fermentation with water, T6: Fermentation and T7: Raw Nonnai grass) were evaluated for their repellent and antimicrobial properties.

Results: Results revealed that fermentation and ethanol extraction notably enhanced fly-repellent activity. In particular, treatments using fermented grass in ethanol (T4) and in water (T5) captured the fewest flies and completely inhibited egg laying. Larvicidal assays showed 100% maggot mortality by day 5 with these treatments, indicating potent larvicidal potential. Antibacterial testing further confirmed that fermented and ethanol-extracted forms exhibited strong inhibitory effects against key pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., and *Salmonella* spp. The water extract (T3) and ethanol-fermented extract (T4) produced the largest zones of inhibition.

Conclusion: These findings highlight the potential of Nonnai grass, especially in fermented or ethanol-based forms, as a viable natural alternative for pest and pathogen control. The study supports its further development as an eco-friendly tool in sustainable livestock and agricultural practices.

Keywords: *Tadehagi triquetrum* L., fly repellent, larvicidal activity, antibacterial, sustainable pest management.

1. Introduction

The *Tadehagi triquetrum* L. Ohashi, commonly known as "Nonnai" grass in Laos, is a traditional herb utilized by local Lao farmers for its herbal repellent properties against flies. Scientific studies have indicated that *T. triquetrum* possesses a diverse chemical profile, with over 70 compounds identified, including significant phytophenols such as rutin and kaempferol, which contribute to its bioactive properties (Tang et al., 2022; Zhang et al., 2021). These compounds are responsible for the herb's potent antioxidant, neuroprotective, and insecticidal effects, making it a promising candidate for various applications. Recent research has extended the understanding of *T. triquetrum* beyond its traditional uses, particularly within the context of veterinary medicine. Essential oils extracted from *T. triquetrum* have been shown to contain organic compounds that contribute to moderate antioxidant and anti-acetylcholinesterase activities, which could be beneficial for managing oxidative stress-related conditions (Song et al., 2023). Furthermore, its phytophenols have demonstrated strong antioxidant activity, suggesting potential therapeutic applications beyond pest control (Zhang et al., 2021).

In veterinary medicine, the herb has been investigated for its antibacterial, antifungal, and insecticidal properties, demonstrating potential as a natural fly repellent (Quattrocchi, 2012). This interest is growing due to concerns about the environmental and health impacts of synthetic chemical pesticides (Nicolopoulou-Stamati et al., 2016). The bioactive properties of *T. triquetrum*, particularly its insecticidal and antimicrobial effects, highlight its promise as a multifunctional herbal remedy in both human and veterinary medicine. The herb's ability to prevent and eliminate flies, along with its antibacterial properties, suggests that it could serve as a valuable natural alternative to synthetic chemicals. This is especially relevant in the current context of increasing antibiotic resistance and the global demand for eco-friendly pest control solutions (Pavela & Benelli, 2016; Ventola, 2015). Therefore, this study aims to scientifically evaluate the efficacy of *T. triquetrum* ("Nonnai" grass) in preventing and eliminating flies, as well as killing pathogenic bacteria. By bridging traditional knowledge with modern scientific research, this work seeks to contribute to the development of safe, effective, and sustainable methods for managing Eliminating Flies and Pathogenic Bacteria in both veterinary and agricultural contexts.

2. Materials and Methods

This experiment evaluated the efficacy of various treatments of *Tadehagi triquetrum* (Nonnai grass) in repelling and eliminating flies, as well as inhibiting the growth of pathogenic bacteria. The treatments were as follows: T1: Control using distilled water, T2: Ethanol extraction (80% ethanol), T3: Water extraction, T4: Fermentation with alcohol for 7 days, T5: Fermentation with water for 7 days, T6: Fermentation with sugar for 7 days, and T7: Raw Nonnai grass

2.1 Preparation of herbal extracts

The herb samples were first washed, dried, and cut into small pieces (approximately 0.50-1.00 cm in length). The pieces were dried at 50°C for 24 hours using a hot air oven. After drying, the samples were finely ground and stored in a plastic bag to prevent moisture absorption and were kept at room temperature until further use.

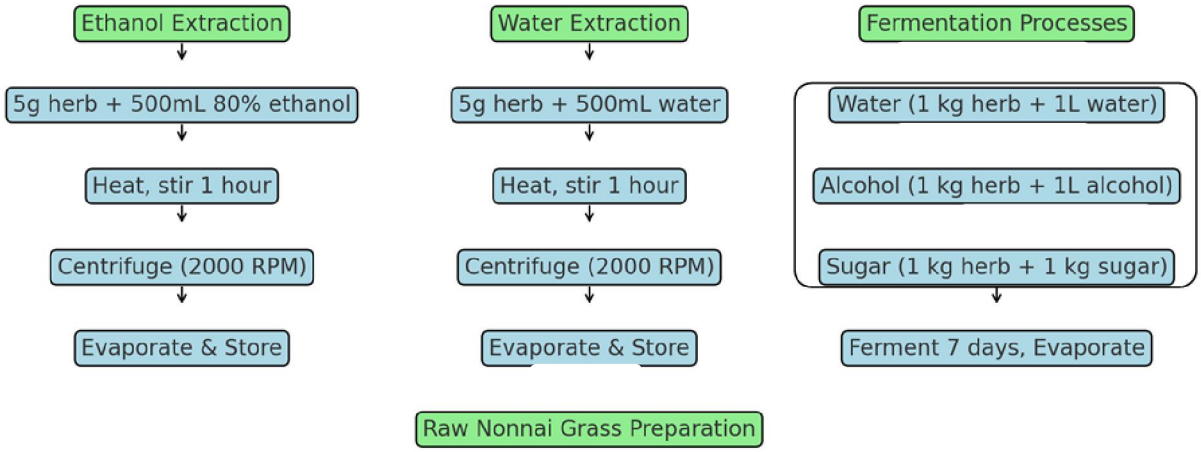

Figure 1. Flowchart of Nonnai grass extract preparation

2.2 Ethanol extraction

Ethanol extraction showed Figure 1 with 5 grams of the ground herb sample was placed in a 500 mL Erlenmeyer flask and mixed with 500 mL of 80% ethanol. The mixture was heated on a hot plate and stirred continuously for 1 hour using a magnetic stirrer. After heating, the extract was centrifuged at 2,000 RPM for 15 minutes, and the supernatant was collected. The ethanol was evaporated using a rotary evaporator to obtain a concentrated extract, which was stored at 4°C in a refrigerator.

2.3 Water extraction

Similarly, Figure 1, with 5 grams of the ground herb sample was mixed with 500 mL of distilled water in a 500 mL Erlenmeyer flask. The mixture was heated and stirred continuously for 1 hour using a magnetic stirrer. The extract was centrifuged at 2,000 RPM for 15 minutes, and the supernatant was collected. The water was evaporated using a rotary evaporator to obtain a concentrated extract, which was stored at 4°C.

2.4 Fermentation processes

Fermentation with Water showed Figure 1 with: One kilogram of ground Nonnai grass was mixed with 1 liter of water and left to ferment for 7 days. After fermentation, the mixture was evaporated to obtain a concentrated substance.

Fermentation with Alcohol Ethanol extraction showed Figure 1 with: One kilogram of ground Nonnai grass was mixed with 1 liter of 50% alcohol and fermented for 7 days. The resulting mixture was evaporated to obtain a concentrated extract.

Fermentation showed Sugar Figure with 1: One kilogram of ground Nonnai grass was mixed with 1 kilogram of sugar and left to ferment for 7 days. After fermentation, the mixture was evaporated to concentrate the extract.

2.5 Raw Nonnai grass

Raw Nonnai grass samples were thoroughly washed, ground into a fine powder, and then evaporated to obtain a concentrated extract for use in the experiments.

Testing the repellent effect against flies

To assess the repellent effect of the Nonnai grass extracts. The fish pieces were washed and cut into small pieces into the dise. Once in place, the various Nonnai grass extracts

were sprayed onto the fish dise daily. The number of flies caught on the plate was observed and recorded every hour for 12 hours (from 8 a.m. to 4 p.m.). Additionally, the number of fly eggs laid on the fish each day was counted.

2.6 Testing the Egg / larvae -killing effect

To assess the *Egg / larvae* -killing effect, small pieces of fish were placed in an ambient environment to attract flies, allowing them to lay eggs on the fish. The various Nonnai grass extracts were applied, and the number of eggs and larvae were observed and recorded over time.

2.7 Larvicidal activity of Nonnai grass

Ten maggots were placed on a plate, and the Nonnai grass extract was sprayed onto the plate twice a day (morning and evening). The mortality of the maggots was observed and recorded every 12 hours.

2.8 Bacterial inhibition test

The antibacterial activity of the Nonnai grass extracts was tested against both gram-positive and gram-negative bacteria, including *Staphylococcus aureus*, *Klebsiella* spp., *Escherichia coli*, and *Salmonella* spp. The bacteria were obtained from the Disease Center and Animal Husbandry Department and cultured to a concentration of 10^8 CFU/mL, equivalent to a 0.5 McFarland standard.

2.9 Well Diffusion Method

Herbal extracts were dissolved in DMSO at a concentration of 100 mg/mL. Sterile 0.45 µL discs were impregnated with the extract, and bacterial cultures were spread on Mueller-Hinton agar. Wells (5 mm in diameter) were drilled into the agar, and 0.1 mL of extract was added to each well. DMSO was used as a negative control, and Gentamicin (10 µg/disc) was used as a positive control. The plates were incubated at 37°C for 18 hours, and zones of inhibition were measured

3. Results

3.1. Fly Protection and egg-laying

The effectiveness of various Nonnai grass treatments on fly capture was evaluated over a four-day period by the various Nonnai grass extracts were sprayed onto the fish dise daily. The number of flies caught on the plate was observed and recorded every hour for 12 hours (from 8 a.m. to 4 p.m.), as shown in Table 1. The control group (T1) consistently captured the highest number of flies, ranging from 12.3 to 13.3 flies per day. In contrast, treatments involving 80% ethanol extract (T2) and water extract (T3) significantly reduced fly captures, with T2 showing the lowest count on day 2 (5.6 flies). Among the fermentation treatments, T4 (fermentation with alcohol) consistently demonstrated the most effective fly repellent properties, with a notable decrease to only 4.6 flies by day 4. Similarly, T5 (fermentation with water) exhibited low fly captures, especially on days 2 and 3, further highlighting its effectiveness. T6 (fermentation with sugar) and T7 (raw Nonnai grass) showed moderate reductions in fly numbers, though they were less effective than T4 and T5.

Table 1. Fly Protection and egg-laying

Day (12h/day)	Number of Flies Captured (Count)							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
day1	12.3^{a}	8^{b}	7.6^{c}	5.3^{c}	6.6^{c}	9^{b}	6.3^{c}	1.33	0.04
day2	10.3^{a}	5.6^{bc}	8.6^{ab}	6^{bc}	4^{c}	7.6^{bc}	4.6^{c}	0.8	0.002
day3	11.3^{a}	6.3^{b}	7.6^{b}	6.3^{b}	3^{c}	7.3^{b}	4.6^{c}	1.52	0.046
day4	13.3^{a}	8.3^{b}	8^{b}	4.6^{c}	3.6^{c}	7^{b}	4^{c}	1.2	0.0006

Note:- means with different letters are significantly different at the 95% confidence level ($P<0.05$).
-Nonnai grass extracts were sprayed onto the fish dise daily. The number of flies caught on the plate was observed and recorded every hour for 12 hours (from 8 a.m. to 4 p.m.)

3.2. Egg laying of flies

The study assessed the effect of different treatments on fly egg-laying behavior over four days, as shown in Table 2. The control group (T1) exhibited a substantial number of fly spawn points, starting at 12.3 points on day 1 and progressively decreasing to 3.3 points by day 4. In stark contrast, all other treatments (T2 to T7) completely prevented egg laying, with zero spawn points recorded across all four days of the study. This dramatic difference in egg-laying activity was statistically significant, with p-values of less than 0.05, indicating the high efficacy of the treatments in preventing egg laying. The absence of egg laying in all treatments except the control (T1) highlights the potency of the bioactive compounds present in Nonnai grass, especially when extracted or fermented. These compounds appear to have a significant impact on fly reproductive behavior, effectively disrupting the egg-laying process.

Table 2. Egg laying of flies

Day (12h/day)	Number of fly spawn points (points)							SEM	P-value
	T1	T2	T3	T4	T5	T6	T7		
day 1	12.3[a]	0	0	0	0	0	0	0.5	0.001
day 2	8.6[a]	0	0	0	0	0	0	0.2	0.001
day 3	5.3[a]	0	0	0	0	0	0	0.5	0.001
day 4	3.3[a]	0	0	0	0	0	0	0.3	0.0001

Note: means with different letters are significantly different at the 95% confidence level (P<0.05).

3.3. Larvicidal activity of Nonnai grass

The larvicidal activity of Nonnai grass showed a clear and significant impact on maggot mortality, as demonstrated in Table 3. By day 5, the treatments T3 (water extract), T4 (fermentation with alcohol), T5 (fermentation with water), and T7 (raw Nonnai grass) exhibited the highest efficacy, with maggot mortality rates ranging from 96.66% to 100%. These treatments significantly outperformed the control group (T1), which did not exhibit any maggot mortality until day 5. The statistical analysis ($p < 0.05$) confirms the effectiveness of Nonnai grass treatments, particularly in their fermented and raw forms, as potent larvicides. The increasing mortality rate over time, especially with treatments T4, T5, and T7, suggests that these preparations significantly enhance the bioactivity of the herb's active compounds.

Table 3. Larvicidal activity of Nonnai grass

Time (day)	Number of Maggots Killed (Units)							SEM	p-value
	T1	T2	T3	T4	T5	T6	T7		
0	0	0	0	0	0	0	0		
day 1	0[c]	1.6[ab]	2[ab]	2.3[a b]	2.6[a]	1.3[b]	2.3[ab]	0.28	0.0004
day 2	0[c]	4.3[ab]	5[ab]	5.3[b]	6.3[a]	3.3[c]	5[b]	0.25	0.001
day 3	0[d]	4.6[bc]	5.3[ab]	5.6[a b]	6.6[a]	4[c]	5.6[ab]	0.35	0.001
day 4	0[d]	6.6[b]	8[a]	8.3[a]	9[a]	6[b]	8.6[a]	0.43	0.001
day 5	1c	7.6[b]	9.6[a]	10[a]	10[a]	7.3[b]	10[a]	0.37	0.001
Alive maggots	10	10	10	10	10	10	10		
Death of maggots (%)	10[c]	76.66 [b]	96.66 [a]	100[a]	100[a]	73.33 [b]	100[a]	3.77	0.000

Note: means with different letters are significantly different at the 95% confidence level (P<0.05).

3.4. Efficacy of Antibacterial assay

The antibacterial assay results demonstrated that Nonnai grass extracts exhibited significant inhibitory effects against all tested bacteria, with efficacy varying based on the preparation method (Table 4). Staphylococcus aureus showed the highest inhibition zones with T3 (water extract, 6.93 mm) and T4 (fermentation with alcohol, 6.51 mm), both of which were significantly greater than other treatments ($P < 0.001$). Escherichia coli was most effectively inhibited by T2 (ethanol extract, 5.06 mm), with significant inhibition also observed for T3, T4, T5, and T7 ($P < 0.001$). Klebsiella exhibited the largest inhibition zones with T4 (11.19 mm), followed by T2 (9.95 mm), highlighting the strong antibacterial activity of fermented and ethanol-extracted Nonnai grass ($P \leq 0.001$). Similarly, Salmonella was most inhibited by T4 (8.05 mm), with significant effects also observed for T2, T3, and T7 ($P < 0.001$).

Table 4. Efficacy of Antibacterial assay

Bacteria	Inhibition area (mm) Antibacterial activity zone of inhibitor							SEM	P-Value
	T1	T2	T3	T4	T5	T6	T7		
Staphylococcus aureus	0.00^{d}	2.77^{bc}	6.93^{a}	6.51^{a}	4.59^{b}	1.78^{c}	4.13^{b}	0.51	0.001
Escherichia coli (E. coli)	0.00^{d}	5.06^{a}	3.39^{b}	3.37^{b}	3.21^{b}	1.45^{c}	3.61^{b}	0.31	0.001
Klebsilla	0.00^{f}	9.95^{b}	9.41^{bc}	11.19^{a}	8.47^{cd}	3.21^{e}	8.04^{d}	0.33	0.001
Salmonella	0.00^{d}	6.64^{ab}	6.98^{ab}	8.05^{a}	5.47^{b}	1.96^{c}	7.18^{ab}	0.47	0.001

Note: means with different letters are significantly different at the 95% confidence level ($P<0.05$).

4. Discussion (Time New Roman size "12" pt, bold)

The lower fly capture rates observed with ethanol and water extracts, as well as fermented preparations of Nonnai grass, suggest that these treatments enhance the availability and potency of active compounds. Ethanol extraction, for example, is known to yield higher concentrations of bioactive compounds than water extraction (Azwanida, 2015). Additionally, fermentation can increase the bioactivity of these compounds by converting complex molecules into more active forms, thereby boosting their concentration and bioavailability as repellent agents (Sadh et al., 2018). The significant reduction in fly numbers observed with fermented Nonnai grass, particularly when prepared with alcohol or water, may be attributed to the enhanced repellent properties of volatile compounds released during fermentation (Isman, 2020; Maia & Moore, 2011). The significant differences in fly counts across all treatments highlight the effectiveness of Nonnai grass, particularly when fermented, in pest management strategies. The effect of different treatments on fly egg-laying behavior over four days, this finding aligns with previous research on plant-derived compounds and their effects on insect oviposition (Isman, 2006). In contrast, the control group, which lacked exposure to these bioactive compounds, allowed for normal egg-laying activity, emphasizing the effectiveness of the treatments. Disrupting the reproductive cycle of insects is a known mechanism by which many botanical insecticides exert their effects (Pavela & Benelli, 2016). The inhibition of egg laying observed in this study suggests that the Nonnai grass treatments—whether prepared through ethanol extraction, water extraction, or fermentation—are highly

effective in preventing fly reproduction. These results demonstrate the potential for Nonnai grass preparations to serve as a natural solution for pest control by targeting not only adult flies but also interrupting their reproductive cycle. The increasing mortality rate over time, especially with treatments T4, T5, and T7, suggests that these preparations significantly enhance the bioactivity of the herb's active compounds. This enhanced bioactivity may be attributed to fermentation or extraction processes that increase the availability and potency of the active components within Nonnai grass. By day 5, the maggot mortality rate in treatments involving fermentation with alcohol or water reached 100%, underscoring the efficacy of fermentation as a method to boost the larvicidal properties of the herb. These results suggest that the bioactive compounds present in Nonnai grass, particularly when fermented or extracted, are highly potent against fly larvae. This finding is consistent with previous studies demonstrating that fermentation enhances the bioactivity of plant-derived compounds, making them more effective against insect pests (Tang et al., 2022). The significant difference in maggot mortality rates between treatments highlights the potential for Nonnai grass as a natural larvicidal agent in pest management strategies.

The fermentation and ethanol extraction processes notably enhanced the antibacterial properties of Nonnai grass, particularly against *Staphylococcus aureus*, *Klebsiella*, and *Salmonella*. These methods likely increased the concentration or bioavailability of the herb's active compounds, consistent with existing research on the efficacy of plant-based antimicrobials (Arifin et al., 2024; Sanjaya et al., 2022). The variation in bacterial susceptibility where *Klebsiella* exhibited the highest inhibition zones may be attributed to differences in bacterial cell wall structure or specific resistance mechanisms, as observed in other studies on bacterial responses to plant extracts (Abd-Alhussain & Alwan, 2024; Wang et al., 2024). This highlights the potential for Nonnai grass preparations, particularly fermented and ethanol-extracted forms, to be developed as natural antibacterial agents.

5. Conclusion

The study demonstrated that *Tadehagi triquetrum* L. (Nonnai grass) exhibits strong potential as a natural agent for pest control and antibacterial applications. Fermentation and ethanol extraction methods significantly enhanced the repellent, larvicidal, and antibacterial properties of the herb. Fermentation with alcohol (T4) and water (T5) proved particularly effective in reducing fly captures and preventing egg-laying, while also showing high larvicidal efficacy. Additionally, both fermentation and ethanol extraction produced notable antibacterial effects, especially against *Staphylococcus aureus*, *Klebsiella*, and *Salmonella*. These findings suggest that Nonnai grass, particularly in its fermented and ethanol-extracted forms, could be developed as an eco-friendly alternative to synthetic chemicals for pest and microbial management.

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Poster Presentation

Effect of Idebenone in Extender on Frozen Semen Quality in Thai Native Chickens

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Abstract

Background: Cryopreservation of rooster sperm leads to damage due to its high content of polyunsaturated fatty acids in the sperm membrane, making it highly susceptible to lipid peroxidation during storage. Lipid peroxidation in sperm cells results in reduced motility, decreased acrosomal reaction capability, DNA damage, and increased sperm mortality, ultimately lowering fertilization rates. This study aimed to compare the effects of idebenone, a quinone antioxidant, on sperm preservation. Idebenone has the ability to inhibit lipid peroxidation, protecting cell membranes and mitochondria from oxidative damage. This study was conducted to investigate the effects of idebenone on the quality of cryopreserved semen in Thai native chickens.

Methods: Semen samples were collected by manual ejaculation from 6 roosters. Each ejaculate was initially diluted at a 1:1 ratio using Schramm's diluent, and then diluted semen samples were pooled together. The pooled semen was further diluted to achieve the final conditions of 1,000 million spermatozoa per milliliter, 6% dimethylformamide (DMF) as the cryoprotectant, and the specified idebenone concentrations (0, 5, 10, and 20 µM). The final diluted semen was then divided into 4 treatment groups accordingly. All semen samples were cryopreserved for at least 3 days before thawing and evaluation. Sperm quality parameters assessed after thawing included total motility, progressive motility, sperm viability, and malondialdehyde (MDA) levels. The experiment was conducted with 8 replicates per treatment.

Results: The highest total and progressive sperm motility were observed in the 10, 5, 0, and 20 µM idebenone groups, respectively. Live sperm percentages ranked as 0, 5, 10, and 20 µM, while MDA levels were highest in the 5, 0, 10, and 20 µM groups. No significant differences were found among groups ($p>0.05$)

Conclusion: The results revealed that idebenone had no significant impact on semen quality. However, this study provides preliminary evidence supporting the practical use of idebenone in rooster semen cryopreservation and highlights its potential as an antioxidant additive for further investigation.

Keywords: Frozen semen, Idebenone, Semen quality, Thai native chicken

Efficacy of Endoscope Assisted Transcervical Artificial Insemination (ETCAI) on Pregnancy Rates in Goats

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Abstract

Background: Conventional artificial insemination (AI) in goats is challenging and highly operator-dependent. This study aimed to evaluate the efficacy of Endoscope Assisted Transcervical Artificial Insemination (ETCAI) in improving pregnancy rates in caprine subjects.

Methods: Does underwent estrus synchronization using intravaginal controlled internal drug release (CIDR) devices for 13 days. On day 12, one day prior to CIDR removal, pregnant mare serum gonadotropin (PMSG) and prostaglandin F2α (PGF2α) were administered. Artificial insemination was performed on day 15. Animals from 4 farms were allocated to two groups: a control group (n=18) inseminated via conventional transcervical AI, and an experimental group (n=19) inseminated using ETCAI.

Results: The ETCAI group achieved a pregnancy rate of 42.11% (8/19), compared to 33.33% (6/18) in the control group. Although the difference in pregnancy rates was not statistically significant.

Conclusion: ETCAI demonstrates potential as a valuable tool to enhance AI efficacy in goats and may mitigate limitations associated with operator expertise.

Keywords: Goats, Artificial Insemination, Endoscope-assisted transcervical artificial insemination

Preliminary Synchrotron SAXS Analysis of Dog Hair with Diverse Characteristics and Surgical Conditions

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Abstract

Objective: This study utilized Small-Angle X-ray Scattering (SAXS) to investigate the nanostructure of dog hair, examining various phenotypic characteristics (color, length, straightness, and curl) and hair from dogs undergoing surgical procedures.

Methods: We analyzed 36 hair samples collected over 3–4 days from ten crossbreed, neutered dogs of varying ages and genders. Six of these dogs (Nos. 1–6) had recently undergone surgery, while four (Nos. 7–10) had not. Samples were triple-washed with absolute ethanol, air-dried, and stored at -20 °C prior to analysis. Electron dispersion signals were plotted as intensity versus scattering vector (q) using PRIMUS software. The d-spacing of the keratin intermediate filaments was calculated from the distinct hump observed in the SAXS graphs.

Results: Consistent SAXS scattering patterns were observed across all dog hair samples, irrespective of the hair's phenotypic appearance or the stress related surgery. All samples exhibited humps at q-values of 0.6 nm^{-1} and 0.8 nm^{-1}, corresponding to d-spacings of 10.5 nm and 7.9 nm, respectively. These findings confirm the typical keratin structure and arrangement within dog hair. SAXS analysis did not reveal distinct phenotypic differences in hair structure. Nevertheless, the hair sample from the enterectomized dog showed a trend of decreased scattering intensity at six days post-surgery, indicating possible nanostructural deterioration.

Conclusion: This study establishes a foundation for the future use of dog hair as a physical biomarker for canine health. However, the one-dimensional nature of SAXS data limits its ability to determine three-dimensional structures. More advanced synchrotron methods are necessary for a comprehensive interpretation of hair nanostructures.

