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Proceedings of

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CUVC 2018 : Research in Practice

April 25-27, 2018
IMPACT Forum Building IMPACT Exhibition and Convention Center, Bangkok, Thailand

Organized by

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Message from Professor Dr. Roongroje Thanawongnuwech  
Dean Faculty of Veterinary Science,  
Chulalongkorn University

Dear Colleagues,

On behalf of the Faculty of Veterinary Science, Chulalongkorn University and the organizing committee, it is my great pleasure to welcome you again to the 17th Chulalongkorn University Veterinary Conference (CUVC): Education serves passion held on April 25-27, 2018 at the Impact Forum Building Impact Exhibition and Convention Center, Thailand. This conference has become an annual veterinary event since 2014 for both local and regional vet practitioners to share the current veterinary trend and knowledge and to discuss scientific matters together. CUVC not only provides the opportunity for the Thai Vets but also for the regional vets to present and share their new findings or novel approaches in the veterinary field based on the CU Vet vision “Research in Practice” addressing the integration of research into field practice. In addition, CUVC will benefit ASEAN vets for its quality continuing education on various current topics related to the questions from our vet society. An expectation from public and consumers on our vet profession is much greater than in the past. Indeed, we all do want to improve the quality of life to all living creatures as best as we could (CU Vet: Responsible for Lives).

Last but not least, certificate of attendance will be prepared for all participants upon requested and the organizing committee would like to thank all supporting sponsors and all speakers and participants for their endless support. Without your contribution, this conference would not be possible. The organizing committee looks forward to welcoming you all and will do its best to make your participation a successful and pleasant event.

Warmly regards,

Professor Dr. Roongroje Thanawongnuwech, DVM, PhD, Dip., TBVP  
Dean of Faculty of Veterinary Science, Chulalongkorn University  
Advisor of CUVC2018
Message from Instructor Dr. Chaiyot Tanrattana
Chairperson, Organizing Committee

Dear Delegates,

We'd like to personally welcome each of you to upcoming 2018 international conference. Faculty of Veterinary Science, Chulalongkorn University will host an annual international conference “The 17th Chulalongkorn University Veterinary Conference, CUVC2018: Education serves passion”. The exciting theme of the conference reflects our pride in being a leading veterinary school in the past, present, and for sustainable future with very strong collaboration to all international institutes, universities and organizations. The conference will bring together the most knowledgeable veterinarians from various fields to disseminate advance knowledge in their fields and also allow young scientists to present their recent research findings. We offer very relevant scientific and societal issues that ask for an answer from the veterinary community. This 3-day conference will be held on April 25-27, 2018 at Impact Forum Building Impact Exhibition and Convention Center, Thailand.

The program consists of the keynote and invited world-class speakers, symposiums, oral and poster sessions, and social events. Our focus will be on all important research topics in swine production, poultry industry, ruminants, aquatic animals and companion and exotic animal practice including infectious diseases, emerging and re-emerging exotic diseases, production management, animal welfare, wildlife conservation, feed and nutrition, antimicrobial resistance, food safety and one health. Furthermore, we attempt to incorporate many a legendary scholars and outstanding researchers presenting their research works. This conference is established in order to be one of the valuable academic resources for veterinary practitioners, researchers, and students.

CUVC2018 consolidates a series of conferences, whose last edition, held in 2017 attracted more than 1,200 participants. We expect this year’s edition to be as successful as the previous one in terms of quality, relevance and attendance.

We look forward to seeing you in Bangkok. For more information, please contact us at info.cuvc@gmail.com or visit our website at http://www.cuvcthailand.com at your convenience.

Sincerely yours,

Dr. Chaiyot Tanrattana, DVM, MSc, MPhil
CUVC2018, Chair
Message from Assistant Professor Dr. Sirilak Surachetpong  
Chairperson, Scientific Committee

Dear Colleagues,

Welcome to the 17th Chulalongkorn University Veterinary Conference 2018 (CUVC 2018): Education serves passion, an annual conference that is organized by the Faculty of Veterinary Sciences Chulalongkorn University. This year the conference will be held at Impact Forum, Impact Exhibition and Convention Center, Thailand from April 25-27, 2018.

The scientific program consists of lectures from invited world class speakers, panel discussions, and oral and poster presentations. The topics will cover current important issues in veterinary medicine including swine and poultry industry, ruminant practice, wildlife, aquatic animals, laboratory animal science, food safety, public health, antibiotic resistance, and companion animals.

On behalf of conference organizing committee, I would like to thank all sponsors, speakers, and participants for their support. I look forward to seeing you in CUVC 2018.

Sincerely yours,

Assistant Professor Dr. Sirilak Surachetpong DVM, MS, PhD  
CUVC2018, Chairperson, Scientific Committee
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### Contents

| Message from Professor Dr. Roongroje Thanawongnuwech | v  |
| Message from Instructor Dr. Chaiyot Tanrattana      | vii |
| Message from Assistant Professor Dr. Siriluk Surachetpong | ix |
| Organizing Committee of CUVC 2018                   | xi |
| Acknowledgements                                   | xv |

### Summary of Speakers

#### Aquatic Animals

- **Advances in Amino Acid Nutrition for Sustainable Farming of *Litopenaeus Vannamei***
  - D. Sangsue, G. Channarayapatna, K. Masagounder
  - S1

#### Companion Animals

- **Cardiology**
  - The effective protocol for treatment acute cardiogenic pulmonary edema in dogs
  - S.D. Surachetpong
  - S5
  - How to treat life threatening cardiac arrhythmia in dog and cat
  - Siram Savarnavibhaja
  - S7
  - Important Clinical Skills for Helping Dogs and Cats with Effusion
  - Tanawan Mangklabruks
  - S9
  - Update in CPR Technique for Successful Life Rescue
  - S. Luckanahasaporn
  - S13

- **Dermatology**
  - Case scenarios of allergic dermatitis approach
  - C. Tanrattana
  - S19
  - Real case approach for dermatophytosis
  - Sivavatchr Panitarangnit, Chaiyot Tanrattana
  - S21

#### Feline Medicine

- **Blood Transfusion in Cats: from A to Z**
  - S. Wadeerat
  - S23
  - Feline Obstruction: Diagnosis and treatment
  - V. Hunprasit
  - S25
  - How to differentiate between cats with respiratory and cardiac problems
  - T. Atiptamvarree
  - S27
  - Jaundice cats: what is your rule out and treatment
  - S. Ritthikulprasert
  - S29
  - Management for Feline Diabetes
  - Pinit Pusoonthornthum
  - S31
What to do with pruritic cats
C. Tanrattana

Imaging
Classification of the intervertebral disc herniations: why are they different? S35
Suwicha Chuthatep

Diagnostic Imaging of the Skull
R. Penchome

Multimodality imaging of vascular disease in dogs and cats
Nardtiwa Chaivoravitsakul

Orthopedics
Experience with the IMEX-SK™ Linear-Circular External Skeleton Fixator Hybrid Constructs and Metal Acrylic for fracture Stabilization in Cats S41
C. Phonsuwan

Long Bone Fracture in Feline: How to Fix it? S45
B. Sutherat

Pelvic Fracture of Feline Fracture
Monchanok Vijarnsorn

Principle of Feline fracture
K. Soontornvipart

Reproduction
Emergency management and surgical considerations for urethral obstruction in tom cats S53
T. Tharasanit, S. Techarungchaikul, T. Pakdeesaneha, T. Thongsima

Surgery
Skin mass, what should be done by surgical management? S57
Worapan Tadadoltip

Treatment and management updates of the oral mass S61
Varisara Tisyangkul

Upper urinary tract obstruction: updates on surgical managements S65
Sukanya Manee-in

Ways out of GI foreign bodies S67
Nilubol Booncharoensombat

Exotic Pets
“Head tilt rabbit” What should I do? S71
Thosaporn Anuntakulnatee

How to approach rabbit gastro-intestinal syndrome S73
Chaowaphan Yinharnmingmongkol

Look at exotic eyes; how different from domestic spp. S75
T. Anusaksathien

Rabbit skull problem; It’s more important than you know S79
N. Suriyakhun
**Food Safety**

Food Risk Analysis: A New Paradigm for Food Safety  
*Suphachai Nuanualsuwan*  
S81

Randomized sampling for AMR monitoring  
*S. Jeamsripong*  
S83

Techniques for isolation and identification of *Campylobacter*  
*T. Luangtongkum*  
S85

**Lab Animal Sciences**

Comparative Medicine and Pathology in Translational Research, Overview of an academic program in the US  
*Cory Brayton*  
S87

Experimental Design and Research Reporting of Translational Research; Addressing the ‘Reproducibility Crisis’  
*Cory Brayton*  
S89

Microbial impacts on contemporary translational preclinical research in mice and rats.  
*Cory Brayton*  
S91

The World’s 1st One-Touch Preclinical Imaging Platform With High-Frequency Ultrasound For Laboratory Animals  
*Shripad Bangale*  
S93

**Poultry**

Genetic characterization of infectious bronchitis viruses isolated from chickens in Thailand, 2016  
*S. Munyahongse, T. Pohuang, J. Sasipreeyajan, A. Thontiravong*  
S95

**Swine**

Analysis of Porcine Immune Responses to Assess Vaccine Performance  
*William T. Golde*  
S97

Dynamic of PED and Control Strategy, Japanese Experience  
*H. Ishikawa*  
S99

Evolution of PRRS in Japan  
*Satoshi Otake*  
S101

The History of Livestock Vaccine Development  
*William T. Golde*  
S103

Gut Health and Immunity: Concept and Implications  
*Sanipa Suradhat*  
S105

Major emerging and re-emerging swine viral diseases in Asia  
*R. Thanawongnuwech*  
S107

New Techniques in Livestock Vaccine Development  
*William T. Golde*  
S109

PRRSV DNA vaccine Lessons learned from vaccine R&D in the developing country  
*Sanipa Suradhat, Wasin Charerntantanakul, Roongroje Thanawongnuwech*  
S111
Oral Presentation

Comparative study of efficacy between live culture and microencapsulation of *Lactobacillus plantarum* 22F on growth performance in experimental piglets.

P. Pupa, Prasert Apiwatsiri, Wandee Sirichokchatchawan, N. Pirarat, N. Muangsin, Tanawong Maisorn, Anantawat Koontanatechanon, N. Prapasarakul

Comparison between diagnosis of animal disease with and without using mobile diagnostic application- POAS

A. Sridetch, N. Pitijareonphong, S. Nobpakul, S. Khawcharak, R. Lohajaroensub, V. Wijitratanagorn, P. Pusoonthornthum, S. Suvarnavibhaja, O. Cheunsuang

Correlation of macroscopic grading of stifle osteoarthritis and clinical parameters with medial patellar luxation in dogs

S. Jahrupatrakorn, W. Banlunara, T. Nedumpun, C. Wangdee

Diagnostic evaluation of the specific-IgG1 to house dust mites (HDM) allergen in atopic dogs by an in-house ELISA

N. Khantavee, C. Chanthick, N. Saelim, A. Tungtrongchitr, N. Sookrung, S. Suradhat, N. Prapasarakul

Effects of the supplementation of encapsulated probiotics on the intestinal morphology and intestinal immune status of Nile tilapia.

Trullàs, C., Pinpimai, K., Pirarat, N.

Immunopathogenesis of Chicken Infectious Anemia Virus Isolated in Taiwan

S. Tongkamsai, H.H. Chung, M.S. Lee, Y.L. Tsai, Y.Y. Lien

A novel recombinant *Circovirus* strain detecting in Thai dogs: vidence for potentially possible evolution role of *Circoviridae* family

C. Piewbang, S. Kesdangsakonwut, A. Rungrassipat, S. Techangamsuwan

Saliva Peptide Fingerprint Analysis of Canine Oral Squamous Cell Carcinoma by MALDI-TOF Mass Spectrometry

S. Ploypetch, S. Roytrakul, J. Jaresitthikunchai, G. Suriyaphol

Tilapia Lake Virus: update and recent research progress

W. Surachetpong, P. Liamnimit, W. Thammatorn, O. Lawhavinit

Unreveling the neglected *Strongyloides* spp. infection in cats in Bangkok, Thailand

W. Jitsamai, T. Powjinda, P. Pornlamfah, P. Harncharoenkul, L. Pluemhathaikij, P. Acharsakwatta, W. Sukhumavasi

Zeta potential of bovine X and Y sperm and its application for sperm sorting

T. Wongtawan, N. Dararatana, B. Oonkhanond, S. Kornmatissuk

Using Mobile Diagnostic App: POAS to Support Veterinary Learning and Diagnostic Competencies

P. Sammamate, A. Lerdngammongkol, N. Sriathongkul, P. Chawkitkul, R. Lohajaroensub, V. Wijitratanagorn, P. Pusoonthornthum, S. Suvarnavibhaja, O. Cheunsuang

Abstracts : Poster Presentation

Assessment of Bacterial Accumulation and Environmental Factors in Sentinel Oysters and Estuarine Water Quality from the Phang Nga Estuary Area in Thailand

S. Jeamsripong, Edward R. Aiwill

Blue light-emitting diode can inhibit scuticociliate (*Mamiensis avidus*) in olive flounder, *Paralichthys olivaceus*

H. Roh, D. Kim
Detection of myxozoans parasite *Henneguya* spp. in gills and mucus of cultured giant gourami (*Ospronemus goramy*)

*P. Liamnimitr, W. Thammatorn, P. Jaemwimon, W. Surachetpong*

Evidence of Anisakiasis in a Stranded Striped Dolphin (*Stenella coeruleoalba*) in Thai Territorial Water

*K. Jongnimitpaiboon, P. Kaewmong, C. Chiakwathanyu, P. Taweethavonsawat, K. Chankow, S. Kesdangsakonwat*

Immersion vaccination of an inactivated whole-cell vaccine coated with chitosan against *Flavobacterium columnare* challenge in red tilapias (*Oreochromis sp.*)

*S. Kitiyodom, T. Yata, C. Rodkhun, N. Pirarat*

Nano Spray TRISWHEAT (Teripang Super Wound Healing Agent) Healing Wound Diabetes Mellitus which is Infected by MRSA (Methicillin Resistant *Staphylococcus aureus*) Bacteria with Sea Cucumber (*Stichopus sp.*) Extract

*N. Hanifah, Y. F. Achmad, M. Permatasari, M. Karniati, D. T. Syafira, S. I. O. Salasia*

Occurrence of antimicrobial resistance in *Vibrio parahaemolyticus* isolated from cultivated oysters and estuarine waters

*W. Khant, M. Kuldee, S. Jeamsripong*

Prebiotic effects of Jerusalem artichoke supplemented diet on growth performance and intestinal morphology in juvenilered tilapias (*Oreochromis sp.*)

*M. Sewaka, C. Trullis, C. Rodkhun, N. Chansue, S. Boonanuntanasarn, N. Pirarat*

A case report: Hepatic cholangiocellular carcinoma with liver fluke in an anorexic cat

*P. Nomaya, A. Sawangwong, K. Chankow, K. Teankum, P. Jongwattanapisan*

Cervical subcutaneous dermoid cyst in domestic cat

*T. Sananmuang, P. Jeeratanyasakul, K. Mankong, K. Chokeshai-usaha*

Confirmation of feline immunodeficiency virus (FIV) infection by provirus PCR

*N. Techakriengkrai, P. Suksamai, N. Wachiratadao*

Conserving domestic cat follicular structure by alginate-fibrin gel in vitro

*A. Chansaenroj, N. Songsasen, K. Chatdarong*

Development of indirect ELISA for detection of feline morbillivirus

*S. Chaiyasak, A. Rungsipipat, J. Ratthanophart, S. Techangamsuwan*

Electropherogram Analysis of TCRG Gene Rearrangement of Feline Lymphoma

*Jedsada Siripoonsub, Somporn Techangamsuwan, Anudep Rungsipipat*

Immunohistochemical Localization of Kisspeptin Receptor in the Cat Ovary on Different Ovarian Stages

*P. Tanyapanyachon, O. Amelkina, K. Chatdarong*

Molecular Detection of Feline Calicivirus Infection in Thailand

*K. Phonggroop, J. Rattanasrisomporn, A. Rungsipipat, S. Techangamsuwan*

Anesthetic Management of Modified Extracapsular Thyroidectomy in Canine Hypothyroidism with Cushing Syndrome: A Case Report

*S. Pattanasittiseree, P. Phoomvithisarn, K. Komin, K. Parvongnukul, S. Durongpongtorn*

Application of The Novel and Rapid Technique: LAMP-LFD *E. canis* DNA Sensor Kit

*P. Jirapiti, M. Sarikapuit, K. Bunroddit, K. Chansiri, N. Viseshakul*

Branching patterns of subclavian arteries in dogs

*D. Durawiroj, R. Chavangyongsanuggool, S. Saingkaew, T. Wongtawan*
Canine pseudomembranous cystitis due to persistent urinary tract infection
T. Sananmuang, P. Jeeratanyasakul, K. Mankong, K. Chokeshai-usaha

A case report: Opened Wound at Nasal Bridge due to Tooth Root Abscess in a Bang Kaew
P. Supannawapat

Detection on the Embryonic transcription factors Oct-4, Nanog, and Sox-2 Proteins in Canine Cutaneous Mast Cell Tumors by Immunocytofluorescence technique
S. Meesuwan, K. Rattanapinyopituk, P. Piyaviriyakul, A. Sailasuta

Effect of dexmedetomidine on isoflurane minimum alveolar concentration in dogs
K. Vinyunantakul, S. Durongphongtorn, M. Kalpravidh

Effect of Recombinant Feline Interferon-ω on Primary Culture of Canine Transmissible Venereal Tumor
C. Setthawongsin, J. Chayapong, S. Techangamsuwan, A. Rungsipipat

Factors influencing anti-Müllerian hormone (AMH) levels in dogs: the effects of body sizes, age and reproductive cycles
T. Thongsima, S. Techarchangchaikul, T. Pakdeesaneha, J. Suwimonteerabutr, T. Tharasanit

Fatigue study of cortical stainless-steel screws with PMMA and SOP plate system for immobilization of vertebral fracture and luxation in canine cadavers
M. Mekavichai, K. Soontornvipart

The first report of the Canine trachea extragenital transmissible venereal tumor
P. Kusolphat, K. Dejyong

Genomic characterization and comparative analysis of Leptospira interrogans isolated from the urine of asymptomatic dogs, Thailand
A. Kurilung, C. Keeratipusana, P. Suriyaphol, N. Prapasarakul

Innovation of Veterinary Anatomical Models by Using Waste Paper
P. Chuesiri, K. Sajjacharoenpong, P. Paisiriuyenyoung, J. Intarapunya, P. Tienthai

Investigation of UVB-Radiation Induced on Canine Keratinocytes Cell Line
N. Assawawongkasem, S. Techangamsuwan, P. Piyaviriyakul, A. Sailasuta

In vitro effects of tetracycline and enrofloxacin on microfilaria motility of Dirofilaria immitis
P. Piromkij, S. Sungrapid, P. Taweethavonsawat

A Novel Targeted Therapy in Canine B-cell Lymphoma
S. Sirivisoot, S. Techangamsuwan, A. Rungsipipat

Occurrence of canine respiratory coronavirus in Thailand
K. Charoenkul, N. Bunpapong, S. Chaiwong, S. Boonyapisitsopa, T. Janetanakit, A. Amosin

Post-intubated tracheal stenosis diagnosed by ultrasonography
K. Chuaychoo

A preliminary report; using bipolar vascular sealant in a canine elongated soft palate resection
K. Channeam, K. Dejyong, K. Dechubol

Prevalence of anticoagulant rodenticide poisoning in dogs and cats submitted to the Faculty of Veterinary Science, Chulalongkorn University between 2008 and 2017
P. Charoenlerk, K. Patthanachai, P. Chantisiripornchai
Remission of secondary disorders of brachycephalic airway obstruction syndrome following primary disorder correction in dogs
S. Thunyodom, P. Brikshavana, C. Kalpravidh, W. Banlunara, M. Kalpravidh

The Successful Treatment of Canine Hepatozoonosis Using a Simple Drug-of-Choice, Sulfa-Trimethoprim
M. Sarikaputi, P. Jirapiti, N. Viseshakul

Surgical Removal of Metastatic Carcinoma in Sublumbar Lymph Nodes: A Case Report
S. Prapaiwit, K. Komin, K. Parvongnakul, N. Choisunirachon, P. Phoomvuthisarn

Theophylline treatment in a Dog with Syncope due to Sinus arrest
P. Parindawong

Upregulation of TGF-β1 and PDGF-B gene expression in periodontitis dogs treated with autologous blood-derived platelet-rich fibrin
C. Kornsuthisopon, N. Pirarat, C. Kalpravidh

The effect of serum concentration on fibrotic gene expression in cultured rat cardiac fibroblasts
W. Korchunjit, O. Wongtawan, T. Wongtawan

Effects of diet containing Thai herbs on reproductive organs sperm production and serum testosterone level in male Wistar rats
W. Sudhirai, R. Jintana, S. Sophon, K. Angkanaporn

Pathogenesis and distribution of duck Tembusu virus in BALB/c mice
N. Yurayart, W. Tumtak, P. Ninvilai, S. Tiawsirisup

Potency of Tomato Paste (Lycopersicon esculentum) Against Histopathological Liver of Mice (Mus musculus) Exposed to Borax
P. A. Wahyuningtyas, V. M. Praharjaya, P. S. Wibowo

Quantitative study of Bat contact in Thailand
K. Suwannarong, K. Suwannarong, P. Kanthaweew, S. Khiewkhern, K. Haraticharnchaul, N. Bunpapong, A. Amornsin

Up-regulation of TGF-β1 and PDGF-A gene expression by bubaline fibrin glue in ligation-induced periodontitis rats
P. Banyatworakul, C. Kalpravidh, C. Rodkhum, P. Brikshavana, N. Pirarat

Campylobacter detection method for samples from broiler farms in Thailand
N. Phetsri, T. Luangtongkum

Genetic Characterization of Newly Emerged Duck Tembusu Virus isolated from Domestic Ducks in Thailand, 2017
P. Ninvilai, K. Oraveerakul, A. Amornsin, A. Thontiravong

Survey of Influenza A viruses in a Live Bird Market in Bangkok, 2016-2017
S. Boonyapisitsopa, S. Chaiyawong, T. Janethanakit, N. Ruangpet, P. Moolthep, A. Amornsin

Accuracy of Pregnancy Diagnosis in Goats Using PAG ELISA Test
S. Thongruay, K. Srisakwattana

Application of Microsatellite Markers for Pedigree Determination in Thai Swamp Buffalo
S. Treembonmuang, W. Nualchuen, K. Srisakwattana, S. Usawang

Assessment of boar sperm motility characteristics by Sperm Class Analyzer (SCA®) diluted in new semen extender “Power-Pit” compared to a commercial Optim-I.A
J. Siwimonterebahut, M. Techakumphu
Boar sperm viability, acrosome integrity, JC-1 and sHOST after dilution in Mid-term (Optim-I.A) and Power-pitboar semen extender

J. Suwimonteerabutr, M. Techakumphu

Bovine Endometrial Cells Secrete Interleukin-8 in Response to Chitosan Oligosaccharide

S. Chotimanukul, T. Swangchan-Uthai, J. Suwimonteerabutr, M. Techakumphu

The effect of melatonin on cardiac differentiation of rabbit induced pluripotent stem cells (iPSCs) under different oxygen tensions

P. Phakdeedindan, P. Setthawon, M. Techakumphu, T. Tharasanit

Effect of Zona Pellucida on Survivability of Bisected Bovine Embryos: A Preliminary Study

K. Tasripoo, T. Tharasanit, K. Srisakwattana

Effects of Mulberry Leaves Supplementation on Goat Performance

W. Nuachuen, W. Suthikrai, K. Tasripoo, K. Srisakwattana, S. Chethasing, R. Jintana and S. Usawang

Effects of supplementation of green tea waste on productive performances In crossbred Saanen lactating goats

R. Sundod, S. Chanpongsang, C. Nuengjiamong

Exploration of the geographic information and proportion of dairy cattle from using the farm management application program

M. Intrakamhaeng

Growth-inhibitory effect of pyroliigneous acid on Bacillus cereus

P. Lazarte Sangunkaew, S. Siwanna, W. Boonri, N. Puttan, K. Nakbuchpa

Key Factors Associated with Sustainability of Small Scale Beef Farms in Thailand

T. Swangchan-Uthai, C. Inchaisri, T. Panbamrungkij, S. Phan-iam

Laparoscopic artificial insemination in goat in smallholder farms, using PMSG/hCG for synchronization program


Ovarian responses on Boer goats with Split-single or Conventional multiple FSH treatment


Rain tree pod (Samanea saman), the miracle feed for ruminants

Thongsuk Jetana, Sirima Thongruay, Kitiya Srisakwattana

Targeted gene functional enrichment analysis of the milk miRNome acquired from dairy cow and nursing mother during 2-6 months of lactation

K. Chokeshai-u-saha and T. Sananmuang

Use of Plasma Progesterone Levels for Monitoring Ovarian Activity in Recipients Beef Cows for Cloned Gaur Embryo Transfer and Early Pregnancy Diagnosis

W. Suthikrai, K. Tasripoo, R. Jintana, K. Srisakwattana, S. Sophon, N. Ketprathum

Administration of non-steroidal anti-inflammatory drugs prior to artificial insemination increases litter size in gilts and sows

P. Pearodwong, N. Jiebna, P. Tummaruk

Association between meconium staining of the skin and incidence of stillborn piglets

J. Udomchanya, A. Suwannutsiri, K. Sriphanta, P. Prachayakul, P. Juthamanee, M. Nuntapaitoon, P. Tummaruk

Concentrations of total immunoglobulin G in colostrum of sows

M. Nuntapaitoon, P. Juthamanee, J. Suwimonteerabutr, P. Tummaruk
Concerning chitooligosacchride levels and term applications as dietary additive in Weaned pigs’ weight gain and small intestinal morphology
B. Thongsong, S. Suthongsa, S. Kalandakanond-Thongsong, R. Pichyangkura

Correlation and agreement between serum and plasma progesterone concentrations in pigs as determine by an enzyme-linked immunosorbsent assay

The development of enzyme-linked immunosorbsent assay for determining plasma progesterone concentration in pre-pubertal and pregnant pigs

Dual Infection between a Thai Isolate HP-PRRSV and the Pandemic H1N1 SIV in Pigs
N. Sangpratum, R. Kedkovid, Y. Woonwong, J. Arunorat, C. Sirisereewan, S. Kesdangsakonwut, R. Thanawongnuwech

Effect of backfat loss during late gestation and lactation on milk yield in sows
S. Thongkhuay, SH. Chuaychut, P. Burarnreak, P. Ruangjoy, P. Juthamanee, M. Nuntapaitoon, P. Tummaruk

Effect of partial supplementation of Everwell™ in nursery pigs performance

Effect of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 and 2 on the Viability of Porcine Endometrial Epithelial Cell Culture
D. Rukarcheep, S. Wattanaphansak, C. Deachapunya, M. Lothong, S. Poonyachoti

Efficacy and safety of Suiseng® in prevention of neonatal diarrhea according to enterotoxigenic E. coli under a mixed infection with PRRSV involved from the field
R. Kitchodok, C. Ananratanakul, T. Kongthong

Evaluation of pig performance after vaccination of weaned pigs with PCV2 vaccine in Southeast Asia pig farms
M. Makhanon

Evaluation the use of oral fluid PRRSV antibody ELISA under the field condition
Y. Panyasing, A. Kittawornrat

Factors associated with colostrum consumption of neonatal piglets
P. Juthamanee, M. Nuntapaitoon, P. Tummaruk

Factors influencing litter size in a modern Landrace x Yorkshire hyper-prolific sows in a swine commercial herd in Thailand
P. Tummaruk, M. Nuntapaitoon

Immunoassay precision of S27 PEDV IgG/IgA ELISA kit for antibody detection against porcine epidemic diarrhea virus in colostrum and milk
A. Srijangwad, P. Jermsutjarit, A. Tantituvanont, J. Luckanakul, D. Nilubol

Molecular characterization, etiology, and, phylogenetic analysis of complete genome sequences of Porcine Deltacoronavirus

Novel Thai unique insertion of Porcine epidemic diarrhea spike gene from the outbreak during 2014
C.J. Stott, K. Sawattrakool, G. Temeeyasen, T. Tripipat, D. Nilubol

Ovulation time in weaned sows did not differ between Landrace and Yorkshire breeds but differ between primiparous and multiparous sows
P. Pearodwong, R. Panyathong, P. Tummaruk
<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEDV monitoring after the PEDV outbreak using oral fluid, fecal and surface swab samples</td>
<td>189</td>
</tr>
<tr>
<td>B. Jantrasakul, J. Arunorat, N. Sangpratun, R. Kedkovid, R. Thanawongnuwech</td>
<td></td>
</tr>
<tr>
<td>Phenotypic and genotypic characteristics of ESBL-producing and colistin resistance in <em>Salmonella enterica</em> and <em>Escherichia coli</em> isolated from pigs in the border provinces between Thailand and Cambodia, Lao PDR and Myanmar</td>
<td>191</td>
</tr>
<tr>
<td>Kyaw Phye Sunn, Sunpetch Angkititrakul, Rungtip Chuanhuen</td>
<td></td>
</tr>
<tr>
<td>Piglet Brooder System in Thailand: Problem Identification and Conceptual Solution Development</td>
<td>193</td>
</tr>
<tr>
<td>T. Panya-ad, P. Anuntavoranich</td>
<td></td>
</tr>
<tr>
<td>Prevalence of <em>Streptococcus suis</em> isolated from pigs in Northern Thailand</td>
<td>199</td>
</tr>
<tr>
<td>W. Mala, S. Angkititrakool, R. Chuanhuen</td>
<td></td>
</tr>
<tr>
<td>Protective Efficacy of Modified-live Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Vaccines Against PRRSV challenge in experimental pigs</td>
<td>201</td>
</tr>
<tr>
<td>A. Madapong, K. Saeng-chuto, A. Boonsoongnern S. Boonyawatana, R. Jolie, Dachrit Nilubol</td>
<td></td>
</tr>
<tr>
<td>Reproductive performance of sows after single fixed-time artificial insemination in a commercial swine herd in Thailand</td>
<td>203</td>
</tr>
<tr>
<td>Reproductive performance of Berkshire sows in Thailand</td>
<td>205</td>
</tr>
<tr>
<td>P. Tummaruk, C. Tretipskul</td>
<td></td>
</tr>
<tr>
<td>Reprogramming porcine somatic cells into transgene-free induced pluripotent stem cells using episomal plasmid vectors</td>
<td>207</td>
</tr>
<tr>
<td>Piyathip Setthawong, Yoko Sato, Mongkol Techakumphu, Theerawat Tharasanit</td>
<td></td>
</tr>
<tr>
<td>Quantitative Microbial Risk Assessment of <em>Klebsiella pneumoniae</em> to Ciprofloxacin from Pork in Bangkok</td>
<td>209</td>
</tr>
<tr>
<td>R. Namkratok, S. Nuamulsuwann</td>
<td></td>
</tr>
<tr>
<td>Quantitative risk assessment of <em>Staphylococcus aureus</em> and methicillin-resistant <em>Staphylococcus aureus</em> from pork in Bangkok</td>
<td>213</td>
</tr>
<tr>
<td>P. Sirotamarat, S. Nuamulsuwann</td>
<td></td>
</tr>
<tr>
<td>T. Janetanakit, K. Charoenkul, N. Bunpapong, S. Chaityawong, S. Boonyawatitsopa, S. Kesdaengsakonwut, P. Assavacheep, S. Wattanaphansak, A. Amonsin</td>
<td></td>
</tr>
<tr>
<td>Synergistic Energy Providing by Lecithin and L-carnitine Supplemented to Pigs in Lactating Period</td>
<td>217</td>
</tr>
<tr>
<td>P. Sanlom, P. Nakbunbh</td>
<td></td>
</tr>
<tr>
<td>Variations of Morphology and Immune Cell Infiltration in the Endometrium of Culling Gilts with Ovarian Cysts</td>
<td>219</td>
</tr>
<tr>
<td>P. Tienthai, P. Tummaruk</td>
<td></td>
</tr>
<tr>
<td>CDC25A Expression on the Immunolocalization of Asian Elephant (Elephas maximus) Testis</td>
<td>223</td>
</tr>
<tr>
<td>A. Sudsukh, Y. Sato, T. Tharasanit</td>
<td></td>
</tr>
<tr>
<td>Histomorphology of reproductive organs and placenta in wild gaur (Bos gaurus)</td>
<td>225</td>
</tr>
<tr>
<td>K. Srisuwatanasagul, W. Mabut, S. Srisuwatanasagul</td>
<td></td>
</tr>
<tr>
<td>Metastatic Squamous Cell Carcinoma in a Richardson’s Ground Squirrel (Spermophilus richardsonii): A Case Report</td>
<td>227</td>
</tr>
<tr>
<td>S. Bhanpattanakul, C. Yinharmingmongkol, P. Punyathi, K. Chankow, S. Kesdaengsakonwut</td>
<td></td>
</tr>
</tbody>
</table>

xxvi
Plastinated specimen from wildlife: a new approach for anatomical study in giraffe reproductive organs
K. Srisuwatanasagul, W. Mahut, S. Srisuwatanasagul

Protective Effect of Hydroxyxanthone on the Leakiness of Intestinal Epithelia Induced by TNF-α
W. Chayalak, C. Deachapunya, S. Suksamrarn, S. Poonyachoti

The effects of Hydroxyethyl starch (HES) on quality of equine sperm following cryopreservation: a preliminary study
O. Nuchanart, D. Srinutiakorn, T. Tharasanit

Effect of lactic acid bacteria strains on reduction of plasmid mediated colistin resistant gene (mcr-1) conjugation in Escherichia coli
P. Apiwatsiri, P. Pupa, K. Lugsomya, J. Yindee, W. Niyomtham, N. Prapasarakul

Authors Index
Efficiency, ecology and food quality & safety constitute three main dimensions of innovation of future sustainability of food value chain. For animal production in particular, efficiency driven by population growth and affordable protein, comprises least cost feed formulation, feed/protein optimization, nutrient utilization and efficient ingredients. Ecology driven by ecological footprint and low emission, comprises modern nutrition concept, improved livestock management and low protein diets. While food quality & safety driven by end-consumer needs and food ethics, comprises animal welfare, gut health solution concepts, safe and healthy food chain and, performance and nutritional additives.

Potential roles of aquaculture in shaping the future
Aquaculture has played and will continue to play an important role in meeting the protein need of growing population. Aquaculture offers a huge potential for growth compared to other livestock industries like poultry and swine. Despite that fact that there is a vast variation among aquatic species/environment and highly diverse industry structure, this challenge comes with the unique opportunities in which innovation is identified as a common key driver. Moreover, exchange of information, best practice sharing, adapting new concepts and technologies are also the essential drivers for sustainable aquaculture production.

Challenges and goals for global aqua feed industry
Aquaculture is a significant protein source for humans and its growth rate is not going to decrease anytime soon. In fact, it should be enhanced by a growing population, increased affluence within the population, and increased per capita consumption. Additionally, governments are beginning to recognize the health benefits of consuming a diet containing aquaculture, which will support even more growth. Not surprisingly, with such rapid growth also comes growing pains. The aquaculture industry in particular is dealing with sustainability questions, both from the environmental and productivity standpoints. One example of these growing pains is the use of marine proteins. These proteins are a significant source of protein nutrients (i.e. amino acids) in many commercial aqua feeds as that protein or more precisely amino acids are building blocks of human and animal bodies, and play vital tasks such as digesting food, contracting muscles, protecting against pathogens and assisting many bodily functions. Animal protein contains the full range of essential amino acids needed for a healthy diet. Fishmeal known to aquatic animal nutritionists that it does not only provide a balanced amount of essential amino acids but also phospholipids, fatty acids and attractants. Unfortunately, there is greater demand for fishmeal for use in aqua and livestock feeds than there is supply. As a result, the cost of fishmeal has increased substantially over the past decades.

Feeding high levels of fishmeal is not environmentally sustainable either as these feeds typically contain excess nitrogen. This excess nitrogen is a result of over-formulation or dietary deficiencies of protein and amino acids, and since inside animal body there is no system to allow for storage of amino acids per se, they must be de-aminated and the nitrogen excreted. This excess nitrogen is as costly to aqua producers as it potentially detrimental to the ecosystem. The excess nitrogen and/or deficiency of essential amino acids is partly due to limited information about these aquatic species nutrient requirements for growth. However, this issue is also driven by lack of understanding about the raw materials being used in these feeds, and truly knowing their nutrient content. Moreover, nowadays there is a need to shift from protein to amino acid based feed formulation because of several advantages such as allowing more flexibility in raw materials and feed formulation in such a way that generates cost savings while optimizes animal performance, reduced safety margin, less ammonia waste and consistent performance. Feed cost savings can be managed by the use of alternative protein sources such as the fishmeal replacement concept. Raw material evaluation and management assists in more flexibility of formulating aqua diets as well as minimization of safety margin. Sustainability and pollution control can be achieved through adopting the practice of degrading nitrogen and other wastes.

In shrimp aquaculture in Southeast Asia (SEA), feeds for vannamei shrimp may contain crude protein levels from 32% to 38%. From farmers’ perspective, one of the major concerns is whether to feed shrimps with high or low protein. Some farmers choose to feed high quality and protein diets with an expectation of faster growth, albeit costly and probably uneconomical. On the other hand, feeding less expensive protein sources can be a false economy as these feeds may have unbalanced nutrients, low palatability and less digestible, often leading to environmental as well as economic setbacks. Regardless of insufficient information on digestibility values such as digestible
protein (DP) and digestible energy (DE), the feed of choice farmers opt for their shrimp farm is generally based on the following technical reasons such as sizes of shrimp, stocking density and pond carrying capacity. In addition, their choices also depend on the level of their experiences, feed price, current farm gate prices of shrimps, etc. In most cases, a combination of both technical and general reasons influence decision making on which feed to use in a certain period of time. Similar to other farmed animals, in fish and shrimp farming, nutrition is critical because feed contributes a major part of the total production costs. Better knowledge of diet formulations continues to support the shrimp farming industry as it is driven to satisfy growing demand for affordable, safe, and high-quality products. Therefore, the scope of this paper is to address two important issues which are efficiency improvement and cost savings for vannamei shrimp feed.

**Table 1:** The total farmed shrimp production (tonnes) in 2014 from the major producers globally as reported by FAO (2016) (1)

<table>
<thead>
<tr>
<th>Region</th>
<th>Country</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total world</td>
<td>China</td>
<td>3,993,500</td>
</tr>
<tr>
<td></td>
<td>Indonesia</td>
<td>613,900</td>
</tr>
<tr>
<td></td>
<td>Vietnam</td>
<td>385,700</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td>340,000</td>
</tr>
<tr>
<td></td>
<td>Thailand</td>
<td>130,200</td>
</tr>
<tr>
<td></td>
<td>Bangladesh</td>
<td>74,600</td>
</tr>
<tr>
<td></td>
<td>U.S.A</td>
<td>65,100</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>65,100</td>
</tr>
<tr>
<td></td>
<td>Malaysia</td>
<td>61,900</td>
</tr>
</tbody>
</table>

With an approximate feed conversion ratio (FCR) of 1.3, the total shrimp feed requirement will be nearly 9 million tonnes or 3.8 million tonnes if the production from China is excluded. This is the amount required to grow shrimp to a market size of 16.67 g/shrimp in 2014. This would mean that the farmed shrimps consumed approximately 3 million tonnes of crude protein, mainly from plant and animal sources, in 2014.

**How protein content in diets are measured?**

Kjeldahl (or similar methods) has been the common analytical method for determining nitrogen content (AOAC, 2000). Nitrogen content is then multiplied by a factor of 6.5 based on an early determination basis by which the average nitrogen is found to be about 16% of protein, in order to come up with the crude protein content. This calculation is based on two assumptions first, dietary carbohydrates and fats contain no nitrogen and secondly, almost all of the nitrogen in the diet is present as amino acids in proteins. However, not all nitrogen in diets is available as proteins some are in the form of non-protein nitrogen (NPN) in which aquatic species has limited ability to utilise. Therefore, amino acids, not protein per se, are more precisely required for growth of the animal.

**Protein and amino acids for starter feeds**

Evonik complete feed surveys, which have regularly been conducted with a purpose to help improve the understanding of the nutritionists, reveal amino acid profiles of Vannamei starter feed produced by some producers in SEA (Table 2)

**Table 2:** A summary of complete feed analysis conducted from January 2014 to December 2015 by Evonik AMINOLab (vannamei starter phase, n 10)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Met</th>
<th>Cys</th>
<th>M+C</th>
<th>Lys</th>
<th>Thr</th>
<th>Arg</th>
<th>Ile</th>
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<tbody>
<tr>
<td></td>
<td>0.85</td>
<td>0.46</td>
<td>0.21</td>
<td>1.31</td>
<td>1.37</td>
<td>2.23</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.04</td>
<td></td>
<td></td>
<td>0.05</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>1.37</td>
<td>2.23</td>
<td>1.43</td>
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<td>0.06</td>
<td>0.04</td>
<td>0.05</td>
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</tr>
<tr>
<td></td>
<td>1.68</td>
<td>0.79</td>
<td>1.57</td>
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<td>0.05</td>
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1. Mean and standard deviation values are reported on the 88% standardized dry matter basis 2. CP (crude protein) was analysed by Dumas combustion method 3. Amino acids were analysed by chromatographic method (wet chem)

**Table 3:** Ratios of the 9 essential amino acids (EAAs) on Lys and their total contents in the vannamei starter diet formulated based on ideal protein ratio recommended by AMINOShrimp®

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Met</th>
<th>Cys</th>
<th>M+C</th>
<th>Lys</th>
<th>Thr</th>
<th>Trp</th>
<th>Arg</th>
<th>Ile</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.38</td>
<td>1.31</td>
<td>2.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.38</td>
<td>1.31</td>
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1. The values are reported on a 88% standardized dry matter basis 2. AMINOShrimp® (first released in the year 2016) is an interactive and easy to use software calculating and giving amino acid recommendations for white leg shrimp diets.

In Table 3, taking into consideration the dietary Lys 2.04±0.07% in table 2, the dietary levels of the other 9 remaining EAAs were calculated using the ratios on Lys recommended by AMINOShrimp®.

**The amino acid-balanced diet and its advantages**

According to NRC 2011 (2), “the definition of ideal protein is the amino acid profile of the diet that meets exactly the requirement of the animal with no excess or deficit where optimum dietary EAAs levels are considered as proportions relative to total essential
amino acids (EAAs) rather than the whole diet”. In practice, ideal protein, or more precisely ideal amino acid patterns of the diets are usually indicated as the ratio of the nine other EAAs to Lys this is because Lys is mostly utilized for protein synthesis, stable and easy to analyze while the other amino acids are not.

**Table 4**: Comparison of the two diets which are formulated based on amino acid (commercial vs ideal protein ratio)

<table>
<thead>
<tr>
<th>INGREDIENTS, % of the diet</th>
<th>Commercial (Table 2)</th>
<th>Ideal protein (Table 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of feed, (USD/tonne)</td>
<td>710.02</td>
<td>679.38</td>
</tr>
<tr>
<td>SBM 48%</td>
<td>35.96</td>
<td>38.7</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>25</td>
<td>28.59</td>
</tr>
<tr>
<td>Fishmeal 61%</td>
<td>17.8</td>
<td>14.13</td>
</tr>
<tr>
<td>Poultry by product 63%</td>
<td>4.62</td>
<td>4.97</td>
</tr>
<tr>
<td>Rice bran</td>
<td>5.17</td>
<td>2.37</td>
</tr>
<tr>
<td>Squid meal</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>Tuna extract 67%</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>Soy lecithin</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Fish oil</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Premixes</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Choline Chloride 75%</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Monocaph</td>
<td>0.5</td>
<td>0.79</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>0</td>
<td>0.51</td>
</tr>
<tr>
<td>Salt</td>
<td>0.69</td>
<td>0.78</td>
</tr>
<tr>
<td>Binder</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.36</td>
<td>0</td>
</tr>
<tr>
<td>AQUAVI® Met-Met</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>0.24</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>ThreAMINO®</td>
<td>0.02</td>
<td>0.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NUTRIENTS, % of the diet</th>
<th>Commercial (Table 2)</th>
<th>Ideal protein (Table 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>88.43</td>
<td>88.37</td>
</tr>
<tr>
<td>Gross Energy, kcal/kg</td>
<td>4,400</td>
<td>4,400</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>39.01</td>
<td>37.86</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>7.5</td>
<td>6.89</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>3.44</td>
<td>3.36</td>
</tr>
<tr>
<td>Ash, %</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Starch, %</td>
<td>16.28</td>
<td>17.66</td>
</tr>
<tr>
<td>Lys, %</td>
<td>2.11</td>
<td>2.11</td>
</tr>
<tr>
<td>Met, %</td>
<td>1.04</td>
<td>0.93</td>
</tr>
<tr>
<td>Cys, %</td>
<td>0.44</td>
<td>0.45</td>
</tr>
<tr>
<td>M+C, %</td>
<td>1.48</td>
<td>1.38</td>
</tr>
</tbody>
</table>

**Table 5**: Comparison between the ratios of 9 EAAs on Lys of the diet formulated based on commercial amino acid levels vs those formulated based on ideal protein ratio.

<table>
<thead>
<tr>
<th>Ratios on Lys, in %</th>
<th>AMINOShrimp®</th>
<th>Table 2</th>
<th>Table 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>44</td>
<td>49</td>
<td>44</td>
</tr>
<tr>
<td>Cys</td>
<td>18</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>M+C</td>
<td>62</td>
<td>70</td>
<td>65</td>
</tr>
<tr>
<td>Thr</td>
<td>72</td>
<td>67</td>
<td>72</td>
</tr>
<tr>
<td>Trp</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Arg</td>
<td>121</td>
<td>116</td>
<td>114</td>
</tr>
<tr>
<td>Ile</td>
<td>61</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>Leu</td>
<td>108</td>
<td>129</td>
<td>127</td>
</tr>
<tr>
<td>Val</td>
<td>71</td>
<td>82</td>
<td>81</td>
</tr>
<tr>
<td>His</td>
<td>44</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>Phe</td>
<td>86</td>
<td>80</td>
<td>79</td>
</tr>
</tbody>
</table>

1. Prices for March 2016 in most of the ingredients
2. DL-Methionine for Aquaculture® (Evonik, Germany) is 99% DL-Met which has the optimized particle size distribution for Aqua feed
3. AQUAVI® Met-Met (Evonik, Germany) is a dipeptide of DL-Methionine and has about 200% relative biological efficacy higher than that of DL-Methionine on a product basis.
4. ThreAMINO® (Evonik, Germany) is L-Threonine feed grade 98.5% produced in a fermentation process using high-potential microbial strains.
5. In the Table 3 diet, DL-Methionine for Aquaculture® was replaced by AQUAVI® Met-Met that resulted in allowing more room for the cost improvement in the formulation.

The above math and simulation (Table 2 – Table 5) explains that upon using the same set of ingredients except DL-Methionine, the amino acid-unbalanced 39.01% crude protein diet (commercial or Table 2 diet) can potentially be improved by reformulating and applying ideal protein ratios recommended by AMINOShrimp®. In doing this, the 39.01% crude protein diet was fine-tuned into a more amino acid-balanced diet which contained 37.86% crude protein (ideal protein ratio or Table 3 diet) coming with a 20% lower in fishmeal inclusion and resulting in a cost reduction of 4.31%. More importantly, this lower fishmeal and crude protein but more amino acid-balanced diet can benefit from having better protein deposition as well as less catabolism of excess amino acids.
References

1. FAO 2016. The State of World Fisheries and Aquaculture 2016. 29
2. NRC 2011. Nutrient Requirements of Fish and Shrimp. 90
The effective protocol for treatment acute cardiogenic pulmonary edema in dogs

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Pulmonary edema is a common condition occurring secondary to the left-sided congestive heart failure (CHF). The assessment and recognition of cardiogenic pulmonary edema are important steps for management of this condition. Common clinical signs associated with cardiogenic pulmonary edema in dogs including dyspnea and cough. On physical examination, murmurs and crackle lung sounds can be heard. Pulmonary crackles should be interpreted with caution because many lower airways and lung parenchymal disease such as pulmonary fibrosis may result in this finding. Dogs with CHF usually have rapid heart rate with regular rhythm due to sympathetic stimulation. An increase of respiratory rate at rest is considered a reliable screening tool for diagnosing of CHF. Evidence of pulmonary edema can be confirmed by thoracic radiography. Dogs with CHF may not tolerate a stressful condition from handling; therefore, make sure that dogs are in good condition before performing radiography. The use of NT-proBNP assay, a cardiac biomarker, in combination with thoracic radiographs can significantly improve diagnostic accuracy in the diagnosis of CHF. However, the commercial assay for NT-proBNP measurement is not available in Thailand yet.

Dogs with severe pulmonary edema should be supplemented with oxygen to increase arterial PO\textsubscript{2}. Sedation some dogs with cardiogenic pulmonary edema may help to reduce respiratory distress and air hunger. Dogs with respiratory failure or respiratory muscle fatigue may need the artificial ventilation.

The drug that should be always supplemented dogs with acute cardiogenic pulmonary edema is furosemide, a loop diuretic. There is a venodilating effect when furosemide is given intravenously. The cardiovascular effects begin within 15 minutes post-injection. The starting dose is 2-4 mg/kg. The dose of furosemide can be titrated up to 6-8 mg/kg to increase the diuretic effect. The serial bolus 1-4 mg/kg every 6-8 hours or more frequently can be given. The constant rate infusion (CRI) of furosemide (0.66 mg/kg/h) can be used to maintain diuretic effects and increase urine output. CRI furosemide provides more diuresis and fewer electrolytes loss than repeated IV boluses of furosemide. Pimobendan is another drug of choice for CHF treatment. The recommended dose of pimobendan is 0.25 mg/kg bid. There is an intravenous preparation of pimobendan available but not in Thailand. The oral preparation has a rapid onset. Thus, pimobendan oral preparation can be used for treating CHF in the emergency situation also, if dogs can be handled and administer the drug orally. Nitroglycerine patch can be used for inducing systemic vasodilation. An angiotensin converting enzyme (ACE) inhibitor is recommended to use in combination with diuretics to reduce the activation of the renin-angiotensin aldosterone (RAAS) system. ACE inhibitors can be started later on after dogs are stable and ready for receiving oral drugs. The licensed ACE inhibitors for veterinary medicine use in Thailand include benazepril (0.25-0.5 mg/kg sid), rampril (0.125-0.25 mg/kg sid) and imidapril (0.25 mg/kg sid). The vasodilators may supplement in case of severe CHF dogs. Make sure that dogs still have normal blood pressure before using vasodilators. Effective vasodilators in the hospital setting include sodium nitroprusside (1-5 mcg/kg/min IV CRI) and hydralazine (1-2 mg/kg q12h). Vasodilators help in decreasing afterload resulting in an increase in stroke volume and a reduction pulmonary edema. Dogs with severe systemic hypotension dobutamine (2.5-10 mcg/kg/min) or dopamine (2-10 mcg/kg/min) can be given.

Intensive monitoring is very important for management dogs with cardiogenic pulmonary edema. All vital signs heart rate and rhythm, respiratory rate, and temperature should be measured for evaluating the response of patients to the treatment. Electrolytes imbalance and dehydration can occur due to an administration of high doses of furosemide. Vasodilators can cause systemic hypotenion; therefore, blood pressure should be monitored continuously during treatment.

In conclusion, dogs with acute cardiogenic pulmonary edema must be treated carefully and aggressively after diagnosis. Oxygen supplementation and potent diuretic and positive inotropic administration are the mainstays of treatment in dogs with CHF.

References
How to treat life threatening cardiac arrhythmia in dog and cat

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Although the majority of cardiac emergency case are acute pulmonary edema induced by acute congestive heart failure, the cardiac arrhythmia is still the interesting problem that challenged the criticist. We commonly found arrhythmias that originate from ventricular muscle rather than the atrium. Most of them are secondary from abdominal organic diseases i.e. splenic mass, post-splenectomy, gastric dilation and volvulus.

Ventricular arrhythmia is more likely to be secondary from any visceral problem while the atrial arrhythmia is usually associate with an organic heart disease. If the underlying disease is treated properly, secondary ventricular arrhythmia may disappear subsequently. Sympathetic nervous system seems to underlie many arrhythmias. Certainly, catecholamine plays a role of proarhythmia which increase activity particularly in painful conditions. The majority of ventricular arrhythmia are accelerated idioventricular rhythm. Such arrhythmia is classified as a non-pathogenic arrhythmia which may not need any treatment.

To classify between pathogenic and non-pathogenic (benign) arrhythmia, we need to guesstimate the impact of such arrhythmia on cardiac output. If the cardiac output is not altered significantly, such arrhythmia should not be treated by any antiarrhythmic drugs. The principle is to weight out between pros and cons of the drug comparing to an arrhythmia.

Arrhythmia is the Latin word means no rhythm but it commonly used to explain an irregular rhythm rather than no rhythm. For this reason, dysrhythmia seems to be a better word. Dysrhythmia can be classified into two groups based on heart rate. Tachyarrhythmia refers to increase in heart rate and bradyarrhythmia refers to decrease in heart rate. Clinically, the tachyarrhythmia occurred more common than bradyarrhythmia in canine and feline patients.

Regarding the mechanism, arrhythmia can be classified as disorders of electrical impulse conduction and disorders of impulse formation. To understand of arrhythmogenesis, please review the electrocardiography textbooks elsewhere.

Selection of antiarrhythmics relies on anatomical target of treatment. If the arrhythmia originates from sinoatrial (SA) and atrioventricular (AV) nodal tissues calcium channel blocker e.g. diltiazem is rather used. The arrhythmia that is originated from the atrial and ventricular myocardium usually been treated with many classes of antiarrhythmics such as sodium channel blockers (NCBs) e.g. lidocaine, potassium channel blockers (KCBs) e.g. sotalol or combinations in mechanism e.g. amiodarone.

In an emergency situation, the animal arrives with urgent need of assessment and resuscitation. Veterinarians have to survey of the patient’s ABC which stand for Airways, Breathings and Circulations. Focusing on the circulation, an interrupted heart rhythm should be investigated deeper. The electrocardiography will be used to evaluate if arrhythmia is suspicious. Type of arrhythmia will be stated and the origin of arrhythmia will be claimed. Diltiazem (CCB) and esmolol (beta blocker: BB) are used in supraventricular arrhythmias (SVAs) and Lidocaine (NCB) is used in ventricular arrhythmias.

Diltiazem is a calcium channel blocker which inhibits calcium influx in myocardium and conductive tissue especially, pacemaker cells. It decreases heart rate so the supraventricular tachyarrhythmia is an indication of diltiazem. In cats, it also indicates for hypertrophic cardiomyopathy. In dogs, it indicates for atrial fibrillation and can be combined with digoxin for additional control of heart rate. Comparing to verapamil, diltiazem is less negative inotropic so it is preferable to verapamil. Diltiazem is less effective vasodilation than amiodipine does so it is less preferable than amldipine to control hypertension. (Ramsey, 2011)

Esmolol is an ultra-short-acting beta-blocker which comes as 10mg/ml injectable solution. Esmolol is relatively selective beta-1 blocker. It is more specific to an adrenergic receptor of myocardium than other tissues. Esmolol is indicated for supraventricular tachycardias i.e. atrial fibrillation, atrial flutter, and atrial tachycardia. Because of ultra-short-acting, esmolol lasts for only 10-20 minutes after i.v. infusion. Oral preparation of other beta blocker can be used in long-term maintenance. The negative inotropic and chronotropic of esmolol should be aware of CHF patient and cardiogenic shock. Hypotension and bradycardia can be the common adverse effects. (Plumb, 2011)

Lidocaine is the most common antiarrhythmics using in the emergency room because the most common type of arrhythmia is ventricular tachyarrhythmia. Lidocaine is i.v. solution that is given initially by bolus at a dosage...
of 2mg/kg slow IV and can be repeated to 3 times to effect and continued as a constant rate infusion (CRI) to maintain an antiarrhythmic effect. Lidocaine may cause neurologic signs in cats so it should be used with caution in this specie. (Macintire, 2012)

EKG monitoring must be used to follow the benefit and adverse effects of the antiarrhythmics. Wired or telemetric remote EKG monitoring machine was used in the critical care unit. For short term monitoring, EKG diagnostic machine with small digital monitor is also useful. Dose of antiarrhythmics will be adjusted according to what we see on the monitor.

Antiarrhythmics may be helpful in a pathogenic arrhythmia and may be harmful in benign arrhythmia so the veterinarian should learn to identify the type of arrhythmia before considering using the drugs. The knowledge to identify the origin of arrhythmia whether primary or secondary and atrial or ventricular ectopia will help the veterinarian to consider which antiarrhythmics are preferred in a specific situation. EKG monitoring is a must facility in arrhythmic therapy.

Reference
Important Clinical Skills for Helping Dogs and Cats with Effusion

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What is effusion and how to treat?
Effusion is referred to an abnormal accumulation of fluid in the body cavity such as in the pleural space between the lung and the chest wall (pleural effusion), in the abdomen (abdominal effusion), or in the pericardial sac (pericardial effusion). The treatment options for the effusion consist of fluid removal to relieve the clinical signs and treating the underlying condition (1). Removal the effusion or the “centesis” is the important clinical skills for helping the patient’s clinical signs dramatically and the procedure provides the fluid sample for making the list of the possible causes. There are 3 mains procedures for take the effusion off including abdominocentesis, thoracocentesis, and pericardiocentesis. This manuscript will explain how to perform abdominocentesis, thoracocentesis and pericardiocentesis in step by step respectively.

Abdominocentesis
Abdominocentesis (sometime refers to abdominal paracentesis or peritoneocentesis) is the basic procedure aiming to remove of abdominal effusion. From physical examination, flatulent of enlarged abdomen is suggested of the fluid inside; however, ascites should be confirmed with any imaging diagnosis, abdominal radiography or ultrasound, before abdominocentesis. For instance, some conditions, such as pyometra, may result in the fluctuation fluid wave on the abdomen so the blind abdominocentesis in such a condition would make some serious injury.

Abdominocentesis is both diagnostic methods and palliative treatment especially for some patient with difficulty breathing from the large amount of abdominal fluid causing impairment of diaphragm. This procedure helps the patient feel more comfortable and ready for doing further diagnosis. The abdominal effusion is very easy to draw out, in the condition that it should be more than 6 ml/kg to make a positive pressure (2). The negative or false negative puncture therefore, sometime, means there is a few fluids inside the abdomen. The retrospective study about abdominocentesis in 129 dogs and cats with intraabdomen injury or disease. 47% of the patient can be diagnosed with abdominocentesis, while majority is because of the false-negative results (3). So, some patient with negative fluid needs to perform the peritoneal larvae. Or some dogs and cats with small abdominal fluid requires the ultrasound-guide puncture.

Equipment needs
1. Clipper and antiseptic scrub set
2. Sterile surgical gloves
3. 18 – 22 gauge 1.5 inches’ needle, or 16- to 18 IV catheters
4. Extension set
5. 10 to 20 ml syringes
6. EDTA tube, plain tube and草地slide
7. Culturettes for bacterial culture (optional)

Step by step of abdominocentesis (4,5,6)
1. Normally, abdominocentesis is performed in the conscious animals. Sedation may be suggested in some patient.
2. The position is neither on the right or left lateral recumbence. Sometimes, standing position is acceptable. The author prefers right lateral recumbence with one veterinary assistant restrain.
3. Shave and aseptic scrub the ventral abdomen.
4. The puncture is usually performed without the local anesthesia, still the over side needle is request local intradermal anesthesia.
5. With sterile gloved, simple abdominocentesis, caudal to umbilicus at the midline, or caudal and 1-2 cm to the right in order to avoid injury to the spleen. Typically, this site has the positive flow; however, the slightly negative drawn from the syringe might help the unsuccessful fluid flow.
6. With the positive flow, the extension is attached with the needle. Let the fluid continually flows by its movement. Sometimes, the negative pressure makes the possible visceral obstruction of the fluid.
7. If the simple abdominocentesis is unsuccessful, the four-quadrant abdominocentesis may be helpful. With dorsal, right or left lateral recumbency, insert the needle at the side described in the picture, refers the umbilicus as the center point. Begins puncture at the right caudal, right cranial, left caudal and left cranial quadrant orderly one by one until there is absent of effusion. Avoid puncture to the caudal superficial and deep epigastric vessels locate paramedian along the nipple line 7).
8. Once the fluid is collected, kept the sample in EDTA tube, plain tube or transport media as needed.
9. Continue to remove the effusion from the one site puncture until the fluid is absent. Then try another puncture site.

S9
Figure 1: The four-quadrant abdominocentesis at the “x” puncture point, avoid the “dot line” locate the caudal superficial and deep epigastric vessels long both side of the nipple line.

Somehow, the abdominal effusion is not sufficient for the positive result. Therefore, the ultrasound guided may useful for the diagnostic plan. In case of peritonitis, there is very small amount of fluid to obtained and this cytological sample is important to the diagnosis. As the results, the diagnostic peritoneal larvae (DPL) is performed (8).

The step by step of diagnostic peritoneal larvae (DPL)
1. Repeat the step 1-5 as above
2. Insert the needle or catheter into the peritoneal cavity at caudal and to the right of the umbilicus.
3. Infuse warmed 0.9% saline or lactated Ringer’s solution 10-20 ml. During the infusion watch closely for the patient respiratory signs.
4. Remove the catheter
5. Walk the patient or gently massage and roll the patient side to side.
6. Then, scrub the ventral abdomen again and repeat the abdominocentesis as described above. The collected fluid is for bacterial culture and cytological analysis.

The complications of abdominocentesis are minimizing but bleeding from organ puncture, GI perforation, or continued leakage of abdominal fluid may occur.

Thoracocentesis

Thoracocentesis refers to the techniques that use for remove fluid or air from the pleural space. The dyspneic dogs and cats, that is suspected pleural effusion, is appreciated the decrease lung sound at the ventral part and increase lung sound in the dorsal part of the thorax from the physical examination. Unlike the abdominocentesis, the dyspneic patient, suspected plural effusion, might need thoracocentesis without the radiographic confirmation (9). Thoracic radiograph can be very harmful in the severe respiratory distress dogs or cats. Thoracocentesis can palliative this patient once the effusion is removed. Moreover, thoracocentesis is one of the diagnostic methods that help identifying the type of effusion and make the tentative diagnosis. The types of effusion include transudate, modified transudate, exudate, blood, or chyloous, hemorrhage or neoplasia.

Equipment needs
1. Clipper and scrub set
2. Sterile gloves
3. For cat and small dog: 20-22 gauge-butterfly catheter
4. For medium to large dog: 18-20 gauge IV catheter
5. 3-way stopcock, extension, and 10 syringe
6. EDTA tube, plain tube, and culturette

Figure 2: The equipment needs for thoracocentesis including clippers, sterile glove, butterfly (for cats or small dogs) or IV catheter (for medium to large dog), 3-way stopcock, extension, and syringe as well as sample collection tube.

Step by step thoracocentesis (10, 11, 12)
1. Place the patient in sternal recumbence with manual and comfortable restraint. This position will allow the fluid move ventrally to the chest. Sedation may be used to ease patient’s stress or reduce heavy movement of the chest from the respiration distress.
2. Clip the hair left and right lateral thorax from rib 5-9. Make the proposed puncture site between rib 7 and 8, then shave 3 x 3 inches’ square from that point. Scrubbed the area.
3. With sterile glove, connect the butterfly needle with 3-way stopcock and syringe. Make sure the 3-way tuned “off” to the butterfly needle.
4. Localized anesthesia with 2% lidocaine may be infiltrated.
5. Insert the needle at between 7th and 8th intercostal space, cranial to rib 8th in order to avoid trauma to vessels and nerves. To remove effusion, insert at the ventral third of the thorax. Insert the needle slowly at the 45-degree ventral direction.
6. As you advance the needle to the chest, the assistant turns the 3-way tap “on” and slightly draw the negative pressure and obtain the effusion to the attach syringe. Once you collect
the sample, collect is to the EDTA tube, plain tube and culture media.

7. Continued to move the effusion to the syringe, and empty it thru the 3 way tap to the collecting bowl. When the fluid almost empty from the chest, move the needle more ventrally. When finish, you may feel the resist of the lung at the tip of needle in the thorax.

8. Turned 3-ways to “off” to the needle and withdrawn from the thorax.

9. Drained or repeat the procedure at the other side of the chest.

10. Record the total volume obtained.

To use to IV catheter, hand the needle and rest the other on the chest wall. Advance the needle with the dominant hand, at the same point as above, until feel a pop which mean the needle is passed through the pleura. Remove the needle and advance the catheter into the chest. Fix the catheter with thumb and index finger of the non-dominant hand. The assistant attaches the catheter with the extension and 3-way and syringe, slightly apply the negative pressure and draw the effusion as described in the previous paragraph (13).

The complication of thoracocentesis may consist of iatrogenic pneumothorax, hemothorax, intercostal artery or nerve laceration, or lung laceration.

Pericardiocentesis
Pericardiocentesis is referred to the procedure that remove the effusion from the pericardial sac, commonly in the dog and cat with pericardial disease. The patient with pericardial disease might have signs of exercise intolerance, weakness. Sometimes the right sided congestive heart failure develops, which result in ascites and jugular pulsation and distension. From the physical examination, the muffled or decrease heart sound may indicate pericardial disease. The pericardial effusion should be confirmed with both thoracic radiograph and echocardiogram before the pericardiocentesis. Pericardiocentesis helps relief the cardiac tamponade, which cause right congestive heart failure. Besides, the procedure provides the pericardial fluid sample that is the value key for disease diagnosis (9).

Equipment needs
1. Clipper and scrub set
2. Sterile glove
3. Cat or small dog: 18 gauge over-the-needle catheters, 2 inches long
4. Large dog: 16 gauge over-the-needle catheters, 2½ inches long
5. 2% lidocaine for local anesthesia
6. Cat urinary catheter (optional)
7. 3-way stopcock and extension set
8. EDTA tube, plain tube, and culturette

Step by step pericardiocentesis (13, 14)
1. The patient usually placed in sternal recumbency or left lateral recumbency. In this manuscript will explain the sternal recumbency position. Attach ECG on the patient to monitor the heart rhythm.
2. Sedation should be required to reduce the movement.
3. Shave and scrub on an area from 3rd to 7th rib, in the ventral half of the right chest wall.
4. Located the puncture point from the palpation on the thoracic wall for the point of maximum intensity, nearest the heart apex. Then infiltrate 2% lidocaine to create a local anesthesia.
5. Insert the needle at between the 5th-6th, 7th intercostal space where is the nearest apex beat. The direction of the needle is craniodorsally or point toward the opposite scapula direction.
6. Once the needle insert to the pericardial sac, the fluid appears on the hub. Withdraw the needle and advance the catheter to leave in the pericardial sac. Attach the catheter with the extension, 3-way, and syringe.
7. The pericardial fluid mostly in the port-wine color, so collect about 5 ml of the fluid into the plain tube and see any clotting. If there is clotting blood, which means the active bleeding from the puncture, so withdraw the needle or stop the process. If there is no clotting blood, means the pericardial effusion, so continued remove the fluid.
8. With confirmed of effusion, continued remove the fluid while closely monitoring on the ECG. If any arrhythmia occurs, slightly pullback the catheter to avoid the stab to the heart
9. Some text recommends to put the cat urinary catheter into the catheter in order to prevent the bend of the catheter. (13)
10. Remove the pericardial fluid as much as possible, but it does not have to remove it all. The small residual volumes will drain to the thorax at the puncture point.
11. When no longer fluid on the syringe, remove the catheter form the patient.

The complication of pericardiocentesis is cardiac arrhythmia, the severe cardiac arrhythmia can be life threatening so quickly remove the cather out from the patient. Vena cava is punctured caused bleeding.

What to do after the “-centesis”
Lastly, after the effusion removal, there are 2 major diagnostic plan for the patient including the radiograph or imaging and the fluid and cellular examination.

1. First, the post-procedure imaging. After the “-centesis”, the patient would feel much better from the previous suffering signs, so the veterinarian would be able to take the further investigation especially the imaging diagnosis that need some restraint. For example,
   - After thoracocentesis typically take the thoracic radiograph.
After abdominocentesis, re-abdominal radiograph or ultrasound to the further diagnosis.

Nevertheless, echocardiogram of the heart with pericardial effusion would appreciate the picture of mass outside the heart better than after pericardiocentesis. Still, echocardiogram after pericardiocentesis is warranted for access the cardiac morphology and function.

2. Secondary, the fluid and cytological analysis (15, 16).
- The EDTA tube collect the sample that test for total cell count and cytological examination. The slide should be made from this EDTA tube as soon after effusion collection.
- The plain tube collect the sample that need the biochemistry test and total protein analysis.
- The culture media collect sample that need for bacterial culture and drug sensitivity.

In conclusion, abdominocentesis, thoracocentesis and pericardiocentesis; is the procedures that help removing the abnormal fluid in the body cavity. This process is suggestive and important skill for the veterinarians in helping the dog and cat’s clinical signs. In addition to the clinical enhancement, this process delivers the sample for the fluid and cytological analysis, which would classify the type of effusion and limit the possible differentiate diagnosis for the patient.

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Update in CPR Technique for Successful Life Rescue

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Introduction

In 2012, the Veterinary Emergency and Critical Care Society (VECCS) and American College of Veterinary Emergency and Critical Care (ACVECC) developed the new guidelines of cardiopulmonary resuscitation (CPR) in dogs and cats called The Reassessment Campaign On Veterinary Resuscitation (RECOVER) (1). The guidelines cover 5 parts of CPR-related: preparedness and prevention, basic life support (BLS), advanced life support (ALS), monitoring and post cardiac arrest (PCA) care. Each guideline was assigned 2 descriptors as class and level (2).

<table>
<thead>
<tr>
<th>Class</th>
<th>Level</th>
<th>Description</th>
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<tbody>
<tr>
<td>Class I</td>
<td>Level A</td>
<td>Multiple high quality studies in multiple populations support recommendation</td>
</tr>
<tr>
<td>Class IIa</td>
<td>Level A</td>
<td>Chest compression should be provided immediately and if many rescuers are present, airway and ventilation management should not delay commencement of chest compressions.</td>
</tr>
<tr>
<td>Class IIb</td>
<td>Level A</td>
<td>Chest compression technique - At the rate of 100-120 / min in cats and dogs (I-A) - Performed with depth being to 1/3-1/2 the width of the chest (I-A). Multiple human clinical study and an experimental study in chest wall recoil (I-A).</td>
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Basic Life Support

Since delaying the start of BLS are significant with decreasing in survival to discharge and neurological status, thus aggressive administration in patients suspected of being CPR is recommended (I-B). BLS includes the recognition of CPR, chest compression, airway management and ventilation. When apneic and unresponsive patients detected rapid airway, breathing, circulation (ABC) assessment no more than 5-10 seconds is concerned. Chest Compression: Chest compression should be provided immediately and if many rescuers are present, airway and ventilation management should not delay commencement of chest compressions.

Patient position and compressor hand placement
- Should be done in lateral recumbency in both dogs and cats (I-B), due to experiment suggested higher ventricular pressures and aortic flow. Moreover higher rate of ROSC associated with compression in this position (11).
- Place hands over the widest part of the chest in medium, large and giant breed dogs with round chest (IIa-C).
- In dog with barrel-chested conformations, such as English bulldogs, dorsal recumbency maybe considered (IIb-C).
- Cats and small dogs can be achieved by one-hand technique with circumferential compression (IIb-C). However, if patient’s thoracic wall compliance decreased due to age, or obesity, 2-handed technique can be used.

CPR Training
- The initial training and refresher training should be at least every 6 months (I-A) (2,7).

Team Dynamic
- The Studies in human medicine have revealed there does not appear to be improve on CPR outcome from the presence of physician as a team leader (8,9). Veterinarians or technicians are capable as leadership of the team (IIb-B). The communication and team skill training can be improved the CPR effectiveness and specific leadership training is recommended for individual (I-A).

CPR and Anesthesia
- Anesthetic-related CPA patients revealed high survival rate (47%) (10), so high quality CPR should be provided (I-B).

Preparedness and Prevention

The successful of CPR depends on the prompt initiation of rescue (3,4). Minimizing the time from CPA to CPR is crucial to improve CPR outcome. This domain focus on intervention involving both personal and environmental factors.

Equipment Organization and Cognitive Aids: The equipment location, storage, and content should be standardized and auditable (I-A). The both of cognitive aids such as checklists, algorithm charts, and dosing charts (I-B) and the training of personnel in the use of these (I-A) are important to improve the survival rate in dogs and cats (5,6).

CPR Training: The initial training and refresher training should be at least every 6 months (I-A) (2,7).

Team Dynamic: The Studies in human medicine have
- Performed 2 minutes cycle without interruption to maximize coronary perfusion (I-A), compressor should be changed after each cycle to minimize fatigue (I-B).

**Ventilation:** Both hypoxia and hypercapnia can reduce ROSC, simultaneous ventilation should be provided viauffed endotracheal tube at 10 breathes per minute, with tidal volume 10 ml/kg and inspiration time 1 second (I-A). 100% of oxygen ventilation is recommended (Ia-B), however use of the room air may be considered (IIb-B). If endotracheal intubation is unavailable, interposed abdomen may be considered (IIb-B). The use of abdominal compressions interposed with chest compression can facilitate venous return from the abdomen and improve cardiac output. If sufficient trained personnel are available, interposed abdomen may be considered (IIa-B).

**Advance Life Support**

ALS should be performed after BLS until ROSC is achieved. The main ALS recommendations are vasopressor and vagolytic therapy, defibrillation, antiarrhythmic drug therapy, reversal agents, and electrolyte therapy.

**Vasopressor and vagolytic therapy:** Vasopressor and vagolytic therapy are increase peripheral vascular resistance for adequate coronary and cerebral perfusion.

- Epinephrine: The use of low-dose (0.01 mg/kg IV) administered every 3-5 minutes early in CPR is recommended (I-B). Although higher dose (0.1 mg/kg IV) have been associated with increase rate of ROSC, but the experimental data in dog shown no benefit or reduced survival possibly due to the exaggerated adrenergic effects (15,16). However the higher dose epinephrine may be considered after prolong CPR (IIb-B).

- Vasopressin: Unlike epinephrine, vasopressin has no inotropic or chronotropic effects, therefore does not affect heart rate and contractility. At 0.8 U/kg of vasopressin may be considered as substitute or in combination with epinephrine every 3-5 minutes (IIb-B).

- Atropine: Many studies shown no beneficial of its use at standard dosing (0.04 mg/kg). The higher dose (0.1, 0.2, 0.4 mg/kg) have been associated with poor outcome in experimental dogs (17). However it can be used of standard dose in patients CPA-related with pulseless electrical activity (PEA) or high vagal tone (Ia-B). The routine use of atropine (0.04 mg/kg IV) may be considered (IIb-C) due to the lack of clear detrimental effect.

**Defibrillation:** Electrical defibrillation is indicated in ventricular fibrillation (VF) and pulseless ventricular tachycardia (VT).

- The biphasic defibrillator (I-A) at the dose 2-4 J/Kg (Ia-B) is recommended. In patients with VF or pulseless VT, defibrillation energy increment (50% dose increase) is reasonable if the first treatment is unsuccessful (Ia-B).

- The patients should be placed in dorsal recumbency. The paddle should be placed on the thorax over the costochondral junction directly over the heart (2).

- The immediate defibrillation in VF or pulseless VT of duration of 4 minutes or less is recommended (I-B).

- In VF or pulseless VT patients for greater than 4 minutes, BLS should be performed 2 minutes cycle before defibrillation to maximize coronary perfusion (I-B).

- Single shock is recommended to reduce interrupt the compression (I-B).

**Antiarrhythmic drug therapy:** Only amiodarone has shown benefit and may be considered in VF or pulseless VT patients resistant to electrical defibrillation (IIb-B). Antiarrhythmic agents should be considered as adjunctive therapy with electrical defibrillation (I-B).

**Reversal agents:** Only naloxone has been evaluated for use in patient CPA (2). In case of opioid toxicity, naloxone should be administrated during CPR (I-B). In absence of opioid toxicity but recent opioid administration, the use of naloxone may be considered (IIb-B). The risks associated with the using of reversal agents are low therefore in dogs and cats that have received reversible anesthetic drug, administer of reversal agents may be considered (IIb-C).

**Electrolyte therapy**

- Calcium: Although hypocalcemia commonly develop in prolong CPR patients, but several studies shown the no benefit or poor outcome in routine use of calcium during CPR (III-B). Intravenous calcium administration may be considered in dogs and cats with moderate to severe hypocalcemia (IIb-C).