Keywords: dog hair, keratin nanostructure, intermediate filament, surgical stress, X-ray diffraction

1. Introduction

Hair is a defining biological characteristic and a common integumentary feature of mammals. Each hair fiber comprises three concentric layers: the outermost cuticle, the central cortex, and the innermost medulla (Yang et al., 2014). The chemical and physical properties of hair are determined by its complex composition, which includes keratin intermediate filaments, protein matrix, pigmented melanosomes, lipids, and minerals (Stanić et al., 2015). The hair shaft primarily consists of filamentous structural proteins known as keratin. These keratins are densely packed to form intermediate filaments, particularly abundant in the cortex (Stanić et al., 2015). Even subtle changes in one component, such as fat composition, can influence the alignment or stability of others, like keratin fibers, thereby offering a comprehensive view of the hair's structural integrity (Alsop et al., 2016).

Given that health and environmental factors significantly impact the biological properties of human hair (Schmidt et al., 2017), hair analysis has become a fascinating area of research across fields such as forensic science, materials science, and biology. Small-Angle X-ray Scattering (SAXS) is a powerful, non-destructive analytical technique that quantifies nanoscale density differences within a sample. It achieves this by analyzing the elastic scattering behavior of X-rays at very small angles (Kajiura et al., 2006). SAXS can specifically differentiate scattering signals originating from both keratin and lipids within the hair structure. For instance, hair from breast cancer patients exhibits X-ray diffraction characteristics indicative of phospholipid deviations when compared to hair from healthy women (Mistry et al., 2012). More recently, SAXS, when combined with advanced techniques like Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), has been used to demonstrate differences in the size and shape of melanin aggregates in hair affected by conditions such as Alopecia Areata (Coroaba et al., 2020). While there's a growing body of nano-structural studies on human hair, comprehensive databases for animal hair remain limited.

In dogs, the condition of their skin and hair coat serves as a clear indicator of their overall health status or potential underlying systemic diseases. The morphologies and metabolites found in specific segments of a hair strand can reflect both current and past physiological histories (Choi et al., 2019). Dogs undergoing surgery experience considerable environmental and physiological stress (Kisani et al., 2018), often leading to insufficient nutrient intake. We have observed that a dog's coat can grow at an impressive rate of approximately 3 to 5 millimeters per day (personal observation), significantly faster than human hair, which grows at about one millimeter per day (Mesarcova et al., 2017). Furthermore, hair is easily collected, making dog hair a potentially suitable sample for physical biomarker studies. This study aims to provide preliminary insights into the SAXS patterns of dog hair with diverse characteristics (color, length, straightness, and curl) and to investigate the impact of surgical procedures on its nanostructure.

2. Materials and Methods

2.1 *Classification of experimental dogs and hair characteristics*

This study builds upon our previous project (Direksin et al., 2021). The operation was conducted under the approval of the KKU Animal Research Ethics Committee (protocol number IACUC-KKU 88/60). For this investigation, the hair samples were selected from our stock collection. Sequential hair samples were collected from the dorsal neck area of participating dogs by cutting them as close to the skin as possible with

scissors. **Table 1** provides a detailed summary of the experimental dogs and their hair characteristics. All dogs were confirmed to be in good health and were included in the study with informed consent obtained from their owners. We analyzed 36 hair samples sourced from ten crossbreed, neutered dogs that varied in age and gender. Six of these dogs (Nos. 1–6) had recently undergone surgical procedures, while four (Nos. 7–10) had not. There were no bone fractures; the operation was limited to accessing the bone without causing structural damage. Hair samples were collected from each dog over a period of 3–4 days, specifically on Day 0 or 1, Day 3, and Day 6 or 7. Prior to analysis, all samples underwent a triple wash with absolute ethanol, followed by air-drying and storage at -20 °C. Hair samples were then analyzed using Small-Angle X-ray Scattering (SAXS) at the Synchrotron Light Research Institute in Nakhon Ratchasima Province, Thailand.

Table 1. Classification of experimental dogs and their hair characteristics.

Dog No.	Age (year)	Gender (neutered)	Weight (Kg)	Hair appearance	Treatment group	Sampling day/ # Sample No.
1	1	Male	21.0	Long black	Incision to expose femur and Humerus bones	D0, D1, D3, D6 # 1,2,4,7
2	2	Male	18.6	Short black	Enterectomy and enterorrhaphy	D0, D1, D3, D6 # 43,44,46,49
3	2	Female	17.0	Short black	Incision to expose femur and Humerus bones	D0, D1, D3, D6 # 8,9,11,14
4	2	Female	6.4	Long black	Caudal midline laparotomy	D0, D1, D3, D6 # 50,51,53,56
5	1	Male	18.0	Short black	Caudal midline laparotomy	D0, D1, D3, D6 # 57,58,60,63
6	1	Female	20.0	Short black and white	Caudal midline laparotomy	D0, D1, D3, D6 # 64,65,67,70
7	5	Male	18.0	Long white	Non-surgical	D1, D3, D7 # 78,79,81
8	6	Female	20.0	Long white	Non-surgical	D1, D3, D7 # 85,86,88
9	13	Male	20.0	Long brown	Non-surgical	D1, D3, D7 # 92,93,95
10	8	Female	18.0	Long black	Non-surgical	D1, D3, D7 # 99,100,102

*Measured from guard hair, long hair is ≥ 3 cm in length.

2.2 Hair Sample Characterization

For each dog, three to five hair shafts were examined under a microscope and photographed to record their length and color. Samples representing all ten dogs (sample numbers 1, 8, 43, 50, 57, 64, 78, 85, 92, and 99, as detailed in Table 1) were selected for this characterization. We defined long hair as being greater than three centimeters in length. The guard hair, originating from the outermost layer, is notably thicker than the down hair, which is lighter and forms an insulating layer beneath the guard hair.

2.3 Synchrotron X-ray diffraction

X-ray scattering experiments were conducted on hair fibers collected from each dog. For each analysis, 3–5 hair strands (representing a specific sample number) were carefully arranged and mounted onto a specially designed sample holder. Synchrotron X-ray scattering was performed using the Small-Angle X-ray Scattering (SAXS) beamline (1.3 W) at the Synchrotron Light Research Institute, following established methodologies (Schaber et al., 2019; Xing et al., 2013). The hair strands were meticulously positioned perpendicular to the X-ray beam to ensure optimal data acquisition. A standard empty sample holder served as a blank for background subtraction and calibration.

2.4 Data Analysis

Scattering profiles were generated using PRIMUS software, plotting the scattering vector (q-value) in nm^{-1} (or $Å^{-1}$) against intensity (in arbitrary units; a.u.). The shapes of these graphs were then compared. The primary scattering peak or shoulder in each graph was used to calculate the d-spacing (d), representing characteristic distances within the sample. This calculation was performed using the formula: $d=2\pi/q$. The resulting d-spacing values indicate the spacing of the intermediate filaments.

3. Results

Hair Phenotypic Characteristics

The phenotypic characteristics of the dog hair samples, including color, length, and shape (curly, wavy, or straight), were thoroughly examined. Dogs No. 1, 2, 3, 4, 5, and 10 displayed pigmented black hair. Dogs No. 7 and 8 had white hair, while Dog No. 9 exhibited brown hair. Notably, Dog No. 6 presented bicolor hair, with both black and white pigmentation within the same strand. Most dogs—specifically Dogs 2, 4, 5, 6, and 9—possessed curly guard hair and wavy down hair.

It is important to note that macroscopic examination of the hair samples was performed after the synchrotron analyses. This revealed that both guard hair and down hair types were mixed within each collected sample. Consequently, the SAXS analysis for each sample was conducted on a pool containing both guard and down hair types.

Small-Angle X-ray Scattering (SAXS) Analysis

All dog hair samples consistently exhibited similar SAXS graph shapes, where intensity decreased as the q-values increased. Prominent peaks were observed at q-values of 0.6 nm^{-1} and 0.8 nm^{-1}, corresponding to d-spacings of approximately 10.5 nm and 7.9 nm, respectively (**Figure 1**). This yielded an average d-spacing of 9.2 nm. All sample peaks maintained consistent positions across the analyses. While slight variations in intensity were observed across the samples and their error bars overlapped, we primarily focused on self-comparison within individual dogs across chronological sampling days for clear interpretation. To illustrate these findings, we present representative SAXS scattering profiles from Dog 10 (a non-surgical dog) and Dog 2 (a surgical dog). **Figure 2** displays the SAXS scattering profiles for Dog 10, showing samples collected on Day 1, Day 3, and Day 7. All hair samples from Dog 10 demonstrated consistent scattering patterns, with only minor variations in intensity. The prominent peaks were consistently observed around q-values of 0.6 nm^{-1} and 0.8 nm^{-1}. Despite slight variations, the Day 1 sample showed the lowest intensity among the other days (D3, D7). **Figure 3** illustrates the scattering patterns for Dog 2, representing the surgical group. All samples collected over the four-day period maintained the same graph shapes. However, a general tendency for overall intensities to decrease was observed from Day 0 to Day 6, with the intensity of the Day 6 sample being the lowest among the surgical group.

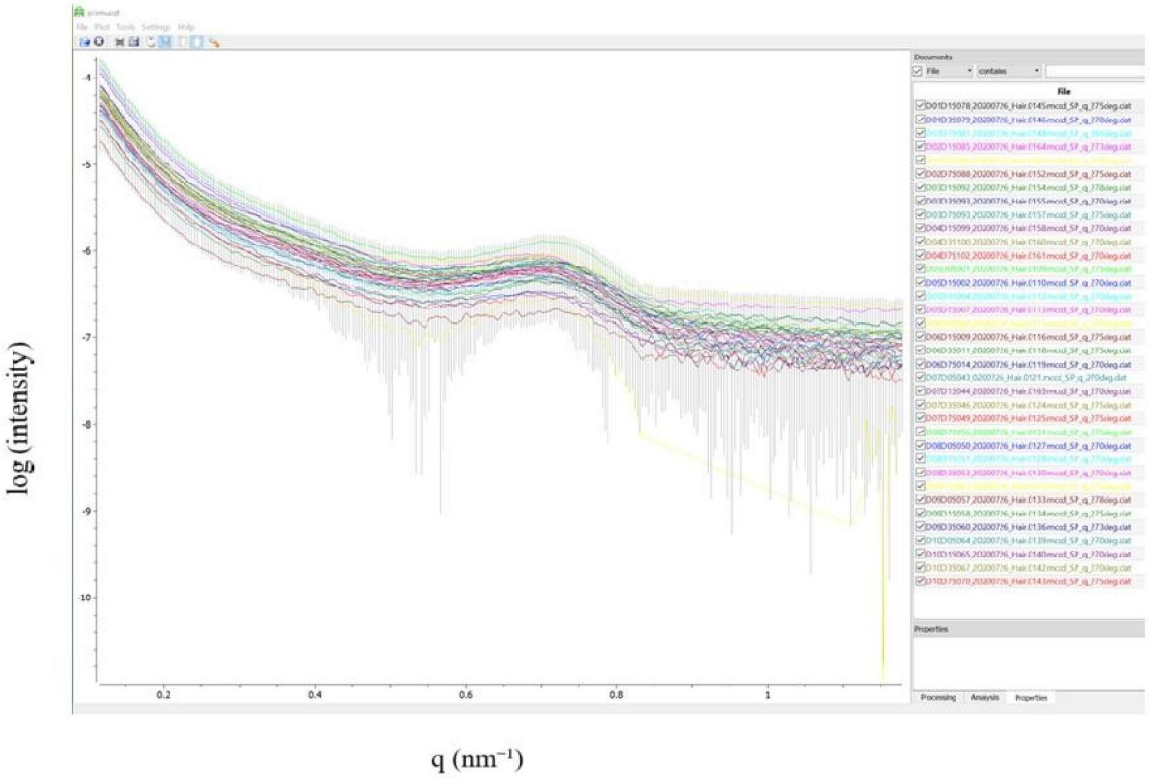

Figure 1. SAXS scattering profiles of dog hair samples. All ten dogs and chronologically collected samples (Day 0–7) are shown. The graph displays log intensity versus q-value (scattering vector). Peaks are observed between q = 0.6 and 0.8 nm^{-1}.

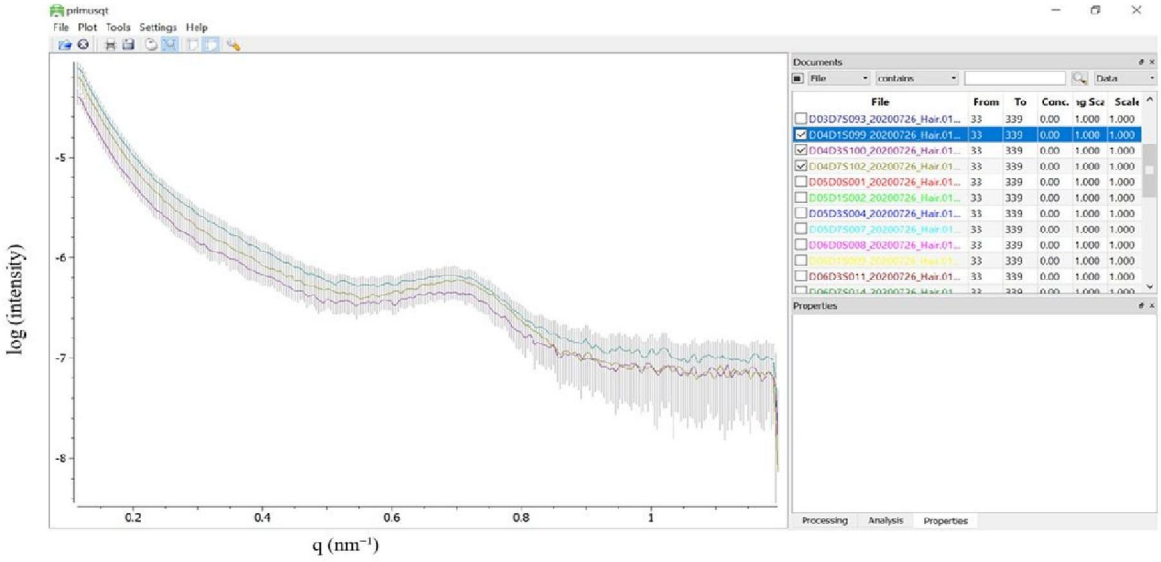

Figure 2. SAXS scattering profiles of dog hair from a non-surgical dog (Dog 10). This graph shows samples collected on Day 1 (green line), Day 3 (purple line), and Day 7 (brown line). All three profiles exhibit consistent scattering patterns with peaks at q-values of 0.6 and 0.8 nm^{-1}. Despite slight variations, the Day 1 sample showed the lowest intensity among the other days (D3, D7).

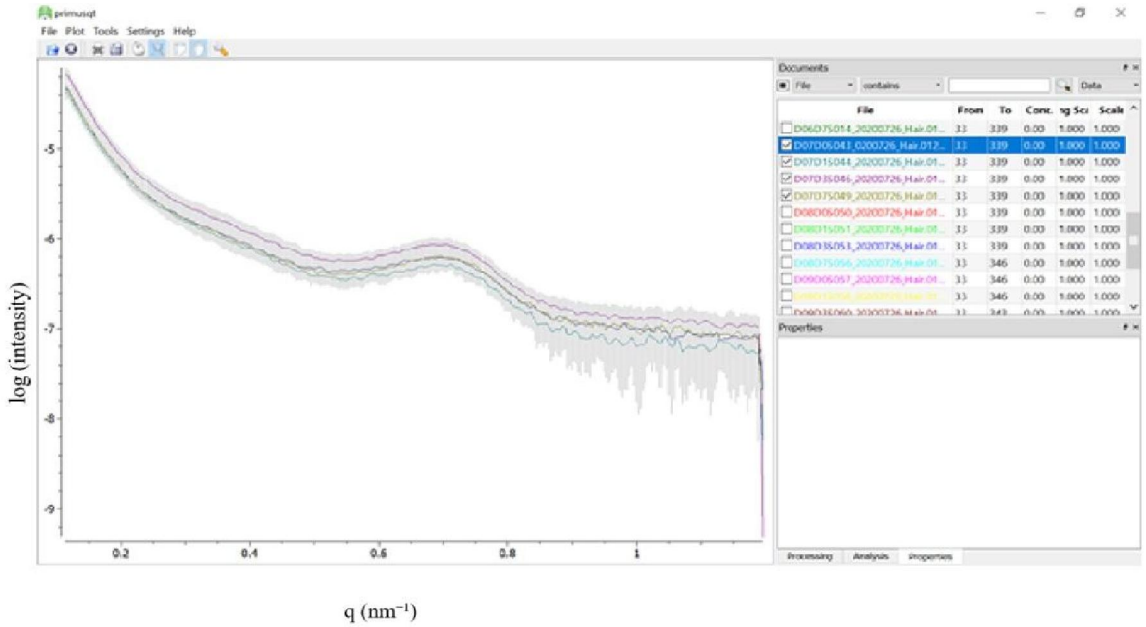

Figure 3. SAXS scattering patterns of hair samples from a surgical dog (Dog 2). This graph displays data collected before (Day 0, blue line) and after surgery (Day 1 (green line), Day 3 (purple line), Day 6 (brown line). All four profiles maintain similar shapes with prominent peaks at q-values of 0.6 and 0.8 nm^{-1}. A clear trend of decreasing overall intensity is observed from Day 0 to Day 6, with the Day 6 sample (brown line) showing the lowest intensity.

4. Discussion

Small-Angle X-ray Scattering (SAXS) is a well-established technique commonly employed in the analysis of polymers (Chu and Hsiao, 2001) and biological tissues (Blanchet et al., 2015). Given that hair shafts are filamentous structures, SAXS applications in the biomedical field have proven valuable, for instance, in differentiating human hair samples from healthy individuals and patients with Alopecia areata (Coroaba et al., 2020). SAXS can similarly reveal distinctive scattering patterns indicative of changes in dog hair structure. The characteristic shape of a SAXS graph provides critical information about the size, composition, and arrangement of nanostructures within the hair.

In this study, the scattering patterns of all dog hair samples exhibited consistent shapes, regardless of phenotypic appearance (e.g., color, length, or waviness). The observed humps in the SAXS graphs are characteristic of the regular arrangement of keratin intermediate filaments and their associated matrix structures within the hair cortex. The stable d-spacing values recorded across samples indicate a consistent molecular structure of dog hair keratin fibers. It is noteworthy that the d-spacing of animal hair fibers can vary across species, likely due to differences in the hierarchical structure of keratin filaments and the presence of diverse keratin-associated proteins (Wang et al., 2016). Similarly, studies on human hair have found consistent keratin structures but variations in lipid content and keratin-associated proteins among different ethnic hair types (Wade et al., 2013). In this study, the keratin structure of dog hair was confirmed using SAXS analysis. Due to technical limitations, additional investigations with other advanced techniques could not be conducted. The average d-spacing of dog hair in our study was 9.2 nm (ranging from 7.9 nm to 10.5 nm), which is comparable to the 9.5 nm reported for human scalp hair by Yang et al (2014)

Domestic dogs (*Canis lupus familiaris*) display a remarkable range of coat types across breeds, exhibiting variations in color, texture, length, curliness, and growth patterns (Parker et al., 2020). Canine coats are typically categorized into two layers: the outer "guard" hairs (long and thick) and the inner "down" hairs (short and soft). For the SAXS analysis in this study, both guard and down hair types were pooled together. Subsequent macroscopic examination confirmed that the analyzed samples were indeed a mix of both hair types. The inherent characteristics of these two distinct hair types could introduce variability in scattering intensities even within samples from the same dog. Therefore, to minimize confounding factors, we focused on comparing samples within individual dogs and selected the most representative dogs from each group for detailed description.

A reduction in scattering intensity at a specific q-value often suggests loose or less ordered hair structure. Our scattering profiles for hair samples from Dog 2, belonging to the surgical group, revealed slight changes in the samples collected six days post-operation (D6). A noticeable reduction in scattering intensity was observed when comparing samples from Day 0 to Day 6. These alterations suggest that post-operative stress or metabolic changes might have impacted the structural integrity of hair proteins at the nanoscale. It is well-documented that surgical procedures induce pain and stress in dogs, evidenced by elevated levels of blood cortisol, glucose, total protein, albumin (ALB), globulin (GL), and altered ALB/GL ratios just two hours after surgery (Kisani et al., 2018). Furthermore, our previous research indicated a surge in hair cortisol levels in Dog 2 approximately three days after enterectomy (Direksin et al., 2021). Postoperatively, the

dog was unable to consume food orally for one week and received only intravenous fluid therapy. This may have contributed to the alterations observed in scattering intensity than other dogs. While SAXS offers valuable insights into hair structure and health, it is crucial to acknowledge its limitations. The technique provides low-resolution data, which may not capture all intricate structural details. However, when integrated with other advanced methods, SAXS remains a vital tool for elucidating the biological implications of hair structure. For a more comprehensive understanding, future studies should consider examining thin sections of hair using techniques such as Transmission Electron Microscopy (TEM) and Energy-Dispersive X-ray Spectroscopy (EDX).

5. Conclusion

This preliminary study suggests a tendency for reduced hair integrity following surgery, although the structural impact on the hair cortex six days post-operation appeared minimal. Nevertheless, this research establishes a foundational basis for future investigations into utilizing dog hair as a simple pathological marker. While this study lays the groundwork for using dog hair as a physical biomarker for canine health, the one-dimensional nature of SAXS data inherently limits its ability to determine complex three-dimensional structures. Consequently, more advanced synchrotron methods will be essential for a truly meaningful interpretation of hair nanostructures.

Acknowledgements

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Retrospective Analysis of Risk Factors and Postoperative Management of Surgical Site Infections in Canine Patients at Chiang Mai University's Small Animal Hospital (2017–2024)

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Abstract

Background: Surgical site infection (SSI) is a common postoperative complication that increases morbidity, mortality, and healthcare costs. This study aims to identify risk factors associated with SSI in dogs.

Methods: Data was collected retrospectively from January 1, 2017, to December 31, 2023, covering a seven-year period, and prospectively from January 1 to November 30, 2024. The diagnosis of SSI was based on the Centers for Disease Control and Prevention (CDC) criteria (CMU – ACUC Ref. No. S1 / 2567).

Results: In the retrospective analysis of 43 cases, SSI was diagnosed in 13 dogs (30.23%). Administration of steroids was identified as a statistically significant risk factor for SSI ($p < 0.05$). In the prospective analysis of 39 cases, SSI was diagnosed in 29 dogs (74%); however, no statistically significant risk factors were identified. Bacterial cultures from surgical sites yielded 35 isolates from 28 samples. The most common pathogens were *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterobacter taylorae*. Among the 35 isolates, 29 were identified as multidrug-resistant (MDR) bacteria. The most commonly resistant antibiotics were Amoxicillin/Clavulanic acid, Cephalexin, and Sulfa-trimethoprim, respectively.

Conclusion: This study concludes that steroid administration significantly increases the risk of SSI in dogs. Veterinarians should focus on strict infection control measures during and after surgery and use antibiotics responsibly to prevent the rise of drug-resistant bacteria.

Keywords: Dog, Multidrug-resistant (MDR), Surgical site infection

Reproductive Tract-Derived Extracellular Vesicles Improve Bovine Embryo Development During In Vitro Culture

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Abstract

Background: *In vitro* embryo production (IVEP) is a cornerstone technique in animal biotechnology that enables rapid genetic improvement in livestock. However, embryos generated under in vitro conditions often exhibit lower developmental competence and viability compared to their *in vivo* counterparts, due to the absence of maternal-derived molecular signals. To address this limitation, extracellular vesicles (EVs), which are nano-sized particles secreted by epithelial cells and known to carry functional proteins, mRNAs, and miRNAs, have emerged as promising bioactive supplements. This study aimed to evaluate the effects of EVs derived from bovine oviduct epithelial cells (BOECs), cultured under two different conditions, monolayer (BOECs-M-EVs) and vesicle-shaped suspension (BOECs-V-EVs) on the development and quality of bovine embryos during *in vitro* culture (IVC).

Methods: EVs were isolated by ultracentrifugation and characterized by nanoparticle tracking analysis. The EVs were supplemented into culture media at concentrations of 2 × 10^6, 4 × 10^6, and 8 × 10^6 particles/mL.

Results: Results demonstrated that embryos supplemented with BOECs-derived EVs showed significantly higher cleavage and blastocyst rates, increased embryo diameter, and greater total and trophectoderm cell numbers. Moreover, gene expression analysis revealed that EV supplementation downregulated the pro-apoptotic gene BAX and upregulated IFNT, a marker for implantation potential, indicating reduced stress and improved embryo viability.

Conclusion: These findings suggest that BOECs-derived EVs help mimic the maternal environment and enhance cell signaling pathways related to survival and development. This study highlights the potential of reproductive tract-derived EVs as a practical and effective supplement for improving the outcomes of bovine IVEP systems.