- Potassium: Hyperkalemia in prolong CPA patients is common. Hyperkalemia should be treated during CPR (I-B). In unavailable hemodialysis, medical administration would be reasonable (18) (IIb-C).
**Other Therapies**

- **Corticosteroids:** Because the lack of evidence of advantage and potential for deleterious side effect from corticosteroids especially in poor condition patients, the routine use of these agents is not recommended (III-C).
- **Alkalization therapy:** Several studies in dogs have documented improved survival with bicarbonate administration in prolong CPA dogs (>10 min). Base on the evidence, bicarbonate therapy (1 mEq/q of sodium bicarbonate) in prolong CPA patients (>10-15 minutes) may be considered (IIb-B).
- **Intratracheal drug administration:** Intratracheal route for epinephrine, vasopressin, or atropine may be considered in intravenous or intrasseous access is not possible (IIb-B). Drugs should be diluted with saline or sterile water and applied via catheter longer than the endotracheal tube (I-B).
- **Supplemental oxygen administration:** oxygen supplementation should be titrated to achieve normoxemia (PaO\(_2\) of 80-105 mmHg). Therefore the use of room air may be considered (IIb-B) and hyperoxemia should be avoided (I-A) (2,19). However this approach is best used in which arterial blood gas analysis is possible so FiO\(_2\) can be titrated. In absence of arterial blood gas data, the risk of hypoxemia likely outweigh the risk of hyperemia, and the use of 100% oxygen is reasonable (IIa-B).
- **IV fluid administration:** Administration of IV fluid in euvelemic patients is associated with decreased coronary perfusion pressure (20). This is due to predominantly increase central venous pressure, opposing blood flow to coronary and cerebral circulation. Therefore during CPR in hypervolemic or euvelemic dogs and cats, routine administration of intravenous fluid is not recommended (III-B). Patients with preexisting hypovolemia are likely to benefit from increase circulating volume and administration of IV fluid is reasonable (IIa-C).
- **Open-chest CPR:** In significant intrathoracic diseases, promptly performing open-chest CPR may be considered (IIb-C).

**Monitoring**

Monitoring is subdivided into diagnosing CPA, monitoring patients during CPA, monitoring patients at risk of CPA, and monitoring patients after ROSC.

**Diagnosing CPA:** Several monitoring techniques and initial assess ABCis recommended. Pulse palpation is widely employed by veterinarians as initial assessment. However many human studies have shown that this technique is unreliable and only 2% of recuers correctly recognize the lack of pulse within 10 seconds (21). Apneic patients are done, the use of pulse palpation to support a diagnosis of CPA is not recommended (III-B). Doppler blood pressure monitoring can be useful in early detect of CPA in risk patients, but given time to place sensor in CPA patients is not recommended (III-C). ECG may appear rhythms despite the presence of CPA, therefore the use of ECG as sole parameter to detect CPA is not recommended (III-B). The low EtCO\(_2\) is expected in CPA because of the decrement of pulmonary blood flow. However in dog with CPA, initial EtCO\(_2\) can be higher than prearrest mean value. Thus the immediate EtCO\(_2\) value should not be used for diagnosis CPA (III-B), although subsequent value can be used.

**Monitoring patients at risk of CPA**

- **Verification of endotracheal intubation:** EtCO\(_2\), direct visualization, auscultation, or observation of chest excursion to verified correct ETT placement (IIa-B).
- **Electrocardiogram:** Evaluation ECG during intercycle pauses of chest compression is recommended for obtain rhythm and guide ALS therapy (I-C). However chest compressions should not be stopped during a complete cycle to allow ECG monitoring (III-B). In VF patients, ECG must be done rapidly to determine if VF resolved, but should not delay resumption of chest compression for another cycle (IIa-B).
- **End tidal CO\(_2\):** There is strong evidence supporting the use of EtCO\(_2\) monitoring during CPR as an indicator of ROSC (I-A) and measure the efficacy of CPR (IIa-B). The data suggesting that higher EtCO\(_2\) value during CPR (>15 mmHg in dogs, >20 mmHg in cats) may be associated with an increased rate of ROSC.
- **Electrolyte and blood gas monitoring:** The routine potassium and calcium monitoring in prolong CPR may be considered (IIb-B) due to hyperkalemia and hypocalcemia commonly developed in this condition. In the patients with electrolyte disturbances related CPA, monitoring electrolytes will help guide therapy is recommended (I-C). Mixed-venous or central-venous blood gas analysis may more accurately reflect tissue acid–base status during CPA. Therefore use of these values to evaluate CPR effectiveness may be considered (IIb-B). CPA causes poor perfusion so peripheral or arterial blood gas is not recommended (III-A).

**Monitoring patients after ROSC:** Basic of monitoring critical patients should be applied. Moreover monitoring should be adequate to detect the reoccurrence of CPA and to guide the proper therapy(I-C). Minimum monitoring should be include ECG, intermittent arterial blood pressure, oxygenation, and ventilation (I-B). Other monitoring may be considered depending on individual patient status and underlying disease (IIb-B).

**PCA Care**

Only 2% to 10% of dogs and cats CPA victims achieving CPR will survive to hospital discharge, despite ROSC in 35%-45% of the animals (11,22). Patients with ROSC are likely to have multiorgan failure, cardiogenic shock, anoxic brain injury, and the sequelae of preexisting disease.
Hemodynamic optimization strategies: The primary resuscitation endpoints of central venous saturation (70%) or lactate (<2.5 mmol/L), and the secondary endpoints including arterial blood pressure (SAP100-200 mmHg, MAP 80-120 mmHg), central venous pressure (10 mmHg), PCV (>25%), and arterial oxygen saturation (SpO2 94%-98%, PaO2 80-100 mmHg) (IIb-B). To achieve these parameters, IV fluid therapy is often indicated. However, large volume administration is not recommended unless hypovolemia is suspected (III-C). The use of vasopressor and/or positive inotropic drug with persistent hypertension and/or cardiovascular instability is reasonable (IIa-B). Hypertension (MAP>150 mmHg in the immediate PCA period in dogs and cats is beneficial (IIa-B). Control of respiratory function: It is reasonable to target normocapnia (PaCO2 32-43 mmHg in dogs and 26-36 mmHg in cats) (IIa-B). Intermittent positive pressure ventilation (IPPV) may be necessary to maintain normocapnia, but one veterinary study found that the use of IPPV is associated with poor survival rate (23). Routine IPPV in all PCA is not recommended (III-B). Oxygen supplementation should be titrated to maintain normoxemia early after CPR (Ia-A).

Hypothermia and rewarming: The increasingly use of mild therapeutic hypothermia (MTH; core body temperature of 32-34°C) in human PCA seen today. The evidence suggests that MTH has organ protective effects in PCA patients, leading to improve cardiac and neurological outcomes. Based on evidence from experimental studies in dog and human, MTH should be apply with IPPV and advance critical care in initial after ROSC and maintained for 24-48 hours (Ia-A). However if mild accidental hypothermia is present, it is reasonable to not rapidly warm these patients. The guideline suggests a slow rewarming rate at 0.25-0.5°C/h (IIa-A). The rewarming rates of >1°C/h should be avoided (III-A).

Drug therapies:
- Corticosteroids: Routine administration of corticosteroids during PCA care is not recommended (III-C). However there is a good evidence in human study that hemodynamics, ScvO2, and survival to discharge have been improved when treated with low-dose of hydrocortisone. Therefore administration of hydrocortisone (1 mg/kg followed by either a mg/kg q6hr or an infusion of 0.15 mg/kg/hr and tapered as the patient’s condition) to dogs and cats that remain hemodynamically unstable despite administration of fluids and inotropes during PCA care may be considered (IIb-C).
- Hyperosmotic therapy: Both hypertonic saline and mannitol for treating cerebral edema with/without neurologic signs may be considered (IIb-C).
- Seizure prophylaxis: Seizure prophylaxis with barbiturates (eg, phenobarbital) may be considered in dogs and cats during PCA care (IIb-B) based on the study in cats (24).
- Metabolic protection: No clinical guidelines can be developed at this time.

Referral center care: It is reasonable to refer PCA patients to a facility with 24-hour care, intensive monitoring, and advanced critical care capabilities hospital (IIa-B).

Conclusion
The RECOVER initiative created the first guideline on veterinary resuscitation, which provided a standard for CPR. The readers are encouraged to review these guideline combined with standardized training in order to practice the best standard of care for PCA patients.

References
Case scenarios of allergic dermatitis approach

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Introduction
Allergic skin disease are common skin problems seen in veterinary practice. Dogs with allergic skin diseases are usually pruritic with clinical lesions related with causes and types of allergy. There are 4 types of allergic skin diseases in small animal practice; Atopic dermatitis (environmental allergy), food allergy, flea allergic dermatitis and contact hypersensitivity.

Types of allergy

Flea Allergic dermatitis (FAD) is a common allergic problem especially in flea endemic area. Clinical signs include pruritus, alopecia and excoriation in which usually seen at dorsal lumbar area, ventral abdomen and tail base. Complete eradication of fleas from the animals and environment is necessary for effective treatment, as a single bite will be enough to start the problem. A pruritic dog with suggestive clinical presenting signs and history must be thoroughly treated for fleas before a diagnosis of FAD can be confirmed or ruled out other pruritic skin disease

Allergic contact dermatitis is a relatively rare skin disease that can occur when a dog or cat makes physical contact with substances. Lesions generally appear on areas that are not well-protected or hairless. Most allergic reactions require a period of repeated physical contact and skin sensitization.

The primary symptom of both types of contact dermatitis is a skin rash characterized by itchy red bumps and inflammation. The patients are usually pruritic so they may scratch or lick excessively. This may lead to secondary bacterial infection.

Food allergy is the least common allergic skin disease of dogs and cats. Clinical signs usually include various degree of pruritus and skin inflammation. Common affected areas are peri-orbital area, ears, muzzle and paws. Lesions at ventral neck, axilla and inguinal areas can also be seen especially in chronic cases or with malassezia dermatitis. Diagnosis of food allergy can only be confirmed with food elimination diet in which the patient can either be placed on a novel protein or hydrolysed protein diet for 8-10 weeks. If improvement is observed after food elimination diet trial, food challenge should further perform to identify causative food allergen so owner should refrain from giving those food to animal.

Atopic dermatitis is an allergic reaction to environmental allergens which cause allergic reaction and inflammation of skin. The common allergens to cause atopic dermatitis in dogs and cats are house dust mites, grass, pollens, insects etc. Diagnosis of atopic dermatitis can be made based on ruling out other possible pruritic skin diseases. These include ectoparasites, infection (bacterial and fungal skin diseases) and other allergies. Once diagnosis has been made, allergic control with medical treatment or further allergen identification will be performed. Identification of environmental allergens by intradermal skin test or serum specific IgE is beneficial to educate owner for allergen avoidance and for allergen specific immunotherapy (ASIT).

Treatment
Allergen avoidance is very crucial in all types of allergy so exposure to causative allergens will decrease leading to lower allergen sensitization. Hyposensitization and medical treatment of allergic patients become necessary to control allergic reactions when allergen avoidance is unable to perform. Hyposensitization has been reported to decrease itchiness in 60 to 80 percent of dogs, but may approximately take six months to a year to see an improvement.

Treatment choices for allergic dermatitis include

Essential fatty acids (EFAs) – These can reduce itchiness in 30-40% of dogs. Synergistic effect can be obtained when use EFAs with antihistamine. EFAs may take several weeks until clinical improvement of pruritic signs seen.

Antihistamine (AH) – AH is considered as low efficacious antipruritic agents. It can be effective in 25% of cases but maximum effect may be seen in 2 weeks after initiation of therapy. There are several types of antihistamine in which animals may respond to each type antihistamine differently and may vary among individual one.

Corticosteroids – It has been use as a main treatment for allergic dogs for yeas with very good efficacy to reduce pruritus in dogs and cats. However, its side effects are of concern including weight gain, pu/pd, liver and kidney damage. For long term use, the dose should be kept as low as possible with regular monitoring of liver enzymes and other possible side effects.

Cyclosporins – It can be as effective as steroids at reducing pruritus without having long term side effects. However, some side effects can occasionally be seen.
These include vomiting, diarrhea, gingival hyperplasia and papilomas.

**Oclacitinib** – It is a specific JAK1 inhibitor designed to inhibit inflammatory cytokines, thereby, helping to reduce is 0.4-0.6 mg/kg twice a day for 2 weeks, followed by once a day for long term use.

In summary, veterinarians should prescribe the right choice of treatment for individual dogs based on price, efficacy, severity of disease and animal’s health status.

Decision making should be done by owner and veterinarian to obtain the best solution.

**References**

Real case approach for dermatophytosis

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Introduction
Dermatophytosis is a common fungal skin infection of the hair, nail or stratum corneum of dogs and cats. It is caused by Microsporum, Trichophyton or Epidermophyton. Infection requires actively growing hair to survive and infect. Infective arthrospores often seen at hair shaft especially with anagen hair with rapid growing cells. Hair shafts containing infectious arthrospores may remain infectious in the environment for many months. Dermatophytosis can be diagnosed by a variety of diagnostic tests. However, Fungal culture is the most reliable diagnostic test among all skin tests and is the only way to identify the specific dermatophyte (1). Spontaneous resolution or self-limiting may occur within 3-4 months without any treatment depending on animal immune status. Therapy can also be prescribe aiming to maximize the patient response to dermatophyte infection, reduce contagion and fasten resolution of infection (1,2).

Clinical sign
The most common clinical signs include focal or multifocal area of hair loss, scaling, and crusting (1). Some animals may demonstrate the classic circular lesion with central healing, fine follicular papules, and raised skin and crusts at the periphery. Dogs tends to develop classic circular lesions more than cats. However, cats are more likely to shown generalized lesions (1). Pruritus is variable and is usually minimal or absent. M. canis is the most common cause of dermatophytosis in either dogs and cats. Longhaired cats, especially Persians, are predisposed to this infection (1,3).

Diagnosis
Wood’s lamp is a useful screening test for only M. canis infection that will show positive apple green fluorescence in 50% of infected cases which is a result from metabolites of fungal organisms. False positive results can be seen in some cases with oily skin or history of cream or ointment use (4).

Direct microscopic examination of suspected cases can be useful. Microscopic examination aims to examine fungal hyphae or spores which may provide rapid confirmation of fungal infection even though it has very low sensitivity (1). The best results are obtained by examination of affected hairs mounted in liquid paraffin, or in 10% KOH, or chlorphenolac.

Fungal culture of affected material (scale and hairs) is the most reliable and used method for confirmative diagnosis of dermatophytosis (1). However, both false-positive and false-negative results are possible (4). Proper sample collection is also crucial to obtain good sample materials leading to reliable test results. Specimens are best collected by hair plucking or Mackenzie brush technique from edge of lesions with active inflammation. Dermatophyte medium containers are best incubated loosely covered and kept at room temperature and protected from light. Dermatophyte colonies may appear within 5 to 7 days of inoculation. Confirmative diagnosis of dermatophytosis can be made by change of medium color, appearance of colonies and confirmation of fungal elements under microscopic examination.

Histopathological examination is a definitive confirmation of fungal infection. It is indicated for nodular lesions, non-healing lesions, dermatophytic pseudomycetoma or auto-immune skin diseases. The technique has a high specificity but appear to be low sensitivity for most of the cases (1).

Treatment
Hair clipping is recommended for infected animals to remove infected hair that may cause of contamination to animals and environment (1,5). However, some reports suggested not to clip hair as it may spread the spores into environment. In short-haired or few lesions (<5 lesions) patients, hair clipping may not be necessary.

Topical treatment
Topical treatment should be prescribed to all confirmed cases of dermatophytosis (1,2,5). Topical treatment should be continued until 2-3 negative fungal cultures at weekly interval (5). Rinses are preferred because the entire body surface can be treated and should be applied twice weekly. Lime sulfur 2%, enilconazole 0.2%, and miconazole 2% plus chlorhexidine 2% are the most effective agent (2,5).

Lime sulfur has shown superior antifungal activity. Application of a 1:16-30 dilution of lime sulfur has demonstrated to be effective against dermatophytes when combine with hair clipping and aggressive environmental control. The higher concentration was more efficacious and need shorter treatment periods (6). Lime sulfur is virtually nontoxic if applied properly and safe to use in young dogs and cats.
Enilconazole is effective in treating dermatophytosis and can be used as a sole, whole-body dipping. Enilconazole is safe for cats but elizabethan collars should be worn until the animal gets dry. It is an effective topical product against dermatophytosis with 100% sporicidal activity (7). However, it is only licensed for dogs and horses.

Miconazole can be used as a sole therapy or in combination with chlorhexidine. Basically, it should be used as adjunctive therapy to systemic therapy rather than as a sole treatment. 2% miconazole combination with 2% chlorhexidine shampoo in dilution 1:10(8) has been confirmed synergistic. Skin contact time of 10 minutes is recommended to ensure its maximum antiseptic activity.

Systemic treatment
All confirmed cases with multifocal lesions or long-haired animals should be treated with systemic antifungal therapy. Moreover, systemic therapy is recommended for patients that are not responding to topical therapy after 2-4 weeks of topical treatment. Systemic therapy should be continued until 3 negative fungal culture at weekly intervals (5).

Itraconazole is considered as fungistatic at low concentrations and fungicidal at high concentrations. It possesses a dose-dependent antifungal activity (9). Rarely hepatotoxicity has been documented in cats, and idiosyncratic cutaneous vasculitis has been seen in dogs. Itraconazole has been used in kittens as young as 6 weeks of age. Itraconazole persists in the skin and nails for months after administration (10). In humans, pulse therapy is frequently prescribed for use in onychomycosis and may also be useful in animals (11).

Fluconazole may be less effective than itraconazole against fungal infections, and at high concentrations. It has not been successfully treated dermatophytosis and no longer recommended for the treatment or prevention of dermatophytosis(15).

Vaccination is licensed for use in USA. A killed M.canis Vaccine does not prevent infection. It can reduce the severity of the lesions and fastens the resolution of clinical lesions, but not mycological cure (16).

Monitoring of treatment
Clinical cure of dermatophytosis is considered when complete resolution of clinical sign has been accomplished. A mycological cure needs two or three consecutive negative weekly fungal culture (5). Long term control of and heavy vacuum cleaning should be performed to reduce chances of reinfection or spreading to other animals in the household.

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Blood Transfusion in Cats: from A to Z

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Keywords: blood transfusion, blood type, cross matching, feline

Abstract
Feline blood transfusion can be a potential treatment for critical patient. At the present, 3 blood groups have been identified in cats which are A, B and AB type. To avoid incompatibility transfusion, blood types of both donors and recipients should be determined or at least the cross matching should be performed in all cases. The syringe technique is commonly applied for blood collection from feline donor. Since feline blood component processing is limited, whole blood is used for feline blood transfusion by intravenous or intraosseous route. However, the blood transfusion safety protocol should be considered for both the donors and the recipients. Furthermore, the cause of the problem should be diagnosed in order to eliminate the problems entirely and to avoid more blood transfusion.

Introduction
Blood transfusion is the appropriate treatment in severe clinical sign cases in cats such as lethal accident, high rise syndrome, blood loss from critical surgery and other hemolysis conditions. However, blood transfusion in cat are less frequently performed than in dogs which may due to fewer hemolytic or bleeding problems, the less amount of the potential cat donors and the difficulties in blood transfusion process compared to dogs. In general, cats have three main blood types: A, B, and AB. Siamese cats are always type A blood. Type B blood is more frequently found in some pure breeds especially, British Shorthair, Ragdoll, Birman and Rox breeds while AB blood type is rare [1]. Regarding the presence of natural alloantibodies on each blood type, only AB-matched transfusions are effective and safe or it can be stated that cats with AB blood type are considered universal RBC recipients as these cats do not have anti-A or anti-B alloantibodies [2]. Therefore, these AB type cats can receive RBC units of type A, type B, or type AB. Cats which are required as blood donors must have general good health and temperament, complete vaccination and they should live only indoor. The ages of donors have to be between 1-8 years (ideally 1-5 years). A minimum weight should be at least 4 kg with pack cell volume more than 35%. Moreover, hematology and blood chemistry should be in normal range including that they should be free from other lethal diseases which are Feline Immunodeficiency virus (FIV), Feline Leukemia virus (FeLV), Feline infectious peritonitis (FIP) and Hemoplasma. For female cats, they should be already spayed in order to avoid any surgery which may occur in the future. In addition, the donor cats must not receive any blood transfusion before.

Blood transfusion management
1. Blood types determination and cross matching
In order to prevent incompatibility of mismatched transfusion, both donors and recipients should be determined for their blood types. However, due to the blood typing test kits are not easily available, the cross matching process is the standard procedure before any transfusion. In general, there are two categories of cross matching i.e. the major cross matching and the minor cross matching. The major cross matching helps determining compatibility between alloantibodies in the recipient’s serum/plasma against donor RBCs while the minor cross matching is performed in order to determine compatibility between alloantibodies in the donor plasma and the recipient RBCs [3]. The cross matching test can help avoiding the incompatible transfusion which can cause life threatening immune-mediated hemolytic transfusion reactions in the recipients. However, compatible cross matching does not eliminate other adverse effects which may occur such as delayed transfusion reactions.

2. Blood collection from the donor cats
Most of the donor cats require sedative treatment before blood collection. The technique of blood collection in cats is called “syringe technique” since only about 60 cc. of blood can be collected by using syringe from each cat. The donor cats should be given fluid therapy for a total of twice the volume of blood removed intravenously. Blood is collected aseptically from the jugular vein via a butterfly needle attached to the T-port and the syringe which was already contained anti-coagulant. Thereafter, collected blood from the donors will be transferred to a transfer blood bags which attached a blood filter giving set for administration to the patient.

3. Administration of collected blood to recipient
In general, the fresh whole blood is used for feline blood transfusion since the availability of blood components is limited. In addition, only small amount of blood (about 60 cc) can be collected from the donor.
cats which make it difficult to process for the blood components. The patient cats can receive the whole blood by intravenous route and intraosseous route by using the blood filter giving set in order to avoid the cell debris as well as blood clots. The whole blood for feline patient should be used or transfer to the patient within 4 hours otherwised it may be contaminated with bacteria. Moreover, the recipient should be observed for any adverse effects such as tachycardia urticaria, hypotension, and haemoglobinuria etc. Though, severe hemolysis from transfusion reaction is rare, it still occurs in some recipient cats. In case with severe transfusion reaction, the blood transfusion need to be stop immediately and other medication such as steroid administration should be considered.

**Conclusion**

Feline Blood transfusion can be a life-saving treatment which becomes increasing in number. However, the blood transfusion safety should be considered for both the donors and the recipients. The pre-transfusion test should be performed in order to avoid the transfusion reaction and other adverse effects which may occur. In addition, patient should be monitored throughout the transfusion procedure. Furthermore, the most important criterion is that the recipient should be diagnosed for the primary cause of blood loss in order to eliminate the problems entirely and to avoid more blood transfusion.

**Acknowledgements**

The authors would like to thanks the staffs from CU VET blood bank, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University.

**References**

Feline Obstruction: Diagnosis and treatment

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Obstruction of the urinary tract in cat results in azotemia, uremia, electrolyte and acid-base disturbance and also is a life-threatening problem. The severity of clinical signs for feline obstruction depends on site, duration, completeness of obstruction and cause. The most common site of obstruction is urethra; however, ureter is another one that is usually overlooked and causes renal failure if left untreated.

Urethral obstruction: the obstruction in urethra can be caused by idiopathic, urethral plug, urolithiasis, or neoplasia. Once it occurs, the urinary bladder distends, and cats show signs of stranguria, licking excessively at perineum and vocalization. The diagnosis should be done before unblocking unless decompression cystocentesis is suggested. It is noted that while unblocking the urethra, the clinicians must aware of the further injuries on urethra and urinary bladder mucosa, therefore, ones must flush and gently advance the urethral catheter simultaneously. Once unblocked, bladder should be flushed for removing blood clot or debris. Indwelling catheterization should be considered based on ability of voluntary urination and characteristics of urine stream. Prevention for recurrence should be discussed with owner about modification of environment and household by introducing the environmental enrichment.

Ureteral obstruction: the obstruction at ureter can occur unilaterally or bilaterally. This condition is hardly to diagnosis because of no specific clinical sign. The obstruction is close to kidney; however, the clinical signs are usually similar to signs of acute renal injury. It is suggested that cat with abruptly changes to abnormal and show sign of uremia, ureteral obstruction should be in the differential list. The means of diagnosis is medical imaging, but ultrasonography is more sensitive than radiography. The dilatation of renal pelvis as well as ureter alerts the clinician for the presence of obstruction. Ureterolith is the common causes of ureteral obstruction. Once obstruction site is detected, surgical intervention or placement of ureteral stent with varying stent types and techniques should be performed. Ideally, kidney function should be measured and if it is severely non-function, nephrectomy is suggested.

Both urethral and ureteral obstruction are life-threatening condition in cat, the clinician must acknowledge the treatment protocol because if unblock earlier, the less injuries occur in kidney and increase the survival rate. However, the recurrence is common, therefore, cause of obstruction must be identified and modified.

References
How to differentiate between cats with respiratory and cardiac problems

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Dyspnea or respiratory distress in cats may cause by cardiogenic or noncardiogenic disease. Dyspneic cats are very fragile and anxiously patients. Therefore handle them with minimal stress is the most important thing to be concerned.¹

There are 5 steps to differentiate primary cardiogenic from primary respiratory causes of dyspneic cats.

Step 1: Patient’s history and a family history of hypertrophic cardiomyopathy, sudden death, arterial thromboembolism, or signalment of breed predisposed to develop familial heritable hypertrophic cardiomyopathy (i.e. Maine Coon, Ragdoll, Scottish fold, British shorthair, American shorthair, Sphynx, Rex, Turkish Van and Siberian cat) may increase suspicion for primary heart disease.¹,² A historical evidence of coughing, retching or wheezing is more consistent with airway disease more than primary congestive heart failure, since cats with heart failure are rarely cough.¹ History of trauma, anticoagulant medication and heartworm prevention should be also undertaken. Heartworm associated respiratory disorder(HARD) in cats is clearly evidence.²

Step 2: Physical examination may reveal variable range of mucous membrane color regardless of the degree of distress.² The murmur heart sound may be auscultated in 60% and 20% of cats with hypertrophic cardiomyopathy and dilated cardiomyopathy respectively. However murmurs can be found in cat with and without underlying heart disease.³ A gallop heart sound is commonly presented in 80% of cats with dilated cardiomyopathy but only 33% in hypertrophic cardiomyopathy cats.¹ Thoracic auscultation will decreased in lung sound if evidence of pleural effusion or pneumothorax.² Crackle lung sound is possibly arise by severe bronchial problems or pulmonary edema (cardiogenic and noncardiogenic causes). Jugular venous pulsation indicated elevated right atrial pressure.³

Breathing pattern identification is the key to localize the origin of respiratory problem.²

a) Rapid, labored, synchronous (chest and abdomen moving together) inspiratory effort pattern is most likely to problem of pulmonary interstitial region which include congestive heart failure pulmonary edema and primary pulmonary problems (i.e. interstitial pneumonia, neoplasia, noncardiogenic pulmonary edema or parenchymal bleeding).

b) Rapid, labored, asynchronous (chest and abdomen moving opposite from each other) inspiratory effort pattern is refer to pleural space problems which could be pleural effusion, pneumothorax or soft tissue occupied (i.e. mass, diaphragmatic hernia).

c) Increased expiratory effort pattern is the result of lower airway problems include acute bronchial constriction, tracheobronchial inflammation and alveolar infiltration (can cause by congestive heart failure lung edema).

d) Loud and slow breathing pattern is markedly refer to upper airway problems (nose to distal trachea).²

Step 3: Thoracic ultrasonography is benefit to evaluate for significant pleural and/or pericardial effusion, assessment of atrial size, left atrial and aortic diameter ratio (greater than 1.8 is possibly related to heart failure), left ventricular function, cardiac wall thickness and cranial mediastinum.¹,² Thoracic focused assessment with sonography for trauma (TFAST)⁴ technique is the most usefulness in dealing with critical respiratory distress cats.

Step 4: Thoracic radiography should be performed only when cat was stabilized enough. The radiographic findings sometime can present of pathognomonic primary pulmonary patterns of bronchial disease or lobar consolidation. Cardiogenic pulmonary edema in cats is most often show diffusive and typically asymmetrical alveolar lung pattern. Valentine shaped heart on dorsoventral or ventrodorsal position is most likely to identify of atrial dilatation.¹ Although the thoracic radiographs is reasonably specific but has a low sensitivity when identifying cardiomegaly in cats with mild structural heart disease. Quantitative and qualitative evaluation of the cardiac silhouette, pulmonary vessels, pulmonary parenchyma and pleural effusion are the most useful diagnostic tools for identification of cardiomegaly and congestive heart failure and for distinguish the cause of respiratory distress in cats.⁵

Step 5: Secondary diagnostic modalities used to investigate the cause of dyspnea include an advanced echocardiogram, computed tomography, bronchoscopy, bronchoalveolar lavage, and additional diagnostic tests.
such as heartworm antigen and antibody test or fine needle aspiration and cytologic evaluation of pulmonary or mediastinal mass. A plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) concentration above 270 pmol/l in cat with respiratory distress is more likely refer to primary cardiac problem. According to one study of NT-proBNP concentration in 167 dyspneic cats, showed higher level in congestive heart failure cats (medians of 754 pmol/l) than non-cardiac cats (medians of 76.5 pmol/l). Although NT-proBNP has a very high negative predictive value (i.e. heart disease is unlikely if NT-proBNP level is normal), other conditions such as hyperthyroidism, hypertension or renal disease can elevate NT-proBNP concentrations. Thus cardiac biomarker testing results should be interpreted in conjunction with other clinical informations.

**References**

Jaundice cats: what is your rule out and treatment

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Jaundice, or icterus, is defined as yellow discoloration of the skin, mucous membranes, and serum. It is caused by increased levels of bilirubin in the blood, or hyperbilirubinemia. Jaundice is clinically detected when the bilirubin is greater than 3 mg/dL.

Pathophysiology

Bilirubin is primarily derived from the breakdown of hemoglobin, which is released from damaged red blood cells in the reticuloendothelial system. Bilirubin is bound to a transport protein, transferred to the liver, and taken up by the hepatocyte, where it is conjugated with glucuronic acid and excreted mostly into bile canaliculi by active transport. Some of unconjugated bilirubin is bound to a protein in the circulation or excreted into the urine. The conjugated bilirubin in the bile is excreted into the duodenum where it is converted to urobilinogen by bacteria in the small intestine. Almost of urobilinogen is excreted into the feces but only a small amount is reabsorbed by the enterocyte, which is then removed from the circulation by the liver or the kidney.

Hyperbilirubinemia can be caused by excessive bilirubin production, impaired hepatic uptake/conjugation, impaired hepatic excretion, or intrahepatic/extrahepatic cholestasis. Extravascular hemolysis is the most common causes of an excess in bilirubin production, but jaundice is only found when moderate or severe red blood cell destruction is present. Hepatocellular dysfunction from any cause can impair the process of uptake, conjugation, or excretion of the liver, resulting in hyperbilirubinemia and icterus. A decrease in bilirubin excretion from either intrahepatic cholestasis or obstructive diseases of the gall bladder, cystic duct, common bile duct, or duodenum can impair bile flow and cause icterus. In cats, hyperbilirubinemia usually precedes bilirubinuria due to their higher renal threshold when cholestasis progresses. Possible causes for jaundice in the cat are listed in table 1.

Table 1 Possible causes for jaundice in the cat

<table>
<thead>
<tr>
<th>Hemopoietic (Prehepatic hepatic)</th>
<th>Hepatobiliary (Hepatic/post-hepatic)</th>
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<tbody>
<tr>
<td>Primary IMHA</td>
<td>Cholangitis</td>
</tr>
<tr>
<td>Secondary IMHA</td>
<td>Hepatic lipidosis</td>
</tr>
<tr>
<td>Hemoplasmas</td>
<td>Neoplasia</td>
</tr>
<tr>
<td>FIV</td>
<td>FIP</td>
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<tr>
<td>FeLV</td>
<td>Toxin</td>
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<tr>
<td>Blood transfusion</td>
<td>Sepsis</td>
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<tr>
<td>Lymphoma</td>
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<td>Cholelithiasis</td>
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<td>Biliary carcinoma</td>
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<td>Biliary rupture</td>
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<td></td>
<td>Liver fluke</td>
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<td>Pancreatitis</td>
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<td>IBD</td>
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</tbody>
</table>

Clinical signs

Jaundice can be detected in the sclera, conjunctiva, mucous membranes, such as gingiva, hard palate, vulva or penis during physical examination. It can also be seen on the inside surfaces of the ears or on the ventral abdomen. Other clinical signs are related to the primary cause of jaundice. For example, weakness and lethargy are common in cats with hemolytic anemia. Anorexia, weight loss, vomiting, diarrhea, abdominal distention with or without pain, polyuria/polydipsia, encephalopathy, or bleeding disorders are clinical signs of cats with hepatic or post-hepatic jaundice.

Diagnosis

A minimum database to evaluate a cause of jaundice as well as concurrent disease or metabolic disorder should include CBC, biochemistry, urinalysis, and testing for FeLV/FIV infections. The first diagnostic step should determine if hemopoietic cause is present. Hemolytic jaundice is often indicated as moderate or severe anemia with normal total protein, and less commonly, hemoglobinemia, hemoglobinuria, or autoagglutination. Further evaluation is performed to determine a cause of hemolysis, such as a blood smear or a PCR assay for hemoplasmosis, or spherocytosis or a Coombs’ test for IMHA. Either hepatic or post-hepatic jaundice is indicated in cases with normal or a mild decrease in hematocrit, and increases in liver enzymes. A marked increase in ALP with less significant increases in ALT and/or GGT might suggest hepatic lipidosis. However, greater increases in ALT and GGT than ALP might indicate cholangitis. A dramatic increase in bilirubin is common in a biliary obstruction while a mild to moderate elevation in bilirubin alone may be seen in cholestasis secondary to sepsis (1).

Differentiating hepatic jaundice from post-hepatic jaundice is more difficult, but it is important for decision of a specific treatment and prognosis. Diagnosis can be performed with a minimally invasive method like abdominal ultrasonography. It is helpful for assessment of the liver, cystic duct, common bile duct, gall bladder, pancreas, and duodenum. More invasive techniques, including a fine needle aspiration, liver biopsy, or laparotomy may be needed if a definitive diagnosis is not indicated by abdominal ultrasonography (2).

Additional diagnostic tests may include bile acids and coagulation profile to assess liver function, thoracic radiographs to assess metastasis, and abdominocentesis with fluid analysis and culture to assess bile peritonitis.
Treatment

Treatment goals should address the primary disease and supportive care. Antimicrobials susceptible to the causative agent of hemoplasmosis, neutrophilic cholangitis, and sepsis are required (3). With severe hemolytic cases, blood transfusions are warranted. Glucocorticoids may be used with lymphocytic cholangitis, IMHA, or IBD (4). Chemotherapy is considered in cases of lymphoma. Surgery is required for bile peritonitis, biliary obstruction, or resection of tumor. Supportive treatment dependent on individual cases may include fluid therapy, electrolyte supplementation, antiemetics, antioxidant, cholerectics, and enteral nutrition.

References

โรคเบาหวานในแมว เป็นโรคที่เกิดความผิดปกติของฮอร์โมนที่สำคัญโรคหนึ่งในแมว ซึ่งสามารถเกิดได้ทั้งแมวเพศผู้และเพศเมีย เป็นได้ทุกช่วงอายุ แต่พบมากในแมวอายุมาก ในต่างประเทศมีรายงานว่า แมวที่มีโรคนี้ในประเทศไทย คือแมวพันธุ์ Burmese แต่ในประเทศไทย พบว่า พันธุ์ Domestic short hair เป็นโรคนี้ได้ โรคเบาหวานในแมวส่วนใหญ่เป็นชนิด non-insulin dependent diabetic mellitus (NIDDM) เมื่อเทียบกับในคนคือเบาหวานชนิด Type II ซึ่งหมายความว่า ร่างกายของแมวหรือของคนมีฮอร์โมน อินซูลินอยู่ แต่อินซูลินที่มีอยู่ไม่ออกฤทธิ์ได้อย่างเหมาะสม จึงทำให้เกิดอาการของโรคเบาหวานได้ โดยปัจจัยที่ทำให้อินซูลินออกฤทธิ์ไม่ดีได้แก่ ความอ้วน กิจกรรมที่ลดลง หรือถูกเลี้ยงให้อยู่ภายในบ้านตลอดเวลา หรือแมวได้รับยาโรคกลุ่มสเตอรอยด์เป็นต้น

อาการของแมวที่เป็นโรคเบาหวาน ได้แก่ กินน้ำมากกว่าปกติ (polydipsia) ปัสสาวะมากกว่าปกติ (polyuria) กินอาหารมากกว่าปกติ (polyphagia) อวัยวะที่ทำงานเกี่ยวกับการควบคุมระดับน้ำตาลในเลือด (hyperglycemia) ร่วมกัน ปัสสาวะมีน้ำตาล (glucosuria)

การรักษาโรคเบาหวานในแมว ประกอบด้วย 3 ส่วน
1. การใช้อินซูลิน
2. การควบคุมด้วยอาหาร
3. การออกกำลังกาย

1. การใช้อินซูลิน ส่งเสริมการควบคุมน้ำตาลในเลือด โดยให้อินซูลินที่มีความถูกต้อง เลือกใช้อินซูลินที่ออกฤทธิ์นาน เช่น Glargine หรือ Determir ที่มีการใช้ในมนุษย์ แต่ไม่ได้ใช้ในแมวอย่างมาก ดังนั้นการใช้ Determir ที่มีสภาวะน้ำตาลในเลือดสูงเป็นวิธีที่ดีที่สุด แต่การใช้ Glargine ที่มีความถูกต้องเป็นวิธีที่ดีที่สุด แต่เป็นการใช้ในมนุษย์

ตารางที่ใช้ในการปรับปรุงอินซูลิน

<table>
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<th>Parameter</th>
<th>การปรับปรุงอินซูลิน</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. อาร์มาร์ค่าต่ำสุดของกลูโคส (Nadir) = 90-160 mg/dL.</td>
<td>ไม่ปรับปรุงอินซูลิน</td>
</tr>
<tr>
<td>2. อาร์มาร์ค่าต่ำสุดของกลูโคส (Nadir) &gt; 160 mg/dL.</td>
<td>เพิ่มอินซูลิน 0.25-1.0 U เช่น เลือกใช้อินซูลิน 2.5 U ให้เพิ่มอินซูลินเป็น 2.75-3.5 U</td>
</tr>
<tr>
<td>3. อาร์มาร์ค่าต่ำสุดของกลูโคส (Nadir) &lt; 90 mg/dL.</td>
<td>ลดอินซูลินลง 50% เช่น เลือกใช้อินซูลิน 2.5 U ให้ลดอินซูลินเป็น 1.25 U</td>
</tr>
<tr>
<td>4. อาร์มาร์ค่าต่ำสุดของกลูโคส (Nadir) = 90-160 mg/dL.</td>
<td>ไม่ปรับปรุงอินซูลิน</td>
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</table>

การตรวจภาวะภาวะน้ำตาลในเลือด (hypoglycemia)
การรักษาโรคเบาหวานในแมวโดยใช้อินซูลินชนิด Glargine หรือ Detemir โดยเฉพาะแมวที่วินิจฉัยได้ก่อน 6 เดือนที่จะเริ่มรักษาพบว่าการให้อินซูลินชนิดนี้ประมาณ 1-1½ เดือนสามารถหายป่วยได้ ซึ่งเรียกว่า Diabetic remission

2. การใช้อาหารร่วมในการรักษาโรคเบาหวานในแมว หลังจากการวินิจฉัยโรคเบาหวานในแมว ควรเตรียมทำอาหารสำหรับโรคเบาหวาน เพราะแมวจะเริ่มหิวเพื่อโอกาสเป็น Diabetic remission ได้ก็ต่อเมื่อหาอาหารที่เหมาะสมสำหรับโรคเบาหวานในแมว ควรเป็นอาหารที่มีส่วน carpbohydate และ high protein โดยมี carpbohydate < 15% ME (Metabolizable energy) หรือ 45% dry matter ซึ่งถ้านำมาเปรียบเทียบกับการใช้อินซูลินชนิด Glargine จะทำให้เกิด Diabetic remission ได้ถึง 85% เหตุผลที่ไม่ควรใช้อาหารที่มีอัตราส่วนของ carpbohydate สูงเพราะว่า อาหารที่มี carpbohydate สูงจะทำให้ระดับน้ำตาลในเลือดอย่างต่อเนื่อง ทำให้เกิดภาวะ glucose toxicity ต่อ β-cells จนทำให้ผลิตอินซูลินจากβ-cells ลดลง ทำให้แนวโน้มอาการของโรคเบาหวานได้ และอาหารที่มีคาร์โบไฮเดรตสูงจะทำให้แมวอ้วนได้ ส่วนอาหารที่มีโปรตีนสูงนั้นช่วยป้องกันการสูญเสียกล้ามเนื้อในขณะที่กำลังลดน้ำหนักของแมวอ้วน

รูปแบบของการกระทำอาหารเป็นมากกว่าอาหารเม็ด เหลือเพียงแค่ในกระบวนการผลิตอาหารมีส่วนสำคัญ อาจไม่สามารถทำให้อาหารเป็นอันแน่น สำหรับอาหารเปียก อาจทำให้อาหารเป็นน้ำในอาหารทำให้เกิดความเสียหายได้เร็ว ส่วนอาหารเม็ดที่กินต่อวันซึ่งถูกแมวเลาะลั่น บางตัวใน 4 ถึง 6 วัน บางตัวกินมากกว่านั้น แนะนำให้คำนวณปริมาณพลังงานที่กินต่อวัน แล้วแบ่งตามจำนวนเม็ดที่ให้ โดยทั่วไปนั้นควรให้ในปริมาณ 40 Kcal/กิโลกรัม/วัน

หลังจากแนวคิด Diabetic remission แล้ว ยังคงแนะนำให้กินอาหารประเภทนี้ตลอดไป เพื่อป้องกันไม่ให้กลับไปเป็นโรคเบาหวานใหม่ และพยายามอย่าให้มีน้ำหนักมากขึ้น

3. การออกกำลังกายแมวที่ถูกเลี้ยงไว้ในลักษณะภายในบ้านตลอดเวลา มักจะอาศัยและทำกิจกรรมต่างๆประจำวันน้อย ปัจจัยเหล่านี้มีความเสี่ยงที่ทำให้เกิดโรคเบาหวานในแมวได้ ดังนั้น เจ้าของแมวควรจัดกิจกรรมเพิ่มขึ้นให้แมวมีแนวโน้มเลี้ยงในลักษณะนี้ เช่น มีของเล่นเพิ่มขึ้น เจาของเล่นกับแมวที่เล็กกว่าแมวตัวนั้น

สรุปการจัดการโรคเบาหวานในแมวควรทำ 3 สิ่ง ได้แก่ การให้อินซูลิน การให้อาหารชนิด low carbohydrate high protein และการทำกิจกรรมเพิ่มขึ้น เพื่อเพิ่มโอกาสในการ Diabetic remission

เอกสารอ้างอิง
What to do with pruritic cats

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Introduction

Dealing with pruritic cats is considered as a difficult approach for general practitioners as there is no distinctive pattern for feline skin diseases. Individual cat may present with varied clinical presentations with same disease. Pruritic cats, therefore, can be a challenge for veterinarians and systematic approach is essential for dermatological approach in cats. Pruritic cats always show clinical signs of excessive licking and compulsive over grooming. However, this may be misinterpreted by owner as normal self-grooming behavior. Veterinarian should evaluate pruritic levels to obtain information on how the owner concern, severity of disease, differentials and appropriate antipruritic drugs.

Clinical lesions

Pattern analysis and recognition of relevant lesions is important for creating objective and reasonable differential lists. In contrast to dog’s skin diseases that may have some common findings for certain diseases (For example, dogs with flea bite hypersensitivity usually have lesions at lumbosacral and inguinal areas), cats tend to have an inexplicit skin patterns. The common skin patterns in cats are miliary dermatitis, feline traumatic alopecia, eosinophilic granuloma complex, feline acne and head and neck dermatitis.

Miliary dermatitis is one of the most common feline skin lesions. It is characterized by papules that may develop a crust. The lesion is most commonly seen at truncal region and may be combined with alopecia and hypotrichosis. The most common diseases to cause miliary dermatitis in cats include flea bite hypersensitivity, atopy, food allergy and dermatophytosis. The less common diseases such as ectoparasitism, pemphigus foliaceus and bacterial infection may also, at least extent, present with miliary dermatitis and ectoparasitism.

Feline traumatic alopecia is a consequence of excessive grooming. It is a key feature to indicate pruritus of the cats. Concurrent erythema and popular lesions may be seen although the skin often appears unaffected in most of the cats with traumatic alopecia. The associated allergic causes are feline atopy, food allergy, flea-bite and insect-bite hypersensitivity. Ectoparasites are also common causes of pruritic skin disease of the cats including cheyletiellosis, feline scabies and demodicosis (D. cati or D. gatoi).

Eosinophilic dermatoses are usually seen in pruritic cats caused by ectoparasitism or hypersensitivity. Presenting lesions include eosinophilic plaque, indolent ulcer and eosinophilic granuloma.

Chin dermatitis or feline acne is often seen in male and older cats. The chin lesions may be associated with allergic skin diseases and progressive to be furunculosis or secondarily infected by bacteria.

Head and neck pruritus are typically associated with lesions restricted to face, head and neck. The lesions include excoriations, self-induced alopecia, erythema, crusts, erosions, and/or ulcerations. The types of lesions seen on clinical examination will vary according to the pruritus severity. Etiology of this pattern include hypersensitivities (most commonly food allergy but may also be seen in atopic dermatitis, flea bite allergy, and mosquito bite allergy), parasitic dermatoses (i.e. otoacariosis, nootedes, and trombiculosis), and infectious (i.e. dermatophytosis and herpes virus dermatitis).

Systematic approach

Obtaining complete history and physical examination: A detailed history is a key step to gather all useful information regarding animal’s basic care (diet, snack, bath, vaccination, ectoparasite prevention etc.) lifestyle and environment (indoor/outdoor, yard, other animals in contact). Moreover, complete disease history (pruritic levels, onset, progression, severity, previous diagnosis and treatment and concurrent diseases) is also essential and very useful for diagnostic procedures. Physical examination findings are beneficial for veterinarians to evaluate whether the animal is pruritus (keystone for dermatological approach) especially when owners are not able to describe the clinical signs. A comprehensive general examination and dermatological examination should be performed to indicate type and distribution of lesions, severity, chronicity including possible concurrent/underlying diseases

Building up reasonable differentials: Veterinarians normally use history and physical examination findings to build up a sensible differential diagnosis. This will narrow down the scope of possible causes leading to a time- and cost-saving selection of diagnostic tests for patient.

Selection of appropriate tests: Diagnostic tests should be chosen based on reasonable differentials that derived from detailed history and physical examination.
Veterinarians should consider to perform the tests with most important consideration first regarding usefulness, availability, cost, type of diseases, disease severity and progression. Skin scrapings, comb brushing and cellophane tape are basic skin tests to detect ectoparasites, flea and flea feces, bacteria and yeast. Trichogram may reveal broken hair tips indicating itchiness of the cat or arthrospores of dermatophytosis. Wood’s lamp has limited usefulness as only 50% of cat with M. canis infection will show positive apple-green fluorescence. Dermatophyte test medium (DTM) may be carried out to confirm the diagnosis of dermatophytosis. Other tests such as FIV and FeLV tests, hematology and blood chemistry profiles are also useful to find out any concurrent diseases or general health status of the cat. Skin biopsy has a benefit when basic tests cannot give a useful information or confirm the diagnosis. Allergy testing is a useful test to identify causative allergens and a prerequisite for allergen specific immunotherapy (ASIT).

Choosing appropriate antipruritics: Antipruritic therapy normally used in cats include antihistamine, omega-3, glucocorticoids, cyclosporine and allergen specific immunotherapy.

- **Antihistamines** have unpredictable antipruritic effects. Clinical benefit may be seen in 20-30% of cases. Assessment of antihistamine effects should be followed after 2 weeks of medication.
- **Glucocorticoids** There have been reported that cats have fewer steroid receptors than dogs resulting in higher dosage, approximately twice that of the dogs. Moreover, cats tend to tolerate glucocorticoids better than other species so that longer acting glucocorticoids may be used in some cases which would not be used in the dogs. Blood glucose and urine glucose should be regularly monitored as the cats may develop diabetes mellitus especially when using injectable form.
- **Cyclosporine** is an immunosuppressive drug that has been used for the treatment of atopic dermatitis and, in some cases with autoimmune diseases. that was developed for veterinary application as Atopica®, so named for the disease being treated in the dog. Both formulations are extra-label in cats with the extrapolation of the dosage from the dog where 5mg/kg is minimal. The recommendation is to give medicine daily for 4-6 weeks then decrease to every other day and eventually 2-3 times per week depending on clinical response. According to immunosuppressive action, some associated diseases such as toxoplasmosis has been observed in some cases. This drug has been considered as an excellent alternative to glucocorticoid therapy and beneficial to lower dosages of glucocorticoids.

- **Allergen specific immunotherapy (ASIT)** is an excellent alternative to other choices or in conjunction with combined therapy for feline atopy. 50% of atopic cats showed improvement of clinical signs making it a good choice especially when owners are unable to pill the cats. It is often more appealing than oral therapy because of avoidance of oral administration. Modification of allergen not to exceed more than 0.5 ml of maintenance allergen solution at any single injection is recommended. Most cats do well with an injection once a week of 0.3 mis. or 0.15 mis. twice weekly. ASIT usually takes several months up to a year to achieve clinical improvement, therefore, other antipruritics may be initially prescribed.

In summary, clinical approach for cats with skin diseases can be complicated as it tends to be no specific clues for the diagnosis. Therefore, veterinarian should employ systematic approach to ensure complete history and physical examination findings, rational differential diagnosis then select proper confirmative diagnosis and treatment regimens for cats. Antipruritics are necessary to relieve pruritic signs but, most importantly, it is crucial to identify underlying or primary causes and solve the problems leading to a complete resolution of clinical signs.

References
Classification of the intervertebral disc herniations: why are they different?

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Intervertebral disc herniation (IVDH) is described as an abnormal localized displacement of the intervertebral disc beyond the related intervertebral disc space. IVDH in dogs are usually detected in dorsal or dorsolateral direction which effect on structures within the intervertebral foramen and spinal canal (spinal cord, cauda equine and/or nerve roots) (1). IVDH commonly occurs in cases of intervertebral disc degeneration but sometimes IVDH can occur in nondegenervative intervertebral disc dog with exogenous trauma. In veterinary medicine, IVDH is currently classified as intervertebral disc extrusion (IVDE, Hansen type I), intervertebral disc protrusion (IVDP, Hansen type II), acute non-compressive nucleus pulposus extrusion (ANNPE), hydrated nucleus pulposus extrusion (HNPE) and intramedullary intervertebral disc extrusion (IM-IVDE) based on morphology, gross pathology and magnetic resonance imaging (MRI). The dogs with different types of IVDH can associate with different signalment, history, clinical signs, onset of neurological deficits, treatment plans and potentially predicted outcome with prognosis to the owner. Consequently, it is important to identify the type of IVDH that can be investigated by using MRI diagnosis. MRI is widespread accepted as a modality of choice for evaluation the type of IVDH and pathological changes of the affected spinal cord. Spinal MRI protocols commonly include T2W sagittal images, followed by T2W and T1W transverse images at the selected locations based on information from initial sagittal images and previous clinical neurolocalization. Additional MR sequences such as STIR, T1W and post-contrast T1W chemical fat suppression are sometimes preferred to increase the sensitivity and detectability of IVDH evaluation.

Intervertebral disc degeneration with herniation was defined by Hansen as structural failure of the intervertebral disc associated with abnormal or accelerated changed in aging. In dogs, histological degenerative changes are classified as chondroid or fibroid degeneration. The degree of disc degeneration in dogs can be defined based on the signal appearance in nucleus pulposus from T2W images. IVDE or Hansen type I or chondroid degeneration is mostly found in young chondrodystrophoid dogs and usually presented with acute onset neurological deficit. Clinical signs appear from variable degrees of spinal hyperesthesia to paraplegia with loss of nociception. IVDP or Hansen type II or fibroid degeneration usually presents in aging dog with insidious clinical onset and clinical signs are less severe as ambulatory tetra/paraparesis and proprioceptive ataxia. To improve diagnosis accuracy of thoracolumbar intervertebral disc extrusion and protrusion, proposed MRI guidelines (lateralized intervertebral disc herniation, partial instead of complete intervertebral disc degeneration, multiple intervertebral disc herniations and herniated disc material confined to intervertebral disc space) are effectively used to differentiate both types of IVDHs (2).

Acute non-compressive nucleus pulposus extrusion (ANNPE) is a type of IVDH that commonly present with acute onset of clinical signs. ANNPE has been referred to as Noncompressive disc extrusion, Gunshot or Bullet-like IVDH, High velocity-low volume IVDH or traumatic disc extrusion because the small nondegenerated nucleus pulposus extrudes during hard exercise or trauma. These can effect on spinal cord contusion with mild or without spinal cord compression. MR images usually present a decrease of hydrated nucleus pulposus volume, localized hyper-signal intensity within the affect spinal cord, clotted blood/ hematoma and minimal spinal cord compression in T2W images. Several studies report that the severity of neurological signs and MRI lesions (extent of the intramedullary hyper-signal intensity on sagittal and transverse T2W images and maximal cross sectional area of the intramedullary hyper-signal intensity on transverse T2W images) can help to predict of outcome in dog with ANNPE. ANNP in dogs are frequently detected in the T3-L3 spinal cord segment and in especially T12-T13, T13-L1 and L1-L2 intervertebral disc spaces are most commonly affected.(3)

Hydrated nucleus pulposus extrusion (HNPE) is identified by the displaced hydrated disc material which
presents iso-signal intensity to hydrated nucleus pulposus compress on adjacent spinal cord in sagittal and transverse T2W images. Tearing of dorsal annulus, decreasing of nucleus pulposus and narrowing intervertebral space of affect intervertebral disc are possibly detected. The decision of surgical decompression or conservative treatment in dogs is based on severity of neurological dysfunction and spinal compression. Most of the compressive HNPE in dogs are represented in the cervical spine with acute onset, symmetric non-ambulatory tetraparesis or tetraplegia and respiratory dysfunction is sometimes found in dogs. The median age at the point of diagnosis is 9 years (range from 8 to 13 years) and most dogs are male and non-chondrodystrophic breeds. The most frequent affect intervertebral disc space in dogs with are C4-C5 and C3-C4 respectively (4).

Intramedullary intervertebral disc extrusion (IM-IVDE) is defined as part of intervertebral disc material (possibly from nucleus pulposus) powerful injection into spinal cord by penetrating the meninges. MRI findings include an appearance of hyper-signal intensity linear tract in vertical direction above the affect intervertebral disc space on T2W images. Localized spinal cord contusion and swelling can also be detected. The prognosis of the dog with IM-IVDE is guarded with severe clinical signs and absent of nociception.

References
Diagnostic Imaging of the Skull

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Due to complex anatomy, consisting of numerous soft tissues, air and osseous structures, and involving multiple systems, therefore, investigation and diagnosis of the abnormality of the head are challenging and essential.

Radiography is the first modality widely available in veterinary practice. Indication for skull radiography include traumatic injuries, nasal discharge, facial deformation, otitis, oral and dental disease, exophthalmos and neurological problem. Radiography gives 2D information, well-suited for imaging bones and air structures but causes anatomic superimposition. Sedation or general anesthesia, proper positioning and exposure setting are required to obtain high-quality image.

Using low kVp, high mAs and a small focal spot can improve geographic sharpness and ability to see fine osseous detail. For an average dog, the mAs is 10 to 15, with 55 to 65 kVp (1).

**Basic positioning for skull, sinuses, TMJ and tympanic bullae:**
- Lateral projection; the nose pararel to table and central beam to the mid cranium.
- Ventrodorsal/ dorsoventral projection; stabilize the head, set the central beam to the midlevel of the zygomatic arch.

**Special projection for nasal cavity:**
- Ventrodorsal open-mouth projection; mandible is caudal to maxillary premolar, center the beam to the midline of hard palate.

**Special projection for frontal sinus:**
- Rostocaudal oblique skyline projection; the patient is in dorsal recumbency position, the nose is perpendicular to the table, center the beam between the eyes.

**Radiographic interpretation**

**Nasal chamber**
Rhinitis appears as diffusely increased nasal opacity with blurring of turbinates and preferentially affects the rostral aspect of nasal cavity without septal deviation. The nasal foreign body is usually radiolucent (eg. grass awn) and appears as focal increased soft tissue opacity with minimal turbinate destruction. Acute rhinitis, foreign body, viral, bacterial and allergic rhinitis may appears as normal radiographs.

Initially, nasal neoplasia is unilateral, often originate from caudal aspect, and may affects both sided in later stage. Destruction of the ethmoturbinates with lysis of septum are the most specific findings. The tumor may erode through the nasal and maxillary bone or cribiform plate into orbit or cranium.

**Frontal sinus**
Traumatic injury might show loss integrity of frontal bone, and should be considered opened fracture due to connection to nasal cavity.

Increased opacification of the sinus is often affected by extension of the nasal disease, but tumor expansion or retention of secretion due to blockage of drainage cannot be differentiate radiographically. Aggressive frontal bone lysis is usually caused by neoplasia, which may originates within sinus or extends from adjacent tissue.

**Aural**
The external ear canals and tympanic bullae are air-filled structures and should be symmetry. Increased in opacity may caused by accumulation of secretion or luminal masses. Mineralization of aural cartilage with narrowing of the lumen occurs with chronic inflammation and hyperplastic mucosa. The bulla wall should be thin and uniform in thickness with breed variation in shape. Thickening of the wall usually occur from infection, indicating otitis media. Cat may have signs of middle ear disease, secondary to nasopharyngeal polyp. Expansion of the tympanic cavity usually due to slow-growing luminal mass eg. cholesteatoma. Lysis of the osseous bulla and the adjacent TMJ or calvaria is highly suggestive of neoplasia.

**Oral and dental structures**
Periodontal disease result in widening of lucent periodontal ligament space combine with horizontal or vertical loss of alveolar bone, furcation exposure and periapical lucency. Other signs of dental diseases include widening of the pulp cavity and irregularity outline of the tooth root. Pathological fracture of mandible is possible in severe case. Oronasal fistulas are not always evident on radiographs.
Benign oral and dental tumors (e.g. dentigerous cyst, odontoma, fibromatous epulis), characterized by expansile lytic mandibular or maxillary lesion with well defined margins. Malignant oral soft tissue neoplasia (e.g. squamous cell carcinoma, fibrosarcoma, malignant melanoma) are predominantly lytic while some are osteoproductive.

**Cranial vault**

There are marked variation in conformation of the cranial vault, the suture lines are clearly visible and should not be mistaken for fractures. In some individuals, the bregmatic fontanelle may persists into adult life. (2) Skull fracture may be visible radiographically if displacement occurred, usually accompany by soft tissue swelling with subcutaneous gas in case of dog bite. Hydrocephalus is excessive accumulation of cerebrospinal fluid within the ventricular system of the brain, characterized in radiographs by doming of calvaria and cortical thinning, persistent fontanelle and homogeneous appearance to the brain. Breed predisposition to congenital hydrocephalus include Chihuahua, Pomeranian, Pug, Yorkshire Terrier. (3)

Most primary tumor arises from skull are malignant. Osteosarcomas of the cranial vault do not resemble those from appendicular skeleton, because they tend to be osteoblastic, have well-defined border, contain granular area of calcification and are less likely to metastases. (3)

**Other imaging modalities**

Ultrasonography is the only modality that does not use electromagnetic radiation. Ultrasonic examinations are now widely used and become a necessary diagnostic imaging test. Ultrasound waves have good penetration to soft tissues and fluid, therefore they are useful to evaluate orbital diseases and brain disorder.

The brain and ventricular system of small animal can be imaged through the fontanelle, the most common application of neurosonography is to measure ventricular size when diagnosing hydrocephalus. The normal lateral ventricular size, measured in dorsoventral dimension on transverse images, is range from 0.4 to 3.5 mm. The normal ratio of dorsoventral dimension of lateral ventricle to the brain (dorsoventral percentage) is 0.14%. The degree of ventriculomegaly ranges from moderate (15-25%) to severe (>25%) which occupy most of the brain.

Color flow Doppler imaging can be used to image the basilar artery or the rostral cerebral artery. Evaluation of vessels may be helpful when the brain is injured or damaged by disease. Velocities of the blood flow in these vessels are measured and the resistance index (RI) are calculated with the following equation:

\[
\text{Resistance index (RI)} = \frac{\text{peak systolic} - \text{end diastolic velocity}}{\text{peak systolic velocity}}.
\]

RI at the basilar artery is higher in dog with clinical hydrocephalus. (2)

Ultrasound examination is also useful for diagnosis of intra-ocular and extra-ocular diseases eg. differentiates between the retrobulbar cellulitis, abscess and neoplasia, identification of foreign body, assessment of the eye in cases with anterior segment opacity or excessive soft tissue swelling.

Cross sectional imaging techniques of Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are being used commonly for imaging of the head, due to the ability to provide images without superimposition of the structures and higher contrast resolution compared to radiography. These methods aid in determine the extension of the disease, differentiate between benign and malignant disorders and evaluate metastasis, which are valuable for surgical planning.

CT images originate from x-ray beam, represent the mean x-ray attenuation, so the interpretation is resemble to radiography. CT is the method of choice for evaluation of bony diseases of the head and intracranial hemorrhage. 3D reconstruction image allows the true extension of the complex lesions to be evaluated.

MRI is the technique of choice for intracranial disease and most diseases affecting soft tissue of the head eg. abnormality of the brain and ventricular system, meninges, cranial nerves, vascular condition, extensive of extracranial tumor, the middle and inner ears, the musculatures, orbital structures and globe, salivary gland, lymph nodes and oral cavity.

Individual imaging modality provides different diagnostic informations, veterinarians should select the suitable examination(s) for each patient and disease condition accordingly.

**References**

Multimodality imaging of vascular disease in dogs and cats

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Introduction
Vascular disease can occur throughout the body and can be critical and acute death. If the abnormality occurs at the superficial, it could be easily detected by a general physical examination such as a focal area of tissue swelling due to congestion, cyanosis because of lacking blood supply. If these vascular diseases occur inside the body, it would be difficult to diagnosis by physical examination alone. It requires more complex procedures to investigation and the diagnostic imaging play an important role in the diagnosis of vascular disease. There are several vascular disorders, but the common disorders are bleeding, vascular occlusion and vascular malformation as will be discussed later.

Bleeding
Bleeding can occur from traumatic or non-traumatic in origin. Common causes of traumatic bleeding are blunt trauma in road traffic accident or dog fighting and penetrated wound with organ rupture or vascular injury. Non-traumatic cause, also called spontaneous bleeding, is frequently caused by malignant neoplasia and coagulopathy. In case of trauma, particularly thorax, the radiography is the first line imaging because it can detect hemothorax and other associated abnormalities such as diaphragmatic hernia, lung contusion or skeletal fracture at the same time. While ultrasound is a modality of choice in detection of the abdominal bleeding because ultrasound has a higher sensitivity and specificity than radiography in detection of free fluid in the abdomen, which is the important indicator of abdominal bleeding. The hemorrhagic effusion usually appear anechoic or echogenic with swirling of particles within the fluid. The ultrasound probably used for localize the source of bleeding such as visceral organ tear or mass rupture. Currently, The use of the Thoracic and Abdominal Focused Assessment with Sonography for Trauma (TFAST and AFAST) is increasing in emergent and critical care unit. TFAST and AFAST provide a rapid triage for patient suspicion of internal bleeding. Serial examination by using a FAST scoring system is helpful for monitoring and help better make decision regarding progressive bleeding, resolving bleeding, and need for blood transfusion and/or exploratory laparotomy. However, in some conditions, the ultrasound can not provide good sensitivity and specificity enough to define the source of bleeding, particularly if the bleeding came from vascular injury or there are multiple bleeding sources in the abdominal cavity. CT angiography provides excellent information about anatomical abnormality such as organ laceration The different attenuation values can distinguish hypoproteic fluids, such as ascites (<15HU) from hemoabdomen (~40 HU). The characteristic of extravascular high-attenuating area is evident of an active bleeding or presence of clotted blood adjacent to the bleeding site, called sentinel clot sign represent the source of active bleeding. Apart from bleeding issues, CT scan enables the consequent outcome such as organ infarct or ischemia.

Vascular occlusion
Vascular occlusion is usually underdiagnosed until the clinical sign is present. Vascular thrombosis is one of the most common abnormalities in dogs and cats. Radiography has poor sensitivity and specificity because it detect only the indirect signs of complete vascular occlusion, such as peripheral edema, ascites or organomegaly. Ultrasound is a great non-invasive tool in the diagnosis of vascular thrombosis because its ability in directly detects the thrombus by using B-mode and color-doppler function. Ultrasonographic findings of vascular thrombosis are absence of flow or unorganized flow around an immobile mildly echogenic structure within the affected vessel lumen. Besides, ultrasound can provided information about the effects of thrombosis such as the extent and degree of parenchymal ischemia and coexisting abnormalities. However there is limitation of ultrasound, it is inability or difficulty visualizing thrombosis in small parts or pulmonary vasculature and the competency in diagnosis depends on machine quality. These would be overcome by the CT angiography. CT not only provides excellent information about the size, shape, location of thrombus but also provide a clear visualization of subsequent collateral circulation. Another vascular occlusion is torsion or volvulus. Torsion is defined as twisting of organs and its vessels resulting in lacking of the blood supply. Radiographic findings associated with torsion are not specific. Similar to thrombosis, ultrasound is a good noninvasive diagnostic tool in unstable patient. Splenomegaly with diffuse, lacy, hypoechoic echotexture and complete absence of flow at splenic hilus is highly suggestive of splenic torsion. In some condition
such as mesenteric volvulus and lung lobe torsion, the radiography and ultrasonography give an inconclusive result. CT angiography has been used to confirm the diagnosis. The supportive CT feature for mesenteric volvulus is the whirl sign due to bowel loops, blood vessels, and fat twisting around the mesenteric attachment. Whereas abruptly interrupted patency of a bronchus is a significant feature of lung lobe torsion.

**Vascular malformation**

Vascular malformation is a general term of abnormal connection between artery, vein or portal system. These lesions can be congenital or acquired, single or multiple and can be identified throughout the body. There are several types of these abnormalities. The main vascular malformation that have clinical significant are portosystemic shunt, arteriovenous malformation or arterioportal fistula and vascular anomalies. The radiography is failure to reach conclusive diagnosis in these kinds of abnormalities. Ultrasound is often used for the initial procedures in diagnosis of intra-abdominal vascular malformation because it is quick and non-invasive. The use of color Doppler is essential because many shunts are too small to be resolved using B mode alone. For the portosystemic shunt, Ultrasonographic findings include identifying single or multiple anomalous shunt vessel drainage into enlarged caudal vena cava. The liver is often small and decreases visibility of intrahepatic portal vein in the congenital portosystemic shunt. The portal vein size has a significant predictive value in shunt investigation. The congenital extrahepatic shunt is suspected if the main portal vein is smaller in diameter compare with its tributaries. From the study, ratio comparing the luminal diameter of the portal vein (PV) with the maximal luminal diameter of the aorta, (at cranial abdomen) or PV-aorta ratio < 0.65 predicts the presence of an extrahepatic shunt, whereas a ratio > 0.8 excludes this type of PSS. Decrease portal vein velocities to less than 10 cm/s in dogs and presence of free fluid in peritoneal cavity indicate concurrent portal hypertension. CT angiography have been described to better characterize vascular ring anomaly and confirm aortic arch abnormalities.

Another important vascular anomaly is vascular ring anomaly. Vascular ring anomaly is congenital aortic arch abnormalities constrict of the esophagus at the level of heart base resulting in dilation of the cranial esophagus, and leading to regurgitation. Because of its position in the thoracic cavity, the trachea may also be entrapped within the ring. The typical clinical history and signalment of young animal with frequent regurgitation beginning at the time of solid food introduction and radiography reveal an esophageal pouch cranial to the heart base highly suggestive of vascular ring anomaly. The positive contrast esophagram can be performed to confirm evidence of external esophageal compression at heart base region. From one study, In all 52 dogs with persistent right aortic arch had leftward deviation of the trachea at heart base. However, CT angiography have been described to better characterize vascular ring anomaly and confirm aortic arch abnormalities.

**Conclusion**

Even if almost the vascular disease are better defined with CT angiography, other conventional imaging methods are important and can be used for the initial diagnostic imaging tool because it is quick and non-invasive and can be reach a final diagnosis in some vascular disease. CT angiography can be used aid in diagnosis in case of advance vascular disease,because it can provide useful and extensive information. However potential nephrotoxicity, the need of general anesthesia limit the use of CT in critically ill patient.

**References**

Experience with the IMEX-SK™ Linear-Circular External Skeleton Fixator Hybrid Constructs and Metal Acrylic for fracture Stabilization in Cats

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External skeleton fixator (ESF) is a widely accepted method for stabilization of long bone fractures and non-union. ESF with increased stiffness have been shown to improve the rate and quality of fracture healing while decreasing pin-tract complication (1,2). The Kirshner-Ehmer (KE) ESF has been the most widely used device in small animal fracture stabilization and several studies have documented its mechanical characteristic (1,3,4,5,6,7) and clinical efficiency (4,8,9,10,11,12,13). The weakest component of the KE system is the connecting rod and the majority of the elasticity of this system is due to connecting rod flexibility (2,5,14). In order to achieve adequate bone segment stabilization, numerous authors have recommended the use of more complex type II and type III frames (2,13,14,15). The IMEX-SK™ develop ESF system utilizes larger diameter connecting rods, therefore overcoming one of the weaknesses of the original KE system, the study results demonstrated that larger connecting rods have a significant effect on overall frames stiffness. The use of two or more full-pins, as in type II and III techniques nullified the contribution of the connecting rod diameter and frame stiffness was more a factor of pin size, number and orientation (16).

The IMEX-SK™ external skeleton fixator has two types, linear and circular ESF system (17, 18). Linear – circular external skeleton fixator hybrid constructs are the combination of both system (19).

The IMEX-SK™ external skeleton fixation system are described. The mini SK™ and mini hybrid SK™ can be used in cat. The components of mini SK™ are single clamp can be connecting rods, connecting rods 3.2 mm diameter, utilizes 7 mm angle and combination wrench and double clamp connecting rod to rod. The components of mini hybrid ESF are full ring made from aluminum are slotted for wire position freedom and can be cut at holes to convert into arches or half-rings. Hybrid rod with 4 mm thread for mini SK™, fastener are cannulated hex bolt, 4 mm hex nut, 4 mm flat washer, 4 mm nylon nut, 4 mm spherical nut and 4 mm spherical washer. Instruments are 7 mm combination wrench, 7 mm angle open socket wrench and 7 mm double wrench for nylon nut.

ESF pins are K-size wire, shank diameter 0.9 mm- 2.4 mm negative - profile thread and 2.0 – 2.4 mm positive profile end-thread and center-thread pins. Stopper wires 1.0 mm for hybrid ring. Other ESF pin are SCAT™ (single cortex negative thread) fixation half pins are similar in designs to the Ellis™ (Kirschner Medical), and Steinmann intramedullary pins.

ESF may be applied after closed or open reduction of the fracture. The ESF should be designed to have appropriate stability for the severity of the fracture and the anticipated healing time. Frame stability depends on several variables, such as frame configuration, number of pins inserted per fragment, pin size and length, and location and size of the connecting bar. Unilateral (type Ia), unilateral biplanar (type Ib), bilateral (type II), and bilateral biplanar (type III) frames are increasingly stiffer (24). A type I construct produces sufficient stability in many feline fractures, especially if a large connecting bar is used. Low – stiffness frames were used in radial and tibial fractures (Ia, Ib,IIb) in cats with no fixation failure (25). Bilateral frames have the disadvantage that the transosseous pins have to be inserted through areas with large muscle mass, which may cause morbidity. They should therefore only be used in heavy cats and fractures which are expected to heal slowly. Insertion of an intramedullary pin and connecting its end to the external bar is another option to enhance frame stability (9). This tie-in configuration is especially useful in fractures of the humerus and femur (26, 27).

The number of pins influences ESF stability which increases when up to four pins are used per fragment (28). A minimum of two pins per main fracture fragment should always be used. Pins are placed according to the far-near, near-far principle. The stiffness of pin is inversely proportion to the third power of pin length; the external bar is therefore placed as near as possible to the skin to reduce pin length.

Using transosseous pin with a diameter of 20-25 % of bone diameter is generally recommended. Pins are inserted in the areas with the least muscle mass and should not interfere with joint and muscle function. The pin-bone interface is the weakness point in most ESF constructions. Holding power of the pin is influenced...
by pin-type and insertion technique. The use of threaded pin enhances the pin-bond interface and therefore pin-holding strength (29). Smooth pins should be inserted with an angle of around 70° to the long axis of the bone to reduce the risk for pulling out of the bone. Thermal and structural damage to the cortex during pin insertion is reduced by predrilling a hole with a drill bit and by inserting the pin using low-speed drilling(30). Predrilling is recommended for positive-thread pins, and is performed with a drill bit 10 % smaller than or the same size as the core diameter of the pin.

A safe corridor is a longitudinal region through which pins can safely be inserted as they do not contain important neurovascular or musculotendinous units; hazardous corridors contain musculotendinous unit but without important neurovascular structures, and finally, unsafe corridors contain both musculotendinous unit and neuromuscular structures (31). Proximal limb bones, such as the femur and humerus, do not have safe corridors for pin insertion; therefore hazardous corridors are identified as the safest areas (32). It is important that the difference in anatomy between the canine and feline distal humerus are understood as they dictate position, method of application and size of implant used. The feline humerus is generally straighter and smaller than the canine. There is a supracondylar foramen located proximal to the medial epicondyle in the cat and a true supratrochlear foramen is absent. The median nerve and brachial artery pass through the supracondylar foramen before continuing into the antebrachium and distal limb(33). There are no safe corridors for ESF pin insertion but hazardous (also known as safe area) exits in the proximal humerus bordered by the omotransversarius and insertion of the supraspinatus dorsally, the deltoideus caudolaterally and the cleidobrachialis cranioderally. The safe area ends in a ‘V’ approximately one third of the way down the humerus where the acromial head of the deltoidius meet the cleidobrachialis. A distal safe area is present in the region of the lateral epicondyle. When placing an ESF pin in the distal humerus it should be angled in a distolateral to proximomedial direction so that it penetrates the bone at least 20 mm proximal to the medial epicondylar avoiding the supracondylar foramen medially and the radial nerve laterally. When placing pins in a transcondylar fashion, a pin of between 1.5 mm and 2.2 mm diameter should be used. The pin should be angled from just cranial to the lateral epicondyle to exit the bone craniodistal to the medial epicondyle. If passage of an IM pin into the medial condyle of the humerus is required, confirmation that this can be achieved should be gain by assessing the size of the intramedullary canal radiographically in this region. A pin of not greater than 1.6 mm in diameter should be used.

The use of linear-circular ESF has a significant advantage in allowing stabilization of small juxta-articular fracture segments where limited bone stock is available (18, 20). This technique has been applied to distal and proximal antebrachial and crural fractures (19, 20, 21, 22), and fixation of intra-condylar humeral fracture with supra condylar comminution (23).

2,4,6 tri(diethylaminomethyl) phenol and biphenol A-Epichlorhydrin reaction product(Selley’s Steel Kneadit), an acrylic steel repair paste is a readily available easy-to-use acrylic. It is packaged as a roll containing inner and outer polymer that are activated by hand-kneading, and consistently from a smooth and malleable putty within 120 second and is practically immobile in six to eight minutes. The material sets with a mild exothermic reaction that is not malodorous. A 25 mm segment is as strong as medium size KE clamps (34).

References

29. Bennett RA et al. 1987. VET surg. 16: 207-211
Long Bone Fracture in Feline: How to Fix it?

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Cat is not a little dog which it has different bony anatomy. Long bone fractures are common and occur approximately 50% of all feline fractures. It is usually caused by road traffic accidents, but may also result from falls, fights and gunshot wounds. Fractures of the hindquarters predominate, with reports of 73% of fractures involving the hind limbs, pelvis or sacrum. In ninety-three cats with traumatic fractures, 38.7% of animals had radiographic evidence of thoracic trauma, the most concurrent injuries are lung contusion and pneumothorax (1).

Table 1 Characteristics Influencing feline fracture repair. The characteristics that have influences on choosing feline fracture repairing methods, which will be discussed below.

<table>
<thead>
<tr>
<th>Favourable characteristics</th>
<th>Unfavourable characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small size and low bodyweight</td>
<td>Lightweight bones with thin cortices mean that fractures are often comminuted</td>
</tr>
<tr>
<td>Naturally athletic</td>
<td>Propensity to climb and jump</td>
</tr>
<tr>
<td>Undemanding lifestyle</td>
<td>Independent lifestyle</td>
</tr>
<tr>
<td>Ability to redistribute weight and protect an injured limb</td>
<td>Prone to the same range of complications as other species</td>
</tr>
<tr>
<td>Ability to compensate for impaired function</td>
<td>May be intractable and uncooperative</td>
</tr>
<tr>
<td>Consistent skeletal morphology</td>
<td>Skeletal morphology differs from that of dogs</td>
</tr>
</tbody>
</table>

**Principle of Fracture Repair**

The chosen method of fixation should achieve adequate alignment and stability of the fragments for the duration of the healing period following consideration of the likely disruptive forces at the fracture site. The surgical approach should as far as possible preserve soft tissue attachments and the blood supply to the fracture fragments.

**Fixation Methods**

It is impossible to identify the ideal fixation method for a given bone due to variations associated with the patient, the fracture configuration, technical expertise, equipment and finances. For some fractures, more than one method of fixation may be equally appropriate and the final choice will be based on the preferences of the surgeon.

**External Coaptation**

This method of fracture repair is indicated for minimally displaced greenstick or stable transverse fracture configurations where there is interdigitation of the fragment ends, especially in young animals. Coaptation is cheap and readily available, but requires substantial maintenance and is prone to complications.

**Intramedullaries Pin**

Intramedullary pin technique is relatively easy, and the equipment and implants are cheap and readily available. IM pin counteract to bending force. This is only force that IM pin can act. The usual recommendation is that the pin should occupy approximately 70% of the diameter of the medullary canal although, in general, the larger the pin, the better in terms of the rigidity of the repair and implant strength. An intramedullary pin rarely provides adequate fracture stability when used alone, but can be employed successfully in combination with cerclage wiring techniques for selected long oblique or spiral fractures. Intramedullary pinning can also be used for ancillary fixation in combination with external skeletal fixation (ESF) as an ESF tie-in or with a plate and screws as a plate–rod. This combination of implants with complementary characteristics is especially useful for non-reconstructable fractures.

**Cerclage Wire**

Orthopaedic wire is cheap and readily available. The technique is best suited to long oblique or spiral fractures combined with pin or plate fixation when a minimum of two wires are employed. Application is unforgiving of technical errors, which predispose to wire loosening and delayed union or non-union of fractures. Cerclage wire has 3 methods. First method is called Twist knots. This method can generate 70-100 N. Second method is Single loop. Single loop can generate 150-200 N. The last method is called Double loop. Double loop can generate 300-500 N. Rules of thumbs are minimum of 2 wires, length of oblique need at least twice the bone diameter, placed at least 0.5cm from fracture end, wire should place 1.0cm of each other, wire lays directly on bone and place perpendicularly to the long axis and never use wire as a sole means of fracture repair. Tension band wire is a pin and wire technique used in orthopaedic surgery to counteract the tensile force of the pull of muscle or tendon attached to bone. Common indications are epiphysyal avulsion fracture, sprain avulsion fracture, patellar fracture and arthrodesis.
External Skeletal Fixation
External Skeletal Fixator (ESF) is a versatile method for both fracture repair and treatment of other orthopaedic conditions. Comminuted and open fracture are the main indication for fracture stabilization with ESF. Temporary fracture and joint immobilization, stabilisation after soft tissue repairs, correction of limb deformities and distraction osteogenesis are other indication. ESF works well against tension, bending, rotation and compression force. ESF is a more expensive form of fracture fixation than intramedullary pinning, but the connecting bars and clamps can be reused to help reduce costs. Fixation pins, clamps and connecting bars are available from a number of manufacturers. In acrylic and pin external fixator (APEF) devices, the clamps and connecting bars are replaced by acrylic columns. Intramedullary Steinmann pins and Kirschner wires are not ideal as they are not sufficiently stiff. When using ESF alone for fracture repair, three or more fixation pins, including at least one positive profile pin, should be placed on either side of the fracture for optimum stability. Positive profile pin sites should be pre-drilled using a drill bit of a slightly smaller diameter than the pin. In order to avoid iatrogenic fracture, fixation pins should not be greater than 25% to 30% of the bone diameter at the site of insertion. The use of ESF for humeral and femoral fractures is inevitably associated with some morbidity because the pins must penetrate the overlying large muscle masses. ESF is best suited to the repair of fractures of the tibia and to a lesser extent the radius and ulna. The primary disadvantage of this technique is the necessity for rigorous postoperative care.

Plates and Screws
Plates and screws are suitable for the repair of all long bone fractures in cats. Plate and screw works against to bending, rotation, compression and tension force. Application generally requires extensive surgical exposure of the fracture site, which further disrupts the blood supply to the fracture fragments and risks the introduction of infection. Nevertheless, when used correctly, plating allows an early return to function, is associated with low morbidity and requires minimal postoperative maintenance. Bone plates that are suitable for use in the cat include round hole, reconstruction and dynamic compression plates, which are available to accommodate 1.5, 2.0, 2.4 and 2.7 mm screw sizes. The veterinary cuttable plate (VCP) is a unique speciality plate commonly indicated for feline fracture repair. The plates are available in various sizes to accommodate either 1.5/2.0 mm screws or 2.4/2.7 mm screws and can be custom cut to the appropriate length at the surgery table. Lock plate system is the most helpful and secure stability of fracture. This system creates rigid fracture stability and, reduces the disruption of soft tissues and blood supply surrounding the fracture region. There are various size 1.5, 1.7, 1.9, 2.0, 2.4, 2.5 and 3.0 mm of screws available which suite for cat bone. 30% of bone diameter in the side of plate placement is the best size of screw.

Plate–rod
The addition of an intramedullary pin to a plate to form a plate–rod is indicated for the repair of non-reconstructable fractures of the tibia, femur and humerus. The pin protects the plate from the strain associated with bone defects and/or empty screw holes. Screws can usually be inserted to engage both cortices in the metaphyses where the bone is flared. Rod size 50% of medullary cavity reduce plate stress by 50% which increases 10-fold plate fatigue life. Rod size 25% of medullary cavity decrease plate stresses by 10% and is not recommended. Therefore, rod size 30% to 50% of medullary cavity at its narrowest point are recommended.

Table 2 Repair methods and control of disruptive forces The summarises of disruptive forces which are controlled by each repairing method.

<table>
<thead>
<tr>
<th>Fixation method</th>
<th>Bending</th>
<th>Shear</th>
<th>Torsion</th>
<th>Compression</th>
<th>Tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>External coaptation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Intramedullary pinning</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Single pin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stackled pins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cortico wire</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>External skeletal fixation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>External skeletal fixation and intramedullary pinning</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone plates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lag screws</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Plate–rod</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Interlocking nails</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Good control of disruptive force, - Variable control of disruptive force, + Poor control of disruptive force

Treatments and Approaches

Humeral Fractures
The feline humerus is straighter and more slender than its canine counterpart. A unique feature is the presence of a supracondylar foramen on the distomedial aspect containing the brachial artery and the median nerve. Distally, there is an olecranon fossa analogous to the canine supratrochlear foramen and the condyle is straighter and wider than that of the dog. The tension side of the bone is craniolateral. Humeral fractures constitute 5 to 10 per cent of all feline fractures. Fractures frequently involve the mid and distal portions of the diaphysis and are often comminuted.
Radial and Ulnar Fractures
Fractures of the radius and ulna account for 3 to 8 percent of all feline fractures. Most involve the midshaft and distal portions of the radius and ulna. The feline ulna is relatively large and straight compared with the canine ulna and the radial diaphysis is narrowed in the craniocaudal plane. The tension side of the radius is cranial. The anatomy of the feline antebrachium allows considerable pronation and supination and it is therefore important to try to preserve motion between the radius and ulna when undertaking orthopaedic procedures on the antebrachium.

Femoral Fractures
The femur is the most commonly fractured long bone, accounting for 18 to 38% of all fractures in cats. The feline femur is a straight tubular bone with a large medullary canal. There is an abundant extraosseous blood supply, which contributes towards rapid healing. Implants are applied laterally on the tension side of the bone. The majority of fractures involve the diaphysis and the distal shaft. Sciatic nerve injury in association with femoral fractures is rare.

Tibial Fractures
The tibia is generally straighter and more tubular than its canine counterpart. The fibula is relatively larger in the cat than in the dog, but it does not require stabilisation unless it is fractured at its proximal or distal extremities. The tibia is the second most commonly fractured long bone, accounting for 5 - 14 % of all feline fractures. The majority of tibial fractures involve the mid- and distal diaphysis (20% of all tibial fractures) (2,3), and open fractures are common distally because of the minimal soft tissue coverage. The tension side of the tibia is craniolateral, but most surgical approaches are made medially where the bone lies subcutaneously.
Table 3 Choice of fixation method in long bone fracture repair

The summary of the level of suitability of each fixation method in long bone fracture repair

<table>
<thead>
<tr>
<th>Fixation method</th>
<th>Humerus</th>
<th>Radius/ulna</th>
<th>Femur</th>
<th>Tibia</th>
</tr>
</thead>
<tbody>
<tr>
<td>External coaptation</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Intramedullary pin</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+/ cerclage wire</td>
<td>−</td>
<td>++</td>
<td>−</td>
<td>++</td>
</tr>
<tr>
<td>External skeletal fixation</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>External skeletal fixation and intramedullary pin tie-in</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Plates</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Plate-rod</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Interlocking nails</td>
<td>++</td>
<td>−</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

− Unsuitable, + Suitable only for reconstructable and/or stable fractures, ++ Suitable for all fracture types

Conclusion

Every technique has pros and cons that it can lead to success or failure depending on the decision made by surgeons. Therefore, surgeons have to make a keen planning that can correct all of biomechanics, which occurs around bone’s surface. Patient assessment- score must also be considered because it is one of the factors that will lead to success or failure. Following the principle on each standard technique is a must for successful outcomes.

References

4. Courtesy picture to Dr. C. Phonsuwan, Vet4 Polyclinic
Pelvic Fracture of Feline Fracture
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Pelvic fractures are traumatic injuries that account for approximately 20-32 % of appendicular fractures in cats (1). Among these feline patients, fractures of the pelvic floor are commonly found (90%), while the incidences of the sacroiliac luxation and the ilial body fractures have been reported as high as 60% and 48.5% (2, 3). The causes of pelvic fractures usually involve high impact trauma predominantly road traffic accidents. The patients can suffer significant morbidity and mortality if the concurrent soft tissue injuries were not appropriately diagnosed and managed. The initial assessment of the vital organ systems should be performed to identify life-threatening injuries. The concurrent non-orthopedic injuries can occur as high as 59-72% of the cases and it is of important that the injured patients need to be stabilized before primary repair of the pelvic bone (1).

The urinary tract disruption and neurological damage are most commonly found among pelvic fracture cases (4). The hindlimb deep pain sensation, motor and sensory function of the tail, pudendal nerve reflexes (urethral sphincter tone, anal sphincter, bulbocavernousus, and perineal reflexes) should be carefully evaluated in every patient. Subluxation or luxation of the coccygeal vertebrae at the base of tail, so called “tail pull injury” can occur when the tail was trapped underneath a car wheel. The traction force related to the struggle of the animal in the accident may stretch the sacrococcygeal nerve root of cauda equina leading to nerve damage (4). The presenting signs include flaccid tail, bladder paralysis and urinary incontinence. The feline patients with intact anal reflex and perineal sensation at 48 hours after trauma are likely to return to normal urinary function. However, the tail function may gain fully recovery by 7-150 days, therefore early tail amputation is not recommended (4).

The single fracture of the pelvic bone is very uncommon because of the rigid box-like structure of the pelvis. The displacement at one side will cause the displacement of the others (5). In sacroiliac fracture-luxation cases, a unilateral luxation is usually accompanied by at least 1 pelvic fracture or a symphyseal separation (6). The current concepts of feline pelvic fracture management have been shifted from conservative treatment in the past to surgical management in recent years. The consideration criteria for surgical treatment of the pelvic fracture include; the integrity of the weight bearing axis (acetabulum, ilial body and sacroiliac joint), pelvic canal diameter, fracture duration, patient comfort level, concurrent orthopedic injuries, expected treatment outcome and financial limitation (5). The surgical stabilization of the pelvic bone will provide the rapid alleviation of pain and discomfort due to the instability of the bone fragments. The anatomical reduction and appropriate fixation of the ilial wing will prevent medial displacement of the bone fragments thus the width of pelvic canal can be maintained. Several techniques for pelvic fracture repair in cats have been described. The lateral and dorsal plating for the fixation of ilial body fracture have been studied in cats using 2.0 mm (1.0 or 1.5 mm thickness) dynamic compression plate (2, 3). The straight ilial body of the cats offers the possibility to apply dorsal bone plate. Comparing to lateral plating, the dorsal plating technique enables the use of longer plate and a better screw purchasing onto the bone leading to rigid fixation with lesser chance of screw loosening (3). The composite fixation of comminuted ilial wing fracture in cat has been documented using the combination of pin, screws, wire and composite resin (polyethylmethacrylate). The successful outcome with uneventful bone healing can be achieved (7). The acetabular fracture fixations by 2.0-2.7 mm bone plate as well as tension band stabilization have been successfully reported in felines (8). However, the comminution of the acetabulum may prevent the anatomical reconstruction of the articular surface. In such cases, the femoral head and neck excision is an affordable method of choice (5). Alternatively, the surgical repair to allow the secondary bone healing can be performed prior to the consideration of the total hip replacement (5). The sacroiliac joint fracture or luxation are common in cats that encountered a blunt trauma to the hind quarter. The surgical intervention is indicated if the feline patients are in painful condition, nonambulatory or demonstrate severe neurological deficit or pelvic canal narrowing. The fixation methods include screw insertion in lag fashion, translilial pins or combination of transiliosacral rod with tension band technique. Pelvic floor fractures are classified as symphyseal separation, fracture of the pubic body or ramus and ischial body (5, 6, 9). The fixations of these parts are indicated if the marked instability of fragments is detected. Fixations of pelvic floor fractures can be performed using bone plate and screw (10).
A long-term follow up study in 43 cats undergone surgical management has reported excellent outcomes with 22% complication rate (1). The most common complication is transient neurapraxia associated with sciatic impairment (1). Activity restriction and effective pain control are advisable during the first month of the post-operative period. The physical rehabilitation can be used with caution to restore and maintain the joint range of motion and muscle strength. The radiographic examination should be performed at 4-6 weeks post-operatively to assess the stability of the implants and bone healing (5).

References
Fractures occur commonly in both dogs and cats and, therefore, are frequently seen in general practice. Cats’ curious nature and its independent behaviors always let them be in the dangerous situations such as trauma as the most common causes of orthopedic problems. Car accidents, Falling from the height, and Bite wounds, are common found in cats rather than in dogs, therefore other orthopedic problems with severe and multiple injuries to the musculoskeletal and soft tissues are also concerned. Many of the fractures encountered in cats are due to high velocity trauma therefore; high comminuted fracture always found in mature cats. Pathological fractures such as paper bone disease or metabolic bone pathology are always found in kitten especially in strayed kitten. Territorial fights between cats often results in bite wounds and open fractures. Orthopedic injuries are addressed only after other severe soft tissue and life threatening problems injuries have been ruled out and stabilized.

Open fractures are more common in cats than in dogs and usually occur distal to the elbow and stifle joints where the bone is less surrounding soft tissues. Soft tissue reconstruction and bone stabilities of the open fracture seems to be serious problems in cats rather than in dogs due to small soft tissue coverage. Even though the External skeletal fixation (ESF) is one of the most suitable for the open fracture treatment, application in cats is more difficult than in dogs due to the cats behavior, quality of cat bones, and special postoperative cares.

Basic principles of fracture repairs are similar for cats and dogs, but there are some clinically important. Cats bones are straighter and more slender than dogs bones. The small size of feline bones limits the choice of implant size and strength, and meticulous technique is required when working with small bone fragments. Strength and Stiffness of implants are required, even though it makes longer bone healing. Small hard callus is predicted when internal fixation is applied in cats to create minimal disturbance to the muscle and tendon functions.

General data including signalment, history, general, orthopedic, and neurological examinations are required in order to perform effective Fracture- Patients Assessment Score (FPAS). History obtained from the cat owner may not be a helpful as for the other species because cats with mild injuries or illness often do not show the obvious or specific clinical signs and it may take longer for the owners to realize the abnormalities.

Cats living outdoor spend whole day unobserved by their owners. The causes of injuries are always inaccurate history. Previous systemic diseases such as FIP, FeLV, kidney problems should be concerned because many cats have suffered without showing the clinical signs.

The examinations of cat patients are likely same as in dogs but with greater calm, appropriate handling and restrain to make cats cooperate and be agreeable patients. Many musculoskeletal abnormalities such as osteochondrodysplasia, femoral neck metaphyseal osteopathy, metabolic bone diseases, retroviral infection, hereditary metabolic musculoskeletal diseases, other bone diseases should be ruled out before treatments or performing FPAS.

Many disorders or diseases are subclinical problems in cats. These disorders may interfere the FPAS and the fracture treatment outcomes when they are not concerned and observed before making the treatment plan including Osteochondrodysplasia in the Scottish Fold.

It is now accepted that the folded ear is an outwards sign of generalized defective cartilage formation. Abnormal longitudinal growth of the skeleton and in turn to shortened metatarsal and metacarpal bones and shorter and wider vertebrae of the proximal tails can be detected. Exostosis and periosteal reaction as well as joint degeneration are also reported. Nutritional secondary hyperparathyroidism

Diets low calcium and high phosphorus causes by malnutrition and parasitic infestation interfered to the strength of the bone especially in strayed cats living outdoor. Even though the paper bone diseases or pathological fracture can be detected in the radiograph, bone density and bone quality cannot show in normal plain film.

Slipped capital femoral epiphysis also known as femoral neck metaphyseal osteopathy, spontaneous femoral capital physeal fracture, and femoral capital physeal dysplasia syndrome is nontraumatic, slow, progressive displacement of the proximal femoral metaphysis from the femoral epiphysis through the physis. This disease is most common in cats but has also been reported in dogs, and the cause is unknown. It is not the same disease as a Salter-Harris type 1 fracture of the femoral capital physis, which is acute and traumatic in origin. The most common age at
presentation is 4.5 to 42 months. Maine coon and Siamese cats may be overrepresented; in one study, Maine coon cats were 12 times more likely than other breeds to develop this condition. Overweight, neutered male cats appear to be at risk. Diagnosis is based on signalment, history, physical examination findings, and radiographic findings. History and physical examination findings include acute or chronic pelvic limb lameness, pain on manipulation of the coxofemoral joint, and an inability to jump. Approximately one quarter of cases are bilateral. Radiographic findings include a widening and lateral displacement of the capital femoral growth plate with progressive resorption and sclerosis of the femoral neck.

Others hereditary metabolic diseases of the musculoskeletal systems including alpha-mannosidosis, mucopolysaccharidoses type I, VI, and VII, mucolipidosis type II, osteogenesis imperfecta, hypothyroidism, rickets and other congenital skeletal malformations are also concerned when dealing with the feline fractures. FPAS should be carefully scored.

Alpha-mannosidosis is a chronic progressive disorder with a chronic severity. Severe cerebellar dysfunction is a predominant sign. The clinical findings included hepatomegaly, corneal changes, clavicular abnormalities, and the cerebellar signs (tremor, ataxia, dysmetria, dementia). Progressive loss of balance is the most common signs when presented.

Mucopolysaccharidoses type I, VI, and VII and mucolipidosis type II or MPS are characterized by the impaired function of one of 11 enzymes required for normal GAG degradation, resulting in many abnormal radiographic signs including Pectus excavatum, Deformed vertebrae, fusion of the cervical vertebrae, bilateral hip subluxation, shallowed acetabulum and femoral head dysplasia, and epiphyseal dysplasia of the long bones. Most of affected cats may present with clinically normal but they always suffered from the OA changes.

Osteogenesis imperfecta is a disorder of bone that lead to spontaneous fractures or in response to minimal trauma. Sometimes it may be confused with the feline nutritional secondary hyperparathyroidism. Affected cats always presented with lameness and pain in the pelvic limbs, walking slowly with cautiously, and avoided jumping on the tables or other objects. All clinical signs seem to be normal until they got the trauma, the greenstick fracture occurred, they reduced appetite or anorexic, avoided the urine box for defecation, resulting in constipation leading to intestinal paralysis probably occurred. Radiographic changes include thin cortical bone, kinks and deformities of long bones, lyerated shape of pelvic bones are indicated.

Other bone diseases including secondary hyperparathyroidism, renal secondary hyperparathyroidism, vitamin D deficiency, hypervitaminosis A, bone cyst, hematogenous osteomyelitis, solitary osteochondromas, hypertrophic osteopathy, osteocartilagenous exostosis, , feline osteochondromatosis and other joint diseases such as osteoarthritis should be considered and investigated in order to make properly FPAS.

References
Emergency management and surgical considerations for urethral obstruction in tom cats

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Abstract
Urethral obstruction (UO) is a life-threatening condition that commonly occurs in male cat. The etiology of this disorder is frequently unknown, but it frequently involves with several disorders such as anatomical abnormality, urethroliths, urethral stricture and urethral plugs. The incidence of the UO is predominantly found in tom cats as the urethra is narrower and longer when compared with female cats. Furthermore, health status, types of food and conditions of urinary tract also become the major predisposing causes of the UO. Cats with UO demonstrate recognizable signs of urinary discomfort, straining and vocalization. The cats with partial UO can be treated medically. However, patients with complete obstruction of the urethra must be considered as emergency conditions. These conditions potentially develop several consequences such as hyperkalemia, metabolic acidosis, azotemia, hyperphosphatemia, decreased ionized calcium and hyperlactatemia, all of which are life-threatening. Surgical intervention to eliminate the cause of obstruction is preferable to reestablish urinary functions, especially when the catheterization is unsuccessful. Of several surgical techniques, perineal urethropotomy is most commonly performed. The prognosis of the UO is frequently good unless the cats develop other complicated conditions such as acute kidney injury, recurrent urinary tract infection and cardiovascular disturbance. Short- and long-term managements are required to prevent recurrent UO.

Anatomical and physiological aspects of feline urinary tract
The lower urinary tract of the cats is composed of the urinary bladder, ureters, urethra and penis. The male urethra is divided into two parts, pelvic and penile urethra. At the distal part of the pelvic urethra, bulbourethral glands are located at the level of ischial arch. The urethra of male cats is longer than that of female cats. The urethra diameter also become narrow at the transition of pelvic to penile urethra, approximately at the bulbourethral apposition. This anatomical difference between male and female results in higher incidence of urethral obstruction in male cats when compared with female cats. Normal micturition requires a neuromuscular coordinator that results in reflex evacuation of the urinary bladder. This reflex is triggered by high pressure within the urinary bladder and thereby resulting in the relaxation of the urethral sphincters. The cats therefore can deliberately control (to initiate or to suppress) the micturition. Anatomical or neurological abnormality result in either partial or complete failure of micturition. These conditions frequently lead to complete urethral obstruction that require emergency attention.

Etiology of urethral obstruction
Urethral obstruction can be classified as partial or complete block of the urination. In majority of the cases, the primary cause of this condition is frequently unknown. However, it is quite commonly associated with feline lower urinary tract infection/inflammation such as feline interstitial cystitis (1, 2). The obstruction can also be caused by physical or functional abnormality of the urinary tract. The physical obstruction includes idiopathic urethral plugs, calculi, urethral stricture and tumor. The neurological muscular disturbance may also result in abnormal micturition, all of which potentially develops secondary urinary tract infection. Other conditions may also predispose to develop urinary blockage are health status, water intake, types of food (dry or wet formula), anxiety, stress condition and surrounding environment (3).

Clinical sign
The clinical signs of the cats are variable due to several factors such as severity of urinary blockage (complete or partial obstruction) and duration of the condition. At an initial phase of urethral obstruction, the cat is easily recognizable by extensive vocalization with abnormal posture during urination. The cats frequently walk to litter box and try to urinate with abdominal straining. In severe case of complete urethral obstruction, the cats become depressed and the abdominal enlargement and cramp may be seen. The clinical signs of cats with urethral obstruction include dehydration and abnormal vital signs such as increased respiratory rate, bradycardia and cardiac arrhythmia. The decreased urination potentially results in an elevation of blood potassium that results in cardiac arrhythmia and bradycardia (4). In addition, this condition also induces metabolic disturbance especially metabolic acidosis due to decrease excretion of hydrogen ions via the kidneys. Decreased blood calcium can be developed with the consequences of cardiovascular and muscular impairments. The decreased muscular blood perfusion caused by decreased cardiac output and shock can also increase lactate production from poorly blood profuse tissues (hyperlactatemia). Prerenal, intrarenal and
postrenal azotemia can be developed in cats with urethral obstruction.

**Diagnosis**

Diagnostic setup should include clinical symptoms of the cats and also the history of urination such as frequently, straining and general health. The plain radiography and ultrasonography of the lower abdomen is recommended to rule out the abnormality of musculoskeletal systems and also the presence of calculi. The neurological sign should also be examined. Complete blood count and serum chemistry must be performed to evaluate health status in particular the renal functions. Cats with complete urinary obstruction should be thoroughly monitored especially the electrolyte (calcium and potassium) levels. The electrocardiogram (ECG) must be evaluated in patients with hyperkalemia as this condition leads to abnormality of ECG and cardiac functions such as prolonged PR interval and widening of the QRS complex. When hyperkalemia is severe (>9 mmol/L), the patients must be seriously care. This condition develops ventricular arrhythmias and fibrillation, all of which are life-threatening condition.

**Treatment**

Treatment of urethral obstruction is essential for life-threatening condition especially when complete urinary obstruction is present. The treatment procedures also depend on the underlying causes. Patients having partial urinary obstruction may need only medical treatments. This treatment includes drugs that decrease urethral tones (α-1 adrenergic antagonists: Phenoxynbenzamine at 0.25 mg/kg) or diazepam at dose 0.5 mg/kg to induce urethral relaxation. In animal with detrusor hyporeflexia or bladder atony can be treated with 2.5-7.5 mg/cat bethanechol chloride. The antibiotics may be required if cats develop any signs of lower urinary tract infection. Fluid therapy is essentially required as cats normally excrete through the kidney such as ketamine may be contraindicated in kidney malfunction with development of azotemia.

Catheterization of urethra is a critical step to ameliorate the urethral obstruction. The main objective of urethral catheterization is to salvage the urine from the urinary bladder. However, the catherization can only be placed for a short period of time as catheter itself can be irritating and ascending infection may be occurred. Type and diameter of the catheter is very important. The small diameter and soft catheter should be used in the beginning of the procedure. The catheter must be lubricated with lubricants such as gel containing lidocaine hydrochloride prior to use. Hydropropulsion with saline solution can be performed to flush the obstruction. Open-ended catheter is the catheter of choice for this procedure as the fluid force can be directedly approach to the obstruction. If catheterization is successful, the catheter is then fixed with the prepuce and skin surrounded the penile urethra. The catheter should be connected to a sterile urine bag to prevent ascending bacterial infection.

**Surgical intervention (urethrostomy)**

Indication of urethrostomy is to remove affected urethra, often the narrowing part of the urethra and/or penis of the tom cats (5). This procedure is normally performed in complete obstruction of the urethra and or when the cats do not response well with the medical treatment. Other indications includestenosis of the urethra, urethral rupture, neoplasia and priapism. Several types of urethrostomy can be performed including prepubic, subpubic, transpelvic and perineal urethrostomy. A latter surgical technique is frequently used as a first line surgery in case of urethral plugs and urethral calculi at distal part of the urethra. Regardless the surgical techniques used, the urethra is exposed and dissected from surrounding tissue and anastomosed with the subcutaneous and skin of the cats. However, clinical outcomes and short- and long-term complications can be different among the techniques used. For perineal urethrostomy, an elliptical-shaped incision is made around the scrotum and prepuce. The subcutaneous tissue is dissected to identify penis and penile urethra. The penis and penile urethra is dissected free from surrounding connective tissue until ischiocavernosus and ischiourethralis muscles are clearly seen. The muscles are cut to free the muscle attachments. This procedure can be aided with electrocautery and is required to access the urethra at the position of bulbus glandis as the diameter of urethra at this region is larger than caudal penile urethra. The ventral ligament and dorsal retractor penis muscle are cut free from the penile urethra. Longitudinal incision is
made along the dorsal urethra starting from the penile urethra until the middle part of the bulbo-urethral glands. The distal part of urethra and penis is cut leaving approximately 1 to 1.5 cm long (from the bulbo-urethral glands). The Foley’s catheter (6 to 10 G) is then inserted into the urinary bladder and the balloon is infiltrated. The urethral mucosa is finally anastomosed with the skin along the urethra opening. The Foley’s can be kept for approximately 1-2 weeks to by pass the urine into urine bag. This catherization decreases wound contamination with urine and allows the vet to examine the urine conditions. Modified perineal urethrostomy can also be performed (6). This technique is similar with conventional urethrostomy, except that the mucosa of the prepuce is completely anastomosed with the urethra (instead of skin). The most critical part of the modified urethrostomy involve appropriate anastomosis between urethra and mucosa of the prepuce. Urine leakage after anastomosis may occur, and re-suturing is therefore required.

Complications after perineal urethrostomy

Complications following urethrostomy have been reported. These complications can be occurred as short- and long-term consequences (7, 8). Short-term complications such as hemorrhage, urinary tract infection, subcutaneous leakage of urine, surgical site infection and wound dehiscence are normally developed with in a week after surgery. To minimize urine leakage and surgical site infection that may occur after perineal urethrostomy, the cats should be catheterized with soft and flexible catheter such as Foley’s catheter for approximately 1-2 weeks. The cats with previous records of lower urinary tract infection should be on susceptible antibiotics and occasionally anti-inflammatory drugs. The long-term complications such as stricture of urethral opening, urinary tract infection, urinary incontinence and dermatitis can also be found.

Conclusion

Urethral obstruction can be medically or surgically treated. While treatment procedures depend on underlying causes and clinical symptom, complete urinary obstruction is life-threatening condition that requires emergency treatment. Cats developing azotemia and electrolyte imbalances resulting in urinary and cardiovascular disturbances require intensive care and treatments. Surgical intervention to restore urinary functions using perineal urethrostomy is frequently performed in stable patients with complete or recurrent urinary obstruction. Short and long-term consequences following perineal urethrostomy should be concerned for veterinarians and owners.

References

Skin mass, what should be done by surgical management?

Worapan Tadadoltip

Surgery is an essential component for diagnosis and treatment of most solid tumors in small animals. Surgeons must have a good understanding of surgical oncology principle, cancer biology and the goals of surgery. The method of biopsy and tumor removal has a significant impact on patients’ outcomes.

Tumors of skin and soft tissues are the most frequently diagnosed in domestic animals because they can be identified easily. Skin is a complex structure composed of epithelial, mesenchymal, neural and neuroectodermal tissues, all of theseis structures can develop distinctive tumors. Skin tumors can behave identified as benign neoplasm whichis means that the mass is localized, non-infiltrative, surrounding by a capsule and easily removed. Neoplasm of intermediate malignancy is locally infiltrative and difficult to remove but doesn’t metastasize. Malignant neoplasm is infiltrative and metastatic potential. Knowledge of type, stage and possible grade of tumor are necessary information for surgical planning.

Preoperative evaluation

Fine-needle aspiration is the first step to get near your diagnosis, although is prone to inaccuracy because of the small sample of cells lacking in vivo orientation but it is a noninvasive technique and it is easy to perform. Before a major surgical procedure, a histologic diagnosis is recommended. Biopsy technique may be tru-cut, incisional or excisional biopsy, decision making for biopsy technique depends on the size, the location and the cytology diagnosis of the mass. Unplanned incision or excision without knowledge of tumor type are often associated with an excision that larger than necessary or not large enough to remove all microscopic disease. Staging for local and distant metastasis is necessary for surgical planning. Radiography and three-dimension imaging are useful in screening metastatic disease. Computer tomography (CT) scan of the mass, thorax, abdomen and sentinel lymph nodes to local the invasiveness and distant metastasis and incisional biopsy mass or lymph node after the evaluating the images is a highly effective way to obtain potential information about extent of disease and definitive diagnosis.

Surgical treatment

Surgical plan can be made based on histologic or cytologic diagnosis, grade and extent of disease. In cases with prior preoperative treatment such as chemotherapy or radiotherapy, the timing should be developed before surgical intervention. The goals of surgery may be palliative or curative. Palliative-intent surgery is intended to improve quality of life and relieve clinical sign even if complete excision is not possible, cytoreduction of tumor cell by intra-lesional or marginal excision can be useful to allow chemotherapeutic agents or radiation to have greatest impact on residue disease or tumor cell as part of multimodal therapy (1).

Surgical oncology principles

Decision making for surgery therapy depends on the type of tumor, size, location and signalment of the patients. For benign neoplasms with no associated clinical dysfunction or ulceration, no therapy may be the treatment option, especially in aged patients. For malignant neoplasms or benign neoplasms that inhibit normal function or cosmetically unpleasant, surgical intervention with complete excision is the best chance to cure with least cost and often with the fewest adverse effects. The goal of surgical excision for malignant skin tumors is to achieve widely and complete en bloc excision of the primary tumor including its marginal of normal tissue in three dimensions. The exact distance recommend for surgical margin will depend on the tumor type and location. Even an approach with wide or complete en bloc will not guarantee the complete removal of all tumor cells because some malignant tumor cells might infiltrate the surrounding normal tissue in the form of ‘satellite’ of metastasis (Fig.1). Surgical excision of skin tumors should be done with aseptic technique and sterile instruments. Gentle tissue handling and maintenance of blood supply, minimization of dead space and tension are important. Previous biopsy site should be removed with the resected tissue. Ideally, intraoperative principle of surgical oncology should be removing the entire tumor cells without entering the tumor capsule to avoid seeding and contaminating of neoplastic cells at surgical sites, changing gloves and instruments before closure is unlikely to results in a difference outcome if tumor capsule was entered during resection. Changing gloves and instruments and lavages with large amounts of saline at surgical site before closing should be done if the tumor capsules was penetrated. Prevention of seroma formation is important because seroma formation can contaminate tissue surrounding tumor bed with neoplastic cell if there is a marginal excision and/or dirty margins and can be difficult to accurate area that should be re-excision or radiation therapy. Penrose drains should be avoided because drains are tunneled from tumor bed to distant ventral sites, especially with dirty margins, contamination of...
draining tract should be treated with resection or radiation.

Figure 1. This diagram illustrates typical tumor anatomy of soft tissue sarcoma and demonstrates the presence of metastases outside the main tumor mass (2).

Surgical margins

Therefore, basic types of skin tumor resection depend on the margin of the tissue resected, based on the theoretical Ennecking system which is developed for musculoskeletal tumor excision (3). Determine the width of the surgical margins by tumor and grade, biological behavior, anatomical location and tissue barrier (tumor resistance fascia layers) (Fig 2).

Intracapsular resection

The tumor is removed from its capsule and leaving behind microscopic and/or macroscopic loci of tumor (4). This method is only suitable for benign conditions. For malignant tumours this procedure should be followed by radiation therapy or other adjuvants therapies to prevent or control the local recurrence of malignant tumors, unless the purpose of surgery is for palliative treatment and to improve the clinical signs. Debunking or cytoreductive procedure is a type of intralcalional resection to reduce the size of tumor when the tumor cannot be removed totally and is usually followed by other adjuvants therapy.

Radical resection

The tumor is removed with and entire anatomical structure. The example of radical resection is such as an amputation for a high grade soft tissue sarcoma of extremities. Surgeons have an important role to communicate with pathologists to identify the specimens margin by providing detailed information on submission form and attempting to maintain tissue architecture using ink or suture to orient tissue margins. If surgical margins are incomplete, the entire surgical scar should be removed and larger margin of surgery should be performed, if not possible adjuvant therapy such as chemotherapy or radiation therapy are indicated.

Reconstructive surgery

Reconstructive procedures may be indicated to reconstruct skin defects after tumor removal, depending on anatomical location and extent of surgical resection. Surgeons should plan the reconstructive procedure before tumor excision by using a wide range of reconstructive techniques including local or pedicle skin flaps, skin grafts, the main aim is to restore the body function without or less surgical wound complication.
**Surgery as part of multimodal therapy**

Even if complete excision is not possible, surgery can be effectively utilized as part of multimodal approach in combination with radiotherapy and or chemotherapy. By using multimodal therapy in the treatment plan, patients will gain more benefit and will have less side effects compared to single treatment option. It is important to always discuss a case with an oncologist before surgical procedure to develop the most appropriate treatment plan.

**In summary**

Surgery is the best tool to treat most neoplasms. Appropriate patient evaluation, surgical planning and tissue handling are crucial to achieve positive outcome and well defined surgical planning should consistent with the owner’s wishes and capable to improve the patient’s quality of life.

**References**

Treatment and management updates of the oral mass

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Abstract
This paper reviews an update on information of oral mass in dogs and cats. Melanoma is the most common malignant oral tumor in dogs and is followed by squamous cell carcinoma. In cats, squamous cell carcinoma is the most common malignancy oral tumor followed by fibrosarcoma. Canine acanthomatous ameloblastoma is the most common benign oral tumor in dogs. Local disease control is the goal of treatment for most animals with oral tumors. Surgery and radiation therapy are the most common treatments used for local control. Surgical resection is the most economical, fastest, and most curative treatment available for most localized tumors. Radiation therapy plays a role in the treatment of oral tumors when the tumor is not surgically resectable, the tumor has been removed with incomplete margins, and/or the tumors has metastasized to local lymph nodes without further distant metastasis. Chemotherapy is indicated for some tumors associated with high metastatic potential. Prognosis of malignant oral tumors is poor to guarded. Benign oral tumors are good in prognosis.

Introduction
Oral cancer in dogs accounts for 6% of canine cancer and is the fourth most common cancer overall. In the cat, it accounts for 3% of all cancers. Melanoma is the most common oral tumor in the dog; additional neoplastic differentials include squamous cell carcinoma, fibrosarcoma, epulides/odontogenic tumors and others (osteosarcoma, mast cell tumor, etc.). Squamous cell carcinoma is the most common oral tumor in cats, followed by fibrosarcoma.

Malignant melanoma
The most common oral malignancy in the dogs is melanoma. Oral melanoma is primarily a disease of older dogs without gender predilection but may be seen in younger dogs. A large incisional biopsy is often required for a definitive diagnosis. Cytologic touch or aspiration preparations are usually not rewarding and can result in an incorrect diagnosis because many oral tumors are associated with a high degree of necrosis and inflammation (1). The biologic behavior of canine oral melanoma is extremely variable and best characterized on the basis of anatomic site, size, stage, and histologic parameters. Melanomas in the oral cavities of dogs are found in the following locations by order of decreasing frequency: gingiva, lips, tongue, and hard palate. For dogs with oral melanoma, primary tumor size has been found to be extremely prognostic. The World Health Organization staging scheme for dogs with oral melanoma is based on size, with stage I = <2-cm-diameter tumor, stage II = 2-cm-to <4-cm-diameter tumor, stage III = 4 cm or greater tumor and/or lymph node metastasis, and stage IV = distant metastasis. (Table 1)

<table>
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<th>Node</th>
<th>Metastasis</th>
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<td>II</td>
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<tr>
<td>IV</td>
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Table 1 Clinical Staging (TNM) of Oral Tumors in Dogs and Cats (2)

Many reports suggest that stage I oral melanoma treated with standardized therapies including surgery, radiation, and/or chemotherapy has longer median survival time than the other stages and most dogs dying of distant metastatic disease, not local recurrence. The staging of dogs with melanoma should include a thorough history and physical examination, complete blood count, biochemical profile, urinalysis, three-view chest films and local lymph node aspiration (ipsilateral and contralateral nodes) with cytology whether lymphadenomegaly is present or not. There is a report that dogs with oral melanoma have a metastasis with lymphadenomegaly but some of them have a metastasis when no lymphadenomegaly is present. Additional considerations should be made for abdominal compartment testing (eg, abdominal ultrasound) in all cases of canine melanoma, because melanoma may metastasize to the abdominal lymph nodes, liver, adrenal glands or other sites. The treatment for dogs with melanoma without distant metastatic disease on staging starts with local tumor control. Surgery and radiation therapy are the most common treatments used for the local control. Surgical resection is the most economical, fastest, and most curative treatment available for most localized tumors. The type of oral surgery depends on tumor type and location. Most oral tumors have some underlying bone involvement and surgical resection should include bony margins to increase the rate of local tumor control. Mandibulectomy, segmental maxillectomy, and...
orbits are generally well tolerated by cats and dogs. These procedures are indicated for most oral tumors, particularly lesions with extensive bone invasion, poor sensitivity to radiation therapy. Margins of at least 2 cm. are usually necessary for malignant cancers include malignant melanoma. Blood loss and hypotension are the most common intraoperative complications, particularly during caudal or aggressive maxillectomy procedures. Presurgical blood typing and cross-matching to ensure adequate availability of blood products during surgery is recommended. Postoperative complications include incisional dehiscence, epistaxis, increased salivation, mandibular drift and malocclusion, and difficulty prehending food, particularly after bilateral rostral mandibulectomy caudal to the second premolar teeth. Enteral feeding tubes are not usually required following oral surgery in dogs but are often recommended for cats treated with any type of mandibulectomy as eating can be difficult for 1 to 2 months following surgery. Local disease control is the goal of treatment for most animals with oral melanoma. Regional lymph node resection has been described in cats and dogs, although it adds to clinical staging information, its effectiveness in controlling local and metastatic disease is unknown. If postoperative adjuvant therapy (e.g., radiation therapy or chemotherapy) is being contemplated, regional nodes having confirmed or suspicious metastasis should be extirpated to achieve minimal residual disease. Radiation therapy can be effective for locoregional control of oral tumors. Radiation therapy plays role in the treatment of canine melanoma when the tumor is not surgically resectable, the tumor has been removed with incomplete margins, and/or the melanoma has metastasized to local lymph nodes without further distant metastasis. Malignant melanoma is known to be radiation responsive. Coarse fractionation schemes for canine melanoma utilizing 6 to 9 Gy weekly to every other week to a total dose of 24 to 36 Gy have been reported by a variety of investigators with complete remission rates of 53 to 69% and partial remission rates of 25 to 30% (3). Acute effects are common but self-limiting. Depending on the tissues irradiated, these include alopecia and moist desquamation, oral mucositis, dysphagia, and ocular changes, such as blepharitis, conjunctivitis, keratitis, and uveitis (1). Chemotherapy is indicated for some tumors associated with high metastatic potential but oral melanoma is chemoresistant in both dogs and cats (1). There many studies report that chemotherapy plays an insignificant role in the adjuvant treatment of canine melanoma (3). Malignant melanoma is generally an immunogenic tumor. Immunotherapy represents one potential systemic therapeutic strategy for melanoma. Immunotherapy strategies to date in canine melanoma have used autologous tumor cell vaccines, allogenic tumor cell vaccines transfected with interleukin-2 or GM-CSF, liposomal-encapsulated nonspecific immunostimulators (eg, L-MTP-PE), intralesional Fas ligand DNA, bacterial superantigen approaches with granulocyte macrophage colony-stimulating factor or interleukin 2 as immune adjuvants, and last, canine dendritic cell vaccines loaded with melanosomal differentiation antigens. Although these approaches have produced some clinical antitumor responses, the methodologies for the generation of these products are expensive, time-consuming, sometimes dependent on patient tumor samples being established into cell lines, and fraught with the difficulties of consistency, reproducibility, and other quality-control issues (3). The advent of DNA vaccination circumvents some of the previously encountered hurdles in vaccine development. DNA is relatively inexpensive and simple to purify in large quantities. Although DNA vaccines have induced immune responses to viral proteins, vaccinating against tissue-specific self-proteins on cancer cells is clearly a more difficult problem. One way to induce immunity against a tissue-specific differentiation antigen on cancer cells is to vaccinate with xenogeneic (different species) antigen or DNA that is homologous to the cancer antigen. The results of the used of xenogeneic DNA vaccination in canine malignant melanoma is safe, leads to the development of antityroosinase antibodies (3), is potentially therapeutic, and is an attractive candidate for further evaluation. Preliminary results suggest immunotherapeutic approaches, in combination with either surgery or radiation therapy, are promising in the management of oral melanoma (1). The prognosis for dogs with oral melanoma is guarded. Metastatic disease, particularly to the lungs, is the most common cause of death. Surgery and radiation therapy can provide good control of local disease, but strategies to manage the high metastatic potential of malignant melanoma, such as chemotherapy and immunotherapy, require further investigation.

Squamous cell carcinoma

Squamous cell carcinoma (SCC) is the most common oral tumor in cats and the second most common in dogs. Oral SCCs are categorized into 2 groups: tonsillar or nontonsillar. Tonsillar SCC is reported to be highly metastatic, with frequent local tumor recurrence following surgical or radiation treatment. Fifty percent to 78% of oral SCCs arise from nontonsillar region. The metastasis rate for nontonsillar SCC is quite low; therefore, achieving local tumor control is considered the most important factor. Despite a reported low metastatic rate, full staging is required prior to treatment recommendation for nontonsillar SCC (Oral tumors in dogs are staged according to WHO classification system as malignant melanoma above). Local tumor control may be achieved by using surgery, radiation therapy, or combination of surgery and...
radiation. To maximize the probability of removing all cancer cells, surgical margins of at least 2 cm. are recommended for SCC in dog (4). If possible, SCC in the cat should be treated with surgical margins greater than 2 cm. because of high local recurrence rates (1). Full-course radiation therapy is indicated, if surgery is not feasible because of tumor size or location, and if surgical margins are incomplete. Due to the low metastatic potential of nontonsillar SCC, chemotherapy is not routinely recommended after surgery or radiation therapy (4). The prognosis for dogs with oral SCC is good, particularly for rostral tumor locations. Local tumor control is usually the most important challenge, although metastasis to the regional lymph nodes is reported in up to 10% of dogs and to the lungs in 3% to 36% of dogs (1). In contrast, SCC of the tonsils and base of the tongue are highly metastatic, with metastasis reported in up to 73% of dogs, and local or regional recurrence is common (1). The prognosis for cats with oral SCC is poor (1). There is no known consistently effective treatment. Local control is the most challenging problem. The use of esophagostomy or gastrostomy tubes is important to provide supplemental nutrition in these cats postoperatively. Radiation therapy alone is generally ineffective in the management of cats with oral SCC. However, the combination of radiation therapy with radiation sensitizers or chemotherapy improves response rates and survival times. Palliative radiation protocols, consisting of 8 Gy fractions on days 0, 7, and 21, are not recommended because of poor disease control and radiation-induced adverse effects (1).

Fibrosarcoma

Oral fibrosarcoma (FSA) is the second most common oral tumor in cats and the third most common in dogs. A unique form, histologically low-grade, yet biologically high-grade tumors, is seen in the oral cavity, has a tendency to grow to quite a large size, and invades deeper structures, including bone. Metastasis can be seen in up to 20% of the cases (1). Metastasis is rare, but these tumors are infiltrative with microscopic tumor cells invading along fascial planes and often recurrence after surgical excision. This tumor, which is common on the hard palate and maxillary arcade between the canine and carnassial teeth of large breed dogs (1). Even with a biopsy result suggesting fibroma or low-grade FSA, the treatment should be aggressive, especially if the cancer is rapidly growing, recurrent, or invading bone. Larger resections, including hemimandibulectomy, hemimaxillectomy, orbitectomy, and radical maxillectomy, may be necessary for more aggressive tumors, especially fibrosarcoma. Radiation therapy can be used as an adjunct for incompletely resected tumors or tumors with an aggressive local behavior. The prognosis for dogs with oral fibrosarcoma is guarded. These are locally aggressive tumors and local control is more problematic than metastasis. Surgery is the most common treatment for oral fibrosarcoma.

Canine acanthomatous ameloblastoma

Canine acanthomatous ameloblastoma is the recent term of Acanthomatous epulis. “Epulis” are the most common benign tumors of the canine oral cavity which is originate from odontogenic origin. While fibromatous and ossifying epulides are confined to the gingiva, acanthomatous epulides often extensively invade adjacent bone. Canine acanthomatous ameloblastoma presents as an exophytic, irregular gingival mass on either side of the dental arcade, with the predilection for the rostral region of the mandible (5). Bone involvement is common. There is no report of metastasis to regional lymph node or other distant organs (5). Computed tomography or magnetic resonsance imagine is recommended prior to surgical or radiation treatment to accurately determine the extend of the primary tumor (5). Because of the highly infiltrative behavior of this tumor, wide local excision of adjacent soft tissue and bone at least 2 cm. is necessary for cure and it is the treatment of choice. Radiation therapy is indicated for dogs with tumors that are not curable with surgery alone. Prognosis of this tumor is good. Local recurrence rates following bone-removing surgery are less than 5% (1). Megavoltage radiation therapy, consisting of an alternate day protocol of 4 Gy per fraction to a total of 48 Gy, results in a 3-year progression-free survival rate of 80% in dogs (1).

General discussion

The oral tumors in dogs and cats are the most common cancer overall. Melanoma is the most common oral tumor in dogs. Squamous cell carcinoma is the most common oral tumor in cats. Most of oral tumor in dogs and cats are malignant and have a bone involvement. Oral tumors need to be treated with wide surgical therapy at least 2 cm. margin or radiation therapy. Surgical treatment (maxillectomy, mandibulectomy) is the treatment of choice for control localized tumor because it is the most economical, fastest and most curative treatment. Radiation therapy plays role in the treatment of oral tumor when the tumor is not surgically resectable, the tumor has been removed with incomplete margins, and/or the tumor has metastasized to local lymph nodes without further distant metastasis. There are some postoperative complications include incisional dehiscence, epistaxis, increased salivation, mandibular drift and malocclusion, and difficulty prehending food. Radiation therapy also have complications. Acute effects are common but self-limiting. Depending on the tissues irradiated, these include alopecia and moist desquamation, oral mucositis, dysphagia, and ocular changes, such as blepharitis, conjunctivitis, keratitis, and uveitis. The prognosis of malignant oral tumor is poor. Metastatic disease, particularly to the lungs, is the most common cause of death.
**Conclusions**

Oral tumors are the most common tumor in dogs and cats. Melanoma is the most common tumor in dogs and squamous cell carcinoma is the most common tumor in cats. Others oral tumors are fibrosarcoma, osteosarcoma, mast cell tumor, and odontogenic tumors which are benign. The World Health Organization staging scheme for dogs with oral tumors is based on size, with stage I = \(<\)2-cm-diameter tumor, stage II = 2-cm-to \(<\)4-cm-diameter tumor, stage III = 4 cm or greater tumor and/or lymph node metastasis, and stage IV = distant metastasis. The treatment for dogs and cats with oral tumors starts with local tumor control. Surgery and radiation therapy are the most common treatments used for the local control. Surgical resection is the most economical, fastest, and most curative treatment available for most localized tumors. The type of oral surgery depends on tumor type and location. Most oral tumors have some underlying bone involvement and surgical resection should include bony margins to increase the rate of local tumor control. Mandibulectomy, segmental maxillectomy, and orbitectomy are generally well tolerated by cats and dogs. These procedures are indicated for most oral tumors, particularly lesions with extensive bone invasion, poor sensitivity to radiation therapy. Margins of at least 2 cm are usually necessary for malignant cancers such as SCC, malignant melanoma, and fibrosarcoma. Local disease control is the goal of treatment for most animals with oral tumors. Radiation therapy plays a role in the treatment of oral tumors when the tumor is not surgically resectable, the tumor has been removed with incomplete margins, and/or the cancer has metastasized to local lymph nodes without further distant metastasis. Chemotherapy is indicated for some tumors associated with high metastatic potential but oral melanoma is chemoresistant in both dogs and cats and non tonsillar SCC has the low metastatic potential. The prognosis for dogs with oral melanoma is guarded. Metastatic disease, particularly to the lungs, is the most common cause of death. The prognosis for cats with oral SCC is poor. There is no known consistently effective treatment. Local control is the most challenging problem.

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Associate Professor Chanin Kalpravidh

**References**

Upper urinary tract obstruction: updates on surgical managements

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Background
Upper urinary tract obstruction is a frequently problem in veterinary practice. Urololiths, mass and congenital defects can be defined as causes of complete and partial obstruction. Data from Prasu-Arthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University demonstrate approximately 50% of upper urinary tract obstruction cases caused from uroliths (struvite and calcium oxalate uroliths are mostly presented).

Pathophysiology of upper urinary tract obstruction
The physiological response of the obstruction depends on multiple factors including animal’s age, degree and duration of obstruction (1, 2, 3). After 1-1.5 hours, complete unilateral ureteral obstruction renal blood flow begins to decrease and gradually decrease until 2 weeks after obstruction the renal blood flow is 20% of normal dog (4). Moreover, hydrourerter and smooth muscle hypertrophy are induced by obstruction and continuously altered into fibrosis (5, 6, 7).

Return of glomerular filtration rate to 46% of normal after obstruction are resolved is 2 weeks. In addition, obstructive period more than 4 weeks can cause a permanent impairment of concentration ability (1, 2, 3).

Clinical presentation
Clinical signs of ureteral obstruction consist of hematuria, stranguria, pollakiuria and abdominal pain. Vomiting, polyuria and polydipsia due to azotemia also present. In dog, abdominal pain, and hematuria are common in upper urinary obstruction (8). In cat, nonspecific clinical signs including decrease appetite, lethargy and weight loss are commonly found (9).

Patient evaluation
Laboratory findings
Routine complete blood count, serum chemistry panel, urinalysis, urine culture and diagnostic imaging should be done.

Diagnostic imaging
Plain radiography: Abdominal radiography is useful for evaluate size, shape and location of radiopaque calculi. Radiolucent or small radiopaque calculi and hydroureration cannot be seen from plain film.
Intravenous excretory urography: Intravenous pyelography is commonly used for ureter and renal pelvis dilation assessment. In cat, antegrade pyelography is useful for ureteral obstruction diagnosis. However, contrast media leakage to renal parenchyma or perirenal tissue may lead a misinterpretation (10).

Ultrasonography: Ultrasonography is suggested to diagnose upper urinary tract obstruction together with survey radiography. It is a preferable method to diagnose hydronephrosis and hydrourerter (11).

Management of upper urinary tract obstruction
Treatment of nephroliths should be performed in only problematic case including complete obstruction, parenchymal compression, pain, recurrent infection. Medical dissolution of nephroliths should be done in struvite nephroliths with ureteral stenting for relief ureteral obstruction, facilitate urine flow and promote ureteroliths to medicate urine exposure. If calcium oxalate uroliths, which cannot be dissolved is presented, surgical management is suggested (12).

Surgical management
Nephrotomy and pyelolithotomy: Data from ACVIM consensus (2016) (12) suggested minimal invasive procedure for nephroliths removal including dissolution, extracorporeal shockwave lithotripsy (use only in dog, cat’s kidney is more sensitive to shock wave and induce tissue injury) and endoscopic nephrolithotomy, due to the renal function damage of bisectonal nephrotomy which decreasing renal function to 30-50%. Bisectional nephrotomy is performed in nephroliths that dilation of renal pelvis and proximal ureter does not extend beyond the renal parenchyma. The alternative procedure pyelolithotomy can be performed when renal pelvis and proximal ureter dilate.

Nephrectomy: Nephrectomy is the last choice of upper urinary obstruction treatment. It has to perform in no kidney function, end-stage hydronephrosis and severe infection with function of remaining kidney (10).

Surgery of the ureter: For ureteral obstruction, both of partial and complete obstruction minimal invasive procedure should be immediately done to prevent renal function impairment. Ureteral stenting is the most effective technique to relief ureteral obstruction in dog, while subcutaneous ureteral bypass is the first choice for cat from a small ureteral diameter cause. The first step of ureteroliths correction is try to retropropulse the ureteroliths to renal pelvis, if it cannot be pass ureterotomy has to be done. In cat, ureterotomy is suggested in case found ureterolith at proximal third of ureter. Ureteroneocystostomy is an implantation of distal ureter to correct ectopic ureter or distal ureteral
lesions excision (e.g., ureterolith at distal two-thirds of the ureter in cat). In dog, intravesical technique (mucosal apposition technique) is appropriate for dog, whereas extravesical technique is preferred in cat due to the minimal postoperative ureteral swelling. Ureteroureterostomy is a reapposition of ureter usually perform when proximal end of the ureter is disable implanted into urinary bladder. Urine leakage from surgical site and ureteral stricture should be monitored as a common postoperative complication (10, 11).

References
Ways out of GI foreign bodies

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Abstract

This study aims to review and update on information about gastrointestinal foreign body in small companion animal. Gastrointestinal foreign body is considered as an emergency case. Ways out of these GI foreign bodies are difference in each organ, which include of esophagus, stomach and small intestine. The clinical signs, degree of severity and outcome after removal depend on location, type, duration and degree of obstruction.

Introduction

Gastrointestinal foreign bodies are commonly present in small companion animal practice which considered as emergency cases. The clinical signs and degree of severity are variety which depend on the type and size of foreign body, duration and degree of obstruction, location, and the presence of perforation of foreign material. Clinical signs of gastrointestinal foreign body are commonly associated with gastrointestinal signs such as hypersalivation, anorexia, vomiting, diarrhea, abdominal discomfort and lethargy. Gastrointestinal obstruction results in disturbances of fluid balance, acid-base status and serum electrolyte concentrations due to hypersecretion and sequestration within the gastrointestinal tract which is exacerbated by vomiting and reluctance to consume adequate volumes of food fluid can also affect the intravascular volume and hydration (1). Diagnosis of gastrointestinal foreign body can be achieved in most of cases by history, physical examination, and plain radiography. Further investigation to diagnose gastrointestinal foreign body might be required in complicated cases such as positive contrast radiography or ultrasonography. Treatment plan of gastrointestinal foreign body is depended on type of material, location and obstruction signs of gastrointestinal tract. Occasionally, ingested material can pass through the gastrointestinal tract without causing any damage. Some foreign material in upper gastrointestinal tract can be removed by rigid or flexible endoscopy. Surgical intervention will be required in unsuccessful removal of foreign material by noninvasive ways. In non-or partial obstructive foreign body in stomach and intestine can be removed by gastroscopy and enterotomy. Intestinal resection and anastomosis will be determined by surgeon for nonviable tissue of damaged and obstructed gastrointestinal tract. Mostly, in uncomplicated gastrointestinal foreign body cases are excellent prognosis with rapid recovery, but in some animal with secondary metabolic signs need to be hospitalized and treated to return to normal condition. Postoperative examination may be required of any leakage of gastrointestinal tract (4).

Esophageal foreign body

Esophagus is a tube that connect and transport ingesta and fluid from pharynx to stomach. Dysfunction of phase of swallowing of the esophagus caused by inflammatory conditions, functional lesions and mechanical lesions. Mechanical obstruction results in accumulation of food and secretions cranially to the obstruction with segmental distension, which disrupts normal neuromuscular function and decrease peristalsis. Common clinical signs are regurgitation of food, gagging, hypersalivation or respiratory distressed. Completely release of obstruction may not fully resolve the normal function of the esophagus. Esophageal foreign body obstruction considered as an emergency cases. The most common foreign body in dog are ingested bones. Airway assessment is importance for an airway compromised patient. History taking and physical examination is crucial for diagnosis and treatment. Radiography with or without positive contrast esophagography is a diagnosis choice to localized and evaluate the degree of obstruction. Initial attempt to retrieve foreign body is by endoscopy or fluoroscopy with grasping forceps. Surgical removal is in consideration after endoscopic removal is failed (2,7). Endoscopic removal with retrieval instruments can be performed by rigid or flexible video endoscope under anesthetized patient. Fluoroscopy with grasping forceps is also useful in esophageal foreign body cases. The foreign material either be removed or pushed distally in to stomach. After foreign material was removed, the esophagus should be inspected for any damage or perforation. If there is a presentation of perforation, it should subjectively determine for size and patient complications such as decrease oxygen saturation, hypotension and pneumothorax. Small perforations can be sealed by itself with prohibited of food and water consumption for at least 72 hours. Surgical intervention is indicated for any risk of lacerations of esophagus, vessel rupture or tracheal tear, perforated esophageal foreign body and failure to remove foreign material endoscopically (2,3). Surgical removal of esophageal foreign body is performed by esophagotomy or esophageal resection and anastomosis. Cervical esophagus is approached by a ventral midline incision, separating sternohyoid muscles and retracting trachea to the right. The intrathoracic esophageal approached can be performed by a left-sided intercostal thoracotomy depends on the location of foreign materials. Esophagotomy can be
perforation with secondary septic and determine degree meal should be avoid. Ultrasonography is also suspected perforation, positive contrast with barium hallow organs rupture such as stomach. Animal of abdomen. Pneumoperitoneum is an indicative of foreign body in most case is taken by plain radiographs aware in sharp foreign body. To diagnose gastric perforation with septic peritonitis should be and secondary regurgitation dur to esophageal irritation. content in stomach with reflux of content by vomiting results in abdominal distension due to accumulation of partial or complete obstruction of pyloric outflow consequence in electrolyte imbalance. Patient with foreign body mostly presents with vomiting which occur in dog more than cat. Animal with gastric foreign body obstruction. The proximal part of foreign body is recommended as a primary attempt to remove foreign body in stomach. Gastrotomy for removal of foreign material is also recommended in case that is failed by endoscopy or cases that material cannot be remove by endoscopy due to size, shape and other factors. Gastrotomy incision length depends on foreign body size and made in the middle of the ventral body of stomach between the branch of the gastric and gastroepiploic vessels. Placement of stay sutures between incision help prevent content spillage. A routine closure of gastric incision can be achieved by continuous double-layer inverting closure with monofilament absorbable material that resist to acid and enzyme rich environment such as polyglyconate and poliglecaprone 25. Post-operative rehydration and correction of electrolyte imbalance is crucial. Oral intake of food and water may start after surgery for 12 hours. Prognosis of gastric foreign body case that is removed by either endoscopy or gastrotomy without devitalization and perforation of GI tract is considered very good (3,4).

**Gastric foreign body**

Stomach is a temporary reservoir and grinding of food where digestive juices are added. During digestion, stomach dilates and increase of motility in order to mix food and push content into duodenum. The remaining food content, inappropriate-sized particle or foreign materials are forced retrograde and repeat in digestion. In veterinary companion practice, gastric foreign bodies represent most cases of gastrointestinal foreign bodies which occur in dog more than cat. Animal with gastric foreign body mostly presents with vomiting consequence in electrolyte imbalance. Patient with partial or complete obstruction of pyloric outflow results in abdominal distension due to accumulation of content in stomach with reflux of content by vomiting and secondary regurgitation due to esophageal irritation. Gastric perforation with septic peritonitis should be aware in sharp foreign body. To diagnose gastric foreign body in most case is taken by plain radiographs of abdomen. Pneumoperitoneum is an indicative of hallow organs rupture such as stomach. Animal suspected perforation, positive contrast with barium meal should be avoid. Ultrasonography is also recommended for diagnosis of radiolucent material, perforation with secondary septic and determine degree of obstruction. Endoscopic removal of gastric foreign body is recommended as a primary attempt to remove foreign body in stomach. Gastrotomy for removal of foreign material is also recommended in case that is failed by endoscopy or cases that material cannot be remove by endoscopy due to size, shape and other factors. Gastrotomy incision length depends on foreign body size and made in the middle of the ventral body of stomach between the branch of the gastric and gastroepiploic vessels. Placement of stay sutures between incision help prevent content spillage. A routine closure of gastric incision can be achieved by continuous double-layer inverting closure with monofilament absorbable material that resist to acid and enzyme rich environment such as polyglyconate and poliglecaprone 25. Post-operative rehydration and correction of electrolyte imbalance is crucial. Oral intake of food and water may start after surgery for 12 hours. Prognosis of gastric foreign body case that is removed by either endoscopy or gastrotomy without devitalization and perforation of GI tract is considered very good (3,4).

**Intestinal foreign body**

Intestinal foreign body obstruction is one of the most frequent indication for celiotomy in dog and cat due to smaller size of luminal diameter compare to upper GI tract. The clinical signs are varying depend on the location, duration, severity of obstruction and secondary complications. Mechanical intestinal partial or complete obstruction effects on fluid, acid base and electrolyte balance. The non-linear foreign body compromises blood supply due to excessive luminal distension and hypersecretion lead to intestinal wall edema and necrosis. These factors cause ileus and intraluminal bacterial proliferation and translocation leading to systemic endotoxemia (5). Hypochloremia, metabolic alkalosis, hypokalemia and hypoponatremia have been reported in dogs with gastrointestinal foreign bodies, dogs with linear foreign body were more likely demonstrated hemoconcentration, an increase in blood urea nitrogen concentrations, and higher pH (1,5). Diagnosis of mechanical intestinal foreign body obstruction can be done by radiography and ultrasonography. Radiography of abdomen with obstruction may present segmental or generalized loops of gas dilated. In linear foreign body, the intestinal appears plicated. Ultrasonography is considered more accurate and produces more confident results than abdominal radiography (6). Linear foreign body is more common in cat and creates a unique form of foreign body obstruction. The proximal part of foreign body typically found at tongue base. The intestine presents in accordion-like pleats along the object, and the object become embedded into mesentery side as the peristalsis continue. Development of perforation, peritonitis and sepsis may result from a delay diagnosis and surgical removal. For intestinal obstruction with linear or non-linear foreign material, surgical management is recommended, and the entire gastrointestinal tract should be examining thoroughly for the presence of
peristalsis, vascular pulsations. Objectively determining viability of tissue are surface oximetry or fluorescein infusion. For non-linear foreign body with relatively healthy tissue, enterotomy and removal of foreign material can be done. In unhealthy or necrosis of effected bowel should be resected, and functional end-to-end anastomosis is performed. Dogs with linear foreign body required significantly more gastrotomies, enterotomies, and intestinal resection and anastomosis, than dogs with non-linear foreign body (5).

**Conclusion**

Animal with gastrointestinal foreign body is common and urgent case in veterinary practice. Assessment and diagnosis are crucial for further treatment plan. Secondary changes from obstruction should be concern and corrected. Post-endoscopic or surgery management also should be accounted.

**Acknowledgements**

Asst. Prof. Pasakorn Brikshavana. DVM. Ms

**References**


“Head tilt rabbit” What should I do?

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Abstract
Head tilt rabbits are common neurological disorder patients that come to clinics. Vestibular system is the system control balance of body, head and eye movement. Central vestibular and peripheral vestibular disorders are abnormal condition in this system. Success of treatment is to understand what happen to the vestibular system and veterinarians should know what is the cause of the disease.

Introduction
“Head tilt” or “Torticollis” in rabbit can found with more frequently. Some pet rabbits showed only head tilt sign but most of them presented with other neurological signs. There are many causes can make rabbit head tilt. Vestibular system is the neurological system that was affected.

Vestibular system
Vestibular system is the system that provides balance of movement and equilibrium of eyes, head and body. This system can be anatomically and functionally divided into two components these are central vestibular system and peripheral vestibular system. The peripheral vestibular system detect rotational head movement, linear acceleration and gravity. These system will stimulate the central vestibular system for symmetrically activity. Separation of diseases affecting these two main system is very important for differential diagnosis, treatment and prognosis in rabbits with head tilt.

Central vestibular disorder
There are many causes can make abnormality of these area. Infectious diseases are the problem usually found in patient rabbits. Bacteria, fungi, virus and protozoa can also affect the brain stem. *Encephalitozoon cuniculi* is an intracellular protozoa parasite. This protozoa found to be widespread in pet rabbit. There are a lot of reports about this protozoa in many countries. Besides, trauma to the head of the rabbit such as accidental falling are common in pet rabbits. In addition, cerebrovascular disease especially problem from heat stroke can cause the vestibular disorder. Because rabbits do not sweat and very susceptible to heat stroke when they affected it will lead to critical condition. Heat stroke can lead to ischaemia and edema of brain stem. Heat stroke rabbit was showed variety of neurological signs. Moreover, metabolic diseases and toxins eventually affect the brain function and may lead to neurological disorder in pet rabbit. Most of rabbit with these problem often show other systemic illness signs such as anorexia and lethargy depend on the cause of abnormality. Lymphoma is the most commonly reported tumor in the central nervous system in rabbits. Clinical signs are rely on the size and site of the tumor.

Peripheral vestibular disorder
The peripheral vestibular system composed of inner ears and middle ears. Abnormality in these areas can be divided to inner ear disorders and middle ear disorders according to anatomical and functional structures. Causes of middle ear diseases are the same as for inner ear diseases, but prognosis for middle ear is better. Microorganisms can penetrate the middle ear and inner ear by the Eustachian tube or external ear downward. Trauma can make an ear drum rupture or tympanic bulla fracture. Besides, foreign bodies can occasionally occur in the ear canal and can make otitis interna in worst case. Also, toxins and some medications can affect the ear by pass through the ear canal or blood stream such as gentamicin.

Diagnosis and treatment
Diagnosis of head tilt rabbits should be followed the history and clinical signs of patient rabbits. Physical and neurological examinations are very important to find causes of the disease. A positive serology result can indicate exposure to *Encephalitozoon cuniculi* but cannot conclude the cause of the disease. Blood results can rule out the other systemic illness from neurological abnormality. Radiographs and computed tommographs are very useful to detect the problem of skull, middle ear, inner ear and brain diseases. Treatment of head tilt rabbit depend on the causes of vestibular disorders. Antibiotic drugs, antiprotozoals and anti-inflammatory drugs can be used in patient rabbits but beware of corticosteroids using because its side effects in rabbit. Prognosis for rabbit head tilt is variable depend on the cause of the disease. Rabbit with peripheral vestibular disorder have good to guarded prognosis. Furthermore, prognosis for the rabbit that still eating and have alert mental condition is better.

Conclusions
There are many causes can affect the vestibular system. Differential diagnosed the cause of the problem is very useful for treatment and prognosis for patient rabbits. Therefore, It’s very important to understand what happened to the head tilt rabbits.
References


How to approach rabbit gastro-intestinal syndrome

Chaowaphan Yinharnmingmongkol
Animal Space Exotic pet hospital

Rabbits are very popular pets in last ten years ago and become to domestic pets in the future. The commons disease of rabbit including teeth problems, gastrointestinal problem, skin problem, ocular and others problem but the most important are gastrointestinal problems. When the rabbit become ill for any reason there will show gastrointestinal sign such as pain, gastrointestinal hypomotility/gastrointestinal stasis. When rabbit stop eating 50% of rabbit will be diagnosis with gastrointestinal sign and as you know when rabbit stop eating only 24 hours this clinical sign very critical for rabbit. The rabbit’s GI tract requires fiber for stimulate their GI tract and water for pass the nutrient to microflora at cecum

Clinical sign of gastrointestinal tract disorder
1. Anorexia or stop eating
2. Sign of pain
3. Abnormal fecal size
4. No feces
5. Diarrhea

Clinical diagnosis
1. Physical examination
2. Radiography
3. Ultrasound

Therapeutic plan
1. Restore hydration
2. Relieving pain
3. Restoring GI motility
4. Correct underlying cause

Fluid therapy is the most important to treat GI problem and aggressive treatment should be done. GI tract disorder in rabbits are poor prognosis but if early detection and aggressive treatment will successes to solve this disease

<table>
<thead>
<tr>
<th>Key Drug</th>
<th>Drug Class</th>
<th>Dose Range</th>
<th>Frequency</th>
<th>Route</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoclopramide</td>
<td>Dopamine agonist</td>
<td>0.5mg/kg</td>
<td>q.8-24</td>
<td>SC or PO</td>
<td>This is a useful promotile drug in rabbits with ileus. Do not administer in the presence of an obstruction.</td>
</tr>
<tr>
<td>Cisapride</td>
<td>5-HT agonist</td>
<td>0.5mg/kg</td>
<td>q.8-12h</td>
<td>PO</td>
<td>Promotile agent is available only as a compounded formula</td>
</tr>
<tr>
<td>Dimethicone</td>
<td>Antifoaming agent</td>
<td>20-40mg/kg</td>
<td>q.6h</td>
<td>PO</td>
<td>In cases with much gas accumulation, this may relieve gas distension and pain</td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>Adsorbent</td>
<td>2g in 20mL water</td>
<td>q.24h</td>
<td>PO</td>
<td>Binds bacterial toxins to prevent/manage endotoxemia or clostridial toxemia</td>
</tr>
</tbody>
</table>

Dosages and Indications
<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loperamide</td>
<td>Anti-motility agent</td>
<td>0.1 mg/kg q.8h X 3 days, then q.24h for days 4 and 5</td>
<td>PO</td>
<td>3 days</td>
<td>Use with caution in treating diarrhea. Could cause ileus.</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>NSAID</td>
<td>0.1-0.6 mg/kg q.24h</td>
<td>SC or PO</td>
<td></td>
<td>Use for GI pain, but evaluate for secondary ulceration with chronic use</td>
</tr>
<tr>
<td>Carprofen</td>
<td>NSAID</td>
<td>2-4 mg/kg q.24h SC, IV, or PO</td>
<td>q.24h</td>
<td>4 days</td>
<td>Use for GI pain, but evaluate for secondary ulceration with chronic use</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>H2 blocker</td>
<td>2-5 mg/kg BID</td>
<td>PO</td>
<td></td>
<td>For suspected GI ulceration</td>
</tr>
<tr>
<td>Famotidine</td>
<td>H2 blocker</td>
<td>0.5 mg/kg q.12-24h</td>
<td>PO</td>
<td></td>
<td>For suspected GI ulceration</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>Narcotic agonist/antagonist</td>
<td>0.01-0.05 mg/kg q.6-8h</td>
<td>SC</td>
<td></td>
<td>Use for GI pain</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>Narcotic agonist/antagonist</td>
<td>0.1-0.5 mg/kg q.2-4h</td>
<td>SC</td>
<td></td>
<td>Use for GI pain. May be more sedating than buprenorphine</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>Anthelminic</td>
<td>10-20 mg/kg q.14 days</td>
<td>PO</td>
<td></td>
<td>For treating enteric parasites</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>Sulfamethoxazole</td>
<td>30 mg/kg BID for 10 days</td>
<td>PO</td>
<td></td>
<td>For the treatment of coccidia. Can be administered BID for antimicrobial use.</td>
</tr>
<tr>
<td>Critical Care for Herbivores</td>
<td>Nutritional support</td>
<td>5-20 mL q.2-6h</td>
<td>PO</td>
<td></td>
<td>When rehydrated is a slurry that can be syringe-fed. 1.9 kcal/mL when mixed 1:1.5 powder:water</td>
</tr>
</tbody>
</table>

**References**

Look at exotic eyes; how different from domestic spp.

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The similarity between eyes of different species means that the basic techniques of ocular examination can be extrapolated from those of dogs and cats. The differences generally occur because of the small size of many eyes of exotic animal species, be they rodents, rabbit, birds and reptiles.

Ancilliary ophthalmic tests include measurement of tear production (Schirmer tear test; STT) and determination of intraocular pressure (IOP). Two key tests same in dogs and cats which should be undertaken as routine ocular examination. For some species groups do have data, but for all many species have no values for normal animals. (Table 1 and Table 2)

Table 1 Normal values for tear quantification tests in Exotic species

<table>
<thead>
<tr>
<th>Species</th>
<th>Schirmer tear test values (mm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>15 - 25</td>
</tr>
<tr>
<td>Cats</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Ferret</td>
<td>5.31 +/- 1.32</td>
</tr>
<tr>
<td>Rabbit</td>
<td>5 - 8</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>0.5 - 1</td>
</tr>
<tr>
<td>Chinchilla</td>
<td>1 - 1.5</td>
</tr>
<tr>
<td>Long-eared hedgehogs</td>
<td>1.7 +/- 1.2</td>
</tr>
<tr>
<td>Birds (Psittaciformes)</td>
<td>5 - 8</td>
</tr>
<tr>
<td>Eurasian Tawny owls</td>
<td>3.12 +/- 1.92</td>
</tr>
<tr>
<td>Little owls</td>
<td>3.5 +/- 1.96</td>
</tr>
<tr>
<td>Common buzzards</td>
<td>12.47 +/- 2.66</td>
</tr>
<tr>
<td>European kestrels</td>
<td>6.20 +/- 3.67</td>
</tr>
</tbody>
</table>

Table 2 Normal intraocular pressure values in Exotic species

<table>
<thead>
<tr>
<th>Species</th>
<th>Intraocular pressure values (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>15 - 25</td>
</tr>
<tr>
<td>Cats</td>
<td>15 - 25</td>
</tr>
<tr>
<td>Ferret</td>
<td>14.50 +/- 3.27</td>
</tr>
<tr>
<td>Rabbit</td>
<td>20</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>18.27 +/- 4.55</td>
</tr>
<tr>
<td>Chinchilla</td>
<td>4.7 - 14.7</td>
</tr>
<tr>
<td>Long-eared hedgehogs</td>
<td>20.1 +/- 4.0</td>
</tr>
</tbody>
</table>

Ocular pharmacology. Mydriatic drugs can be used and a significant problem in different exotic animals. The mammals have autonomically innervated iris musculature which means that parasympatholytic agents such as atropine and tropicamide are generally effective in providing pupil dilation. In dog and cats, tropicamide is used diagnostically owing to its rapid onset (20-40 minutes) but the rabbit and rodents with pigment irides often show poor or slowly acting pharmacologically mediated mydriasis. Some contain atropinase, an enzyme that breaks down atropine. The lack of mydriasis mediated by these muscarinic parasympatholytic agents in reptiles and birds occurs because here the muscles of the iris are not smooth muscles, autonomically innervated as in the mammals. Another important pharmacological factor in many exotic animal species concerns the fact that with such a small globe size, and a small blood volume, using the same size of eye drop that one would employ in a dog a more frequency times as as big as a small rodent or a bird, must give a high risk of substantial systemic toxicity.