Keywords: Bovine embryos, Extracellular vesicles, Bovine Oviduct epithelial cells, In vitro culture, Embryo development,

1. Introduction

In vitro embryo production (IVEP) is a crucial technology in livestock genetic improvement programs, facilitating the rapid production of embryos from genetically superior animals. Despite its considerable advantages, embryos generated through IVEP typically exhibit lower developmental competence and reduced survival rates than embryos produced *in vivo*. These shortcomings primarily result from artificial culture conditions that inadequately replicate the maternal reproductive tract's complex biochemical and cellular environment, leading to cellular stress, mitochondrial dysfunction, and activation of apoptotic pathways (Leal et al., 2022). Various strategies have been investigated to enhance *in vitro* culture (IVC) systems, including the supplementation of culture media with bioactive molecules. Recently, extracellular vesicles (EVs) have gained attention as promising supplements due to their essential role in intercellular communication (Feliciano et al., 2014; Qiao et al., 2018). EVs transport crucial bioactive molecules involved in embryo development, implantation, and pregnancy processes. Studies in multiple species, including mice and sheep, have demonstrated beneficial effects of EV supplementation, although research in cattle remains relatively limited. Previous studies indicate that EV supplementation can enhance embryo quality by improving the inner cell mass (ICM) to trophectoderm (TE) ratio, promoting cellular proliferation, and reducing apoptosis. For instance, EVs derived from bovine uterus and oviduct epithelial cells (BOECs) have shown the potential to upregulate genes associated with blastocyst development, improve mitochondrial activity, and reduce lipid accumulation in embryos (Leal et al., 2022; Lopera-Vasquez et al., 2017; Qiao et al., 2018; Qu et al., 2017; Wei et al., 2022). These effects collectively contribute to higher embryo viability and developmental potential. This study specifically explores the effects of EVs derived from bovine oviduct epithelial cells cultured either as monolayers (BOECs-M-EVs) or as vesicle-shaped structures (BOECs-V-EVs) on the developmental outcomes and quality of bovine embryos produced through *in vitro* fertilization (IVF). Given the unique capacity of BOECs-derived EVs to mimic physiological conditions within the oviductal environment, this research aims to evaluate their impact on embryo developmental competence, embryo quality, and the expression of critical genes involved in embryo survival, development, and implantation. Specifically, the study focuses on genes such as *BAX*, associated with apoptosis; *BCL2,* related to cell survival; and *IFNT*, essential for maternal recognition and successful pregnancy establishment.

The objectives of this study were to evaluate the effects of BOECs-M-EVs and BOECs-V-EVs supplementation in IVC medium on the developmental rate and quality of bovine embryos produced via IVF, and to examine the influence of these EVs on the expression of genes associated with apoptosis (*BAX, BCL2*), implantation (*IFNT*), and pluripotency in IVF-derived embryos.

2. Materials and Methods

2.1 Reagents and Ethics Statement

All reagents described in the materials and methods will be bought from Sigma-Aldrich (St. Louis, MO, USA), except otherwise noted.

2.2 Ethical statement

The Animal Ethics Committee of Suranaree University of Technology (IACUC-67-42), Thailand, obtained ethical approval for using bovines in this study.

2.3 Experiment design

Experiment 1

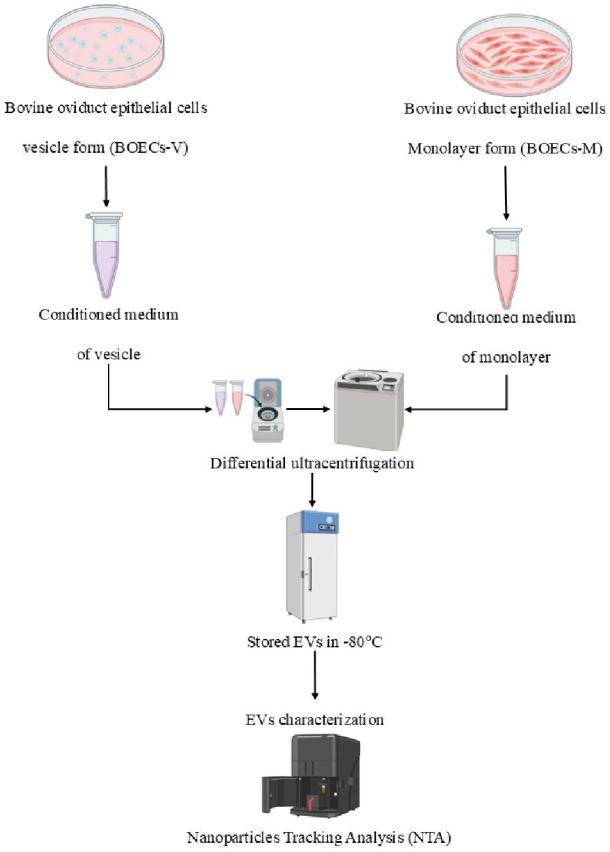

Figure 1. Workflow for isolating extracellular vesicles (EVs) from bovine oviduct epithelial cells cultured in vesicle (BOECs-V) or monolayer (BOECs-M) forms. EVs were collected by differential ultracentrifugation and analyzed by nanoparticle tracking analysis (NTA).

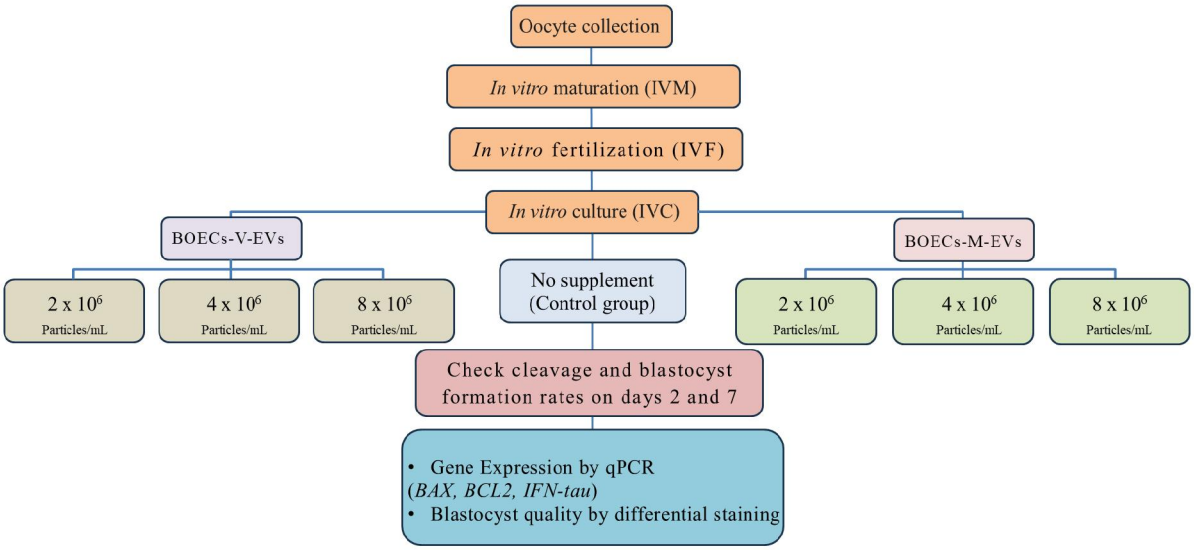

Figure 2. Experimental design of *in vitro* embryo production (IVF) supplemented with BOECs-derived EVs at varying concentrations. Embryo development and quality were assessed by cleavage and blastocyst rates, qPCR, and differential staining

2.4 Preparation of EVs-depleted fetal bovine serum (dFBS)

EVs were separated from heat-inactivated FBS as previously detailed (Aswad, Jalabert, & Rome, 2016). In brief, FBS was heat-inactivated (30 min at 56°C in a water bath), followed by two rounds of ultracentrifugation (90 min at 120,000 × g at 4°C), and the supernatant was collected, aliquoted (in conical tubes), and stored at −20°C until usage.

2.5 Preparation of bovine oviduct epithelial cells (BOECs)

Healthy bovine oviducts at the post-ovulatory stage (days 1–5 of the estrous cycle) were collected from a slaughterhouse, as described by Almiñana et al. (2017). Briefly, the oviducts were thoroughly rinsed with 0.9% (w/v) NaCl solution, placed in sterile plastic bottles containing the same solution, and stored at 4°C. Upon arrival at the laboratory, they were rinsed again with 0.9% NaCl to remove blood and debris, then soaked in phosphate-buffered saline (PBS). To reduce contamination, the oviducts were briefly submerged in 70% ethanol, followed by additional rinses with PBS and two washes in 70% ethanol. Connective tissues and blood vessels were carefully removed inside a biosafety cabinet. Subsequently, the oviducts were rinsed with a washing solution consisting of TCM199-HEPES supplemented with 0.2% (w/v) bovine serum albumin (BSA). Oviductal epithelial cells were collected by pressing the oviductal segments into 1.5 mL microcentrifuge tubes using sterile forceps. At least three oviducts were pooled. The harvested cells were dissociated by repeatedly aspirating and expelling the cell suspension ten times using a 1 mL syringe fitted with 21-gauge needles. The resulting cell suspension was transferred to a 15 mL conical tube containing 10 mL of TCM199 with 10% FBS and incubated at 38.5°C for 5 minutes. After incubation, the supernatant was discarded, and the cells were washed twice with 10 mL of washing medium.

Subsequently, 100 µL of the recovered cells were diluted in 10 mL of culture medium (TCM199 supplemented with 10% FBS) and seeded in 100 mm culture dishes (Nunc, Denmark; 10 mL per dish). The cultures were incubated at 38.5°C in a humidified atmosphere containing 5% CO_2 for 48 hours. The BOECs were then cryopreserved in liquid nitrogen until further use.

2.5.1 Preparation of conditioned medium from monolayered bovine oviduct epithelial cells (BOECs-M)

BOECs from 3.5 were seeded in 100 mm culture dishes containing TCM199 medium -the cell-free medium was discarded and replaced with 10 mL of fresh TCM199 medium supplemented with 10% dFBS. The cells were maintained at 38.5°C in a humidified atmosphere with 5% CO_2 in air until they reached confluence. Conditioned media were collected on days 2, 4, and 6 post-incubation for EVs isolation. After each collection, 10 mL of fresh TCM199 medium supplemented with 10% dFBS was added to the culture dishes. The harvested conditioned media were stored at –80°C until further use.

2.5.2 Preparation of conditioned medium from vesicle-shaped bovine oviduct epithelial cells (BOECs-V)

BOECs from 3.5 were propagated in culture medium in 100 mm culture dishes and incubated at 38.5°C for 48 hours, as previously described. To obtain EVs exclusively from suspended BOECs-V, the conditioned medium was transferred to a new 100 mm culture dish and incubated at 38.5°C for another 48 hours. Subsequently, the conditioned medium containing suspended BOECs-V was pipetted into 5 mL of TCM199 medium supplemented with 10% dFBS and incubated at 38.5°C for 48 hours. The suspended BOECs-V were washed twice in TCM199 washing solution containing 10% dFBS. After washing, the cells were cultured in 60 mm dishes with 5 mL of TCM199 supplemented with 10% dFBS. Conditioned media were collected on days 2, 4, and 6 post-incubation and stored at –80°C, as previously described.

2.5.3 EVs isolation

EVs were isolated from the conditioned media collected from BOECs monolayers (BOECs-M-EVs) and suspended vesicle-derived BOECs (BOECs-V-EVs) using a protocol based on Théry et al. (2006), with minor modifications. The conditioned media containing BOECs-M-EVs and BOECs-V-EVs were separately centrifuged at 300 × g for 15 minutes at 4°C to remove cells and debris. The resulting supernatants were collected and centrifuged again at 2,000 × g for 15 minutes at 4°C, followed by a third centrifugation step at 12,000 × g for 15 minutes at 4°C to eliminate large vesicles and apoptotic bodies. The final supernatants were subjected to ultracentrifugation at 100,000 × g for 90 minutes at 4°C to pellet the EVs. The pellets were resuspended in PBS (without calcium and magnesium; PBS–) and filtered through a 0.22 µm filter to remove remaining contaminants. Both BOECs-M-EVs and BOECs-V-EVs were stored at –80°C until further use.

2.5.4 The particle size distribution by nanoparticle size analyzer

Particle size distribution of the isolated EVs was analyzed using NTA with the NanoSight Pro instrument (Malvern Panalytical, UK), following the manufacturer's instructions. Briefly, EVs pellets were resuspended in PBS(–). The EVs suspension was then transferred into a clean glass cuvette and loaded into the instrument for particle size and concentration analysis using the NTA system.

2.6 In vitro embryo production (IVEP)

2.6.1 Oocyte collection and IVM

Bovine ovaries were collected from a local abattoir and transported to the laboratory in 0.9% (w/v) NaCl solution at room temperature. Upon arrival, the ovaries were rinsed thoroughly with 0.9% NaCl solution. Cumulus oocyte complexes (COCs) were aspirated from 2–8 mm follicles using an 18-gauge needle attached to a 10-mL syringe. The retrieved COCs were examined under a stereomicroscope, and those exhibiting at least three layers of compact cumulus cells and homogeneous cytoplasm were selected. Selected COCs were washed in modified Dulbecco's phosphate-buffered saline (mDPBS) and subsequently transferred into a 60 mm culture dish containing IVM medium (20 COCs per 100 µL drop), covered with mineral oil. The IVM medium consisted of TCM-199 supplemented with 10% FBS, 1 IU/mL human chorionic gonadotropin (hCG; Intervet, Netherlands), 0.02 IU/mL follicle-stimulating hormone (FSH, Antrin R10; Kyoritsu Seiyaku Co., Tokyo, Japan), and 1 µg/mL 17ß-estradiol. Oocytes were cultured for 23 h at 38.5°C in a humidified atmosphere 5% CO_2 in air.

2.6.2 Sperm preparation and IVF

Frozen fertile semen from a Wagyu bull (Pornchai Intertrade Ltd., Ratchaburi, Thailand) stored in 0.25 ml straws was thawed in air for 10 seconds and subsequently immersed in a 37.5°C water bath for 1 minute. The thawed semen was transferred to the bottom of a 5 ml snap-cap tube (Corning, Glendale, AZ, USA) containing 2 ml of TALP medium (Lu et al., 1987) and incubated at 38°C under a humidified atmosphere of 5% CO_2 for 30 minutes. After incubation, the upper 1.8 ml layer was gently collected and transferred to a 15 ml conical tube (SPL Life Sciences) containing 5 ml of TALP medium. The sperm suspension was centrifuged at 400 × g for 5 minutes, and the supernatant was discarded. The sperm pellet was resuspended and adjusted to a final concentration of 2 × 10^6 sperm/ml using TALP medium. A 50 µl aliquot of the sperm suspension was placed into 35 mm culture dishes overlaid with mineral oil and incubated at 38°C in a humidified atmosphere of 5% CO_2 for 10 hours. Following co-incubation, presumptive zygotes were denuded of cumulus cells and excess sperm. The zygotes were then cultured in CR1aa medium (Rosenkrans et al., 1993) supplemented with 5% fetal bovine serum (FBS) under a gas phase of 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C for 7 days. Embryo development was assessed on Day 2 and Day 7 to evaluate cleavage (2–8 cell stage) and blastocyst formation, respectively.

2.6.5 IVC of presumptive zygotes

After 10 hours of co-incubation of sperm and COCs in the fertilization medium, the presumptive zygotes were denuded by gentle pipetting in TCM199 HEPES supplemented with 10% FBS. The denuded zygotes were then cultured in CR1aa medium supplemented with 5% dFBS and either BOECs-M-EVs or BOECs-V-EVs at

three different concentrations (2×10^6, 4×10^6, and 8×10^6 particles/mL). The CR1aa medium supplemented with 5% dFBS, but without EVs supplementation, served as the negative control. The culture medium was covered with mineral oil, and the zygotes were cultured in 35 mm culture dishes at a ratio of 12 zygotes per 80 µL of culture medium. Incubation was carried out under a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N2 at 38.5°C for 7 days. Embryo development was assessed on day 2 and day 7 post-culture.

2.6.6 Embryo quality

On day 7 post-insemination and cloning, blastocysts were first measured for embryo diameter before fixation and staining. Bright-field images were captured at 200× magnification using an inverted microscope. Embryo diameter was measured using ImageJ software (NIH, USA) by drawing a straight line across the widest axis of the blastocyst, excluding the ZP. Each measurement was calibrated using a built-in scale bar, and each group's average diameter was recorded.

Following size measurement, the embryos were stained with 0.1 mg/ml propidium iodide (PI) and 0.2% Triton X-100 in mDPBS supplemented with 0.1% PVP for 1 min. Afterward, embryos were placed into Hoechst 33342 solution dissolved in 99.5% ethanol for 5 min before being mounted with glycerol on a glass slide. Using an inverted fluorescent microscope, counts of TE and ICM were conducted. ICM cells appeared blue due to Hoechst uptake, while TE cells were stained pink red.

2.7 Quantitative real-time polymerase chain reaction (qPCR)

Twenty blastocysts from each group were washed three times with PBS (-) and stored at -80°C until further use. The manufacturer extracted Total mRNA using the FavorPrep™ Tissue total RNA Mini Kit (Favorgen Biotech Crop., Pingtung, Taiwan). cDNA synthesis was performed using biotechrabbit™ cDNA Synthesis Kit (Biotechrabbit, Berlin, Germany), and the expression of specific genes was assessed using the KAPA SYBR FAST qPCR Master Mix (Applied Biosystems) on the CFX Opus 96 real-time PCR system (Biorad, Hercules, California, USA). Melting curve analysis was performed for all primers, which were optimized. The primer sequences are provided in Table 3.9.1. GAPDH was used as a housekeeping gene to normalize the expression of target genes. qPCR was performed in triplicate, and statistical analysis was conducted using the 2^-ΔΔCt method. GAPDH was used as the housekeeping gene to normalize the target genes.

Table 1. Genes used for real-time qPCR of blastocysts.

Genes	Primer sequences	Product length (bp)	Accession numbers
Bax	F:(5′–3′) TCTGACGGCAACTTCAACTG R:(5′–3′) TCGAAGGAAGTCCAATGTCC	135	NM_173894.1
BCL2	F:(5′–3′) ATGCGGCCCCTGTTTGATTT R:(5′–3′) GCCTGTGGGCTTCACTTATG	116	NM_001166486.1
IFN-tua	F:(5′–3′) CTGGCCCGAATGAACAGACT R:(5′–3′) AGAGGTTGAAGCACTGCTGG	151	XM_024989143.2
*GAPDH**	F: (5′–3′) CTCCCAACGTGTCTGTTGTG R: (5′–3′) TGAGCTTGACAAAGTGGTCG	222	NM_001034034.2

2.8 Statistical analysis

Data are expressed as mean ± SEM. Numbers of embryo development and total cell number were tested for normality and homoscedasticity before statistical analysis. Differences in embryo development and total cell number were analyzed using a one-way ANOVA followed by Dunnett's Multiple Comparison for post-hoc comparison Gene expression data were obtained from at least three biological replicates, each performed in triplicate, and analyzed using one-way ANOVA, followed by Tukey's multiple comparison test to determine statistical differences among groups. Differences were considered statistically significant at a probability level of $P < 0.05$. All statistical analyses were performed using GraphPad Prism software (GraphPad Prism 5.01 Inc., La Jolla, CA, USA). The level of significance for all analyses was $P < 0.05$.

3. Results

3.1 EVs isolated from oviduct epithelial cells in this study

As shown in Fig. 6, extracellular vesicles (EVs) were isolated from two distinct BOECs culture systems: monolayer-attached cells (BOECs-M) and vesicle-like suspended structures (BOECs-V). The BOECs-M group consisted of cells adherent to the culture dish, typically measuring 50–100 µm in diameter. In contrast, BOECs-V cells formed floating spheroid-like structures that were not attached to the surface and ranged from 100–200 µm in size. These two culture strategies successfully generated morphologically distinct EV populations.

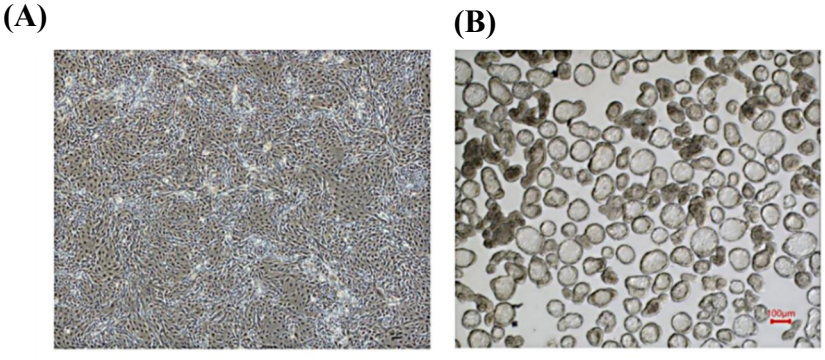

Figure 3. Morphology of oviduct epithelial cells grown in TCM199+10 % dFBS in this study: A) monolayer culture (BOECs-M), and B) vesicle culture (BOECs-V). A: Scale bar 10 µm, B: Scale bar 100 µm.

EVs were successfully isolated from both types of cultured cells: BOECs-M and BOECs-V (Figure 6). According to nanoparticle tracking analysis (NTA), the concentration of BOECs-M-derived EVs was 1.89 × 10^{12} particles/mL, whereas BOECs-V-derived EVs exhibited a lower concentration of 2.42 × 10^{11} particles/mL. Both BOECs-M and BOECs-V EVs appeared as spherical particles with size distributions ranging from 118 nm to 684 nm. The predominant particle sizes were approximately 118 nm for BOECs-M EVs and 131 nm for BOECs-V EVs, as illustrated in Figures 7A and 7B.

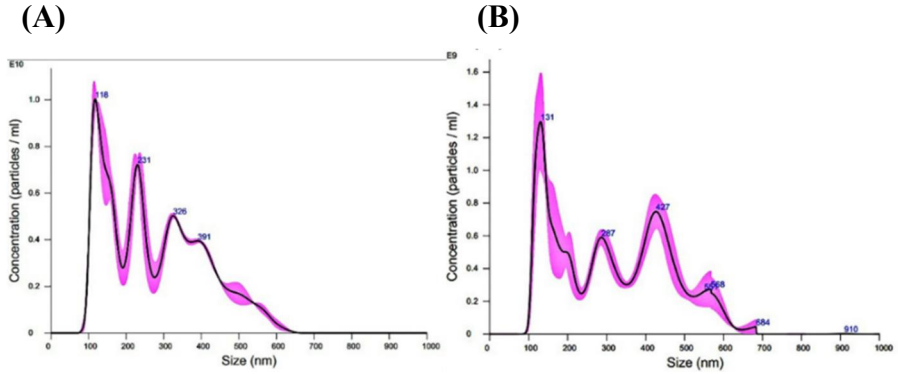

Figure 4. Average particle size of BOECs-M-EVs (A) and BOECs-V-EVs (B) using the NTA technique

3.2 Embryo development

The cleavage rates of IVF embryos showed no significant differences among treatment groups, ranging from 69.10% to 78.91%. However, blastocyst formation rates on day 7 were significantly higher in embryos treated with BOECs-M-EVs and BOECs-V-EVs, particularly at concentrations of 4 × 10^6 and 8 × 10^6 particles/mL, compared to the control group ($P < 0.05$). The highest blastocyst rate was observed in embryos treated with 4 × 10^6 particles/mL BOECs-M-EVs (M4), reaching 36.12%, followed closely by the V8 and M8 groups. In addition, the proportion of hatching and hatched blastocysts was significantly increased in the M2, M8, and V4 groups ($P < 0.05$), with the M8 group exhibiting the highest hatching rate at 50.92%.

Table 2. Effect of BOECs-derived extracellular vesicles and monolayer co-culture on bovine embryo development *in vitro* fertilization.

ANOVA –test (10 replicates) %: Mean ± SEM. [a b] values with different superscripts are significantly different at $P < 0.05$

Type of cells	Treatment	No. IVC	Cleaved D.2 (%)	Blastocyst D.7 (%)	Hatching D.7 (%)
-	Control	468	340/468 (72.65)	121/468 (25.85)	53/121 (43.80[b])
Monolayer	2×10^6	468	338/468 (72.22)	150/468 (31.32)	72/150 (48.00[a])
	4×10^6	468	378/468 (80.76)	173/468 (36.12)	77/173 (44.51[a])
	8×10^6	468	331/468 (72.01)	163/468 (34.03)	83/168 (50.92[a])
Vesicles	2×10^6	468	344/468 (73.50)	142/468 (29.65)	58/142 (40.85[a])
	4×10^6	468	339/468 (72.43)	151/468 (31.52)	73/151 (48.34[a])
	8×10^6	468	348/468 (74.35)	167/468 (34.86)	71/167 (42.51[a])

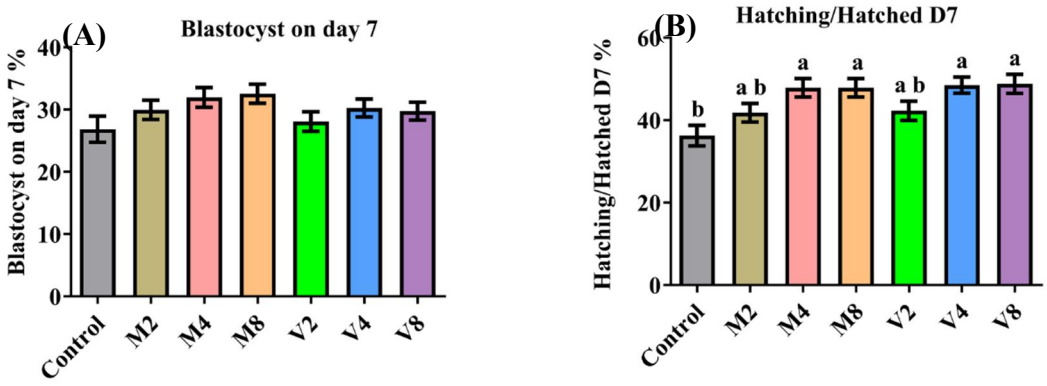

Figure 5. Effects of BOECs-derived EVs supplementation on blastocyst formation ($P > 0.05$) and hatching rates of IVF-derived embryos cultured *in vitro.* Data are presented as mean ± SEM, a b values with different superscripts are significantly different at $P < 0.05$ (ANOVA test), M2 = BOECs-M-EVs 2×10^6 Particle/ml, M4 = BOECs-M-EVs 4×10^6 Particle/ml, M8 = BOECs-M-EVs 8×10^6 Particle/ml, and V2 = BOECs-V-EVs 2×10^6 Particle/ml, V4 = BOECs-V-EVs 4×10^6 Particle/ml, V8 = BOECs-V-EVs 8×10^6 Particle/ml

3.3 Embryo quality in IVF-derived blastocysts

The average diameter of day-7 blastocysts was significantly larger in embryos treated with BOEC-derived EVs compared to the control group. Specifically, blastocysts in the BOEC-M-EVs and BOECs-V-EVs groups measured 190.5 ± 16.59 µm and 179.3 ± 15.93 µm, respectively, whereas those in the control group averaged 151.7 µm ($P < 0.05$). Embryo quality was further evaluated by assessing total cell numbers, trophectoderm (TE), and inner cell mass (ICM) counts in IVF-derived blastocysts. All EVs-treated groups, including 2×10^6, 4×10^6, and 8×10^6 particles/mL from both BOECs-M and BOECs-V sources, exhibited significantly higher total cell numbers compared to

the control group ($P < 0.05$), although no significant differences were observed among the EVs-treated concentrations. Regarding TE cell number, embryos treated with 4×10^6 and 8×10^6 particles/mL of BOEC-M-EVs, as well as 8×10^6 particles/mL of BOEC-V-EVs, showed significant increases relative to the control group ($P < 0.05$). Treatments with 2×10^6 particles/mL from both sources resulted in intermediate values. Notably, embryos supplemented with 8×10^6 particles/mL of BOEC-V-EVs exhibited the highest TE cell counts among all groups. In contrast, ICM cell numbers did not differ significantly among the groups. However, embryos supplemented with EVs, particularly at 4×10^6 and 8×10^6 particles/mL, tended to show slightly higher ICM values compared to the control. These results suggest that EVs supplementation at higher concentrations improves blastocyst quality by increasing total and TE cell numbers without adversely affecting ICM allocation.

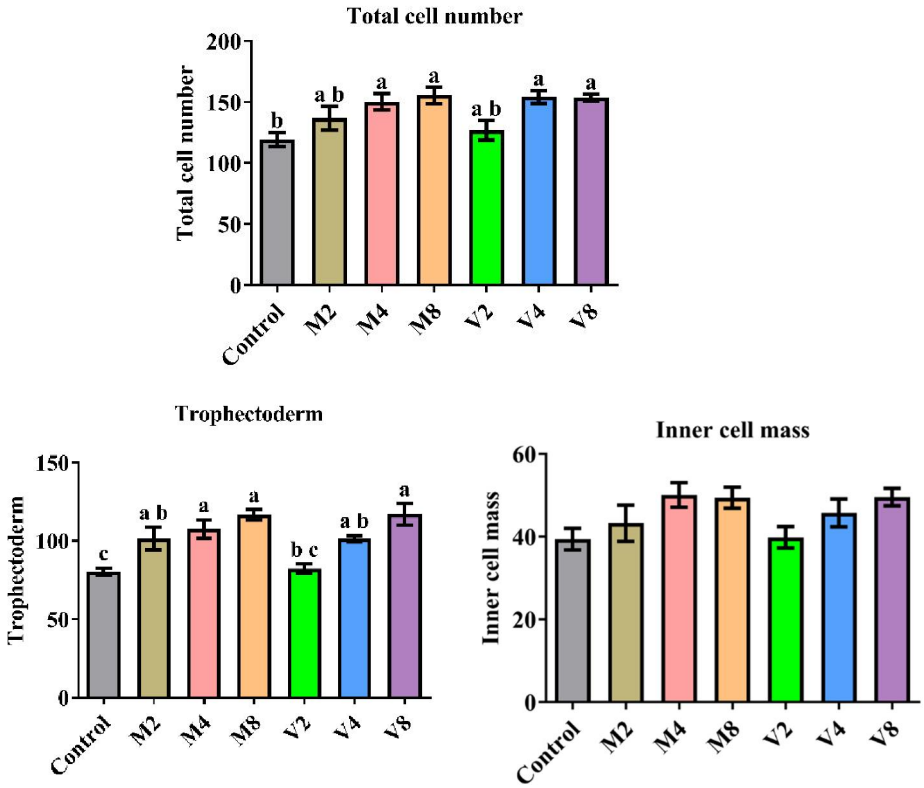

Figure 6. Analysis of the TE, ICM, and total cell number of embryos cultured in IVC with or without BOECs-M-EVs and BOECs-V-EVs. A total of 10 embryos per group were analyzed. Data are presented as mean ± SEM, $P < 0.05$ (ANOVA test). M2 = BOECs-M-EVs 2×10^6 Particle/ml, M4 = BOECs-M-EVs 4×10^6 Particle/ml, M8 = BOECs-M-EVs 8×10^6 Particle/ml, and V2 = BOECs-V-EVs 2×10^6 Particle/ml, V4 = BOECs-V-EVs 4×10^6 Particle/ml, V8 = BOECs-V-EVs 8×10^6 Particle/ml

3.4 Gene Expression in IVF-derived Blastocysts

To evaluate the molecular effects of BOECs-derived EVs on embryonic development, we analyzed the expression of key genes associated with apoptosis (BAX, BCL2) and implantation (IFN-tau) in day-7 IVF-derived blastocysts. The pro-apoptotic gene BAX exhibited significantly lower expression in embryos treated with BOECs-V-EVs compared to the control group ($P < 0.05$), indicating reduced apoptotic activity. In contrast, the anti-apoptotic gene BCL2 was significantly downregulated in both BOECs-M-EVs and BOECs-V-EVs groups relative to the control ($P < 0.05$), possibly reflecting a more balanced and less stressed *in vitro* environment. Additionally, the expression of IFN-tau, a gene critical for maternal recognition of pregnancy, was markedly upregulated in both EV-treated groups ($P < 0.05$), suggesting improved implantation potential following EV supplementation during *in vitro* culture.

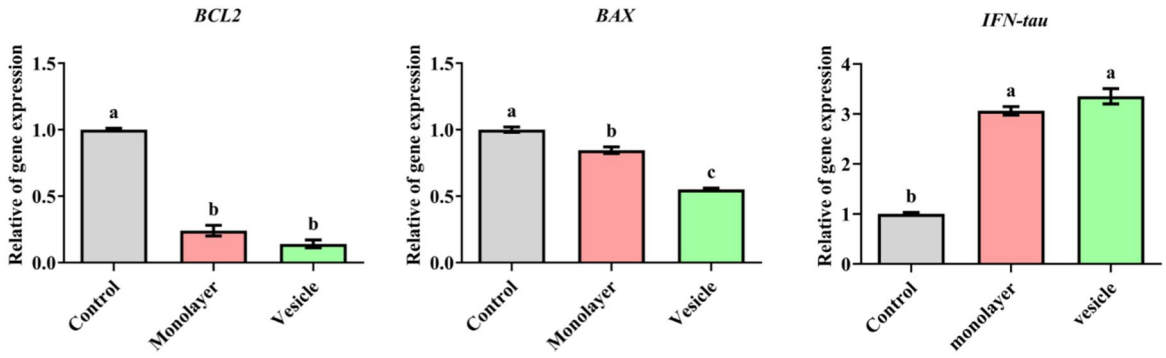

Figure 7. Expression levels of *BCL2*, *BAX*, and *IFN-tau* genes in bovine embryos produced *in vitro*. A total of 20 blastocysts per group were analyzed. Data are presented as mean ± SEM. a b Values with different superscripts are significantly different at $P < 0.05$ (ANOVA test). Monolayer = BOECs-M-EVs 4 × 10^6 Particle/ml, V4 = BOECs-V-EVs 4 × 10^6 Particle/ml

4. Discussion

This study is the first to investigate the effects of EVs derived from vesicle-shaped bovine oviduct epithelial cells (BOECs-V-EVs) on bovine embryo development. To our knowledge, no previous research has applied BOECs-V-EVs in embryo culture systems in cattle. This novel approach expands the potential application of reproductive tract-derived EVs in assisted reproductive technologies. Our findings demonstrate that supplementation of IVC medium with BOECs-derived EVs significantly enhances embryo developmental competence and quality in IVF systems. The optimal supplementation levels led to improvements in blastocyst formation, hatching rates, and embryo quality parameters, including total cell number and balanced allocation between the ICM and TE lineages. EVs were effectively separated from attached monolayer cells (BOECs-M) and floating vesicle-like cells (BOECs-V), two morphologically different types of BOECs. NTA revealed that while BOEC-M produced a higher concentration of EVs, both types exhibited sizes consistent with small EVs (around 118–131 nm) as shown in Figure 3, supporting their classification based on biophysical properties (Bastos et al., 2022; Lopera-Vasquez et al., 2016). Although surface marker profiling (e.g., CD9, CD63, CD81) was not performed, the combination of ultracentrifugation and NTA characterization was

considered sufficient for functional evaluation in embryo culture. Nevertheless, future studies should include molecular profiling following the Minimal Information for Studies of Extracellular Vesicles guidelines to enhance EVs characterization (Leal et al., 2022; Sidrat et al., 2022; Wei et al., 2022). Supplementation with BOEC-M-EVs and BOEC-V-EVs during IVF culture significantly increased blastocyst formation and hatching rates, particularly at 4×10^6 and 8×10^6 particles/mL (Figure 6). These results align with previous reports demonstrating that reproductive tract-derived EVs can improve embryonic development and mimic physiological conditions (Leal et al., 2022; Sidrat et al., 2022; Wei et al., 2022). Consistently, Wei et al. (2022) confirmed that supplementation with BOECs-derived exosomes enhanced the developmental competence and implantation potential of bovine embryos. The average diameter of the embryos supplemented with EVs produced from BOECs was noticeably greater than that of the control group. This increased size may indicate an increased total cell number. Hoechst 33342 nuclear staining revealed a correlation between an increase in diameter and a greater total number of cells. These results are in line with earlier research that suggested embryo size is a good indicator of viability and developmental potential. The findings are consistent with the theory that EVs increase embryonic development by delivering bioactive chemicals that promote cell division and metabolic support during the culture period. These enhancements could be explained by the bioactive cargo that EVs carry, such as proteins and miRNAs that can affect intracellular pathways, including MAPK and PI3K/AKT. These signaling cascades control mitochondrial function, apoptosis suppression, and cell proliferation, all of which are important factors in blastocyst growth and a rise in the overall number of cells. Therefore, during IVC, EVs probably function as modulators of embryonic physiology, improving cellular and morphological development (Gurung et al., 2021; Qu et al., 2020; Sidrat et al., 2022).

Embryo quality analysis further confirmed the beneficial effects of EVs, as total cell numbers and TE cell counts were significantly higher in EVs-treated groups compared to the control (Figure 6). Although ICM cell numbers did not differ substantially, there was a trend toward higher ICM values with increasing EVs concentrations, suggesting that EVs supplementation supports balanced lineage allocation (Lopera-Vasquez et al., 2017). Gene expression profiling revealed that EVs supplementation modulated key apoptosis and implantation genes. In Figure 7, the pro-apoptotic gene BAX was downregulated in BOEC-V-EVs supplemented embryos, indicating reduced cellular stress, whereas the anti-apoptotic gene BCL2 also showed lower expression across EVs-treated groups, reflecting a less stressful environment *in vitro*. Moreover, IFN-tau, critical for maternal recognition of pregnancy, was upregulated, suggesting enhanced implantation potential.

Although both BOECs-M-EVs and BOECs-V-EVs significantly improved embryo development and quality compared to the control, subtle functional differences were observed between the two EVs sources. BOECs-M-EVs tended to promote higher blastocyst and hatching rates, whereas BOECs-V-EVs demonstrated a more pronounced anti-apoptotic effect through stronger BAX suppression. These findings suggest that the bioactive cargo composition of EVs may differ depending on the origin of cellular architecture. The differential outcomes observed between BOECs-M-EVs and BOECs-V-EVs raise the possibility that these vesicles carry distinct molecular cargos, such as microRNAs, tRNAs, or proteins, which influence developmental signaling pathways. Further proteomic and transcriptomic analyses are warranted to elucidate the underlying mechanisms (Sheta et al., 2023).

5. Conclusion

This study demonstrates that supplementation of IVC medium with EVs derived from BOECs significantly enhances the developmental potential and quality of bovine embryos produced via IVF. Embryos treated with BOECs-derived EVs showed increased blastocyst formation rates, larger diameters, and higher total cell numbers compared to untreated controls, indicating improved developmental competence. These findings support the role of EVs as mediators of maternal signals, capable of delivering bioactive molecules such as proteins, mRNAs, and miRNAs that activate key signaling pathways involved in proliferation, cell development. The ability of BOECs-derived EVs to mimic physiological conditions *in vitro* highlights their potential as a valuable supplement for improving current ART. Their integration into embryo culture protocols could enhance IVEP efficiency, embryo viability, and overall outcomes in cattle breeding programs. This advancement opens new opportunities for refining *in vitro* systems and developing more reliable, biologically relevant models for reproductive biotechnology applications in livestock.

Acknowledgement

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Impact of Resveratrol in Culture Media on the Development and Cryotolerance of *In Vitro* Produced Bovine Embryos

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Abstract

Background: Resveratrol is known for its strong antioxidant characteristics, which mainly reduce oxidative stress and greatly improve the quality, developmental competence, and cryotolerance of mammalian oocytes and embryos. This study assessed the impact of 0.5 µM resveratrol supplementation in *in vitro* culture (IVC) and warming media on the developmental potential of bovine embryos produced *in vitro*, both before and after vitrification.

Methods: In the resveratrol group, the rates of cleavage and blastocyst formation (80.45% and 36.75%, respectively) were significantly higher ($P < 0.05$) compared to the control group (which did not receive resveratrol), where the rates were 70.86 % and 28.24%, respectively. The blastocysts, both with and without resveratrol supplementation, were vitrified and later warmed in media with or without resveratrol before being cultured in IVC medium for an additional 48 hours.

Results: The results indicated that resveratrol supplementation exclusively in the culture medium, rather than in the warming medium, resulted in significantly higher ($P < 0.05$) hatching rates (78.75%) compared to those with supplementation only in the warming medium (47.11%).

Conclusion: These findings highlight the beneficial function of resveratrol in enhancing embryo resistance to cryopreservation stress and promoting embryonic developmental competence. The results suggest 0.5 µM of resveratrol is optimal for enhancing blastocyst quality and post-warming survival. Future research should clarify the patterns of gene expression related to apoptotic pathways, oxidative stress, and the development of embryos.

Keywords: *In vitro* fertilization, Resveratrol, Blastocyst, Viability, Vitrification, Bovine

1. Introduction

In assisted reproductive technologies (ARTs), cryopreservation is essential because it allows for the preservation of female fertility by preserving oocytes and embryos for future use, in addition to assisting in the conservation of genetic material from livestock and endangered species (Woods et al., 2004). Cryopreservation is a cost-effective and practical alternative to the transportation of live animals in the livestock industry, as it enables the transportation of oocytes and embryos over long distances. Vitrification, a rapid cryopreservation technique that transforms cells into a glass-like, amorphous state, has proven effective in preventing the formation of ice crystals, which can otherwise damage cellular structures (Rall & Fahy, 1985). This technique has become more popular in the cryopreservation of oocytes and embryos because it is easy to use, effective, and has greater post-thaw survival rates than traditional slow-freezing methods.

Although these developments, as evidenced by lower survival rates in culture and lower pregnancy rates following embryo transfer, *in vitro* produced (IVP) embryos have lower post-warming survivability than their *in vivo*-derived embryos (Kaidi et al., 1998; Lonergan et al., 2003; Massip et al., 1995). This disparity is mainly because of oxidative stress, which is the result of an accumulation of reactive oxygen species (ROS). ROS can negatively impact mitochondrial function, affect calcium signaling during fertilization, induce apoptosis, and affect embryo development. Although cells can regulate ROS and utilize their signaling potential, excessive ROS can be detrimental (Morado et al., 2009). The female reproductive system offers protective antioxidants *in vivo* to lessen damage caused by ROS (Guerin et al., 2001). However, such protective systems are often absent or insufficient in *in vitro* conditions, leaving gametes and embryos vulnerable to environmental stressors (Guerin et al., 2001; Marques et al., 2007). To overcome this challenge, the supplementation of culture media with exogenous antioxidants has been formulated as a method to enhance cellular defense mechanisms, counteract ROS, and maintain embryo viability.

To prevent oxidative damage, recent studies have explored various antioxidant agents, including enzymes, thiol compounds, vitamins, flavonoids, amino acids, and their derivatives. Among these, the naturally occurring polyphenolic chemical resveratrol (3,4,5-trihydroxy-trans-stilbene) has demonstrated significant promise in enhancing the quality of oocytes and embryos in a variety of animals, including cattle, pigs, goats, and mice (Piras et al., 2019; Itami et al., 2015; Chinen et al. 2020). Due to its chemical structure, resveratrol exhibits both intracellular and extracellular effects, making it an efficient antioxidant (Gambini et al., 2015). In addition to lowering reactive oxygen species (ROS) levels and increasing intracellular glutathione (GSH) content, resveratrol supplementation during *in vitro* maturation (IVM) has been shown to improve cumulus cell expansion, polar body extrusion, blastocyst yield, and total cell number per blastocyst in cattle (Wang et al., 2014). According to previous studies carried out in our lab, adding resveratrol to culture media and vitrification solutions enhanced mouse blastocyst cryotolerance and changed the expression of genes related to implantation and apoptosis (Puangjit, 2019). Likewise, supplementing with resveratrol in a short-term recovery medium before IVF improved blastocyst growth and decreased oxidative stress (Chinen et al., 2020). Additionally, there is evidence that embryo culture conditions with 0.5 µM resveratrol may improve cryotolerance at the cellular level (Salzano et al., 2014). This study aimed to investigate whether 0.5 µM resveratrol supplementation *in vitro*

culture medium (IVC) and post-warming medium affects the survivability and gene expression of post-warmed vitrified bovine embryos. Additionally, the evaluation of developed competence (based on blastocyst yield) and quality of blastocytes is done by counting total cell numbers through IVF and embryo culture.

2. Materials and Methods

Ethical approval for the use of bovine specimens in this study was obtained from the Animal Ethics Committee of Suranaree University of Technology, Thailand (IACUC-67-39).

2.1. Chemicals and reagents

All chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless specified otherwise. Cell culture media were purchased from Gibco (Paisley, UK), and plasticware for cell culture was supplied by SPL Life Sciences (Gyeonggi-do, Republic of Korea).

2.2. Oocyte collection and in vitro maturation

The ovaries were collected from the slaughterhouse and stored at room temperature in 0.9% NaCl solution during transportation to the laboratory. Subsequently, the ovaries were washed with 0.9% NaCl solution and follicles with a diameter of 2-8 mm were collected using 18G needle connected with 10 ml syringe. The collected cumulus oocytes complexes (COCs) were examined under a stereomicroscope, and those exhibiting at least three layers of compact cumulus cells and homogeneous cytoplasm were selected. After that, the COCs were cultured in 60 mm culture dish containing IVM medium covered with mineral oil (20 COCs/100 µl of IVM medium) at 38.5 °C in a humidified atmosphere of 5% CO_2 in air for 23 hours. The IVM medium composed of tissue culture medium 199 (TCM-199) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 50 IU/ml human chorionic gonadotropin (HCG, Intervet, Netherlands), 0.02 AU/ml follicle-stimulating hormone (FSH, Antrin R10; Kyoritsu Seiyaku Co., Tokyo, Japan), and 1 µg/ml 17ß-estradiol.

2.3. Sperm preparation and in vitro fertilization (IVF)

The frozen fertile semen of a Wagyu bull (Pornchai Intertrade Ltd., Ratchaburi, Thailand) in 0.25 ml straw was thawed in the air for 10 seconds and then immersed in a water bath at 37.5°C for 1 minute. The semen was subsequently placed at the bottom of a 5 ml snap tube (Corning, Glendale, AZ, USA) that contained 2 ml of TALP medium (Lu et al., 1987). The snap tube was incubated at 38.5°C in a humidified atmosphere of 5% CO_2 in air for 30 minutes. After that, the top 1.8 mL layer of the medium was collected and transferred to a 15 mL conical tube containing 5 mL of TALP medium. The supernatant was discarded after the sperm suspension was centrifuged at 400 x g for 5 minutes. The sperm pellet was diluted with TALP and adjusted concentration of 2×10^6 sperm/ml. Then, 50 µl of the sperm suspension was transferred to 35 mm culture dish covered with mineral oil. The sperm and COCs were co-incubated at 38°C in a humidified atmosphere of 5% CO_2 in the air for 10 hours.

2.4. In vitro embryo culture (IVC)

After the co-incubation of sperm and COCs, the presumptive zygotes were stripped of cumulus cells and excess sperm. The presumptive zygotes were subsequently cultured in an IVC medium (CR1aa medium, Rosenkrans et al., 1993) supplemented with 5% FBS with or without 0.5 µM resveratrol in 35 mm culture dish covered with mineral oil (10 oocytes/50 µl of IVC medium) for 7 days in a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C. To cleavage and blastocyst stages were examined on days 2 and 7, respectively.

2.5. Evaluation of trophectoderm (TE) and inner cell mass (ICM), and total cell numbers

The eight blastocysts from each group were stained by immersing them in a solution containing 1 mg/ml propidium iodide (PI) and 0.2% Triton X-100, which was prepared in mDPBS supplemented with 0.1% PVP, for 1 minute. The blastocysts were subsequently transferred to a solution of 25 µg/ml Hoechst 33342 in 99.5% ethanol for 5 minutes. Lastly, the stained blastocysts were mounted on glass transparencies using glycerol. The TE and ICM cells of the blastocysts were subsequently examined under an inverted fluorescence microscope (IX70, Olympus, Tokyo, Japan).

2.6. Vitrification and warming of embryos

The grades 1 and 2 blastocysts were rinsed with TCM199-HEPES supplemented with 20% FBS (base medium, BM). After that, the blastocysts were incubated at 24-25°C for 3 minutes in an equilibration solution (ES), which was composed of BM supplemented with 7.5% (v/v) ethylene glycol (EG) and 7.5% (v/v) dimethyl sulfoxide (DMSO). Subsequently, the blastocysts were transferred to vitrification solution (VS), which was composed of BM supplemented with 15% (v/v) DMSO, 15% (v/v) EG, and 0.5 M sucrose, for 1 minute. Finally, 2-3 blastocysts were placed on a Cryotop® (Kitazato BioPharma, Fujinomiya, Japan) and promptly immersed in liquid nitrogen within 1 minute of being exposed to the vitrification solution. The blastocysts were preserved in liquid nitrogen for a minimum of one week before their utilization. For warming, the Cryotop® tip was directly inserted into a 35 mm culture dish containing 2.5 ml of warming solution, which was constituted of BM supplemented with 1 M sucrose and 0.5 µM resveratrol, for 1 minute at 38.5 °C. Subsequently, the blastocysts were sequentially transferred to BM that was supplemented with 0.5 µM resveratrol and 0.5 M, 0.25 M, and 0 M sucrose for 3, 5, and 5 minutes, respectively. After warming, all blastocysts were cultured in post-warming medium (with or without resveratrol) under a humidified atmosphere of CO_2, 5% O_2 and 90% N_2 at 38.5°C. The development of the blastocysts was examined after 24 and 48 hours of culture.

2.7. Statistical analysis

The results were analyzed using a t-test and one-way analysis of variance (ANOVA), followed by a Tukey-Kramer Honest Significant Difference (HSD) as a post-hoc test, all executed using GraphPad Software (version 5; San Diego, CA, USA). The significance level was determined as $P < 0.05$.

3. Results

3.1. Effect of Resveratrol in IVC Medium on Embryo Development.

Table 1: Effect of resveratrol supplementation during IVC on cleavage and blastocyst rates in bovine embryos

Groups	No. Oocytes	No. (%) Cleavage (Mean ± SE)	No. (%) Blastocyst (Mean ± SE)
Control	402	290 (70.86 % ± 5.07)[b]	109 (28.24% ± 2.39)[b]
0.5 µM Resveratrol	402	323 (80.45 % ± 4.30)[a]	142 (36.75 % ± 2.86)[a]

ANOVA –test (6 replicates) %: Mean ± SEM

Different superscripts (a, b) within a column indicate significant differences at $P < 0.05$.

Table 1 shows the cleavage and blastocyst formation rates following treatment with 0.5 µM resveratrol during IVC. The embryos treated with resveratrol exhibited a significantly higher cleavage rate of 80.45% compared to the control group, which had a cleavage rate of 70.86% ($P < 0.05$). The blastocyst developmental rate in the group treated with resveratrol (36.75%) was significantly higher ($P < 0.05$) compared to the control group (28.24%). The results indicated that adding 0.5 µM resveratrol to the IVC medium improves early embryonic development by enhancing cleavage and blastocyst rates.