The rabbit

The eye are placed very prominently and laterally. The rabbit has a circular pupil (liked dogs), and a heavily pigmented iris in pigmented species. The rabbit eye has several anatomical peculiarities that differentiate it from that of dogs and cats. An important feature is the retrobulbar venous plexus, vital to note during enucleation. The vasculature of the merangiotic lagomorph fudus is unlike that of any other mammal, in that the vessels extend from the optic disc with myelinated nerve fibres horizontally. Another anatomical difference concerns the nasolacrimal duct. There is a single nasolacrimal punctum in the rabbit and a duct which has a convoluted passage through the lacrimal and frontal bones, passing close to the molar and incisor tooth roots. Malocclusion of the molar arcades in particular results in retropulsion of the tooth into the weakened maxillary bone, with subsequent nasolacrimal occlusion.
 Conjunctivitis and dacryocystitis are thus common and potentially problematic conditions in domestic rabbits. Another ocular problem in rabbit included blepharitis, keratitis, retrobulbar abscessation, entropion, corneal epithelial dystrophy, corneal ulceration, conjunctival centripetalisation or pseudopterygium, nictitans gland prolapse, cataract, glaucoma and uveitis (encephalitozoon-associated lens induced uveitis).

**The guinea pig**

The guinea pig eye is not dissimilar to that of other rodents of similar size but with one major difference, that of the retinal vasculature which is paurangiotic with its lack of blood vessels. The guinea pig is incapable of synthesising its own vitamin C. However, quite remarkable and with potential effects on the lens and retina. The guinea pigs shows that 45% had some ocular anomaly, many of them lens-related. These guinea pigs are subject to levels of dietary ascorbate sufficiently low to lead to lens opacities.

Diseases of the guinea pig eye include conjunctivitis, keratoconjunctivitis sicca, keratitis, corneal lipidosis, anophthalmos, cataract, entropion, anangiotic retina, trichiasis, excess lipid deposition in the inferior conjunctiva, heterotopic bone formation and glaucoma.

**The rat and mouse**

Rats and mice have three lacrimal structures, the intraorbital gland, situated deep in the orbit, the extraorbital gland, located near the base of the masseter muscle, and the Harderian gland positioned as a U shape around the optic nerve behind the globe. The cornea is very thin in these species and the lens is spherical, taking up a large amount of the intraocular volume, in order to focus light on the retina in such a small eye. Almost all rodents have a round pupil, but chinchillas have a slit pupil somewhat like that of a cat. Rats have an orbital venous plexus which is important to note when performing an enucleation (same rabbit). The fundus of the rat and mouse is holangiotic with retinal venules and arterioles radiating from the optic nerve head.

Diseases of the rat and mouse eye include chromodacryorrhoea, ocular surface trauma, conjunctivitis and systemic disease, microphthalmos and anophthalmos, non-ulcerative corneal lesions, corneal ulcers, exposure keratopathy, uveitis, lens luxation, glaucoma, retinal degeneration and ocular neoplasia.

**Other rodents: the chinchilla and hamster**

**The Chinchillas**

The orbit is a shallow, a rudimentary nictitating membrane, a large cornea, a densely pigmented iris with a vertical slit pupil, and an anangiotic fundus with variable vascularization of the optic disc.

Diseases of the Chinchillas eye include glaucoma with lens luxation, bilateral posterior cortical cataracts and asteroid hyalosis. Epiphora and exophthalmos.

**The hamster**

Normally globes can be readily repositioned but prolonged globe prolapse will lead to exposure keratitis and ocular surface damage. A common problem with hamsters is globe prolapse either through trauma. Cataracts are relatively common in hamsters either solely in older animals.

**The ferret**

The ferret eye and visual system appear less well developed than in other carnivores, although it may be that the eye’s diminutive size as well as its behaviour in dim light. Ferret kits are born, with their eyes closed but unusually, compared with the dog or cat, the eyelids do not open until as late as 28–34 days postnatally. The ferret globe is small with a relatively large lens and a wide cornea for optimal light gathering in low light conditions. The ovoid pupil as with that of the cat. Ferrets have a holangiotic fundus with retinal blood vessels radiating from the optic disc. Pigmented ferrets have a dog-like tapetum lucidum with a green–blue reflective eye-shine. As with the cat, the ferret has an area of increased photoreceptor density superiotemporal to the optic disc, the area centralis.

Diseases of the ferret eye include ophthalmia neonatorum, conjunctivitis, keratitis, cataract, entropion, uveitis, lens luxation, glaucoma and retinal disease.

**The hedgehogs**

The small size of the eye of the European hedgehog or the African pygmy hedgehog, more commonly kept as a pet animal. The shallow orbit of these species also contributes to the high incidence of globe protrusion. Diseases of the hedgehog eye include conjunctivitis, anophthalmos, exophthalmos, entropion and epiphora.

**The avian**

The globe is big compared to body size, with a posterior segment disproportionately larger than the anterior segment. Upper and lower eyelids and nictitating membrane are present, both eyelid are mobile but the lower lid is more mobile than the upper. The nictitating membrane is well developed, actively mobile the cornea during blinking and the menace response, near transparent, thin and covered by a papillary layer of epithelium. The Meibomian glands are also absent and the lacrimal gland is present inferotemporal to the globe with an additional hardenian gland acting as a second lacrimal gland at the base of the nictitating membrane. Inferior and superior nasolacrimal puncta drain the lacrimal secretions into the nasal cavity. The orbits are separated only by a thin bony structure or a septum of connective tissue. The orbit is open and is occupied predominantly by the globe in most birds. For this reason the extraocular
muscles are not particularly well developed in those species. The most important feature of the orbit is the close proximity of the globe to the infraorbital diverticulum of the infraorbital sinus. Different bird species have quite variable globe shape. The cornea of avian species is likely of mammals but for its considerably reduced thickness. The anterior chamber of most avian eyes is considerably shallower than that of mammals. The avian iris is thin and, importantly, contains striated muscle rather than the autonomically innervated dilator and constrictor muscles of the mammalian eye. The retina of the bird differs markedly from that of the mammal. Without retinal blood vessels or a choriocapillaris to provide a direct vascular supply of oxygen and nutrition for the retinal photoreceptors, a portion of the choroid, the pecten, protrudes into the fluid posterior vitreous. There is no tapetum in the avian fundus.

Diseases of the avian eye include infraorbital and periocular sinusitis, microphthalmos and anophthalmos, cryptophthalmos, poxviral blepharitis, hypovitaminosis A, lid neoplasia, conjunctivitis, corneal ulceration, uveitis, cataract, len luxation, keratitis, retinal disease and Horner’s syndrome.

The reptile

The eyes of lizards, snakes, crocodilians and chelonia are surprisingly different, these variations giving considerable insight into both the diversity of their evolutionary origins and their current residence in different ecological niches. The large orbits contain the extraocular muscles, the large Harderian gland which curls around the globe at its posterior aspect, the lacrimal gland and an orbital sinus which connects to the internal jugular vein via the vena cerebralis, clearly an important fact to be borne in mind when considering enucleation. The orbits meet medially with only a cartilaginous septum between them. The eyelids are unequal with the upper eyelid, although containing smooth muscle, being relatively immobile. The eye opens predominantly through downward movement of the lower eyelid which contains a large cartilaginous plate.

In several families of lizard and in all snakes the eyelids are fused to form a transparent spectacle. The cornea has a thin stroma but a relatively thick Bowman’s layer covered by a thin corneal epithelium. The eyes of all snakes are covered by a spectacle formed from fused transparent eyelids. The iridocorneal angle is poorly developed compared with that of higher vertebrates. Diseases of the reptile eye include hypovitaminosis A in cheloniens, periocular lesions infection and inflammation, conjunctivitis, periocular masses, opacity of the spectacle, retained spectacle, subspectacular abscessation, corneal ulceration, corneal opacification, uveitis, cataract, glaucoma, microphthalmos and anophthalmos.

References
Rabbit skull problem; It’s more important than you know

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When we mention about skull of the rabbit, the most important organs are likely dental and ocular system. Because of a close anatomical relationship between the roots of maxillary teeth and the structures of the eye and nasolacrimal duct. Whenever root elongation and periapical infections are part of the syndrome of acquired dental disease that is so common in pet rabbits and dental disease is often manifested by ocular symptoms. Therefore detailed knowledge of the normal, topographic and anatomy of the eye with nasolacrimal duct and glands, the teeth and skull with a focus on mandible and maxillar are important for understanding.

The rabbits have large, prominent eyes that are position on the side of the head with a cornea that occupies approximately 25% of the globe. These features give rabbits a visual field of nearly 360°, they have a blind spot under the chin. The aperture to the nasolacrimal duct, the punctum lacrimale, is situated in the anteromedial aspect of the lower eyelid. The punctum opens into a short canaliculus that opens into lacrimal sac. At the root of the primary maxillary incisor, the duct makes an abrupt mediodorsal bend and its diameter is reduced from approximately 2 to 1 mm. At this point, the duct is compressed between the alveolar bone surrounding the root of the primary maxillary incisor and the nasal cartilage. Elongation of the root of the incisor can easily occlude the duct at this point. The duct then courses medially, alongside the incisor tooth root and emerges in the nasal cavity at ventromedial aspect of the alar fold. There are the three glands within the orbit: the lacrimal gland, the accessory lacrimal gland and the deep gland of the third eyelid. The lacrimal gland is situated in the caudodorsal sector of the orbit. The large accessory lacrimal gland lies in the lower part of the orbit and has three lobes: the orbital lobe, retro-orbital lobe and infraorbital lobe. The infraorbital lobe rests between the zygomatic bone, the superficial gland of the third eyelid and the globe. It is close proximity with the alveolar bulla that contains the roots of the maxillary cheek teeth. (see figure 1) (5)

Figure 1  Nasolacrimal duct and glands of the eye

The most common skull problem is the dental disorder, considered to be congenital but it has now become evident that other factors are involved and altered the relative position of a tooth, even by a fraction, can result in development of malocclusion and the

Because of a close anatomical relationship between the roots of the maxillary teeth and the structures of the eye and nasolacrimal duct. Elongated tooth roots especially of the upper primary incisors can block the nasolacrimal duct and interfere with drainage of tears from the conjunctival sac. Epiphora is often the first indication of acquired dental disease (ADD). (3, 5) Examination of the teeth and surrounding structure may reveal other signs of dental disease or palpable swelling along the ventral border of the mandible. Blockage of the nasolacrimal duct and impaired drainage of the tear from conjunctival sac allow bacteria and debris to accumulate. Secondary bacterial can causing conjunctivitis and/or infection and inflammation of the lacrimal sac so called dacryocystitis. The dacryocystitis is characterized by a profuse mucopurulent ocular discharge that is the most marked at the medial canthus. Root elongation and periapical infection of the upper cheek teeth are sometimes manifested by epiphora, conjunctivitis, dacryocystitis or exophthalmos. The elongated, distorted premolar roots penetrate the maxilla and/or the lacrimal bone can obstruct the nasolacrimal duct also and they can also grow into the orbit, causing ocular pain or infection of the periorbital structure. Retrobulbar abscesses or osteomyelitis are usually the result of periapical infection. (3)
formation of elongated crown. There is a progressive syndrome of acquired dental disease (ADD) characterized by deterioration in tooth quality, acquired malocclusion and elongation of the tooth roots. Periapical abscesses frequently occur. The exact aetiopathogenesis is not clear and several factors are probably involved, including metabolic bone disease, dietary texture and genetic predisposition. The rabbits will express the signs of bone pain and toothache such as reduced appetite, lassitude, unkept coat and uneaten caecotrophs including lack of grooming, digestive disorder, ribbed teeth, epiphora with or without dacryocystitis, abscess together with incisors and cheek teeth malocclusion. (5) Elongated roots of the upper first premolar can penetrate the nasal cavity and occasionally the nasolacrimal duct is involved at this site. Even if elongated roots of the upper molar penetrate the alveolar bulla into the zygomatic gland in the retrobulbar space and can cause retrobulbar abscess later on. The end-stage of ADD will perform osteomyelitis together with abscess formation and become progressively calcified and embedded in the surrounding bone. (see figure 2)

Abscesses are typically firm, cool, and nonpainful on palpation. Early small masses are usually missed by the owners because of their location and the presence of fur, especially in long-haired rabbits. However, they may increase to considerable size. Occasionally part of the overlying skin is necrotic and a fistula or rupture may occur. Medical therapy alone is unrewarding although it is an important adjunct to the dental and surgical treatment, which is usually a combined intraoral and extraoral approach. (1,3,4)

This finding is easy to understand if the 3 distinguish traits of periapical infections and abscesses of rabbits are considered: the presence of a capsule, the osteomyelitis and the disease tissue (soft tissue, dental and bony) acting as a sequestrum. Treatment of all 3 pathologic conditions must be pursued and addressed in order to obtain long-term therapeutic success and prevent frequency reoccurrence. The combined dental and surgical treatment is designed to remove the entire capsule and affected tooth/teeth (see figure 3), and to thoroughly debride the osteomyelitic bone. (3,4) Marsupialization of the soft tissues around the area of the affected bone is the procedure that associated with high percentage of successful outcomes and long-term postoperative follow-up, particularly in cases of deep or severe osteomyelitis. (see figure 4) (4) The surgical technique should allow postoperative flushing and debridement of surgical site, application of antiseptics or other products to promote healing and constant direct monitoring of healing. This outcome ultimately facilitates the efficacy of antibiotic therapy. In addition to antibiotic therapy, the key point of medical treatment should include supportive such as fluids and nutrition with assisted feeding formulas for herbivores and analgesic therapy when indicated.(1,2,4)

Abscesses are typically firm, cool, and nonpainful on palpation. Early small masses are usually missed by the owners because of their location and the presence of fur, especially in long-haired rabbits. However, they may increase to considerable size. Occasionally part of the overlying skin is necrotic and a fistula or rupture may occur. Medical therapy alone is unrewarding although it is an important adjunct to the dental and surgical treatment, which is usually a combined intraoral and extraoral approach. (1,3,4)

Prognosis should therefore be tailored to the specific patient and case. The first critical element for proper prognosis is diagnostic accuracy. The diagnosis must be correct and as detailed as possible, specifically with regard to dental and bony involvement. Therefore diagnostic imaging is of paramount importance. Several practical, even nonmedical, factor should be considered when formulating the prognosis. Including management during postoperative period, the owner’s understanding with compliance (in particular with regard to advanced and chronic disease) and cost.(2,4)

References
Food Risk Analysis : A New Paradigm for Food Safety

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So far, food safety has been fundamentally about practices of controlling foodborne pathogens from farm to fork. Stakeholders in various steps of food chain independently work to minimize the foodborne disease pathogen. Yet the problematic microbes are still around in both domestic and international trades. Codex Alimentarius Commission has launched a new concept of connecting various steps of food chain with one ultimate goal so-called Appropriate level of SPS protection (ALOP). This term is about the risk or number of foodborne illness that is set out to be acceptable under the country's jurisdiction. Quantitative risk assessment is a scientific tool to evaluate the likelihood of getting foodborne illness upon consuming food. Therefore the output of risk assessment (risk estimate) was used to compare with ALOP to see whether the food products are acceptable or not. If the risk estimate was higher than ALOP, the risk management options for food production were deemed necessary.

However ALOP is not practical for risk managers to work with food industry. ALOP was then converted to be Food Safety Objective (FSO) which is basically the prevalence or concentration of foodborne disease pathogen at the point of consumption that is corresponding to the ALOP. FSO is compatible with the food safety standard of international trade. However FSO has to be working closely with food industry to set out the contamination levels of foodborne disease pathogen in earlier steps in food chain. The measures applied to control the contamination in food chain steps to achieve FSO are parts of risk management. The information regarding risk management and risk assessment should be conveyed to all stakeholders and consumers. This practice is called risk communication. Conclusively, food risk analysis is composed of risk assessment, risk management and risk communication.
Randomized sampling for AMR monitoring

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Abstract
This presentation will review sample size determination and sample strategies for maximizing the sensitivity of AMR monitoring system for detecting a current situation on antimicrobial resistance (AMR) and monitoring trend of resistance in the national level. Randomized sampling and sample size determination should be done prior to perform AMR monitoring and surveillance to achieve representativeness of population based on objectives of the study. Cross-sectional study or survey would be appropriate approach to select for determination of sample size. Furthermore, probability sampling such as simple random sampling, stratified sampling or multi-stage sampling should be implemented for better statistical inference.

Introduction
The emergence of AMR have been a major public health concern that affect both human and animal health. Antimicrobial agents have been used in many settings such as medicine, veterinary medicine, community and aquaculture. The occurrence of AMR issues associated with overuse and improper use of antimicrobial agents. As a result, AMR monitoring and surveillance especially in food-animals are being developed and implemented in a national level in many countries (1-4). For public health perspective, monitoring and surveillance on AMR in zoonotic and commensal food-borne microorganisms is derived from healthy animals collected from farms, slaughterhouses and retails/outlets. Healthy animals are used for AMR monitoring because they are intended for human consumption.

The main purposes of national monitoring and surveillance of AMR in foodborne microorganisms from healthy food animals are to observe the current situation of AMR and to monitoring trends within or between countries (5). Furthermore, another objective for national AMR monitoring is to observe the emergence of AMR that are not previously described in the animals and their products (6). A common challenge for AMR monitoring and surveillance in the national level is to insure that sample collection from farms, slaughterhouses or retails are a representative for the targeted population. Furthermore, proper determine sample size determination and sampling strategy are need to define based on the objective of AMR monitoring plan.

Determination of sample size for AMR monitoring
Prioritization pathogenic and enteric bacterial species, identification of target sample, and selection of sampling location are important components to define prior to perform sample size determination for AMR monitoring. For AMR monitoring, parameters affected sample size in a cross-sectional study or survey are estimated prevalence, desired precision or margin error (e) and confidence level (CL) assuming infinite number of target population. Minimize desired precision and maximize confidence level are associated with more reliable data. Generally, the margin of error and CL are considered as 5% (1-10%) and 95% (either 90% or 95%), respectively. However, precise estimated prevalence of resistance bacteria on national strategic plan remains unknown, so expected prevalence should be critically reviewed from publications.

Sample size determination is an important issue, since too small samples would lead to inaccurate results and cannot be generalized to the population while too large samples would be waste time, budget and resources. Calculation of sample can be performed by using either sample size formula or available statistical software. For example, assuming 5% of marginal error, 50% of expected prevalence and 95% CL, the sample size for target bacterial isolates equal 385 specific bacterial isolates. The number of isolates should be adjusted after calculation in order to adjust the total number of sample based on the prevalence of specific microorganism. Furthermore, the sample size per time would further justify based on human resources, logistic, economical practically sampling as well as statistical plausibility.

Sampling strategy
Sampling strategy is a sampling technique to draw a sample from a target population. This technique can be classified as non-probability sampling (i.e. convenience, purposive and quota sampling) and probability sampling (i.e. simple random sampling, stratified sampling, cluster sampling and multi-stage sampling). A major impact on reliability of sample inferences associated with a sampling design. Thus, performing a probability sampling can be achieved better statistical inferences from sample to population than non-probability sampling because a sample from probability sampling can be drawn with an equal probability.
Randomized sampling is a method of selection sample randomly. The proper sampling method should be implemented for AMR monitoring to select a sample from a targeted population. Additionally, types of sample need to be a represent of specific targeted bacteria. For example, caecum sample is an appropriate sample for *Escherichia coli* detection in a slaughterhouse for AMR monitoring.

**References**

Techniques for isolation and identification of *Campylobacter*

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*Campylobacter* species particularly *C. jejuni* and *C. coli* have been recognized as important causes of bacterial gastroenteritis in humans since the late 1970s (1). Food-borne campylobacteriosis is mainly associated with foods of animal origin particularly poultry meat and poultry products. Consumption of undercooked poultry and other foods that are cross-contaminated with raw poultry meat during food preparation is considered a major risk factor of this food-borne disease (2).

Unlike other recognized food-borne pathogens, *C. jejuni* is strictly microaerophilic meaning that they require low level of oxygen for growth. *C. jejuni* grows well in environment containing approximately 3 to 8% O₂ and 5 to 15% CO₂. Although most *Campylobacter* grow at 37 °C, *C. jejuni* and *C. coli* show optimal growth at 42 °C (3). In order to isolate *Campylobacter* from samples that may contain varieties of bacteria such as fecal samples, selective media should be used. Over the last several decades, a number of *Campylobacter* selective media have been described including blood-free media such as modified charcoal cefoperazone deoxycholate agar (mCCDA), charcoal-based selective medium (CSM) and Karmali agar; semi-solid blood-free selective motility medium; and blood-containing media such as Skirrow's medium, Butzler's medium, Blaser's medium, Preston agar and Campy CVA agar (4). In addition to *Campylobacter* selective media, a number of enrichment broths have been formulated to enhance the recovery rates of thermophilic *Campylobacter*. These enrichment broths are beneficial when low number of *Campylobacter* are expected. The most widely used *Campylobacter* enrichment broths include Bolton broth, Preston broth, Doyle and Roman broth, Park and Sanders broth, Hunt and Radle broth and Exeter broth (4). Since each selective medium and enrichment broth contains different combination and amount of antibiotics, a combination of media should be used to increase success rates of *Campylobacter* isolation.

In general, direct plating method and selective enrichment method can be used for *Campylobacter* isolation, depending on sample types and number of *Campylobacter* present in those samples. Because feces or intestinal/cecal contents from chickens usually contain high number of *Campylobacter*, these samples can be directly plated onto *Campylobacter* selective agar. On the other hand, food and environmental samples seem to have low number of *Campylobacter*. Thus, selective enrichment method should be used. For optimal recovery, samples should be enriched in enrichment broth no longer than 24 hours because a prolonged enrichment can reduce *Campylobacter* recovery rate (4). In addition, when samples contain high number of fast-growing background microflora, such as in the case of fecal samples, selective enrichment method should not be used because the background flora may overgrow *Campylobacter* during the enrichment step leading to the reduction of *Campylobacter* recovery rates (4).

Although presumptive identification of *Campylobacter* can be made by typical colony morphology, other phenotypic tests should be performed to confirm the identification results. The most common biochemical tests for initial identification of thermophilic *Campylobacter* include oxidase test, catalase test, ability to grow at 37 or 42 °C but not at 25 °C and hippurate hydrolysis test (5). Hydrolysis of sodium hippurate is a main biochemical test that is used to differentiate *C. jejuni* from other *Campylobacter* species because only *C. jejuni* can hydrolyze sodium hippurate and give a positive result to this test. However, some strains of *C. jejuni* may occasionally be hippurate-negative (3, 5). In the past, nalidixic acid susceptibility test was also commonly used for differentiation of thermophilic *Campylobacter* species. However, due to the emergence of quinolone/fluoroquinolone resistance among *C. jejuni* and *C. coli*, the identification of thermophilic *Campylobacter* using nalidixic acid susceptibility test can become a problem. If nalidixic acid susceptibility test is used for identification of thermophilic *Campylobacter* species, the results must be carefully interpreted. At present, polymerase chain reaction (PCR) is commonly used for confirmation and identification of *Campylobacter* species. This method significantly improves rate of *Campylobacter* detection from samples containing low number of this organism (5, 6). In addition, it can help differentiate hippurate-negative *C. jejuni* from *C. coli*, which cannot be differentiated by phenotypic methods. Although PCR method provides rapid detection as well as high sensitivity and specificity, it is unable to discriminate between viable and non-viable *Campylobacter* cells, which may be essential in some epidemiological studies.

In this presentation, an overview of *Campylobacter* isolation and identification methods will be discussed. Also, recommended methods for isolation and identification of *Campylobacter* from different sample types will be provided.
References

Comparative Medicine and Pathology in Translational Research, Overview of an academic program in the US

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Abstract
- Academic research program in a medical school;
- Large census, diverse species;
- Research support by clinical veterinarians and veterinary pathologists, includes 9 veterinary faculty board certified by ACLAM and or ACVP, 12-15 veterinarians in training for specialty board certification, actively participating in research and research support, as well as veterinary student externs and visiting scientists;
- Faculty and veterinarians in training participate in course and one on one training of researchers;
- The ‘Phenotyping core’ provides project specific support specializing in experimental design, model selection, clinical and anatomic pathology, and coordinates with other cores and resources in diverse research areas.

Acknowledgements
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Experimental Design and Research Reporting of Translational Research; Addressing the Reproducibility Crisis

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Abstract
What’s wrong (and right) with research reporting? Increasingly, reputable journals require adherence to guidelines such as the ARRIVE guidelines, ILAR guidance, NIH or FASEB recommendations, or specialty based minimum reporting recommendations. The Materials and Methods section of research publications will be emphasized, from the point of view of the author/scientist, and of a reviewer/editor. The importance of accurate nomenclature, and descriptions of animals, husbandry and procedures will be discussed, with contemporary examples.

Acknowledgements
The financial support of travel was provided by....

Topics and Selected References

Guidance for reporting

Guidance for experimental design

Assessments of reporting

Nomenclature of mice, rats, genes, proteins
Microbial impacts on contemporary translational preclinical research in mice and rats.

Cory Brayton

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Abstract

Implications for Research: EVERYTHING immunomodulates; research relevance and concerns regarding ‘pet store mice’ and wild rodents, and their microbiota.

Implications for Animal Health and Welfare: In immune sufficient mice, sick mice or rats are unusual today, and ‘Detection’ is more common than Disease. In immune deficient mice or rats, sick or dead animals are not so unusual; and beware of biological materials.

Implications for Human Health: Be aware of immune deficient or humanized animals as reservoirs of potential pathogens for humans or non human animals.

The discussion will include selected common and concerning microbial friends, frenemies, and pathogens.

Aims and Conclusions:
1. Expect the common agents and be aware of potential research complications;
2. Consider the possibility new and interesting, emerging or re-emerging agents;
3. Beware of ‘pet store’ mice and wild animals;
4. Beware of Biological Materials, and test them.

Acknowledgements

The financial support of travel was provided by....

Topics and Selected References

General


Viruses

Parvoviruses

Murine Norovirus

Murine Astrovirus

Rat polyomavirus

Seoul virus (hanavirus)

Bacteria

Corynebacterium bovis NHAC

Bordetella hinzii, pseudohinzii

P pneumotropica

Fungi

Pneumocystis spp

Protists

Enteric flagellates and immunomodulation

Metazoa


Pet Store & Wild rodents

**Microbiome**


**Segmented Filamentous Bacteria (SFB)**


The World’s 1st One-Touch Preclinical Imaging Platform With High-Frequency Ultrasound For Laboratory Animals

Shripad Bangale

Abstract
FUJIFILM VisualSonics designs and manufactures ultra high frequency in vivo imaging systems, for both research and clinical use with specific focus on developing ultrasound technology that has been scaled to much higher frequencies than commonly found in many of the conventional ultrasound systems on the market today. As a result, our ultrasound platform provides images at resolutions that far exceed any other system available on the market; as fine as 30 micrometers, clearly differentiating our company from our competitors. Our preclinical customers are mostly academic researchers, often involved in the fields of cardiovascular and cancer research. Neurobiology and developmental biology are other key areas that are among an ever growing number of applications. For these customers, funding for purchasing capital equipment comes mainly through grants. Obtaining funding is often a challenge and sources of funding may change from time to time so we constantly need to be aware of these dynamics and help potential customers navigate through this.

Introduction
VisualSonics was founded in 1999 by medical physicist Dr. Stuart Foster, a Senior Scientist at Sunnybrook Research Institute, who had been involved in the development of high-frequency ultrasonic systems since 1983. The company’s intellectual property was based on research supported by the Canadian Institutes of Health Research (CIHR), Ontario Research and Development Challenge Fund (ORDCF), the Terry Fox Foundation, and venture capital investment, with infrastructure support from the Canada Foundation for Innovation and Ontario Research Fund.

Originally, Dr. Stuart Foster and his team started using this technology in preclinical research, in small animal models of human disease (e.g. mice or rat models of cancer and cardiovascular disease). By using high frequency ultrasound, researchers were able to study their live animals in real-time, longitudinally, and with no issues of safety or side effects. “From the inception of the company, we always envisioned that the technology would eventually find a home in human clinical applications and it is exciting that that day has finally arrived,” says Dr. Foster. In June of 2010, VisualSonics was acquired by SonoSite, Inc. (based in Bothell, US), a leader in hand-carried and mountable ultrasound, and impedance cardiography equipment. SonoSite, Inc., was then subsequently acquired by Fujifilm Holdings in December of 2011. Vevo LAZR-X (High Resolution Multi-modal In vivo Imaging Platform)
The Vevo LAZR-X combines high frequency ultrasound and photoacoustics into one platform for high resolution anatomical, functional and molecular imaging.

The new Vevo LAZR-X features:
- New laser technology for faster, more sensitive image acquisition at a wider wavelength range (680 - 970 nm and 1200 - 2000 nm)
- Versatility through flexible, application-specific light delivery
- Accessories and software tools for a variety of applications
- Hands-free scanning with Vevo Voice
Vevo 3100 (The Ultimate Preclinical Imaging Experience)

The Vevo 3100 Imaging System helps you visualize your data at high resolution like never before.

Benefits

- Vevo HD - Visualize your data like never before
- Intuitive touchscreen interface for all user types
- Customizable workflow for rapid data acquisition
- Anatomical, hemodynamic, functional, and molecular data all in one platform
- Compact, maneuverable system
- Hands-free scanning using Vevo Voice
- State-of-the-art Ultra High Frequency - up to 70 MHz
- 3D & 4D Imaging
- Photoacoustic imaging using the LAZR-X cart
- Resolution down to 30 µm

The unique, gesture-driven user interface can be personalized by each user, and is easily controlled with the glide of a finger - no keys or buttons. Customization of workflow drives rapid data acquisition, providing extremely high-throughput.

In vivo imaging has never been easier!

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Genetic characterization of infectious bronchitis viruses isolated from chickens in Thailand, 2016

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Keywords: Chicken, infectious bronchitis viruses, genetic characterization, Thailand

Introduction
Infectious bronchitis (IB), caused by infectious bronchitis virus (IBV), is a highly contagious viral disease in chickens, posing a significant threat in poultry industry worldwide (1). The disease is characterized by respiratory signs, decrease in egg production and quality and occasional nephritis. IBV belongs to the genus Gammacoronavirus in the family Coronaviridae. The IBV genome is a positive sense single stranded RNA molecule, which encodes four structural proteins, including the nucleocapsid (N), membrane (M), envelope (E), and spike (S) proteins (2). Among them, S protein, in particular S1 subunit, has been widely used to classify IBV serotypes and genotypes due to the high sequence diversity (3). Currently, a large number of new IBV variants continuously emerged worldwide due to the high frequency of mutation and recombination in the S1 gene, causing vaccination breaks (4). In Thailand, five groups of IBV genotypes, including group I (QX-like IBV), group II (THA50151), group III (unique Thai IBV or THA001), group IV (4/91) and group V (Massachusetts), were reported to circulate in chickens during 2008-2013. (5, 6). To better understand the evolution of IBV currently circulated in Thai chicken flocks, we genetically characterized IBV field strains isolated from chickens in Thailand during January to June 2016 by analysis of complete S1 gene.

Material and Methods
From January to June 2016, 475 pooled organ samples were collected from IBV suspected chickens on different locations in Thailand. All samples were initially screened for the presence of IBV by RT-PCR with S1 gene specific primers (7). All IBV positive samples were then tested for differentiation between Massachusetts vaccine strain and variant IBV strain by restriction fragment length polymorphism (RFLP) analysis (7). All variant IBV positive samples were inoculated into 10-day-old embryonated SPF chicken eggs for virus isolation. Five variant IBV positive samples were randomly selected for complete S1 gene sequencing. The RNA was converted to cDNA by using a random-priming strategy (Improm-II™ reverse Transcription System, Promega, USA). The complete S1 genes were amplified by using two S1 gene specific primers (5). The nucleotide sequences were assembled and analyzed by using DNASTAR software (DNASTAR Inc., Wisconsin, USA). The complete IBV S1 gene sequences identified in this study, previously reported Thai IBVs and other selected IBV reference strain sequences available in GenBank database were subjected to phylogenetic analysis. Phylogenetic tree was constructed in MEGA6 v.6.0 using neighbor-joining algorithm with the Kimura-2 parameter model applied to 1000 replications of bootstrap (8). The nucleotide identities among our 2016 IBVs, previously reported Thai IBVs as well as other selected IBV reference strains were examined using the MegAlign software v.5.03 (DNASTAR Inc., Wisconsin, USA).

Results and Discussion
A total of 475 samples were collected from chicken farms located in all regions of Thailand, including the central, the eastern, the northern, the northeastern, the western and the southern regions of Thailand. A total of 86 (18.1%) out of 475 samples tested positive for IBV by S1-specific RT-PCR. Ten IBVs were randomly selected for complete S1 gene sequencing. In contrast to previous reports in Thailand during 2008-2013, our results based on the phylogenetic analysis of S1 gene showed that the 2016 Thai IBVs characterized in this study were clustered into four groups, including group I (QX-like IBV) (n=7), group IV (4/91) (n=1), group VI (Massachusetts) (n=1) and group VII (n=1), which is a novel genotype of IBV firstly identified in this study. It is noted that group II and III of IBVs previously reported in Thailand were not detected in this study. In addition, our result showed that most of the 2016 IBV isolates were clustered into group I (QX-like IBV), with 93.7% - 96.6% nucleotide identities. This finding indicated that QX-like IBV was predominantly circulating in Thai chicken flock in 2016. Interestingly, a novel genotype of IBV (group VII) was first identified in this study. This virus shared only 88.3% - 89.9% nucleotide identities with QX-like IBVs commonly circulating in Thailand. Based on field observation, chickens infected with this novel IBV genotype had higher mortality rate (5.56%) than those infected with...
commonly circulated QX-like IBVs (2.14%), indicating that this novel IBV genotype is possible increased virulence. In conclusion, our results demonstrated that the QX-like IBV was a dominant genotype currently circulated in Thai chicken flocks; however, a novel genotype of IBV could also be detected in chickens in Thailand. This study indicated that Thai IBVs have been continuously evolving, possibly affecting the effectiveness of vaccination. Thus, continued IBV surveillance should be conducted to monitor the genetic evolution of IBVs for effective disease control and prevention.

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Analysis of Porcine Immune Responses to Assess Vaccine Performance

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Abstract
Vaccines based on formulation of killed pathogen in adjuvant have the common characteristic of inducing antibody, or humoral responses, with an absence of a cellular immune response. This is a characteristic of the killed virus vaccine for foot-and-mouth disease virus (FMDV). However, the infection of swine by FMDV results in a readily detectable cytotoxic T lymphocyte (CTL) response. New flow cytometric technologies have allowed for the analysis of CTL responses in outbred species like swine. The recently developed recombinant vaccine for FMDV, adenovirus 5 delivered FMDV capsid proteins or AdFMD, induces a humoral immune response very similar to the killed virus vaccine. We redesigned AdFMD to target a CTL response. Using flow cytometry, we showed that CTL killing of virus-infected cells is correlated with class I MHC tetramer staining of FMDV specific T cells. Further, the specificity of the CD8 T cell the modified AdFMD vaccine varies between cohorts of genetically identical animals. And finally, we demonstrate epitope specificity of CD8 T cells expands following multiple immunizations with such adeno5 virus vector expressing the structural proteins of FMDV.

Introduction
FMD is a highly contagious and economically important viral infection of cloven hooved livestock species including swine. Control and eradication of this disease is a high priority to enable large and smallholder farmers in developing countries to have a reliable source of protein for consumption as well as supplemental income from the sale of products of livestock farming. Present killed virus vaccines have limited value in eradication efforts due to narrow protection across the many clades of viral strains and minimal duration of immunity. There is no detectable cellular immune response, induction of a CTL response. The recently licensed virus vectored vaccine for FMDV utilizes replication defective human adenovirus to deliver an “empty capsid”. This recombinant vaccine suffers the same performance shortfalls as the widely used killed virus vaccine, a strong antibody response and no CTL induction (1). However, this vaccine platform presents an intriguing opportunity for addressing these performance deficits.

To analyze vaccine performance based the immune response, colleagues have developed bioinformatic algorithms for the human leukocyte antigens (HLA) (NetMHCpan) to predict antigenic epitopes for T cells derived from any pathogen proteome (2). This is based on the HLA molecules of that individual to bind pathogen derived peptides and “present” those to T cells. We have now applied this technology to the homologues of these proteins in swine (swine leukocyte antigens; SLA)(3).

Results
Analysis of the proteome corresponding to structural proteins (capsid) of FMDV, strain A24, identified 60 potential T cell epitopes for CD8 expressing, cytotoxic T lymphocytes (CTL) predicted to be bound by two swine class I SLA proteins. Further biochemical binding analysis determined 20 of the predicted peptides were bound (4). Using these results, we constructed MHC tetramers and tested animals vaccinated against FMDV for virus specific CD8 beta expressing T cells in peripheral blood. In three separate cohorts of genetically identical NIH minipigs vaccinated with the modified AdFMD (T cell inducing), we detected virus specific CD8 T cells correlating with the presence of CD8 cells that kill FMDV infected target cells. Of interest is the fine specificity of these cells was consistent within each cohort, but differed between the cohorts. Finally, we show that following multiple boosts with the modified AdFMD vaccine, the fine specificity of the T cell response expanded. This result is the opposite of B cell responses following multiple boosts, which narrow, becoming less crossreactive (5).

Conclusions
Results presented here have critical connotations for FMDV vaccine design. The ultimate vaccine for this disease would have high cross-protection from the antigenically diverse viruses not only across serotypes, but within a given serotype. Our data indicate that designing a vaccine to target cytotoxic T lymphocyte responses has the inherent characteristic of an expanding antigen specificity following multiple boost vaccinations. The T cell epitopes, short peptide fragments of viral proteins, are much more likely to be shared across clades of FMD viruses than the larger, three dimensional epitopes that antibodies bind. As such, we propose similar vaccine designs for viral infections of swine will hold greater potential for vaccines that can protect against a broad spectrum of viral strains.
References
Dynamic of PED and Control Strategy, Japanese Experience

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Introduction
Porcine epidemic diarrhea (PED), a highly contagious diarrheal disease caused by PED virus (PEDV). PED occurred in Japan after interval of seven years in 2013, spreading throughout the country in 2014. The initial outbreaks were almost confined in southern Kyusyu area. However, the disease suddenly spread across almost all area of Japan from March 2014. By January 2015, a total of 1.3 million pigs died (reports by MAFF). PEDV is relatively more difficult to control or eliminate than TGE virus from the point of view of environment resistance, and stability under wide range of pH. From this fact, PED is more likely to normal inhabitants in a herd [1,2]. In this study, we report the measures and strategy we tried to control and eradicate PED/PEDV in pig farms.

PED in Japan
The first case of PED was reported in 1980s in Japan and the initial outbreaks were almost confined in southern Kyusyu area. In 1994 and 1996, many outbreaks were confirmed and domestic live vaccines were approved in 1996. After that the outbreaks had settled down and there were few outbreaks in early 2000s through 2012. However this disease has re-emerged since October 2013 and spread worldwide and more than 390,000 pigs died in total as January 12, 2015 (Fig-1). Genetic analysis of PEDV strains collected in Japan from 2013 to 2014 showed the presence of two types of PEDV strains as similar to those of PEDV strains detected in other countries in recent years. One is highly virulent (North American) type and other is INDELs type [3]. And what was even worse, a new type of coronavirus, which is called delta coronavirus, was detected.

Control and eradication of PED
There are more than 100 animal health and sanitation stations (AHSS) and about one thousand veterinary officers work there. PED is mainly diagnosed at (AHSS). PED is one of reportable diseases in Japan. Framers and veterinarians have a duty to report outbreak when occur. So we can know when and where outbreaks occur as soon as possible. Independent veterinary practitioners who work with pigs play important roles to control and eradicate PED for their clients. Commercial PED vaccines are available and the vaccination program is that sows are vaccinated twice one month before farrowing, one month interval between the first time and the second time. Unfortunately the vaccines do not work well enough. Successful control and eradication of PEDV relies on stimulation of natural immunity via live virus exposure and reduction of environmental contamination through thorough cleaning, disinfection, and drying of facilities. There is unique balance between natural immunity and lowering of environmental challenge necessary to achieve containment and exclusion.

Eradication of PED in a pig farm in Japan: case report
The case farm was an one-site, farrow-to-finish production with 100 large white sows. Watery diarrhea and vomiting were observed on the 23rd of April in 2014 in seven sows and lactating piglets in the farrowing room. The diarrhea spread through all sows and lactating piglets in the farrowing room immediately. We necropsied three piglets showed diarrhea, and intestine and feces were collected for histopathological examination and PCR testing. The diarrhea was diagnosed as PED.

Eradication procedures were started as below;
1. Practical feedback (natural planed exposure): Feedback materials contained feces and intestinal homogenate from suckling piglets with clinical sings. The material were given to all sows for three days.
2. All the piglets which developed diarrhea in farrowing room were euthanized.
3. Once clinical findings were not observed, we washed and disinfect pigpen with didecyldimethylammonium chloride (2%).
4. Herd closure: Introduction of any pigs into the farm was stopped for 6 months.

We monitored the PED situation after the outbreak. We collected blood samples from 12 sows monthly for 4-month period of time following the onset of outbreak, and tested to measure the neutralization antibody titers against PEDV. Also, we collected fecal samples from slurry pit monthly. The fecal and manure samples were tested by PCR for the PEDV. Pigs that were free of PEDV were introduced into the farm as sentinel five months after the onset of the clinical break, clinical signs and checked the neutralization titer against PEDV one month after introduction.

Piglets that were born after May 12th 2014 were not suffered from diarrhea. PEDV was not detected by the PCR the feces collected from sows and suckling piglets at 2-month following the clinical onset. PEDV virus was detected from slurry in the manure pit until 6 months following the clinical onset, but was not detected at 7-month. Sentinel pigs did not show any diarrhea and stayed all negative for neutralization antibodies during
period of 1 month of testing.

The results confirm that PEDV successfully eradicated from this farm.

References
Evolution of PRRS in Japan

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant diseases in swine industries worldwide. The annual economic loss due to PRRS was reported as $641 million USD in USA (1), and ¥28 billion JPY ($373 million USD) in Japan (2). Prevention and control of PRRS have to be comprehensive approach, which should include the components as below:

1) Pig flow
2) Herd immunity
3) Medication
4) Husbandry / management
5) Testing
6) Biosecurity

PRRS virus can be successfully controlled and eliminated from individual farms; however, re-introduction of new PRRS virus into the farms is still a frequent event. Therefore, biosecurity is one of the most critical component of the fundamentals of PRRS resilience. The objective of this paper is to focus on the importance of biosecurity in particular. Topics we discuss are as follows:

- What is biosecurity?
- How is PRRS virus transmitted?
- What can we do to reduce a risk of each transmission route?
- How can we audit and measure biosecurity?
- Conclusions & summary

What is biosecurity?

Definition of biosecurity is “the protection/security of susceptible animal herds from the introduction and transmission of infectious pathogens” (Saunder’s Veterinary Dictionary, 1999). Biosecurity has to be:

1) Science based
2) Practically feasible (simple, organized)
3) Effective (cost vs. benefit)
4) Committed to continue (execution)
5) Measurable

Components of biosecurity include as follows:

1] Internal biosecurity (within-farm)
   - To minimize the transmission of pathogens that already exist within a farm

2] External biosecurity (Between-farms)
   - To prevent new introduction of pathogens into a farm

3] Execution
   - Monitoring, auditing, education, and communication

Transmission routes of PRRS virus

Transmission routes of PRRS virus are classified as below:

1) Direct transmission (porcine vectors)
   - Live animals (pigs)
   - Semen

2) Indirect transmission (non-porcine vectors)
   - Needles
   - Personnel
   - Coverall and boots
   - Fomites
   - Transport
   - Carcass disposal
   - Birds
   - Rodents
   - Wild animals
   - Insects
   - Manure processing
   - Water
   - Air
   - Feed

Measure biosecurity risks on your farms

Biosecurity should be numerically measurable. Here are some examples of tools that are able to assess biosecurity risks on farms:

- PADRAP (AASV: American Association of Swine Veterinarians, North America) (3)
- BioCHECK (Ghent University, EU) (4)
- BioAsseT (P-JET: PRRS-Japan Elimination Team, Japan) (5)
Area regional approach

In some regions, economic significance of PRRS has let producers and veterinarians to initiate area regional approach in order to control or eliminate such diseases (6). To initiate PRRS area regional control/elimination in Japan, P-JET (PRRS-Japan Elimination Team) has been founded since July 2011. Missions of P-JET are the followings:

• To organize a working group that consists of swine practitioners, researchers, and industrial partners who focus on PRRS area regional control/elimination in Japan.
• To establish and provide a network, technical know-how, and educational support for pig producers and veterinarians who are active in their PRRS area regional control/elimination projects in Japan.
• To create and publish a hands-on manual of PRRS control/elimination, which will be tailored to the some of the specifics of the Japanese pig industry. The manual will be available for pig producers and veterinarians in Japan.

To date, P-JET has created some technical tools to contribute PRRS control and elimination both in individual farm level and regional level in Japan, such as:
1) P-JET PRRS herd classification
2) P-JET PRRS sampling/testing manual
3) P-JET biosecurity educational brochure
4) P-JET biosecurity risk assessment tool (BioAsseT)

This is the first initiative of PRRS area regional control/elimination in the history of Japanese swine industry. P-JET has supported PRRS area regional control/elimination projects as well as each farm case. Currently, 10 Japanese projects in 8 prefectures have been started under the influence of P-JET, including approximately 50,000 sows and 300 sites: Farrow-to-grow (20%) and Farrow-to-finish (80%) sites (Figure 1). To ensure and extend them, P-JET activities will be continued.

Figure 1:

Conclusions & summary

PRRS control and prevention have to be comprehensive approach. Biosecurity is an only way of the “true” proactive approach of disease prevention, and it is one of the most important fundamentals of PRRS resilience. Collaboration (such as area regional approach) and execution is the key of prevention/control of PRRS through successful biosecurity.

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The History of Livestock Vaccine Development

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Abstract

The technology of vaccination against infectious diseases is one of the oldest technologies in medical and veterinary science. The term vaccine is derived from the Latin word, vacca, which means cow. This technology was first tested by Richard Jenner in 1796, who, despite not understanding why it worked, was able to inoculate individuals with the material from cowpox lesions and those individuals would get a mild, non-life threatening illness. They were then “protected” from smallpox disease, which is often fatal. Over the next century, vaccine formulations were developed using a similar approach, cross-reactive protection, as well as new approaches of isolation of live, attenuated “vaccine” strains from serial passage in vitro, chemically killed vaccines and eventually recombinant vaccines. Livestock vaccines continued to be critical to the evolution of many of these vaccine technologies and remain a critical area of vaccinology to the present day.

Livestock vaccine development

Jenner’s first use of “vaccination” was based on scrapings of cowpox to inoculate humans for protection against smallpox. As the understanding of the microbial world developed, vaccine scientists came to understand vaccinia protected against smallpox due to the cross-reactivity of the immune response to the two viruses. Better vaccines for smallpox were made and deployed leading to the disease being the first to be eradicated.

The second disease to be officially eradicated worldwide was cattle plague. Caused by Rinderpest virus, this disease was highly contagious and characterized by high rates of mortality, and thus devastating to cattle farmers. In 1897, Robert Koch demonstrated passive immunization of naïve animals with immune sera from convalescent animals protected against disease (Reviewed in 1). In the 1950’s, Walter Plowright and colleagues developed a method to grow Rinderpest virus in the laboratory using primary bovine kidney cell monolayers (2). Serial passage of the virus for many years resulted in attenuated strains that were tested for use as a vaccine. These viral derivatives had to retain the infectious state, induce immune responses, but no longer cause disease pathology.

Once these live, attenuated “vaccine strains” of Rinderpest were established and tested, they began to be deployed in a disease control strategy in Africa. Many times, the attenuated vaccine reverted to virulence and the vaccine caused outbreaks. Further development of more completely attenuated viral strains continued until a much safer vaccine was eventually isolated and characterized. The final stages of eradication were significantly enhanced by the development of a better method for lyophilization (freeze drying) that allowed transport of vaccine stocks without the requirement of a cold chain (3). This was one of many critical advances allowing vaccination to reach remote areas of the world and the declaration of eradication of Rinderpest in 2011 by the United Nations.

Another vaccine developed in the early stages of the field was a vaccine for human tuberculosis. In the 1800’s, it was established that, like humans, cattle get infected with a bacteria that causes tuberculosis. Robert Koch was the scientist that distinguished the human pathogen, Mycobacterium tuberculosis from the cattle pathogen, Mycobacterium bovis. Following Jenner’s experience with smallpox and cowpox, scientists proposed “vaccinating against human tuberculosis by inoculating with M. bovis. Unfortunately, they soon learned M. bovis is also pathogenic for humans causing a similar disease to M. tb.

At the Pasteur Institute in Lille, Albert Calmette and Camille Guerin developed a method of culturing Mycobacteria. After more than a decade of subculturing M. bovis, they isolated an attenuated strain known as bacillus Calmette Guerin (BCG), still used today. This vaccine confers substantial protection to vaccinated individuals, but there are many characteristics of the vaccine that restrict its use in multiple specific cohorts of the human population (reviewed in 4).

In the 1950’s, Sabin developed an attenuated strain of polio virus that became central to the vaccination program to eradicate this terrible human disease. Up until the present day, there are still problems with the vaccine strain reverting to virulence and causing disease. At the same time, Salk developed a killed virus vaccine for polio. As long as the chemical inactivation was done effectively, this provided a much safer vaccine. This approach was applied to another picornavirus that effects livestock, foot-and-mouth disease virus (FMDV). Growing the virus was the biggest challenge as the original vaccine was made from virus grown in the tongues of cattle. The animals were euthanized and the virus harvested from the tongue epithelium (reviewed by Doel, 4). The most significant advance in FMD vaccine technology since
it’s beginning was a tissue culture system to grow the virus (4).

A different approach was taken for another cattle disease, East Coast fever, caused by the parasite, *Theileria parva*. Despite intense efforts to develop a classic vaccine, researchers in Kenya settled on an infect and treat method (ITM) of vaccination (5). Cattle are infected with a lethal dose of parasites and immediately treated with antibiotics to control the infection. Successful vaccination results in solid, protective immunity in these animals. There are many problems with this strategy making it hard to deploy, not the least of which is the threat of development of resistance to treating antibiotic. Efforts continue to design a safer, recombinant vaccine for *T. parva*.

More effective and safe vaccines for infectious diseases of livestock are being developed using 21st century technologies to be discussed in the next talks.

**References**

For million years, animals have developed biological networks and strategies to coordinate functions of the body systems upon encounter with threats and challenges. The ultimate goal is to maintain host homeostasis that will eventually increase the chance of survival. The immune system comprised of networks of numerous cells working in an orchestrated manner to protect the organisms from both external and internal challenges. Three layer including physical barrier, innate, and adaptive immunity form intricate protective layers against microbial invasion. Improper activation of the immune system can be detrimental, and therefore, must be strictly controlled. Immune regulation, via cytokines and effector T lymphocytes, plays critical role in controlling the immune functions and homeostasis. In addition, the immune-neuroendocrine network also served as critical network for maintaining of host homeostasis, and controlling of the immune activation. It is now widely accepted that immunological, pathological, physiological, behavioral, and psychological states of the animal are closely linked in a dynamic manner. Factors affecting any of the system can significantly influence the behavior and functions of the entire network. For example, immunological stress (pro-inflammatory cytokines) can activate the endocrine system (through Hypothalamus-Pituitary Axis; HPA axis) and the central nervous system, leading to downregulation of the immune functions. This could benefit the host for efficiently restore the homeostatic condition after successful clearance of infection. On the other hand, chronic stress, i.e. immunological, pathological, psychological stresses, can also lead to suboptimal immune functions and secondary immunodeficiency due to prolonged activation of the neuroendocrine network within the affected host. On the other hand, excessive immune activation can also alter host’s behavior, metabolism, and nutrient utilization resulted in reduced animal production and performance (reviewed in [1]).

The mucosal immune system represents a large unique immune compartment in the body. As mucosal tissues and organs also have vital physiological functions, both physiological functions and immunological protection must be sustainably maintained. The mucosal immune compartment possesses a unique immune regulation where by immune exclusion at the mucosal surface is preferred over the active inflammatory reaction. The main mechanisms of mucosal protection include various protective mechanisms at the mucosal barriers, preferential induction of IgA, and suppression of inflammatory reaction with in the mucosal tissues [2, 3]. The mucosal immune system plays important roles in immunity against pathogen invasion and epithelial barrier restoration [3]. One of the key components for maintaining the gut health is the gut microbiota, a collection of microorganisms that live within the gut. The microbial communities, including their components and metabolites, are crucial for development, function and maintenance of the immune homeostasis. Gut microbiota has numerous benefits to the host including digestion and fermentation of carbohydrates, production of certain vitamins, promote epithelial cell growth and maintaining of mucosal barrier integrity, regulation of the immune homeostasis, and protection against pathogenic bacteria [4, 5]. It should be pointed out that both local and systemic immune compartment are influenced by gut microbiota. For example, the short chain fatty acids (SCFA), produced from colonic microbial fermentation of the undigested dietary fibers, can be transported to other systemic organs, such as the brain and lungs, in which they directly or indirectly act on local or resident antigen-presenting cells to decrease inflammatory responses [6]. Dysbiosis, an abnormal microbiome structure which affects the taxonomical composition and functions of microbial community, often leads to impaired intestinal functions and immune dysregulation. Several factors, including genetic, diet, hygiene, stress, infection and inflammation, vaccine and medication particularly antibiotics, can affect the structure of the intestinal microbiome [7]. The effect of gut dysbiosis on gastrointestinal physiology included increased intestinal permeability, altered intestinal motility, increase intestinal sensitivity and inflammation, immune dysregulation, and modulation of gut-brain axis [8].

The gut-brain axis represents the bidirectional interaction between brain and gut, whereby reciprocal signals between the 2 systems are exchanged for maintaining of host homeostasis. The interactions, in responses to various environmental signals, are mediated through several pathways, including neurologic, immunological, metabolic and endocrine mediators [3]. Indeed, it has been well appreciated that the immune system is the master regulator of brain–gut and gut–brain communications [3]. The microbiota-gut-brain axis has been indicated to have crucial role on controlling of host homeostasis. For example, gut microbiota dysbiosis has been indicated as the leading cause of post weaning diarrhea and gastrointestinal
infection in piglets [9]. Dietary transition and environmental changes during weaning period are linked to gut dysbiosis and impaired mucosal defense, which leads to enteric infection in the piglets [5].

Apart from genetic selection and improving farm management and animal hygienic, several alternative products have been applied to maintain and restore the intestinal balance [9]. Among these, probiotics and prebiotics has emerged as potential alternatives for restoration of the gut health. The benefit of probiotics include direct inhibition of the pathogenic bacteria, modulation of the resident microbiota composition and activities, immune regulation, and improving the intestinal barrier functions [4, 9].

In summary, healthy gut environment is crucial for shaping of the immune system and maintaining of host health and homeostasis. Improper activation of immune responses in the gut can profoundly affect animals’ health and performances. Thus, minimizing stress, promoting gut health and reduction of intestinal infection/inflammatory reactions can promote overall host homeostasis and performances.

References

Major emerging and re-emerging swine viral diseases in Asia

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Several swine viral diseases including foot-and-mouth disease (FMD), classical swine fever (CSF) and Aujeszky’s disease (AD) etc. are still endemic in Asia and sporadically re-emerge, particularly, FMD when having biosecurity and/or vaccination failures. FMD trans-boundary disease movement risk assessments should be considered and collaboration among neighboring countries, authorities, social network, stakeholders and farmers must identify risk factors in order to reduce FMD outbreaks and spreading to others. CSF still causes high mortality in back-yards and small holders decreasing household-incomes and food security for many rural villagers and the causative virus may spill into the industrial farms causing more or less economic impact depending on the herd immune status. Similarly, AD seems to be under control for so many decades but the recent outbreaks in China appear to be a warning of emerging lethal variant AD in the regional areas [1]. Those countries have been spending a lot of money on vaccination and test kits for prevention and control those infectious swine viral diseases.

Unavoidably, many emerging swine diseases including porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus associated diseases (PCVAD) etc. have been introduced into the Asian countries possibly from imported breeders and semen in the last 3 decades [2]. Those diseases currently become endemic due to previously unrestricted animal movement and biosecurity failure between and within the countries. Current PRRSV control strategies are not predictably successful either using conventional control strategies or commercially available vaccines due to its severe immunosuppressive effects. Eradication and rigorous biosecurity could be the ultimate tool for PRRSV control. However, PCV2 vaccination strategy has been successfully implement in most affected farms. Recently, PCV3 associated with many syndromes has been reported in many countries including Thailand [3].

Emerging porcine epidemic diarrhea (PED) virus was noticeable in China and South Korea a few years before spreading to other countries and across the continents [4]. Currently, the Chinese-like PED virus becomes endemic and sporadically causes problems in the suckling piglets of subpopulation gilts and sows. PED vaccines are still not effective enough to provide fully lactogenic immunity to suckling piglets. Either eradication and rigorous biosecurity or maintaining the sow immunity by whole herd feed-back and later regular gilt acclimatization can be the alternative tool depending on the farm location and disease prevalence. Factors involving in inter- and intra-countries spreading are discussed. However, animal products and animal movement (legally and illegally) among neighboring countries plays a major role in this incidence. These trans-boundary swine diseases provide Asian pig producers with a unique challenge in disease diagnosis and control. Swine veterinarians can better control those diseases by utilizing more suitable screening tests and acquiring a better knowledge of the pathogenesis and modern diagnostic techniques. In addition, the changes in herd size and operational systems are further challenges in the disease control internally. The impact of globalization on these trans-boundary swine diseases demonstrates that any emerging disease occurred in one country may emerge in others sooner or later similar to the pandemic H1N1 2009 [5] depending on the pathogen nature. This talk discusses on current emerging swine viral diseases mainly in South East Asia along with major factors involving in disease spreading and important means to prevent and control the diseases as ‘One World, One Health’ reflecting the impact of globalization on these trans-boundary swine diseases.

References
New Techniques in Livestock Vaccine Development

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Abstract
Traditional approaches to vaccine development have long been time consuming and lacking approaches informed by the understanding of the immune response. Deriving attenuated vaccine strains by serial passage of the virus in tissue culture has yielded excellent vaccines in the case of Rinderpest and yellow fever, or bacterial vaccines like BCG, but such development took many years. As new pathogens rapidly become health and economic issues, like porcine reproductive and respiratory syndrome virus (PRRSV) and Middle Eastern respiratory syndrome coronavirus (MERS CoV), technologies applied to vaccine development need to utilize the enhanced understanding of livestock and human immune responses, cease testing vaccines in mice when they are not a natural host of the virus, and use 21st Century technologies to rapidly develop and deploy effective vaccines against emerging and remerging infectious diseases.

Introduction
Vaccines against viral diseases of livestock are mostly developed by trial and error. Analysis of vaccine efficacy is mostly limited to protection from clinical disease, not infection and assessed by measuring antiviral serum antibody, as it is readily accessible. Using old technologies, the development of vaccines is slow and labor intensive and tend to have poor performance characteristics such as a narrow range of coverage if the virus is antigenically diverse. Finally, these vaccines commonly have very minimal duration of protection since the antigenic payload disseminates and clears rapidly. This results in little or no generation of immunologic memory and so boosting is required to maintain immunity. Multiple boosts with the same vaccine antigen leads to antibodies with a narrowing specificity range (1) as B cells can somatically mutate the genes for antibody during proliferation and expansion, leading to higher affinity, less cross-reactive antibody responses (2, 3).

The primary adaptive immune response to viral infections in particular, is the CD8+ cytotoxic T lymphocyte (CTL) response. Virus specific CTLs arrest virus proliferation by killing infected cells before viral progeny can be produced (4). Most vaccines against viral pathogens of livestock induce only antibody responses and no measurable CTL. Induction of the CD8 T cells (CTLs), requires stimulation of the antigen receptor on those T cells, the TcR, by major histocompatibility complex (MHC) proteins and peptide fragments of viral proteins bound by the MHC (5).

New Technologies
The evolution of new vaccine technologies has accelerated in the past few years. There are many new adjuvants that can be formulated into vaccines to induce specific aspects of an immune response that developers are targeting. A more sophisticated understanding of innate immune responses has led to the knowledge of how vaccine adjuvants work. Different components of the adjuvant stimulate different innate receptors and these mediate specific innate responses.

Next generation sequencing technologies make it relatively cost effective and simple to generate whole genome sequences of pathogens. This knowledge significantly enhances understanding of infectious agents. There has been a tremendous expansion of the available delivery systems for vaccines including replication defective viral vectors. These include lentivirus vectors, adenovirus, vaccinia virus and a series of pox viruses. Because they are viruses, they “infect” cells and stimulate the same immune response pathways as the virus infection. However, because they are replication defective, they are designed to lack capacity for viral replication, but rather they only express the vaccine antigen payload, making them safe.

Effective vaccines have been designed using canary pox virus expressing proteins of bluetongue (6), human adenovirus 5 expressing the capsid proteins of foot-and-mouth disease virus (7) and vaccinia virus (modified vaccinia virus Ankara; MVA) expressing proteins of the rabies virus (8). Another critical advantage of vaccine vectors is the simple and direct design of diagnostic tests that can differentiate infected from vaccinated animals (DIVA). Thus, the design balances understanding of viral antigens that induce antibody responses during infection and/or mediate protection. Thus, viral vector vaccine technologies can provide safe and effective vaccines.

References
Since the emergence of Porcine reproductive and respiratory syndrome virus (PRRSV) almost 30 years ago, PRRSV continues to be a significant pathogen leading to a serious economic impact to the swine industry, worldwide. Infection with PRRSV induces slow and weak anti-viral immune responses, leading to persistent infection and secondary complication [1], leading to Porcine Respiratory Disease Complex (PRDC). Unfortunately, the traditional control strategies and available vaccines fail to provide sustainable disease control, as they suffer from both antigenic heterogeneity and inadequate vaccine-induced immunity. The lack of anamnestic responses in the previously vaccinated pigs following PRRSV exposure implied that even well-primed immune system was severely impaired during active PRRSV infection.

Numerous evidences indicate that PRRSV can evade and suppress host immune system through several mechanisms [1]. Among these mechanisms, induction of interleukin-10 (IL-10) is believed to be one of the focal mechanisms leading to the unique PRRSV-induced immunological outcomes and interference of PRRS vaccine efficacy [2]. The PRRSV nucleocapsid (N) protein plays an important role in induction of IL-10 following PRRSV infection [3]. During PRRSV-2 infection, the numbers of PRRSV-specific regulatory T lymphocytes (Treg) in the circulation [4], lungs, and tracheobronchial lymph nodes significantly increased. In addition, IL-10 is essential for the development of PRRSV-specific Treg [3]. We previously proposed that removal or reduction of the viral-induced negative immunomodulatory effects especially during the first 2 weeks following infection is essential to establish proper anti-PRRSV during the infection period [5].

To test the hypothesis, our research team had developed a novel DNA vaccine to reduce the PRRSV-induced immunosuppressive effects. The novel DNA vaccine, encoding truncated nucleocapsid protein (pORF7t), was designed to primarily induce cell-mediated, but not humoral, immunity against the PRRSV nucleocapsid protein. The vaccine could reduce PRRSV-induced immunomodulatory activities, i.e. IL-10 and Treg induction, and enhance long-term anti-PRRSV immunity in vaccinated pigs [6, 7]. To enhance PRRSV-specific protective immunity, heterologous DNA-MLV prime-boost immunization was further explored. Our results demonstrated that DNA-MLV prime-boost immunization significantly improved the quality of PRRSV-specific cellular and humoral immunity in the vaccinated pigs [7]. Our data suggested that this novel vaccine concept could potentially benefit the development of PRRSV management and control strategies, particularly in the endemic herds.

Recently, the highly pathogenic PRRSV (HP-PRRSV) with high mortality and severe clinical outcomes has complicated the PRRS management and control strategies in the field. The commercially available modified live vaccines (MLV) do not provide complete clinical protection against the HP-PRRSV [8, 9]. We explored the potential use of the heterologous DNA-MLV prime-boost immunization for improving the protective immunity against HP-PRRSV in the vaccinated-challenged model. Although complete clinical protection was not achieved, the heterologous prime-boost immunization significantly improved PRRSV-specific innate, cell-mediated, and humoral immune responses following the HP-PRRSV challenge. In addition, DNA-MLV immunization resulted in reduction of negative immunomodulatory factors (Treg, IL-10), which were usually observed following PRRS-MLV vaccination.

Our study indicated that DNA vaccine was a powerful tool for priming and modulating of PRRSV-specific immune responses. DNA vaccine can be incorporated with other vaccines, for improving PRRSV-specific protective immunity. This advantage will benefit the management of PRRSV-specific immune responses and the pig’s general health. Our results confirmed the benefit of incorporating the strategy to reduce viral-induced immunomodulation in the PRRSV vaccine designs.

Although the novel DNA vaccine has demonstrated its potential use for PRRSV management and control, the path for vaccine delivery to the end users has been somewhat obscure. This is due to the lack of vaccine-graded, upscale production system and know-how. The available upscale production service is limited to the medical field and the estimated cost/dose is not feasible for veterinary vaccine usage. In addition, the application process for international patent requires an experienced legal team and takes time. Even in collaboration with the experienced international corporate, it took 10 years for our DNA vaccine to

PRRSV DNA vaccine

Lessons learned from vaccine R&D in the developing country

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obtain the US patent. Thus, despite proving the hypothesis, the direction of DNA vaccine R&D in term of licensing and delivery to the market is not promising.

Global veterinary vaccine market continuously expands during the past decade and expected to reach 6.8 billion USD by 2021, posting a compound annual growth rate (CAGR) of approx. 5% globally and 10-15% in Asia (Source: https://www.businesswire.com). Inactivated and MLV vaccines remain the dominant product types in the swine vaccine market. Other product types include toxoid, recombinant vaccine, conjugate vaccine and DNA vaccines. Interestingly, DNA vaccine segment is expected to be a high-growth segment in the near future, due to the stronger demand and increase in R&D investments (Source: https://www.transparencymarketresearch.com).

Developing new vaccine requires extensive research and development. The timeframe from discovery of the potential vaccine candidate to delivery of the vaccine to the target species can take at least 8-10 years. In addition, vaccine manufacturing is a complex process including a large scale vaccine production system with numerous quality control tests. The process also involves stringent evaluation and assessment by the regulatory authorities.

In February 2016, the vaccine manufacture capability among the South East Asia (SEA) countries was analysed. Through the survey platform, the vaccine manufacturers in the targeted Asian countries were identified and profiled. Among the major swine producers in SEA, the countries with veterinary vaccine manufactures included Thailand (2), Indonesia (4), and Vietnam (5). Interestingly, Vietnam and Indonesia also export the vaccines to other countries. In addition, Vietnam already has a manufacturer with technology to produce reverse genetic vaccines. Despite the increased demand in porcine vaccine in the regions, the capability to manufacture vaccines in SEA countries is still very limited. This has also impeded the progress in vaccine R&D and manufacturing in the region.

Despite the strengths in swine production and research, Thailand has limited infrastructure and capacity for veterinary vaccine R&D and manufacturing. Currently, there is only one government owned vaccine manufacturer with limited capacity. An infrastructure for upscale vaccine production, and quality control system is fairly immature and primarily allocated for the human vaccine production. Thus, there is an urgent need for capacity building on veterinary vaccine R&D in parallel with establishing relevant policies, and regulatory framework. Public-Private-Partnership in collaboration with the existing veterinary vaccine networks can help accelerating the process of veterinary vaccine R&D in the country. In addition, a business model of a spinoff company, under the authorized and good manufacturing standard, should be explored for sustainable veterinary vaccine production and disease control.

References
Comparative study of efficacy between live culture and microencapsulation of Lactobacillus plantarum 22F on growth performance in experimental piglets

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Keywords: Thai Lactic acid bacteria, Spray-drying, Growth performance, Piglets

Introduction

For decades, Lactic acid bacteria (LAB) have been proposed to be alternative substances for swine production (1). They produce beneficial effects to the host including improve gut integrity and microbiota ecosystem, enhance intestinal immunity system, control intestinal pathogens and reduce diarrhea sign, improve digestibility and promote growth performance (2). The most commonly used LAB in pig such as Lactobacillus spp., Bifidobacterium spp., Enterococcus spp. and Pediococcus spp. (1). From previous study, we isolated Thai LAB strains from pigs which showed high performance in in vitro studies, especially Lactobacillus plantarum 22F (3). However, applying live culture of LAB strains into the field are troublesomedue to inconvenient preparation and transportation or loss of the number of LAB during storage and digestion process (4). As consequence, microencapsulation technologies especially spray-drying method has been recommended for field application since its advantages such as simplex process to prepare, homogenous distribution of LAB in final product and small diameter sizeand handy feature of product (5,6). Nevertheless, there was no information to confirm that microencapsulated LAB can confer positive outcomes on growth performance as good as live culture of LAB in the host. The propose of study was to evaluate an efficacy of live culture of L. plantarum 22F comparing to spray-dried L. plantarum 22F on growth performance including average daily gain (ADG), feed conversion rate (FCR), morbidity and mortality in experimental piglets.

Materials and Methods

*L. plantarum* 22F were cultured in MRS agar and incubated at 37°C for 18-20 hr, then the final concentration was prepared at 10⁹ CFU/mL (7). Alginate (1% w/v) and chitosan (0.4% w/v) were used as inner and outer wall materials for microencapsulation, respectively. Amount 9 log CFU/mL of LAB was added at ratio 1:5 (v/v) to alginate solution then mixture was atomized through spray dryer (inlet temperature at 130°C), then alginate coated powder were collected and added to chitosan solution before atomizing through spray dryer again (6). Alginate-chitosan coated powder were stored at room temperature for six months. A total of 90 healthy Large White × Landrace × Duroxneonatal pig were nursed and cross-fostered before randomly distributing into 3 experimental groups. Control group; pigs were fed with 3 ml of sterile peptone water. Three millilitres of live culture of *L. plantarum* 22F suspension (10⁹ CFU/ml) and 6-month-storage encapsulated *L. plantarum* 22F mixture were fed to pigs in live culture and microencapsulated groups respectively. Administration were performed every three days for 5 times (at D0, D3, D6, D9 and D12 after cross-fostering). After weaning period, piglet body weight and feed intake were recorded weekly throughout the experimental period. Data were analyzed by one-way ANOVA and comparison of means were tested by Tukey’s multiple range tests using SPSS version 22.0. The effects were considered to be significant at *P*<0.05.

Results and Discussion

Piglets in all groups had no clinical abnormality and survived until 8-week age. After 5-week experimental period, the results showed that both piglets of group receiving live *L. plantarum* 22F and spray-dried *L. plantarum* 22F mixture exhibited greater ADG than piglets of the control group (*P*<0.05). Nevertheless, there was no difference on ADG between both treated groups (Fig.1). Moreover, piglets fed with LAB in all applications had lower FCR than piglets of the control group (*P*<0.05). However, there was no significant difference of FCR range between live culture and...
microencapsulated group (Fig.2). These results were in agreement with previous studies (8,9) confirming the positive effect to enhance growth performance in pigs by our candidate strain. Use of single and combined LAB strains such as *L. fermentum, Enterococcus faecium* NCIMB 10415 and mixture of *L. amylovorus* and *E. faecium* were also successful to improve growth performance in nursery period (10,11). The improvement of growth performance may involve several mechanisms including stimulation of intestinal immune response, maintaining intestinal microbiota homeostasis, remedying gut health consequently enhance digestion and utilization nutrients (1,2).

![Fig.1 Comparison the ADG of control, live culture and microencapsulated during wk3-8.](image1)

![Fig.2 Comparison the FCR of control, live culture and microencapsulated during wk3-8.](image2)

Interestingly, our results showed that application by microencapsulated LAB could perform as good as those of live culture. These results indicated that spray-drying method could preserve amount and prolong LAB life span and properties. In microencapsulation process, alginate and chitosan polymers showed protective effect to shield LAB from detrimental environment (7,12). Moreover, application microencapsulated LAB was very practical for the industrial field in term of storage and transportation. In conclusion, live culture of *L. plantarum* 22F could be a very good candidate probiotic in piglets which improved growth performance as well as its microencapsulated form.

**Acknowledgements**

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**References**

Comparison between diagnosis of animal disease with and without using mobile diagnostic application- POAS

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Keywords: Mobile diagnostic application, POAS, veterinary diagnosis

Introduction

One of the key success in animal treatment requires correct and rapid veterinarian’s diagnosis. Accurate diagnosis depends on clinical skills and experiences of veterinarians. Furthermore, there are many contributing factors affecting the pets’ health problems, such as age, types of care and environment. When veterinarians take the signalment from pet owners, they have to define, rearrange and analyze the problems then give differential diagnosis from history, signs and laboratory results. Occasionally, owners may hide crucial information or misunderstand the signs that their pets demonstrated, leading to misdiagnosis. Veterinarians who have little experience or newly graduated veterinarians are often anxious (1). At present, Smartphone has become a useful tool for searching or accessing medical information rapidly. Using a mobile diagnostic application can also help veterinarians to perform differential diagnosis from clinical signs. It can reduce nervousness and quickly guide to treatment. According to Ventola (2), using mobile diagnostic application enhances clinical performance such as reducing fault information while communicating with pet owners or rapidly accessing useful data or information. Learning from clinical signs can guide veterinarians to give differential diagnostic for possible cause of disease and plan to treatment (3), which is the principle of mobile diagnostic application, “POAS” that was used in the present study. This name derives from the principle of Problem Oriented Approach course in the Doctor of Veterinary Medicine Programme of Chulalongkorn university.

Materials and Methods

Retrospective analysis of the data from the animal medical records, Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn university during April 2012 - September 2017 was performed in this study. The cases presented with 10 common clinical symptoms including anemia, cough, diarrhea, edema or effusions, jaundice, PUPD, pruritus, seizure, vomit and weight loss, were re-diagnosed step by step using POAS. The final diagnoses were compared to the diagnosis initially made by attending veterinarians.

Results and Discussion

The total of 780 cases that had one of the 10 symptoms were re-analyzed using POAS and the final diagnoses were compared to those made by veterinarians. The results (Table 1) show that 73.33% of POAS assisted diagnoses were similar to the diagnoses made by veterinarians, 20% were different, and 6.67% were inconclusive.

Table 1. Comparison of Diagnoses of 10 common symptoms with and without using POAS

<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>Number of cases</th>
<th>Similar results</th>
<th>Different results</th>
<th>Inconclusive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>95</td>
<td>68 (71.58%)</td>
<td>10 (10.53%)</td>
<td>17 (17.89%)</td>
</tr>
<tr>
<td>Cough</td>
<td>100</td>
<td>58 (58%)</td>
<td>17 (17%)</td>
<td>25 (25%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>93</td>
<td>89 (95.6%)</td>
<td>4 (4.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Edema</td>
<td>75</td>
<td>64 (85.3%)</td>
<td>11 (14.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Jaundice</td>
<td>67</td>
<td>37 (55.2%)</td>
<td>30 (44.8%)</td>
<td>0</td>
</tr>
<tr>
<td>PUPD</td>
<td>76</td>
<td>55 (72.3%)</td>
<td>21 (27.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>98</td>
<td>88 (89.7%)</td>
<td>10 (10.3%)</td>
<td>0</td>
</tr>
<tr>
<td>Seizure</td>
<td>78</td>
<td>42 (58.8%)</td>
<td>36 (48.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Vomit</td>
<td>63</td>
<td>38 (62.32%)</td>
<td>1523.81%</td>
<td>10 (15.87%)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>35</td>
<td>33 (94.2%)</td>
<td>2 (5.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>780</td>
<td>572 (73.33%)</td>
<td>156 (20%)</td>
<td>52 (6.67%)</td>
</tr>
</tbody>
</table>

The symptoms that had the highest matched diagnoses were diarrhoea (95.6%), followed by weight loss (94.2%) and pruritus (89.6%), respectively. The lowest
matched diagnoses were jaundice (55.2%).