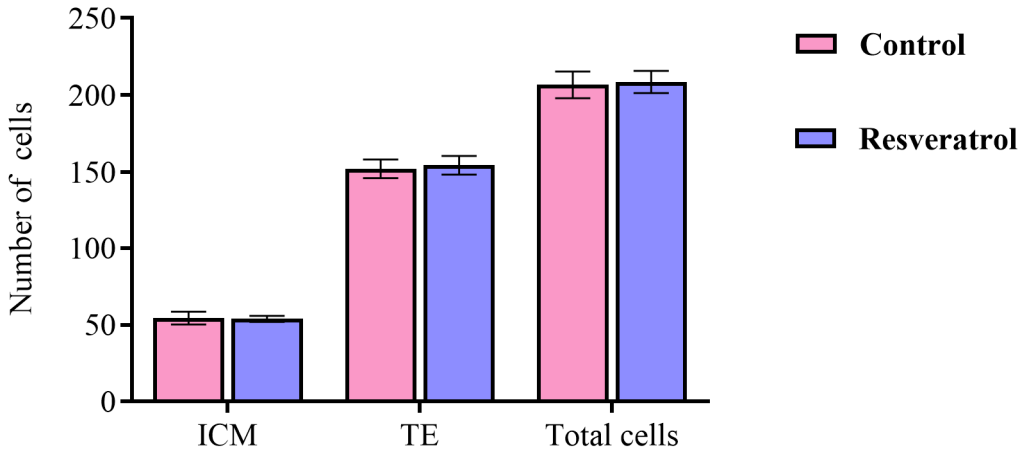

Figure 1. Analysis of the TE, ICM, and total cell numbers in blastocysts following resveratrol supplementation in the IVC medium. A total of 8 embryos per group were analyzed. Data are presented as mean ± SEM. No significant difference at $P > 0.05$ (ANOVA test).

On day 8 of IVC, the numbers of TE, ICM, and total cells in blastocysts were evaluated using staining techniques, with the results displayed in Figure 1. No significant differences were observed in the TE, ICM, or total cell counts between the control and resveratrol-treated groups ($P > 0.05$). These findings suggest that although 0.5 µM resveratrol supplementation during IVC enhances cleavage and blastocyst formation rates, it does not have a significant impact on the TE, ICM, or total cell number.

3.3. Effect of post-warming resveratrol treatment on embryo developmental competency

Table 2: Effect of resveratrol supplementation in the post-warming medium on embryo developmental competence after warming

Res in IVC	Res in post-warming culture medium	No. Blastocyst	Post-warming culture					
			24 h.			48 h.		
			No. (%) Survival	No. (%) Development	No.(%) Hatching and hatched	No. (%) Survival	No. (%) Development	No. (%) Hatching and hatched
-	-	43	39 (91.43 ± 5.09)	25 (60.16 ± 7.13)	14 (31.27 ± 5.91)	35 (80.32 ± 9.26)	10 (21.43 ± 7.85)	25 (58.89 ± 4.23)^{c}
-	+	43	37 (86.15 ±2.24)	24 (55.68 ± 6.15)	13 (30.48 ± 8.22)	32 (73.53 ± 7.26)	12 (26.43 ± 9.04)	20 (47.11 ± 6.76)^{b}
+	-	49	47 (97.50 ± 2.50)	26 (53.06 ± 12.02)	21 (44.45 ± 12.07)	46 (96.25 ± 3.75)	9 (17.50 ± 6.29)	37 (78.75 ± 7.74)^{ac}
+	+	49	46 (95.00 ± 2.89)	28 (53.61 ± 17.27)	18 (41.39 ± 16.16)	43 (89.72 ± 4.09)	11 (18.75 ± 10.87)	32 (70.97 ± 11.10)^{c}

ANOVA –test (4 replicates) %: Mean ± SEM

Different superscripts (a, b) within a column indicate significant differences at $P < 0.05$.

As shown in Table 2, the survival and developmental competence of blastocysts were not significantly affected by the supplementation of 0.5 µM resveratrol in the post-warming medium. At 24 hours after warming, the survival rates were comparable among all experimental groups, with a range of 91.43% to 95.00% ($P > 0.05$). Similarly, the proportion of re-expanded blastocysts did not significantly differ between the treatments, with results ranging from 53.06% to 60.16% ($P > 0.05$). The survival rates among the groups were also comparable at 48 hours post-warming, with a range of 73.53% to 96.25% ($P > 0.05$).

It is important to note that resveratrol supplementation only in the culture medium, rather than in the warming medium (+R/-R), resulted in significantly higher ($P < 0.05$) hatching rates (78.75%) compared to those with supplementation only in the warming medium (-R/+R) (47.11%).

4. Discussion

In the past few years, there has been an increasing amount of interest in the function of resveratrol in the *in vitro* development of bovine embryos, which has contributed to the investigation of its underlying mechanisms and its effect on the quality of the embryos. This study evaluated the effects of 0.5 µM resveratrol supplementation during IVC, using quantitative developmental measurements (Table 1) to examine the effect on embryonic development and quality. These results support previous studies indicating that low amounts of resveratrol do not affect early cell division (Piras et al., 2020). At the blastocyst stage, embryos in the control group displayed normal expansion and compaction patterns, but those treated with resveratrol showed enhanced structural integrity, especially in the ICM) and TE layers. This aligns with previous studies indicating that resveratrol improves blastocyst quality by reducing oxidative stress and enhancing mitochondrial activity (Piras et al., 2019). The absence of developmental delays or morphological defects in the treated embryos additionally suggests resveratrol's capacity to enhance embryo viability (Gaviria et al., 2019). Collectively, these results suggest that supplementation with 0.5 µM resveratrol has no effect on early cleavage and may enhance blastocyst quality, highlighting its potential in increasing embryo quality for reproductive biotechnology applications. At 24 and 48 hours after warming, no significant differences in blastocyst survival or developmental competence were seen across treatment groups, even when 0.5 µM resveratrol was added to the post-warming culture media. However, the results revealed that in the supplemented resveratrol only in IVC medium but not in warming medium group (+R/-R) had significantly higher hatching rates at 48 hours than without resveratrol in IVC medium and with resveratrol in warming medium group (-R/+R), suggesting that resveratrol's usefulness may vary depending on the situation. It seems to promote better hatching potential when used during IVC and then absent during the post-warming medium. These findings align with earlier research indicating that resveratrol's effects depend on cellular context, timing, and concentration because of its regulatory function in mitochondrial function and oxidative stress (Hara et al., 2018). Notably, resveratrol has been shown to have pro-oxidant and antioxidant properties, affecting developing embryos via pathways linked to mitochondrial homeostasis and apoptosis. The +R/-R group increased hatching could be because of a beneficial reduction in reactive oxygen species (ROS) before cryopreservation, which would promote mitochondrial function and developmental results (Gaviria et al., 2019; Takahito et al., 2017). Conversely, resveratrol exposure following warming, as in the -

R/+R group, may induce a shift to a pro-oxidative state, disrupting the redox balance and diminishing its protective effects. According to de la Lastra and Villegas (2007), these complications demonstrate the dual nature of resveratrol and the crucial interaction between mitochondrial activity, redox control, and embryo survival. Overall, these results contribute to the understanding of the importance of timing antioxidant treatments in promoting the optimal development of embryos.

5. Conclusion

In summary, this study demonstrates that supplementation with 0.5 µM resveratrol during IVC significantly enhances early embryonic development by increasing both cleavage and blastocyst formation rates. Although the addition of resveratrol solely during post-warming did not significantly affect blastocyst survival or re-expansion, embryos exposed to resveratrol during IVC and cultured without it post-warming (+R/-R group) exhibited a significantly higher hatching rate. These results suggest that the efficacy of resveratrol is context- and timing-dependent, likely mediated through its role in modulating oxidative stress and mitochondrial function. The dual nature of resveratrol as both an antioxidant and pro-oxidant underscores the importance of precisely timing its application to optimize embryo viability and developmental potential. Overall, resveratrol appears to be a promising supplement for enhancing bovine embryo production, particularly when applied during the IVC period. It may contribute to improved cryotolerance and developmental outcomes following warming, which are critical factors in ARTs. Future research should focus on elucidating the molecular mechanisms underlying these effects, including the regulation of genes involved in apoptosis, oxidative stress, and embryonic development. Additionally, evaluating pregnancy outcomes after the transfer of vitrified blastocysts treated with resveratrol will be essential for determining its practical application in bovine embryo production systems.

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OCT-4 Activating Compound 1 (OAC1) Enhanced In Vitro Development of SCNT Porcine Embryos

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Abstract

Background: Somatic cell nuclear transfer (SCNT) is an assisted reproductive technique involving a donor cell is transferred into an enucleated oocyte. Its efficiency remains low, due to abnormal nuclear reprogramming. Previous studies have shown that SCNT blastocysts exhibit aberrant expression of *OCT-4* and related genes. *OCT-4* transcription factor is crucial for embryonic development, maintaining pluripotency, and regulating primordial germ cell formation. Enhancing *OCT-4* expression in SCNT embryos may improve their reprogramming efficiency.

Methods: In this study, we investigated the optimal concentration and effects of OCT-4 Activating Compound 1 (OAC1) that was reported to induce the expression of *OCT-4* and *NANOG* in bovine SCNT embryos. Comparisons were made with porcine embryos derived from *in vitro* fertilization (IVF).

Results: The results of this study indicated that treatment with OAC1 at a concentration of 1.5 µM significantly increased the blastocyst formation rate and total cell numbers in SCNT embryos ($p < 0.05$). Furthermore, OAC1 enhanced the expression of *OCT-4*, *SOX2*, and *NANOG* specifically at the 8-cell stage, but did not significantly affect gene expression at other developmental stages.

Conclusion: In conclusion, OAC1 appears to exert beneficial effects on embryonic development in SCNT embryos by promoting *OCT-4* and related genes expression.

Keywords: Porcine SCNT, OAC1, Embryo development, Reprograming

1. Introduction

Somatic cell nuclear transfer (SCNT) involves transferring a donor cell into an enucleated oocyte. Currently, SCNT has been successfully applied to a range of species. Animal production based on SCNT offers a range of opportunities in basic and applied research, in agriculture, genetic conservation, and human medicine (Campbell et al., 2007). However, SCNT efficiency is still low because of incomplete epigenetic reprogramming of donor cell nuclei (Long et al., 2014). Consequently, the improvement of epigenetic reprogramming is a crucial factor in enhancing the developmental efficiency of SCNT embryos (Qiao et al., 2018).

OCT-4 (Octamer-binding transcription factor 4) is a major transcription factor in embryonic stem cells and primordial germ cells (Looijenga et al., 2003) The *OCT-4* gene is essential for complete embryo development (Nichols et al., 1998) and regulating pluripotency in embryonic stem cells (Niwa et al., 2000). Previous SCNT-related studies reveal that the expression level of the *OCT-4* gene in donor cells improves developmental efficiency, promotes nuclear reprogramming of SCNT embryos in bovine and porcine (Goissis et al., 2013) and enhances the expression level of the *OCT-4* during cleavages resulting in a higher developmental rate of mouse SCNT embryos (Pfeiffer et al., 2010). Oct4-activating compound (OAC1) has been widely applied to increase the expression activity of the *OCT-4* in mouse (Li et al., 2012). Moradi-Hajidavaloo et al. (2023) reported that using 1.5 µM of OAC1 in IVC medium improves the quality of bovine SCNT embryos. However, the effects of OAC1 on porcine SCNT embryos have not yet been reported.

This study aimed to determine the optimal concentration and the effects of OAC1 as a small-molecule supplemented in IVC medium on cleavage rate, blastocyst formation rate, expression level of *OCT-4*, *SOX2*, and *NANOG* genes of porcine SCNT embryos.

2. Materials and Methods

2.1 Chemicals

Chemicals used in this research were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA), if not, specify additionally.

2.2 Preparation of oct-4 activating compound 1 (OAC1) solution

Commercially available OAC1 was dissolved in 1 ml dimethylsulphoxide (DMSO) to produce a 21.07 mM 1,000X stock solution. Porcine zygote medium-3 (PZM-3) was used to dilute the stock solution to obtain a working solution.

2.3 Donor cell preparation

Fibroblast cells were isolated from the ear skin tissues of a male piglet. The skin was manually cut into small pieces after washed with 70% alcohol and removing root hair and cartilage. The small pieces of ear skin tissue were placed on culture dishes and covered with a sterilized glass slide. Then add 4 ml of culture medium in the culture dish and culture under a humidified atmosphere of 5% CO_2 in air at 37°C. The culture medium consisted of Minimum Essential Medium Eagle, Alpha modification (αMEM) supplemented with 10% fetal bovine serum (FBS, Gibco, 10270-098), 1mM L-glutamine, and 100 IU/ml penicillin-G, and 100 µg/ml streptomycin sulfate). The medium was changed every 3 days. After the fibroblasts reached at least 80% confluency, they were harvested using trypsin/EDTA and then passaged until reaching the third passage. In the third passage, the harvested cells were resuspended in a freezing medium, loaded in 0.5 ml straw. Then they were kept at -80 °C overnight and subsequently placed in liquid

nitrogen until used. Fibroblasts were prepared for donor cells by thawing and cultured on a culture dish with 3 ml of culture medium under a humidified atmosphere of 5% CO_2 in air at 37°C for 2-3 days. Porcine fibroblasts at the fourth passage were used as donor cells.

2.4 Oocyte collection and in vitro maturation (IVM)

Porcine ovaries were obtained from a local slaughterhouse and kept in 0.9% NaCl solution at room temperature within 2 h during transport to laboratory. Then cumulus-oocyte complexes (COCs) were collected from antral follicles using an 18-gauge needle equipped with a 10 ml syringe. COCs with a homogeneous cytoplasm and surrounded by at least 3 layers of intact cumulus cells were cultured with IVM-I medium (50 COCs/500 µl) in a 4-well dish covered with mineral oil under humidified atmosphere of 5% CO_2 at 38.5 ºC in air for 23 h, and then, cultured in IVM-II medium under humidified atmosphere of 5% CO_2 at 38.5 ºC in air for 22 h.

2.5 Somatic cell nuclear transfer (SCNT)

SCNT was performed following the protocol described in a previously published paper (Wei et al., 2013). Briefly, Cumulus cells were removed from the COCs at 42-44 h in IVM medium. Only A and B-graded metaphase II (MII) oocytes were cut the zona pellucida above the 1st polar body and enucleated with a glass needle squeeze. Complete enucleation was confirmed by staining the squeezed-out cytoplasm and 1st PB with 5 µg/ml Hoechst 33342 and visualizing under a fluorescence microscope (IX71, Olympus, Tokyo, Japan). Passage 4 of the donor cell was inserted into the perivitelline space of the enucleated oocyte. The oocyte-donor cells complex was fused using an electro-cell fusion machine (SUT F-1, Suranaree University of Technology) with two direct current (DC) pulses of 24V, 16 µsec. The reconstructed embryos were activated by incubated in 3 µM Ionomycin diluted in TCM199H for 4 min at room temperature. After that cultured in 2mM 6-Dimethylamino purine (6-DMAP) diluted in PZM-3 under a humidified atmosphere of 5% CO_2 in air for 3 h.

2.6 In vitro embryo culture with OAC1 treatment

After the processes of SCNT, 10 oocytes were cultured with 500 µl of PZM-3 medium (Cao et al., 2012) added with 0, 1.0, 1.5, and 3.0 µM of OAC1 in 4-well dish covered with mineral oil at 38.5 ºC under a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2 for 6 days. Development of the embryos was examined on days 3 and 6 to record cleavage and development to the blastocyst stage, respectively.

2.7 Total cell number of porcine SCNT embryos

Total cell number measurements were performed using the Hoechst 33342 staining method (Chazotte, 2011). After the IVC process at 144 h post activation, 10 blastocysts collected from each treatment were incubated with 25 µg/ml Hoechst 33342 diluted in PBS (-) (Phosphate Buffered Saline without calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions.) for 5 min at room temperature. The stained embryos were mounted on a glass slide and observed under a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

2.8 In vitro fertilization (IVF)

Embryos from the IVF group were used for positive control (Normal control). After IVM culture (2.4) for 44 h, the oocytes were washed three times in a modified pig-FM medium (Suzuki et al., 2002) containing 10 mM HEPES, 2 mM caffeine, and 5mg/ml BSA. To prepare sperm, fresh semen from the SUT farm was diluted in a BTS extender (Bwanga et al., 1990) at 15-20 ºC. After that sperm were preincubated in sperm washing medium (TCM 199 (Earle's salts, Gibco), 4.12 mM calcium lactate, 3.05 mM glucose, and 12% FBS pH 7.8 under a humidified atmosphere of 5% CO_2 in air at 37°C for 30 min. Then sperm were centrifuged and removed a supernatant and the pellet was resuspended and adjusted with pig-FM to a final concentration of 1.0×10^6/ml. To fertilize spermatozoa with oocytes, a 50 µl droplet of sperm containing 10-15 oocytes in a 35 mm culture dish covered with mineral oil was co-incubated under a humidified atmosphere of 5% CO_2 in the air at 38.5°C for 4-6 h. After fertilization, cumulus cells were removed by gentle pipetting and cultured in PZM-3 medium in a 35 mm culture dish covered with mineral oil at 38.5°C under a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2. The embryos at PN, 2-, 4-, 8-cells, and blastocyst stages were evaluated on days 1, 2 and 5-6, respectively.

2.9 Effect of OAC1 on the expression level of OCT-4, SOX2, and NANOG by qPCR

IVF and SCNT embryos at PN, 2-, 4-, 8-cells, and blastocyst stages produced from all treatment groups were harvested in PBS (-), and stored at -80 ºC until RNA was extracted. Embryos at PN stage (n=150), 2-cell stage (n=120), 4-cell stage (n=60), 8-cell stage (n=40), and blastocyst stage (n=20) were lysed by lysis buffer and mRNA were extracted using FavorPrep™ Tissue Total RNA Mini Kit (Favorgen Biotech Crop., Pingtung, Taiwan) according to the manufacturer's instructions. Next, mRNA was converted to complementary DNA (cDNA) using biotechrabbit™ cDNA Synthesis Kit (Biotechrabbit, Berlin, Germany). Finally, cDNAs were stored at -20 °C until use. To analyze gene expression (specific primers as shown in Table 1) quantitative real-time PCR (qPCR) was then the mixer was incubated at 25 °C for 30 sec and 52 °C for 30 min respectively. After that, the mixer was incubated at 99 °C for inactivating the reverse transcriptase enzyme. Finally, cDNAs were stored at -20 °C until use. To analyze gene expression (specific primers as shown in Table 1) quantitative real-time PCR (qPCR) was performed using the KAPA SYBR-Green PCR Master mix (Applied Biosystems, Carlsbad, CA, USA). The examination was performed on the CFX Opus 96 real-time PCR system (Biorad, Hercules, California, USA). Finally, the relative gene expression of embryos under different culture conditions was normalized with GAPDH (reference gene) and compared with the IVF embryos (normal control) with $2^{-\Delta\Delta Ct}$ method.

Table 1. Genes used for real-time qPCR

genes	Primer Sequence5' to 3'	Product Length, bp	Accession No.
genes related to embryo development			
OCT4	F: TTTGGGAAGGTGTTCAGCCAAACG R: TCGGTTCTCGATACTTGTCCGCTT	198	NM_001113060
SOX2	F: ATGCACAACTCGGAGATCAG R: TATAATCCGGGTGCTCCTTC	130	NM_001123197
NANOG	F: GGTTTATGGGCCTGAAGAAA R: GATCCATGGAGGAAGGAAGA	98	NM_001129971
genes related to epigenetic reprogramming			
DNMT1	F: TCGAACCAAAACGGCAGTAC R: CGGTCAGTTTGTGTTGGACA	215	NM_001032355
DNMT3a	F: CTGAGAAGCCCAAGGTCAAG R: GTACTGATACGCGCACTCCA	200	NM_001097437
HDAC1	F: CGCATGACTCACAATTTGCT R: AGCCATCAAATACCGGACAG	211	XM_013999116
HDAC2	F: ACAGGAGACTTGAGGGAT R: CACATTTAGCGTGACCTT	232	XM_001925318
HDAC3	F: GCTGCTGGACGGATGAGA R: CTGGATGGAGCGTGAAGT	108	NM_001243827
GAPDH	F: GTCGGTTGTGGATCTGACCT R: TTGACGAAGTGGTCGTTGAG	207	NM_001206359

2.10 Statistical analysis

Statistical analysis was performed by GraphPad version 5 (GraphPadSoftware, San Diego, CA, USA), and data were represented as the mean ± SEM. A value of $p < 0.05$ was considered significant with different superscript-case letters. The differences between data were indicated using a one-way analysis of variance (ANOVA), followed by the Tukey–Kramer Honest Significant Difference (HSD) Post hoc test to compare differences between the two groups.

2.11 Ethical Approval Statement

This study was approved by the Institutional Animal Care and Use Committee, Suranaree University of Technology

3. Results

3.1 Cleavage and blastocyst rate of porcine SCNT cultured with OAC1

To evaluate the optimal concentration of OAC1 supplemented in IVC medium on porcine SCNT embryos development. After the SCNT process, the reconstructed embryos were cultured in IVC medium supplemented with 0, 1.0, 1.5, and 3.0 µM OAC1. The results from Table 2 showed that supplemented with 1.5 µM OAC1 significantly higher cleavage rate than the untreated group (86.33 ± 1.2% vs 74.2 ± 1.8%, $p < 0.05$). The blastocyst rate of the 1.0 and 1.5 µM OAC1 was significantly higher than untreated group (42.87 ± 1.9% and 48.07 ± 0.8% and 36.1 ± 0.9% $p < 0.05$, respectively) (Table 2)

3.2 Total cell number of porcine SCNT cultured with OAC1

In the next step, we examine the effect of OAC1 supplemented in IVC medium on the total cell number in the blastocysts. The results showed that total cell number of supplemented with 1.5 µM OAC1 was significantly higher than the other group (Table 2).

Table 2 The effect of different concentrations of OAC1 on the development of porcine SCNT embryos

OAC1 concentration (µM)	No. of embryos cultured	No. of cleaved (mean ± SEM, %)	No. of blastocysts (mean ± SEM, %)	Total cell number in blastocyst (mean ± SEM)
0	156	117 (74.2 ± 1.8) ^{c}	57 (36.1 ± 2.9) ^{c}	53.8 ± 1.6 ^{c}
1.0	158	124 (79.0 ± 1.7) ^{bc}	68 (42.8 ± 1.9) ^{b}	61.1 ± 1.2 ^{b}
1.5	160	140 (86.3 ± 1.2) ^{a}	76 (48.1 ± 1.8) ^{ab}	66.0 ± 1.8 ^{a}
3.0	158	130 (82.5 ± 1.6) ^{bc}	66 (41.53 ± 2.0) ^{bc}	58.2 ± 1.1 ^{b}

9 replicates were performed

$^{a, b,c}$ Values with different superscripts in the same column are significantly different ($P<0.05$)

3.3 OAC1 affected the expression of genes related to the development and pluripotency of porcine SCNT embryos

We investigated the OAC1 that regulated the mRNA expression level of *OCT-4*, *SOX2*, and *NANOG* genes at PN, 2-, 4-, 8- cells and blastocyst stages between untreated SCNT embryos group as a negative control (SCNT-Untreated), IVF group as a positive control (normal control) and SCNT embryos treated with 1.5 µM of OAC1 as a treatment group (SCNT-OAC1) with the results shown in Figure 1

The expression level of the *OCT-4* gene in the treatment group showed variation across the developmental stages. At PN and 8-cell stages, the *OCT-4* expression level of the SCNT-OAC1 group was significantly higher than the SCNT-Untreated group (p < 0.05), but no significant difference when compared with the IVF group. At 2- and 4-cell stages, the *OCT-4* expression level of the SCNT-OAC1 group was significantly higher than the SCNT-Untreated and IVF groups (p < 0.05). Finally, at the blastocyst stage, the *OCT-4* expression level of the SCNT-OAC1 group had no significant difference when compared with the SCNT-Untreated and IVF groups. The expression level of the *SOX2* gene in the treatment group showed variation across different stages. At PN stage, the *SOX2* expression level of the SCNT-OAC1 group was significantly higher than SCNT-Untreated and IVF groups (p < 0.05). At the 2-cell stage, the *SOX2* expression level of the SCNT-OAC1 group was significantly lower than the SCNT-Untreated, but no significant difference when compared with the IVF groups. At 4-cell and blastocyst stages, the *SOX2* expression level of the SCNT-OAC1 group was significantly lower than the IVF group (p < 0.05), but was significantly higher than the SCNT-Untreated group (p < 0.05). Finally, at 8-cell stage, the *SOX2* expression level of the SCNT-OAC1 group was significantly higher than SCNT-Untreated and IVF groups (p < 0.05), but the IVF group was significantly higher than the SCNT-Untreated group (p < 0.05).

The expression level of the *NANOG* gene in the treatment group showed variation across different stages. At PN and 4-cell stages, the *NANOG* expression level of the SCNT-OAC1 group was significantly lower than the IVF group (p < 0.05), but no significant difference when compared with the SCNT-Untreated group. At the 2-cell stage, the *NANOG* expression level of the SCNT-OAC1 group was significantly lower than

the IVF group ($p < 0.05$), but was significantly higher than the SCNT-Untreated group. Finally, at 8-cell and blastocyst stages, the *NANOG* expression level of the SCNT-OAC1 group was significantly higher than the SCNT-Untreated group ($p < 0.05$), but no significant difference when compared with the IVF group.