The inconclusive results were generally due to incomplete history or missing data. For instance, in some cases veterinarians did not record character of dyspnea, therefore we were unable to finish the application process. These signs are important to make a differential diagnosis, for instance the characteristics of the cough intracheal disease may bedry cough and dyspnea, in bronchiolar disease it may becoughing with expiratory dyspnea and in alveolar disease it may bemild cough with dyspnea (4). In vomiting, some patients did not have radiography or ultrasonography which may be essential for finding the cause of this symptom. Although some cases had sufficient clinical history, there were no inflammatory lesions, partial or complete obstruction and motility disorders while blood profile was normal. Therefore we could not finish the application process and confirm the veterinarian’s diagnosis. The different diagnostic results may be due to the nature of such illness that is often chronic and complicated by other problems, hence accurate diagnosis requires communication skill to get necessary information, extensive laboratory tests and expertise of skilled veterinarian. Unfortunately, sometimes careful history taking and complete examination were neglected because the patients came with the same pattern of sickness. Thus some important information or some signs of illness was missed. We believe that if thorough history taking, complete physical examination, laboratory results and radiography/ultrasonography were complete, it may help to increase percentage of similar diagnosis between veterinarian and application. However, it may be concluded that POAS can be a useful tool to guide particularly young veterinarians in making differential diagnosis bearing in mind that clinical knowledge and veterinary skills are indispensable.

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References
Correlation of macroscopic grading of stifle osteoarthritis and clinical parameters with medial patellar luxation in dogs

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Keywords: correlation, macroscopic grading, medial patellar luxation, osteoarthritis, synovium

Introduction
Osteoarthritis (OA) is the most common problem of arthritis in dogs. OA is characterized by degeneration of articular cartilage, variable degree of synovitis, osteophyte formation and subchondral bone sclerosis. Most dogs can be identified as secondary OA and stifle joint is one of the most affected joint occurrence following medial patellar luxation (MPL). Repetitive medial luxation of the patella can cause synovitis and erosion of articular cartilage both on patella and femoral condyle leading to progression of OA. The study was aimed to determine if the stifle macroscopic morphology score (OA score) of the stifle joint with MPL was associated with clinical parameters including degree of luxation, body weight, age, lameness score and duration of disease.

Materials and Methods
Thirty-four stifles presented at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University for surgical correction had grades 2, 3, and 4 MPL in 13, 13, and 8 stifles, respectively. Five stifles 3 dogs normally examined by radiographic and macroscopic finding were used as a control group. Age, body weight, lameness score and duration of disease were recorded. All stifles were examined and scored for the gross pathologic features of femoral condyle and patella by using modified macroscopic morphology grading system (OA score). OA score was divided into 3 categories including synovial score, cartilage score and osteophyte score (5-6). This study was approved by the Laboratory Animal Care and Ethics Committee (No 1731041). The correlation of OA score and 3 subcategories score was determined with duration of disease (DOD), body weight (BW), age, patellar luxation (PL) grading and lameness score by spearman-rank test, and the statistical significance between groups were determined using the Kruskal-Wallis test with a p value < 0.05 followed by Dunn’s correction for multiple comparison test (PISM® ver. 6.01, GraphPad, Inc.).

Results and Discussion
The mean ± SD age of control group, grade 2, 3 and 4 MPL groups were 97.6 ± 50.1, 17.6 ± 12.9, 37.3 ± 27.2 and 24.5 ± 18.5 months, respectively. Median scores (range) of OA, synovial, cartilage, osteophyte and lameness of control and MPL groups showed in Table 1.

Table 1 Median scores (range) of osteoarthritis, synovial, cartilage, osteophyte and lameness of control and MPL groups

<table>
<thead>
<tr>
<th>Scores</th>
<th>control</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoarthritis</td>
<td>0</td>
<td>3°</td>
<td>3°</td>
<td>3°</td>
</tr>
<tr>
<td>Synovial</td>
<td>0°</td>
<td>1°</td>
<td>1°</td>
<td>1°</td>
</tr>
<tr>
<td>Cartilage</td>
<td>1.7°</td>
<td>0.7°</td>
<td>0.6°</td>
<td></td>
</tr>
<tr>
<td>Osteophyte</td>
<td>0.2°</td>
<td>0.2°</td>
<td>0.1°</td>
<td></td>
</tr>
<tr>
<td>Lameness</td>
<td>1</td>
<td>2°</td>
<td>2°</td>
<td></td>
</tr>
</tbody>
</table>

* Different letters a, b, c indicate in the same row represent significant differences (p<0.05).

Figure 1 The proximal femoral condyle has severe synovitis, grade 2(A). The medial femoral condyle has severe full thickness cartilage erosion, grade 4(B). The osteophyte formation and severe articular cartilage erosion occur at the proximal femoral condyle, grade 2(C). Full thickness cartilage erosion on the apex of patellar articulation, grade 4(D).

O5
The correlation of OA score, synovial score, cartilage score, osteophyte score and lameness score with duration of disease, body weight, degree of PL, age at surgery and lameness score showed in table 2.

There was a significant difference of OA score between control group and all grades of MPL. Synovial score was significantly different between control and grades 3 and 4 MPL and between grades 2 and 4 MPL. Cartilage score was significant difference between control and grades 2 and 3 MPL. The significant difference of osteophyte score was found only in grade 3 MPL. Lameness score was significant difference between control and all grades of MPL.

These findings were similar with other studies that observed induced osteoarthritis both in cranial cruciate ligament transection and in medial patellar luxation models. Asimilar correlation was found in previous study, where age at surgery was positively correlated with severity of osteoarthritis in the stifles surgically treated as well as the study of found that age and degree of PL were risk factors related to cartilage erosion. The heavier dogs are, the more osteophyte formation occurs which could be explained by the influence of BW causing mechanical loading to the abnormal structure of the joint. Articular structure is composed of articular cartilage, subchondral bone and synovial membrane. Not only synovium but also subchondral bone has vascular, lymphatic vessel and rich nerve supplies while articular cartilage covering subchondral bone and comprising of chondrocyte is avascular and avascular. Therefore, injury of these structures can cause pain and lameness which illustrated by the correlation of lameness score and synovial score in this study. In summary, all dogs with MPL in this study had osteoarthritis even the dogs did not show sign of lameness.

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**References**

Diagnostic evaluation of the specific-IgG1 to house dust mites (HDM) allergen in atopic dogs by an in-house ELISA

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Keywords: atopic dogs, IgG1, IgG2

Introduction
Atopic dermatitis (AD) is one of common skin diseases in dogs resulting in inflammatory and chronic-relapsing pruritic dermatitis. The common clinical appearance including pruritus and erythema, are often associated with IgE specific to common allergens such as mites, ticks, dust, pollen, and microbes (1). *Dermatophagoides farinae* and *D. pteronyssinus* are common house dust mites (HDM) in which to act as causative allergen in atopic dogs (2). Intradermal skin testing (IDST) and allergen-specific IgE serology testing (ASIS) are the technique of choices for specific allergic detection in dog patients. ASIS is non-invasive and more practical than intradermal testing which does not need to take withdrawal times of anti-inflammatory drug and risk of anesthetic condition. To date, not only IgE, but the level of canine IgG1 subclass also could be triggered by parasitic infestation, atopic dermatitis and allergen-specific immunotherapy (ASIT) (5). Previously, a level of allergen-specific IgG subclasses was able to identify specific allergens and indicate the causative allergens in canine AD (6). This study aimed to evaluate the precision of our in-house ELISAs via variety of specific immunoglobulin markers comprising IgE, IgG, IgG1 and IgG2 against *D. farinae* and/or *D. pteronyssinus* between atopic dogs with positive and negative to HDMs by IDST.

Materials and Methods
Total of 46 serum samples derived from atopic dogs included 31 positive and 15 negative to HDMs by IDST. All subjects were also tentatively diagnosed by the authorized veterinarians using the approve criteria (1). For allergen preparation, protein antigens of *D. farinae* (Df) and *D. pteronyssinus* (Dp) were separately prepared by sonication and filtration. The protein concentration in supernatant was measured by Bradford assay (7). Two micrograms of each protein antigen were coated onto each well of 96-well plate. The micro-well plate was blocked with phosphate buffer saline contained 1% BSA and 0.05% Tween 20 for 1 hour. The sera were diluted at 1/5 and 1/500 for IgE and IgG detection, respectively, and incubated for 1 hour at 37°C. After washing, the mouse-anti dog IgE was added, then the plates were washed before adding with goat anti-mouse IgG-AP. Sensitest Canine IgE Substrate were used to develop chemical reaction and OD intensity was measured at 405 nm. For IgG and it subclasses, the plate was reacted to anti-dog IgG1-HRP, anti-dog IgG2-HRP or anti-dog IgG-HRP. ABTS® peroxidase substrate was finally reacted and measured OD intensity at 405nm. The mean OD plus two SD values in negative HDM groups were used as positive cut-off value in our in-house ELISAs. The precision of all in-house ELISAs were analysed by SPSS software and IDST was considered as a standard method in this study. The performance of our ELISAs were presented via the percentage of sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and accuracy.

Results and Discussion
The specificity, sensitivity and predictive values to HDM allergen detection in AD dogs comparing between in-house ELISAs and IDST were shown in Table 1 and 2. All immunoglobulin markers showed the different levels of evaluation calculation. In Df detection, the high sensitivity was found in IgE and IgG1 markers whereas, all markers showed specificity from 69.2-93.3%. IgE and IgG1 markers also showed the high percentage of PPV at ≥80 percentage. PPV were used to confirm the feasibility of positive ELISA detection represented dogs allergic with the relevant allergens. Thus, IgE and IgG1 could screen only the true positive subjects but the level should not be observed for healthy or the subjects negative to Df.

For specific Dp-allergen detection, use of specific IgE to Dp was also acceptable for atopic dogs positive to Df allergen. Interestingly the specific IgG1 gave the highest sensitivity and PPV
percentage over the other markers including IgE. There was a slight difference between accuracy rates of Df and Dp detection by IgG1 ELISA base. This may be due to different allergenic proteins or different concentration of major allergen between two species of HDMs (8). Various antigenic substances between Df and Dp may trigger different levels of IgE and IgG1 response in AD dogs. Antigen of Df may induce a stronger IgE than Dp antigens (8,9), so sensitivity of Dp- IgE was lower than Df-IgE. Moreover, some of AD dogs in this study had double positive IDT to Df and Dp. The cross-reaction between Df and Dp allergens have been reported (10), thus this could interfere the accuracy of serology detection in IgG1 ELISA base between Df and Dp allergy.

Table 1 The precision of in-house ELISAs with different markers to indicate Df sensitive in AD dogs

<table>
<thead>
<tr>
<th>Specific markers to D. farinae</th>
<th>%</th>
<th>IgE</th>
<th>IgG</th>
<th>IgG1</th>
<th>IgG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>63.3</td>
<td>10.0</td>
<td>60.0</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>78.6</td>
<td>93.3</td>
<td>69.2</td>
<td>93.3</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>86.4</td>
<td>75.0</td>
<td>81.8</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>50.0</td>
<td>34.1</td>
<td>42.9</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>68.2</td>
<td>66.7</td>
<td>69.8</td>
<td>66.7</td>
<td></td>
</tr>
</tbody>
</table>

In our study, neither allergen-specific total IgG nor IgG2 were not a good marker to indicate HDM allergy, because they could be found in both positive and negative HDM groups. It suggested that IgG and IgG2 levels were not associated with allergic condition.

The results confirmed that HDM specific-IgG1 could detect the allergic dogs that positive to HDM as the indication of ASIS. All detective markers by ELISA gave the low NPV reflecting a caution for the subject negative to HDM or healthy dogs. To use of IgG1 subclass, dog serum is required a hundred times lesser than that of IgE detection. Thus, this platform is more practical, user friendly and non-invasive when compared to specific IgE detection by IDST and ASIS (1).

Table 2 The precision of in-house ELISAs with different markers to indicate AD dogs positive to D. pteronyssinus.

<table>
<thead>
<tr>
<th>Specific markers to D. pteronyssinus</th>
<th>%</th>
<th>IgE</th>
<th>IgG</th>
<th>IgG1</th>
<th>IgG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>36.7</td>
<td>3.3</td>
<td>60.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>92.9</td>
<td>93.3</td>
<td>100</td>
<td>93.3</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>91.7</td>
<td>50.0</td>
<td>100</td>
<td>75.0</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>40.6</td>
<td>32.6</td>
<td>45.5</td>
<td>34.1</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>68.2</td>
<td>66.7</td>
<td>75.0</td>
<td>66.7</td>
<td></td>
</tr>
</tbody>
</table>

In our study, neither allergen-specific total IgG nor IgG2 were not a good marker to indicate HDM allergy, because they could be found in both positive and negative HDM groups. It suggested that IgG and IgG2 levels were not associated with allergic condition.

Acknowledgements

We thank the Dermatology Clinic, Small Animal Hospital, Kasetsart University for serum sample collection and Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University for house dust mite antigen. We are also thankful to the funding grants; Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program for student scholarship fund, (RSA5980056) Thailand Research Fund and STAR; Detection and Monitoring Animal Pathogen, Chulalongkorn University.

References

Effects of the supplementation of encapsulated probiotics on the intestinal morphology and intestinal immune status of Nile tilapia

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Keywords: Lactobacillus rhamnosus GG; Saccharomyces cerevisiae; Nile tilapia; probiotic; encapsulation

Introduction

Nile tilapia, Oreochromis niloticus, is one of the most valuable cultured species in Thailand. However, in tilapia farms, outbreaks of disease causing high and acute mortalities occur due to the culture intensification, environmental fluctuations and continuous exposure of fish to different microorganisms.

Probiotics have become widely used in many developing countries as an environment-friendly approach to control disease and improve the production of cultured fish (1, 2). In Nile tilapia, studies demonstrated the potential of Lactobacillus rhamnosus GG (LGG) (3) and the yeast Saccharomyces cerevisiae (4) to reinforce the immune function, improve the growth performance and feed utilization and/or the protection against bacterial infections. Probiotics can increase intestinal villi height enhancing the area for nutrient absorption (5) and have beneficial effects on the gut-associated lymphoid tissue, rising the number of intestinal intra epithelial leukocytes (IEL) and the presence of mucous cells (MC) (2, 5). IEL interact with the epithelial cells and protect the mucosa by killing infected cells and attracting other immune cells (6), while MC produce mucin, the first defence against microbes (7).

In order to efficiently apply probiotics in aquaculture, they need to remain viable at the site of action in the gastrointestinal tract of fish (4). Among the techniques available for immobilizing living cells, alginate remains the most commonly used bio polymer for microencapsulation (8).

Taking all of this into account, the present study was aimed to assess the effects of dietary encapsulated probiotics LGG, S. cerevisiae, or their combination, on the intestinal morphology and amount of immune cells in Nile tilapia. The inclusion of a dietary combination between the two probiotics was particularly interesting, given their numerous individual benefits reported in cultured fish.

Materials and Methods

Probiotics preparation: The probiotic LGG (ATCC 53103) was cultured in de Man, Rogosa and Sharpe (MRS) broth at 37°C for 48 h. The probiotic cells were centrifuged at 4°C and then washed three times with PDS. The probiotic S. cerevisiae (JCM 7255) was cultured in Sabouraud dextrose broth at 30°C for 48 h. The probiotic cells were centrifuged in refrigeration and washed with sterile peptone dilution saline (PDS) three times. Both probiotics were encapsulated under sterile conditions. The viability of the two encapsulated probiotics during storage and during simulated gastric conditions and 10% tilapia bile were confirmed following the methodology described by (3) for LGG and (4) for S. cerevisiae.

Fish husbandry and experimental diets: all the procedures were approved by the ethics committee of Chulalongkorn University Animal Care and Use Committee (CU-ACUC) (No. 11310010). Nile tilapias (26.11±0.88 g) were randomly placed in fifteen tanks of 90 l of capacity (30 fish per tank). Five experimental diets were used: a commercial diet (C), diet C supplemented with 10⁶ cfu LGG/g diet (LGG), diet C supplemented with 10⁶ cfu S. cerevisiae/g diet (SC), diet C supplemented with a combination of LGG and S. cerevisiae (10⁵ cfu LGG/g diet and 10⁶ cfu S. cerevisiae/g diet) (LGG+SC), and diet C supplemented with free microcapsules (Cap). Each experimental diet was randomly assigned to three replicate tanks and fish were fed the experimental diets once per day with 3% of the body weight for 30 days.

Intestinal histology: proximal, middle and distal intestinal samples from 6 fish fed each of the experimental diets were collected at the end of the feeding period. Samples were fixed in 10% buffered formalin, processed according to standard histological techniques. For the intestinal villus height measurement, the ten highest villi were selected per section, and their length was measured from tip to bottom. The average of ten villi per section was expressed as the mean intestinal villus height of each section. For IEL, the same villi previously selected were given an arbitrary score from 0 to 3 based on the frequency and population number: 0 - none, 1 - mild, 2 - moderate and 3 - marked IEL. Finally, for MC, the tissue sections were stained with a combination of Alcian blue and periodic acid-Schiff reagent. The 10 highest villi were then selected and the positive mucous cells were averaged and scored on frequency and population number as 0 representing none, 1 representing 1-10, 2 representing 10 - 30 and 3 representing >30 cells per villus.
Statistical analysis: data were analysed using one-way ANOVA followed by the Bonferroni-type multiple t-test. Differences were considered significant at P < 0.05 in all the cases.

Results and Discussion

Fig. 1. Average intestinal villus height (µm) in fish fed the experimental diets (C: control; LGG: 10⁶cfu LGG/g diet; SC: 10⁹cfu S. cerevisiae/g diet, and LGG+SC: 10⁶cfu S. cerevisiae and 10⁹cfu LGG/g diet) for 30 days (n = 6). Bars belonging to the same group (proximal, middle, or distal) with different signs or letters are significantly different (P<0.05), according to ANOVA.

Table 1. Intraepithelial lymphocytes and acidophilic granulocytes in intestine of Nile tilapia fed the experimental diets.

<table>
<thead>
<tr>
<th>Int. portion</th>
<th>Diets</th>
<th>IELs (mean score)</th>
<th>AGs (average number)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>LGG</td>
<td>SC</td>
</tr>
<tr>
<td>PI</td>
<td>1.30±0.35a</td>
<td>1.37±0.15a</td>
<td>1.83±0.21a</td>
</tr>
<tr>
<td>MI</td>
<td>1.03±0.06</td>
<td>1.33±0.29</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>DI</td>
<td>1.0±0.0</td>
<td>1.13±0.15</td>
<td>1.44±0.44</td>
</tr>
<tr>
<td></td>
<td>76.0±5.29a</td>
<td>43.0±13.5a</td>
<td>14.7±4.93</td>
</tr>
<tr>
<td>MI</td>
<td>35.7±2.5a</td>
<td>24.0±2.65a</td>
<td>11.7±8.02</td>
</tr>
<tr>
<td>DI</td>
<td>37.2±5.91a</td>
<td>24.0±8.43a</td>
<td>36.3±6.51a</td>
</tr>
</tbody>
</table>

Regarding the intestinal villus height, proximal intestine of fish fed SC presented the highest villus height (P<0.05) (Fig.1 A). In middle intestine the highest villus height (P<0.05) was that of fish fed Cap. Finally, in distal intestine, fish fed C and LGG+SC had the lowest villus height (P<0.05). Many studies on the inclusion of different probiotics in diets for different species of cultured fish reported higher villus height in fish fed probiotics compared to those fed control diets (5, 6). In the present study, an effect of probiotics on the improvement of villus height was observed in proximal and distal intestines. Dietary S. cerevisiae and LGG gave the highest villus height (P < 0.05) in proximal and in distal intestines, respectively. The single probiotic diets seemed to have an overall stronger effect on the intestinal villus height than the probiotic combination diet. Despite studies reporting that single strain probiotics are less effective than those based on combinations, others reported that a dietary combination of probiotics does not always provide advantages in tilapia. The amount of IEL in proximal intestine was significantly higher (P < 0.05) in fish fed diet SC than in those fed diets LGG+SC and Cap (Table 1). No significant differences (P > 0.05) were observed in middle and distal intestines. Fish fed diet C had the highest number of AG in the three intestinal portions. This was higher (P < 0.05) than in proximal intestines of fish fed SC and LGG+SC and distal intestines of fish fed LGG+SC. The probiotic S. cerevisiae had a positive effect on the villus height and presence of IEL in proximal intestine.

As a conclusion, in the present study dietary-encapsulated S. cerevisiae presented more advantages regarding intestinal morphology and IEL than LGG, the probiotic combination or the control diet in Nile tilapia. The combination of LGG and S. cerevisiae did not have an advantageous effect on any of the parameters assessed.

References

Immunopathogenesis of Chicken Infectious Anemia Virus Isolated in Taiwan

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Keywords: Immunopathogenesis, Chicken anemia virus

Introduction
Chicken infectious anemia (CIA) is an important immunosuppressive disease of poultry. The disease caused by chicken anemia virus (CAV), which is classified in the genus Gyrovirus of the family Anelloviridae (1). The virus targets hemocytoblasts in the bone marrow and precursor lymphocytes in the thymus. This virus causes clinical diseases of high mortality characterized by anemia. Immunosuppression caused by thymus atrophy leads to aggravation of co-infections. The disease was first reported in Japan (2) and has been reported worldwide via virus detection by PCR, molecular characterization, isolation and experimental pathogenicity studies (3,4,5). However, there was a few report and also immunopathogenesis studies regarding this virus in Taiwan. Therefore, the present work was undertaken with the aim to investigate the relationship among clinical symptom, mortality, lymphocyte depletion and antibody response.

Materials and Methods
The 104-05 CAV isolate used in these experiments had been passaged 4 times in MDCC-MSB1 cells. One-day-old SPF chicks were inoculated 0.1 ml CAV intramuscularly with 10^4 (n=8) and 10^5 (n=9) TCID50 and an uninoculated group was used as control (n=8). Blood samples were taken, at 7 day intervals to determine the haematocrit level and CAV antibodies. Fourteen and twenty-one days after inoculation, two chickens from each group were killed for thymic lymphocyte depletion examination. The experiment was terminated at twenty-eight days after inoculation. Antibodies to CAV were detected by commercially available ELISA kit. Lymphocyte depletion were evaluated by morphometry of thymus stained section.

Results and Discussion
Both treatment groups developed anemia after 14-day post infection (dpi). A chicken died 10 dpi in 10^5 TCID50 of CAV (high dose) group. By 21 dpi, the high dose group had lower hematocrit level compared with low dose group (10^4 TCID50) (Fig1). The ability of CAV to produce anemia in experimentally inoculated chicks is dose dependent. These results were supported in previous report (6). Antibodies to CAV were present in both treatment groups after 14 dpi. CAV antibodies of the high dose group peaked at 21 dpi (Fig2). These results suggest that higher virus levels corresponded to higher antibody levels. This is a consequence of greater virus stimulation also described in another 4 week-inoculated experiment (7). A chicken died at 20 dpi in the low dose group. Another chicken died at 16 dpi in the high dose group while presenting very low hematocrit level. These results were similar to a previous experiment (8) where dead chickens were characterized by aplastic bone marrow or hematocrits of 25 or less. Hematocrit values increased after 21 dpi and returned to normal at 28 dpi. According to previous published paper, there was no recorded bone marrow hypoplasia or aplasia lesion after 24 dpi (9).

Figure 1 Hematocrits of SPF chickens inoculated at 1 day of age with different doses CAV intramuscularly
The low dose group showed thymic lymphocyte depletion earlier at 14 dpi, while the high dose group presented nonsignificant depletion. By 21 dpi, both treatment groups showed significant lymphocyte depletion in the thymus (P<0.05) as compared with the control group. At 28 dpi, repopulation of the thymus had occurred in both treatment groups (Fig3). These circumstances were explained by the possibility of CAV persisting in thymic lymphocytes where they may not be affected by antibodies from 14-21 dpi. Until infected lymphocytes finally undergo apoptosis, antibodies can protect new cells from infection. This allows an efficient thymic repopulation at 28 dpi (10). The pathological effects of different CAV dosage on lymphocyte depletion are now knowledge available for future applications.

References
A novel recombinant Circovirus strain detecting in Thai dogs: Evidence for potentially possible evolution role of Circoviridae family

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Keywords: Circovirus, Dog, Genetic analysis, Recombination, Thailand

Introduction
Circoviruses are non-enveloped small circular DNA viruses belonging to the family Circoviridae. This genus harbors viruses that infect domestic and wildlife animal species, including porcine circoviruses (PCV-1 and -2), canary circovirus (CaCV) and beak and feather disease virus (BFDV) of birds (1). Next generation sequencing (NGS) has recently allowed the discovery of additional mammalian circoviruses including PCV-3 in pigs and canine circovirus-1 (CanineCV-1) in dogs that has been associated with several disease entities accompanied by manifestations like vasculitis, hemorrhages, thrombocytopenia, neutropenia and diarrhea (2). Genetic mutations and recombination are major drivers of cicovirus evolution, as several previous studies have shown genetic recombination within the Circoviridae family. However, no genetic recombination of CanineCV genomes has been previously documented. In the present study, we have molecularly characterized CanineCV strains from Thai dogs by NGS with special emphasis on the occurrence of genetic recombination.

Materials and Methods
Animal description and routine virological testing: Fresh tissue samples were collected from vital organs of three dogs with respiratory symptoms in Bangkok, Thailand, that were also subjected to routine post-mortem examination at Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. In addition, twenty nasal or oral swabs were collected from other Thai dogs showing various degrees of respiratory problems. Extracted nucleic acids from the fresh tissues and swabs were subjected to routine laboratory investigations. Common viruses of canine infectious respiratory disease complex (CIRDC) including canine influenza virus (CIV), canine parainfluenza virus (CPIV), canine distemper virus, (CDV), canine respiratory coronavirus (CRCoV), canine adenovirus type 1 and 2 (CaHV1-2) and canine herpesvirus type 1 (CaHV-1) were screened for by multiplex PCRs (3).

Sequence independent single primer amplification and NGS: Fresh lung samples of dogs were prepared for NGS using a modified sequence-independent single-primer amplification (SISPA) protocol as previously described (4). A DNA Library was constructed following the Nextera XT protocol (Illumina, USA). Samples were then deep sequenced on an IlluminaMiSeq system using MiSeq Reagent kit V3 (300x2 cycles). Raw reads were initially screened using an in-house metagenomics pipeline to identify interesting viral reads (4).

Genome sequencing and CanineCV-specific PCR: PCRs with Sanger sequencing were performed to confirm the presence of specific viral sequences. Nearly complete CanineCV genomes were obtained by PCR amplification. The genetic diversity of CanineCV's was further investigated in the lung tissue sample from other one dog and 20 respiratory swabs, using specific CanineCV PCR amplification. Primers were designed based on CanineCV strains available in GenBank, targeting 517 bp of partial capsid gene.

Sequence analysis and genomic recombination: CanineCV sequences from all positive samples were aligned using MAFFT alignment version 7 and MEGA 7. Phylogenetic trees were constructed using a cocktail of neighbor-joining methods and bootstrap analysis was performed using 1000 replicates. Recombination events of CanineCV’s TH/2016 were detected by using a range of recombination detection methods including RDP, GeneConv, Bootscan, MaxChi, Chimera, SiScan and 3Seq. Similarity plot and bootscan analysis in SIMPLOT software package version 3.5.1 were also performed on CanineCV sequences.

Molecular detection of CanineCV in canine tissues: Primers targeting the capsid gene of CanineCV were also used to assess the distribution of this virus in the dogs. To confirm the presence of CanineCV, in situ-hybridization (ISH) was performed on FFPE tissues.

Results and Discussion
No viruses had been detected using the multiplex PCR screen in the lungs and swabs. For NGS, over 900,000 trimmed reads were obtained from individual samples, of which more than 380 and 10 reads were detected with highest homology to CanineCV. Moreover, conventional PCR specific for CanineCV showed positive results in six out of the 20 respiratory swabs. In situ hybridization revealed single weakly positive cells in a lymph node and strongly positive cells within the follicle center of the tonsils from one dog (Fig. 1). All
CanineCV’s TH/2016 contained 2 main open reading frames (ORFs), which encode a putative replication associated (Rep) and capsid (Cap) proteins. Moreover, one additional ORF3 was detected. Pairwise distances revealed that the CanineCV’s TH/2016 differed by 10.5-16.2% from the USA-origin CanineCV’s UCD3-478 and Italy-origin AZ5586-13. Interestingly, most of the CanineCV’s detected in this study were most closely related to CanineCV_UCD3-478. All of the CanineCV’s-TH/2016 strains were identified as recombinants at average $p$-value = $1.42 \times 10^{-4}$. Among these viruses, CanineCV’s UCD3-478 and JZ98/2014 were identified as major and minor parents, respectively. Furthermore, bootscan analysis confirmed the disparities observed in phylogenetic analyses of CanineCV’s TH/2016. CanineCV UCD3-478 served as a parental template for the Cap gene.

In the present study samples from dogs with acute respiratory disease in Thailand were subjected to additional analyses. The detection of partial CanineCV genome sequences by NGS, PCR and ISH detection of CanineCV were positive in various organs, prompted us to perform molecular analyses of CanineCV genomes generated from the positive dogs. Here, we showed a recombination breakpoint at base positions 380-820, located in the Rep gene, which overlaps the putative ORF3 region. The Rep gene encoded by ORF1, plays a critical role in viral replication (5). Analysis of genome sequences of the novel Thai CanineCV’s identified in this study, showed the presence of ORF3, a recently recognized overlapping anti-directional region of ORF1 gene that is dispensable for virus replication (6) but has been linked to virus-induced apoptosis in PCV2. The recombination event documented in the present study adds to our understanding of the diversity of the recently recognized CanineCV’s. The identification of such genetic recombination events among CanineCV’s also supports whole genome sequencing as an alternative to phylogenetic analysis based on only single genome regions.

**Acknowledgements**

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**References**

Saliva Peptide Fingerprint Analysis of Canine Oral Squamous Cell Carcinoma by MALDI-TOF Mass Spectrometry

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Keywords: canine oral squamous cell carcinoma, peptide mass fingerprint; MALDI-TOF mass spectrometry; saliva

Introduction
Canine OSCC was the second most common oral cancer, accounting for 17 - 25% (1). Biopsy technique is used as the gold standard to evaluate the oral lesion but it is an invasive technique and not suitable for cancer screening and monitoring (2). Rapid screening test and early diagnosis can improve survival rate of dogs with OSCC after treatment (1). Saliva is selected as an alternative for diagnostic or therapeutic purposes due to its non-invasiveness and easy collection (3). Matrix absorbed laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) is a rapid technique commonly used to identify peptide mass fingerprint (PMF) in patients. MALDI-TOF MS provides high throughput, high reproducibility and high resolution (4). This is the first study to identify salivary PMF by MALDI-TOF MS for rapid screening of canine OSCC.

Materials and Methods
Six saliva samples (3 OSCC and 3 normal oral health controls) were provided in the study. The histological results were determined by pathologist. Ultraflex III TOF/TOF was used to classify PMF in a linear positive mode with a mass range of 1,000-20,000 Da. Principle component analysis (PCA), a multivariate method, was used to analyze the variance of a data set MS spectra and the PCA were analyzed by Flex Analysis and ClinPro Tool software, respectively.

Results and Discussion
PMF results showed distinct MS spectra peaks in OSCC (4,070 – 4,080, 5,180 – 5,190, 7,320 – 7,330, 10,360 - 10,370 and 14,640 - 14,650 Da) from controls (3,160 – 3,170 and 6,870 – 6,880 Da) (Fig. 1). Some individual variations were also found in this study. The PCA of OSCC samples were clustered together and separated from that of the controls (Fig 2). For the future work, selected peaks should be analyzed by MALDI-TOF MS/MS to identify protein biomarkers.

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Figure 1 Peptide mass fingerprint of saliva in OSCC and normal gingiva in range of 1,000 – 20,000 Da

Figure 2 The 3-dimensional principal component analysis (3D PCA) scatterplot of oral squamous cell carcinoma (OSCC) and control groups
Science, Chulalongkorn University and Prasu Arthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University for samples collection.

References
Tilapia Lake Virus: update and recent research progress

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Keywords: Tilapia Lake Virus, update, coinfection, tilapia

Introduction

Tilapia Lake Virus (TiLV) is an emerging viral disease in tilapia. Currently, the virus has been identified in eight countries; Israel, Ecuador, Colombia, Egypt, Taiwan, Thailand, Malaysia, and India. The virus may cause clinical infection in Nile and red hybrid tilapia with mortality rate ranging from 20 to 90%. For the diagnosis of TiLV, the molecular techniques including reverse transcriptase polymerase chain reaction (RT-PCR), nested RT-PCR, RT-quantitative PCR and in situ hybridization as well as virus isolation in cell culture have been applied for TiLV detection. Electron micrographs of TiLV particle showed that the virus contain envelope with virions at approximate size of 50-80 nm. Comparison of the whole genome sequences of Thai virus and Israel virus revealed that both viruses shared 95-99% sequence similarity [1]. Although vertical transmission has not been confirmed for TiLV, horizontal transmission via infected fish mucus has been demonstrated in the cohabitation challenge model [2]. In this study, the co-infection of TiLV and other bacteria were investigated in red tilapia rearing in an open environment.

Materials and Methods

A total of 20 red hybrid tilapia were brought for routine disease investigation. Skin scraping and gills biopsy were performed in a drop of 0.85% normal saline under light microscope for parasite investigation. Bacteria were isolated from anterior kidney using aseptic technique. The samples were streaked on trypic soy agar (TSA) and incubated at 28°C for 24-48 hr. The bacteria colonies were identified using conventional biochemical tests. Pools of liver were collected for TiLV detection using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) following the published protocol [3]. For Francisella detection, DNA was extracted from spleen, liver or anterior kidney of fish using commercial DNA extraction kit. Amplification of a specific product follow the published protocol [4].

Results and Discussion

The gross signs of moribund red tilapia include corneal opacity, skin redness and erosion around the mouth. The RT-qPCR results revealed that TiLV could be amplified in the liver of infected fish. Interestingly, co-infection with bacteria in the genus Aeromonas or Franciscella was detected in all fish.

Figure 1. (A) Gross lesions of red tilapia with clinical signs of TiLV infection. Note; corneal opacity, redness around the mouth, skin hemorrhages and erosion

Figure 2. Multiple granulomatous inflammation in the liver and pale gills appearance.
Figure 3. Bacterial isolation from anterior kidney of affected fish (n=3). The bacterial colony on TSA incubated at 28°C for 24 hrs.

For additional studies, we performed laboratory challenge of susceptible tilapia via intraperitoneal injection with isolated virus causing high mortality within 7-10 days [5]. The cohabitation of infected fish with naïve fish also led to virus infection as confirmed using RT-qPCR and virus isolation in E-11 cell culture [2]. Given to the importance of tilapia aquaculture, strategies including biosecurity and vaccine will improve the control of TiLV.

Acknowledgments

This study was financially funded by the National Research Council of Thailand (NRCT) and the Agricultural Research Development Agency (ARDA) under the Thailand Research Organizations Network (TRON) (grant number PRP6005020450).

References

Unrevealing the neglected *Strongyloides* spp. infection in cats in Bangkok, Thailand

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**Keywords:** Bangkok, Cats, Scanning electron microscopy, *Strongyloides*, Thailand

**Introduction**

Threadworms, *Strongyloides* spp., are a soil-transmitted parasitic nematode infecting a wide range of domestic animal species including human worldwide. Cats can be experimentally infected by *Strongyloides stercoralis*, a zoonotic species, and naturally infected by *S. tumefaciens, S. planiceps* and *S. felis*. Major routes of transmission for *S. stercoralis* are percutaneous and mucosal penetration via oral route whereas the mode of infection for other species in cats is not completely known. Most of cat threadworms reside in the small intestine except for *S. tumefaciens* that inhabits the large intestinenodules. Parasitic females produce larva stage 1 (L1) or partially embryonated eggs, only for *S. planiceps*, and shed in feces. Although most *Strongyloides* infections in cats seem to be asymptomatic but potential zoonotic capacity has not yet been determined. In Thailand, *Strongyloides* spp. infections in cats were previously reported as uncommon findings but these might be underestimated due to the low sensitivity of the fecal examination techniques. Also, the species of *Strongyloides* was not further explored. So, the objectives of this study were to determine the prevalence of *Strongyloides* infection in cats and to reveal the species of *Strongyloides* infecting cats in Bangkok using morphological and molecular identification.

**Materials and Methods**

The cross-sectional study was conducted in 2016 to collect samples from veterinary neutering services unit, the Center for Animal Reservoir Control, Department of Livestock Development, Bangkok. The project was approved by Chulalongkorn University Institutional Animal Care and Use Committee (Animal Use Protocol No. 1631039). A total of 327 fecal samples of clinically-healthy cats from Bangkok and vicinities were collected. Fecal samples were collected by rectum and or colon flushing using sterile normal saline. All samples were examined for the presence of parasitic stages using conventional microscopic methods including PBS-ethyl acetate centrifugal sedimentation and or ZnSO4 (specific gravity 1.18) centrifugal flotation. Larva-positive samples were cultured using modified agar plate culture method to observe development of larvae. Larvae and free-living adults were then preserved in 2.5% glutaraldehyde. Samples were subjected to cleaning, dehydration with graded series of ethanol, critical point drying and gold coating prior to observe morphology under scanning electron microscope (JEOL JSM-IT300, Japan). In addition, larvae preserved in ASL lysis buffer were subjected for PCR. Specific primers of *S. stercoralis* were manually designed by comparing 18s rDNA of several *Strongyloides* spp. using Clustal Omega program. The primers were searched for specificity using blastn program of NCBI database prior to detection of the parasite samples. Amplification of positive control (*S. stercoralis*) showed a positive band at 269 bps an expected size.

**Results and Discussion**

From a total of 327 cat fecal samples, rhabditiform larvae were found 3.1% (10/327). Measured under the light microscope, the average dimensions of larva were 243.5 ± 8.8 μm in length and 20.3 ± 6.8 μm in width (Fig. 1). From our differential diagnostic list, hookworm larva was initially excluded as fresh fecal samples were examined within 24 hours followed by lungworm due to the absence of kink tail and inconsistent dimension. Thus, larva with a straight tail and a prominent genital primodium, arrow in Fig. 1, pertained morphological characteristics compatible with *Strongyloides* spp.
A rhabditiform esophagus, straight tail and discernible genital primordium (arrow) were shown in this larva. Wet fecal smear was performed from an infected cat. Scale bar, 50 µm.

Only 6 larva-positive samples were subjected to culture using modified agar plate culture method due to insufficient mass of fresh feces. A free-living male (Fig. 2A) and female (Fig. 2B) adult of Strongyloides spp. recovered 2 days after inoculation was demonstrated. Their average length was 745.8 ± 116.1 µm and 1229.9 ± 126.2 µm, respectively. Postvulva constriction was distinct in female adults (Fig. 2B arrow).

Using scanning electron microscope, the ultrastructure of free living female adults revealed a hexagonal stoma surrounded by the circumoral elevation with six lobes at the cephalic extremity (Fig. 3A). The anus to tail distance is 77.9 ± 3.3 µm. (Fig. 3B.)

The cephalic extremity of free living male adult was hexagonal stoma surrounded by the circumoral elevation with six lobes as well as in female (Fig. 4A). At terminal end, four pairs of papillae were seen including 2 pairs at the cloaca level on the ventral side, 1 pair on ventral subterminal end of tail and 1 pair on dorsal subterminal end of tail (unilateral side was shown). One pair of spicules associated with gubernaculum was observed (Fig. 4B).

In this study, post vulva constriction was seen in free living female adult in which it was in line with the characteristics of Strongyloides felis. The result from conventional PCR using primers targeting 18S rDNA of S. stercoralis and S. procyonis was found negative hence ruling out the zoonotic species of Strongyloides stercoralis. Further PCR will be conducted to target hypervariable regions of Strongyloides in order to establish the novel DNA sequence of Strongyloides if there is no match with previous database.

To enhance the sensitivity of larva detection, fresh fecal smear, agar plate culture and Baermann techniques should be implemented as well as molecular typing of Strongyloides species. Based on previous studies in S. felis-infected cats, no evident clinical signs were observed except for some cats having acute watery diarrhea. However, in experimental infection, some infected cats had focal granulomatous, subpleural inflammatory plaque and vasculitis associated with larva migration. Although infected cats in this study seemed to be clinically healthy, they may have experienced respiratory signs at the beginning of infection. Therefore, Strongyloides should not be neglected from differential diagnosis list of feline respiratory signs. Also, further study may help address if the association of Strongyloides in cats with disseminated infection during immunosuppressive status, is possible. Strongyloides infection might be responsible for subclinical disease in cats and could be aggravated if cats are infected with retrovirus which is common infections in Thailand.

Figure 1. A rhabditiform esophagus, straight tail and discernible genital primordium (arrow) were shown in this larva. Wet fecal smear was performed from an infected cat. Scale bar, 50 µm.

Figure 2. Free-living male (A) and female (B) adult were observed under light microscope. Scale bar, 200 µm.

Figure 3. Cephalic extremity (A) and tail (B) of a free-living female adult observed under scanning electron microscope. Scale bar, 2 µm and 10 µm, respectively.

Figure 4. Cephalic extremity (A) and tail (B) with four pairs of papillae (arrow head) of free-living male adult observed under scanning electron microscope. Scale bar, 2 µm and 10 µm, respectively.
Acknowledgements
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Zeta potential of bovine X and Y sperm and its application for sperm sorting

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Keywords: Bovine, Sperm, Zeta potential

Introduction

Zeta potential (ZP) or membrane charge is the charge that develops at the interface between a solid surface (cell membrane) and its liquid medium (1). The initial study of human sperm shows that X and Y sperm are different in zeta potential (2). This phenomenon causes some sperm to adhere to surface of materials, then it is recently applied to sort mature sperm with intact DNA from immature or DNA damaged sperm in human (3) and cow (4). Although the zeta potential of human X and Y sperm has been known, the separation of human X and Y sperm based on this method has never been developed. It is well-known that sperm from each species are different, but the zeta potential measurement is previously performed only in human. The objectives of this study were to investigate whether the membrane charge of bovine X differed from Y sperm, and to apply the strategy to sort X and Y sperm.

Materials and Methods

Unsorted frozen bull semen was obtained from Daily Farming Promotion Organisation of Thailand. Sex sorted sperm was purchased from Sexing Technologies (USA). Frozen semen was thawed (37°C) and diluted in three buffers, TRIS, TALP or HEPES. Sperm concentration number was adjusted to 50,000, 100,000 or 150,000 cell/mL. Ten millilitres of sperm solution were injected into Zetasizer 3000 (Malvern, UK) to measure zeta potential. Zeta potential was measured 10 times per injection and the experiment was twice replicated. Dielectrophoretic chips were made to sort sperm according to a previous publication (5). Peristaltic pump (model Minipuls3, Gilson, USA) was used to control microfluidics and the electricity was controlled by arbitrary function generator (model AFG3021B, Tektronik, USA).

Results

The effect of buffer on zeta potential was shown in Figure 1. From three different buffers, Y sperm exhibited more negative charge than X sperm. Zeta potential of Y sperm in TALP buffer showed very higher variation (SD) than in TRIS and HEPES buffers. The difference in zeta potential of X and Y sperm tend to be higher in HEPES (90.83 mV) than in TRIS (72.42 mV). The effect of sperm concentration on zeta potential was found only in TALP buffer, particularly X sperm (Figure 2). At 50,000 cell/mL, X sperm exhibited positive charge of zeta potential while increasing cell number (> 100,000 cells/mL) the zeta potential of X sperm became negative charge. The highest difference in zeta potential between X and Y sperm was found at 150,000 cells/mL.

Figure 1 Effect of buffers on zeta potential of X and Y sperm

When using 150,000 cells the zeta potential of sperm in all buffers seem similar. The difference in zeta potential of X and Y sperm was between 72 to 90 mV (Figure 3). Then we applied this knowledge to sort X and Y sperm using dielectrophoretics and microfluidics chip (Figure 4) to trap Y sperm in the chip and permit Y sperm went through. However, we found that TRIS, TALP and HEPES buffers were not suitable to use with dielectrophoretics due to the conductivity was too high (>100 mS/m). Then we tried low-conductivity tubal fluid medium (LHTF) (40 mS/m) which can be used with sperm (6); however, this buffer could not trap any sperm due to contains BSA.
Thereafter, we used our conducting medium (8.5% sucrose, 3%glucose, 1M KCl) that previously used in our lab (5). We found that at 4V, 1KHz of electricity, and flow rate 8 µL/min, most Y sperm were trapped in the chip, and allow most X sperm to pass the chip. The percentage of Y sperm was reduced from 50% to 33%(SD=4). However, sperm viability in conducting medium (45±5%) was significantly (p<0.05) lower than LHTF (70±3).

**Discussion**

The present study revealed that the zeta potential of bovine X and Y sperm was very different. Based on the difference in membrane charge, we could use dielectrophoretic chip to separate bovine X and Y sperm. Although conducting medium was better for sorting sperm, it was toxic compared to others. An alternative buffer is required to preserve sperm viability and can be used to sort sperm.

The difference in zeta potential of human X and Y sperm from previous publication (2) seem to be lower than bovine sperm. Y human sperm have zeta potential approximately -16 mV, whereas X sperm are -20 mV (2). Based on this knowledge, two methods are already invented to isolate healthy mature human sperm called zeta method (7) and electrophoretic mobility (8). However, one report reveals that zeta method may cause altering the offspring sex ratio (9).

The difference in membrane charge of X and Y sperm may explain by the different protein composition in cell membrane between X and Y sperm as shown in previous reports (10-11).

**Acknowledgements**

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Using Mobile Diagnostic App: POAS to Support Veterinary Learning and Diagnostic Competencies

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Keywords: Mobile diagnostic application, POAS, veterinary diagnosis skill, problem oriented approach (POA)

Introduction
History taking and physical examination are the initial steps to gather information of patient’s background then combined with laboratory investigations to analyze and differentially diagnose the possible cause(s) before making a treatment plan. However, the teaching of veterinary medicine usually starts from a disease, its etiology followed by symptoms, diagnosis and treatment. Consequently, students get used to thinking in such pattern, which is the reversed sequence in the actual practice. Therefore, it may lead to some problems for instance, unable to systematize the diagnostic plan, unable to make a scope of differential diagnosis or some may not be confident to make their own diagnosis, or simply do not know where to start particularly during emergency.

In modern veterinary education, the problem based learning (PBL) method is increasingly applied in order to solve the previously mentioned problems. PBL method starts with patients’ clinical signs leading to differential diagnosis and treatment plans, which has similar thinking process to that in the actual practical situations (1-2). Recently, a number of mobile applications have also been created to support learning and skill development in medical and veterinary fields (3-5). POAS is a mobile diagnostic application, developed by Faculty of Veterinary Science, Chulalongkorn University, based on PBL concepts for differential diagnosis. It aims to assist veterinary students and newly graduated veterinarians establishing skills in systematic differential diagnosis thus enhances veterinary learning.

The present study was designed to ascertain whether using POAS application can enhance veterinary learning and diagnostic competency, and how it affects user’s thinking process while making diagnosis. Undertaking survey is the chosen method to collect users’ opinions and another respond needed for analyzing the final conclusion.

Materials and Methods
The survey was developed by studying principles of creating questionnaire, settle the questions according to the objectives of our study and gathering and analyzing opinions from veterinary students who had used POAS. The questionnaire questions were made based on POAS users’ opinions from focus-group interview. The questionnaire has 3 parts; respondents’ personal information, opinion toward POAS, and competency for making appropriate diagnosis of the symptoms in POAS. The third questionnaire has 2 sets in order to compare the results before and after using each POAS sub-applications (Anemia, Coughing, Diarrhea, Edema/effusion, Jaundice, Polyuria or polydipsia, Pruritis, Seizures, Vomiting and Weight loss). Reliability and validity of the questionnaires were verified by professors and veterinarians of Small Animal Teaching Hospital, Chulalongkorn University before distributing to the target group; 46 fifth year students of the Faculty of Veterinary Science, Chulalongkorn University, who attended the Problem-oriented approach (POA) subject course number 3107519. These students had an opportunity of using POAS during their clinical practice from October to early December 2017. They were given the first set of questionnaires in early October before clinical practice and the second set in late December after using the apps for approximately 2 months during their practice. All questionnaire responses were collected, analyzed and discussed to accomplish the conclusion of this study.

Results and Discussion
46 fifth year students of the Faculty of Veterinary science, Chulalongkorn University, completed two sets of questionnaires, before and after using POAS in order to ascertain whether using POAS can support veterinary learning and diagnostic competencies. The results show that 69.57% of students used the application via their own smartphone/tablet, while 26.09% and 15.22% of students were using the application via their own personnel computer and shared computer/tablet respectively. Majority of students reported using the application 1-2 times per week (47.83%), 39.13% used the application less than once per week and 13.04%...
used the application 3-4 times per week and the three most used application were Diarrhea application (73.91%), Vomit application (63.04%) and Anemia application (54.35%). Prior to using the apps, the most expected objective from using this application was to use this application for diagnosis in urgent time (26.09%) and the others were to develop differential diagnostic skill (22.22%), to build up more confident on making diagnosis (19.57%) and to help recalling differential diagnostic concepts (17.39%). After using the apps, the highest achieving objectives were developing diagnostic skill and increasing systematic diagnosis (52.17%), better memorizing differential diagnostic concepts (44.44%), decreasing time for making diagnosis (10.87%) and becoming more confident to diagnose unsure cases (10.87%), respectively.

In addition, 58.7% and 41.30% of students suggest that they are fairly to highly aware more about the principle of making diagnosis after using POAS, respectively, and 84.59% of the students believed that POAS is beneficial to highly beneficial to developing their diagnostic skill.

Regarding the ease of using, Weight Loss application scored the highest (3.69 out of 5), whereas PU/PD application scored the lowest (3.17 out of 5). The applications that the users believed to be the most accurate was Edema/Effusion app (4.5 out of 5) and Weight Loss app (4.5 out of 5), while the least accurate seemed to be PU/PD app (3.83 out of 5). The application that the users thought to be the most and the least beneficial for differential diagnosis were Pruritis app (4.44 out of 5) and Vomit app (3.82 out of 5), respectively (Table 1).

The results of the third part of questionnaires suggested that a number of students improve their problem-oriented approach (POA) analytical skill, which is the foundation of POAS development after using the app, to illustrate, 50% of students improve their skill after using Edema/Effusion app, 48.72% for Diarrhea app, 48.57% for Anemia app, 45.24 for Pruritis app, 42.86 for PU/PD app, 38.46% for Coughing app, 33.33% for Jaundice app, 33.33% for Weight Loss app, 31.43% for Vomiting app and 30.95 for Seizures app, respectively. The result also shows that Edema/Effusion app has the most improved overall score after using the app (4.36%).

Despite the positive results, there are some limitations of using the application such as duration and motivation of using apps as well as the access to the apps, which may affect the results of this study, especially the result from third part of questionnaires. The duration of using this application still less than it should be, 1-2 times per week in 2 months might not enough to make a great impact of developing diagnostic skill and the fact that the application is not available on iOS, which seems to be the most popular platform for most users, might make them less motivated to use the app. Nonetheless, the result of this study still suggested most of students believe that POAS is likely to be useful for developing their diagnostic skill based on PBL concept and can be their supporting tool either for their studying or working in the clinic.

Acknowledgements
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References

Table 1. Users’ opinions on POAS.

<table>
<thead>
<tr>
<th>Apps</th>
<th>Scores (out of 5)</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ease of using</td>
<td>accuracy</td>
</tr>
<tr>
<td>Anemia</td>
<td>3.42</td>
<td>4.16</td>
</tr>
<tr>
<td>Coughing</td>
<td>3.57</td>
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</tr>
<tr>
<td>Diarrhea</td>
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<td>4.21</td>
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<tr>
<td>Edema/effusion</td>
<td>3.58</td>
<td>4.5a</td>
</tr>
<tr>
<td>Jaundice</td>
<td>3.2</td>
<td>4.07</td>
</tr>
<tr>
<td>Pruritis</td>
<td>3.44</td>
<td>4.44</td>
</tr>
<tr>
<td>PU/PD</td>
<td>3.17b</td>
<td>3.83b</td>
</tr>
<tr>
<td>Seizure</td>
<td>3.67</td>
<td>4.17</td>
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<tr>
<td>Vomit</td>
<td>3.64</td>
<td>4.33</td>
</tr>
<tr>
<td>Weight loss</td>
<td>3.69a</td>
<td>4.5a</td>
</tr>
</tbody>
</table>

a: highest score among 10 applications
b: lowest score among 10 applications
Assessment of Bacterial Accumulation and Environmental Factors in Sentinel Oysters and Estuarine Water Quality from the Phang Nga Estuary Area in Thailand

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Keywords: Fecal coliform; oyster; Salmonella; Shigella; Vibrio parahaemolyticus; estuarine water

Introduction
Shellfish products have been recognized as relatively inexpensive and nutritious sources of protein, minerals and vitamins, and there is growing global demand for shellfish products given the wide recognition of their nutritional value (1). In Thailand, bivalve production has benefited from a plentiful supply of natural oyster seed, no excessive influx of fresh water, intact shorelines and an enclosed and protected growing area. The objectives of this study were to (1) measure bioaccumulation of total coliforms (TC), fecal coliforms (FC), Escherichia coli (EC), Vibrio parahaemolyticus (VP), Salmonella, and Shigella in cultured oysters and surroundings estuarine water, (2) quantify concentrations of Mn, Pb and Cd accumulated in oysters and estuarine waters from different aquaculture regions within Phang Nga Bay, and (3) build statistical models to predict the association between biological and chemical contaminants in these bivalve ecosystems and potential environmental factors influencing oyster food safety.

Materials and Methods
Oyster and estuarine water: A total of 240 samples including fresh pooled oyster meats (C. lugubris and C. belcheri) (n=144) and estuarine water (n=96) were collected from March, 2016 to February, 2017 in Thap Put district at Phang Nga Bay along the Andaman Sea from Krabi province in southern Thailand. Three pooled oyster samples along with two 500 mL samples of estuarine water were collected each month for 12 months of four different sampling locations.

Environmental parameters: Ambient air temperature (˚C), relative humidity (RH) (%), season (rainy, winte, summer), the stage of tidal condition (incoming, outgoing), precipitation (present or absent), current wind speed (m/s), maximum wind gust (m/s), and average wind speed (m/s) were recorded by an air flow meter anemometer wind meter monthly. In addition, 7-day averages were calculated for wind speed (m/s), RH (%), precipitation (mm), and ambient air temperature (˚C) by recording values every three h per day and then summarized. The data was retrieved from the Thai meteorological department.

Determination of microbiological and heavy metal contamination: TC, FC, and EC as well as VP concentrations were determined as MPN for oyster meats and estuarine waters according to US-FDA's Bacteriological Analytical Manual (BAM) with slight modifications (3,4). Salmonella and Shigella were detected in both oyster meat and estuarine water samples using the protocol described by US-FDA's BAM methods (5,6). Mn, Pb and Cd levels in oyster meat and estuarine water were quantified using Atomic Absorption Spectrophotometry (AAS: Varian model AA280FS, Agilent, USA) as described by Association of Analytical Communities (AOAC) international (7).

Statistical analyses: Negative binomial regression was used to test the association between levels of EC in oysters (MPN/g) or waters (MPN/100 mL) and daily and 7-day environmental factors: average wind speed (m/s), wind gust (m/s), air temperature (˚C), RH (%), precipitation (mm), stage of tide, season, concentrations of TC, FC and VP, presence of Salmonella or Shigella, and heavy metal levels (ppm). Univariate regression models for all independent variables were first screened for potential significance. Using the P-value for initial inclusion, a backward stepping algorithm was used to build the multivariable mixed-effects negative binomial regression model for oysters and estuarine waters, with a P-value ≤ 0.05 based on a likelihood ratio test for retention in the final model. For statistical inferences, two-sided hypothesis tests were used with a 5% significance level.

Results and Discussion
The prevalence and average mean concentrations (in parentheses) of TC, FC and EC were 99.3% (9.3×10^3),
94.4% \( (6.4 \times 10^5) \) and 93.1% \( (4.6 \times 10^5) \) MPN per g oyster meat, and 94.8% \( (4.7 \times 10^5) \), 79.2% \( (4.2 \times 10^5) \), and 78.1% \( (2.2 \times 10^5) \) MPN per 100 mL of water, respectively. Average concentrations of VP were 8.5 \( \times 10^7 \) MPN per g oyster meat and 4.5 \( \times 10^5 \) MPN per 100 mL of seawater. The prevalence of *Shigella* and *Salmonella* in the pooled oyster meat samples was 7.6% and 30.6%, respectively. In contrast, the prevalence of *Shigella* and *Salmonella* in water was 27.1% and 0%, respectively. The dominant *Salmonella* serotypes \( (n=51) \) in oysters were Paratyphi B (25%), Seremban (11.4%), and Kentucky (0.09%). Environmental factors associated with EC accumulation in oyster meat were concentration of FC \( (P<0.0001) \), 7-day average precipitation \( (P=0.002) \) and temperature \( (P=0.011) \), 24-h average relative humidity \( (P=0.001) \), and presence of *Salmonella* \( (P=0.004) \) (Table 1). In Figure 1, the predicted concentration of EC per g of cultivated oyster meat are calculated for different environmental factors.

The concentrations of Mn, Cd, and Pb in water were very low to non-measurable, while levels in the oyster meats were 3.34, 0.15 and 0.26 ppm, respectively.

**Table 1.** Mixed-effects negative binomial regression model for factors associated with the concentration of *E. coli* in oysters from Phang Nga Bay, Southern Thailand, March 2016 to February 2017

<table>
<thead>
<tr>
<th>Factors</th>
<th>Coefficient</th>
<th>95% C.I. (^a)</th>
<th>( P)-value (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>18.07</td>
<td>9.47 to 26.67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Concentration of FC (MPN/g oyster)</td>
<td>1.49 ( \times 10^4 )</td>
<td>1.28 ( \times 10^4 ) to 1.70 ( \times 10^2 )</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Average precipitation prior 7 days (mm)</td>
<td>1.28 ( \times 10^2 )</td>
<td>4.75 ( \times 10^3 ) to 2.09 ( \times 10^4 )</td>
<td>0.002</td>
</tr>
<tr>
<td>Average temperature prior 7 days (˚C)</td>
<td>-0.28</td>
<td>-0.50 to -0.065</td>
<td>0.011</td>
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<tr>
<td>Relative humidity (%)</td>
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<td>-0.065 to -0.017</td>
<td>0.001</td>
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<td>-</td>
<td>0.004</td>
</tr>
<tr>
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<td>0.068 to 0.36</td>
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</table>

\( \text{AIC}^b = 2402.58 \)

\(^a\) The 95% confidence interval (CI) and \( P\)-values were adjusted for potential intra-group correlation within four oyster sampling locations.

\(^b\) Akaike Information Criteria (AIC)

**Figure 1** Predicted average concentration of EC per gram oyster meat as a function of A: average precipitation over 7 days stratified by presence of *Salmonella* spp., B: relative humidity (%), and C: average temperature over 7 days prior to harvest

This study indicates that the designated shellfish growing area of Phang Nga Bay resulted in relatively safe levels of heavy metals, whereas bacterial contamination was very high for the cultured oysters. Based on these results, we recommend continued monitoring for bacterial contamination and adequate thermal exposure during cooking of oysters to enhance the microbiological safety of these popular shellfish. Increasing sanitary practices, consumption of adequately cooked seafood, avoiding harvesting oyster during heavy precipitation, and applying a rapid early warning system based on these significant environment parameters could function to reduce the risk of bacterial contamination of oysters and enhance the food safety of Thailand seafood.

**Acknowledgements**

This research was supported by the Rachadaphiseksomphot Endowment Fund Chulalongkorn University: Project RGN_2559_038_02_31. The authors would like to thank Somporn Sarakarn for coordination of field sampling and logistics. We also thank to Chilai Kuwatrananukul and Winn Khant for laboratory assistance.
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Blue light-emitting diode can inhibit scuticociliate (*Miamiensis avidus*) in olive flounder, *Paralichthys olivaceus*

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**Keywords**: scuticociliate, LED, photo-inactivation, apoptosis

**Introduction**

Scuticociliates are facultative and histophagous parasites. They usually cause catastrophic biological consequences with high mortality and tremendous economic losses to fish farming industry worldwide. In particular, *Miamiensis avidus*, *Philasterides dicontrarchi*, *Pseudocohnilembus persalinus*, and *Uronema nigricans* are important pathogenic species causing devastating scuticociliatosis in olive flounder (*Paralichthys olivaceus*) (1-3). Several antiprotozoal compounds such as formalin, doxycycline and hydrogen peroxide (H$_2$O$_2$) have been reported as therapeutic agents against scuticociliatosis in farmed fish (4-6). Despite their effectiveness, these chemotherapeutic agents should be controlled thoroughly or restricted to preserve the natural environment and reduce emergence of antibiotic resistance of microbes. In recent years, blue light (400-500 nm) is gaining great attention due to its anti-microbial effects. Blue light have been reported that it does not cause side effect with high antimicrobial activity (7-9). The aim of this study was to determine the antiprotozoal activity of blue light against *M. avidus* in vitro and scuticociliatosis in olive flounder in vivo.

**Materials and Methods**

$10^5$ cells ml$^{-1}$ of *M. avidus* were exposed to 250μ·mol·m$^{-2}$·s$^{-1}$ of 405 nm or 516 μ·mol·m$^{-2}$·s$^{-1}$ of 465nm, respectively. At several hours after exposure (3, 6, 12, 24 and 48hr), ten-microliter suspensions were stained by trypan blue and viable cells were counted. Furthermore, in order to verify the death mechanism (e.g., apoptosis, necrosis) by blue light, blue light exposed ciliates were stained by propidium iodide (PI) and Alexa Fluor® 647 conjugated Annexin V and monitoring both fluorescents using flow cytometry with in a hour.

Two replicated in vivo experiments (Exp.1, Exp.2) were independently performed. Ten and twelve olive flounder (*Paralichthys olivaceus*, average weight = 28.7 ± 6.8 g) were challenged by $10^3$ ciliates based on immersion methods. Subsequently, challenged flounder were moved into each fish tank (70L; 35x50x45 cm$^3$) containing 35 L of water (salinity of 11-12 psu) and promptly exposed to ambient light, 405, or 465 nm LED under a photoperiod of 12D:12L. During the experiment, water temperature was maintained at 20°C. LED was located above the water tank. Emitting intensities of the 405 and 465 nm LED were 198 and 369 μ·mol·m$^{-2}$·s$^{-1}$, respectively, reaching light flux on water surface of approximately 63 and 101 μ·mol·m$^{-2}$·s$^{-1}$, respectively.

**Results and Discussion**

Results of survival of *M. avidus* rate with different wavelengths and exposure time and count with different dose are shown in Fig. 1(A) and Fig. 1(B).

![Fig. 1](image-url) Percent of survival rate at 3, 6, 12, 24 and 48 hour post exposures (A); Survived *M. avidus* count with different dose and wavelength (B) *, P<0.05, **, P<0.01, ***, P<0.001.

Results of flow cytometry analysis are presented in Fig. 3. The lower left quadrant was regarded as living ciliate cells shown as black dots in scattergrams. The rest of the quadrant indicated dead ciliates. The upper and lower right quadrant represented population of non-viable ciliate cells (necrotic cells) and apoptotic cells (stained with only PI and Alexa Fluor® 647 Annexin V), respectively.
In Exp. 1, accumulated mortalities of groups exposed to ambient light, 405 nm LED, and 465 nm LED were 100%, 30%, and 70%, respectively (Fig. 3A). Relative percent survival (RPS) rates in 405 nm and 465 nm LED exposure groups were 70% and 30%, respectively. In Exp. 2, accumulated mortalities of groups exposed to ambient light, 465 nm LED, and 405 nm LED were 100%, 67%, and 25% respectively. RPS of 405 nm LED group was 62.5%, similar to the RPS (70%) of this group in Exp. 1 (Fig. 3B).

To the best of our knowledge, this is the first study that demonstrates that 405 and 465 nm LED illumination can inactivate *M. avidus* by inducing apoptosis. Such LED illumination can also reduce mortality of olive flounder due to scuticociliatosis without causing severe adverse effects on olive flounder. Results of this study strongly indicate that short wavelength blue LED light can be applied to future intensive fish farming system to control scuticociliatosis, a contagious and notorious disease, thus increasing productivity.

**Acknowledgments**

This research was a part of the project titled ‘LED-Marine Technology Convergence R&D Center’ funded by the Ministry of Oceans and Fisheries, Korea.

**References**

Detection of myxozoans parasite *Henneguya* spp. in gills and mucus of cultured giant gourami (*Ospronecus goramy*)

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**Keywords:** *Henneguya* spp., giant gourami, gills, skin mucus

**Introduction**

Giant gourami (*Osphronemus goramy*) is an important omnivorous freshwater and cultured fish in Thailand. They are native to Chao Phraya and Mekong basins in Southeast Asia (1). Although giant gourami is easily to grow in different culture condition, several diseases including bacteria and parasite infections have been occasionally reported. Myxozoans are commonly protozoa parasites found on wild and farm-raised fish. Among different Myxozoan parasites, the parasites in genus *Henneguya* with more than 200 species have been reported to affect various fish species. For example, *Henneguya* spp. could be isolated from marine and fresh water fish (2). The parasite could be detected in the gills, fins, liver, kidney, dermis, gall bladder and peritoneum (3). However, little study shows the persistence of this parasite on fish mucus. In the present work, we recorded in which a *Henneguya* spp. was found in the skin mucus of the giant gourami (*Ospronecus goramy*).

**Materials and Methods**

Lived giant gourami (*Ospronecus goramy*) collected from Ratchaburi province (Fig. 1A) were processed for disease surveillance at fish laboratory, Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkhen campus, Bangkok. Some fish showed clinical signs of lethargy, loss of appetite, and swimming at water surface, skin darkness and redness (Fig. 1B). Severely moribund fish were euthanized using an overdose of eugenol solution (Aquanes, Better Pharma, Thailand). For parasitic investigation, gills filaments were excised by a scissor and skin mucus was scraped using a thick cover glass and placed on a clean glass microscope slide in a drop of 0.85% normal saline. Microscope slides were examined for external parasites under optical microscope with objective lens magnification 10x, 40x and 100x. Photomicrographs of myxospores and plasmodium were detected in the samples.

**Results and Discussion**

Under light microscope, the lanceolate-shaped myxospores were detected in giant gourami mucus. The spore body of parasite was 7 to 10 µm in length and 15 to 20 µm in width (Fig. 2). The plasmodia stage presented on the gills filaments (Fig. 3A, B). Morphological analysis indicated that the observed parasite shared similar morphology to myxozoans parasite in the genus *Henneguya* spp.

![Figure 1. (A) Giant gourami were collected from Ratchaburi province (Western, Thailand). (B, C) Gross signs of moribund fish including skin darkness and redness.](image1)

![Figure 2. Morphology of mature spores of *Henneguya* spp. presented in giant gourami mucus. bar = 10 µm](image2)
Figure 3. (A, B) The oval shape external parasites (plasmodia stage) of *Henneguya* spp. were observed in the gills filaments of giant gourami. bar = 100 μm

Previous studies reported that *Henneguya* spp. could be detected in multiple organs of various kinds of fish including gills, heart, gastrointestinal tract, kidney and liver (4,5). Furthermore, mature spores or cyst of *Henneguya* spp. have been found in skin, gill filaments and muscle (6). However, no reports of *Henneguya* spp. detection in the fish skin mucus especially in giant gourami. Thus, our study revealed that *Henneguya* spp. could be detected in giant gourami mucus which indicated the route of parasite spreading. This study demonstrated a harm of *Henneguya* spp. since *Henneguya* infection could lead to deformity and gill necrosis causing respiratory distress and respiratory failure. (7). Moreover, *Henneguya* spp. has the ability to produce several proteolytic enzymes that result in cyst formation in skin and muscle of infected fish. Up to date, no effective treatment has yet been reported for this parasite (8). However, reduction of stress and improvement of water quality will reduce the impact of this parasite infestation.

Acknowledgments

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References

Evidence of Anisakiasis in a Stranded Striped Dolphin (Stenellacoeruleoalba) in Thai Territorial Water

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Keywords: Anisakis spp., Strand, Striped dolphin, Gastritis

Introduction
Anisakid nematode is an aquatic parasite that required both invertebrate and vertebrate hosts to complete their life cycle (1,2). Anisakis, Pseudoterranova and Contracaecum are the most common anisakid nematode that infect the paratenic host (1,2). Recently, anisakiasis caused by consumption of raw or undercooked fish or squid that contained anisakid larvae in muscle or edible parts, was a public health concern in many parts of the world (1,3,4). Marine mammals, especially dolphins, are considered as definitive hosts for anisakid (1). Anisakis spp. can induce gastric lesions by various stages of parasite (5). In Thailand, human anisakiasis and the presence of anisakid nematode in fish had been reported (6). However, anisakid nematode had not yet been described in dolphins in Thailand. This is the first report of the presence of anisakid nematode in a stranded striped dolphin in Thailand.

Materials and Methods
Case history: An approximately 25-year-old, intact male, striped dolphin (S. coeruleoalba) was stranded on the western coast of Phuket island, Thailand. The dolphin was rescued and transferred to Phuket Marine Biological Center for medical treatment. Physical examination revealed scratches on the body surface, weakness, halitosis, swimming disability, and convulsion. Despite the supportive treatment, the dolphin died within 24 hours after the rescue.

Pathological examination: A complete necropsy was performed. The selected tissues were collected and fixed in 10% neutral buffered formaldehyde, for histopathological examination.

Parasite identification: The adult worms were fixed in 10% neutral buffered formaldehyde for parasite identification.

Results and Discussion
Gross findings: The dolphin had ulcerations on the tongue. Pneumonia was noticed grossly. Multiple abscess-like structures were observed in the pancreas, spleen, mesenteric lymph node and reproductive organ. Interestingly, a myriad of adult nematode (size ~ 4 cm length) was seen in the forestomach (Fig. 1). An adult worm was morphologically identified as an Anisakid nematode. The fundic stomach revealed a cluster of anisakid larvae (Fig. 2) that were embedded in the mucosa (Fig. 2). The gastric mucosa was also congested and covered by the mucus.

Microscopic findings: Suppurative inflammation was recognized in the spleen and lymph node. Pancreatitis accompanied with intra-lesional trematode eggs was seen. Multiple parasitic granulomas were seen in the lung. There were nematode larvae embedded in the gastric mucosa. The larvae were surrounded by bright eosinophilic substance (consistent with Splendore-Hoepli reaction) and necrosis of the adjacent tissue. Lymphocytes and eosinophils predominantly infiltrated the area with occasional neutrophils and macrophages (Fig. 3). The cross section of the larvae was compatible with an anisakid larva as previously described (4). The larva was round, size ~ 300 x 400 um, with thin cuticle. The hypodermal muscle was separated into 4 parts by the lateral cord that showed two wing-like distal lobes(Fig. 4). The excretory cells were banana-shaped, located beneath the esophagus (Fig. 4).

This recent study reports the presence of anisakid parasite in a striped dolphin in Thailand. Anisakid nematode is commonly found in the Pacific and Atlantic oceans (1,2), which have cold temperature. However, the presence of anisakid nematode in dolphins in the Indian ocean indicates that this nematode can also survive in a warmer climate. Anisakid nematode was also detected in fish in Thailand (6). This recent case revealed gastric lesions with intra-lesional nematodes, similar to a previous study (5). Further investigation and public health warning of anisakid nematode should be considered in Thailand.
Figure 1. A myriad of adult Anisakis spp. harbored in the forestomach. Inset (lower panel), the adult nematode under light microscope.

Figure 2. The fundic stomach revealed catarrhal gastritis with embedded anisakid larvae. Inset, the cross section demonstrated the embedded larvae in the gastric mucosa (arrow).

Figure 3. The gastric mucosa was necrotized (N) by embedded larvae (L). Lymphocytes, eosinophils, neutrophils and Splendore-Hoeppli reaction (S) were seen around the larvae. H&E, bar 250 μm.

Figure 4. The larva was round-shaped with thin cuticle. The hypodermal musculator was separated with the lateral cord with two wing-like distal lobes (thick arrow). Gastrointestinal tract (GI) was lined with columnar cells. Excretory cell (thin arrow) had a banana-shape appearance. H&E, bar 100 μm.

Acknowledgements
The authors are grateful to Mrs P.Punyathi for histological technique.

References
Immersion vaccination of an inactivated whole-cell vaccine coated with chitosan against Flavobacterium columnare challenge in red tilapias (Oreochromis sp.)

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Keywords: vaccine, formalin-killed, Flavobacterium columnare, red tilapia

Introduction

Tilapia (Oreochromis sp.) is a great source of high-quality protein worldwide, recognizing as a main culture species of freshwater aquaculture in Thailand (1, 2). However, bacterial infection caused by Flavobacterium columnare, the causative agent of columnaris disease, is now concerning as one of the most serious infectious diseases in farm tilapias. This bacterium affects both cultured and wild freshwater fish worldwide. F. columnare is gram negative, rod and slender filamentous bacterium with gliding motility and yellow rhizoid colony formation (4, 5). 

Materials and Methods:

Bacteria and vaccine preparation

F. columnare CUVET 1214 was isolated from gill of diseased tilapias at Phetchaburi province (5). Bacterial cultures used for vaccine preparation, antigen preparation, and challenges were grown in 100 to 1000 ml batches in Tryptone Yeast Extract Salt Medium: TYES Broth (pH7.2) and incubated at 25-28 ºC for 48 h. Final concentrations were approximately by log colony forming units (CFU) ml/L (8). For vaccine preparation, Bacterial cells were harvested by centrifugation at 3,000g at 4ºC for 40 min. The cells were resuspended in phosphate-buffered saline (PBS) with 0.2% formalin and incubated at 4ºC for 20 h. The formalin-killed bacteria were washed three times by centrifugation and resuspended in PBS to 10⁷ cfu/mL. For formalin killed and coated in chitosan vaccine (FKC vaccine) preparation, Chitosan 22 kDa was dissolved in acidic solution. Bacterins were encapsulated by the addition chitosan 1% to formalin killed vaccine 1:1 (v/v).

Fish challenge

Fingerling tilapias (10 g) were divided into 3 groups; control, formalin-killed, and formalin-killed with chitosan groups (15 fish each) with 2 replicates. Fish were immersed with formalin killed bacteria with 10⁷ CFU/mL for 30 mins. At 30 and 60 days after immersion vaccination, Fish were challenged with 1x10⁶ CFU/mL lethal concentration of a virulent strain of F. columnare for 1 h. Cumulative mortality and survival rate were recorded for 14 days after immersion challenge. Relative percent survival (RPS) was calculated as 1 - (mortality rate of vaccinated fish/mortality rate of control fish) (9).

Results and Discussion

The cumulative mortality after 14 days post challenge was 90 and 83 % (30 and 60 days after vaccinated fish) in control fish which was significantly higher than the cumulative mortality of 17% and 30% (30 and 60 days after vaccinated fish) in vaccinated fish respectively (Fig.1)
Figure 1. The cumulative mortality of control and vaccinated groups after challenge with 1x10^6 CFU/mL F. columnare

Formalin-killed bacteria has been widely used as antigens for fish vaccination. In this study, we prepared the vaccine with a high virulent strain of F. columnare (5), high concentration (10^7) and long-time immersion. Bacteria in vaccine were killed by formalin with a short time and low concentration, and carefully washed 3-times to remove the formalin as quick as possible. The result revealed an immersion exposure of formalin killed F. columnare coated with chitosan was capable to induce a high-level of protection. It might be noted that the high antigen amount in the vaccine preparation influenced the high antigenic uptake by an immersion route. Inactivation of the bacteria by short time exposure and low concentration of formalin might impact the antigenic properties of F. Columnare by the protein cross-linking formation (7,8). Chitosan has been extensively investigated for its immunogenic activities, especially via the mucosal routes. The main mechanism of chitosan mucoadhesion appears to be electrostatic interaction between the positively charged polymer and negatively charged materials such as mucus and cell surface (9,10). Chitosan in FKC increases the contact time with the mucosa thereby increasing the potential of enhancing antigen uptake by the Antigen presenting cell.

In conclusion, inactivated F. Columnare encapsulated in chitosan immersion vaccine can protect red tilapia fingerlings from columnaris disease with a high RPS. However, the vaccine has short protection time (30-60 days) and the immune response of fingerling red tilapia after vaccination should be further focused.

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Nano Spray TRISWHEAT (Teripang Super Wound Healing Agent) Healing Wound Diabetes Mellitus which is Infected by MRSA (Methicillin Resistant Staphylococcus aureus) Bacteria with Sea Cucumber (Stichopus sp.) Extract

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Keyword : sea cucumbers, diabetes melitus, nano spray, bacteria

Introduction
One of the degenerative which can be a big problem in Indonesia is diabetic mellitus (DM), a disease with a symptom of the height of the glucose followed by carbohydrate metabolism disorder, lipid and protein due to the discharge of insulin activity which is followed or unfollowed by insulin resistance (1). Problem appears when the second type of DM patient especially gets wound.

The wound in diabetic is the wound which happen in the diabetic patient which involve peripheral neuron and autonomic disorder (2). In the wound healing process, there are 3 phase: inflammation phase, proliferacy phase and maturation phase (3).

To resolve, there have been medicines in market in oral service (tablet, capsule, and syrup), or topical (cream or ointment). Besides, it is not available yet service in spray which can increase the comforts of the users.

To take the advantage of the potency from one of the sea biota, Sea Cucumber (Stichopus sp.), it’s used as a wound healing for the second type of DM patient. Sea cucumber has many uses in medicine; one of the uses is for wound healing. Sea cucumber has some contents which is usable in wound healing, such as fatty acid, triterpene glycoside, chondroitin sulfate, glycosaminoglycan, (GAG), and flavonoid (4).

The purpose of research is prove nano spray extract sea cucumber capable of heals the wound in people with DM type 2 and was detected concentration steady. So obtained an easy preparation applied and achievable for heals the wound.

Materials and Methods
Sea cucumber extraction: Sea cucumber cut into some part, while digestive organ as the large intestine that contains impurities and sand disposed. Sea cucumber weighed and extracted by means of maceration. Filtrat obtained put into squash steam then volatilized by using vaccum rotary evaporators at a temperature 40 degrees centigrade with speed 900 rpm to thickened to extract 100 %

Preparation of Bacteria Methicillin Resistant Staphylococcus aureus (MRSA): Methichilin Resistant Staphylococcus aureus comes from the culture which is planted on Plat Agar Darah (PAD) or blood agar plate agar base (Oxoid, Germany). The bacteria then planted on Todd Hewitt Broth (THB) (Pronadisa, Spain) for 18-24 hours, after being vortexed, the bacteria culture centrifuged with the speed of 3000 rpm for 10 minutes. Pellets washed with 10 ml of Phospate Buffered Saline (PBS), resuspension done then centrifuged with the speed of 3,000 rpm for 10 minutes. Pellets added as much as 2 ml PBS and the value of Optical Density (OD) determined with Spectrophotometer with 10% absorbency on λ 620 nm so it can be gotten solution as much as 108 cells of bacteria. Suspension used on the wound infection is 10 ul 10^8 bacteria cells in PBS (5).

Dilution Examination: Principally, it is antibiotic concentration dilution series. It can be used for determining MIC (Minimum Inhibition Concentration) of an antibiotic. An antibiotic dilution series inoculated in a in a tube contents liquid media and inoculated with examiner bacteria then observed the turbidity level or the growth. The highest dilution from the clear liquid media is stated as MIC, while the clear tube is scratch inoculated on plate agar media, incubated and observed the availability of colony growth on the agar plate media surface (6).

Nano Spray Making: Sea cucumber as the result of extraction made into the shape Nano emulsion oil type in water or o/w. sea cucumber extract mixed with paraffin oil and Polisorbate 80. In the other side, it is made the mixture of glycerin and methyl paraben. Then the mixture of glycerin methyl paraben poured into the mixture of sea cucumber extract. Mixing and magnetic stirrer is done and continued with sonification and ultrasonic.

In Vivo Examination: The trial animal used is male Wistar Mouse with 100-160 gr weight. In the first 1 week, the mouse is adapted in the trial animal laboratories, faculty of Veterinary UGM. Then the mouse is fasted for 12 hours and inducted with diabetic with Streptozotocin (STZ) with dose of 55 mg/KgBB.
intramuscularly. STZ diluted with buffer solution sodium citrate, pH 4, after 3 days mouse get its glucose checked with glucose test. The mouse which has glucose level above 200 mg/dl used in this research. After it is stated as a diabetic, mouse is fasted for 12 hours. Mouse then weighed to determine the most correct dose. Mouse anesthetized, its back hurt incisional with punch biopsy with diameter of 0.8 cm. afterward, its infected with MRSA bacteria (Methicillin Resistant Staphylococcus aureus ) which is injected into the wound by means of intradermal. Mouse then grouped according to the treatment they are 6 control (-), 6 sea cucumber extract nano spray and 6 control (+). The wound is cured every day for 14 days according to the treatment group.

Histopathology preparation making : Histopathology preparation making is made from skin texture, routine necropsy result day of 3, 7 and 14. Texture fixed with formalin 10%, the proceeded and embedded with paraffin, sliced as thick as 4 micron, and colored with hematoxylin Eosin (HE). Preparation is checked under a microscope.

General Discussion

Result of Dilution Test
Sea cucumber extract dilution test results with multilevel concentration ranging from 1% to 80% with multiples of 10

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Bacteria MRSA Tube 1</th>
<th>Bakteri MRSA Tube 2</th>
<th>Explanation</th>
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<td>1</td>
<td>+</td>
<td>+</td>
<td>Growing</td>
</tr>
<tr>
<td>10</td>
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<tr>
<td>80</td>
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<td>Not Growing</td>
</tr>
</tbody>
</table>

Dilution examination result show on Sea cucumber extract 40% is already effective used as MIC (Minimum Inhibition Concentration).

3.2 Induction of Diabetes in Rats (In vivo)
The results of the induction of diabetes with STZ in mice showed that all mice have blood sugar levels above 200 mg/dl that showed that rats suffered diabetes.

3.3 Histopathology Analysis
The following image analysis of histopathology of the skin of mice of diabetes mellitus that is infected with MRSA.

<table>
<thead>
<tr>
<th>Table 2 The identification histopathology the skin rat with a scaled up a microscope ( 4x10 )</th>
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</thead>
<tbody>
<tr>
<td>Negative control (K-) no medicine given</td>
</tr>
<tr>
<td>Sea Cucumber Extract Nano Spray (T)</td>
</tr>
<tr>
<td>Penicillin Nano Spray (P)</td>
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</table>

On the preparation of Histopathology control seen there are so many leucocytes which show inflammation, epithelization grow imperfectly, content some MRSA bacteria colony. On the treatment 1 or T (Teripang; Sea Cucumber) lymphocytes shown rare and it doesn’t show inflammation. On the treatment 2 or P (Penicillin) epithelization doesn’t grow well, on that preparation show leucocytes in the large amount, this marks that there is inflammation. Besides, it’s also done the observation of wound diameter macroscopically.
On table 2 we can see that the wound with Control; Kontrol (K) treatment isn’t healed. On the Sea cucumber extract nano spray (T) the wound diameter gets smaller and healed in the day of 14. On the Penicillin Nano spray (P) the wound gets smaller but it doesn’t cover as well as on the Sea cucumber extract nano spray (T).

**Table 3** The development of the health of in macroscopic

<table>
<thead>
<tr>
<th>Treatment</th>
<th>K</th>
<th>T</th>
<th>P</th>
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</thead>
<tbody>
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<td>Days-3</td>
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<tr>
<td>Days-7</td>
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<td></td>
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<tr>
<td>Days-14</td>
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</table>

**Note**: Control; Kontrol (K) no medicine given, Sea Cucumber Extract Nano Spray (T), Penicillin Nano Spray (P)

On table 2 we can see that the wound with Control; Kontrol (K) treatment isn’t healed. On the Sea cucumber extract nano spray (T) the wound diameter gets smaller and healed in the day of 14. On the Penicillin Nano spray (P) the wound gets smaller but it doesn’t cover as well as on the Sea cucumber extract nano spray (T).

**Conclusions**

According the the explanation of analysis result can be seen that Nano spray TRISWHEAT (Teripang Super Wound Healing Agent) made from sea cucumber extract with 40% concentration can heal the diabetic mellitus wound which is infected by bacteria (Methicillin Resistant Staphylococcus Aureus) more effective than penicillin nano spray within 14 days treatment.

**Acknowledgements**

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**References**

Occurrence of antimicrobial resistance in *Vibrio parahaemolyticus* isolated from cultivated oysters and estuarine waters

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**Keywords:** Antimicrobial resistance, estuarine water, oyster, *Vibrio parahaemolyticus*, virulence genes

**Introduction**

*Vibrio parahaemolyticus* is an important seafood-borne pathogen causing gastroenteritis in humans. This pathogen is associated with the consumption of raw or undercooked seafood products (1). Thermostable direct hemolysin (*tdh*) and TDH-related hemolysin (*trh*) have been considered as important virulent factors leading gastroenteritis in human cases (2). In Thailand, *V. parahaemolyticus* infection occurred at the highest rate up to 78% of food poisoning cases associated foodborne disease outbreaks (3). Quinolones, tetracycline, cephalosporin, cefotaxime, ceftazidime, and penicillin are antimicrobial drugs of choice for *Vibrio* treatment in human infection, and these antimicrobial agents are considered to be highly susceptible to many clinical cases (4). Over the past few decades, antimicrobial resistance (AMR) has been recognized as an important, serious and urgent health threat, and multidrug-resistant strains have disseminated into the environment due to the imprudent use of antimicrobials in human medicine, veterinary medicine, community and aquaculture settings. The objectives of this study were to determine the occurrence of antimicrobial resistance and to detect the virulence genes of *V. parahaemolyticus* isolated from cultivated oysters and estuarine waters during one year period in Southern Thailand.

**Materials and Methods**

A total of 594 environmental *V. parahaemolyticus* isolates from pooled cultivated oysters (n=361) and estuarine waters (n=233) were received from stored isolates. Samples were collected monthly between April 2016 and March 2017 from Thup Pud district, Phang Nga province in Southern Thailand. Vibrios were isolated according to the US-FDA's BAM method (5). Minimum Inhibitory Concentrations (MICs) of erythromycin, sulfamethoxazole, trimethoprim, ampicillin, streptomycin, tetracycline, chloramphenicol, and ciprofloxacin were determined using a serial two-fold agar dilution method according to the Clinical and Laboratory Standard Institute (CLSI, formerly NCCLS) guidelines (6).

*V. parahaemolyticus* isolates were phenotypically tested on Kanagawa phenomenon for the presence of thermostable direct hemolysin (*tdh*) and TDH-related hemolysin (*trh*) (7). The isolates were grown on Wagatsuma agar containing 2% washed sheep erythrocytes. After overnight incubation, strains producing a clear hemolytic zone around the colonies were identified as Kanagawa phenomenon-positive and isolates without hemolytic zone were identified as Kanagawa phenomenon-negative.

For statistical analysis, independent sample-test (SPSS, version 22.0) was conducted to compare the mean difference of particular antimicrobial resistance rate between oysters and estuarine water. Statistical testing was performed under two-sided hypothesis with *P*<0.05.

**Results and Discussion**

*V. parahaemolyticus* isolates from pooled oysters were resistant to erythromycin 53.7% (194/361) followed by sulfamethoxazole 33.5% (121/361), trimethoprim 27.4% (99/361), and ampicillin 10.2% (37/361), respectively. The resistant rates to antimicrobial drugs of vibrios in estuarine waters were found in erythromycin 54.9% (128/233), sulfamethoxazole 36.5% (85/233), trimethoprim 28.8% (67/233), and ampicillin 12.4% (29/233). Only 1.4% (5/361) of *Vibrio* isolates from oysters were resistant to streptomycin and 1.3% (3/233) of *Vibrio* isolates from estuarine waters were resistant to tetracycline. No chloramphenicol and ciprofloxacin resistant were detected in all oyster and estuarine water isolates. Seventy-four percent (441/594) of all isolates were resistant to at least one antimicrobial agent. Furthermore, multidrug-resistant bacteria was found in 13.8% (82/594) which was resistant to at least three classes of antimicrobial agents. Although totally 20 different resistance patterns for both oysters and estuarine water were observed, the individual resistance patterns were very similar as shown in Figure 1.
Many classes of antimicrobial agents were previously detected in the environmental samples such as sewage, effluents, surface water, and wastewater treatment plants (8). The resistance revealed that the sewage management system plays an important role in the occurrence of AMR in the environment that can impact on oysters and estuarine water quality. A previous study reported that the virulence strains of *V. parahaemolyticus* were commonly found higher in clinical samples than environmental isolates (9). In this study, the presence of *tdh* and *trh* were not found, since all isolates were derived from the environmental samples which are less likely to observe those virulence genes as the clinical cases. Monthly distribution of AMR in erythromycin, sulfamethoxazole, trimethoprim, ampicillin, streptomycin, and tetracycline were observed (Fig 2).

The results of independent-samples t-test on each antimicrobial agents compared between oyster and estuarine waters showed no significant difference \((P>0.05)\). These results revealed that AMR patterns are equally distributed throughout the year. These findings demonstrated that estuarine waters can be used as representative observing AMR pattern in oysters to reduce the cost of sampling on fresh cultivated oysters in AMR monitoring and surveillance system.

**Conclusions**

In this study, AMR of *V. parahaemolyticus* was observed in both oyster and estuarine water isolates. Although the virulence genes *tdh* and *trh* were not detected, it does not mean that the oysters are safe for consumption. This demonstrated that AMR strains of *V. parahaemolyticus* have been disseminated through the environment. For a further study on AMR, estuarine waters could be used as representative of the environmental samples for AMR monitoring and surveillance of oyster to enhance the effectiveness of sampling and logistics. Therefore, restrictive policies on the use of antimicrobials in human medicine, veterinary medicine, and aquaculture together with good practices on wastewater management are mandatory to minimize the dissemination of AMR in the environment. Increasing of sanitary practices and consuming of adequate cooked oysters are also recommended to promote seafood safety and to reduce the risk of seafood-borne illnesses.

**Acknowledgements**

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**References**

Prebiotic effects of Jerusalem artichoke supplemented diet on growth performance and intestinal morphology in juvenilered tilapias (Oreochromis sp.)

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Keywords: Jerusalem artichoke, Intestinal villi, Growth performance, Juvenilered tilapia

Introduction

Red tilapia(Oreochromis sp.) is an important cultured freshwater fish in Thailand. Red tilapia farming in the country is done in extensive, semi-intensive and intensive culture systems in ponds, pens, and cages. To sustainable and eco-friendly red tilapia farm management and production of safe aquaculture foods, several methods including prebiotic supplementation have been focused (1). Prebiotics are defined as non-digestible ingredients which beneficially affect the host health by selectively stimulating the growth and/or activity of the bacterial microflora (2). Jerusalem artichoke (JA) or Kantawan is one of the most common prebiotics together with inulin and fructo-oligosaccharides (3). It has been reported that the JA had beneficial effects on the growth performance, disease resistance and immune parameters of Nile tilapia (Oreochromis niloticus)(4). However, there is no information about the effect of the prebiotic from Jerusalem artichoke in the growth performance and intestinal morphology of red tilapia. Therefore, the objective of this study was to assess the effect of the JA in the growth performance and intestinal morphology in juvenile red tilapia.

Materials and Methods

Experimental diets: The three experimental diets were: 1, basal diet (control, C); 2, 5.0 g kg⁻¹ kantawan-supplemented diet (5K); 3, 10.0 g kg⁻¹ kantawan-supplemented diet (10K). Thirty male red tilapia (10–15 g body weight) were distributed into 1000-litre tanks containing water under continuous aeration. Air and water temperatures were measured and were 25–33ºC and 25–28ºC, respectively. Dissolved oxygen (DO) content and pH values were within acceptable ranges of 5.24–5.98 mg L⁻¹ and 7.48–8.16, respectively. Fish were fed the experimental diets for 4 weeks.

Histology: At the end of the feeding period, three parts of the intestines, the foregut, midgut and hindgut from fish of experimental diets were collected and fixed in neutral buffered 10% formalin. After that, tissues were processed routinely, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). The villous height and width were measured to calculate the absorptive area using the formula: absorptive area = villous height x villous width (5).

Growth performance: Final weight, weight gain (WG%), specific growth rate (SGR), and feed conversion ratio (FCR) were calculated according to standard formulae.

WG (%) = 100 x (final mean body weight-initial mean body weight) / initial mean body weight
SGR = [(ln (final body weight) – ln (initial body weight) / days)] x 100
FCR = feed intake (g) / Weight gain ADG = (gain %)/(number of days)

Statistical analysis: Results were analyzed by one-way analysis of variance (ANOVA) using SPSS version 22 software for Windows (SPSS Inc., Chicago, USA) and the significance of the differences between means was tested by Tukey’s test. Differences were considered significant when P<0.05.

Table 1 Growth performance and feed utilization of red tilapia fed with the experimental diets: 1, basal diet (control, C); 2, 5.0 g kg⁻¹ kantawan-supplemented diet (5K); 3, 10.0 g kg⁻¹ kantawan-supplemented diet (10K) during 4 weeks.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>5K</th>
<th>10K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>14.68±3.26</td>
<td>13.68±5.15</td>
<td>13.88±3.61</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>24.52±5.67</td>
<td>28.76±5.37</td>
<td>27.73±6.18</td>
</tr>
<tr>
<td>WG (%)</td>
<td>67.03±7.01</td>
<td>125.70±45.46</td>
<td>106.38±13.79</td>
</tr>
<tr>
<td>FCR g d⁻¹</td>
<td>2.32±0.55</td>
<td>1.36±0.11</td>
<td>1.61±0.35</td>
</tr>
<tr>
<td>SGR (%)</td>
<td>1.76±0.14</td>
<td>2.74±0.70</td>
<td>2.49±0.23</td>
</tr>
<tr>
<td>ADG (% day⁻¹)</td>
<td>2.31±0.24</td>
<td>4.34±1.57</td>
<td>3.67±0.48</td>
</tr>
</tbody>
</table>
Values represent Mean±SD of 25 fish fed each experimental diet. Different letters indicate statistical significance obtained (P<0.05), according to ANOVA. If significant differences were found among treatments, Tukey’s Honestly Significant Difference was used to rank the means.

Table 2 Effect of experimental diets on intestinal morphology of red tilapias

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>5K</th>
<th>10K</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Proximal part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus height (μm)</td>
<td>280.2±4.96</td>
<td>253.8±4.34</td>
<td>263.3±5.98</td>
</tr>
<tr>
<td>Villus width (μm)</td>
<td>77.0±3.28</td>
<td>76.7±3.20</td>
<td>80.0±1.45</td>
</tr>
<tr>
<td>Absorptive area (villi length x villi width (μm²))</td>
<td>0.0222±0.006</td>
<td>0.0235±0.0005</td>
<td>0.0233±0.0002</td>
</tr>
<tr>
<td>2. Middle part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus height (μm)</td>
<td>262.2±6.72</td>
<td>263.8±9.47</td>
<td>265.1±4.39</td>
</tr>
<tr>
<td>Villus width (μm)</td>
<td>91.5±1.42</td>
<td>94.2±0.97</td>
<td>91.8±5.00</td>
</tr>
<tr>
<td>Absorptive area (villi length x villi width (μm²))</td>
<td>0.0240±0.0017</td>
<td>0.0240±0.0017</td>
<td>0.0242±0.0006</td>
</tr>
<tr>
<td>3. Distal part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus height (μm)</td>
<td>172.9±4.81</td>
<td>182.0±6.42</td>
<td>178.2±4.62</td>
</tr>
<tr>
<td>Villus width (μm)</td>
<td>99.2±0.73</td>
<td>101.4±4.06</td>
<td>104.1±5.37</td>
</tr>
<tr>
<td>Absorptive area (villi length x villi width (μm²))</td>
<td>0.0154±0.0009</td>
<td>0.0155±0.0003</td>
<td>0.0155±0.0009</td>
</tr>
</tbody>
</table>

Values represent Mean±SD of 6 fish fed each experimental diet. Different letters indicate statistical significance obtained (P<0.05), according to ANOVA. If significant differences were found among treatments, Tukey’s Honestly Significant.

Results and Discussion

The results of the 4-wks prebiotic supplementation revealed that the WG (%), SGR and ADG of fish fed the JA-supplemented diets (5K and 10K) were significantly higher (P < 0.05) than in fish fed the control diet. The FCR of fish fed the JA-supplemented diets (5K and 10K) were significantly lower (P < 0.05) than in fish fed the control diet (Table 1). Similar results were observed in effects of JA-supplemented diets on the Nile tilapia for 2 months (6). The villus height and absorptive area in the proximal, middle part of red tilapias fed the JA-supplemented diets (5K and 10K) were higher (P > 0.05) than those fed the control diet but were not statistically significant, while the absorptive area in the distal part were significantly different (P < 0.05) between red tilapias fed the JA-supplemented diets (5K and 10K) and those fed the control diet (Table 2). The results suggested the JA-supplemented diets (5K and 10K) had beneficial effect to the absorptive area in the distal part intestine of juvenile red tilapias. According to (6), the JA-supplemented diets fed in the juvenile Nile tilapia during 8 weeks improved the villus height in proximal and middle part of intestine (6) suggesting that the difference on the duration of feeding might be involved to the difference of the results. The JA can increase the growth performance in red tilapia by the direct supplementation of nutrients such as iron, calcium, potassium, vitamin B, vitamin C and vitamin A (7-8). In addition, the JA contains the prebiotics such as inulin and FOS which improve the growth of beneficial bacteria in the host, enhance the digestive enzyme activities (9-11) and ultimately effect to growth performance.

Altogether, the results in the present study extended the knowledge that the JA-supplemented diets had beneficial effect on growth performance in juvenile red tilapias by improving the intestinal villus morphology and absorptive area.

Acknowledgements

We would like to thank the 90th ANNIVERSARY OF CHULALONGKORN UNIVERSITY FUND (Ratchadaphiseksomphot Endowment Fund), Faculty of Graduate School, Chulalongkorn University for funding of this study. We also thank the staff from the Department of Pathology, the Department of Veterinary Microbiology and the Department of Veterinary Medicine from the Faculty of Veterinary Science of Chulalongkorn University for their support and contribution in this work.

References

A case report: Hepatic cholangiocellular carcinoma with liver fluke in an anorexic cat

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Keywords: Cat, Hepatic cholangiocellular carcinoma, Liver fluke, Histopathology

Introduction
Cholangiocellular carcinoma is a malignant tumor of bile duct epithelium and also a major cause of primary liver tumors in cats (1). There are no predisposing breed (2) and sex (3) for this cancer but mainly affect animals older than 10 years (1). It has been reported that liver fluke infection, prolong exposure of o-aminoazotoluene containing insecticide and hepatitis B virus infection may cause cholangiocellular carcinoma in human, cat, and experimental animals (1,4,5,6). However pathophysiology of the disease remains unclear. This case report described the cat presenting at the Small Animal Teaching Hospital, Chulalongkorn University and its necropsy examination revealed cholangiocellular carcinoma with liver fluke infestation.

Materials and Methods
A 16-year-old, castrated male, domestic shorthaired cat with a 1-week history of anorexia and weight loss was presented in order to follow up its previous treatment. The cat lived currently 100% indoor after a history of going missing for a year. He was regularly vaccinated and had a history of hunting behavior, especially geckos and cockroaches. Test for FIV antibody and FeLV antigen were negative. On physical examination, the cat was depression, dehydrated, normal heart rate and respiratory rate. He had moderate abdominal distension without pain and abdominal mass by palpation. The thoracic auscultation found decreasing in heart sound and lung sound. He also had mild icteric skin which was not observed on the previous visits. Radiographic findings showed microcardia, mild bilateral pleural effusion, and moderate abdominal effusion (Fig.1a and b). Abdominocentesis was done to get 400 mL of clear yellowish fluid which was modified transudate with no growth of bacteria and negative to Rivalta test. Ultrasonographic results revealed that the cat had hepatomegaly with a hepatic nodule (Fig.1c), moderate to severe bile retention with both intra and extrahepatic biliary tract obstruction (Fig.1d), 1.4 x 1.7 cm mass at the right renal and peritoneal effusion. Blood works (Table 1) indicated that the cat had gradually decreased in hematocrit, slightly leukocytosis, neutrophilia, hypoproteinemina, hypoalbuminemia, and normal liver enzymes. Symptomatic and supportive treatments were given with crystalloid fluid, antacids and antibiotics (Marbofloxacin 2.1 mg/kg PO q 24 hours). However, the cat died on the next day and its body was sent to the Department of pathology for further investigation.

Figure 1a and b Lateral view of thoracic and abdominal radiography. Ultrasonography showed hepatic nodule (arrow head) (c), and dilated common bile duct (d).

Table 1 Complete blood count and blood chemical profile of the cat from the 3 consecutive visits

<table>
<thead>
<tr>
<th>Blood Profile</th>
<th>Range</th>
<th>23/10/60</th>
<th>1/11/60</th>
<th>6/11/60</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>10^6/mm³</td>
<td>6-10</td>
<td>7.58</td>
<td>6.64</td>
</tr>
<tr>
<td>Hct %</td>
<td></td>
<td>29-45</td>
<td>34.3</td>
<td>27.4</td>
</tr>
<tr>
<td>WBC 10^3/mm³</td>
<td></td>
<td>5.5-19.5</td>
<td><strong>20.08</strong></td>
<td><strong>22.03</strong></td>
</tr>
<tr>
<td>Neutrophils Absolute</td>
<td>2.5-12.8</td>
<td>18.6</td>
<td><strong>20.04</strong></td>
<td><strong>17.6</strong></td>
</tr>
<tr>
<td>Eosinophils Absolute</td>
<td>0-1.5</td>
<td>0.12</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>ALT IU/L</td>
<td></td>
<td>5-60</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>ALP IU/L</td>
<td></td>
<td>10-150</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>BUN mg/dL</td>
<td></td>
<td>15-34</td>
<td><strong>103.7</strong></td>
<td><strong>46.5</strong></td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>0.8-2.3</td>
<td>2.1</td>
<td>1.9</td>
<td>1</td>
</tr>
<tr>
<td>Total protein g/dL</td>
<td>5.1-7.8</td>
<td>6.5</td>
<td>5.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Albumin g/dL</td>
<td></td>
<td>2.6-4.3</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>Globulin g/dL</td>
<td></td>
<td>2.3-4.5</td>
<td>3.5</td>
<td>3.3</td>
</tr>
</tbody>
</table>


Results and Discussion
The necropsy findings showed that there were 50 and 410 mL of yellowish fluid in pleural and abdominal...
The hepatic parenchyma was affected by multifocal to coalescing of neoplastic epithelial cells that differentiated toward biliary epithelium and formed acini, tubules, and packets. The neoplastic cells ranged from low cuboidal to columnar with pale amphophilic, vacuolated and sometimes eosinophilic cytoplasm (Fig. 3a) (1). The nuclei were large, round to oval and contained 1-2 prominent nucleoli. Mitotic figures were scarcely seen. Some neoplastic ducts and acini contained neutrophils and cell debris. Therefore, its histopathological diagnosis was hepatic cholangiocellular carcinoma. Surprisingly, some trematode eggs were found in hepatic bile ducts (arrow head) (Fig. 3b) and a crossed section of its adult was also found from the H&E slide (Fig. 3b).

**Figure 2** White firm masses were found at right caudal lobe of the liver (a) and right kidney (b).

Histopathology of the hepatic mass demonstrated that the hepatic parenchyma was affected by multifocal to coalescing of neoplastic epithelial cells that differentiated toward biliary epithelium and formed acini, tubules, and packets. The neoplastic cells ranged from low cuboidal to columnar with pale amphophilic, vacuolated and sometimes eosinophilic cytoplasm (Fig. 3a) (1). The nuclei were large, round to oval and contained 1-2 prominent nucleoli. Mitotic figures were scarcely seen. Some neoplastic ducts and acini contained neutrophils and cell debris. Therefore, its histopathological diagnosis was hepatic cholangiocellular carcinoma. Surprisingly, some trematode eggs were found in hepatic bile ducts (arrow head) (Fig. 3b) and a crossed section of its adult was also found from the H&E slide (Fig. 3b).

**Figure 3** Cuboidal to columnar neoplastic epithelial cells were showed in hepatic parenchyma (a). Golden brown trematode eggs were also found in hepatic bile ducts (b).

There is no pathognomonic sign for both cholangiocellular carcinoma and liver fluke infestation. Inappetence, lethargy, vomiting, and weight loss have been reported in many case reports of cholangiocellular carcinoma and liver fluke infestation (1,7,8). The macroscopic findings of cholangiocellular carcinoma are usually described as white, firm, well delineated mass and its sectioned surface may contains necrotic and hemorrhagic areas (1). Metastatic organs of cholangiocellular carcinoma include the peritoneum, mesentery, lungs, kidneys, spleen, bone marrow, etc (5) and its microscopic features show well-differentiated cubic and cylindrical neoplastic cells which form acini or bile duct epithelium. Even though the cancer does not normally invade healthy tissue, it is a high malignant potential tumor (5). There are several possible causes of cholangiocellular carcinoma in human and cat including liver fluke infestation. Most of the trematodes infection in cats in Thailand cause by *Platynosomum fastosum* and *Opisthorchis viverrini* and both of which were reported to associate with cholangiocarcinoma in human, cat, and experimental animals (4,6). Although both of the trematodes cause similar pathologic changes in bile duct epithelium and also induce bile ducts obstruction (4), life cycles and their intermediate hosts are different. Dogs and cats become infected with *O. viverrini* by eating uncooked or undercooked infected cyprinid fish (9) whereas *P. fastosum* infects by eating its infected paratenic hosts such as lizards, reptiles, amphibians, and possibly cockroaches (10). *P. fastosum* was likely to be a cause of trematode infection in this cat because its 34.0–50.0 µm × 23–35 µm golden brown, thick-shelled operculated eggs, (10) and size of the adult fluke which were found from histopathology (Fig. 3). Since liver fluke is an important zoonotic trematode in Thailand and cats can serve as reservoir of this parasite for human, diagnosis and prophylactic treatment (Praziquantel 20 mg/kg PO q 24 hours for 3-5 consecutive days) of liver fluke infection should conduct in a cat with vague liver diseases.

**Acknowledgements**

The authors acknowledge Asst. Prof. Waraporn Sukumavasi for her excellent help in trematode egg identification. We wish to thanks staff of Pathology unit and Department of Veterinary Pathology, Radiology unit, General Medicine unit, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University.

**References**

Cervical subcutaneous dermoid cyst in domestic cat

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Keywords: Subcutaneous dermoid cyst, neck, domestic cat

Introduction
Dermoid cyst is an uncommon tumor arising from developmental anomaly. It is contributed to the failure of the skin and neural tube to separate completely during embryonic growth (1). The lesion is usually isolated and shows progressive enlargement due to accumulations of hair, keratin and sebum inside the cyst (2). In domestic cat, there are few reports about dermoid cyst by which the congenital form commonly involved with cornea, conjunctiva, and eyelids (3). Interestingly, this report demonstrated the rare subcutaneous dermoid cyst in cat at the neck area closed to the jugular vein.

Case report
A 1-year-old, 4.45 kg, domestic shorthair, intact male cat presented with dark brown-colored elastic band protruding from the skin at the neck area closed to the jugular vein (Fig.1). Physical examination revealed the palpable small tract deep in the subcutaneous tissue connected to the opening of the thin elastic band—suspected as the sebum. It gradually developed for an unknown period of time with neither pain nor inflammation. The cat appeared bright, alert and responsive with no evidence of trauma or infection. Seven months later, the cat was brought to the hospital with the enlarge mass at the same area. The palpable, firm and round-shaped mass (1.0 x 1.0 x 1.0 cm) was located deep in the subcutaneous tissue connecting the sinus. Complete blood count showed thrombocytopenia (159,000 cell/µl) with normal blood chemistry. The mass and sinus were removed by surgical excision and were sent for histopathological examination. Gross findings of the mass revealed the accumulation of some necrotic hair shaft, sebum and tissue debris within the encapsulated mass (Fig.2).

Figure 1 The dark brown-colored band of sebum protruding outside the skin (red arrowed) closed to the jugular vein.

The histopathological findings revealed dermal cystic structure lined by thin layers of mature stratified squamous cells and keratin. The cyst lumen contained necrotic hair shaft and tissue debris by which was diagnosed as dermoid cyst (Fig.3). The surgical wounds healed completely without any complications. There was no recurrence of the lesion in the excised area after a 12 months follow-up.

Figure 2 The palpable firm, round-shaped mass (1.0 x 1.0 x 1.0 cm) with accumulated sebum and hair shaft inside (red arrowed) connected to the protruding band of sebum via the sinus (asterick).
Discussion
Cutaneous or subcutaneous dermoid cysts were considered rare in cats and were usually solitary masses—appeared either congenital or acquired (4). But only 10% of this tumor like lesion is believed to be acquired due to the trauma (5). Considering the age of the affected cat, dermoid cyst seemed to be congenital disorder in this case. Based on limited cases reported, no breed predilection was documented in cats. However, this report also occurred in domestic shorthair breed similar to the other previous ones (1, 5).

Figure 3 The histopathological findings revealed necrotic hair shaft and tissue debris within the cyst.

This report was different from the others due to the lesion’s location at cervical area close to the jugular vein. As far as we know, we found no other previous report in the same location. Most congenital dermoid cysts are usually asymptomatic until they become distended or infected. Due to the progressive enlargement of the cyst and sinus in this case, surgical excision was thus performed without recurrence nor complication.

References
Confirmation of feline immunodeficiency virus (FIV) infection by provirus PCR

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Keywords: feline immunodeficiency virus, FIV, provirus, confirmation

Introduction
Feline immunodeficiency virus (FIV) is a contagious lentivirus, causing immunodeficiency and malignancies in domestic cats. As the efficacy of FIV vaccine remains controversial, the most efficacious preventive measures are detection and segregation (1, 2). Currently, point-of-care tests, which detect FIV-specific antibodies in the circulation, are extensively used in the clinics as the first line of FIV diagnosis. Although these tests are with excellent sensitivity and specificity, secondary tests are highly recommended for the confirmation of FIV infection. In this study, we reported the use and performance of FIV provirus PCR as the confirmatory test for FIV infection.

Materials and Methods
One hundred EDTA-anticoagulated blood samples used in this study were submitted to the Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University for the diagnosis of feline immunodeficiency virus (FIV) and/or feline leukaemia virus (FeLV) infections. Forty-two samples were collected from FIV/FeLV suspected, clinically sick cats in the Bangkok Metropolitan Region between January and June 2017. Fifty-eight samples were collected from clinically healthy cats enrolled for neutering service in Bangkok, Trat province in October 2017, and were submitted to estimate FIV/FeLV prevalence on the island. Upon arrival, plasma was collected and used for the detection of FeLV p27 antigen and FIV antibodies, using rapid point-of-care test (Witness® FIV/FeLV, Zoetis). Genomic DNA (gDNA) was purified from the buffy coat using QIAamp® DNA blood Mini Kit (Qiagen) and used as template in nested FIV provirus PCRs. The primers were designed to amplify conserved region of FIV gag and env based on FIV clade A, Petaluma and clade D, Shizuoka sequence (Genbank Accession no: M25381 and AY679785, respectively). The primers for gag were as followed: outer primers; F211F: 5’-AGGGAGAAGTTTGAGTTAGAC-3’ and F602R: 5’-TCCCTATCTGCTGACACAACCT-3’, and inner primers; F380F: 5’-AGGTAGAGGAGCCTCCACA-3’ and F514R: 5’-GTGGGACCTCCTCCTCCTCCT-3’. The primers for env were: outer primers; F7317F: 5’-GATTCTTGAGGTAC-3’, and inner primers; F7125F: 5’-CACCAATGTGGATGGGAACC-3’ and F8282R 5’-ACCATCCTAGCAGTGCC-3’. Amplicons were subjected to direct Sanger sequencing for confirmation.

Table 1 Demographic data of the studied population

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n = 58)</th>
<th>Sick (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIV Ab*</td>
<td>FIV+</td>
<td>FIV-</td>
</tr>
<tr>
<td>gender</td>
<td>male:female</td>
<td>15:33</td>
</tr>
<tr>
<td>age (median &amp; range)</td>
<td>3 years (3m–5y)</td>
<td>3 years (9m–10y)</td>
</tr>
<tr>
<td>complete:neutered</td>
<td>58:0</td>
<td>15:1</td>
</tr>
</tbody>
</table>

*Presence of FIV antibodies was determined by Witness® FIV/FeLV test, Zoetis (sensitivity 94.7%, specificity 100% (4)).

Using nested PCR targeting FIV proviral gag and env, 17 and 15 samples were positive, respectively (table 2). Of the 16 seropositive cats, 2 were negative by gag and env provirus PCR, respectively, rendering their sensitivity to be equally at 87.5% (table 3). Nevertheless, when both gag and env provirus PCRs were performed together, all 16 seropositive samples were positive for FIV by using point-of-care test. All FIV positive samples were free roaming and most FIV seronegative cats were healthy cats, enrolled for neutering service (58/84), it was not possible to determine whether outdoors access and being complete were risk factor for FIV infection.

Results and Discussion
Of 100 samples tested in this study, 16 samples were positive for FIV by using point-of-care test. All FIV seropositive cats were adult male (Table 1). This finding was in agreement with several previous studies reporting being adult male as risk factor for FIV infection (1, 2, 3). Additionally, FIV seropositive cats were 3.23 times more likely to appear clinically ill (Fischer’s exact test, p < 0.0001). However, as all cats were free roaming and most FIV seronegative cats were healthy cats, enrolled for neutering service (58/84), it was not possible to determine whether outdoors access and being complete were risk factor for FIV infection.
Interestingly, there were 3 samples that tested negative for FIV antibodies by point-of-care test but were positive by gag provirus PCR. Of these 3, 2 were also positive by env provirus PCR, ascertaining the validity of the detection. These 3 samples were repeated twice. Their sequences confirmed the presence of FIV provirus in their genomes, with approximately 93-94% identity with FIV isolates previously reported in Thailand (5).

### Table 3 Performances of gag and env provirus PCR as compared with Witness® FIV/FeLV point-of-care test

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Result</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>gag</td>
<td>14/16</td>
<td>87.5%</td>
<td>100%</td>
</tr>
<tr>
<td>env</td>
<td>14/16</td>
<td>87.5%</td>
<td>100%</td>
</tr>
<tr>
<td>gag &amp; env</td>
<td>16/16</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

The discrepancy between presence of FIV antibodies and FIV provirus in the genome observed in this study most likely resulted from the stage of infection. In most experimental study, FIV provirus could be detected as early as 2 weeks post inoculation (6, 7). Similarly, antibodies against capsid p24 antigen, the most immunodominant target of FIV, could also be detected as early as 3 weeks, followed by matrix p17 and envelope glycoprotein at 6 and 10-12 weeks, respectively (7). The point-of-care Witness® FIV/FeLV test used in this study, was designed to detect antibodies against GP40 subunit of the FIV envelope glycoprotein. Although GP40 detection enables Witness® FIV/FeLV test to differentiate between FIV vaccination and infection (8), it might not be able to detect recently infected cats and those still at the early stage of infection. In conclusion, herein we reported the use of nested PCRs, specifically designed to detect gag and env of FIV provirus, for the confirmation of FIV infection. These tests were not only able to confirm results of point-of-care test with excellent sensitivity and specificity, but also able to detect cats, tested negative for FIV-specific antibodies. Moreover, as FIV provirus detection is the only mean to separate vaccinated cats from naturally infected cats, it is highly recommended in all suspected cases, especially in those with unknown history of exposure and vaccination.

### References

Conserving domestic cat follicular structure by alginate-fibrin gel in vitro

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Keywords: alginate-fibrin, domestic cat, follicle, in vitro culture

Introduction

The ovarian follicle consists of an oocyte surrounded by layers of granulosa and theca cells. During follicular development, the cells surrounding the oocyte undergo proliferation and differentiation. Bi-directional communication between oocyte and somatic cells through the gap junctions supports oogenesis and folliculogenesis (1, 2). Several hydrogels, including alginate and alginate-fibrin have been utilized to support and maintain the normal follicular architecture during in vitro culture (3, 4). The previous study indicated that pre-antral follicles encapsulated in alginate hydrogels are able to grow and produce fertilizable oocytes and embryos generated from such gametes are capable of developing into live offspring (5). However, the oocytes from follicles cultured within interpenetrating alginate-fibrin matrix resumed meiosis higher than in the alginate alone in the mouse (6). According to this success, we tested the ability of alginate-fibrin in maintaining cat follicle’s structural integrity during in vitro incubation.

Materials and Methods

For each donor cat, two-layered (characterized by two layers of granulosa cells and diameter less than 150 µm), multi-layered (diameter more than 150 µm without antral formation) and secondary follicles were collected from one ovary by being crushed on the stainless steel mesh and filtered with 40 µm Falcon cell strainer (BD Bioscience Discovery Labware, Durham, NC, USA). The remaining ovary was sliced into cortical strips. Insulin-gauge needles were used to dissect early antral follicles (≤ 500 µm). Minimum essential medium (MEM) supplemented with 3 mg/ml bovine serum albumin (BSA), 2 mM L-glutamine, 25 mM Heps, and 10 IU/ml penicillin G sodium and 10 mg/ml streptomycin sulfate was used as collection medium. Individual follicles were encapsulated in 3 µl of alginate-fibrin (FMC BioPolymers, Philadelphia, PA, USA). Alginate-fibrin was cross-linked with thrombin in 40 mM CaCl2 in Tris-buffered saline solution to form gel beads. The encapsulated follicles were washed in the collection medium before moved into 4-well culture dish. Three encapsulated follicles were cultured in a well containing 500 µl MEM medium (supplemented with 0.05 IU/ml equine chorionic gonadotropin (eCG), 3 mg/ml BSA, 2mM L-glutamine, 10 ng/ml activin, 10 µg/ml insulin, 1.9 µg/ml transferrin, 5 µg/ml selenium and 10 IU/ml penicillin G sodium and 10 mg/ml streptomycin sulfate). The follicles were then incubated at 38.5°C in 5% CO2 in humidified air for 12 days. Follicle and oocyte diameters were measured on Day 0 and Day 12.

**Results and Discussion**

The diameter of follicles did not change after 12 days of culture in all developmental stages (P > 0.05) (Table 1). The oocyte diameter of the two-layered secondary follicles were significantly decreased (Table 1) indicating the inability to maintain oocyte viability after 12 days of culture. However, the oocyte diameter of the multi-layered secondary follicles and early antral follicles were remained their initial size (Table 1). For the follicle extrusion rate, the two-layered secondary follicles did not present follicle extrusion (0%) while the follicles of the multi-layered secondary and early antral follicles did extrad 2.7% and 8.3%, respectively. According to the previous study (7), domestic cat preantral follicles can be cultured for few days and it can grow up to 250 µm, as same as, in the cows (8) presented at 2 days of culture, most of the oocytes in the preantral follicles were degenerated. Therefore, the major problem for small preantral follicle culture is to maintain oocyte viability.

**Table 1. Growth data of the follicles at Day 0 and Day 12 (Mean ± SEM).**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Follicle diameter (µm)</th>
<th>Oocyte diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 12</td>
</tr>
<tr>
<td>TS</td>
<td>126.4±4.3</td>
<td>123.1±5.4</td>
</tr>
<tr>
<td>MS</td>
<td>193.5±7.0</td>
<td>191.8±8.7</td>
</tr>
<tr>
<td>EA</td>
<td>402.1±13.3</td>
<td>419.7±22.2</td>
</tr>
</tbody>
</table>

*Two-layered secondary follicle.
**Multi-layered secondary follicle.
***Early antral follicle.

In conclusion, the alginate-fibrin gel can preserve the domestic cat follicle morphology based on their initial size. However, other gonadotropins or growth factors supplementation may be required to promote follicle growth and to maintain oocyte viability.
Acknowledgements
2. The Research Unit for Obstetrics and Reproduction in Animals
3. Smithsonian Institution.

References
Development of indirect ELISA for detection of feline morbillivirus

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Keywords: feline morbillivirus, matrix protein, indirect ELISA

Introduction
Feline morbillivirus (FmoPV, FeMV) or feline paramyxovirus (FPaV) is the new member of genus Morbillivirus, family Paramyxoviridae. FmoPV was detected in stray cats since 2012 and might associate with chronic kidney disease (1). This viral antigen can be detected by molecular assay from various sources of samples such as urine, feces, blood and formalin fixed embedded kidney tissues (FFPE) from cats (2-10). However, the serological status of FmoPV infection is fragmentary (11-12). The matrix (M) protein is selected for this study based on the fact that it interacts between hemagglutinin (H) enveloped protein and nucleocapsid (N) protein. Moreover, the M protein plays a key role in virus budding of infected cell. This research aimed to develop the indirect ELISA assay by producing the recombinant M protein of FmoPV and to evaluate the sensitivity and specificity of established ELISA in a cohort cat serum.

Materials and Methods
Production of recombinant M protein
Nucleic acid from urine of FmoPV positive cat (No.16) (13) was reverse transcribed to cDNA and further amplified with specific primers for matrix (M) gene (FemorbiMatrix1011_F: 5'-ATA GAA TTC ATG ACT GAG ATA TTC ACT CGT GAT GAG AGC-3'; FemorbiMatrix1011_R: 5'-TAT CTC GAG TTT AAT CTT GAA GAG ACC ATT GTC AAT AAT-3'); the restriction enzyme site is underlined containing EcoR1 and Xho1 respectively. The specific band of M gene (1,011 bp) was cloned into pGEM- T easy vector (Promega, USA) and transformed into competent cells (DH5α; ThermoFisher Scientific, USA). Positive white colonies were identified by plating on ampicillin-containing plates. After that, M gene was subcloned into the pet24a(+) expression vector (Novagen, Germany) and transformed into competent cells (DH5α; ThermoFisher Scientific, USA). Positive white colonies were identified by plating on ampicillin-containing plates. After that, M gene was subcloned into the pet24a(+) expression vector (Novagen, Germany), harboring M gene with his-tagging protein and transformed into BL21 (DE3) (Novagen, Germany). The constructions, pet24_M was confirmed by sequencing. Purification of the His-M was performed with the His Bind Kit (Novagen, USA) according to the manufacturer’s protocol. The Ni-NTA agarose beads were affinity with his-tag (Affinity purification). The purified His-M was analyzed by SDS-PAGE analysis in 12% polyacrylamide gel. Final concentrations of purified proteins were determined with a NanoDrop ND-1000 Spectrophotometer (ThermoFisher Scientific, USA).

Immunoblot assay
Twenty-six randomized cat sera were tested (13). After PVDF membrane contained appropriately transmitted protein, then the cat sera (dilution 1:100) was probed on the membrane and incubate at 37°C overnight. All blots were incubated with anti-cat IgG horseradish peroxidase (HRP)-conjugated secondary antibodies (dilution 1:2000) (Invitrogen, ThermoFischer Scientific, USA), the specific protein band was detected with DAB kit (CWBIO, China) with molecular weight of 38 kDa of M protein. The M protein transblotted PVDF membrane probed with distilled water was used as negative control.

Indirect ELISA assay and antigenic efficacy test
The 96-well flat bottom ELISA plates were coated with 0.625, 1.25, 2.5, 5 and 10 µg/mL of recombinant M protein antigen solution at 4°C overnight. Thereafter, blocking with 100 µL of 1X ELISA assay diluent (BioLegend, CA) was done at room temperature for 2 hours and plates were washed with PBS containing 0.05% Tween-20 (PBST). One-hundred µl of 2 dilutions (1:100 and 1:150) of 26 cat sera were added triplicately in each well and were incubated at 37°C for 1 h. After incubation and washing, 100 µl of HRP-conjugated with rabbit anti-cat IgG (Sigma-Aldrich®, USA) was added in a serial dilution of 1:2000, 1:4000 and 1:8000 and incubated at 37°C for 1 h. After another washing, 100 µl of TMB substrate was added and the reaction was observed carefully. Finally, 100µl of 1N HCl was added to stop reaction. The plates were read at 450 nm with ELISA reader and expressed as the reciprocal of the maximum dilution showing positive absorbance. The cut off value was analyzed by mean of negative group ± 2SD at 95% CI.
Results and Discussion

The constructed recombinant M protein showed the sequencing data compatible with GenBank accession no. JQ411016 (FmoPV strain M252A). The results from immunoblot assay revealed positive group (n=12) and negative group (n=14). The positive samples showed the specific band at 38 kDa of M protein; while the negative samples displayed non-specific band or absence of M protein band. Our results showed that the optimized conditions of indirect ELISA were the concentration of 0.625 µg/ml of coated M protein, the diluted serum at 1:150 and the dilution of secondary anti-cat IgG antibody was 1:8000. After reading the reaction at OD 450 nm, the cut off value was set at 0.25 which were able to discriminate between positive group (n=11/12; OD>0.25) and negative group (n=13/14; OD<0.25). However, there were 2 samples that remained ambiguously (Figure 1). The sensitivity and specificity of indirect ELISA test were 91.67% and 92.86%, respectively, at 95% CI compared with immunoblot assay. We suggested that the M protein of FmoPV can be used as the target protein to monitor the antibody titer of FmoPV infection from cat serum as similar as the previous studies in other morbillivirus (14-17). For the further research, other structural proteins such as phosphoprotein (P) or nucleocapsid (N) protein should be performed for comparison the suitable target protein to diagnose the FmoPV infection in cat population.

Acknowledgements

S. Chaiyasak was supported by The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship. This research was partially granted by the Chulalongkorn University-Veterinary Science Research Fund (RG 2/2561) and STAR Feline Health and Infectious Diseases Research Center, Chulalongkorn University. We were also cordially grateful to Ban Nang Fah, the cat shelter in Saraburi province, for sample collection.

References

Electropherogram Analysis of TCRG Gene Rearrangement of Feline Lymphoma

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Keywords: Electropherogram, Feline, Lymphoma, T-Cell, TCRG gene,

Introduction
Lymphoid neoplasms are the most common neoplasms in cats. The expansions of lymphoid cells cannot be diagnosed by cytology alone. Immunohistochemistry is a valuable tool to evaluate protein expression in tumor tissues (1). CD-3 and Pax-5 antibody was performed to confirm the diagnosis for T-cell and B-cell, respectively (1). PCR-based clonality assessment is developed for detection IgH and TCRG gene rearrangement (3,6). Furthermore, biopsy could not be done in some cases especially mediastinal mass. The aim of this study was to develop and analyze the electropherogram pattern of TCRG gene rearrangement for feline lymphoma diagnosis.

Materials and Methods
Fine needle aspiration from submandibular lymph node, pleural effusion by thoracocentesis and lymph node biopsy were collected from lymphoma cat. Cell pellets were kept into PBS-contained Eppendorf at -80°C until used. Immunohistochemistry (IHC) will be performed to detect an immunophenotype of PAX-5 (B-cell marker, Dako-PAX5, Dako) and CD3 (T-cell marker, LN 10, Leica).

DNA was extracted from fresh tissue and FNA-cells of both affected lymph nodes, mediastinal mass and pleural effusion. PCR was performed by using one primer pair for T-cell and 2 primer pairs for B-cells (Table 1) (4,6). PCR products were applied into QIAxcel Advanced System capillary electrophoresis using the QIAxcel DNA high resolution Kit and the QX alignment marker 15 bp/1000 bp. Electropherogram analysis showed single (monoclonality), two (biclonality) or more than three (oligoclonality) positive peak of T-cell lymphoma. These results were compatible with CD3 positive immunophenotype.

All samples showed single peak by using TCRG gene primer (Fig 4,5). IgH gene primer showed multiple non-reproducible peaks (pseudoclonality) of B-cell lymphoma. Like PAGE analysis, smear band of B-cell lymphoma was noted.

In conclusion, electropherogram analysis is new method to apply for precise diagnosis with TCRG primer (T-cell lymphoma). Samples used in this study were tissue biopsy, cell from fine needle aspiration and cell pellets from pleural effusion gave a same result.

In case of B-cell lymphoma diagnosis, these primers were not covered all VH and JH segments. On the other hand, the primers that amplify rearranged portion of IgK and IgL or the kappa deleting element should be designed for improving the sensitivity and specificity for feline lymphoma diagnosis.

Table 1 Primer for T-cell and B-cell lymphoma

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>5'-AAGAGCGAYGAGGMGTGT-3'</td>
</tr>
<tr>
<td>J</td>
<td>5'-CTGAGCAGTGCCAGSACC-3'</td>
</tr>
<tr>
<td>FR2</td>
<td>5CCA GGC TCC AGG GAA GGG-3'</td>
</tr>
<tr>
<td>FR3</td>
<td>5'TCC AGA GAC AAC GCC AAG AAC-3'</td>
</tr>
<tr>
<td>J2</td>
<td>5'TGAGGACACTGTGACTATGGTTC-3'</td>
</tr>
<tr>
<td>JD</td>
<td>5'-GGACACCGTCACYAKGVYTCC-3'</td>
</tr>
</tbody>
</table>

Results and Discussion
This study findings revealed negative nuclear immunostaining of B-cell lineage of Pax-5 protein whilst that of CD3 showed intense cytoplasmic immunostaining of T-cell lineage (Fig 1,2). PCR products were range from 80-120 bp which were detected by agarose or polyacrylamide gelelectrophoresis (Fig 3). Electropherogram analysis showed single (monoclonality), two (biclonality) or more than three (oligoclonality) positive peak of T-cell lymphoma.
Fig. 1 Immunophenotypes showed negative nuclear immunostaining of Pax-5 protein of B-cell lineage.

Fig. 2 Immunophenotype showed intense cytoplasmic immunostaining of CD3 of T-cell lineage.

Fig. 3 PAGE analysis with PCR products of T-cell and B-cell lymphoma with smear background and unclear results. L1: Ladder 50 bp L2: Ladder 25 bp N: Negative control 1, 2, 6, 7: T-cell lymphoma, 2, 4, 5: B-cell lymphoma.

Fig. 4 Electropherogram showed three positive peaks at 80-120 bp (FNA-cells of lymph node).

Fig. 5 Electropherogram showed positive peak at 80-120 bp (Cell-pellet of pleural effusion).

Acknowledgement
J. Siripoonsub was granted by The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship.

References
Immunohistochemical Localization of Kisspeptin Receptor in the Cat Ovary on Different Ovarian Stages

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Keywords: GPR54, KpR, domestic cat

Introduction
Kisspeptin has been recognized as a central regulator of reproductive processes, exerting its function via stimulation of GnRH secretion in the hypothalamus (1). Apart from the brain, kisspeptin and its receptor (KpR) have also been reported in the ovary, indicating the possible local roles (2). The objective of this study was to investigate the localization of KpR in relation to ovarian stages in the domestic cats.

Materials and Methods
Twenty ovaries were obtained from domestic cats (age from 7 months to 5 years; body weight from 2.7 to 4.5 kg) after routine ovariohysterectomy in the Small Animal Hospital, Bangkok (the reason for surgery was not related to the study). Ovarian stages were classified by ovarian gross morphology, vaginal cytology and blood progesterone level. Ovarian samples were classified into inactive (n = 7), follicular (n = 7) and luteal stages (n = 7). Immunohistochemistry was performed using polyclonal rabbit anti-KpR (NLS1926, Novus Biologicals, CO, USA) as a primary antibody. Immunoreactive signal was visualized by avidin-biotin-peroxidase system. The dog uterine tissue was used as a positive control due to the previous report on KpR labeling using the same antibody (3). Primary antibody was substituted with PBS and normal rabbit IgG as negative and isotypic negative controls, respectively. In addition, primary antibody was incubated with KiSS1R/GPR54 Blocking Peptide (NLS1926PEP, Novus Biologicals, CO, USA) overnight and applied for preabsorption test.

Results and Discussion
In this study, immunolabeling of KpR was detectable in the cat ovaries of all ovarian stages with no remarkable changes between the stages.

Figure 1 The immunolabeling of KpR in cat ovaries (arrowhead). (A) Ovarian cortex with primordial follicles; (B) Secondary follicle; (C) Antral follicles; (D) Luteal cells. Scale bars = 20 µm (A, B), 50 µm (C, D). OSE = Ovarian surface epithelium, TC = Theca cells, GC = Granulosa cells.

Immunoreactive KpR was demonstrated in the oocytes of both preantral and antral follicles (Fig. 1A, B, C). In the secondary follicles, the KpR staining was present in the granulosa and theca cells (Fig. 1B). In the antral follicles, KpR was present in theca cells (C). Immunostaining of KpR was detected in the luteal cells, whereas no staining was observed in the nonsteroidogenic cells of corpus luteum (Fig. 1D). In addition, immunostaining was detected in the ovarian surface epithelial cells (Fig. 1A). In the positive control, immunolabeling was observed in dog uterine glands (Fig. 2A), as previously reported (3). No immunostaining was detected in the negative and isotypic negative controls (Fig. 2B, C). Little to no immunolabeling was present in ovarian stroma in the preabsorption test (Fig. 2D). The light staining in the ovarian stroma might either indicate non-specific staining of this structure or insufficient concentration of the specific blocking peptide. The present findings of KpR localization indicated the potential involvement of...
kisspeptin in local regulation of ovarian function at all ovarian stages in the domestic cats, possibly in oocyte survival, folliculogenesis and steroidogenesis. In addition, the results of present study showed a similar staining pattern to other species (4, 5, 6), suggesting a conserved role of ovarian kisspeptin in mammals.

**Figure 2** Control of immunohistochemistry (A) Positive control (arrowhead); (B) Negative control; (C) Isotypic negative control; (D) Preadsorption test, faint staining in the ovarian stroma. Scale bars = 100 μm. CL = corpus luteum.

**Acknowledgements**

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**References**

Molecular Detection of Feline Calicivirus Infection in Thailand

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Keywords: cats, feline upper respiratory disease complex, feline calicivirus, Thailand

Introduction
Feline upper respiratory tract disease (FURTUD) is a complex disease typified by rhinotracheitis, conjunctivitis, gingivitis, stomatitis and faucitis. There are several pathogens involved with FURTUD including Feline herpesvirus (FHV-1) (1), Feline calicivirus (FCV), Chlamyophila felis (C.felis), Bordetella bronchiseptica (B. bronchiseptica) and Mycoplasma felis (M.felis) (2). FCV is recognized as one of main viral pathogens for FURTUD. Most of FCV infected cats present acute respiratory disease and oral ulceration as common sign (3). Moreover, many cats associated with severe chronic oral inflammation; as known lymphoplasmacytic gingivitis stomatitis complex (LGSC), are also positive for FCV (4). Infected cats mostly become the carriers following recovery by shedding viral for more than 30 days (4,5). Prevalence of FCV infection of many countries in both household and shelter cats have shown between 8% (6) to 50% (7), and unclear results for being probable risk factor of age, sex, and sterilization (6,8,9) are demonstrated. As a present, a presumptive diagnosis for FCV infection seem likely base on such characteristically sign as clinical sign of upper respiratory tract, hypersalivation, oral ulcer, and stomatitis. However, there is some remarkable overlapping clinical sign between FCV infection and LGSC. As a result, causal pathogen should be identified using to guide for appropriate treatment. In Thailand, there are widespread of imported catteries and cat shelters that will accumulate many pathogens in event of inappropriate sanitation. Furthermore, the study of epidemiology and pathogen diversity causing FURTUD never been reported. Therefore, the aim of this study was to be the preliminary investigation of the FCV infection in Thailand using reverse transcriptase (RT) PCR technique.

Materials and Methods
Patients: Samples were collected by convenient sampling from 4 regions of Thailand including; North, South, North East and Center. Information of age, sex, sterilization and housing were also documented. Owner’s consent was asked on each cat before performing sample collection. All procedures were approved by the Chulalongkorn University Animal Care and Use Committee (No. 1631002).

Sampling: Individual sterile cotton swab was used to collect samples from nasal passage and oropharyngeal mucosal area. Each swab was placed in 1% sterile phosphate buffer saline (PBS) and kept in -80°C until analysed.

RT-PCR: QIAamp® cador® Pathogen Mini Kit (QiagenGmbH, Hilden, Germany) was used for RNA extraction by 100 μl of Buffer VXL and 20 μl of protein kinase K were added in 200 μl of sample solution (1% PBS) and incubated at room temperature (20-25°C) for 15 minutes, then was followed the manufacturer’s protocol. Reverse transcription was performed using Omniscript® RT Kit (QiagenGmbH, Hilden, Germany) according to manufacturer’s guidelines. The RT-PCR were performed as briefly, each 20 μl reaction contained 2 μl cDNA, 10 μl GoTaq® Green Master Mix (Promega, USA), 6 μl nuclease-free water and 1μl each of forward and reverse primers. The thermal cycling consisted of DNA denaturation (95°C, 5 min), followed by 40 cycles of denaturation (95°C, 30 s), primer annealing (60°C, 30 s) and primer extension (72°C, 30 s). Final extension was performed at 72°C (5 minutes).

Statistical analysis: GraphPad Prism was used for statistical analysis. Fisher’s exact test was used for all comparisons by setting level of significance as P < 0.05.

Results and Discussion
Signalment and clinical sign: 77 cats (45 domestic shorthair cats, 4 persia, 2 American shorthairs, and 26 data not available) from 4 regions of Thailand were included in this study. Thirty-nine cats (50.65%) were female and 28 cats (36.36%) were male. The age of cats was between 2 months and 11 years 6 months (median 2 years 2 months). Of all 77 cats, 29 cats (37.67%) showed gingivitis, 29 cats (37.66%) were asymptomatic, and other 19 cats (24.68%) could not retrieve the data.

Detection rate of Feline calicivirus: Totally, 154 samples from 2 different sampling sites obtainable from 77 cats. Forty-eight of 77 cats (62.34%) revealed FCV positive result by RT-PCR. Detection rates of each region; Central, North, South, North-East, were shown in Figure1.
Figure 1. Percentage of cats from each region in Thailand; Central, North, South, and North-East, which testing positive to FCV by RT-PCR were shown as 54.2%, 14.6%, 22.9%, and 8.3% respectively.

Of all 48 positive cats, only 29 clinical data were available (Table 1). As a result, although cats revealed FURTD clinical signs; especially stomatitis and gingivitis, 44.83% was represented negative for FCV detection by RT-PCR.

Table 1. Detection rate of FCV in cat with and without clinical sign.

<table>
<thead>
<tr>
<th>Clinical Sign</th>
<th>(+) FCV</th>
<th>(-) FCV</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>16 (55.17)</td>
<td>13 (44.83)</td>
<td>0.56-4.13</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Absent</td>
<td>13 (44.83)</td>
<td>16 (55.17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* sign: stomatitis, gingivitis

Values are given as n (%); CI = confidence interval

Number of positive swabs from 2 locations; nasal and oropharynx, were shown in Figure 2. Of all 48 cats, 27 cats (56.25%) were FCV positive from both sites (NS and OS). Whereas, 35 cats (72.92%), and 40 cats (83.33%) were the number of FCV detection from NS and OS respectively. Moreover, 12 (25%) and 7 (14.58%) cats revealed FCV positive only at OS and NS, respectively.

Figure 2. Detection of FCV from 2 different sampling sites. NS: Nasal Swab, OS: Oropharyngeal swab.

Feline calicivirus had ever been reported in many countries in Asia such as Japan. Cai et al (2002) reported the prevalence of FCV in 66 domestic cats in which 21.2% showed clinical sign of FURTD. In China, 37.6% of stray cats were FCV seropositive investigated in 2017 (11). Besides, this study was the first document of FCV prevalence in Thailand as amount of 62.34% which was higher than others. For the preliminary of geographic distribution, Central area demonstrated a highest detective rate following by South, North and North-East, respectively. For a further study, the geographic data and animal habitat should be collected for risk analysis. Although there was no statistical significantly different of FCV detection between clinical sign and non-clinical sign, it raised awareness of overdiagnosis of FCV infection (44.83%) by clinical observation only or those cats are in carrier stage. In this study, oropharyngeal mucosal area tended to be a detective site of FCV more often than nasal area as similar to previous study. Kahn et al (1975) demonstrated the result of viral isolation, from 6 experimental cats infected with FCV, showed more frequently FCV isolates from pharyngeal swab than conjunctival swab and nasal swab (12).

Acknowledgements

We thank veterinarians from hospital and private clinics on each region; Tak, Saraburi, KhonKhen, Phuket, and Dusit Zoo, for collecting samples. K. Phongroop was granted by Chiang Mai University.

References

Anesthetic Management of Modified Extracapsular Thyroidectomy in Canine Hypothyroidism with Cushing Syndrome: A Case Report

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Keywords: Anesthesia, Thyroidectomy, Cushing’s syndrome

Introduction
Anesthetic considerations for hypothyroidism and hyperadrenocorticism (Cushing’s syndrome) are important as the imbalance of these hormones leads to a deregulation of metabolism in the body. Canine hypothyroidism results in a decrease of metabolic rate. The most common cause of canine hypothyroidism is primary hypothyroidism which resulted from the destruction of thyroid gland such as thyroid carcinoma (1). These non-functional thyroid carcinomas have been considered as an aggressive cancer that usually invade adjacent tissues, such as the larynx, tracheal, cervical muscles, nerves, and esophagus with a high rate of pulmonary metastasis (2). According to the surgical treatment, removal of both lobes of the thyroid gland (total thyroidectomy) are recommended. Intraoperative complications following hypothyroidism including a decrease in respiration rate, bradycardia, hypotension, and hypothermia can cause life-threatening conditions (1). Canine hyperadrenocorticism resulted from excess cortisol in circulation. This high level of cortisol leads to muscle atrophy, hepatomegaly, hypertension, and lethargy (3). Thus, these complications should also be managed for successful anesthetic outcome.

Materials and Methods
A 10 years old mixed breed dog, weight 6.8 kg was presented to the Chulalongkorn University small animal teaching hospital regarding cervical mass over thyroid gland area. From history taking, the dog underwent surgical removal of the right lobe of thyroid gland last year which was confirmed later as thyroid carcinoma. The dog has been given thyroxine. In addition, the dog has a concurrent disease as hyperadrenocorticism which has been treated with trilostane until the presentation. The retained left thyroid gland mass was further visualized by Computerized Tomography (CT scan). The reduction of intrathyroidal iodine concentration was demonstrated, therefore the mass is likely to be non-functional thyroid mass. In addition, bilateral adrenomegaly with pituitary gland enlargement were found. However, no lung metastasis or other lymph node involvement were found. Blood profile and serum chemistry showed hypothyroidism (T4 level was <1 ug/dl) and hypercholesterolemia (cholesterol level was 350 mg%), while other parameter were all normal. Electrocardiogram was further performed and no abnormality was found. Patient was assigned physical status of an ASA3 (American Society of anesthesiologists Classification).

Surgical procedure
The skin incision was made and the thyroid mass (Fig 1) was removed by modified extracapsular technique. The parathyroid glands were preserved and secured with monofilament absorbable suture. The subcutaneous and skin layer were then closed carefully.

Figure 1. Left thyroid mass

Anesthetic Management
The patient was premedicated with morphine (0.3 mg/kg) and acepromazine (0.01 mg/kg), intramuscularly (IM) followed by the placement of 20G catheter into cephalic vein. Patient was preoxygenated before an induction of general anesthesia with midazolam (0.2 mg/kg) together with propofol (4mg/kg), intravenously (IV). After tracheal intubation, anesthesia was maintained with isoflurane adjusted End tidal CO2 (35-40 mmHg) with intermittent positive pressure ventilation. The Bair hugger system was used to maintain a patient’s body temperature. Intravenous fluid therapy with crystalloids (10 ml/kg) and colloids (5ml/kg) were administered intraoperatively. Cephazolin (25 mg/kg, IV) was administered every 90 minutes throughout operation. The vital signs were monitored using electrocardiography, pulse oximetry, capnography, and electronic oscillometric device (Fig.2). The depth of anesthesia was checked continuously via palpebral/corneal reflex, pedal/pain reflex, and eye positioning. During operation, patient has moderate bradycardia (60 bpm), thus atropine (0.02 mg/kg) was administered IV to increase heart rate into
100-120 throughout the operation. On the other hand, blood pressure was normal during the same time.

**Pain management**

Analgesia was provided using MLK (morphine (3.3 mcg/kg/min), lidocaine (50 mcg/kg/min), and ketamine (10 mcg/kg/min) in acetar solution 500 ml) with constant rate infusion (CRI) of 10ml/kg/hr. Carprofen (2.2 mg/kg) was administered subcutaneous (SC) after operation.

**Results and Discussion**

Anesthesia times were 270 minutes and surgery times were 180 minutes. The patient recovered from anesthesia in the critical care unit (CCU). Post-operative assessment of vital sign, urinary output, calcium, phosphorus, T4 level was continued. Pain management was done by giving morphine twice a day (0.3mg/kg) and then changed to tramadol (4mg/kg). The Patient had no other complications until discharge. The biopsy result confirmed the thyroid carcinoma.

Anesthetic management in total thyroidectomy is challenging due to the large thyroid mass. Any airway obstruction present in the awake patient will be exacerbated with sedation and anesthesia until an endotracheal tube is successfully placed. Moreover, hyperadrenocorticism can cause respiratory compromise from hepatomegaly and muscle weakness which affect the diaphragm (2). Thus, the use of capnometry can assist in monitoring ventilation. Hypertension is common due to cortisol’s enhancement of epinephrine’s vasoconstrictive effect (3). Short-acting drugs that require minimal or no metabolism or that are readily antagonized are preferred. Opioids and benzodiazepines usually produce adequate preanesthetic sedation in the lethargic elderly patient. Propofol and isoflurance are suitable for canine hypothyroidism (1).

Hypothyroidism leads to bradycardia, therefore ECG monitoring for heart rate and potential arrhythmias was carefully observed and the bradycardia that occur during anesthesia was managed by atropine administration. Temperature should be monitored to avoid hypothermia, which causes recovery time can be prolonged. In addition, measurement of ionized calcium is also recommended postoperatively following bilateral thyroidectomy to identify damage to or loss of parathyroid function. Multimodal analgesia was used as the co-administration of morphine, ketamine, and lidocaine, the latter also for MAC sparing (4) and antiarrhythmic properties. The NSAID was not given intraoperatively because total thyroidectomy can potentially cause severe hemorrhage and NSAIDs can impair platelet function.

In conclusion, anesthetic management of hypothyroidism concurrent with hyperadrenocorticism was done successfully. The proper monitoring devices and drug selection are important for anesthetist.
Application of The Novel and Rapid Technique: LAMP-LFD E. canis DNAsensor Kit

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Keywords: LAMP-LFD, E. canis DNAsensor Kit, molecular diagnosis, dogs.

Introduction

Ehrlichia canis, responsible for Canine Monocytic Ehrlichiosis, is difficult to be diagnosed accurately from the standard method of thin blood smear and staining. The microscopic method yields less neither sensitive nor specific compared to DNA-based molecular detection such as PCR (1). This study aims to validate a novel, highly sensitive and specific, and cost effective technique to detect E. canis from canine whole blood samples by loop-mediated isothermal amplification with lateral flow dipstick (LAMP-LFD). Amplification of E. canis using the LAMP-LFD technique was successfully achieved by using specific set of primers in conjunction with LFD. The obtained amplicons were probed and applied on dipstick to give a chromatographic banding output. This procedure may help to measure the prevalence of CME and to validate the technique in where there is no data available as a rapid screening at point-of-vet-care purpose.

Materials and Methods

In this study, 10 blood specimens obtained from dogs attending at Veterinary Teaching Hospital of Chulalongkorn University either as surplus amount of blood samples when veterinarian collected in routine practice or the leftovers from hematological laboratory work in EDTA containing tubes. Loop-mediated isothermal amplification (LAMP) is used following the previous assay (2). Together with Lateral flow device, (LFD), the kit is named E. canis DNAsensor. It was developed by Kespunyavee Bunroddith, Nareerat Visesakul, Somchai Santiwatanakul, Thongchai Kaewphinit and Kosum Chansiri in 2016 (Development of DNA biosensors for detection Ehrlichia canis dissertation for Doctor of Philosophy program in molecular biology, petit patent 1503000654 signed by Srinakharinwirot University, 1 May 2015, Department of Intellectual Property, The Ministry of Commerce, Thailand.

Results and Discussion

Time of process between LAMP-LFD and PCR is compared and shown in Table 1.

Table 1. The process comparisons of LAMP-LFD and direct PCR.

<table>
<thead>
<tr>
<th>Assays</th>
<th>Time of process per sample</th>
<th>Limit of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAMP-LFD</td>
<td>1.5 hours</td>
<td>8 pg/µL</td>
</tr>
<tr>
<td>direct PCR</td>
<td>3 hours</td>
<td>80 pg/µL</td>
</tr>
</tbody>
</table>

Results suggested that LAMP-LFD E. canis DNAsensor Kit is successfully create a rapid screening test for the first time ever in the Veterinary Teaching Hospital Chulalongkorn University. The complete process is only 1.5 hours suitable for point-of-vet-care purpose.

References


Branching patterns of subclavian arteries in dogs

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Keywords: Branching pattern, Dog, Subclavian arteries

Introduction

The knowledge of an anatomical variation which is important to understand animal physiology and diseases is rarely demonstrated in reference textbooks for teaching canine anatomy (1-3). The canine left and right subclavian arteries are the major vessels circulating blood from the heart to the forelimbs, neck, thorax as well as the spinal cord. A subclavian artery gives rise to four branches: vertebral artery, costocervical trunk, superficial cervical artery, and internal thoracic artery (1-3) before continuing to the forelimb as axillary artery. The branching order of the subclavian arteries in the dog recently described in detail in two specific breeds, the German shepherds (4) and greyhounds (5) are different in the frequency of pattern and probably related to some abnormalities of canine subclavian arteries either developmental or inherited diseases (6-7). The objectives of this study were to investigate the branching pattern of subclavian arteries of dogs in Thailand, and to comparatively analyse the pattern’s frequency in dog from all studies.

Materials and Methods

This research project has been approved by Animal Care and Use Committees of Mahidol and Chulalongkorn Universities. The branching patterns of subclavian artery and the frequency of each pattern were modified by combination of two previous reports (4-5) with our results. Our study was conducted in 58 anatomy teaching-purposed cadavers. There were 43 Thai mixed breed dogs and 15 other breeds. The data of present study (58 dogs) was combine to previous data (4, 5) which studies in 87 dogs.

Results and Discussions

The illustration of all branching patterns and occurrence of pattern in the present study were shown in Figure 1 and 2. The highest frequency of branching pattern in this study was type I (50.9%), particularly type Ib (32.8%), which is similar to that of German shepherd’s study (4) but not that of greyhounds (5) which type V is the most common pattern.

The frequency of branching patterns from all studies is shown in Table 1. According to the textbooks (1-3) the common pattern is type Ib which is consistent with our analysis from all studies with 26.9% of the occurrence from all studies. The most common patterns of left artery were type b and type c, but the most common pattern of the right artery was type Ib.

Several textbooks (1-3) mentioned that the internal thoracic artery branched off the subclavian artery opposite to the origin of the superficial cervical artery, this pattern was shown in type Ib, IIb, IIIb, IVb, and Vf which found 46.2% (n=134) from all studies, and 57.7% (n=67) of our study. These textbooks also stated that the vertebral artery is the first branch, and the second branch is the costocervical trunk (1-3).
However, the chance of vertebral artery was the first branch (type Ia-c, Vc-f) was 62.9% (n=73) from our study, and 63.7% (n=185) from all studies. The common trunk of vertebral artery and costocervical trunk (type IIIa-c) was found 10.2%, and the percentage that both arteries arose at the same level (IIa-c) was 18% from all studies. Surprisingly, type Vc-f found in the present study was the pattern never been reported.

In conclusion, type Ib is the most common pattern but it is found less than a half of cases. The symmetric pattern of left and right subclavian arteries is not common. Therefore, teaching canine anatomy need to be more concerned on the anatomic variation in the branching of subclavian arteries due to it is not mentioned in the textbooks.

Figure 2. The alternative branching pattern of subclavian arteries and the frequency of each pattern found in the present study. All the alternative pattern is not related to classical pattern. Reference textbooks also state that the branching patterns of the left and right subclavian arteries are symmetric (1-3). However, the symmetric pattern of subclavian artery was found only 31% in the present study and about 38% from previous studies (4-5).

Table 1. The frequency of branching pattern of subclavian arteries from all studies.