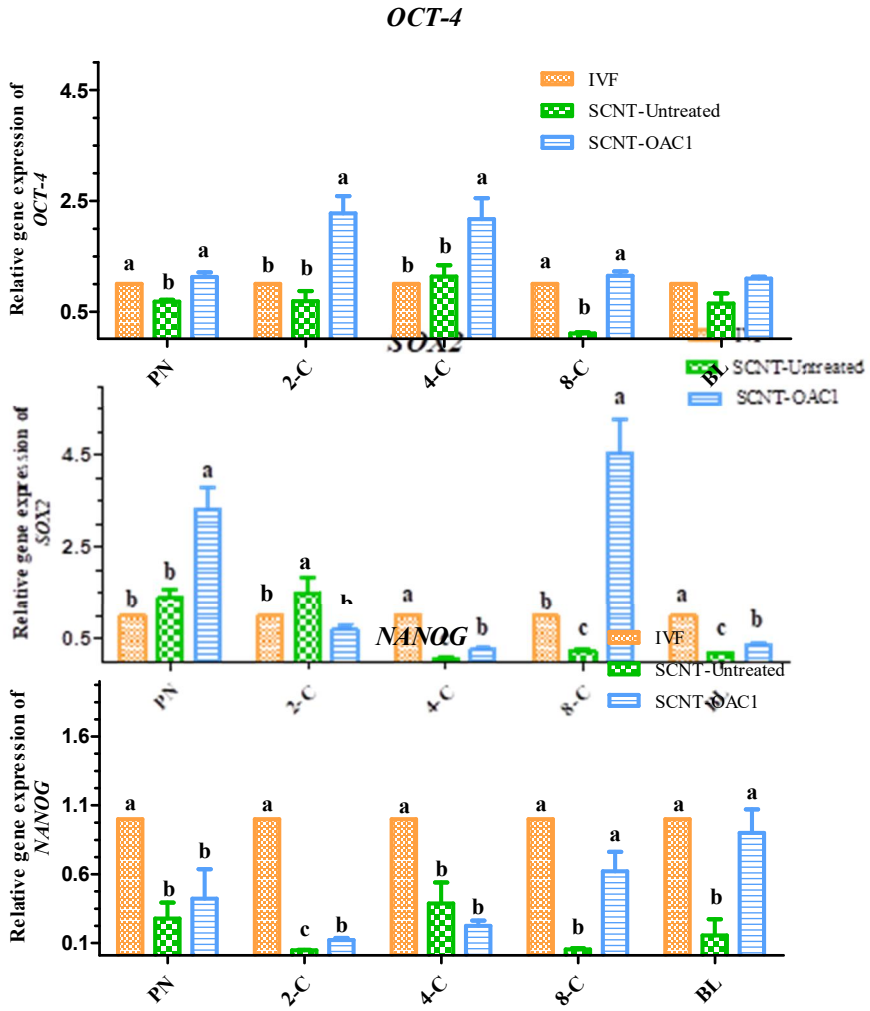

Figure 1 Comparison of mRNA expression levels (mean ± SEM) of genes related to development and pluripotency of porcine embryos between the SCNT-OAC1, IVF, and SCNT-Untreated groups at PN, 2-cell, 4-cell, 8-cell, and blastocyst stages: (A) Expression levels of *OCT-4* gene, (B) Expression levels of *SOX2* gene, and (C) Expression levels of *NANOG* gene. [a, b, and c] Values with different superscripts indicate a significant difference ($p < 0.05$). Error bar = standard error of the mean.

4. Discussion

In our study, first of all, we treated various concentrations of OAC1 supplemented in IVC medium and cultured for 6 days to examined the optimal concentration by measuring the quality and development of porcine SCNT embryo (cleavage rate, blastocyst rate, and total cell numbers) between treated and untreated OAC1. The results showed that 1.5 µM of OAC1 gives a significantly increased the embryo development particular at cleavage and blastocyst rates, and the total cell numbers of blastocyst-derived porcine SCNT. The results of this experiment are similar to a SCNT bovine

embryo that was treated with 1.5 µM of OAC1, showing an increased developmental rate (Moradi-Hajidavaloo et al., 2023). We used 1.5 µM of OAC1 to investigate the mRNA expression of *OCT-4*, *SOX2*, and *NANOG* genes that pluripotency markers of embryonic cells play an important role in self-renewal and maintaining the pluripotency of cloned embryos (Keramari et al., 2010; Park et al., 2012). The effect of pluripotent markers induced by OAC1 has not yet been reported in a porcine SCNT embryo. However, the *OCT-4* expression in our study showed an increase in the SCNT-OAC1 group at the PN until 8-cell stages than the untreated and IVF group but no significant difference was observed at blastocyst stages between the untreated and IVF group. This result is consistent with the report in SCNT porcine embryo, the *OCT-4* expression level is detected until the 8-cell stage from transcription using maternal mRNA, and the zygote *OCT-4* expression being from 8 – 16-cell stage (Kirchhof et al., 2000), In addition, in mouse embryos, the loss of *OCT-4* expression showed at the blastocyst stage due to the occurrence of differentiation of totipotent cells to somatic lineages (Pesce & Schöler, 2001). During early embryonic development, transcription factors (TFs) play a crucial role in determining the fate of blastomere by regulating the activation or suppression of *OCT-4,* the key development gene is a core component of the pluripotency regulation network, guiding blastomere to ICM, which later gives rise to embryonic stem cell or toward differentiation into trophoblast cell, with contribution to extra-embryonic structure (Niwa et al., 2000). Several studies have demonstrated a function of *OCT-4* in the embryos development (Boiani et al., 2002). Further studies on the transcription regulation of *OCT-4* in porcine improved the developmental efficiency of nuclei transfer embryos, suggesting that high *OCT-4* expression in donor cells are more efficient promote nuclei reprogramming (du Puy et al., 2011; Lee et al., 2014). However, the mRNA expression of *OCT-4* in porcine and bovine SCNT embryos was significantly lower than that derived by IVF (Garcia-Canovas et al., 2024). Previous studies have made progress on identifying an OCT-4 promoter-activating compound to promote the *OCT-4* expression to enhance the efficiency of reprogramming (Li et al., 2012) and differentiated cells into pluripotent cells (Huang et al., 2016; Li et al., 2012; Moradi-Hajidavaloo et al., 2023).

The *SOX2* expression level of SCNT-OAC1 group showed higher than untreated and IVF groups at PN and 8-cell stages, lower than IVF group at 4-cell and blastocyst stage but no difference at the 2-cell stage. *Sox2* acts cooperatively with *OCT-4* at promoters activating the transcription of several genes, which play important roles in embryo development (Nishimoto et al., 1999). The downregulate of *SOX2* expression is possible to decreasing *OCT-4* expression levels influence several transcription factors and induce developmental arrest (Kirchhof et al., 2000).

The *NANOG* expression level in the OAC1 treatment group was lower than in the IVF group at the PN stage until the 4-cell stage, and there was no difference at the 8-cell to blastocyst stages. The results were consistent with the previous study, the *OCT-4* and *SOX2* network stimulates the expression of *NANOG* has shown that the expression of *OCT-4*, *SOX2*, and *NANOG* follows the same trend, with high expression level of *NANOG* at the 8-cell stage to the blastocyst stage (Kalmar et al., 2009; Rodda et al., 2005) and the distinct expression pattern of *OCT-4* compared to *NANOG* at certain stages of embryo development supports previous studies indicating that OAC1 promotes the expression level of *OCT-4* and *NANOG* (Li et al., 2012).

5. Conclusion

The optimal concentration of OAC1 at 1.5 µM could enhance cleavage and blastocyst rates, as well as the total cell numbers. Additionally, it improved the expression of genes related to the pluripotency of SCNT embryos, including *OCT-4*, *SOX2*, and *NANOG* at the 8-cell to the blastocyst stages. However, more of the OAC1 dynamic on the development and epigenetic modification of porcine SCNT embryos need to be confirmed in the future. The suggestion of the study might be to investigate such as the optimal time to culture OAC1 after the SCNT process, the suitable period of treatment, the IVM or IVC period, the mechanism of OAC1, co-culture with another small molecule, another position of histone modification and the number of healthy offspring after embryo transfer.

Acknowledgement

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16S rRNA Metagenomic Analysis of Fecal Microbiota in Dairy Cattle with Different Body Condition Score

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Abstract

Background:

Holstein Friesian cows are globally recognized for their milk production, and Body Condition Score (BCS) is a key health indicator. Low BCS linked to reduced milk output and fertility. Gut microbiome is found to potentially influence the cow's physiology. This study aimed to investigated and compare the fecal microbiome profiles of Holstein Friesian cows with normal versus low BCS.

Methods:

Fecal samples were collected from non-pregnant Holstein Friesian heifers across 8 dairy farms in Maha Sarakham province, Thailand. Samples were divided into two groups of 16 each: "thin" cows (BCS < 3.0) and "normal" cows (BCS ≥3.0). The 16S rRNA gene sequencing was performed on the Illumina Hiseq 2500 platform. Amplicon sequence variants (ASVs) and taxonomic annotation, calculation of Alpha diversity indices and ADONIS value were performed using QIIME2. Principal Component Analysis (PCA) was conducted for beta diversity analysis. Statistical analyses included t-tests and Wilcoxon rank-sum tests to compare relative abundances of individual taxa.

Results:

12,031 ASVs were identified. Alpha diversity was not significantly differences between groups ($p > 0.05$). Although the PCA plot revealed unclear clustering between groups, ADONIS analysis indicated significant differences in the fecal microbiota composition ($p = 0.035$). The predominant phyla in both groups were Firmicutes, Bacteroidota, and Spirochaetota. Taxonomic composition differed between two groups with significant differences in the abundance of certain genus ($p < 0.05$). Thin cows had significantly higher levels of *Turicibacter,* while normal cows had significantly higher levels of *Alistipes* and *Bacteroides*.

Conclusion:

The study revealed different pattern of bacterial community between normal and thin cows. We propose that, in addition to genetic and nutritional factors, gut microbiome may contribute to subclinical health conditions that lead to lower BCS values.

Keywords: Body Condition Score (BCS), Fecal microbiome, 16S rRNA, Targeted sequencing

Heart Rate and Heart Rate Variability in the First and Second Trimester Pregnant Mares

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Abstract

Background: The autonomic nervous system play a major role in physiological adaptaion during pregnancy. The balance of autonomic nervous system activity can be assessed using heart rate variability (HRV). The changes in autonomic nervous system activity during early pregnancy in mares remain unclear. This study aims to compare heart rate (HR) and heart rate variability among healthy non-pregnant mares and mares in the first and second trimesters of pregnancy.

Methods: A total of 45 Thai native crossbred mares without cardiac abnormalities were included in the study. Animals were divided into three groups: the non-pregnant mare group (n = 5), the first trimester pregnant mare group (n=18) and the second trimester pregnant mare group (n = 22). An ECG Holter system was used to record data and obtain HR and time-domain HRV parameters, including SDNN, SDNN index, rMSSD, SDANN, pNN50, and vasovagal tonus index (VVTI). VVTI was calculated to assess short-term heart rate variability using 20 consecutive R-R intervals.

Results: HR was significantly higher in first-trimester pregnant mares compared to non-pregnant mares ($p < 0.05$), and even higher in second-trimester mares compared to those in the first trimester ($p < 0.05$). However, time-domain HRV parameters showed no significant differences among the three groups.

Conclusion: This study suggests that physiological adaptation begins in the early stage of pregnancy in mares, even though imbalances in autonomic nervous system activity are not yet apparent.

Keywords: heart rate, heart rate variability, horses; pregnancy, vasovagal tonus index

Seasonal Variation and Antimicrobial Resistance of *Salmonella* Isolated from Pork at Slaughterhouses and Markets in Northeast of Thailand

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Abstract

Background: Salmonellosis is a foodborne zoonotic disease that poses a significant threat to human health. This study aimed to determine seasonal variation and antimicrobial resistance patterns of *Salmonella* isolated from pork at slaughterhouses and markets in northeast of Thailand.

Methods: During 2022-2023 period, a total of 458 samples were collected and analyzed according to ISO 6579:2002, with 326 samples sourced from slaughterhouses and 132 from markets.

Results: In the summer season, *Salmonella* was detected in 17.5% (22/126) of slaughterhouse samples and 74% (17/23) of market samples. During the rainy season, prevalence was 13% (13/100) in slaughterhouses and 75.4% (43/57) in markets. In winter, *Salmonella* was detected in 19% (19/100) of slaughterhouse samples and 59.6% (31/52) of market samples. The predominant Salmonella serovars identified were Rissen in the summer, Anatum in the rainy season, and Rissen again in winter. The highest levels of antimicrobial resistance were observed in summer, with *Salmonella* exhibiting 100% resistance to ampicillin, streptomycin, and trimethoprim/sulfamethoxazole. In the rainy season, resistance was most prevalent to ampicillin (38%), tetracycline (38%), and streptomycin (19%). In winter, resistance rates were highest for ampicillin (100%), streptomycin (84%), and tetracycline (55%).

Conclusion: These findings highlight the high prevalence of *Salmonella* contamination in pork and the concerning antimicrobial resistance patterns. Effective prevention and control measures in pork processing are essential for safeguarding public health and ensuring the well-being of both pigs and consumers.

Keywords: antimicrobial resistance, *Salmonella*, pork, slaughterhouse, market

Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Poultry in Thailand

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Abstract

Background: *Salmonella* spp. is a leading cause of foodborne zoonotic disease globally, with non-typhoidal *Salmonella* serotypes being the primary etiological agents of salmonellosis in poultry. Contaminated poultry eggs and meat products served as major sources of human *Salmonella* infection.

Methods: In this study, 689 poultry samples were collected from slaughterhouses and markets in Khon Kaen Province, Thailand during the 2022-2023 period and were isolated and identified according to ISO 6579:2002.

Results: The prevalence of *Salmonella* spp. contamination in slaughterhouses and markets varied by season. In the summer, contamination rates were 13% and 26%, respectively; in the rainy season, 49% and 52%; and in the winter, 25% and 58%. The predominant *Salmonella* serovars identified were Saintpaul in summer, Singapore in the rainy season, and Agona in winter. Antimicrobial resistance patterns varied across seasons and locations. In summer, resistance was observed to ampicillin (38%) and a combination of ampicillin and streptomycin (54%) in slaughterhouses and markets. In the rainy season, resistance to nalidixic acid (45%) and ampicillin (37%) was noted, while in winter, resistance was most prominent to streptomycin (84%) and ampicillin (55%).

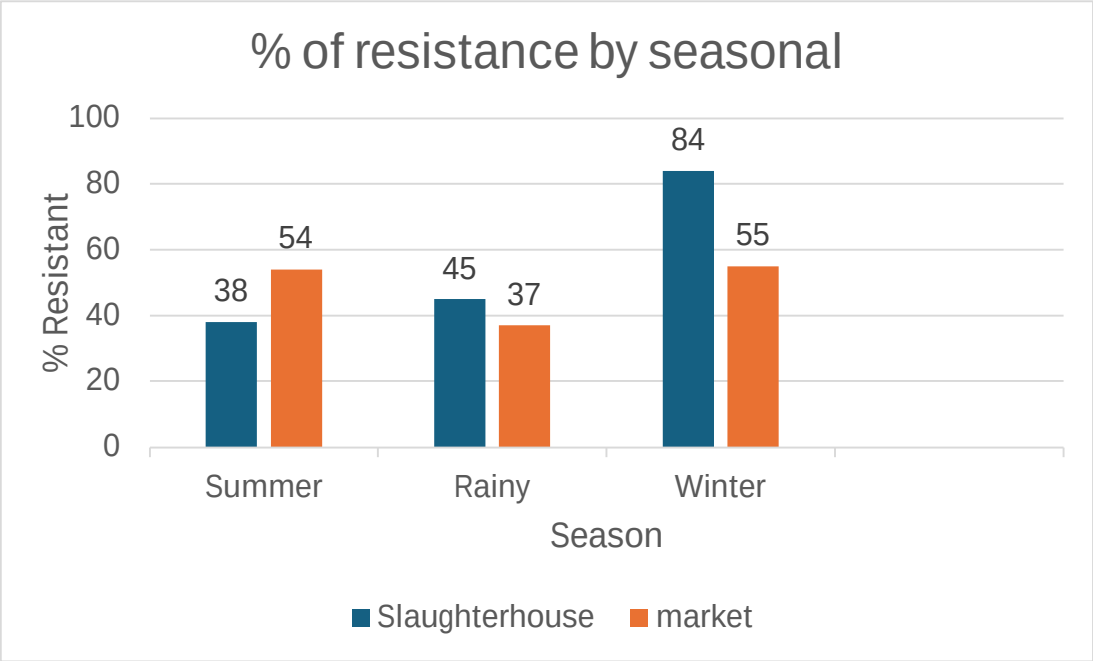
Conclusion: To reduce *Salmonella* contamination and antimicrobial resistance, the adoption of Good Agricultural Practices (GAP) and Hazard Analysis Critical Control Point (HACCP) principles is recommended. As the consequence, establishment of a standard pathogen and AMR monitoring system in poultry production, personnel training on personal hygiene, biosafety and farm biosecurity for all farm attendants and visitors; education on zoonotic pathogens; and restrictions on the uncontrolled use of antimicrobials; are recommended.

Keywords: antimicrobial resistance, poultry, prevalence, *Salmonella*

Season	Location	Number	Positive (%)	Serovars
Summer	slaughterhouse	101	13 (12.87)	Molade (3), Agona (2), Apeyeme (2), Covallis (2), Bareily (1), Stanley (1), Rissen (1), Krefeld (1)
	market	67	26 (38.81)	Saintpaul (8), Bareilly (4), Hvittingfoss (4), Mbandaka (3), Stanley (2), Enteritidis (1), Agona (1), Corvallis (1), Rissen (1), *S*. enterica subsp.enterica ser.4,5,12:i:- (1)
Rainy	slaughterhouse	149	49 (32.8)	Singapore (21), Bovismorbificans (11), Oslo (8), Saintpaul (6), Agona (2), Kentackey (1)
	market	80	52 (65%)	Agona (7), Mbandaka (7), Molade (7), Stanley (6), Bareilly (5), Enteritidis (4), Brancaster (4), Bredeney (3), Rissen (3), Covallis (2), Singapore (1), Anatum (1), Derby (1), Albany (1)
Winter	slaughterhouse	166	25 (15.06)	Saintpaul (18), Hvittingfoss (6), *S*. enterica subsp.enterica ser.4,5,12:i:- (1)
	market	126	58 (46.03)	Agona (22), Bareilly (9), Mbandaka (5), Enteritidis (4), Rissen (4), Corvallis (3), Apeyeme (3), Hvittingfoss (2), Stanley (2), Saintpaul (2), Infantis (1), Virchow (1),

Number of Resistance															
	N	S	Nor	TE	GM	NA	SXT	IPM	CTX	C	CIP	AM	CAZ	CRO	AMC
Summer S	13	3	0	3	1	1	1	0	0	0	0	5	0	0	0
Summer M	26	14	0	6	13	6	2	0	0	1	0	14	0	0	3
Rainy S	49	18		1	7	22	1					6			1
Rainy M	52	7	0	11	2	7	8	0	4	7	0	19	3	3	5
Winter S	25	21	0	18	18	14	0	0	0	0	0	15	0	0	9
Winter M	58	24	0	28	5	18	17	0	2	2	0	32	0	1	10

% of Resistance															
	N	S	Nor	TE	GM	NA	SXT	IPM	CTX	C	CIP	AM	CAZ	CRO	AMC
Summer S	13	23	0	23	8	8	8	0	0	0	0	38	0	0	0
Summer M	26	54	0	23	50	23	8	0	0	4	0	54	0	0	12
Rainy S	49	37	0	5	14	45	2	0	0	0	0	12	0	0	2
Rainy M	52	13	0	21	4	13	15	0	8	13	0	37	6	6	10
Winter S	25	84	0	72	72	56	0	0	0	0	0	60	0	0	36
Winter M	58	41	0	48	9	31	29	0	3	3	0	55	0	2	17


% of resistance by seasonal
100
80
60
40
20
0
% Resistant
38
54
45
37
84
55
Summer
Rainy
Winter
Season
Slaughterhouse
market

Development of detection of *Escherichia coli* O157 in cow milk using Loop Mediated Isothermal Amplification (LAMP) assay

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Abstract

Background: *Escherichia coli* O157 is a foodborne pathogen frequently identified in milk, noted for its low infectious dose and significant pathogenic potential. Therefore, developing a rapid, highly sensitive, and convenient detection method for this bacteria is essential. This study focused on assessing the efficacy of the loop-mediated isothermal amplification (LAMP) diagnostic test, which targets the *stx2* gene for the rapid and specific detection of *Escherichia coli* O157 isolated from raw milk samples.

Methods: Twenty raw cow milk samples with somatic cell counts exceeding 600,000 cells/mL were examined for *Escherichia coli* O157 using PCR targeting the *Z3276* gene and LAMP targeting the *stx2* gene. LAMP assay was performed and sensitivity was determined by serial dilution.

Results: A total of three *Escherichia coli* O157 isolates were tested using the LAMP assay. DNA amplification was visualized as turbidity visible to the naked eye and confirmed by 1% agarose gel electrophoresis. The assay demonstrated high sensitivity and specificity, and it was also 10-fold more sensitive than the conventional PCR assay; sensitivity was determined by serial dilution. LAMP assay showed high accuracy in detecting *Escherichia coli* O157.

Conclusion: The LAMP assay is a simple, rapid, and highly specific gene amplification technique suitable for use as a screening tool in basic laboratories and field testing for the detection of *Escherichia coli* O157 in raw milk.

Keywords: *Escherichia coli* O157, LAMP, PCR, Raw milk

Seroprevalence and Risk Factors of Brucellosis in Goats in Muang District, Nakhon Phanom Province

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Abstract

Background: Brucellosis is a major bacterial zoonosis affecting public health. It causes serious economic losses in livestock production such as mastitis, metritis, epididymitis, orchitis, and infertility. The objectives of this study were to determine seroprevalence and risk factors of brucellosis in goats in Muang District, Nakhon Phanom Province.

Methods: The study was carried out from July to September 2024 using a cross-sectional study. A total of 264 goat serum samples from 15 goat farms were collected. The serum samples were tested for brucellosis antibodies using the Rose Bengal Test (RBT). Information on risk factors was obtained through interview using a questionnaire.

Results: The results of seroprevalence showed that at the individual level was 0.76% (2/264), whereas 0.88% (2/227) in female and 0.00% (0/37) in male. Which seroprevalence was not different between genders ($P>0.05$). The herd level was found only in Kuruku as 6.67% (1/15). There was no significant difference in prevalence among the locations ($P>0.05$). However, the factor of introduction of new goats into herd was significantly associated with the risk of brucellosis ($P\leq0.05$).

Conclusion: The results indicated that goats raised in Muang District, Nakhon Phanom Province are at risk of brucellosis. Therefore, farmers should be educated about the control and prevention of brucellosis to reduce the risk of infection to human and goats.

Keywords: Brucellosis, Goat, Risk factors, Seroprevalence

1. Introduction

Goats farming contribute to the economy in terms of capital storage, saving and income for smallholder farmers (J-Khaza'leh et al., 2015). Goat stock in Thailand from 2023 to 2024 decreased from 1,547,544 to 1,489,917 (3.72%). Similarly, goat stock in Nakhon Phanom Province decreased from 9,296 to 8,852 (4.78%) (Department of Livestock Development; DLD, 2024). However, the major problems affecting economic goat production are high feed costs, high sterility rates, high prenatal mortality rates, and multiple reproductive failures (J-Khaza'leh et al., 2015; Rerkyusuke et al., 2024). Therefore, enhancement of feed use efficiency, reproductive management and herd health are suggested.

Brucellosis is a major bacterial zoonosis with affecting public health. It causes serious economic and reproductive losses in livestock production as mastitis, metritis, epididymitis, orchitis and infertility. In human, brucellosis is also known as undulant fever with symptoms includes fever that fluctuates, night sweats, headache, joint pain, and back pain (Center for Food Security and Public Health; CFSPH, 2018). *Brucella melitensis* is caused of brucellosis in goats. It is spread with the placenta, fetus, amniotic fluid, udder secretion, vagina discharges, semen, milk, and urine by ingestion and exposure. The organism can be isolated from reproductive organs such as uterus, epididymis and testes, as well as lymph nodes from the head, spleen and from arthritic lesion (World Organisation for Animal Health; OIE, 2022). *B. melitensis* is the main *Brucella* species involved in acute and chronic forms of brucellosis. The acute form causes macroscopic placentitis in pregnant females. In males necrotic epididymis and orchitis can be detected. The chronic form can infect other animals and human, but the clinical sign may not occure (Quintas et al., 2019). The study of 443,561 of goats and sheep in Thailand covering the period from 2013 to 2015 reported that prevalence at herd level was 9.85% (Peck et al., 2018). In Northeastern Thailand, the study of seroprevalence in caprine brucellosis reported that prevalence at herd level was 6.73% (Rerkyusuke et al., 2024). In addition, the study of seroprevalence of brucellosis form 431 of meat goats in Khon Kaen Province reported that prevalence at individual level was 2.78% (Jansod et al., 2019).

The objectives of this study were to determine seroprevalence and risk factors of brucellosis in goats in Muang District, Nakhon Phanom Province.

2. Materials and Methods

2.1. The study location and design

The Institutional Animal Care and Use Committee of Nakhon Phanom University (project code NPU009/2566) reviewed and officially approved this study (date 13.12.2023). From July to September 2024, the study was carried out by a cross-sectional study. The sample size was determined using Krejcie & Morgan (1970) formula as follows: $[s = X^2 NP (1-P) \div d^2 (N-1) + X^2 P (1-P)]$ where s = required sample size, X^2 = 3.841, N = 750, P = assumed to be 0.5, and d = 0.5. The sample size was increased from 254 to 264. All goats aged at least three months were included in the study. The samples were collected from 15 herds and located in 5 villages as followed Banklang (2 herds), Dongkhang (1 herd), Khamthao (1 herd), Kuruku (7 herds), and Thakho (4 herds) (**Figure 1**). A pretested questionnaire was used to collect information related to risk factors associated with brucellosis seropositivity at herd level. The risk factors evaluated were

herd size, rearing management, vaccination, deworming, shared rams with other farm, previously tested for brucellosis, introduction of new goat into herd, and history of abortion.