<table>
<thead>
<tr>
<th>Type</th>
<th>% case (n)</th>
<th>% left (n)</th>
<th>% Right (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>10.5% (31)</td>
<td>4.1% (12)</td>
<td>6.6% (19)</td>
</tr>
<tr>
<td>Ib</td>
<td>26.9% (79)</td>
<td>9.7% (28)</td>
<td>17.6% (51)</td>
</tr>
<tr>
<td>Ic</td>
<td>15.0% (44)</td>
<td>9.7% (28)</td>
<td>5.5% (16)</td>
</tr>
<tr>
<td>Total</td>
<td>52.4% (154)</td>
<td>23.4% (68)</td>
<td>29.7% (86)</td>
</tr>
<tr>
<td>IIa</td>
<td>1.7% (5)</td>
<td>0.3% (1)</td>
<td>1.4% (4)</td>
</tr>
<tr>
<td>IIb</td>
<td>8.8% (26)</td>
<td>5.2% (15)</td>
<td>3.8% (11)</td>
</tr>
<tr>
<td>IIc</td>
<td>7.5% (22)</td>
<td>6.6% (19)</td>
<td>1.0% (3)</td>
</tr>
<tr>
<td>Total</td>
<td>18.0% (53)</td>
<td>12.1% (35)</td>
<td>6.2% (18)</td>
</tr>
<tr>
<td>IIIa</td>
<td>2.0% (6)</td>
<td>1.4% (4)</td>
<td>0.7% (2)</td>
</tr>
<tr>
<td>IIIb</td>
<td>5.4% (16)</td>
<td>2.8% (8)</td>
<td>2.8% (8)</td>
</tr>
<tr>
<td>IIIc</td>
<td>2.7% (8)</td>
<td>2.4% (7)</td>
<td>0.3% (1)</td>
</tr>
<tr>
<td>Total</td>
<td>10.2% (30)</td>
<td>6.6% (19)</td>
<td>3.9% (11)</td>
</tr>
<tr>
<td>IVa</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
<td>0.0% (0)</td>
</tr>
<tr>
<td>IVb</td>
<td>4.1% (12)</td>
<td>3.8% (11)</td>
<td>0.3% (1)</td>
</tr>
<tr>
<td>IVc</td>
<td>0.3% (1)</td>
<td>0.0% (0)</td>
<td>0.3% (1)</td>
</tr>
<tr>
<td>Total</td>
<td>4.4% (13)</td>
<td>3.8% (11)</td>
<td>0.7% (2)</td>
</tr>
<tr>
<td>Va</td>
<td>8.8% (26)</td>
<td>2.8% (8)</td>
<td>6.2% (18)</td>
</tr>
<tr>
<td>Vb</td>
<td>3.1% (9)</td>
<td>1.0% (3)</td>
<td>2.1% (6)</td>
</tr>
<tr>
<td>Vc</td>
<td>0.3% (1)</td>
<td>0.0% (0)</td>
<td>0.3% (1)</td>
</tr>
<tr>
<td>Vd</td>
<td>0.3% (1)</td>
<td>0.3% (1)</td>
<td>0.0% (0)</td>
</tr>
<tr>
<td>Ve</td>
<td>0.7% (2)</td>
<td>0.3% (1)</td>
<td>0.3% (1)</td>
</tr>
<tr>
<td>Vf</td>
<td>0.3% (1)</td>
<td>0.3% (1)</td>
<td>0.0% (0)</td>
</tr>
<tr>
<td>Total</td>
<td>13.6% (40)</td>
<td>4.8% (14)</td>
<td>9.0% (26)</td>
</tr>
</tbody>
</table>

References
Canine pseudomembranous cystitis due to persistent urinary tract infection

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Keywords: Pseudomembranous cystitis, persistent, urinary tract, infection, canine

Introduction
Pseudomembranous cystitis is defined as the presence of shaggy layers of necrotic material covering a hemorrhagic and ulcerated bladder mucosa (1). It is uncommon in animal previous reports in cats, cattle and pigs with ambiguous etiology. Previous report in feline indicated that the accumulated necrotic tissue could contribute to the obstruction of the urine outflow (2). Since there had been no pseudomembranous cystitis reported in dog, this report demonstrated the rare case of canine pseudomembranous cystitis.

Case report
A 2-year-old, 3.35 kg, mixed breed, spaying female dog presented with paraparesis and intermittent hematuria. Chronic cystitis was previously diagnosed and the dog had been treated with Enrofloxacin (Baytril®) (dose 5 mg/kg, sid, pc) along with urethral catheterisation and repeated bladder flushing every day for two months. Concomitantly, wet food for junior (Mini Junior, Royal Canin®) together with dry food for urinary bladder (Urinary SO, Royal Canin®) were also replaced. The dog, however showed the progressive signs of stranguria, vomiting and lethargy. Contrast radiography was later performed and revealed thickening of the urinary bladder’s wall with unusual mucosal surface (Fig.1). Abdominal ultrasonography revealed multiple hyperechoic luminal septations of the urinary bladder wall (Fig.2). Since the hematology was normal, exploratory cystotomy was thus performed—revealing extensive thickening of the bladder with abnormal thick grayish-yellow membranes on the mucosal surface of bladder (Fig.3). Histopathological examination of the bladder biopsy was performed. The content within the bladder was also acquired for bacterial culture and drug sensitivity test.

Histopathological examination revealed mucosal epithelial necrosis and hemorrhages with diffused segmented neutrophils’ infiltration in the submucosal layer of the bladder. Fibrino-hemorrhagic necrotic cystitis was hereby diagnosed. Pseudomonas spp. infection—sensitive to Amoxicillin /Clavulanic acid, cephalexin, ceftriaxone, cefazolin, amikacin, imipinem, clindamycin and enrofloxacin was indicated by the bacterial culture and drug sensitivity tests. After surgical correction, the hematuria was present again two months later.
**Discussion**

As far as we know, this case demonstrated rare case of canine pseudomembranous cystitis contributed from persistent or recurrent urinary tract infections (UTIs). Persistent or recurrent UTIs were uncommon disorders—occurring in dogs with other underlying disorders, such as abnormal micturition, anatomical defects, alteration of urothelium, altered urine composition and impaired immunity (3). Among such cases, *Enterococcus spp.* and *Pseudomonas spp.* also show a higher prevalence in persistent or recurrent UTIs when compared with uncomplicated UTIs (4).

At the beginning, conventional antimicrobial therapy was performed in this case. However, the infection was likely to be persisted in the urinary bladder. Since most UTIs are contributed to ascending infections, the paraparesis in the case should be the major contributing factor of persistent UTIs in this case (4).

**References**

A case report: Opened Wound at Nasal Bridge due to Tooth Root Abscess in a Bang Kaew

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Keywords: Tooth root abscess, extraction, opened wound, canine teeth

Introduction
Tooth root abscess (periapical abscess) is the abscess formation surrounding the tooth apex causes by chronic bacterial infection in the pulp cavity. Predisposing cause of pulp infection is tooth cracked. Often founded the occurrence at the maxillary canine, maxillary 4th premolar and maxillary molars teeth. (1) In dogs, face swelling with presence of draining sinus tracts under ocular area and nasal bridge can be found. (2) This case report aims to elaborate the symptom, diagnosis, and treatment.

Materials and Methods
The 8 years old, castrated male, Bang Kaew dog which was raised in the house area with complete history of vaccination, was referred to Prasuarthon animal hospital, Mahidol University. The illness history showed chronic nasal bridge wound for 3 months. He was treated with antibiotic administration with bacterial sensitivity test and had wound dressing daily. The dog did not response for the mentioned treatment. General physical examination found 2.5×1.5 cm opened wound at the nasal bridge with 3 cm wide subcutaneous cavity, and purulent discharge. The dog had normal blood profile, both CBC and blood chemistry. General anesthesia was performed for oral examination and skull radiography. The oral examination result showed sign of all 4 canine teeth were cut with pulp necrosis and gingivitis. Draining sinus tracts were found at muco-gingival junction of both maxillary canine teeth. (104 and 204) (Fig. 1). The skull radiograph found undifferentiated structures around the root of the tooth at rostral part of maxilla and mandible (Fig. 2). The dog was treated by open extraction of 104 and 204 (Fig. 3) and curetted around tooth socket for remove granulation tissue. Retrograde flushing with NSS. The flushing fluid was able to reach to the opened wound at nasal bridge. Oronasal fistula was rule out by probing. The gingiva was sutured with simple interrupt by 4-0 polydioxanone (PDS®). The post operation care was daily injection of Amoxycilin and clavulanic acid (Synulox®) 8.75 mg/kg SID subcutaneously for 7 days oraly. Additionally, soft feed and daily mouth rinsing after meal with 0.12% chlorhexidine was performed.

Results and Discussion
There was a series of follow-up appointment; 9 days after the operation, the outcome was no significant size change of the wound but less amount of discharge. Oral sutures were clean. Gingival inflammation was reduced. In 30 days after the operation, the nasal bridge wound was completely closed. (Fig. 4). No sign of gingivitis was found. In this case, the reason of tooth root abscess was believed coming from canine teeth cut lead to pulp infection.
The frequent signs are face swelling under-eye area with or without draining sinus tract. (2) The swelling usually responds to antibiotic treatment and recur after stop antibiotic administration. (1) Treatment options are root canal treatment and extraction. These options are different in expenses, treatment tools and equipment, and post operation care. Root canal treatment indicated in the tooth without extensive periapical structural damage and need follow up by dental radiography. (4) This case the treatment of choice is tooth extraction because of severe damage of periapical structure and the dog is aggressive that may cause the hardship of post operation care.

Cutting canine teeth was not a proper method to reduce aggressive, which is a behavior problem. Thai veterinarian should be concern about animal welfare and encourage society and owner to stop this malpractice.

**Acknowledgements**

I would like to thanks Dr. Panithi Sukho for her kind support and all staff of Surgery Unit, Prasuarthon Animal Hospital, Mahidol University.

**References**

Detection on the Embryonic transcription factors Oct-4, Nanog, and Sox-2 Proteins in Canine Cutaneous Mast Cell Tumors by Immunocytofluorescence technique

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Keywords: Canine cutaneous mast cell tumor, Immunocytofluorescence, Oct-4, Nanog, Sox-2

Introduction

The three major Embryonic transcription factors (ETFs) Oct-4, Nanog and Sox-2 are the essential genes that regulated self-renewal and pluripotency of normal stem cell: embryonic and adult stem cells (1, 2). There are many reports on putative cancer stem cells (CSC; small subpopulation of cancer cells that have self-division and pluripotency properties (3). Many cancers such as human breast cancer, human seminoma and canine mammary gland tumors, have demonstrated the existence of ETFs Oct-4, Nanog and Sox-2 that can used for prognosis, and develop treatments by target therapy (4,5,6).

The canine cutaneous mast cell tumor (MCT), the common skin tumor in dogs, which has been reported that they expressed Oct-4 Immunohistochemistry (IHC) staining (7). The result may be suggested that MCT has a potential to be the putative cancer stem cell. Immunocytofluorescence technique (ICF) can be applied for determined ETFs Oct-4, Nanog and Sox-2 protein in normal stem cells and putative CSC tumors (8,9). In this experiment, the objective of this study is to determine major ETFs Oct-4, Nanog and Sox-2 proteins by ICF in MCT cells.

Materials and Methods

MCT tissue samples were collected from operation room, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. MCT cells were aspirated from fresh MCT tissues. The MCT single cells were washed with wash buffer (BD bioscience®, USA) and adjusted volume to 1 ml by wash buffer. Approximately, 1x10⁶ MCT cells were washed with wash buffer before increased permeability with permeabilisation buffer (BD bioscience®, USA) for 45 minutes at 4°C. After that the samples were incubated in primary antibodies: Mouse anti-human Oct-4 monoclonal antibody conjugated PE, Mouse anti-human Nanog monoclonal antibody conjugated PE and Mouse anti-human Sox-2 monoclonal antibody conjugated PE (BD bioscience®, USA) for 45 minutes at 4°C. Then wash with wash buffer, after that the cells were stained with 4,6-diamidino-2-phenylindole (DAPI). Images were then captured by inverted microscopy. The canine keratinocyte cell line (CELLnTECAdvanced Cell Systems, Switzerland) was used for positive control.

Results and Discussion

The results demonstrated that ICF technique can be determined the Oct-4 Nanog and Sox-2 proteins. The nuclei of keratinocyte cell line and MCT sample were stain with DAPI, Oct-4 Antibody, Nanog antibody and Sox-2 antibody (Fig.1). The result suggested that MCT sample express Embryonic transcription factors Oct-4, Nanog and Sox-2 proteins.

Further study, on the number of positive cells which expressed ETFs Oct-4, Nanog and Sox-2 expression was localized in the nuclei of cells (arrow).

Further study, on the number of positive cells which expressed ETFs Oct-4, Nanog and Sox-2 proteins should analyzed. It is suggested the possibility on the prognosis of MCT.
Acknowledgements
The financial supported by Rachadaphiseksomphoph Endowment Fund, Part of the “Strengthen Chulalongkorn University Researcher’s Project”, the 90th anniversary of chulalongkorn university fund 2017 and Oncology Clinic, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, 2014 till present.

References
Effect of dexmedetomidine on isoflurane minimum alveolar concentration in dogs

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Keywords: dexmedetomidine, dogs, isoflurane, MAC

Introduction
Cardiovascular depression related to high anesthetic concentration is one of the main concerns of inhalation anesthesia. Benefits of the administration of sedative and analgesic drugs prior to or during surgery are a reduction in minimum alveolar concentration (MAC) of a volatile anesthetic required to maintain surgical anesthesia, then reduction of cardiovascular and respiratory complications.

Dexmedetomidine is an α2-adrenergic agonist widely used in small animal anesthesia to provide sedation, anxiolysis and analgesia (1). Dexmedetomidine provided sedation in dogs (2), decreased concentration of isoflurane for dogs receiving a bolus followed by CRI of the agent (1, 3). This study aimed to determine isoflurane MAC and cardiorespiratory variables after giving a bolus of dexmedetomidine IM.

Materials and Methods
Animals: This study (Protocol No. 1731052) was approved by the Animal Care and Use Committee of Chulalongkorn University, Bangkok, Thailand. Twelve client-owned, adult male dogs scheduled for castration at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, were enrolled in the study. All dogs had a good health and were withheld food for at least 12 h and water for 6 h prior to injection of the tested drugs. The dogs were randomly allocated into Group 1 (n = 6) receiving distilled water as placebo IM at the same quantity as if dexmedetomidine given, or Group 2 receiving 5 µg kg⁻¹ of dexmedetomidine IM.

Anesthesia and monitoring: Respiratory rate (fR), ECG, heart rate (HR), oxygen saturation (SpO2), and systolic arterial pressure (SAP) were recorded before the tested drug injection (except ECG), at 15 min after the injection, and during determination of isoflurane MAC. Blood gases were analyzed before the tested drug injection and following the last noxious stimulation by clamping the third or fourth digit of the hind limb. At 15 min after drug injection, sedation according to the sedation scoring scale (Table 1) and response to the noxious stimulation were assessed. Then, anesthesia was induced via face mask with 4% isoflurane in 4 L min⁻¹ of oxygen. After intubation, anesthesia was maintained with isoflurane in oxygen at a flow rate of 2 L min⁻¹ using a Bain coaxial circuit. End tidal (ET) isoflurane concentration and ET carbon dioxide were determined by DatascoperMindrayPassportV and Gas Module 3™ Multi-Gas Analyzer. The fR was set at 12 breaths min⁻¹ on the ventilator. Acetated Ringer’s solution was administered at a rate of 10 ml kg⁻¹ h⁻¹. Body temperature was maintained by a warm air mat.

Table 1 Sedation scoring scale modified from others (2,4).

<table>
<thead>
<tr>
<th>Score</th>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Alert with normal startle reaction (head turn towards noise/cringe), standing</td>
</tr>
<tr>
<td>1</td>
<td>Less alert but still active, reduced startle reaction (reduced head turn/minimal cringe), if recumbent, animal can rise normally</td>
</tr>
<tr>
<td>2</td>
<td>Drowsy, minimal startle reaction, difficult rising if recumbent</td>
</tr>
<tr>
<td>3</td>
<td>Very drowsy or stuporous, absent startle reaction, unable to rise</td>
</tr>
</tbody>
</table>

Determination of isoflurane minimum alveolar concentration (MAC): The ET isoflurane concentration had been initially set at 1% for at least 15 min for anesthetic equilibration before SAP, HR, SpO2, isoflurane vaporizer setting, inspired isoflurane concentration, ET isoflurane concentration, and ET carbon dioxide were recorded. Then, the noxious stimulation was carried out as previously reported (3,4), by clamping the third or fourth digit of the hind limb using a 20-cm hemostat of which jaws protected by a rubber sleeve to prevent tissue damage. The jaws were closed to the first ratchet until positive motor response was detected or 1 min elapsed. A positive response was considered when there was gross purposeful movement of the head or extremities, including jerking or twisting of the head or movement of the extremities. The ET isoflurane concentration was increased or decreased by 0.1 - 0.2%, depending on degree of response, once the response was positive or negative, respectively. The new ET concentration was maintained for at least 15 min for anesthetic equilibration before the noxious stimulation was repeated. The isoflurane MAC was the...
The ET concentration of isoflurane between the highest concentration at which the positive response was detected and the lowest concentration at which the response was negative. After the isoflurane MAC determined, Group 1 was premedicated with sedative and analgesic drugs apart from this study protocol and was discarded. Caudal abdomen of Group 2 dogs was aseptically prepared for castration. The ET concentration of isoflurane was initially set at 1.5 times of the determined isoflurane MAC and adjusted accordingly to the anesthetic depth.

**Results and Discussion**

Data are presented as mean±SD, median and/or range, and analyzed by t-test using Microsoft Excel. A p-value of less than 0.05 was considered significant. Breeds of dogs in both groups were shown intable 2. There were no significant differences (p>0.05) in age and weight of dogs between Groups 1 and 2 (table 2). All dogs responded to the noxious stimulation before anesthesia induction. Sedation score of Group 2 was significantly greater (p<0.05) than that of Group 1 which had no dogs sedated (table 2), indicating dexmedetomidine provided sedation as reported by Grint et al (2).

This study revealed dexmedetomidine had a sparing effect on the isoflurane MAC. Mean isoflurane MAC of Group 2 was significantly less than that of Group 1 (table 2). A mean basal isoflurane MAC of 1.34±0.11% was previously reported by a study using techniques of anesthesia induction and noxious stimulation similar to the current study (4). In addition, another study reported a mean isoflurane MAC of 1.58±0.28% for inhibiting response to the noxious stimulation similar to the present study in dogs receiving propofol for anesthesia induction (3). The isoflurane MAC of Group 2 was less than the two reported means (3,4), supporting the existence of the sparing effect on the isoflurane MAC of dexmedetomidine. Furthermore, the sparing effect of dexmedetomidine on the ET isoflurane concentration was reported in dogs undergone soft and orthopedic surgery while receiving CRI following a bolus of the same dose rate as this study (1).

For castration, the ET concentration of isoflurane was significantly greater than the isoflurane MAC. The ET isoflurane concentration for surgery/the isoflurane MAC ratio was 1.73, indicating the ET isoflurane concentration for castration was 1.73 MAC (table 2). Light to deep level of anesthesia usually require 1 to 2 MAC of an inhalant anesthetic (5). The ET concentration for surgery was higher than the isoflurane MAC possibly due to more severe pain of castration than clamping the digit and the over-time reduction of serum concentration of dexmedetomidine.

Means±SD (medians) of FR of dogs before and 15 min after injection of the tested drug were 62±24 (56) and 59±25.52 breaths min⁻¹, respectively, in group 1, 61±49 (42) and 23±6 (24) breaths min⁻¹, respectively, in group 2. The FR before drug injection was not significantly different between groups. The rate at 15 min after drug injection in Group 2 was significantly less than the rate before drug injection and the rate in Group 1 at the same measuring point. However, the FR and blood gases were within the clinically acceptable limit.

Means±SD (medians) of HR of dogs before and 15 min after injection of the tested drug, and after the isoflurane MAC determined were 129±22 (130), 136±28 (136), and 108±15 (110) beats min⁻¹, respectively, in Group 1; 110±25 (98), 60±18 (57), and 61±12 (60) beats min⁻¹, respectively, in Group 2. HR were not significantly different between groups before drug injection but were significantly different at all measuring points after drug injection. HR at 15 min after drug injection and after the isoflurane MAC determined in Group 2, but not in Group 1, were significantly decreased from the rate before drug injection. The decreases in the FR and HR were the clinically adverse effects of the α₂-adrenergic agonist (6). Mean±SD (median) of HR after the ET isoflurane concentration for surgery determined in Group 2 was 89±21; 83) beats min⁻¹ and was not significantly different from the rate before drug injection.

**Table 2** Dogs of each breed, median (range) of age and weight, mean±SD (median) of sedation scores, isoflurane MACs (Iso MAC), ET isoflurane concentrations for surgery (Iso surgery), and ratio between Iso surgery/ Iso MAC of dogs in Group 1 receiving placebo and Group 2 receiving dexmedetomidine.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>3.5 (1-5)</td>
<td>3.5 (1-5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.0 (2.1-5.4)</td>
<td>6.2 (2.3-6.4)</td>
</tr>
<tr>
<td>Dogs of each breed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chi Hua Hua</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Crossbreed</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pomeranian</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Poodle</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Shih Tzu</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Sedation score (0-3)</td>
<td>0 (0)</td>
<td>0.8 (0.4-1)</td>
</tr>
<tr>
<td>Iso MAC&lt;sub&gt;η0&lt;/sub&gt;</td>
<td>1.63±0.23 (1.60)</td>
<td>1.16±0.27 (1.20)</td>
</tr>
<tr>
<td>Iso surgery&lt;sub&gt;η0&lt;/sub&gt;</td>
<td>ND</td>
<td>1.90±0.13 (1.85)</td>
</tr>
<tr>
<td>Iso surgery/Iso Mac</td>
<td>ND</td>
<td>1.73±0.48 (1.58)</td>
</tr>
</tbody>
</table>

*paired significant difference (p<0.05) in the same row, · paired significant difference in the same column, ND, not determined
groups. SAP after the isoflurane MAC determined in Group 1 was significantly less than those before and 15 min after drug injection, probably due to the effect of isoflurane. Mean±SD (median) of SAP after the ET isoflurane concentration for surgery determined in Group 2 was 137±16 (140) mmHg. No significant differences in the SAP between before and after drug injection at all measuring points were observed in Group 2. The pressure after the isoflurane MAC determined in Group 2 was significantly greater than that of Group 1. This might be due to vasoconstriction effect of the \( \alpha_2 \)-adrenergic agonist though the HR was low (6).

In conclusion, dexmedetomidine had sedative and sparing effects on the isoflurane MAC. Respiratory variables and SAP were within the clinically acceptable limit.

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**References**

Effect of Recombinant Feline Interferon-ω on Primary Culture of Canine Transmissible Venereal Tumor

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Keywords: CTVT, interferon, in vitro study, primary cell culture

Introduction

Canine transmissible venereal tumor (CTVT) has a special regression features which spontaneous occurring due to the immune reaction between CTVT itself and tumor infiltrating lymphocytes (TILs). During regression phase, the host interleukin-6 (IL-6) from itself and tumor infiltrating lymphocytes (TILs). During occurring due to the immune reaction between CTVT special regression features which spontaneous Canine transmissible venereal tumor (CTVT) has a (SPL Life Sciences, Korea) to obtain uniform single was minced in HBSS and crushed using cell strainer (biopsied mass (approximately sized 1.5×1.5×1.5 cm) Purification of CTVT cells

Interferons (IFNs) are the signaling proteins which produced by the host cells in response to the pathogens as well as tumor cells. IFNs play roles in antiviral, antiproliferative and immunomodulatory effects. The type I IFNs, include IFN-α, β, δ, τ, and ο, have ability to inhibit tumor cell growth and induce apoptosis, activate the activity of Natural killer (NK) cells, and increase the expression of MHC class I molecules. The type II IFN, only IFN-γ, has effect to increase the expression of MHC on antigen presenting cells and activate NK cells (2). Normally, tumor cells evade the immune system by decrease expression of MHC class I molecules (1,2,4). A recombinant feline IFN-ω (rFeIFN-ω) has been proved that it has the in vitro growth inhibition activities on various canine and feline tumor cell lines (2,3,5). The aim of this study was to evaluate the in vitro effect of rFeIFN-ω on primary CTVT cells.

Materials and Methods

Animal: Genital CTVT mass of male dog was diagnosed by cytology and polymerase chain reaction (PCR) (6). The tumor tissue was biopsied with sterile technique. Sampling procedures were approved by the Chulalongkorn University Animal Care and Use Committee (No. 133100077). Tissue sample was immersed in Hank’s buffered salt solution (HBSS; Gibco Lab, USA) containing 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco Laboratories, USA).

Purification of CTVT cells from TILs: The sterile CTVT biopsied mass (approximately sized 1.5×1.5×1.5 cm) was minced in HBSS and crushed using cell strainer (SPL Life Sciences, Korea) to obtain uniform single cell suspension. The single cell suspension was overlaid on a gradient of 42% Percoll (GE Healthcare, Sigma-aldrich, USA) and centrifuged at 820×g, 4˚c, for 25 min. After centrifuged, CTVT cells appeared at the interface were isolated and harvested; then washed 3 times with Dulbecco’s Modified Eagle Medium (DMEM, Gibco Lab, USA) (1). CTVT cells were counted and seeded in a 24-well plate (2.78×10⁶ cells/well) in DMEM media supplemented with 10% fetal bovine serum (FBS, Gibco Lab, USA) and incubated at 37˚C in 5% CO₂.

Interferon and viability of CTVT cells: Serial dilutions of rFeIFN-ω (Virbac, France) between 10⁴ and 2×10⁶ IU/100µl were added to each well in the same day after initial isolation. Cell viability was counted with a hemocytometer using trypan blue dye and reported as the total number of viable cell per well in 24, 48 and 72 hours (hrs) after treated with rFeIFN-ω. The results from triplicate wells were averaged and expressed as the percentage of the viability of treated CTVT cells compared with the untreated CTVT control cells counterpart at the same time point. Means and standard error of mean (SEM) of the percentage of the viability of control cells were plotted with varied rFeIFN-ω concentration to generate a dose response curve. A one-way analysis of variance (ANOVA) was used to compare the means, followed by the Tukey’s Multiple Comparison test (P<0.05 is considered significant)

Results and Discussion

The mean percentage (±SEM) of viability of primary CTVT culture treated with varied concentration of rFeIFN-ω at 24, 48 and 72-hour post treatment were shown in figure 1-3, respectively. CTVT cells were sensitive to rFeIFN-ω at concentration higher than 10⁶ IU/100 µl at 24 and 48 hrs after treatment. However, CTVT cells showed the sensitive effect at 72 hrs at lower concentration (10⁵ IU/100µl) after treatment. These findings indicated that CTVT cells were sensitive to the effect of rFeIFN-ω in a dose-dependent manner and might be involved with the duration time of exposure. This study was the first observation in order to evaluate the in vitro effect of rFeIFN-ω in various concentration on CTVT cells. However, the antiproliferative effect, the role of MHC expression induction and the combination effect with IL-6 from
TILs are remained obscure and need to be clarified in the future study. This commercial feline-derived IFN might encourage trials in vivo study to evaluate the efficacy of this optional treatment. Similarly, rFeIFN-ω has been used for safety study in 20 feline fibrosarcoma cases. At the final evaluation (day 360), 10 cats were disease free. Moreover, the efficacy of this treatment has to evaluate by performing the placebo-controlled trials in the further study (2).

Figure 1 A dose response curve between rFeIFN-ω and cell viability at 24 hours after treatment. (*,**, p<0.05)

Figure 2 A dose response curve between rFeIFN-ω and cell viability at 48 hours after treatment. (*,**, p<0.05)

Figure 3 A dose response curve between rFeIFN-ω and cell viability at 72 hours after treatment. (*,**, ***, p<0.05)

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References
Factors influencing anti-Müllerian hormone (AMH) levels in dogs: the effects of body sizes, age and reproductive cycles

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Keywords: age, anti-Müllerian hormone (AMH), dog, estrous cycle, size

Introduction
Anti-Müllerian hormone (AMH) is a dimeric glycoprotein of the transforming growth factor-β superfamily that involves in growth and differentiation of reproductive organs (1). The AMH is generated from gonadal somatic cells. This hormone is synthesized and secreted by Sertoli and granulosa cells in male and female animals, respectively (2). In female animals, the AMH specifically expresses in preantral and antral follicles within the ovarian cortex. The levels of AMH expression have been demonstrated to detect in different types of ovarian follicles. The highest levels of this hormone were found in small antral follicles (less than 4 mm in diameter). However, it did not obviously detected in large and preovulatory follicles (3). The AMH functions to suppress and control the growth and numbers of preantral and small follicles. This hormone has therefore been used to detect ovarian resting follicle pools or ovarian reserve (4). Several factors have been demonstrated to affect the levels of AMH such as reproductive status, age and size (5). However, information on factors associated with the level of this hormone in domestic dogs has been limited. This study aimed at determining the factors that affected the levels of AMH in dogs. The parameters to be examined included stage and status of reproductive cycle, size and age of dogs.

Materials and Methods

Animals: A total of 32 female dogs with different breeds, ages, and reproductive status and cycles, were carried out at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. In experiment 1, the effects of size were examined. Female dogs were divided into 3 groups according to their body weight as follows: small size (<15 kg; n = 15), medium size (15 – 40 kg; n = 7) and large size (>40 kg; n = 10). Blood was collected via cephalic vein. The blood samples were allowed to coagulate, and the serum was collected and kept at -80 °C for AMH determination. Experiment 2, the serum levels of AMH in small size dogs (n=15) with different ages (1-3, 4-10 and >10 years old) were determined. The effects of estrous cycles (anestrus, diestrus and estrus) on serum AMH in 23 dogs were examined in experiment 3. In addition, the effects of reproductive status on the levels of AMH (experiment 1) on AMH levels were determined in experiment 4. The blood samples were collected as previously indicated at before (day 0) and after neutering (day 4 post ovariohysterectomy, OVH). Hormonal assay: Serum AMH concentrations were measured by Canine Anti-Müllerian hormone ELISA test kit (Neoscientific®), which has been validated for dogs. A serial dilution of canine serum was parallel to the standard curve of the assay. Samples were run in duplicate and measured at OD 450. Sensitivity of this ELISA test was 0.1 ng/ml and the species cross-reactivity has not been specifically detected. Statistical analysis: the data were present as mean ± standard error of the mean (SEM). The statistical differences for the effects of age, size, and reproductive cycles on AMH levels were analyzed by One-way Analysis of Variance (ANOVA) with Turkey Post-hoc test. Dependent T-test was used to compare AMH levels between intact and neutered dogs. The statistical analysis was performed using SPSS version 22, and the p value less than 0.05 was considered statistical difference.

Results and Discussion

In experiment 1, the mean levels of serum AMH were found to be different in dogs with different sizes. The dogs with size between less than 15 and 40 Kg (small to medium size) had similar AMH levels (4.02 ± 1.37 ng/ml and 4.39 ± 0.54 ng/ml, respectively). However, large size dog with weight more than 40 Kg had significantly lower AMH levels compared with small and medium size dogs (P<0.05). Experiment 2 demonstrated the effects of age on AMH levels. We found that the AMH levels were highest during young to medium ages (less than 4 year to ten years) and become low in aged animals. Dogs with age less than 4 years had mean AMH level similar to that observed in dogs aged between 4 and 10 years (2.76 ± 0.55 ng/ml vs. 1.86 ± 0.15 ng/ml, P>0.05). This findings was contradictory with the results obtained from senior dogs (>10 years) in that they significantly had lower AMH levels (1.31 ± 0.13 ng/ml) compared with other ages (P<0.05). This supports the principles that the AMH is secreted by the active antral follicles. This AMH is therefore used as a marker to indicate ovarian reserve in humans and animals (1,6). As this hormone involves the reproductive status, the experiment 3 defined the effect of reproductive status on AMH levels. We found in both breed sizes (small and medium size) that the reproductive cycles significantly affected the AMH
levels. The serum AMH levels did not significantly change during diestrus to anestrus. These AMH levels obtained from these reproductive statuses were significantly lower as compared with estrous period as shown in Table 1. It is likely that the AMH may increase simultaneously when the follicle stimulating hormone initiates and recruits the small follicle from resting pool of the preantral follicles (4). The experiment 4 demonstrated convincingly that the ovaries is a major source of this hormone as the levels of AMH significantly reduced soon after OVH when compared with intact animals (0.86 ± 0.13 ng/ml vs. 3.76 ± 0.95 ng/ml, P<0.05). The findings in this study as well as previously reports confirm that AMH plays a role in reproductive physiology and AMH levels can be further used as a biomarker for determining ovarian activity in domestic dogs (7,8). Further study should use this hormone as a diagnostic tool to determine the ovarian reserve for ovary stimulation and also the remaining of ovarian tissue (ovarian remnant syndrome) after OVH.

**Table 1:** The effects of reproductive cycles on AMH levels in dogs with different body size. The AMH levels (ng/ml) were determined in dogs during different stages of reproductive cycles. The values are presented as Mean ± SEM.

<table>
<thead>
<tr>
<th>Body size</th>
<th>Reproductive cycle</th>
<th>Anestrus</th>
<th>Diestrus</th>
<th>Estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small size (&lt;15 Kg)</td>
<td></td>
<td>2.19±0.41</td>
<td>1.98±0.36</td>
<td>4.38±1.03*</td>
</tr>
<tr>
<td>Medium size (15-40 Kg)</td>
<td></td>
<td>2.35±0.26</td>
<td>1.45±0.17**</td>
<td>5.97±0.54*</td>
</tr>
</tbody>
</table>

a,b With in row, superscripts denote values that considerably significant difference (P<0.05

**Acknowledgements**
Authors would like to sincerely thank to staff of Division of Obstetrics, Gynaecology and Reproduction, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University for assistance in sample and data collection.

**References**
Fatigue study of cortical stainless-steel screws with PMMA and SOP plate system for immobilization of vertebral fracture and luxation in canine cadavers

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Keywords: cycles to failure, failure mode, Polymethylmethacrylate, stress concentration, SOP plate system

Introduction
Vertebral fracture and luxation (VFL) is one of the most common neurological damages in dogs and cats globally (4,7). One of the commonly cause of VFL is vehicular injury, with attacks on the lumbar vertebrae. The prevalence of these cases in dog has been showed in 39%(6). Lumbar VFL causes severe clinical outcomes such as both hindlimbs paralysis. In case of the patient with severe neurological deficit, acute compression of the spinal cord or an instable spine, should be treated with a surgical procedure. The standard treatment for vertebral bodystabilisation of canine VFL are screws with polymethylmethacrylate (PMMA) and the string of pearls (SOP) plate system fixation (1, 3, 5, 7). Moreover, the VFL with implants are usually exposed to repetitiveforce or cyclic loading fashion. Indeed, stress on the implant cyclically at a load significantly less than its ultimate tensile strength can cause fatigue failure, one of the major causes of implant breakage. Therefore, the aims of this study were to compare the failure mode of two different implants using a specifically designed fatigue testing machine (FTM).

Materials and Methods
A total of 10 lumbar vertebral specimens (L1-L6) were collected from mature canine cadavers (15-25 kg body weight in range) which died at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The specimens which had a history of vertebral column disease were excluded. Laterolateral and dorsoventral radiographs were done to confirm the skeletal maturity and the absence of vertebral pathology of the specimen. Hypaxial and epaxial muscle, spinal ligaments and joint capsules were stored. Specimens were kept moist with 0.9% saline solution during preparation, storage, and testing (2). The vertebral column specimen was secured in the FTM by inserting Steinman pins (4 mm in diameter) transversely through the L1 and L6, passing through the hole on the specimen’s fixator part of the FTM. Then, all the specimens were assigned equally to one of two testing construction which were Cortical screws with syringe application PMMA (CorP) and Cortical screws with SOP plate (SOP) group, matched to the body weight of the cadaver. The two different implants were constructed as follows: 3.5 mm cortical screws with syringe application of PMMA (CorP) and 3.5 mm cortical screws with a SOP plate (SOP). All specimens were subjected to fatigue testing by a specifically designed FTM after fixation of the L3-L4 vertebral bodies. The mean number of cycles to failure and the failure mode of all fixation methods were compared(8).

Results and Discussion
The results revealed that the SOP implant was more resistant to failure than CorP group, with the higher number of cycles before the implant failure. The point of weakness, as defined by the failure mode, occurred in PMMA at the screw neck position of CorP group and 3.5 mm cortical screw in SOP group. Consequently, SOP plate system possessed the greater fatigue properties in this study, thus the failure mode still occurred at the cortical screw neck. This implies that the SOP plate system failed due to the stress concentration effect. Whereas the failure mode of the CorP construction arisen at the PMMA bridge, at the junction between cortical screw and PMMA. This mixed material construction was also affected by fatigue limit, which was lower than stainless steel,
indicating that the PMMA bridge was the weakest point of CorP construction. In conclusion, based on the results of this study, it is recommended that the SOP system should be used as the standard treatment for VFL in dogs as it is most resistant to implant failure.

**Table 1** Mean ± SD of cycles to failure and failure mode of each construction group

<table>
<thead>
<tr>
<th>Group</th>
<th>Cycles to failure</th>
<th>Failure mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bridging compartment</td>
<td>Bone anchoring compartment</td>
</tr>
<tr>
<td>CorP</td>
<td>49,550 ± 6,392 a</td>
<td>PMMA Cortical screw</td>
</tr>
<tr>
<td>SOP</td>
<td>112,820 ± 7,562 b</td>
<td>SOP plate Cortical screw</td>
</tr>
</tbody>
</table>

*Different letters (a, b) with in the same column represent significant differences (p<0.05).

**Acknowledgements**

This study was supported by a research grant from the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). Moreover, we would like to thanks for all staffs from the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University for data and specimens.

**References**

The first report of the Canine trachea extragenital transmissible venereal tumor

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Keywords: Transmissible venereal tumor, tracheal resection and anastomosis, canine

Introduction
Mass in the tracheal dog is uncommon but cause of respiratory distress (1). such as coughing, cyanosis, inspiration dyspnea that is life-threatening. This mass possible to benign or malignant. Benign tracheal tumors include chondroma, osteochondroma, ecchondroma-osteochondroma dysplasia and leiomyoma. Malignant tracheal tumors include Osteosarcoma, Chondrosarcomas, Mast cell Tumor, Lymphoma, and Squamous cell carcinoma (2)., 82% were malignant and 18% were benign (3). In this case, report extragenital transmissible venereal tumor in tracheal dog without a history of the abnormal mass in a genital area.

Materials and Methods
Donut, a 7.8 kg, 10-year-old male crossbreed dog was brought to the Prince of Songkla University Veterinary teaching hospital because he was having difficulty breathing, noisy breathing, vomit, intermittent cyanosis and exercise intolerance. From physical examination found generalize mild increase lung sound, cyanosis when palpating around trachea. Hematology analysis found thrombocytopenia and ELISA based kit (IDEXX Laboratories, Inc, USA) resulted positive Anaplasma spp. and Ehrlichia canis. Cervical and thoracic radiographs present mass within the middle of trachea (Fig. 1) then surgical removal was considered.

Figure 1 The radiography presented an intra tracheal soft tissue radiopacity mass size 1x1.5 cm with rooted at the dorsal tracheal border (white arrow).

The patient was surgical remove the mass together with two invaded tracheal rings (fifth and sixth rings) (Fig. 2A) by tracheal resection, then end to end anastomosis (Fig. 2B).

Figure 2 Post-tracheal resection, the mass adhered to the trachealis muscle along with two tracheal rings (A) and Tracheal resection, then end to end anastomosis (B)

After the recovered the patient recovered without abnormal respiratory signs. A month after the surgery, the patient presented normal respiration and appetite. However, the patient will be appointed for tracheal and chest radiography every 2-3 months for the recurrence. Microscopic findings of tracheal mass biopsy presented the mass consisted of dense sheets and nests of neoplastic round cells. Some lymphocyte and plasma cells were seen around the edge. Definitively, the biopsy was reported transmissible venereal tumor (Veterinary Pathology Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University) (Fig. 3).
These cells had round, slightly eccentric nuclei prominent nucleoli (White arrow) and moderate amounts vacuolated of pale basophilic cytoplasm (black arrow). (Hematoxylin and Eosin stain, 100X magnificent)

Figure 3 Microscopic findings of tracheal mass biopsy.

Results and Discussion
Although tracheal tumors are rare in dogs but this can be found include chondroma, osteochondroma, lymphoma, and squamous cell carcinoma, mast cell tumor, extramedullary plasmacytoma, and leiomyoma (4). This transmissible venereal tumor may be a tumor, which should be listed in the differential diagnoses for tracheal neoplasia in dogs even without a history of an abnormal mass in the genital area and that surgical treatment alone may be curative.

Acknowledgements
This case accomplished by the cooperation of the surgery and ICU unit veterinarians and veterinary nurses of the veterinary teaching hospital, Faculty of Veterinary Science, Prince of Songkla University and We would like also thanks, Dr. Katriya Chankow for histological interpretation support.

References
Genomic characterization and comparative analysis of *Leptospira interrogans* isolated from the urine of asymptomatic dogs, Thailand

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**Keywords**: Genome, *Leptospira interrogans*, Dogs, Thailand

**Introduction**

*Leptospira* are Gram negative with spiral-shaped bacteria caused worldwide important zoonotic disease especially in tropical country including Thailand (1, 2). *Leptospira* can infect most of mammalians and produce a wide range of clinical manifestation from acute to chronic infection. To date, *Leptospira* is classified to 3 groups; pathogenic, intermediate and non-pathogenic group with 22 species base on *rrs* phylogeny. Previously, two pathogenic *L. interrogans* were isolated from the urine of asymptomatic dogs in Thailand (2), and the epidemiological important of these isolates was remarkable in term of genetic content associated with chronic infection. Since the next-generation sequencing was popular in the last decade, several *Leptospira* species have been sequenced and published in the database. Nevertheless, the evidence of Thai *Leptospira* strains is still lacking. Herein, we report the draft genome sequences of two *L. interrogans* and compare them with four representative published strains. This information might be allowed us to understand insight of genetic content related to latent infection in animals.

**Materials and Methods**

**Bacterial strains**: In 2014, two *L. interrogans* strains CUDO5 and CUDO8 (Thai strains) were isolated from the urine of asymptomatic dogs in Nan province, Thailand, and were identified species by the *rrs* sequencing and phylogeny (2). These isolates were passedage less than five times, purified and stored as a stock culture in -80 °C. Five hundred microliters of stock cultures were inoculated in 5 mL of liquid EMJH with 3% rabbit serum and incubated at 28 °C for two weeks. To check other bacterial contamination, 300 µL of each culture was spread on to blood agar and incubated at 37 °C for two days. Purified leptospires were then centrifuged, extracted for DNA isolation and kept at -20 °C before library preparation and genome sequencing.

**Genome sequencing and de novo assembly**: Genomic DNA was evaluated either quality or quantity using spectrophotometer (NanoDrop™, Thermo Fisher Scientific, USA) and Qubit™ Fluorometric Quantitation (Thermo Fisher Scientific, USA) before library preparation. Library preparation was generated using Nextera XT DNA Library Preparation kit (Illumina, USA) followed manufacturer’s protocol. Genome sequencing was carried out using Illumina-MiSeq with 250 paired-end run cycles. De novo genome assembly was conducted by A5-Miseq pipeline (3). Scaffolds were then oriented and ordered in comparison of the whole genome sequence of *L. interrogans* serovar Lai strain 56601 using ABACAS (4). Gaps among scaffolds were closed by IMAGE program (5).

**Genome annotation**: Draft genome of two *L. interrogans* were annotated using rapid prokaryotic genome annotation (PROKKA) (6) and Rapid Annotation using Subsystem Technology (RAST) version 4.0 (7).

**Comparative genome analysis**: To identify the core genes sharing among of *Leptospira* genus and strain-specific genes, the genome of two Thai *L. interrogans* strains were compared with the four representative published complete genomes of other *Leptospira* species comprising *L. interrogans* strain 56601, *L. interrogans* strains Fiocruz L1-130, *L. borgpetersenii* strain L505 and *L. biflexa* strain Patoc using OrthoVenn web service with the default parameters (8).

**Results and Discussion**

The summary results of *de novo* genome assembly are shown in **Table 1**. The final assembly processes generated 163 contigs with 124.47× coverage, and the genome size was estimated ~4.93 Mbp with 35.1% of GC content in strain CUDO5 whereas strain CUDO8,
83 contigs were obtained with 109.34× coverage after assembly process, and the genome size was estimated ~4.90 Mbp with 35.1% of GC content.

**Table 1** Summary of genome assembly in two Thai *Leptospira* isolates

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. Contig</th>
<th>Coverage</th>
<th>Size (Mbp)</th>
<th>No. CDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CUDO5</td>
<td>163</td>
<td>124.47</td>
<td>~4.93</td>
<td>4040</td>
</tr>
<tr>
<td>CUDO8</td>
<td>83</td>
<td>109.34</td>
<td>~4.90</td>
<td>4013</td>
</tr>
</tbody>
</table>

PROKKA annotation identified 4,040 and 4,013 protein-coding sequences (CDS) in strain CUDO5 and CUDO8, respectively. They contained one copy of three ribosomal RNA (rRNA) genes including 5S, 16S and 23S. RAST predicted 323 and 320 sub-systems in strains CUDO5 and CUDO8, respectively. CDSs of both strains were mostly related in Cofactors, Vitamins, Prosthetic Groups, Pigments group, RNA Metabolism group, Motility and Chemotaxis group and Amino Acids and Derivatives group.

In comparative genome analysis, 1,860 orthologous proteins were shared among representative *Leptospira* species. Protein-encoded genes that related to rRNA binding were enriched in this analysis (P<0.05) and associated with survival of *Leptospira* (Figure 1).

Eighteen and 21 strain-specific genes were identified in strain CUDO5 and CUDO8, respectively. Genes related to stress responses and rhamnose metabolisms were mostly identified in both of strains which might be associated with specific function leading *Leptospira* infection in chronic infection. To response in stress condition such as temperature, humidity, ionizing radiation or even kidney milieu, *Leptospira* might use these genes for adaptation (9). Rhamnose is one of the components in lipopolysaccharide (LPS) structure of several gram negative bacteria including *Leptospira*. Increasing expression of LPS associated with chronic infection in mice model and might aid *Leptospira* to attach renal cell and to avoid immune response (10). Our analysis provided genetic information insight of the *Leptospira* genome from asymptomatic dogs in Thailand.

**Acknowledgements**

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Innovation of Veterinary Anatomical Models by Using Waste Paper

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Keywords: Papier Mache, invention, veterinary anatomy, waste paper, canine head,

Introduction

In the past until present, the formalin fixed cadavers have been applied for both medical and veterinary anatomical teaching (1). Although formaldehyde is an outstanding tissue fixative, it might function as an irritant that causes mild eye, mucous membrane complaints as well as might be a carcinogen that reasons the respiratory cancer (2, 3). However, the order of preference for teaching methods was cadaveric dissection by students, prosection, living and radiologic anatomy, computer-aided learning, lectures alone, and the use of anatomical models (4). Up to date, the veterinary anatomical models have been alternatively utilized in the gross anatomy laboratory for teachers and students (5). The advantages of veterinary anatomical models are found in various reasons, for instance, the teachers and students are safe from the toxic agents, the students are able to study all the times they require and they can schedule and reschedule their learning times (6). Furthermore, the prospect of using anatomical models is to replace the formalin fixed specimens, to reduce the cost and quantity of animal specimens (7).

Papier Mache was originated in China, started as a commercial medium in France and has been found all over the world because it is inexpensive and can be sculptured in numerous designs (8). Therefore, the objective of this study was to develop methods to invent veterinary anatomical models with “Papier Mache” fixed formula by using different types of waste paper.

Materials and Methods

A canine half-head was collected from formalin fixed carcass and preserved with good quality for dissection. The plaster of canine half-head molds and the silicone rubber were prepared by artistic techniques. The Paper Mache technique made from various kinds of waste paper including A4 paper, packing paper, core of tissue paper and milk box paper, were performed in this study. Briefly, each type of waste paper was immersed in the water for 24 hours and minced the moisture waste paper with the blender machine. The pulp paper was mixed with tapioca flour and latex adhesive in the proportion of 10:5:3, the mixture was kneaded homogeneously and fills in the canine half-head mold. The molds were left in the room temperature until dry and the canine half-heads from Papier Mache were decorated as present in the original specimens. Finally, the anatomical models were evaluated about the satisfaction indexes by the 2nd year veterinary students (n=20) from Faculty of Veterinary Medicine, Chulalongkorn University. The criteria used in the assessment are the satisfaction levels (0-5). All data were statistically analyzed with t-test independent and ANOVA by experienced statistician.

Results and Discussion

The canine half-heads by A4 waste paper made by Paper Mache method were demonstrated in Figures 1 and 2 whereas the results of the satisfaction indexes from the veterinary students were illustrated in Table 1. The findings indicated that the veterinary anatomical model, i.e. canine half-head, was able to be made from various types of paper with “Papier Mache” method. The waste paper composed of A4 paper, packing paper, core of tissue paper and milk box paper, were applied to be theraw materials and prove that these veterinary anatomical models are effective with various aspects, for example, easy to storage, easy to apply and move, light weight, shape and color are similar to real conditions. Definitely, these models can be used to enhance learning in the veterinary anatomy laboratory.
**Figure 2** Canine half-heads from A4 waste paper made by Paper Mache method before decoration were shown the characteristics on the left lateral (A), right lateral, left medial (C) and right medial (B) sides.

**Figure 2** Canine half-heads from A4 waste paper made by Papier Mache method after decoration were verified the anatomical details in the lateral (A) and medial (B) sides.

**Table 1** The satisfaction indexes of the veterinary anatomical models made by Papier Mache method which were categorized into 5 levels (5, most satisfaction; 4, very satisfaction; 3, moderate satisfaction; 2, scant satisfaction; and 1, dissatisfaction) and evaluated from 2nd year veterinary students (n=20) (Mean±SD).

<table>
<thead>
<tr>
<th>Satisfaction indexes</th>
<th>A4 paper</th>
<th>Packing paper</th>
<th>Core of tissue paper</th>
<th>Milk box paper</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Durable</strong></td>
<td>4.40±0.46a</td>
<td>3.35±0.76b</td>
<td>4.55±0.47a</td>
<td>3.75±0.62b</td>
</tr>
<tr>
<td><strong>Maintenance after use</strong></td>
<td>4.45±0.36a</td>
<td>3.65±0.87b</td>
<td>4.65±0.34a</td>
<td>3.95±0.68b</td>
</tr>
<tr>
<td><strong>Easy to storage</strong></td>
<td>4.65±0.24a</td>
<td>4.35±0.45b</td>
<td>4.65±0.25a</td>
<td>4.25±0.30b</td>
</tr>
<tr>
<td><strong>Easy to apply and move</strong></td>
<td>4.90±0.09a</td>
<td>4.85±0.24a</td>
<td>4.80±0.27a</td>
<td>4.75±0.30b</td>
</tr>
<tr>
<td><strong>Firmness</strong></td>
<td>4.35±0.23a</td>
<td>3.20±0.90b</td>
<td>4.50±0.36a</td>
<td>3.35±0.55b</td>
</tr>
<tr>
<td><strong>Weight of item</strong></td>
<td>4.45±0.47a</td>
<td>4.65±0.24a</td>
<td>4.25±0.62a</td>
<td>4.55±0.36a</td>
</tr>
<tr>
<td><strong>Similarity to real material</strong></td>
<td>4.45±0.47a</td>
<td>4.20±0.48a</td>
<td>4.50±0.47a</td>
<td>4.05±0.68b</td>
</tr>
<tr>
<td><strong>Replace real specimen</strong></td>
<td>4.30±0.43a</td>
<td>3.95±0.58a</td>
<td>4.25±0.72a</td>
<td>3.95±0.79a</td>
</tr>
<tr>
<td><strong>Enhance for studying</strong></td>
<td>4.65±0.45a</td>
<td>3.65±0.87a</td>
<td>4.00±0.95a</td>
<td>3.50±0.68b</td>
</tr>
<tr>
<td><strong>Overall satisfaction</strong></td>
<td>4.50±0.26a</td>
<td>3.75±0.40b</td>
<td>4.50±0.47a</td>
<td>3.30±0.43a</td>
</tr>
</tbody>
</table>

*Different superscripts (a, b, c) differ significantly (p<0.05)*

Considering to the satisfaction indexes, the canine half-head made from the A4 and packing paper demonstrated significantly (p<0.05) differences in the criteria of the durable, maintenance after use, firmness and overall satisfaction compared to the models made from the core of tissue paper and milk box paper. While the other criteria were not found significantly different.

The veterinary anatomical models can be invented by Papier Mache method with the good quality and are definitely replaced the formalin fixed specimens in the anatomy laboratory. Additionally, the advantages of the use of anatomical models made from varieties of waste paper in this study can reduce the number of animal in teaching veterinary anatomy; reduce cost of animal specimens and other models from abroad; manage waste garbage; and reduce the risks of toxic chemicals or carcinogens that harm to human health corresponded to the previous study (2, 3). Importantly, the methods to invent the anatomical models with Papier Mache fixed formula method in this study might be repeated and are able to produce good quality models equally to or better than the models produced from manufacturer with other materials. It is known that the veterinary anatomy education is not only an important part of the veterinary curriculum, but also supports to further the enhancement veterinary professionalism (9). As described in the previous study, the successful teaching and learning anatomy must include the prosected cadaver specimens, life models, radiologic images, telescopic views of the living body maximize learning and other anatomical models(4). Therefore, the innovation of veterinary anatomical models with the good quality as evaluated by veterinary students with satisfaction factors in the present study could be an essential part of the veterinary anatomy teaching and learning in the new era.
Acknowledgements
The authors would like to thank Assoc. Prof. Dr. SompornTechangamsuwan for reviewing the abstract, Mr. Garn Chuesiri for statistical analysis, the 2nd year veterinary students, Faculty of Veterinary Science, Chulalongkorn University for evaluating results. The present study was financially supported by the Faculty of Veterinary Sciences, Chulalongkorn University, Thailand.

References
Investigation of UVB-Radiation Induced on Canine Keratinocytes Cell Line

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**Keywords:** UVB-Radiation, Canine, Keratinocytes, cell culture

**Introduction**

UVB is a well-known cause of cutaneous neoplasia. It produces a characteristic mutation at sites in DNA where two pyrimidine bases (i.e. cytosine and thymine) are found together. The radiation produces a dimer of these molecules that can lead to mutation and subsequently turn to be cancer (1). The majority of neoplasm that are induced by UV irradiation arise in the site of maximal exposure, epidermis. In human, UV exposure increases the risk of skin cancer, i.e. squamous cell carcinoma and basal cell carcinoma. Malignant melanoma may also be related to UV exposure, but the evidence is less persuasive. Squamous cell carcinoma is associated with UV exposure in animals and these tumor overexpress p53 and Ki67 (2). However, more data is needed to determine of carcinogenesis of skin neoplasia induced by UV radiation in animals. This investigation is a part of the research to study using of canine keratinocytes cell line on its cytologic change after UVB-induced.

**Materials and Methods**

**Cell culture:** Canine progenitor epidermal keratinocytes (CPEK) (CELL*TEC Advanced Cell Systems, Switzerland) plate 5x10^5 cells with DMEM without antibiotic at 6-well plate incubated in 37°C and 5% CO2. At approximately 50% of culture confluent were used for investigate UVB-radiation.

**UVB-radiation:** Cultured cells were UVB irradiated (emission peak at 312 nm) with a DNA crosslinker light (ItfLabortechnik, Wasserburg, Germany) (3) at 30 second, 30 mins and 1 hour respectively.

**Monitoring DNA damaged:** to define UV-induced DNA damage. Immunohistochemical technique of anti-gamma H2A.X (phosphoS139) antibody (Abcam®) visualized with DAB counterstaining were used.

**Monitoring cell vitality:** to define the cell vitality and cytotoxicity. Immunofluorescence technique of Fluorescence-base Molecular Probes, LIVE/DEAD™ assay (ThermoFisher) were used.

**Results and Discussion**

The cytologic change of cultured canine keratinocytes cell line were initially detected at 30 min after UVB radiation exposure. The H2AX staining of cultured cells after UVB exposure showed brownish color in diffuse pattern or panuclear, which are detectable under immunohistochemistry and increase intensity with consistently appearance. This appeared due to the quantitative for DNA double-strand breaks in longer UVB exposure time. However, the LIVE/DEAD staining showed reddish color in the nucleus while live cells were greenish. That staining could be detected in low intensity after 10 second and gradually in high intensity after 1 hour exposure respectively.

Phosphorylated H2AX (γH2AX) formation showed the potential tool for study DNA double-strand breaks formation. This investigation reported the ionizing radiation and UVB induced DNA damage in the culture canine keratinocyte cell line. The initially effect was detected with H2ax after 30 mins exposure. Additionally, the vitality of cells by LIVE/DEAD staining assay was obviously detected after 1 hour exposure due to the toxic effect of cultured cell. Upon the investigation, the UVB radiation could produce the effect on DNA damage in the cultured cells after 30 mins exposure. Further investigation should be conducted for the carcinogenesis of skin cancer in dog.

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**References**

In vitro effects of tetracycline and enrofloxacin on microfilaria motility of Dirofilaria immitis

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Keywords: Wolbachia, microfilaria, motility, tetracycline, enrofloxacin

Introduction

Dirofilariasis is caused by filarial nematodes in the genus Dirofilaria, including Dirofilaria immitis, Dirofilaria tenuis and Dirofilariaursi. D. immitis mainly resides in the right ventricle and pulmonary artery of the dogs and cats, while D. repens generally resides in the subcutaneous tissue of dogs, foxes and cats. Recently, Wolbachia, Gram-negative bacterial endosymbionts of arthropods and filarial nematodes, has been considered as a potential target for treatment of filarial infections. There are some in vitro and in vivo experiments have been revealed that treatment of antibiotic with chemotherapeutic efficacy against the bacterial group which Wolbachia belongs, e.g., tetracycline is effective. Unfortunately, informations of proper dosage and treatment protocol in anti-Wolbachia antibiotics in animals are insufficient. Moreover, studies on the efficacy of enrofloxacin has not been established. The objective of this study to optimize dose of tetracycline and enrofloxacin, one of the most common antibiotic used in veterinary field, against Wolbachia spp. in microfilaria of D. immitis, in vitro.

Materials and Methods

Blood samples were collected at the Veterinary Teaching Hospital of Mahidol University. Filarial worm infections were screened by fresh smear technique and microfilariae were morphologically confirmed by using Giemsa staining technique. Microfilaraemia was calculated before using in vitro assay.

5 ml of EDTA-blood were collected from infected dogs. Plasma were washed 3 times with RPMI-1640 medium, then, washed 5 times with RPMI-1640 containing Streptomycin, Amphotericin-B and Penicillin. Microfilarial content were transferred to 96-well plates adding RPMI-1640 supplemented with 25 mM HEPES buffer, 2 mM glutamine, 10% fetal calf serum, Streptomycin, Amphotericin-B and Penicillin at 37°C in a 95% air-5% CO2 atmosphere. Microfilariae were divided into one control and two drug treatment groups (<50 microfilaria) well and performed duplicate experiments. Tetracycline and enrofloxacin at final concentrations between 0.25 to 256 µg/ml, prepared in a serial two-fold dilution will be used. final concentration as 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256. Microfilaria motility and death were assessed visually by inverted microscope. The observations were performed every 12 hours and scored as 0, immotile or dead; 1, slightly active; 2, active and motile; 3, moderately active; and 4, highly active. The data were analyzed by Kruskal-Wallis methods and differences were considered significant with P values <0.05.

Results and Discussion

Microfilaria were cultured with different concentrations of antibiotics. Compared with control group, motility score of microfilaria treated with tetracycline were markedly reduced as shown in table 1. Whereas, only high concentrations of enrofloxacin gradually reduced worm motility relative to control microfilariae cultured in medium without antibiotics.

Table 1. Effect of antibiotics on Dirofilariaimmitis microfilaria motility. Motility was assessed visually and the observations were scored as 0-4. The scoring of motility was compared with the motility of worms in control group.

<table>
<thead>
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<th>Enrofloxacin Concentration</th>
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<tr>
<td>Day 7</td>
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On day 7, these decreases in motility of tetracycline at 16, 64, 256 µg/ml and enrofloxacin at 256 µg/ml compare to control were statistically significant (P<0.05). This present study has confirmed some previous studies on the effects of tetracycline on...
microfilarial motility and suggest new antibiotic, enrofloxacin, as a substitute.

Wolbachia endosymbionts are found in many species of filaria infecting animals and humans, including *D. immitis*. Depletion of Wolbachia bacteria drastically affects worm fertility and motility. These effects led to the definition of Wolbachia as targets for a novel chemotherapeutic approach for canine dirofilariasis with tetracyclines group. The benefits of anti-Wolbachia treatment in canine heartworm disease have been well documented. For instance, some studies revealed that pretreated with tetracycline before melarsomine administration can reduce post-treatment complications and mortality in dogs naturally infected with *D. immitis*. Besides, according to WHO and American Heartworm Society, the increase of macrocyclic lactones resistance in filariasis (e.g. canine dirofilariasis) has been risen up progressively. In conclusions, anti-Wolbachia antibiotic in group tetracyclines should be considered as a future gold standard for prevention and control of canine dirofilariasis while enrofloxacin can be used as a substitute drug.

**Acknowledgements**
Authors would like to thank the Veterinary Teaching Hospital of Mahidol University for providing positive samples.

**References**
A Novel Targeted Therapy in Canine B-cell Lymphoma

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Keywords: ABC subtype, diffuse large B-cell lymphoma, dog, GCB subtype, treatment

Introduction
Diffuse large B-cell lymphoma (DLBCL) is the most common histological subtype in dogs (1). In human study DLBCL was classified into two subgroups according to gene expression profiling; germinal center B-cell (GCB) like and activated B-cell (ABC) like. Patients with GCB DLBCL had better prognosis than ABC DLBCL because they were responsive to standard chemotherapy (CHOP, vincristine, cyclophosphamide, doxorubicin, and prednisolone) with rituximab, an anti-CD20 monoclonal antibody (2). Besides genomic methods, immunohistochemical algorithms have been developed for evaluating cell of origin and correlating to clinical outcome (3-6). Antibody panels that used to determine GCB and ABC subtypes are GCET1, CD10, MUM1, Bcl-6, FOXP1 and LMO2; however, each system presented diverse accuracy, sensitivity, and specificity to the gene expression profiles. Canine DLBCL was also classified into two subtypes similar to human based on microarray analysis and immunohistochemistry. In addition, this study reported that canine ABC DLBCL involved in the B-cell receptors and NF-κB signaling pathways resembling to human (7). On the other hand, the PI3K/Akt/mTOR pathway is probably promoted tumorigenesis in GCB subtype by downregulation of PTEN (8). Therefore, a specific target therapy based on molecular subtype might improve clinical outcome and relapsed/refractory disease.

Even though GCB patients have a good response and prolonged survival time with R-CHOP, mostly relapse or refractory diseases are reported at the first-line treatment. A novel target agent to inhibit PI3K was clinically used to treat human B-cell lymphomas. Copanlisib, a selective pan-class I PI3K isofrom p110α and p110δ, was studied on phase I to treat 6 patients with follicular lymphoma and 3 patients with DLBCL. Seven of them showed clinical response: all six follicular and one DLBCL (9). Furthermore, phase II and phase III trials were studied to treat relapsed follicular lymphoma and relapsed/refractory DLBCL with rituximab. This drug was approved by the US FDA for the treatment of relapsed follicular lymphoma in adults (10). Another therapeutic target drug is mTOR inhibitors. A single-agent everolimus was studied on phase II trial in patients with relapsed/refractory DLBCL and overall response rate (ORR) was 30% (11). Additionally this drug was applied concurrent with R-CHOP in relapsed DLBCL patients and yielded 96% complete response rate (12). Temsirolimus underwent a phase II trial as a monotherapy in DLBCL with 28% ORR (13). Another target pathway of GCB is Akt Nelfinavir and MK-2206, Akt inhibitor, combined with mTOR inhibitor showed synergic cytotoxic effect in DLBCL cell lines (14).

The nuclear factor kappa B (NF-κB) pathway is a therapeutic target in ABC DLBCL. The first-generation proteasome bortezomib combined with DA-EPOCH (dose-adjusted etoposide, vincristine, doxorubicin with cyclophosphamide and prednisolone) showed higher overall response rate and prolonged median overall survival time in ABC patients than GCB patients (15). This drug also combined with R-CHOP and showed 88% ORR in DLBCL; however, the clinical outcomes were not different between ABC and GCB (16). Not only the NF-κB signaling pathway, but the BCR signaling pathway is also a target therapy in ABC DLBCL. The BTK inhibitor ibrutinib was used as a single treated agent in refractory/refractory DLBCL. The ORR was 40% and limited to ABC subtype (17).

A target therapy is limited in veterinary oncology filed. Recently, a novel anti-canine CD20 antibody was generated and developed as a target treatment for canine B-cell lymphoma (18). Acalaibrutinib, a second-generation of BTK inhibitor, was investigated in in vitro and in vivo studies. A phase I clinical trial in 20 dogs with B-cell lymphoma showed 25% ORR with median progression free survival 22.5 days. Only mild degree of adverse effect was noted (19). INCBO40093, a novel, selective, and potent PI3Kδ inhibitor, had inhibitory effect of cell proliferation in canine B-cell lymphoma cell lines (20). A trend on target treatment is not only focused on signaling pathways, but specific mutation genes on each subtype is also one of therapeutic target, such as Bcl-2 inhibitor. However,
further investigation of novel target agents for efficacy needs to study on both canine GCB and ABC DLBCL in clinical trials.

References
17. Wilson et al., 2012. Blood. 120(suppl; abstr 686).
Occurrence of canine respiratory coronavirus in Thailand

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Keywords: canine, respiratory coronavirus, Thailand

Introduction
Canine respiratory coronavirus (CRCoV) is an emerging pathogen and was first detected in dogs with severe respiratory illnesses in UK (1). To date, CRCoV had reported worldwide (2,3). The virus causes mild to severe respiratory signs and occasionally fatal due to bronchopneumonia (4). Genetic characterization of CRCoV revealed that CRCoV is closely related to human coronavirus (HCoV-OC43) and bovine CoV and posed ability to cross-species transmission (5). In Thailand, human and bovine CoVs have been reported but the genetic information of CRCoV is still limited (6). The objective of this study was to survey CRCoV in Thailand during January 2017 – December 2017.

Materials and Methods
Sample collection: During January 2017 to December 2017, a total of 242 nasal swab samples were collected from respiratory illness dogs (sneezing, nasal discharge, ocular discharge, cough and dyspnea) from small animal hospitals. The samples were obtained from dogs of all age and gender.

Virus identification: Viral RNA extractions were conducted by using the QIAmp viral RNA mini kit (Qiagen, Hilden, Germany) following manufacturer’s suggestion. CRCoV was detected by using RT-PCR with gene specific primers.

Genetic characterization: The CRCoV positive samples were selected for whole and partial genome sequencing based on age of dog, breed, severity of clinical sign, location and collection date. The nucleotide sequences were assembled and validated by using SeqMan software version 5.03 (DNASTAR Inc., Madison, WI, USA). Phylogenetic analysis was performed by using MEGA version 6.06.

Results and Discussion
In this study, the occurrence of CRCoV was 12.8% (31/242) from sick dogs. By age group, out of thirty-one CRCoV positive samples, fourteen (45.16%) of positive samples were older age group (more than 5 years old). While, twelve (38.71%), four (12.90%) and one (3.23%) of positive samples were puppy (less than 6 months), adolescent (1-5 years old) and younger (6 months -1 year old), respectively (Figure 1). In relation of CRCoV by month, the positive samples were high during June – September (rainy season) (Figure 2). According to our preliminary result, CRCoV is highly circulating in sick dogs in Thailand. Moreover, the older and puppy dogs might be the susceptible age groups for CRCoV infection. The rainy season is one of the factors of CRCoV circulating in sick dogs. Up to date, the genetic characterization of virus is in-process.
Acknowledgements

We would like to acknowledge Chulalongkorn University for its financial support to the Center of Excellence for Emerging and Re-emerging Infectious Disease in Animals. We would like to thank the Thailand Research Fund for providing its financial support to RGJ Ph.D. scholarship to KC (PHD/0056/2557) and the TRF Senior Scholar to AA (RTA6080012).

References

Post-intubated tracheal stenosis diagnosed by ultrasonography

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Keywords: Dog, tracheal stenosis, ultrasound

Tracheal stenosis is the serious complication followed by prolonged intubation, oversize tube and excessive cuff pressure. The ultrasound can be the useful diagnostic tools to diagnosed the tracheal disease in dyspneic patient. This case report reveals a dog diagnosed tracheal stenosis by using ultrasonography

Introduction

With high incident of cobra bite envenomation in Thailand, the dog that was bitten by cobra will come to the hospital with respiratory paralysis. The dog will be intubated to provided proper respiration and oxygen. One of the most common complication followed endotracheal tube intubation in human is potential development of tracheal stenosis (1). There are only 3 cases in the dogs according to the author’s knowledge (2,3,4). Radiograph, CT scan and endoscope have been use as diagnostic tools for tracheal stenosis. However, sometimes dogs with the loud respiratory sound and respiratory distress are not stable enough to perform those procedures. The tracheal sonography will provide you basic information to plan for the further diagnoses and bring you final diagnosis. (5)

Patient case presentation

3 years old male dog, Neutered, Boxer breed with the history of Cobra snake bite. He was intubated to provided ventilation and oxygen for three days before he was recovered from the venom neurotoxin. One month later, he had been referred to Prasu-arthorn animal hospital, Mahidol University with the clinical sign of dyspnea, tachypnea, stertor and cyanotic mucous membrane. The radiographic image from the private clinic shows the suspicious of tracheal. The dog was sent to repeat radiograph at Prasu-arthorn animal hospital. The radiographic findings state that trachea had focal soft tissue opacity mass at the level of C3-C4. The trachea was circumference by this soft tissue opacity mass. Whereas, tracheal diameter reduced from 2 cm. to 0.8 cm. as you can see in figure 1. Then, the ultrasound was performed. Before that, the hair clipped from the midline at the level of larynx along with the thoracic inlet, with 2 inches wide. An ultrasound machine (GE Logic p6) with a 7.5 MHz linear transducer was used to investigate. Ultrasonic gel was gently applied to clipped area The dog was in dog sitting position and the neck was extended. The ultrasound starts from the larynx, use trachea as landmark and scan throughout the trachea. The result present on figure 2 comparing with normal tracheal wall of the same dog. The trachea cartilage present with hypoechoic layer and the tracheal lumen is described by hyperechoic line with reverberation artifact.

Figure 1: The image from Prasu-Arthorn animal hospital in right lateral projection.

Figure 2: The normal tracheal wall (left) and the thickened tracheal wall (right)

The dog is under general anesthesia to examined trachea by using endoscope. He was premedicated with intravenous 0.3 mg/kg diazepam, inducted with intravenous 4 mg/kg propofol and maintained with propofol intravenous throughout the procedure. Then, tracheal endoscopy was performed carefully to confirm the diagnosis. The tracheal endoscopy revealed focal stenotic tracheal lumen with inflammation, as shown in figure 3. We decided to send him to do the tracheal resection and anastomoses for treatment. The tissue was sent to pathology. He has recovered without any complication within 2 days.
The pathological result showed granulation tissue and interlining bundles of fibrous tissue. After the surgery, the dog came back at 1, 3, 6 weeks to monitor tracheal radiograph. No remission of tracheal stenosis was found.

**Discussion**

In human medicine, there are many complications of tracheal intubation such as upper airway organ trauma, tracheal perforation, tracheal stenosis etc. (Airway management) Either prolonged intubation or over-inflated cuff intubation can be the cause of tracheal stenosis. The severity depends on, how much does the pressure of endotracheal cuff and how long does the dog have been intubated. (6)

In this case, the tracheal mucosa was replaced by fibrous and granulation tissue. The wedge-shaped lumen was generated due to the nature contractility of fibrous tissue. Ischemic tissue of tracheal mucosa at the contact area between cuff and trachea will have higher risk to be infected and necrosis. (6,7)

Because of quicker, less invasive, transportability and lower cost, these may be the reason why tracheal ultrasound can use to determine tracheal wall thickness in case of tracheal stenosis and the result correlate with CT scan. The use of ultrasound has been study to examine various disease in human, such as tracheal deformities, tracheal tuberculosis and tracheal stenosis. (5)

In human beings, there is no different between using ultrasound and MRI to measure transverse plain of tracheal diameter in subglottic stenosis. (8) The histopathological results of tracheal stenosis followed by tracheal intubation in human medicine demonstrate various lesion such as inflammation, epithelium erosion, ulceration and fibrosis. The severity of lesion depends on tube size and the progression of lesion. (9)

The result in canine model show by the study of Zhuquan in 2017. The histological result shows the inflammation cell accumulation, hyperplasia of granulation tissue and collapsing tracheal ring. They reported that the excessive cuff pressure and the size of tube are the risk factor of tracheal stenosis. (7)

The ultrasound can use to detect tracheal collapse and grading the severity in dog. Rudolf study show that the ultrasound can use together with radiograph and tracheoscopy to confirm the diagnosis of canine tracheal collapse (10).

**Conclusion**

The tracheal stenosis is the complication that followed by the prolonged tracheal intubation. In this case, Endotracheal cuff compressed the tracheal wall, obstructed blood flow, cause tracheal necrosis and fibrosis. From this case, the ultrasonography can be the effective tool to diagnose the tracheal stenosis avoid causing stress to dyspneic patient. The appropriate cuff pressure should be study in dog with prolonged intubation. Tracheal ultrasound practice to diagnose the tracheal stenosis is required to improve the knowledge and to establish the correlation with tracheoscopy in advance case.

**Acknowledgements**

Many thank for the veterinary clinician at surgery unit and emergency unit at Prasu-arthon animal hospital.

**References**

A preliminary report; using bipolar vascular sealant in a canine elongated soft palate resection

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Keywords: bipolar vascular sealant, elongate soft palate, canine

Introduction

The elongated soft palate is an anatomic anomaly of soft palate, which pushed caudally against the epiglottis result in inspiration difficulty or dyspnea. The brachycephalic airway syndromes abnormalities including stenotic nares, enlarged tonsils, elongated soft palate, everted lateral saccules of the larynx, narrowed rima glottidis, and collapse of the larynx and trachea(1). The inspiratory stridor is one of the predominant clinical sign occurs by increase negative pressure during inspiration, which brings the elongated soft palate close together within the rima glottides. This obstruction will presents as sleeping asphyxia as well(2).

Generally, brachycephalic syndromes can be surgical treated for the two major defects, stenotic nares widening and elongated soft palate resection(1)(3)(4). The soft palate can be shortened by cutting then suturing or using a CO\textsubscript{2} laser(5). However, the conventional surgery seem to be difficult for an inexperience surgeon due to hemorrhage and narrow surgical field of oral cavity. Because cutting together with vessel sealing performance of bipolar vascular sealant (Caiman\textsuperscript{®}, Aesculap AG, Tuttingen, Germany), which commonly using in minimal invasive laparoscopic surgery. This device is applied in elongated soft palate resection.

Materials and Methods

A 12-years old pug dog with bilateral stenotic nares and elongated soft palate was under the surgical correction of both defects due to exercise intolerance, stridor respiration during the day and intervals dyspnea during the night. Chest radiograph found normal cardiac silhouette and lung appearance.

The patient was operated under isoflurane anesthesia with endotracheal intubation on sturnum recumbency. The upper jaw was pulled upward by a sterile silicone string, then two stay sutures were fixed on the left and right edge of the soft palate. (Fig. 1)

![Figure 1](image1.png)

Figure 1. The presentation of elongated soft palate by two stay sutures.

The middle of the tonsils was marked as a resection level, then a jaw of the bipolar vascular sealant grasped from the right edge to the middle of the soft palate then sealed and cut afterward and repeat on the left side. (Fig. 2)

![Figure 2](image2.png)

Figure 2. The bipolar vascular sealant grasped on the elongated soft palate at the middle of the tonsils level.

Totally, from the soft palate was stayed by sutures to the elongated piece of the palate was resected spent only 2 minutes. There was no occurrence of tissue
burning, hemorrhage and swelling. Moreover, there was no shifting of the landmark line during the cutting. (Fig. 3)

**Figure 3.** The remnant of the resected soft palate.

On the recovery, the patient present excellent breathing compared with the pre surgical period. The patient was observed in the hospital for 5 days and came to the hospital at day 10 without any discomforts.

**Results and Discussion**

According to the author experience, there was no different in time consumption between CO\textsubscript{2} laser surgery technique and bipolar vascular sealant. However, the laser required a vacuum machine and laser protection eye glasses.

The sealing performance of the electrosurgical bipolar vessel sealing was described for short operative time, low intraoperative blood loss and no intraoperative complications in several article for instant, laparoscopic colectomy for cancer surgery(6), hepatic parenchymal transection(7) and pancreatoduodenectomy(8).

Currently, the advanced bipolar instrument was approval for the minimal invasive surgery but there is no using report in the open surgery.

**Acknowledgement**

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**References**

Prevalence of anticoagulant rodenticide poisoning in dogs and cats submitted to the Faculty of Veterinary Science, Chulalongkorn University between 2008 and 2017

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Keywords: cats, dogs, prevalence, rodenticides

Introduction

Anticoagulant rodenticides (AR) have been used for several decades to control the rodent population worldwide. (1) Warfarin and dicoumarol represent the first generation of AR, while bromadiolone and brodifacoum are second generation products (9). AR acts by inhibiting vitamin K - epoxide reductase in the liver, thus causing a decreased synthesis of clotting factors II, VII, IX, and X (2,4,6,7). Vitamin K is a necessary cofactor in the modification of non-functional clotting factors into the functioning factors used in the extrinsic, intrinsic and common pathways of the coagulation cascade. Without the activity of vitamin K-epoxide reductase, vitamin K cannot be recycled and the animal becomes dependent on new, external sources of vitamin K. As vitamin K is depleted and not replaced, the synthesis of factors II, VII, IX, and X stops and these factors disappear from the circulation rapidly (half lives in dogs are 41, 6.2, 13.9, and 16.5 hours, respectively) (6). Animals suffering from AR intoxication always show signs of generalised bleeding (6). Since the vitamin K pathway is common to humans and terrestrial vertebrate species, the clinical effects of anticoagulants are fairly similar across species but susceptibility may vary greatly. The most susceptible species include rodents, hares and rabbits, swine, canids (dog, fox), mustelids (stoats) and birds of prey. The least susceptible species include many herbivores (ruminants, horses, etc.). Other species lie in between these two categories (8). AR poisoning in nontarget species, principally dogs and cats is a problem of increasing concern to veterinarians. To the authors’ knowledge, there are no reports on the prevalence of AR poisoning in dogs and cats in Thailand, despite the availability of general toxic data.