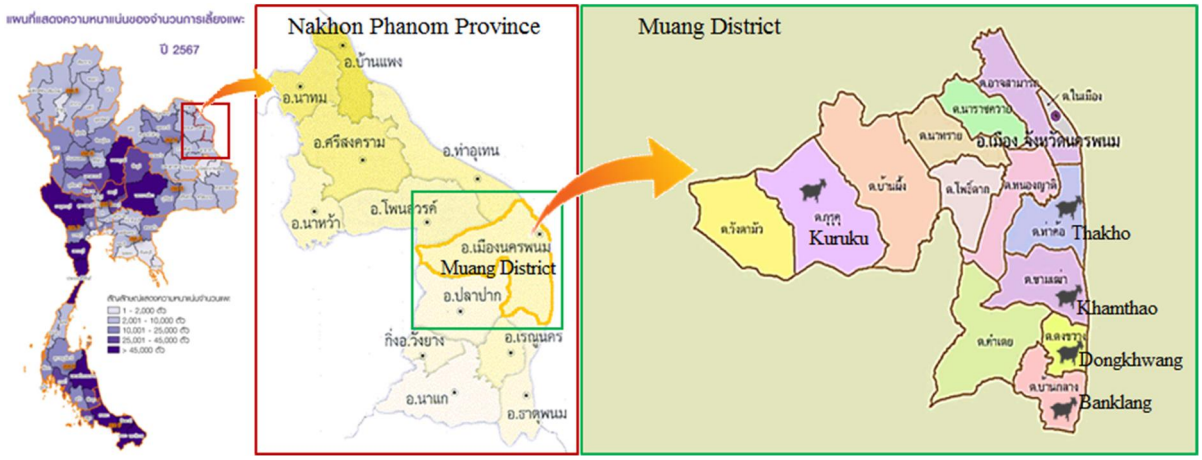

Figure 1 Map of the study location in Muang District, Nakhon Phanom Province
Source: *Land Development Department* (2024); DLD (2024)

2.2. Blood sample collection

Approximately 5 mL of blood sample was collected from the jugular vein of each animal using sterile needles, disposable syringe, and plain vacutainer tubes. Each blood sample was labeled and stored at 4 °C in a cool box with ice packs. Subsequently blood samples were centrifuged at 3,000 rpm for 10 minutes to separate clear sera. Finally, sera were transfered into 1.5 mL of micro-centrifuge tube and stored at -20 °C until used.

2.3. Serological test

The serum samples were tested for brucellosis antibodies using Rose Bengal Test (RBT) with *Brucella abortus* Weybride strain 99 antigen. The testing procedure was carried out following the guidelines described by the World Organisation for Animal Health (OIE, 2022). Briefly, only the required quantities of serum samples and antigen for the day's testing were removed from the refrigeration and allowed to equilibrate to room temperature (25 °C). Subsequently, 30 µL of each serum sample was dispensed onto a glass slide within a pre-marked circle of approximately 2 cm in diameter. The antigen bottle was gently shaken and placed 30 µL of antigen near each of the serum droplet. Thereafter, the serum and antigen were then mixed within the circled area using disposable pipette tip and gently rocked for 4 minutes. Agglutination was assessed immediately after mixing: any visible agglutination was interpreted as a positive reaction, while the absence of agglutination was recorded as negative.

2.4. Statistical analysis

Data were analyzed by Chi-square test (X^2-test). The results are represented as a percentage of seroprevalence. The P- value of statistical significance was at $P \leq 0.05$.

3. Results

3.1. Seroprevalence of brucellosis

A total of 264 serum samples were collected from 15 smallholder farms located across 5 villages in the Muang District. The results of seroprevalence showed that 2 heads of goats were in the positive classification at Rose Bengal (RBT). All other goats were test-nagative (**Figure 2**). At the individual level, the seroprevalence of brucellosis was 0.76% (2/264). The genders were not different between female (0.88%; 2/227) and male (0.00%; 0/37) (P>0.05). The seroprevalence of brucellosis at the herd level was recorded only Kuruku as 6.67% (1/15). There were no significant difference in the prevalence among the locations (P>0.05) (**Table 1**).

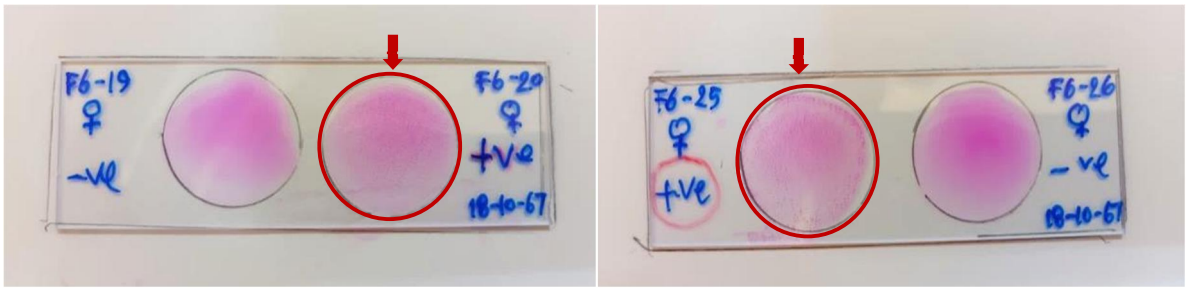

Figure 2 Rose Bengal Test (RBT). Arrows and circles are indicating the positive samples with agglutination.

Table 1 Seroprevalence of brucellosis in goats in Muang District, Nakhon Phanom Province

Variables	Samples (N)		Positive (N)	Negative (N)	Seroprevalence (%)
	Herd	Individual			
Individual					
Female	-	227	2	225	0.88
Male	-	37	0	37	0.00
Location of herd					
Banklang	2	49	0	49	0.00
Dongkhwang	1	9	0	9	0.00
Khamthao	1	9	0	9	0.00
Kuruku	7	101	2	99	1.98
Thakho	4	96	0	96	0.00
Total of individual	**-**	**264**	**2**	**262**	**0.76**
Total of herd	**15**	**-**	**1**	**14**	**6.67**

3.2. Factors associated with brucellosis seropositivity

Data from the questionnaire study showed that almost all farms (9/15; 60.00%) had less than 20 heads. Majority of farms (14/15; 93.33%) had rearing management by grazed and kept in stall. Almost of farms (11/15; 73.33%) had no another vaccination and less than half (6/15; 40.00%) had deworming every 3 to 6 months. Shared rams with other farm was 3/15 (20.00%). All farms had no tested brucellosis. Introduction of new goat into herd was 3/15 (20.00%). Over half all farms (9/15; 60.00%) had history of abortion (**Table 2**).

The reproductive risk factors were analyzed to detect their associated with brucellosis seropositive at herd level (**Table 3**). The results showed that only one among

3 of reproductive risk factors was statistically associated with herd seropositivity. The factor of introduction of new goats into herd was significantly associated with the risk of brucellosis (P≤0.05).

4. Discussion

In the present study, the results of seroprevalence of brucellosis demonstrated that at the individual level was 0.76% (2/264) and at the herd level was 6.67% (1/15). The study by Rerkyusuke et al. (2024), who found a similar result, reported that prevalence at the herd level in northeastern, Thailand was 6.73%. Similarly, the study in 443,561 of goats and sheep in Thailand by Peck et al. (2018) reported that prevalence at the individual level was 0.80% and at the herd level was 9.85%. The study in Chiang Mai Province by Kladkempetch et al. (2017) reported that prevalence at the individual level was 0.60% (3/500), but at the herd level was higher than this study as 16.67% (2/12). On the other hand, Longkae et al. (2021), who studied in 63,162 goats and 4,802 farms of 5 southern border province of Thailand, reported that prevalence at the individual and herd levels were lower than current study as 0.28% and 1.27%, respectively.

Table 2 Questionnaire study on farm management and knowledge of brucellosis

Risk factors	Category	Number	Percentages
Herd size	≤ 20 heads	9	60.00
	21-50 heads	6	40.00
Rearing management	Grazed and the kept in stall	14	93.33
	In stall	1	6.67
Vaccination	Yes (Foot and Mouth Disease)	4	26.67
	No	11	73.33
Deworming	Every 3 month	6	40.00
	Every 6 month	6	40.00
	Annual	3	20.00
Shared rams with other farm	Yes	3	20.00
	No	12	80.00
Previously tested for brucellosis	Yes	0	0.00
	No	15	100.00
Introduction of new goat into herd	Yes	3	20.00
	No	12	80.00
History of abortion	Yes	9	60.00
	No	6	40.00

Table 3 Reproductive risk factors associated with brucellosis seropositive at herd level

Risk factors	Category	Number	Positive	Chi square	*P*-value
Shared rams with other farm	Yes	3	0	0.268	0.605
	No	12	1		
Introduction of new goat into herd	Yes	3	1	4.286	0.038*
	No	12	0		
History of abortion	Yes	9	1	0.714	0.398
	No	6	0		

Note: * Denote significant difference (P≤0.05).

However, the current result is relatively lower than the previous study by Antarasena et al. (2013) reported that prevalence in the western Thailand at the individual level was 5.08% (3/500) and at the herd level was 18.39% (16/87).

Several previous studies have shown the seroprevalence and risk factors of brucellosis in small ruminants in other countries. Sadhu et al. (2015) reported that prevalence at the individual level in District of Gujarat, India was 11.30%. Shafy et al. (2016), who studied in Mymensingh District, Bangladesh, reported that prevalence at the individual level was 9.53%. Alshekh et al. (2024) have studied in Al Jufrah District in Libya and reported that prevalence at the individual level was 1.90% (6/320). Kimera et al. (2025) reported that prevalence at the individual level in Northern Tanzania was 5.31% (11/207). In addition, Martindah et al. (2025) reported that prevalence at the individual and herd levels in Jabodetabek, Indonesia were 5.07% (18/355) and 3.57% (9/252), respectively. However, from this study and previous studies shown that the range of prevalence at the individual level were 0.28 to 11.30 and at the herd level were 1.27 to 18.39.

In our study, we found that the factor of introduction of new goats into herd was significantly associated with the risk of brucellosis (P≤0.05). Similarly, the study by Hussen et al. (2023), who surveyed in Somali regional state, eastern Ethiopia revealed that 13 times increased chance of having seropositive reactors for *Brucella* infection from introduced new animals into the herds (OR = 13.091; 95% CI: 1.241–138.11, P = 0.032). Likewise, the study in Ningxiang, China by Li et al. (2021) reported that introduction was the factor with strong association with disease present (OR= 42.05; 95% CI= 13.29-187.67; P<0.01). On the other hand, Leahy et al. (2020), who studied in eastern India revealed that introduction was not significantly associated with any seroprevalence risk (P=0.658). Rerkyusuke et al. (2024) stated that introduced a new animal in herds within 6 months had not associated with seroprevalence risk (P=0.475). The difference between the results of above studies is because in the occurrence of pathology, some infected animals in the herd may develop self-limiting infection or become asymptomatic latent carriers and may spread the pathogen (OIE, 2022). However, the result of present study is in agreement with findings of several authors who found that introduction of new animals from herds with unknown brucellosis status or non-free brucellosis was a main factor associated with brucellosis in sheep, goat and cattle herds (Musallam et al., 2015; Cardenas et al., 2019).

The factors of shared rams with other farm and history of abortion in this study had no significantly associated with seroprevalence risk (P>0.05). Likewise, the survey by Rerkyusuke et al. (2024) reported that buck circulation between herds was not statistically significant (P=0.286). On the other hand, Musallam et al. (2015) stated that lending or borrowing a ram for reproduction was significantly associated with seropositive status

(OR= 8.9; 95% CI= 3.0-26.1; P<0.01). Similar findings were reported by Thuamsuwan et al. (2023) (OR= 0.1; 95% CI= 0.02-1.1; P=0.05), who studied in Sing Buri Province, Thailand; and Hussen et al. (2023) (OR= 14.7; 95% CI= 1.384-156.179; P=0.026). For the history of abortion, the study by Teshome et al. (2022) reported that herds with history of reproductive problem reports in Southern Oromia, Ethiopia had an approximately 5 times significantly higher chance of seropositive reactor than did not experience group (OR= 5.32; 95% CI= 3.14-9.00; P=0.000). Likewise, the study in Niger by Boukary et al. (2013) reported that approximately 4.5 times increased chance of containing seropositive reactors for herds with abortion than herds that did not experience abortion group. This was statistically significant (OR= 4.5; 95% CI= 2.23-8.95; P<0.001).

This study has some limitations because this study did not use ELISA or complement fixation test to confirm the results. Thus, interpretation of the results should be done with caution.

5. Conclusion

In conclusion, the results indicated that goats those raised in Muang District, Nakhon Phanom Province are risk of brucellosis. Therefore, farmers should be educated on control and prevention of brucellosis to reduce risk of its infection to human and goats.

Acknowledgement

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Ocular disease of captive pinnipeds in the Zoological Park Organization of Thailand

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Background: A high incidence of ocular diseases in captive pinniped species has raised concern regarding their health and welfare. This study investigates the occurrence of ophthalmic diseases and enclosure parameters in captive pinnipeds housed across four zoos managed by the Zoological Park Organization of Thailand.

Methods: A retrospective analysis was conducted using archival records and interviews. The study population consisted of South American fur seals (*Arctocephalus australis*) and South African fur seals (*Arctocephalus pusillus*). Analyzed variables included: (1) age at first ophthalmic diagnosis; (2) activity status; and (3) enclosure characteristics. Descriptive statistics and Fisher's exact test were performed using Microsoft Excel.

Results: Of the 18 fur seals assessed, 11 individuals (7 *A. australis*, 4 *A. pusillus*) had a history of ocular disease, other 7 individuals were reported normal. Five individuals were diagnosed with multiple ocular conditions. The distribution of ocular diseases included: cataracts (n = 6, 54.5%), corneal injuries with lens luxation (n = 2, 18.2%), uveitis (n = 1, 9.1%), nuclear sclerosis (n = 1, 9.1%), and glaucoma (n = 1, 9.1%). The average age at first diagnosis was 11.0 ± 3.82 years. Fisher's exact test showed a statistically significant association between ocular disease and age group ($p = 0.04$). No significant associations were found with species, sex, origin, or enclosure parameters, including pool area, pool depth, pool volume, and land area.

Conclusion: Age group is significantly associated with the occurrence of ocular disease in captive fur seals in Thailand. To enhance animal welfare, routine health examinations for adult pinnipeds should include comprehensive ophthalmic assessments by an ophthalmologist as part of the surveillance protocol.

Keywords: Ocular disease, captive, pinniped

L-Asparaginase Monotherapy as a Salvage Treatment for Chemotherapy-Resistant Extragenital Transmissible Venereal Tumor (TVT) in a Mixed-Breed Dog: A Case Report

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Abstract

Background: Transmissible venereal tumor (TVT) is a naturally occurring, contagious round-cell neoplasm of dogs that typically responds to vincristine chemotherapy. However, extragenital or disseminated forms may exhibit reduced sensitivity, resulting in treatment failure in some cases. This report describes a case of vincristine-resistant extragenital TVT that responded favorably to L-asparaginase monotherapy.

Case Description: A 6-year-old spayed female mixed-breed dog presented with marked buphthalmos and ulcerative, hemorrhagic lesions of the right eye, accompanied by multiple cutaneous nodules. Mild anemia was detected, while serum biochemistry remained within reference limits. Due to unrelenting ocular pain and irreversible vision loss, the right eye was surgically enucleated. Histopathology and cytology confirmed TVT involving ocular and cutaneous tissues. Vincristine chemotherapy (0.6 mg/m² intravenously, once weekly) was initiated with a treatment plan of 6–8 doses. Despite receiving three doses, the patient showed no clinical response. Instead, the lesions progressed and became increasingly ulcerated, suggesting chemoresistance. A salvage protocol with L-asparaginase (5,000 IU/m² intramuscularly every 14 days) was implemented. Clinical improvement, including lesion regression and ulcer resolution, was observed after the second dose. Treatment continued for four total doses. By the end of the course, all visible lesions had resolved. The patient remained in complete remission with no recurrence during a three-month follow-up.

Conclusion: This case demonstrates that four doses of L-asparaginase successfully induced complete remission in a dog with vincristine-resistant extragenital TVT. The findings support its potential as a rational and effective salvage option in refractory presentations.

Keywords: L-asparaginase, chemotherapy resistance, canine transmissible venereal tumor

Acute Stabilization and Management of MMVD Stage C with Pulmonary Hypertension and Tick-Borne Co-infection in a Geriatric Dog

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Abstract

Case Description: A 13-year-old intact male mixed-breed Poodle presented with depression, icterus, abdominal distension, and dyspnea. Physical examination revealed a Grade 5/6 pansystolic murmur, tachycardia, tachypnea, and hind limb proprioceptive deficits. Blood analysis showed anemia, thrombocytopenia, hypoalbuminemia, and *Ehrlichia canis* infection. Imaging confirmed right-sided pleural, pericardial, and abdominal effusions, hepatomegaly, pulmonary edema, LA enlargement, and ascites. Serosanguineous fluid was obtained via thoracocentesis and abdominocentesis.

Initial stabilization involved oxygen, effusions drainage, and diuretics. Diagnostics confirmed MMVD Stage C with PH. EKG revealed normal sinus rhythm (MEA 87). Echocardiography showed mitral/tricuspid regurgitation, LA/LV enlargement, and pulmonary artery distension with pulmonic regurgitation (LA:Ao = 2.89, TR pressure gradient = 71.55 mmHg, PA:Ao = 1.89).

In-hospital treatment included oxygen cage, IV furosemide (1 mg/kg q3-4h), and pimobendan (0.25 mg/kg q12h), with continuous monitoring. After one night, the patient's condition significantly improved, dyspnea resolved, and imaging showed resolved pulmonary edema/effusions.

Conclusion: Discharge was initiated due to marked improvement, decreased RRR, and stress from kennel noise. The patient received sildenafil, ramipril, pimobendane, doxycycline, liver and blood supplement for home care, with owner RRR monitoring and a one-week follow-up. This case emphasizes prompt stabilization and multimodal management for complex cardiac-infectious multimorbidity in geriatric dogs.

Keywords: Anaplasmosis, Ascites, Echocardiography, Ehrlichiosis, Furosemide, Myxomatous Mitral Valve Degeneration, Pimobendan, Pleural Effusion, Pulmonary Hypertension, Tick-borne Disease

Chronic Pancreatitis Presenting with Pancreatic Calcification in a Male Cat: A Case Report

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Abstract

Background: Feline pancreatitis is an inflammatory disease of the pancreas that may occur in either acute or chronic form. Clinical signs are often vague and nonspecific, including lethargy, anorexia, vomiting, and weight loss. Diagnosis typically involves elevated feline pancreatic lipase immunoreactivity (fPLI) and abdominal ultrasonography.

Case Description: An 8-year-old neutered male domestic shorthair cat was referred to the Veterinary Teaching Hospital, Khon Kaen University, for chronic vomiting persisting over six months, with a suspected gastrointestinal foreign body. Despite dietary modification to a hypoallergenic formula and a maintained appetite, the cat continued to experience occasional vomiting. Blood samples were collected for hematological and biochemical analysis. fPLI was markedly elevated at 16.9 µg/L (reference <3.5 µg/L), consistent with feline pancreatitis. Serum creatinine, blood urea nitrogen (BUN), and total calcium were within normal limits. Parathyroid hormone (PTH) measurement was not performed. Abdominal ultrasonography revealed hyperechoic calcification at the body and right limb of the pancreas, anechoic bile, a thin gallbladder wall, and a normal appearance of the intrahepatic ducts. Multiple hyperechoic nodules were noted throughout the liver lobes. Liver function parameters were within normal limits, suggesting age-related hepatic changes. No gastrointestinal foreign body was detected.

Conclusion: Pancreatic calcification in this case suggests a chronic process, such as previous pancreatitis or dystrophic mineralization. However, as ionized calcium and PTH levels were not assessed, a metabolic cause—such as hypercalcemia of unknown origin or primary hyperparathyroidism—cannot be definitively excluded. Management is nonspecific and requires a multifactorial approach, including dietary modification to a highly digestible or hydrolyzed protein formula, symptomatic therapy, and regular monitoring of fPLI.

Keywords: Chronic pancreatitis, Feline, Pancreatic calcification

Comparison of Primary Chicken Embryo Liver and Primary Chicken Embryo Fibroblast Cells for the Efficient Isolation of Fowl Adenovirus Serotype 2, 8 and 11

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Abstract

Background: Continuous outbreaks of inclusion body hepatitis (IBH) have caused significant economic losses to the poultry industry worldwide. Fowl adenovirus (FAdV) serotype 2, 8 and 11 are considered the major serotypes currently circulating in Asia. This study aims to compare the efficiency of two primary cell culture systems in supporting the isolation of FAdV from clinical samples.

Methods: In this study, primary chicken embryo liver (CEL) and primary chicken embryo fibroblast (CEF) cells were evaluated and compared for their ability to support the isolation and propagation of FAdV serotype 2, 8b and 11. Tissue suspensions from 20 confirmed FAdV clinical samples, comprising FAdV serotype 2 (n=11), 8b (n=7) and 11 (n=2), were inoculated into CEL and CEF cells using an absorption method. Typical cytopathic effect (CPE) was observed between 2 and 4 days post inoculation.

Results: Our results demonstrated that FAdV serotype 2, 8b and 11 were successfully isolated from all known FAdV positive clinical samples using primary CEL cells in the first passage and yielded high virus titer in the second passage (average $10^{7.9}$ $TCID_{50}$/ml). In contrast, primary CEF cells supported only the isolation of FAdV serotype 2 in the first passage and produced lower virus titer in the second passage (average $10^{2.45}$ $TCID_{50}$/ml). The presence of each FAdV serotype was confirmed by conventional PCR technique using hexon gene specific primers, followed by DNA sequencing.

Conclusion: In conclusion, our findings supported the use of primary CEL cells as an efficient host system for isolation and propagation of FAdV serotype 2, 8 and 11 from chicken clinical samples.

Keywords: cell cultures, fowl adenovirus, virus isolation, virus propagation

Effects of Crocodile (*Crocodylus Siamensis*) Liver Extracts on Detoxification Enzyme Activities in the Rat Liver and Kidney

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Background: *Crocodylus Siamensis*, a freshwater crocodile native to Southeast Asia region, possesses a unique liver enzyme profile with high detoxification capacity. Previous studies reported significantly higher activities of glutathione S-transferase (GST) and cytochrome P450 (CYP450) enzymes in crocodile liver compared to livestock species. Liver extracts also exhibit antioxidant and anti-apoptotic effects in hepatocellular carcinoma cells.

Objective: To investigate the effects of oral *C. Siamensis* liver extracts on detoxification enzyme activities and kinetics in rat liver and kidney.

Methods: Male Wistar rats (n= 5) were orally administered liver extracts ranged from 50 to 800 mg/kg once daily for seven days. Activities of CYP1A2, CYP2E1, and GST were assessed in liver and kidney tissues. Kinetic parameters (K_m and V_{max}) were calculated using Michaelis-Menten models.

Results: Enzyme activities were modulated in a dose-dependent and tissue-specific manner. CYP1A2 activity significantly increased in both organs, with hepatic V_{max} rising from 0.017 to 0.13 µmol/min/mg protein. CYP2E1 showed moderate hepatic induction, while renal CYP2E1 declined at higher doses. Hepatic GST catalytic velocity increased at 200–400 mg/kg without total activity changes, indicating an increased substrate turnover rate and enhanced catalytic efficiency. In contrast, renal GST velocity increased sharply only at 800 mg/kg, indicating a dose-dependent activation threshold in the kidney. Changes in K_m and V_{max} values among different enzymes suggest that both the efficiency of the enzymes and their ability to bind to substrates were affected.

Conclusion: *C. Siamensis* liver extract enhances detoxification enzyme functions, particularly via CYP and GST modulation, suggesting its potential in regulating xenobiotic metabolism in mammals especially in the rat.

Keywords: *Crocodylus Siamensis*, cytochrome P450, glutathione S-transferase, detoxification, enzyme kinetics, xenobiotic metabolism, aflatoxin B1

Maxillary Osteonecrosis Secondary to Brachycephalic Malocclusion in a Juvenile Pug: Clinical Management and Fungal Isolation Without Disease Progression

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Abstract

Background: Brachycephalic malocclusion alters occlusal biomechanics and predisposes young dogs to early-onset periodontal disease, localized ischemia, and alveolar bone necrosis. This report describes the diagnosis and conservative management of localized osteonecrosis in a juvenile pug, including fungal isolation without antifungal therapy.

Case Description: A 1-year-old intact male pug presented with a three-month history of halitosis and missing maxillary premolars (106–107). Oral examination revealed severe periodontal attachment loss with exposed bone. Intraoral radiographs demonstrated horizontal and vertical bone loss affecting teeth 104–109. Computed tomography confirmed focal osteolysis of the right maxillary body, without sinus, nasal, or oronasal communication. During surgery, sequestrated bone fragments encapsulated by granulation tissue were removed via gentle debridement. Platelet-rich fibrin (PRF) was applied to promote healing, followed by mucogingival flap closure. Postoperative care included amoxicillin–clavulanate (20 mg/kg PO q12h for 14 days) and robenacoxib (2 mg/kg PO q24h for 4 days). Fungal culture was obtained from a necrotic maxillary tooth, with isolation of *Aspergillus niger*. The infection was presumed to be associated with brachycephalic-related structural malformation. Systemic antifungal therapy was not administered, as complete excision of compromised tissue resulted in primary wound healing. At the three-week follow-up, mucosa had healed fully, with no signs of reactivation.

Conclusion: This case demonstrates that brachycephalic malocclusion can lead to focal osteonecrosis even in juvenile dogs. Conservative debridement with regenerative support may suffice for local disease control despite fungal colonization. However, continued clinical monitoring is recommended to assess long-term treatment success.

Keywords: brachycephalic malocclusion, maxillary osteonecrosis, platelet-rich fibrin

A Portable LAMP Assay for Rapid Molecular Detection of *Leishmania* in Resource-Limited Settings

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Abstract

Background: Leishmaniasis is a neglected tropical disease in resource-limited regions. Objectives: to develop and validate a loop-mediated isothermal amplification (LAMP) assay targeting the 18S rRNA gene for rapid, sensitive, and specific detection of *Leishmania* spp.

Methods: Reactions contained four LAMP primers, *Bst* DNA polymerase, dNTPs with GelGreen and hydroxynaphthol blue as the indicators in a 25 µL volume. Analytical sensitivity was determined using ten-fold dilutions of purified *Leishmania donovani* and *L. major* DNA (10^{2} ng to 10^{-4} ng per reaction) and parasite suspensions of two *Leishmania* species (10^{4} to 10^{0} parasites per reaction) at 63 °C for 60 min, with real-time fluorescence and end-point visualization. Specificity was assessed against DNA from non-*Leishmania* protozoa (*Trypanosoma cruzi*, *Plasmodium yoelii*, *P. berghei*, and *Toxoplasma gondii*) and no-template controls.

Results: Robust amplification was achieved down to 10^{-4} ng of both *Leishmania* DNA per reaction, with the highest concentrations detectable within 20–30 min and the lowest dilutions at 60 min. The sensitivity was verified using PCR, which showed a similar result. Remarkably, the assay detected the *L. donovani* and *L. major* parasite suspensions at a concentration of ten and a single parasite per tube, respectively, by 60 minutes. No amplification occurred with any non-*Leishmania* templates or controls, while all *Leishmania* species yielded clear positive signals

Conclusion: These findings confirm that the conventional LAMP assay sensitively and accurately detected both *Leishmania* DNA and direct parasites, with simple visualization, as well as real-time monitoring, supporting its potential as a rapid diagnostic tool in endemic, resource-constrained settings.

Keywords: isothermal amplification; LAMP; Leishmania; sensitivity; specificity.

The efficacy of in straw dilution method on survival rates of vitrified bovine embryos.

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Abstract

Background: The goal of this study was to refine thawing protocols for vitrified, in vitro-derived bovine embryos using the in-straw dilution technique by assessing the postoperative survival and development.

Methods: The bovine oocytes were obtained from slaughterhouse ovaries, and the resulting embryos were cryopreserved at the expanded blastocyst stage with Cryotop vitrification method. The warming solution's sucrose concentration (0.2, 0.3, and 0.4 M) and thawing duration were tested for their effects. Post-thaw, embryos were cultured in CR1aa medium with 5% fetal bovine serum for 24 and 48 hours.

Results: Among embryos thawed with 0.2 M sucrose, survival rates were high for both Grade 1 (95.9%) and Grade 2 (73%) at 24 hours and remained substantial at 48 hours. These outcomes were comparable to the fresh control group and exceeded those of embryos thawed using higher sucrose concentrations or conventional protocols. The optimized protocol involved warming the straw in the first warming solution for 5 minutes. Then, mixing with the second column for 20 seconds. Following this, sealing and inverting the straw for 3 minutes and 20 seconds. Finally, keeping it horizontal on a warm plate for 1 minute and 40 seconds.

Conclusion: The in-straw dilution method with 0.2 M sucrose provides an effective, practical approach for thawing vitrified bovine embryos, supporting high post-thaw survival rates and offering a simple protocol suitable for field application.

Keywords: Bovine, in vitro embryo production, vitrification, in straw dilution

Innovation And Model for Creating Rabies-Free Zones in Kantharawichai Communities

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Background: Rabies poses a significant threat to both animal and human health. Kantharawichai District, Mahasarakham Province, has a growing pet population due to increasing residential density. In 2022, three canines in two sub-districts tested positive for rabies. Consequently, sub-district municipalities recognize the potential public health implications for both animal and human populations. This project sought to develop and implement a mobile-based model and aimed to demonstrate its potential as a public health strategy aligned with the One Health concept.

Methods: A mobile application, termed PAL, has been developed to enable pet owners to register their animals, access news updates, facilitate communication for mutual understanding, receive vaccination schedule notifications, and monitor rabies outbreaks in animal and human populations. Pet population data from the government's existing Rabies One Data system has been imported into the PAL application database, and the application has been subsequently introduced to pet owners in every sub-district, since February 2025. A training course has been provided to enable pet owners to utilize the application to add vaccination data when their animals receive services from government agencies and animal hospitals affiliated with the Faculty of Veterinary Medicine, Mahasarakham university. The project's success was evaluated based on the number of pet owners who consistently used the application, with a minimum threshold of 1,000 users. Consequently, all users brought their pets to receive services as scheduled, resulting in widespread vaccination coverage and the absence of rabies-infected animals in the area.

Results: The PAL application database recorded data for 13,093 dogs and 3,480 cats, of which 564 were unowned and 16,029 were owned. A total of 5,432 pet owners were able to utilize the application to add vaccination data when they received vaccination notifications. The availability of data on each sub-district's animal populations facilitated officials to accurately estimate the number of vaccines required and calculate the procurement budget. In fiscal year 2026, a budget for vaccine procurement was allocated for 16,637 doses to ensure comprehensive vaccination coverage. Throughout the operation, no rabies animals were found in the area. Currently, this application can be used to register animals, provide animal history information to the veterinary team, report symptoms, track animal treatment results, and search for nearby animal hospitals, dog shelters and dogs for adoption. It can also be used to track lost dogs.

Conclusion: The project has been a great success and aims to expand the application to other areas of the country to expand the customer base and connect with other animal hospitals, adding more service formats such as donations, purchasing products, tracking statistics, news, announcements to find animals, announcements to adopt, and tracking animals with active microchips

Keywords: Rabies, Mobile application, Innovation, Disease prevention

Immunosuppressive Therapy for the Management of Fistula Tract in a Cat: A Case Report

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Abstract

Background: Antimicrobial-resistant bacteria pose a significant challenge in managing chronic wound infections, particularly in veterinary medicine. This case report details the diagnosis and treatment of a 4-year-old neutered male Domestic Shorthair (DSH) cat with a chronic abdominal fistula. The infection involved multidrug-resistant Enterococcus faecalis and Aspergillus spp. Our objective is to highlight strategies for complicated infections unresponsive to conventional antimicrobial therapy, where infection control is achieved but clinical lesion improvement remains limited.

Case Description: The DSH cat presented with a chronic abdominal wound and fistula following a bite, with over a month of unsuccessful antibiotic treatment. Diagnostics included bacterial and fungal cultures, antimicrobial susceptibility testing, CT imaging, histopathology with PAS and GMS stains, and cytology. Treatment involved systemic antibiotics, antifungals, an immunosuppressive drug, and advanced wound care.

Despite aggressive antimicrobial and antifungal therapy, the wound showed only temporary improvement and recurrent inflammation. The lesion harbored both Enterococcus faecalis and Aspergillus spp.; however, negative fungal confirmation by PAS and GMS staining suggested the Aspergillus spp. finding from fungal culture was likely due to environmental contamination. Histopathological examination revealed suppurative granulomatous inflammation, and fine needle aspiration showed moderate numbers of mixed small and large lymphocytes and some lymphoglandular bodies.

Bacterial growth was no longer detected in the latest MIC result. Due to persistent inflammation and generalized lymphadenopathy, immunosuppressive therapy with cyclosporine (7 mg/kg/day) was initiated. This led to over 80% lesion reduction and resolution of lymphadenopathy, with no fistula recurrence observed during 4 months of treatment.

Conclusion: In chronic, non-healing wounds exhibiting granulomatous inflammation and negative infectious staining, immune dysregulation should be considered. Immunomodulatory therapy is a valuable adjunct when standard antimicrobial treatments fail.

Keywords: Feline, Chronic wound, Immunosuppressants, Drainage tract

ABOUT VM-KKU


คณะสัตวแพทยศาสตร์
มหาวิทยาลัยขอนแก่น
"คณะสัตวแพทยศาสตร์ระดับเอเชีย
และศูนย์กลางบริการสุขภาพสัตว์เพื่อสังคม"
พันธกิจ
1.จัดการศึกษา มุ่งผลิตบัณฑิตให้มีความรู้ความสามารถตามมาตรฐานวิชาชีพ
2.วิจัยที่ผลิตองค์ความรู้และนวัตกรรม เพื่อแก้ไขปัญหาสุขภาพสัตว์
3.บริการวิชาการเพื่อแก้ไขปัญหาด้านสุขภาพสัตว์ให้กับชุมชนสังคม
4.บริการสุขภาพสัตว์ภายใต้มาตรฐานคุณภาพ
5.บริการหน่วยธุรกิจสัตวแพทย์เพื่อสนองความต้องการของลูกค้าด้านการดูแลและผลิตภัณฑ์สุขภาพสัตว์
ค่านิยม
อุทิศเพื่อสังคม (Social Devotion)
การบริหารจัดการด้วยข้อมูลจริง (Achievement by Fact)
มุ่งความสำเร็จด้วยการทำงานเป็นทีม (Achievement by Teamwork)
มุ่งเน้นลูกค้า (Responsibility for Customer)
มุ่งสร้างเทคโนโลยีและนวัตกรรม (Technology and innovation)
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น ตำบลในเมือง
อำเภอเมือง จังหวัดขอนแก่น รหัสไปรษณีย์ 40002
โทรศัพท์/โทรสาร 043-202150 และ มือถือ 081-6621170
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Facebook : Veterinary Medicine KKU, Official

คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น ก่อตั้งเมื่อวันที่ 13 พฤษภาคม พ.ศ. 2529 เป็นคณะลำดับที่ 13 ของมหาวิทยาลัยขอนแก่น และเป็นคณะสัตวแพทยศาสตร์แห่งที่ 3 ของประเทศไทย ปัจจุบันสอนในระดับปริญญาตรี และระดับบัณฑิตศึกษา มีสิ่งสนับสนุนการเรียนการสอน การวิจัย และบริการวิชาการที่ ทันสมัยโดยเฉพาะอย่างยิ่งมีโรงพยาบาลสัตว์เพื่อการเรียนการสอน ที่ใหญ่ที่สุดในภาคตะวันออกเฉียงเหนือ คณะได้รับการรับรองจากสัตยแพทสภา ผู้สำเร็จการศึกษามีสิทธิ์สอบรับใบอนุญาตประกอบวิชาชีพได้ตามกฎหมาย

วัฒนธรรมองค์กร

ปรับตัวได้ในทุกสถานการณ์และรับผิดชอบในหน้าที่

สมรรถนะหลัก

1.การจัดการศึกษาที่ผลิตบัณฑิตสัตวแพทย์ที่มีความชำนาญด้าน บริการสุขภาพหนึ่งเดียวโดยบูรณาการร่วม ทั้งคนและสัตว์
2.การบริการสุขภาพสัตว์ชั้นสูง ที่มีความเชี่ยวชาญเฉพาะสาขา สัตว์เลี้ยงและปศุสัตว์

การรับนักศึกษา

รับนักศึกษาไทยและต่างชาติที่สามารถใช้ภาษาไทยได้เป็นอย่างดี ที่สำเร็จการศึกษาไม่ต่ำกว่ามัธยมศึกษาตอนปลายสามัญ หรือสำเร็จการศึกษาระดับปริญญาตรี จำนวน 80 คนต่อปี โดยแบ่งการรับในแต่ละรอบ ดังนี้

รอบที่ 1 (Portfolio) แบ่งเป็นกลุ่มต่างๆ ตามคุณสมบัติ เช่น กลุ่มเรียนดีมีคุณธรรม จริยธรรม และ บำเพ็ญประโยชน์ เรียนดี มีคุณธรรม กลุ่มบุตรเกษตรกรผู้เลี้ยงสัตว์ กลุ่มบุตรผู้ประกอบการวิชาชีพการสัตวแพทย์ กลุ่มบุตรผู้ประกอบการที่เกี่ยวข้องกับวิชาชีพการสัตวแพทย์ กลุ่มบุตรผู้ให้การสนับสนุนด้านการเรียนการสอน กลุ่มผู้สำเร็จการศึกษาปริญญาตรี เป็นต้น
รอบที่ 2 (Quota) รอบโควต้าภาคตะวันออกเฉียงเหนือ
รอบที่ 3 (Admission) รับตรงร่วมกัน ผ่านระบบ mช และ ผ่านกลุ่มสถาบันแพทย์ศาสตร์แห่งประเทศไทย(กสพท.)

หลักสูตรและจัดการศึกษา

หลักสูตรสัตวแพทยศาสตรบัณฑิต (สพ.บ.) เป็นหลักสูตร 6 ปี เปิดรับนักศึกษาทั้งไทยและชาวต่างชาติที่สามารถใช้ภาษาไทยได้เป็นอย่างดี ปัจจุบันเป็นหลักสูตรปรับปรุง พ.ศ.2563 ได้รับการรับรองปริญญาจากสัตวแพทยสภา 7 ปี (2563-2569) มีการจัดการศึกษาในระบบทวิภาค จำนวนหน่วยกิต ตลอดหลักสูตร 244 หน่วยกิต มีการจัดการเรียนการสอน ดังนี้

•

กลุ่มวิชากายวิภาคศาสตร์	15	หน่วยกิต
กลุ่มวิชาสรีรวิทยา	15	หน่วยกิต
กลุ่มวิชาเภสัชวิทยาและพิษวิทยา	10	หน่วยกิต
กลุ่มวิชาพยาธิชีววิทยา	27	หน่วยกิต
กลุ่มวิชาอายุรศาสตร์	25	หน่วยกิต
กลุ่มวิชาศัลยศาสตร์	14	หน่วยกิต
กลุ่มวิชาวิทยาการสืบพันธุ์	9	หน่วยกิต
และกลุ่มวิชาสัตวแพทย์สาธารณสุข ผ	18	หน่วยกิต

วิชาสัมมนาและการทำวิจัยทางสัตวแพทย์ เป็นการฝึกทักษะในการเขียนข้อเสนอโครงการ การทำงานวิจัย การเขียน และเผยแพร่ผลงานทางวิชาการ 6 หน่วยกิต วิชาคลินิกเวียน (สัตว์เลี้ยง สัตว์เคี้ยวเอื้อง ม้า สุกร สัตว์ปีก สัตว์น้ำ สัตว์ป่า และชันสูตรโรคสัตว์) 34 หน่วยกิต เพื่อฝึกทักษะทางด้านตรวจวินิจฉัยและรักษาโรค วิชาเตรียมสหกิจศึกษาและสหกิจศึกษาทางสัตวแพทย์ 10 หน่วยกิต

เพื่อฝึกทักษะในการทำงานด้านสัตวแพทย์ในสายงานที่นักศึกษามีความสนใจทั้งในและต่างประเทศนอกจากนั้น ยังมีการสนับสนุนการฝึกงานนอกหลักสูตรในช่วงปิดภาคการศึกษา เพื่อเพิ่มทักษะและประสบการทางวิชาชีพที่นักศึกษาสนใจ

ค่าใช้จ่ายตลอดหลักสูตร

ค่าธรรมเนียมการศึกษา แบบเหมาจ่ายภาคการศึกษาละ 35,000 บาท

ทุนการศึกษา

นักศึกษาที่เข้าศึกษาในหลักสูตรสัตวแพทยศาสตรบัณฑิต มีโอกาสได้รับการพิจารณาให้รับทุนการศึกษาในทุกปีๆ ประมาณปีละ 50 ทุน เช่นทุนสำหรับนักศึกษาที่เรียนดี หรือทำกิจกรรมเด่น

- ทุนเรียนดีแต่ขาดแคลนทุนทรัพย์
- ทุนแลกเปลี่ยนระหว่างประเทศเพื่อไปฝึกอบรมดูงานในต่างประเทศ เช่น เนเธอร์แลนด์ ญี่ปุ่น มาเลเซีย และจีน เป็นต้น
- ทุนทำงานวิจัยในรายวิชาวิจัยทางสัตวแพทย์
- ทุนไปฝึกงานในต่างประเทศในรายวิชาสหกิจศึกษา

แนวทางการประกอบอาชีพ

เมื่อสำเร็จการศึกษาแล้วบัณฑิตสัตวแพทย์สามารถประกอบอาชีพได้อย่างหลากหลาย ดังนี้

1.รับราชการในกระทรวง กรม กองต่างๆของรัฐ เช่น กระทรวงเกษตรและสหกรณ์ (กรมปศุสัตว์ และอื่นๆ) กระทรวงสาธารณสุข กระทรวงมหาดไทย หรือกระทรวงกลาโหม เป็นต้น
2.เป็นอาจารย์/นักวิจัย/เจ้าหน้าที่ในมหาวิทยาลัยของรัฐ และเอกชน
3.ทำงานในองค์กรปกครองส่วนท้องถิ่น เช่น เทศบาล เป็นต้น
4.ทำงานในรัฐวิสาหกิจ เช่น อสค. องค์การสวนสัตว์ ฯลฯ
5.ทำงานในบริษัทเอกชน เช่น นักวิชาการในบริษัทจำหน่ายเวชภัณฑ์สัตว์ อาหารสัตว์ ฯลฯ
6.ประกอบอาชีพส่วนตัว เช่น คลินิกรักษาสัตว์ โรงพยาบาลสัตว์ หรือฟาร์มเลี้ยงสัตว์ ฯลฯ
นอกจากนี้ยังสามารถศึกษาต่อในระดับปริญญาโท และปริญญาเอกของมหาวิทยาลัยต่างๆ ทั้งในประเทศและต่างประเทศ รวมทั้งหลักสูตรระดับปริญญาโท และปริญญาเอกของคณะเองด้วย

MISSIONS

- The faculty organizes education to produce graduates with professional knowledge and abilities, conducts research for knowledge and innovation in solving animal health problems, and provides academic services to address societal animal health issues.
- Additionally, we offer quality animal health services and veterinary business unit services to meet customer needs in animal care and health products.

VALUES WITH SMART

- S = Social Devotion
- M = Management by Fact
- A = Achievement by Teamwork
- R = Responsibility for Customer
- T = Technology and Innovation

VISION

Asian Class of Veterinary School and Animal Health Service Center for Society

MAIN GOALS

- Ready-to-work graduates
- Research for One Health: Strengthen the research for One Health
- Animal Health Hub: Become an animal health center
- Cultural Conservation: Preserve local wisdom and culture.
- Happy Work Place and Enhanced Quality: Be organization with happiness and increasing quality

CONTACT US

Faculty of Veterinary Medicine,
Khon Kaen University,

123 Moo 16 Nai Muang Subdistrict,
Muang District, Khon Kaen 40002.

Telephone/Fax: 043-202150

Website: http:/vet.kku.ac.th

Faculty of Veterinary Medicine, Khon Kaen University
Faculty of Veterinary Medicine
Khon Kaen University

History

- Established in 1986, the Faculty of Veterinary Medicine at Khon Kaen University is the third veterinary faculty in Thailand.
- Currently, we offer comprehensive equipment and tools for research and teaching, including a Veterinary Teaching Hospital that serves as a learning resource for both undergraduate and graduate students.

Graduate programs

1. Doctor of Philosophy (International Program): Ph.D.

• Expertise in each subject group

1) Veterinary Science
2) Veterinary Public Health
3) One Health

• Course format and number of credits

1) Type 1.1 (research only students; graduated from M.Sc. degree) at least 48 credits
2) Type 1.2 (research only; students graduated from bachelor degree) at least 72 credits
3) Type 2.1 (research and coursework; students graduated from M.Sc. degree) at least 48 credits
4) Type 2.2 (research and coursework; students graduated from bachelor degree) at least 72 credits

• Language

English

2. Master of Science (International Program): M.Sc.

• Expertise in each subject group

1) Veterinary Science
2) Veterinary Public Health
3) One Health

• Course format and number of credits

1) Type A1 (research only) at least 36 credits
2) Type A2 (research and coursework) at least 36 credits

• Language

English

Scholarships

The scholarships from the Faculty of Veterinary Medicine are allocated to students every year, including:

1. Scholarship from the Graduate Production and Academic Development Fund, Faculty of Veterinary Medicine
- Free tuition fees, Free international student fees (In the case of international students) and free accommodation on the 6th floor of Pichet Building (in case of necessity)

2. Research grant for graduate students in the amount of 150,000 baht/project.

3. Graduate Teaching Assistant (TA) Scholarship

4. Scholarship for the Economic Cooperation Development Project in the Greater Mekong Subregion (GMS) with Khon Kaen University.
- Free tuition fees, Free international student fees (in the case of international students),
- Free monthly expenses for international students (in the case of international students) and free the 6th floor accommodation (In the case of international students and the cooperative organizations with MOU and Thai students in the case of necessity)

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• โรงแรมฝากเลี้ยงสุนัขและแมว

1. บริการฝากสัตว์เลี้ยงจำพวกสุนัขและแมว ในกรณีที่เจ้าของไม่อยู่บ้าน ติดธุระ หรือฝากดูแลหลังทำหมัน โดยโรงแรมตั้งอยู่ในบริเวณที่แยกสัดส่วนออกจากหน่วยสัตว์ป่วยใน และมีเจ้าหน้าที่แยกส่วนดูแล
2. บริการสระว่ายน้ำสำหรับสุนัข
3. ติดต่อได้โดยตรงที่ Fanpage facebook: โรงแรมสัตว์เลี้ยง มหาวิทยาลัยขอนแก่น

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เครือข่ายสถานพยาบาลสัตว์ในเขตภาคตะวันออกเฉียงเหนือ
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Veterinary Diagnostic Laboratory,
Faculty of Veterinary Medicine , Khon Kaen University

ห้องปฏิบัติการชันสูตรโรคทางปศุสัตว์
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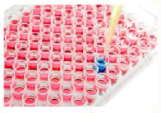
วันจันทร์-ศุกร์ ตั้งแต่เวลา 9.00น.-16.00น.
ด้วยเทคนิคทาง
ซีรัมวิทยา
ไวรัสและอณูชีววิทยา
แบคทีเรียวิทยา
ปรสิตวิทยา
ผ่าชันสูตรซาก
จุลพยาธิวิทยา



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คณะสัตวแพทยศาสตร์มหาวิทยาลัยขอนแก่น

Disease	Sample	Method	Price
โรคอหิวาต์แอฟริกาในสุกร (African Swine Fever - ASF)	EDTA blood ,Meat	Realtime -PCR	800
โรค PCV-2 (Porcine circo virus -2)	EDTA, Serum	PCR	800
โรค อหิวาต์สุกร (Classical Swine Fever -CSF)	EDTA, Serum	RT-PCR	1000
โรค PRRS	EDTA, Serum	RT-PCR	1000

รายการตรวจโรคสัตว์ปีก

Disease	Sample	method	price
Newcastle disease virus (NDV)	serum	HI test	30
	serum	ELISA	55
	Tracheal/Cloacal swab, Organs	RT-PCR	1000
Infectious bronchitis virus (IBV)	serum	ELISA	55
Infectious bursal disease virus (IBDV)	serum	ELISA	55
	Organ: Bursa of Fabricious	RT-PCR	1000
Inclusion body hepatitis (IBH)	liver	PCR	800

การตรวจทางห้องปฏิบัติการ
โรคติดต่อในโคกระบือและแพะแกะ
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

Disease	Sample	Method	Price
โรค Bovine Viral Diarhrea (BVD)	น้ำเชื้อ, เลือด, รก	Real-time RT-PCR	1000 ฿
โรคเลปโตสไปโรซิส (Leptospira spp.)	น้ำเชื้อ, เลือด, ปัสสาวะ	Real-time PCR	800/1200 ฿
โรคข้ออักเสบและสมองอักเสบในแพะ (Caprine arthritis encephalitis ;CAE)	ซีรัม	ELISA	220 ฿
โรคแท้งติดต่อ (Brucellosis)	ซีรัม	Rose Bengal Test	50 ฿

การตรวจทางห้องปฏิบัติการ
เพื่อเคลื่อนย้ายม้าแข่ง
คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

Disease	Sample	Method	Price
โรคภาวโรคแอฟริกาในม้า (African Horse Sickness; AHS)	เลือด (EDTA blood) 1-2 ml	Real-time RT - PCR	1000 ฿
โรคพยาธิในเลือด (Trypanosomiasis; Serra)	เลือด (EDTA blood) 1-2 ml	Thin film , Woo's & Buffy coat smear	60/90 ฿
โรคโลหิตจางในม้า (Equine infectious anemia; EIA)	ซีรัม (Clotted blood) 2-4 ml	AGID	300/350 ฿

Dog and Cat PCR

Disease	Sample	Method	Price
Leptospirosis	Blood 1 cc AND Urine 5-10 cc	Real-time PCR	1200
Feline herpes virus	Conjunctival swab OR Oral swab	Real-time PCR	800
Feline infectious peritonitis virus	Pleural OR Peritoneal fluid 3 cc	Conventional PCR	800
Pythiosis	Serum 1 cc or Biopsy sample	ICT (Ab test) / Realtime PCR	100 / 800

การตรวจทางห้องปฏิบัติการ
โรคติดต่อในจิ้งหรีด
คณะสัตวแพทยศาสตร์มหาวิทยาลัยขอนแก่น

Disease	Sample	Method	Price
โรคติดเชื้ออิริโดไวรัส (Iridovirus)	จิ้งหรีด ถาดไข่ อาหาร	PCR	800
โรคไวรัสอัมพาตจิ้งหรีด (Cricket Paralysis Virus)	จิ้งหรีด ถาดไข่ อาหาร	RT-PCR	1000

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Faculty of Veterinary Medicine


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Muang District
Khon Kaen, 40002