The present study describes the data on the prevalence of AR poisoning in dogs and cats recorded at the Department of Veterinary Pharmacology in the Faculty of Veterinary Science, Chulalongkorn University between January 2008 and December 2017 was considered in this study. All of the cases were suspected to have died from AR intoxication according to history taking, common signs prior to death and pathological investigation. Details regarding animal species (dog or cat), the sexes of the animals and the types of animals’ specimens (organs, stomach content or bait feed) were subjected to study.

All the animal specimens were analysed using the thin layer chromatography (TLC) technique and spectral analysis by derivative spectrophotometry (5). Briefly, the animal specimens were ground and extracted with chloroform in a vaporous condition. The extracts were filtered and the residual material was re-extracted and re-filtered. The residue was reconstituted in 1 ml chloroform. TLC separation was performed using silica gel G plates as the stationary phase and methyl ethyl ketone : benzene (6:120, by vol.) as the mobile phase. Standard solutions and control extracts of the animal samples were also prepared. Quantification was performed by spiking extracts of the animal samples with anticoagulant standards, running them under the standard TLC protocol. For spot detection of AR, a hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) solution was oversprayed on plates and then a FeCl\textsubscript{3} solution was sprayed. AR positive was recorded when both methods showed positive result.

Results and Discussion

AR poisoning is a major issue in veterinary clinical toxicology. Death is a frequent outcome in clinical cases because AR has a delayed onset of signs and poisoning is often only suspected in animals when clinical signs occur (1). In our laboratory, we used the TLC and spectrophotometry to analyse AR in the animal specimens. The limitations of the TLC technique included its inability to identify the specific type and measure the amount of AR in each affected specimen. Therefore, higher specific and more sensitive methods such as high performance liquid chromatography (HPLC) was a more suitable method for identifying the specific type and quantity of AR intake. Incidentally, these methods are a reliable screening test for AR (5).
From the data gathered between January 2008 and December 2017, 122 animal cases were submitted for AR detection. Out of 122 cases, 80 cases (65.57%) showed positive to AR poisoning. In the positive group of 80 cases, 74 cases (92.50%) were dogs and 6 cases (7.50%) were cats (Table 1). This study indicates that dogs were the more affected species at 92.50% which correlates with the reports of previous studies (1).

With reference to the sex of the animals, there was no difference between males and females with AR poisoning positive results (Table 1). In any case, the sample size was too small in this study to investigate the sex distribution of dogs and cats.

As the type of specimens, the liver was the most suitable specimen for AR detection because high levels of AR were accumulated in it (3). Therefore, as far as the direction for specimen submission in our laboratory is concerned, we suggest the collection of whole livers or at least 100 grams of liver for AR evaluation. In this study, livers were the most frequently submitted specimens for AR evaluation in both dogs and cats (Table 2 and 3). However, other specimens such as stomachs, stomach contents and bait feed (Fig. 1) were also submitted together with liver specimens in some cases. According to our data, positive results were clearly shown in liver specimens.

This retrospective study shows the prevalence of AR poisoning in dogs and cats submitted to our laboratory. It indicates the risk of AR in domestic animals which are nontarget species and reveals suitable specimens for AR detection.

References
Remission of secondary disorders of brachycephalic airway obstruction syndrome following primary disorder correction in dogs

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Keywords: airway obstruction, brachycephalic, dog, everted tonsil, laryngeal collapse

Introduction
Brachycephalic airway obstruction syndrome (BAOS) is divided into primary and secondary disorders. Primary disorders, consisting of elongated soft palate, stenotic nares and hypoplastic trachea will increase airway resistance. Chronic abnormal pressure and airflow dynamics in upper airway can lead to secondary malformations or disorders, consisting of everted laryngeal saccules, everted tonsils, laryngeal collapse, and tracheal collapse. Everted laryngeal saccule is the first stage of laryngeal collapse (1) which is composed of 3 stages (Tables 1)(2).

Early correction of BAOS such as stenotic nares and elongated soft palate has favorable long-term outcomes. From a previous study (3), secondary disorders could resolve spontaneously after correction of the primary disorders such as stenotic nares and elongated soft palate. Objective of this study was to determine secondary disorders before and after correction of the elongated palate and stenotic nares.

Table 1 Laryngeal collapse stages (1)

<table>
<thead>
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<th>Stage</th>
<th>Laryngeal collapse</th>
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<tbody>
<tr>
<td>1</td>
<td>Everted laryngeal saccules</td>
</tr>
<tr>
<td>2</td>
<td>Medial displacement of the cuneiform processes of the arytenoid cartilages</td>
</tr>
<tr>
<td>3</td>
<td>Collapse of the corniculate processes of the arytenoid cartilages</td>
</tr>
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</table>

Materials and Methods
This research was approved by the Animal Use and Care Committee of Chulalongkorn University, Bangkok Thailand (approval number 1731028). Ten dogs with BAOS were enrolled in this study. Breed, age, stenotic nares, elongated soft palate, tracheal hypoplasia, laryngeal collapse grade, and everted tonsils were recorded. Each dog was premedicated intramuscularly with acepromazine maleate (0.03 mg/kg), and morphine sulfate (0.5 mg/kg). Anesthesia was induced intravenously with propofol (1-4 mg/kg) to effect, and maintained with isoflurane in oxygen through endotracheal tube. Intravenous cefazolin (25 mg/kg) and dexamethasone (0.5 mg/kg) were preoperatively administered.

All dogs were placed in sternal recency for laryngoscopy immediately performed after anesthesia induction. By conventional technique, the soft palate at the caudal border of tonsils was excised by a scissors. Then, the nasal and oral mucosal layers were sutured with a 4-0 synthetic absorbable monofilament in simple continuous pattern. Alaplasty was performed by wedge shape technique. The nasal mucosa was sutured with a 5-0 synthetic absorbable monofilament suture in simple interrupted pattern. Firocoxib (5 mg/kg) were administered per oral 8 hours after surgery, then once a day for the first 4 postoperative days. Laryngoscopic follow up was performed at day 14 after surgery.

Results and Discussion
Dogs in the present study were 70% French bulldogs, 20% Pugs and 10% Boston terriers. Age (median(range)) were 3.5 (1-9) years. Primary disorders found were 100% and 90% of elongated soft palate and stenotic nares, respectively. There was no hypoplastic trachea found in any dogs. Preoperative and postoperative disorders were shown in Table 2.

Table 2 Preoperative (Pre-op) and Post-operative (Post-op) secondary disorders and % self-remission after correction of primary disorders.

<table>
<thead>
<tr>
<th>Secondary disorders</th>
<th>Number of dogs</th>
<th>%self-remission</th>
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<tr>
<td>Everted tonsil</td>
<td>5 (10)</td>
<td>80</td>
</tr>
<tr>
<td>None</td>
<td>5 (10)</td>
<td>90 (20)</td>
</tr>
<tr>
<td>One site</td>
<td>2 (20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Two sites</td>
<td>3 (30)</td>
<td>1 (10)</td>
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Laryngeal collapse

<table>
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<tr>
<th>Number of dogs</th>
<th>%self-remission</th>
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<tr>
<td>4 (20)</td>
<td>50 (10)</td>
</tr>
<tr>
<td>None</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>0 (0)</td>
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</table>
Spontaneous or self-remission of everted tonsil and laryngeal collapse after correction of elongated soft palate and stenotic nares were 80% and 50%, respectively (Table 2).

In this study, everted laryngeal saccule and everted tonsil were spontaneously resolved in some dogs 14 days after staphylectomy and alarplasty (Figures 1 and 2) and all dogs had postoperative clinical signs better than the preoperative sign. This was identical to the findings of others (3,4) which reported that though everted laryngeal saccules and tonsils were not corrected, they could be clinically improved in some dogs. Moreover, a previous study reported that respiratory signs of some dogs without ventriculectomy were better than those receiving ventriculectomy (5). In conclusion, correction of secondary disorders of BAOS was not necessary for every case.

Figure 1. Preoperative everted laryngeal saccule and everted tonsil in a dog.

Figure 2. From the same dog in Figure 1, the everted laryngeal saccule and everted tonsil were spontaneously solved.

Acknowledgements
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References
The Successful Treatment of Canine Hepatozoonosis Using a Simple Drug-of-Choice, Sulfa-Trimethoprim

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Keywords: Sulfa-trimethoprim, molecular diagnosis, dogs, hepatozoonosis.

Introduction
Hepatozoonosis is a neglected blood parasitic disease of pet dogs and other canid species known worldwide. This disease commonly occurred when dogs are heavily infested by ticks, *Ripicephalus sanguineus*, brown dog tick (1). Although some cases have no clinical signs of illness, however, some could be fatal with secondary complication and untreated. In this study and the attempt to treat hepatozoonosis, a 5-year-old female dog has a natural infection of *Hepatozoon canis* when applied molecular test using PCR specific for 18S rRNA gene. In the past and until now, there were no successful treatment of hepatozoonosis available (2). Even lately, Doxycline, recommended by veterinarian, is ineffectively used to cure hepato-zoonosis (3). We chose sulfa-trimethoprim for the first time and the drug of choice in this case. The therapeutic dose is 15-20 mg/kg PO for 2 weeks. After 14 days of treatment, PCR testing are shown as followings.

Materials and Methods
The amount of 0.5-1 ml of blood was collected from a dog from Dermatology unit of Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The samples were subjected to DNA extraction with Nucleospin® Tissue kit. The extracted genomic DNA were tested, with PCR assay, with in-house designed primers specific for *Babesia canis* and *Hepatozoon canis*. The pGEM®-T Easy plasmids containing 18 rRNA gene fragments derived from *B. canis* and *H. canis* were used as positive controls.

Results and Discussion
This dog was PCR diagnosed with *H. canis* only while there was no *B. canis* infection. Total blood DNA samples were tested as shown in Figure 1. When sulfa-trimethoprim, 15-20 mg/kg was applied per os for 14 days, this dog is recovered healthily and the blood sample was drawn for PCR rechecked.

Figure 1 The PCR products were analyzed by 1.5% gel electrophoresis. Plasmids containing 18SrRNA gene fragments from *B. canis* and *H. canis* were used as positive controls (Lanes 1 and 4). Non-template reactions were applied as negative controls (Lanes 2 and 5). The specifically PCR amplified for *B. canis* is 643 bp and *H. canis* is 1093 bp. The only amplified 1093 bp was detected (Lane 6, arrow) showing the active infection of *Hepatozoon canis* while *Babesia canis* was not found (Lane 3). Lane M is 100 bp DNA ladder.

Figure 2 The PCR products were reanalyzed by 1.5% agarose gel electrophoresis. The products of 1093 bp specific for *Hepatozoon canis* were not detected (arrow) in patient after 2 weeks treatment with sulfa-trimethoprim (Lane 7). Lane 1 is the internal control of...
canine genomic DNA. Lane 2 and 5 are the positive controls of B. canis and H. canis. Lane 3 and 6 are non-template negative controls. Lane 4 and 7 are the sample tested with PCR specific for B. canis and H. canis respectively. Lane M is for 100 bp DNA ladders marker.

When blood sample was reverified with molecular test, there were no H. canis found in Figure 2 (Lane 7 arrow). Results suggested that hepatozoonosis is successfully cured for the first time ever. The complete clearance of parasites from the blood stream is effective using a simple remedy, sulfa-trimethoprim.

Acknowledgements
Associate Professor Dr. Athipoo Nuntaprasert for DH5-alpha E.coli for DNA fragment cloning.

References
Surgical Removal of Metastatic Carcinoma in Sublumbar Lymph Nodes: A Case Report

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Keywords: Carcinoma, Metastasis, Sublumbar lymph node

Introduction
The term sublumbar lymph node refers to all lymph nodes that present in the sublumbar region including, medial iliac, hypogastric, and sacral lymph node. The location of these lymph nodes is close to the external and internal iliac arteries. Their main function is to drain lymph from the hindlimb and reproductive organs (1).

Sublumbar lymph nodes are the common metastatic site of malignant tumours such as anal sac adenocarcinoma. Anal sac adenocarcinoma accounted for 17% of all perianal tumour found in dogs (2). It is locally invasive and has a high rate metastasis to medial iliac lymph node (2-4). Thus, one of the effective treatment is surgical removal of the tumour itself and affected sublumbar lymph nodes (2). Therefore, the purpose of this case report is to describe the diagnosis and treatment of metastatic carcinoma in sublumbar lymph node from an anal sac adenocarcinoma in dog.

Materials and Methods
A 12-year-old male, English cocker spaniel breed dog was referred to Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University regarding chronic constipation. From history taking, the dog underwent surgical removal of anal sac adenocarcinoma before and no recurrence was found until presentation. The dog was normal except the large mass was detected via rectal examination and the murmur heart sound was found on physical examination. Blood collection was further performed to evaluate health status and mild elevate alkaline phosphatase (176 mg/dL; reference range, 3-60 mg/dL) was found, while other values were all in normal range.

Consequently, the computed tomography was performed. The computed tomographic finding showed that two masses were located in sublumbar area with deviated internal iliac vessel and colon. The former large mass was left medial iliac lymph node with the size of 3.9 x 7.2 cm, while the latter small mass was sacral lymph node with the size of 3 x 3.8 cm (Figure 1). Metastatic tumours to L2 lumbar vertebral body was found. In addition, intervertebral disc herniation of T12- T13, loss of left anal sac due to previous surgical removal, small size of left kidney, and single small cyst of right renal cortex were also demonstrated.

Figure 1. The large mass (arrow) was left medial iliac lymph node with the size of 3.9 x 7.2 cm., which deviated internal iliac vessel, and the small mass (arrow head) was sacral lymph node with the size of 3 x 3.8 cm.

Surgical procedure
The patient was pre-anesthetically medicated with acepromacine (0.01 mg/kg) and pethidine (4 mg/kg) intramuscular (IM). The dog was given cefazolin (25 mg/kg) as the prophylaxis antibiotic, intravenously (IV). Anesthetic induction was performed with midazolam (0.2 mg/kg) and propofol (4 mg/kg), IV. Epidural nerve block was done using bupivacaine (0.5 mg/kg) and morphine (0.2 mg/kg). The dog was then intubated and anesthesia was maintained with isofurane. The approach to abdomen was made through cranial to caudal midline incision at lineaalba. Two masses were found and the larger one attached to internal iliac vessel, while the other one placed caudally. Surgical removal of the larger mass was carefully performed due to the attachment of the tumour capsule to internal iliac artery (Figure 2). The smaller mass was totally removed. Then, the lineaalba, subcutaneous, and skin layers were closed as usual. Both masses were then fixed in 10% formalin and sent for histologic evaluation. According to post-operative
care, the dog was detained in the hospital until clinically stable and then discharged.

Figure 2. A. Medial iliac and sacral lymph node (left to right). B. A larger mass (arrow head) was attachment to internalliliac artery (arrow)

Figure 3. The cytoplasm showed many epithelial cells on RBCs background, the cells were anisokaryotic with moderate cytoplasm. The cells were present in clumps and glandular patterns. Numerous lymphocytes were observed. A. Medial iliac lymph node. B. Sacral lymph node.

Result and Discussion

In this case, histological finding reveals both masses were carcinoma (Figure 3). These masses were characterised as medial iliac and sacral lymph node due to their location and histologic appearance. The anal sac adenocarcinoma is suggested as the cause of metastatic carcinoma in these sublumbar lymph nodes due to previous anal sac adenocarcinoma. However, the metastasis adenocarcinoma in sublumbar lymph node is commonly found from anal sac adenocarcinoma, but not metastatic carcinoma (5-6). Therefore, we might suggest to carefully monitor clinical signs of this dog regarding other possible tumour sites.

Acknowledgements
We would like to thank Surgery Unit, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University for their cooperation. Also, we would like to acknowledge Vet and Vitro Central lab for their histologic evaluation.

References
Theophylline treatment in a Dog with Syncope due to Sinus arrest

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Keywords: Theophylline, sinus arrest, sinus pause, Holter ECG, syncope

Introduction

Syncope is defined as a temporary loss of consciousness and spontaneous recovery. Two-thirds of small animals with syncope also have a cardiac disease and arrhythmias represent one of the most frequently reported etiologies (1). Sick sinus syndrome, sinus arrest, advanced atrioventricular heart block, atrial fibrillation, and paroxysmal ventricular tachycardia are common arrhythmias in dogs that have been associated with syncope. However, arrhythmias are not the only mechanism responsible for syncope, and effort to rule out arrhythmias from other causes of syncopal events are important. Ambulatory monitoring can help identify or exclude cardiac arrhythmias as a cause for syncope in small animals.

Sinus arrest are a period where there is no evidence of atrial activity for a period in excess of the two preceding R-R intervals (2). It is usually recognized in dogs with sinus node dysfunction or high vagal tone activity. Periods of sinus arrest without development of an escape rhythm for greater than approximately 3-5 second may lead to neurologic signs such as staggering or syncope (3). If it occurs, the treatment is necessary. Anticholinergic drugs, Theophylline or terbutaline therapy are frequently recommended for management of this disease, therapy with these drugs alone is usually not effective. In the long term, sinus arrest is most effectively treated by pacemaker implantation.

Materials and Methods

A 13-year-old, 20.5 kg, female Thai Bangkaew dog was came to Thonglor Pet Hospital with 3 syncopal events on that day characterized by a sudden transient loss of consciousness, limb stretching and urinary incontinence were observed for few seconds without signs of tonic clonic limb motion, hypersalivation, muscle tremor, or post event disorientation.

On physical examination, the dog was good body condition. The heart rate was 120 breaths per minute, the respiratory rate was 20 bpm with a normal respiratory pattern. Cardiac and pulmonary auscultation were normal.

Initially the thoracic radiographs, echocardiogram, electrocardiography (ECG), systemic blood pressure measurement were performed. Blood samples were collected for complete blood count and blood chemistry profiles. Thoracic radiographs revealed normal cardiac silhouette with normal size of pulmonary veins and arteries. The pulmonary parenchyma showed a generalized mild broncho-interstitial pattern. Two dimensional and M-mode echocardiography showed thickening at tip of both anterior mitral valve leaflet and anterior tricuspid valve leaflet. Normal of left ventricular systolic function (fractional shortening: 45.45%) was observed. Color and continuous wave spectral Doppler revealed no mitral valve insufficiency and no tricuspid valve insufficiency. The peak aortic (0.98 m/s, range: <1.5-1.7 m/s) and pulmonary arteries (0.93 m/s, range: <1.3 m/s) velocities were within normal limits. The blood results (including complete blood count, creatinine, blood urea nitrogen, albumin, total protein, fasting blood glucose) were within normal limits. Elevation of alkaline phosphatase (ALP: 1545) and alanine aminotransferase (ALT: 223) levels were found. The ECG showed sinus rhythms. After all, Holter ECG monitor was performed for 24 hours to determine the cause of the syncope. During the monitoring, atypical heart patterns were frequent and long-lasting; these included sinus pauses (13 to 16 seconds) with supraventricular escape beat (Fig. 1), and sinus bradycardia. The minimum HR recorded was 16 bpm and the maximum HR recorded was 163 bpm.

Figure 1 Holter ECG showed the period of sinus pause (arrow) with supraventricular escape beat (star)
Results and Discussion

The patient presented significant episodes of syncope and based on the clinical results, drug therapy was initiated with theophylline 10 mg/kg BID. There was significant reduction in episodes of syncope after starting treatment, syncope event has gone. Randomized ECG sample during resting stage was done after starting this drug for a week, sinus rhythms were seen. The report of human medicine found 2 of 11 patients who received long term treatment with theophylline failed to maintain its effect on sinus pauses for >6 months despite a significant initial effect and maybe induced SA node dysfunction which led these patients to choose pacemaker implantation (4). However, a year has passed after starting theophylline in this dog. The patient is still well controlled of syncopeal episode without any side effects. Only one time of syncope was presented after the dog was in excitement condition.

In conclusion, the other study said pacemaker implantation is an effective treatment of choice for sinus arrest or sick sinus syndrome (5), but in case of the dogs that have other concurrent disease or in senile stage, they have the high risks for surgical and anesthetic procedure. Therefore, theophylline maybe the best choice for treatment of sinus arrest or sick sinus syndrome even though in long term used.

Acknowledgements

Holter ECG monitoring in this study was supported by Veterinary Cardiology clinic. Small animal teaching hospital, Faculty of Veterinary Science, Chulalongkorn University.

References

Upregulation of TGF-β1 and PDGF-B gene expression in periodontitis dogs treated with autologous blood-derived platelet-rich fibrin

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Keywords: dog, periodontitis, platelet-rich fibrin

Introduction
Periodontitis (PD) is one of the most prevalent inflammatory oral diseases in dog. Non-surgical managements involving scaling, sulcular lavage, and closed root debridement are conventionally performed to obtain healthy oral status (1). With progressive PD, advanced periodontal surgery and also dental extraction become warranted with major disadvantages of trauma, bleeding, prolonged anesthetic time and hospitalization, and wound healing impairment (2). Hence, regenerative periodontology is now emphasized as a novel approach to down-regulate an inflammation, stimulate a periodontal regeneration, and achieve optimum periodontal status (3). Platelet-rich fibrin (PRF), a second generation platelet concentrate, is predominantly composed of platelet and leukocyte cytokines co-operated in fibrin matrix, which synchronously acts as a bioscaffold rich in integrated reservoir of growth factors essential for tissue regeneration (4) (5). Since it was first described by Choukroun et al. in 2001 (6), many researches have been done to study its role on human periodontitis. Nonetheless, its efficacy in canine periodontitis treatment has not yet been fully described (7). The objective of this study was to evaluate the efficacy of autologous blood-derived PRF in an aspect of periodontitis treatment through transforming growth factor-beta 1(TGF-β1) and platelet-derived growth factor B (PDGF-B) gene expression.

Materials and Methods

Experimental animals: Experimental animals were recruited with the inclusion criteria as follows: 1) Mesocephalic dogs aged between 8 months to 5 years 2) Maxillary 4th premolars and mandibular 1st molar with periodontal pocket depth (PD) between 3-5 millimetres 3) Weight between 5-10 kilograms. 4) Healthy based on physical and hematological examination (8) (9). In this study, Split mouth clinical design was used to randomly divide surgical sites. Group 1: healthy gingiva, no periodontitis (n=5) Group 2: Periodontitis group (pocket depth 3-5mm) (n=10) Group 3: Periodontitis (pocket depth 3-5mm) with PRF treatment group (n=10)

Surgical procedure: Dental scaling was performed together with opened root debridement via off-modified Widman flap technique (Modified Widman Flap,MWF) using ultrasonic instruments and Greecycurrette (Hu-FriedyMfg Co. Inc., Chicago) (10). In group 3, PRF harvested from 4ml of autologous venous blood collected from jugular vein and prepared according to the protocol of Kobayashi et al.(11) was positioned over alveolar bone to study the efficacy of PRF. Mucoperiosteal flap was repositioned using a 4-0 monosyn® (B. Braun, Spain) in an interrupted interdental sutures pattern.

Inflammatory cytokine expression analysis: Gingival tissue samples were collected on day 0, day 7, and day 14 to evaluate for TGF-β1 and PDGF-Bgene expression level by real time reverse transcription polymerase chain reaction (qRT-PCR) technique using the SYBR-green fluorescence quantification system (Qiagen, Venlo, Netherlands). The analyses were performed in triplicate and relative mRNA expression levels were calculated with normalization to the internal control (β-actin). The comparison between 3 groups was performed using Kruskal-Wallis H test.

Results and Discussion
The results of TGF-β1 and PDGF-B relative gene expression were respectively showed in Figure 1 and Figure 2. The significant up-regulation of target genes expression was observed in PRF treatment group, with the highest expression on Day 7 (PDGF-B) and Day 14(TGF-β1)

![Figure 1](image-url) Expression of TGF-β1. Different capital letters differ significantly at P < 0.01 among trial periods in each group. Different small letters differ significantly among groups in each period.
Wound healing and inflammatory response are composed of sequential process concerning various cytokines. TGF-β1 and PDGF-B are one of the important ones as they act as anti-inflammatory cytokines co-operating in many phases of wound healing (12). TGF-β1 is considered to play key roles in all phases of wound healing by interacting with other cytokines to recruit fibroblast and inflammatory cells, stimulating proliferation of osteoblast and also collagen synthesis (13). PDGF-Bis a potent activator for migration and proliferation of mesenchymal lineage cells and mainly concerns in inflammatory and proliferative phase of wound healing process. PDGF-B has angiogenesis effect on endothelial cells and also collagen synthesis synthesis (13). PDGF-Bis a potent activator for migration and proliferation of mesenchymal lineage cells and mainly concerns in inflammatory and proliferative phase of wound healing process. PDGF-B has angiogenesis effect on endothelial cells and also collagen synthesis (13). In the present study, PRF showed the efficacy of favoring periodontitis treatment by up-regulation the expression of gingival TGF-β1 and PDGF-B in vivo. Our results were correlated with study of Ling et al., who studied the levels of TGF-β1 and PDGF-B in PRF exudate obtained from human blood at particular time points and evaluate its effect on rat calvaria osteoblast. They reported that the highest amounts of TGF-β1 and PDGF-B released from PRF were found on day 14 and day 7 respectively. Also, osteoblastic cells reached peak mineralization when treated with PRF exudate collected at day 14 (16). In resemblance to this, Wang et al. reported the same period of time that TGF-β1 and PDGF-B reached their highest release concentration from PRF. Moreover, they also found that adipose tissue-derived stem cells expressed maximum proliferation and alkaline phosphatase activity when cultured with PRF exudate of day 14 (17). The highest gene expression of gingival TGF-β1 and PDGF-B found on 7 days and day 14 post treatment with PRF that we found in our study corresponded with the peak concentration levels of TGF-β1 and PDGF-B released from PRF at the same particular day reported in other previously mentioned studies have been suggested that the anti-inflammatory properties of PRF accelerate the expression of these 2 important cytokines, which in turns promotes the wound healing process and also the PD treatment outcome. Therefore, PRF might be a novel modality for PD treatment with the advantages of being simple, autologous, and economical. Further study should be done to determine other parameters regarding PD treatment, for example clinical outcome, histopathological evaluation, together with other essential cytokines concerning inflammatory process and oral wound healing in canine PD.

Acknowledgements
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References
The effect of serum concentration on fibrotic gene expression in cultured rat cardiac fibroblasts

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Keywords: Cardiomyocyte, cardiac, skin, muscle, fibroblast,

Introduction
Cardiac (heart) fibroblasts are a major cell population in the heart tissue, it plays a significant role in development, structure integrity, cell signalling, and electro-mechanical function and repair of the heart (associated with fibrosis) (1). Many studies use cultured rat cardiac fibroblast to study mechanism of myocardium infarction and oxidative stress (2-3). The objective of this study was to investigate the effect of serum concentration on fibrotic gene expression in cultured rat cardiac fibroblast due to it may use as an in vitro model of myocardium/reperfusion.

Materials and Methods
This research project has been approved by Animal Care and Use Committee, Faculty of Veterinary Science-, Mahidol University. Rat fibroblasts from skin, heart and skeletal muscle were isolated and cultured according the previous study (4). For gene expression, RNA was extracted by using Trizol® (Invitrogen, USA) and cDNA was synthesised by SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, USA). Real-time PCR was performed according to previous studies (4-5). Six teen fibrotic genes were analysed gene expression. ANOVA and LSD were used to analyse statistical difference in gene expression using SPSS software.

Results
Most fibrotic gene expression were similar among three fibroblasts, except Mmp9, Postn, and Ctgf. Mmp9 in muscle (16 folds) and dermal (25 folds) fibroblast were very significantly higher (p<0.01) than cardiac fibroblast. Postn and Ctgf in cardiac fibroblast were significantly higher (p<0.05) than muscle and dermal fibroblasts (>3 folds) (see Figure 1).

After altering serum concentration from conventional culture (10% serum) to 5%, the fibrotic gene expression of cardiac fibroblast was not affected. Conversely, increasing serum concentration to 20% resulting in the reduction of expression of most fibrotic genes, but very significantly increased (p<0.01) for the expression of Mmp9 (>42 folds) and Hgf (increased 2.82 folds) (see Figure 2).

Figure 1. Gene expression in three types of fibroblasts. Mmp9 expression was very low in cardiac fibroblast. **Represent significant difference p<0.01. *represent p < 0.05.

Figure 2. Effect of serum concentration on fibrotic gene expression. 10% serum

Mmp9 was known being related to heart diseases, particularly myocardium infarction (6). Then we mimicked the blood block in vitro by removing serum in culture medium (0% serum or starvation). After serum starvation for 24 h, Mmp9 expression was reduced until undetectable, but its expression was significantly elevated (p<0.05) after adding serum to either 10% (2.89 folds) or 20% (4.59 folds) compared to control. Hgf expression was also increased (3.45 folds) after starvation, then substantially increased (p<0.05) after adding serum to 10% (5.20 folds) or 20% (5.80 fold). Timp1 expression was not affected after starvation but its expression was significantly (p<0.05) reduced (2.72 folds) when serum was added to 20%. This substantially change was not observed in muscle and dermal fibroblast (see Figure 3).
Mmp9 (Matrix metallopeptidase 9) is an important protein which plays vital roles in cardiovascular diseases and cardiac remodelling (6). It also involves cardiac fibroblast migration (7). Mmp9 is commonly used as a marker for the severity of cardiovascular disease (6). Not only Mmp9, Mmp1 is previously found decreasing after adding 20% of serum into the medium for culture human heart fibroblast (8). Similar to our study, human cardiac fibroblasts also express low level of Mmp9 (9). High expression of Mmp9 in cardiac fibroblast can be stimulated by oxidative stress, IL-1β, and TNF-α (10-11) which are associated with heart remodelling. The stimulation of Mmp9 expression by high serum concentration may be used as a model study myocardial infarction and reperfusion. Although serum withdrawal did not increased Mmp9 and Timp1 expressions, the previous report show that it stimulate of MMP-2 and TIMP-2 expression (12).

In this study, high serum in culture reduced the expression of Timp1 which is an inhibitor of Mmps. Timp1 plays a regulatory role in post-MI remodelling and accelerated myocardial remodelling (13). Upregulation of TIMP1 and down-regulation of MMP9 in cardiac fibroblast induced by basic fibroblast growth factor (bFGF) lead to inhibited progression of cardiac fibrosis during hypertensive heart failure (14).

Serum Hgf is elevated after acute myocardial infarction (15). The mechanism might be explained by the present study result which Hgf was elevated after starvation and increasing serum concentration in fibroblast culture. Hdf has been shown to be cardioprotective towards acute cardiac ischemia-reperfusion injury (16). Administration of Hgf protein reduces infarct size and increases cardiac performance in a rat (16). In contrast, acute blockade of endogenous Hgf increases infarct size and mortality (16).

This study was concluded that cardiac fibroblast is unique, its fibrotic genes response to the increase of serum concentration in vitro culture. This phenomenon may be related to the instantly increase of blood supply after myocardial infarction (reperfusion) and could be used as in vitro model for study myocardial infarction and reperfusion.

Acknowledgements
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References
Effects of diet containing Thai herbs on reproductive organs sperm production and serum testosterone level in male Wistar rats

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Keywords: Thai herbs, Wistar rat, Testosterone, Sperm, reproductive organs

Introduction
In Thailand, farmers have known how to use plants as food and medicine for domestic animals in order to cure disease, improve animal health as well as quality of products since prehistoric. While, the focus on organic farming methods and complementary veterinary medicine is important to consumers in industrialized countries, who increasingly demand high quality animal food products, locally produced and locally marketed. In the Northern part of Thailand at Nan Province, there are traditional beliefs that five plants as following: *Thunbergia laurifolia* Linn. *Melastoma* sp. *Tadehagi triquetrum* Ohashi *Eclipta prostrate* Linn *Derris sp.* and *Dioscorea bulbifera* Linn. have a biological effect on sexual behavior and thus to giving a better semen production in man and animal. It is worth to investigate whether these plants do help male fertility as the farmers’ belief. Therefore, attempts were made to study effects of Thai herbs on reproductive organs, level of serum testosterone (T) and total sperm count in male Wistar rat.

Materials and Methods
Animals: Healthy, thirty five male Wistar rats aged 9 weeks weighing 275.19 - 362.44 g were purchased from National laboratory Animal Centre, Mahidol University, Bangkok, Thailand. The animals were housed under standard laboratory conditions at 25 ± 2 °C, with a 12-h light/12-h dark photoperiod and feed with standard pellet diet and tap water. All experiments with animals were conducted under surveillance of Chulalongkorn University Animal Care and Use committee (permitted No.71/2549). The animals were randomly divided into 7 groups and each group was fed as follows.

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
</tr>
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<tbody>
<tr>
<td>G 1</td>
<td><em>Tadehagi triquetrum</em> Ohashi leave</td>
</tr>
<tr>
<td>G 2</td>
<td><em>Melastoma</em> sp. Stem</td>
</tr>
<tr>
<td>G 3</td>
<td><em>Thunbergia laurifolia</em> Linn. stem</td>
</tr>
<tr>
<td>G 4</td>
<td><em>Derris sp.</em> leave</td>
</tr>
<tr>
<td>G 5</td>
<td><em>Eclipta prostrate</em> Linn stem</td>
</tr>
<tr>
<td>G 6</td>
<td><em>Eclipta prostrate</em> Linn leave</td>
</tr>
<tr>
<td>G 7</td>
<td>standard pellet diet</td>
</tr>
</tbody>
</table>

Herb: These Thai Herbs were collected from Nan province. Each parts of plants were dried at 40°C in hot air oven (moisture 10-13%). The dried plants were ground, passed through a sieve (No.80) and mix with powdered of standard pellet diet and pressed into pellet and dried at 40°C for 2 days (Figure 1).

Figure 1 Standard pellet diet with herb for feeding animals.

The treatment group were received herb pellets everyday for 52 days (1). The effective dose of *Lasia spinosa* Thw. rhizome was reported as 30 g/bull/day can increase plasma testosterone and libido in buffalo bulls (2) thus 7 g/kg of body weight /animal/day were applied. On the 53rd day of treatment, the animals were killed by an overdose inhalation of anesthetic. Immediately after death the animals underwent laparotomy for the removal and weighing of the following organs; testis, epididymis, vas deference, seminal vesicle, prostate, liver and kidney. Cauda from all males were used for individual analysis the number of sperm counted by using hematocytometer. Blood samples were collected every 15 days from tail vein and separated for serum by refrigerated centrifugation at 1,500 x g for 30 mins. Serum samples were stored at -20°C until assay for T. Level of serum T was analyzed by radioimmunoassay method (3,4). The reliability of the method was tested in three pools of low, medium and high standard T added in the blank plasma pools. The mean percentage of recovery in serum was 88.67% . The sensitivity of the assay was 0.02- 2.0 ng/ml. Specificity of T-antibody (As-T#475) were showed in percentage of cross reaction with T and 5-α DHT were 100 % and 46.67% respectively and no cross reaction with progesterone, androsterone,
17-α hydroxy progesterone, androstenedione, corticosterone, 17-β oestradiol and estriol.

**Results and Discussion**

Effects of herbs on liver, kidney and reproductive organs weights: A summary of the organ weights for each group are presented in Table 1. There were no significantly difference among weight of livers, kidneys and reproductive organs in treatment groups and control group. In the present study, results indicated nontoxicity of thus herbs as evidenced by vital organ which indicate normal weight of liver and kidney and androgen-dependent accessory reproductive organ. (5)

**Table 1** Mean ± SD weight of testis, Vas deference, seminal vesicle, prostate gland epididymis, kidney and liver in male Wistar rat fed with diet added with Thai herbs and control group.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control (n=5)</th>
<th>Treatment (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>2.24±0.28</td>
<td>2.22±0.20</td>
</tr>
<tr>
<td>Vas deference</td>
<td>0.14±0.02</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td>2.02±0.20</td>
<td>1.99±0.41</td>
</tr>
<tr>
<td>Prostate</td>
<td>0.57±0.12</td>
<td>0.54±0.15</td>
</tr>
<tr>
<td>Epididymis</td>
<td>1.48±0.16</td>
<td>1.49±0.24</td>
</tr>
<tr>
<td>Liver</td>
<td>18.59±0.65</td>
<td>17.81±1.43</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.86±0.29</td>
<td>2.84±0.23</td>
</tr>
</tbody>
</table>

Effects of herbs on serum T and total sperm count: Level of serum T in all animal (n=5) in each group were summed together (Table 2). And difference value between total T after and before treatment were 0.26, 0.28, 0.73, -0.17, -0.31, -0.79 and -1.3 ng/ml in animal group 1, 2, 3, 4, 5, 6 and control group respectively.

**Table 2** Levels of serum testosterone (ng/ml) in male Wistar rats before and after fed with diet added with *Thunbergia laurifolia* Linn. stem (G1), *Melastoma sp.* stem (G2), *Eclipta prostrata* Linn. stem (G3), *Eclipta prostrata* Linn. leave (G6), and standard feed (G7).

<table>
<thead>
<tr>
<th>Group of Ani. (n)</th>
<th>Total Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before fed (n=10)</td>
</tr>
<tr>
<td>G1 (n=5)</td>
<td>1.51</td>
</tr>
<tr>
<td>G2 (n=5)</td>
<td>1.69</td>
</tr>
<tr>
<td>G3 (n=5)</td>
<td>1.47</td>
</tr>
<tr>
<td>G4 (n=5)</td>
<td>1.63</td>
</tr>
<tr>
<td>G5 (n=5)</td>
<td>2.83</td>
</tr>
<tr>
<td>G6 (n=5)</td>
<td>2.77</td>
</tr>
<tr>
<td>G7 (n=5)</td>
<td>2.85</td>
</tr>
</tbody>
</table>

There were only three groups of the male Wistar rat responded after taking *Tadehagi triquetrum* Ohashi leave (G1), *Melastoma sp.* stem (G2) and *Thunbergia laurifolia* Linn. stem (G3) which indicated by level of total serum T were elevated. The number of total sperm count (Figure 2 and Table 3) significantly increased (p<0.005) in treated animal G2 and G3 when compared to the control group (G7). And the level of total serum T in group 2 and 3 tend to be corresponding to total sperm count. The results indicated that *Melastoma sp.* stem (G2) and *Thunbergia laurifolia* Linn. stem (G3) can improve male Wistar rats fertility (6,7).

![Figure 2](image-url)  **Figure 2** The difference value of total serum T in male Wistar rat between before and after fed with diet added with Thai herbs and control group.

In conclusion, *Melastoma sp.* stem and *Thunbergia laurifolia* Linn. stem were proved to be useful for enhancing male Wistar rat fertility. However, research for investigation of effective dose and mechanism of action should be studied in further.

**Table 3** The total count (X10^6) of sperm in male Wistar rat with diet added with Thai herbs. The significant differences are indicated by different letters.

<table>
<thead>
<tr>
<th>Group of Ani. (n)</th>
<th>Sperm count (X10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>G1 (n=5)</td>
<td>54.4 ^a</td>
</tr>
<tr>
<td>G2 (n=5)</td>
<td>75.2 ^a</td>
</tr>
<tr>
<td>G3 (n=5)</td>
<td>62.4 ^ab</td>
</tr>
<tr>
<td>G4 (n=5)</td>
<td>45.0 ^b</td>
</tr>
<tr>
<td>G5 (n=5)</td>
<td>52.8 ^b</td>
</tr>
<tr>
<td>G6 (n=5)</td>
<td>44.0 ^b</td>
</tr>
<tr>
<td>G7 (n=5)</td>
<td>44.4 ^b</td>
</tr>
</tbody>
</table>

P<0.005 Different letters mean significance.

**Acknowledgements**

The authors would like to thank Asst.Prof. Wilaiporn Chuanchai and Asst. Prof. Sangkeaw Kumkuans Assit. and Prof. Noparat Chuanchai of technology Lanna, Nan Campus in kindy provides some of herbs. This study was supported by Chulalongkorn University.
References
Pathogenesis and distribution of duck Tembusu virus in BALB/c mice

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Keywords: Pathogenesis, Distribution, Duck Tembusu virus, BALB/c mice

Introduction

Duck Tembusu virus (DTMUV) causes a severe neurological disease in avian hosts, especially in duck and chicken (1). Infected animals show severe neurological signs including ataxia, paralysis, and seizure. Moreover, it causes hemorrhagic ovaritis and salpingitis that causes embryonic death and decreasing in egg production, especially in productive duck (2). DTMUV is a member of the Flavivirus group which is a large group of viral pathogens including Japanese encephalitis, West Nile, and Dengue virus. Most of them cause disease in various hosts such as avian and mammal hosts, and important zoonotic disease between animals and humans (3, 4). Viral transmission can occur in several routes such as fecal-oral and mosquito transmission route. Since 1955 to the present, DTMUV has been isolated from mosquito vector, duck, and chicken in China and Southeast Asia (5, 6, 7). Disease was only reported in avian hosts but was not found in mammal host. On the other hand, antibody against the virus was isolated in saliva and blood from Bornean orangutans and duck farmers (8, 9). It indicated that DTMUV has the ability to infect mammal hosts. In this study, the potential of DK/TH/CU-1 strain of DTMUV, an isolated virus from Thailand, to infect and cause disease in BALB/c mouse was examined.

Materials and Methods

A group of 80 female and 40 male BALB/c mice was equally separated into four subgroups, including two inoculated and two negative control groups. Inoculated groups consisted of intracerebral (IC) and subcutaneous (SC) groups. Both of them were inoculated with 3x10⁴ TCID₅₀ of DTMUV (DK/TH/CU-1 strain) by intracerebral and subcutaneous routes, respectively. Negative control groups were inoculated with free allantoic fluid by the same route with inoculated groups. Clinical signs and oral cavity were daily observed and swabbed for 14 days. On day 1, 3, 5, 7, 9, 12, and 14 post inoculation (PI), three mice from each group were randomly euthanized, and blood and organs were collected. Sampled organs included brain, heart, lung, liver, spleen, and kidney. They were divided into two parts for tissue section and viral detection. Tissue sections were stained by using H&E and immunocytochemistry staining to observe the microscopic lesion and the distribution of virus in infected cells. Viral infection was confirmed by RT-PCR technique. Primer set was specific to E gene of DTMUV and PCR conditions were described by Su et al. (2011) (10). The animal used was conducted in compliance with the Chulalongkorn University Laboratory Animal Care and Use Committee (Animal Use Protocol No. 1773002). Data were analyzed in term of clinical sign, disease severity, pathogenesis, and distribution of the virus.

Results and Discussion

Mice in IC and SC inoculated groups showed clinical signs such as loss of appetite, ruffled hair, and depression on day 3 and 4 PI, respectively. On day 10 PI, IC inoculated group showed hind limb paralysis and blepharitis (eyelid inflammation) that was different from SC inoculated group (Fig. 1A and 1B). The mortality was found only in IC inoculated group which were nine mice (seven female and two male). Macroscopic lesions were found in both inoculated groups such as splenomegaly, enlarged kidney, and brain congestion when compared with negative control group. Immunocytochemistry staining was used to detect the virus in infected cells. Virus were found in internal organs of inoculated mice (Fig. 1C and 1D).

![Figure 1](image-url)
Virus were also detected from all oral swabs and organs of death mice by using RT-PCR technique. This might indicate the spread of virus throughout the body and oral cavity. DTMUV were detected in mice inoculated by IC earlier than mice inoculated by SC. Previous studies showed that other strains of DTMUV could infect BALB/c mice by IC route but could not infect by SC and intraperitoneal routes. Clinical signs in infected mice with different strains of virus were similar (11, 12). However, DTMUV isolated in Thailand can caused disease in BALB/c mice but the pathogenesis in this study was different from other strains of the virus.

Acknowledgements

Duck Tembusu virus was kindly provided by Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University. This research was funded by Chulalongkorn University (CU-GR_60_05_31_02), the Thailand Research Fund (IRN5803PHDW03), and the Special Task Force for Activating Research, Chulalongkorn University (GSTAR 59-001-31-001).

References

Potency of Tomato Paste (*Lycopersicon esculentum*) Against Histopathological Liver of Mice (*Mus musculus*) Exposed to Borax

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**Keywords:** mice, liver, borax, tomato paste

**Introduction**

In the era of globalization there is an increase in consumption of food. Many food industries use chemicals in the form of borax as an effort to reduce production costs (1). Exposure to borax causes poisoning symptoms of dizziness, fever, vomiting, redness of the eyes (2). Borax poisoning also occurs in animals that are dead cows due to borax-based fertilizer poisoning left on peanut plantations (3). Borax causes abnormal liver where the liver will experience hydropic degeneration, fibroblast proliferation, and macroscopically the liver cells of animals try to experience magnification and blackish brown. Provision of antioxidants contained in tomatoes that lycopene can control free radicals 100 times more efficient than vitamin E (4). Tomatoes containing lycopene is higher than other processed tomato ingredients is tomato paste. Therefore, a study of the effects of tomato paste (*Lycopersicon esculentum*) on histopathologic images of mice liver (*Mus musculus*) exposed on orally borax.

**Materials and Methods**

Twenty four mice (*Mus musculus*) with 60-90 day ages and 20 g average of body weight were divided into six groups (P0, P1, P2, P3, P4, and P5). P0 was treated with sterile aquadest 0.1 ml/20g Bw/day, P1 was treated with borax 7.5 mg/20g Bw/day, P2 was treated with tomato paste 300mg/20g Bw/day, P3 was treated with tomato paste 150mg/20g Bw/day and borax 7.5 mg/20g Bw/day, P4 was treated with tomato paste 300mg/20g Bw/day and borax 7.5 mg/20g Bw/day, and P5 was treated with tomato paste 450mg/20g Bw/day and borax 7.5 mg/20g Bw/day. This research has been conducted for 14 days. The data of histopathological liver with lesions of necrosis and degeneration appearance were analyzed with Kruskal-Wallis and continued with Man-Whitney.

**Results and Discussion**

The results in Table 1 showed that there was a significant difference (P <0.05) in the P1 group (borax dose 7.5 mg / 20 g Bw / day) with another group.

**Table 1 Mean Rank histopathologic changes of liver mice on all treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>5.25ab</td>
</tr>
<tr>
<td>P1</td>
<td>22.5c</td>
</tr>
<tr>
<td>P2</td>
<td>10.13ab</td>
</tr>
<tr>
<td>P3</td>
<td>17.5b</td>
</tr>
<tr>
<td>P4</td>
<td>14.5bc</td>
</tr>
<tr>
<td>P5</td>
<td>5.13a</td>
</tr>
</tbody>
</table>

Description: Different superscripts showed significant differences between treatments (p <0.05).

The borax into the body would be a compound 4B(OH)4- contained in the borax enters the cell resulting in increased degeneration and necrosis of histopathologic images of hepatic mice (*Mus musculus*). The damaged liver is caused by damage to the cell wall so that the liquid can not be eliminated and will experience cell swelling (degeneration hidropik) which is reversible. If the lesion continues there will be irreversible damage to the organelle of the cell that ends with overall cell death (cell necrosis), all histopathological changes (P0, P1, P2, P5) are presented in (Fig. 1).
The P2 group was not significantly different from P3 (tomato paste 150 mg / 20 g BB / day and borax dose 7.5 mg / 20 g BB / day) and P4 (tomato paste 300 mg / 20 g BB / day and borax dose 7.5 mg / 20 g BB / day). These three groups can be interpreted that the dosage of tomato paste is still not effective to reduce the damage of histopathologic images of hepatic mice to such a picture of P0 or normal group. In contrast to the P0 group compared with P2 and P5, the histopathologic picture of P0 versus P2 (giving a dose of tomato paste 300 mg / 20 g BB / day) was not significantly different, as did when compared with P5 (dose of tomato paste 450 mg / 20 g BB / day and dose of borax 7.5 mg / 20 g BB / day), it is shown that the provision of lycopene as an antioxidant in tomato paste that serves to catch free radicals, prevent damage to the cell wall and help cell regeneration, so that the histopathologic picture can reduce hepatic damage to almost the same as P0 (normal).

**Acknowledgements**

The authors are grateful to Djoko Legowo, drh., M.Kes. as mentor and staff of histopathology laboratory, Faculty of veterinary medicine, Airlangga University for their technical help and cooperation.

**References**

Quantitative study of Bat contact in Thailand

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Keywords: knowledge; attitude; practice; KAP; bat; contact; interface

Introduction
Bats is a reservoir of several zoonotic pathogens. Currently, there is less number of studies of knowledge, attitude and practice (KAP) study regarding bat-human interface, especially in Thailand. Therefore, this study aimed to determine the KAP toward bat contact activities among residents who have been living nearby high density of bat habitats in within four regions of Thailand during May 2016 to April 2017.

Materials and Methods
An analytic cross-sectional study was conducted by using structured questionnaires and face-to-face interview methods. Simple random sampling of the respondents was used to select potential respondents, aged between 20 – 65 years old, and had been in the areas at least 6 months before the data collection.

Results and Discussion
626 respondents from the four regions of Thailand were interviewed by using a quantitative questionnaire. The results indicated that KAP factors were associated with bat contact behaviors among respondents who lived nearby high density of bat habitats. This also found positive correlation between knowledge, attitude and practice scores. Majority of the respondents had very good practice despite the average level of knowledge and attitude which was compatible with results of other studies[1, 2]. Besides, respondents who didn’t report bats contact activities in past 6 months before the study implementation had better knowledge, attitude and practice score than respondents who reported bat contact activities. These results were consistent with other studies which found that people with more knowledge tried to avoid health risky behaviors [3, 4]. These would recommend that appropriate education to promote proper knowledge, attitude and practice regarding to bats, and bat-borne diseases should be conducted in the communities especially among persons who have frequent contacting with bats via direct and indirect channels.

Figure 1Bats at a study site

Acknowledgements
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References


Up-regulation of TGF-β1 and PDGF-A gene expression by bubaline fibrin glue in ligation-induced periodontitis rats

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Keywords: bubaline fibrin glue, gene expression, periodontitis, oral cavity, rat

Introduction
Periodontitis is the most common clinical condition of periodontium inflammation, spontaneously occurring in small animals in any age. Various techniques in periodontal wound management including regenerative medicine wound care, have been implemented. Fibrin glue, the blood-derived biological product, is highlighted as an alternative choice for periodontitis treatment, reducing the loss of periodontium and decreasing the level of an inflammatory reaction. The properties of fibrin glue are sealing, hemostatic, and enhancing wound healing. Fibrin glue composed of two main components, thrombin and fibrinogen. Since buffalo blood was containing the highest fibrinogen level compared with others (1). Bubaline blood derived fibrin glue may be one of the novel biomedical agents for periodontitis treatment. The aim of this study was to determine the expression level of TGF-β1 and PDGF-A cytokine genes in the ligation-induced periodontitis rats after treatment with bubaline fibrin glue.

Materials and Methods
The bubaline fibrin glue was prepared according to (2) with modification. The experimental Wistar rats were randomly divided into three groups: Control (Group I), Untreated group (Group II), Treated (Group III). Ligation method was performed around the cervix of both mandibular first premolars for seven days. After removing the ligation (at day 7), the bubaline fibrin glue was applied at the ligation sites in Group III rats for 14 days. The rats were euthanized at the 1 and 7 day of ligation (DOL) and at the 1, 7, 14 day after ligation (DAL) to collect the gingival tissues following the method of (3). The gingival tissues were analyzed by qRT-PCR for the evaluation of the anti-inflammatory cytokines (TGF-β1 and PDGF-A) gene expression level.

RNA isolation was carried out with Trizol® reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. Primers were designed using Primer 3 Plus software version 2.3.7 (USA) and BLAST analysis (USA). β-actin gene was used as a house-keeping gene (4). cDNA reverse transcription kit (ImPron-II® Reverse Transcription System, Promega, USA) was used to produce cDNA. KAPA SYBR Fast qPCR Kit Master Mix (2X) Universal (Kapabiosystems, Massachusetts, USA) was chosen to produce real-time PCR products. The relative cytokine gene expression data was expressed as log_{10} (2^ΔΔCt).

The comparison between groups was performed using One Way ANOVA Test. Data were analyzed quantitatively by using SPSS version 22 for Windows program (Version 22, IBM, USA) and differences were considered significant at P<0.05.

Results
Figure 1 Gingival TGF-β1 gene expression. Different small letters differ significantly among groups (p<0.05) in each trial period.

Figure 2 Gingival PDGF-A gene expression. Different small letters differ significantly among groups (p<0.05) in each trial period.
The up-regulation of gene expression was observed in each time-point in both Group II and III. After remove ligation, treated group showed higher expression than untreated group at day 21.

**Discussion**
This is the first study using the bubaline fibrin glue in the treatment of periodontitis. Our results showed the expression of anti-inflammatory cytokines (TGF-β1 and PDGF-A) in the treated group were higher than untreated group. It has been suggested that the fibrin glue could accelerate wound healing process and perform the anti-inflammatory properties as seen in the commercial fibrin glue. This is related to the remodeling phase of the inflammatory response (6). The combination of fibrinogen and thrombin could promote wound healing and sealing effects (7).

Knowledge obtained from this study may be useful for demonstrating of xenologous fibrin glue. In conclusion, our study demonstrates the potency of the novel biomedical agents by promoting wound healing process. The bubaline fibrin glue might be an alternative material for the periodontal diseases.

**Acknowledgements**
This study was supported by the 90th Anniversary of Chulalongkorn University (Rachadapiseksomphot Endowment Fund) (R/F_2559_012_02_31) and the 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship.

**References**
Campylobacter detection method for samples from broiler farms in Thailand

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Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University
*Corresponding author: taradon.1@chula.ac.th

Keywords: Campylobacter, broiler farm, boot swab, cloacal swab, fresh feces

Introduction
Campylobacter is one of the most frequently reported bacterial zoonotic causes of human gastroenteritis (1,2). Most campylobacteriosis cases in human were associated with poultry and poultry products consumption (3,4). Since Campylobacter isolated from broiler farms were closely related to Campylobacter contaminated in chicken meat (5) and since standard protocol for Campylobacter monitoring is still unavailable in Thailand, appropriate Campylobacter detection method is essential for establishing routine monitoring program. Over the years, several methods such as ISO, USDA FSIS, etc are used to detect Campylobacter from food samples. However, these methods may not be suitable for samples with high background flora such as samples from broiler farms. Therefore, the objective of this study was to identify Campylobacter isolation methods suitable for Campylobacter detection in broiler farms in Thailand.

Materials and Methods
Sample collection: Cloacal swabs, fresh feces and boot swabs were randomly collected from 60 broiler flocks. A total of 180 samples, including 60 cloacal swab samples, 60 fresh fecesand 60 boot swab samples, were obtained for Campylobacter isolation.

Campylobacter isolation: Campylobacter were isolated from cloacal swabs and fresh feces using direct plating method with different agar media e.g modified Charcoal Cefoperazone-Deoxycholate Agar (mCCDA), mKarmali, Preston agar and Campy-cefex agar. These agar plates were incubated at 42°C for 48 hours±2 hours under microaerobic condition (5%O2, 10% CO2, 85% N2). For boot swab samples, both direct plating method and selective enrichment method were used for Campylobacter isolation. Four selective enrichment broths including Bolton broth, Preston broth, Exeter broth and blood free Bolton broth were used in this study. Samples in Bolton broth, Preston broth and Exeter broth were incubated at 37°C for 4-6 hours, then at 42°C for 44±4 hours under microaerobic condition, whereas samples in blood free Bolton broth were incubated at 42°C for 48±2 hours under the same condition. After enrichment step, samples in Bolton broth, Preston broth and Exeter broth were subsequently cultured onto mCCDA, while samples in blood free Bolton broth were subcultured onto Campy-cefex agar(6). The chi-square test ($p<0.05$) was used to compare positive Campylobacter isolation rates of each method.

Campylobacter identification: Presumptive Campylobacter colonies were confirmed by biochemical tests and identified to species level by multiplex PCR(7).

Results and Discussion
Among 180 tested samples, 43 samples (22 boot swabs, 12 cloacal swabs and 9 fresh feces) were Campylobacter-positive. The results revealed that mCCDA combined with Preston agar, mCCDA combined with Campy-cefex agar and mKarmali combined with Preston agar gave the highest Campylobacter isolation rate for cloacal swab samples, while only mCCDA combined with Preston agar yielded the highest Campylobacter isolation rate for boot swab samples (Table 1). On the other hand, the highest recovery rate of fresh feces was obtained by using mCCDA alone. No improvement of Campylobacter isolation rate was observed for fresh fecal samples even though different combinations of media were used (Table 1). Although Vidal et al (2013) indicated that Exeter broth gave the highest positive rate for Campylobacter isolation from boot swab samples (8), our study revealed that Preston broth provided better Campylobacter isolation rate than other selective enrichment broths (Table 2). However, Campylobacter isolation rate obtained from selective enrichment method was lower than that obtained from direct plating method. Because selective enrichment broth might promote the growth of other microorganisms, which can obscure Campylobacter colonies, this may be an explanation why the low Campylobacter isolation rate was observed when selective enrichment method was used. Therefore, we recommend that direct plating method should be used for Campylobacter detection from boot swab samples. Based on the results of our study, direct plating method on mCCDA and Preston agar is recommended for detection of Campylobacter from broiler farms.

Table 1 Comparison of Campylobacter recovery rates by direct plating method using different types of media
Table 1 Comparison of Campylobacter recovery rates by direct plating method using different types of media

<table>
<thead>
<tr>
<th>Media used for Campylobacter isolation</th>
<th>Recovery rates by sample type (%)</th>
<th>Clonal swabs</th>
<th>Fresh feces</th>
<th>Boot swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCCDA</td>
<td>7.60 ± 11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.60 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.60 ± 18.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>mKarmali agar</td>
<td>5.00 ± 8.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.60 ± 5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.60 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Preston agar</td>
<td>8.60 ± 13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.60 ± 13.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.60 ± 15&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Campy-cefex agar</td>
<td>6.60 ± 10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.60 ± 3.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>mC-mK</td>
<td>7.60 ± 11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.60 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.60 ± 18.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>mC-P</td>
<td>10.60 ± 16.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.60 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.60 ± 26.7&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>mC-C</td>
<td>10.60 ± 16.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.60 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.60 ± 20&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>mK-P</td>
<td>10.60 ± 16.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.60 ± 15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.60 ± 16.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>mK-C</td>
<td>9.60 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.60 ± 6.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.60 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>P-C</td>
<td>9.60 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.60 ± 13.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.60 ± 15&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

mC-mCCDA, mK-mKarmali, P-Preston, C-Campy-cefex
*Different superscripts indicate significant difference within the same column (p<0.05)

We would like to thank National Bureau of Agricultural Commodity and Food Standards (ACFS Grant 19/2559) for financial support.

References

Table 2 Comparison of Campylobacter recovery rates using selective enrichment method

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Recovery rate by isolation method (%)</th>
<th>Clonal swabs</th>
<th>Fresh feces</th>
<th>Boot swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolton broth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCCDA</td>
<td>4.60 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.60 ±11.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.60 ± 5.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60 ± 1.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Preston broth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCCDA</td>
<td>4.60 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.60 ±11.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.60 ± 5.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60 ± 1.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Exeter broth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mCCDA</td>
<td>4.60 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.60 ±11.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.60 ± 5.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60 ± 1.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Campy-cefex broth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

106
Genetic Characterization of Newly Emerged Duck Tembusu Virus isolated from Domestic Ducks in Thailand, 2017

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Keywords: Duck, duck Tembusu virus, genetic characterization, Thailand

Introduction
Duck Tembusu virus (DTMUV), a mosquito-borne Flavivirus, caused an emerging disease characterized by severe neurological disorders and serious drop in egg production in broiler and layer ducks in several Asian countries, including China, Malaysia and Thailand (1, 2, 3, 4). After the initial outbreak in 2013, DTMUV cases have been continuously detected in several duck farms in Thailand. Previous studies showed that Thai DTMUVs were closely related to Chinese DTMUVs (3). Therefore, the disease surveillance and the genetic characterization of the currently circulating DTMUV in ducks in Thailand are necessary for understanding the genetic diversity of this virus. This study aims to investigate the genetic characteristics of DTMUV circulating in ducks in Thailand during January - May 2017.

Materials and Methods
A total of 23 pooled organ samples including brain, spinal cord and spleen from DTMUV suspected cases were obtained from duck farms during January - May 2017. Organ samples were then homogenized in sterile phosphate-buffered saline at a 10% suspension (w/v). Viral RNAs were extracted from tissue suspensions by using QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany). The samples were then examined for the presence of DTMUV by RT-PCR using E gene specific primers (1). All samples tested negative for common duck pathogens, including Duck Enteritis virus (DEV), Avian Influenza virus (AIV) and Newcastle Disease virus (NDV) (5,6,7). DTMUV positive samples were subjected to the complete E gene sequencing. Subsequently, the nucleotide sequences were assembled using SeqMan software v.5.03 (DNASTAR Inc., Wisconsin, USA). The nucleotide sequences were aligned using Clustal W v.2.0 (8). Phylogenetic analysis was analyzed by comparing the complete E gene sequences from these 6 new Thai DTMUVs identified in this study with previously reported Thai, Chinese, Malaysian DTMUVs and TMUVs isolated from mosquitos (MM1175) and chickens (Sitiawan virus) from GenBank database. Phylogenetic analysis was constructed in MEGA v.6.0 using neighbor-joining algorithm with the Kimura-2 parameter model applied to 1000 replications of bootstrap (9). Nucleotide (nt) and amino acid (aa) identities among Thai, Malaysian, Chinese DTMUVs and TMUVs isolated from mosquitos (MM1175) and chickens (Sitiawan virus) were determined by MegAlign software v.5.03 (DNASTAR Inc., Wisconsin, USA).

Results and Discussion
A total of 6 (26.09%) out of 23 samples were tested positive for DTMUV by E-specific RT-PCR. DTMUV-positive samples were collected from duck farms located in 5 provinces in the eastern (Chonburi, Chachoengsao and Prachinburi), the central (Singburi) and the northeastern (Nakhon Ratchasima) regions of Thailand during January - May 2017 (Table 1). This finding indicated that DTMUV has continuously circulated and caused outbreaks in the duck producing areas of Thailand.

Table 1 Details of DTMUVs characterized in this study

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Province</th>
<th>Duck type</th>
<th>Time of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>DK/TH/CU164</td>
<td>Singburi</td>
<td>Layer</td>
<td>March 2017</td>
</tr>
<tr>
<td>DK/TH/CU170</td>
<td>Chachoengsao</td>
<td>Broiler</td>
<td>April 2016</td>
</tr>
<tr>
<td>DK/TH/CU172</td>
<td>Chonburi</td>
<td>Broiler</td>
<td>May 2016</td>
</tr>
<tr>
<td>DK/TH/CU175</td>
<td>Nakhon Ratchasima</td>
<td>Layer</td>
<td>May 2016</td>
</tr>
<tr>
<td>DK/TH/CU177</td>
<td>Nakhon Ratchasima</td>
<td>Layer</td>
<td>May 2016</td>
</tr>
<tr>
<td>DK/TH/CU178</td>
<td>Prachinburi</td>
<td>Broiler</td>
<td>May 2016</td>
</tr>
</tbody>
</table>

Table 2 Comparison of the nucleotide (nt) and amino acid (aa) identities of the complete E gene sequences of 2017 Thai DTMUVs with reference DTMUVs and TMUVs isolated from mosquitos (MM1175) and chickens (Sitiawan virus).
Thai DTMUVs were most closely related to the 2013-2016 Thai DTMUVs (Figure 1; Table 2). It is noted that all of the Thai DTMUVs were grouped into subcluster II-a, while the Malaysian and most of the Chinese DTMUVs were located in cluster I and subcluster II-b. This finding indicating that subcluster II-a was associated with the current DTMUV outbreaks in Thailand.

In conclusion, our results demonstrated the genetic characteristic of recent DTMUVs circulating in Thailand. Moreover, this finding also indicated the correlation between virus cluster and geographic location. Further DTMUV surveillance should be routinely conducted to provide the useful information on the evolution of Thai DTMUVs for the effective disease control and prevention.

Acknowledgements
We thank all graduated students and the staffs of the virology unit, Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University for their assistance with laboratory works. This research was supported by Thai government budget for 2017. We would like to acknowledge Chulalongkorn University for its financial support to the Center of Excellence for Emerging and Re-emerging Infectious Disease in Animals. We would like to thank the Thailand Research Fund for its financial support to the TRF Senior Scholar to AA (RTA6080012).

References
5. Li et al., 2006. Virus Genes. 33: 221-227.
**Survey of Influenza A viruses in a Live Bird Market in Bangkok, 2016-2017**

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**Keywords**: survey, influenza A virus, live bird market, Bangkok

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**Introduction**

Live bird market (LBM) is a place where live birds are sold for consumption. Due to mixing of different species, sources and the lack of all in–all out system, LBMs are the potential sources of influenza A virus (IAV) transmission (1). The occurrences of IAV in LBMs were reported in many countries. According to recently report, LBMs were linked to the H7N9 outbreaks in human in 2013 (2). In Thailand, the previous reports showed that HPAI-H5N1 and multiple of LPAI viruses could be found in poultry in LBMs in Bangkok (3, 4, 5). The co-infection in the same host in LBMs were reported in many countries. According to previous reports, influenza A virus were isolated from samples collected every months as showed in Figure 1. Interestingly, all positive swab samples were collected from chicken and ducks without any clinical signs.

---

**Materials and Methods**

During August 2016 to October 2017, a longitudinal survey for IAV in a LBM was conducted. Two vendors selling both chickens and ducks were selected for monthly sample collection. Oro-pharyngeal (OP) and cloacal swabs were collected from chickens and ducks. All swab samples were subjected for virus isolation by egg inoculation. The allantoic fluid of each sample was tested by Hemagglutination (HA) test. The samples were subjected to IAV detection by realtime RT-PCR (M gene).

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**Results and Discussion**

Total 780 swab samples were collected from 390 chickens and ducks. The OP and cloacal swabs were collected monthly from August 2016 to October 2017, except February and April 2017 due to the inconvenient of vendors owners. The result showed that 30.38% (237/780) of swab samples were positive for HA test. Out of 780 samples, 186 (23.85%) were positive for IAV by realtime RT-PCR. It noted that IAVs could be isolated from samples collected every months as showed in Figure 1. Interestingly, all positive swab samples were collected from chicken and ducks without any clinical signs. This finding suggested that chickens and ducks are the potential reservoirs of IAV in LBM. In this study, the origin flocks could not be identified. The shop vendors usually bought the poultry from dealers who gathered chickens and ducks from various sources, either backyard or farm. In conclusion, these findings supported that live bird market is a potential source of IAVs transmission. The genetic characterization of isolated viruses should be process to monitor the current status of IAVs circulated in LBMs.

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**Acknowledgements**

We would like to acknowledge Chulalongkorn University for its financial support to the Center of Excellence for Emerging and Re-emerging Infectious Diseases in Animals and the 60/40 Support for Tuition Fee. We would like to thank the Thailand Research Fund for providing its financial support to the TRF Senior Scholar to AA (RTA6080012).

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**References**

Accuracy of Pregnancy Diagnosis in Goats Using PAG ELISA Test

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Keywords: PAG, ELISA, pregnancy, goat

Introduction

Goats are small ruminants, their body size is comparatively smaller than other livestock animals so they don’t require large area for housing which are suitable for small farm. Goats are easily maintained because they can consume all kinds of plant which are generally refused by the other animals thus they can survive in all region of Thailand. Goats are mature enough to start breeding around 7-8 months of age and stay pregnant for 150 days. Goats give newborn kids within a short period thus economic return to the farmer rapidly. However, there are lack of breeding management in small farms, they get a lot of reproductive wastage income due to prolong kidding interval. To increase number of kids produced each year, shorten the kidding interval by diagnosing the pregnant and non-pregnant goats may be useful tool for improving reproductive efficiency. Pregnancy-associated glyco-proteins (PAG) belong to a family of aspartic proteinase (1) synthesized by mono- and binucleate trophoblastic cells in ruminants (2). PAG have been used as a new technology to determine pregnant and non-pregnant animals after artificial insemination (AI). Determination of PAG for pregnancy diagnosis by radioimmunoassay technique was highly accurate as early as day 21 of pregnancy in goats (3). PAG had significantly higher specificity than progesterone for diagnosis of non-pregnant (4). Recently, commercial kits for PAG have been produced for pregnancy diagnosis. IDEXX Bovine Pregnancy Test is a commercial ELISA test has been developed for determination of pregnancy in bovine. The previous study demonstrated that the commercial PAG ELISA test for bovine can determine the PAG levels in goats and can be applied for pregnancy diagnosis on day 30 of pregnancy onwards (5). However, the accuracy of PAG ELISA test for pregnancy diagnosis in goats has not been reported. The aim of the study is to determine the accuracy of a pregnancy-associated glycoprotein (PAG) ELISA test for diagnosis pregnancy status in goats.

Materials and Methods

The study was carried out on 167 female goats at the small farms in NongJok district, Bangkok, Thailand. All of the female goats were kept with the bucks and they gave the last kids for more than 3-6 months. Blood samples were collected from the jugular vein of each animals. Immediately after collection, blood samples were centrifuged at 1,500 g for 15 min and the plasma was stored at −20 °C for PAG analysis. All of the animals were followed up the pregnancy status for 125 days after blood collection. Plasma PAG levels were determined by a commercial ELISA test (IDEXX bovine pregnancy test, IDEXX Laboratories, Switzerland, AG) at Research and Development Center for Livestock Production by Nuclear Technology, Faculty of Veterinary Science, Chulalongkorn University (Bangkok, Thailand). The samples and control were tested according to the manufacturer assay protocol. Optical density (OD) were measured at 450 nm using a microplate reader (EL311 BioTek, USA). The PAG levels of the samples were determined by calculating from the OD of the sample (S) minus the OD of negative control (N); (S-N) value. The cut off values were recommended by the manufacturer, if PAG value was equal or greater than 0.300, the goat was considered pregnant; if PAG value was less than 0.300, the goat was considered non-pregnant. The goats were reexamined by following the parturition within 125 days after blood collection. Days of pregnancy were calculated from gestation period (150 days) minus the days from blood collection to parturition.

Results and Discussion

Among the 167 samples using PAG ELISA test, there was 130 goats whose PAG levels were above the cut off value were considered pregnant and 37 goats whose PAG levels were less than the cut off value were considered non-pregnant. In the present study, the PAG levels increased rapidly from day 25-30 of pregnancy and maintained at high levels at the later stage of pregnancy (Table 1). The results were harmonized with our previous study (5) and showed similarities to the other ruminant species such as cattle (6), sheep (7) and buffalo (8).

Table 1 Plasma PAG levels of 130 pregnant goats by a commercial PAG ELISA test.

<table>
<thead>
<tr>
<th>Days of pregnancy</th>
<th>n</th>
<th>PAG levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-30</td>
<td>8</td>
<td>0.76 ± 0.53</td>
</tr>
<tr>
<td>31-60</td>
<td>30</td>
<td>2.42 ± 0.82</td>
</tr>
<tr>
<td>61-90</td>
<td>46</td>
<td>2.36 ± 0.52</td>
</tr>
<tr>
<td>91-120</td>
<td>35</td>
<td>2.52 ± 0.54</td>
</tr>
<tr>
<td>121-150</td>
<td>11</td>
<td>2.78 ± 0.47</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>2.27 ± 0.84</td>
</tr>
</tbody>
</table>

PAG: Pregnancy-associated glyco-proteins
ELISA: Enzyme-linked immunosorbent assay
IDEXX: IDEXX Laboratories, Switzerland, AG
According to the previous study, PAG levels of pregnant goats were above the cut off value after day 25 of pregnancy (5) thus all of the goats had been reexamined the pregnancy status by following the date of parturition within 125 days after blood collection. Based on PAG ELISA test confirmation by parturition, there were 130 pregnant (correct positive diagnosis), 35 non-pregnant (correct negative diagnosis), 2 pregnant (incorrect negative diagnosis) and there were no non-pregnant (incorrect positive diagnosis), as shown in Table 2. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the PAG ELISA test were 98.5, 100, 100, 94.6 and 98.8 %, respectively. In conclusion, PAG ELISA test are highly accurate tests for pregnancy diagnosis in goats.

**Table 2** Comparison of results from pregnancy diagnosis by PAG levels and confirmation by parturition.

<table>
<thead>
<tr>
<th>PAG ELISA Test</th>
<th>Confirmation by parturition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant</td>
</tr>
<tr>
<td>Pregnant</td>
<td>130</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98.5</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
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<tr>
<td>Positive predictive value</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>94.6</td>
</tr>
<tr>
<td>Accuracy</td>
<td>98.8</td>
</tr>
</tbody>
</table>

**Acknowledgements**

This work was supported by the project of “The Use of Nuclear Technology to Improve Artificial Insemination in Dairy Cattle and Swamp Buffalo” under Thai Government budget.

**References**

Application of Microsatellite Markers for Pedigree Determination in Thai Swamp Buffalo


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*corresponding author: csupitc1@gmail.ac.th

Keywords: parentage testing, swamp buffalo, microsatellite marker, multiplex PCR

Introduction

The correct pedigree and parentage identification information are essential for buffalo breeding programs. The development in the area of molecular genetic by use of multiplex PCR of microsatellite analysis for parentage test in livestock animals (1) was the purpose of this research. The purpose of this research was to use set of 11 microsatellite marker to examine the relationship between families in buffaloes in Thailand.

Materials and Methods

Genomic DNA from 15 family of Thai swamp buffalo was extracted by using commercial kit according to the manufacturer instruction from blood and semen sample. DNAs were assessed and quantified using 2% agarose gel electrophoresis. Eleven microsatellite were selected for this study. Set of microsatellite markers were combined in multiplex PCR using forward primers of each microsatellite used was labeled with fluorescent dye (BM1013, CSSM038, CSSM019, CSSM043, CSM022, CSSM041, BM1818, CSSM057, ETH121, ITLS 033, ITLST006) as shown in Table 1. And the reaction procedure was followed those in our previous study (2) and was analyzed by the ABI prism 3130 Genetic Analyzer. Detection and sizing of separated fragments were performed with genotype Gene Mapper Software V3.7 (Applied Biosystems (USA). Then the results of allele band of offspring will be compared with DNA sample from their parents (mother and father) from 15 families by compared with recorded pedigree. Parentage test was to determine whether two individuals are biologically parent and calf.

Results and Discussion

This study we are able to utilize set of microsatellite markers in order to identity and differentiate each animal. As an example of the application of parentage identification in Thai swamp buffalo since the results of analysis were in accordance with pedigree data of the herds of buffaloes. The results of parentage testing was analysis according to Mendelian fashion. PCR amplification was determined for each marker separately. The amplified products were shown in figure1. It was found 8 loci from set of 11 microsatellite markers. While those 3 loci (Y1, B8 and G7) could be amplified by single PCR. It may be because of the inability of competition in the reaction.

This suggested that the reaction should be separated into 2 sets of microsatellite markers in the next study. In Figure 1 shown that pedigree in a genotype loci by microsatellite analysis of sire, dam and calf were compared with the pedigree data in the herds of Thai swamp buffalo. In Table 2, the representative of 5 family of parentage testing by multiplex PCR comparison of eight multiplex microsatellite analysis are shown. The frequencies of matching loci was 66.66% (10 family) for parentage testing inclusion, frequencies of not matching was 13.33% (2 family) for paternity testing exclusion and frequencies of 20% (3 family) for inconclusive; no decision can be made was shown in table 2.

Adding more family samples and separation of two multiplex set with eight and three of microsatellite markers respectively for parentage testing in swamp buffalo will be further study. As well as the paternity and maternity testing will be performed parallel for highly confirmation.

In conclusion, our results showed that these 11 markers could be possibly used for parentage identification in domestic Thai swamp buffalo. This results support the application of this set of genetic markers for identity testing in domestic buffalo. (3)

Acknowledgments

The current study was supported in part by grants in aid from Research and Development Centre for Livestock Production Technology, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant street, Phatumwan, Bangkok, 10330, Thailand. We thank the buffalo owners in the central parts of Thailand, who allowed us to collect blood and semen sample and we...
are grateful to many people who help us in this research.

References

Table 1: Eleven microsatellite markers of primer was labeled with fluorescent dye

<table>
<thead>
<tr>
<th>Marker</th>
<th>label</th>
<th>probe</th>
<th>dye</th>
<th>size range</th>
<th>name</th>
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<tr>
<td>BM1818</td>
<td>FAM</td>
<td>blue</td>
<td>240-280</td>
<td>B8</td>
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<tr>
<td>CSSM043</td>
<td>A565</td>
<td>red</td>
<td>220-260</td>
<td>R11</td>
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</tr>
<tr>
<td>ETH121</td>
<td>FAM</td>
<td>blue</td>
<td>100-130</td>
<td>B9</td>
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</tr>
<tr>
<td>BM1013</td>
<td>A550</td>
<td>yellow</td>
<td>210-240</td>
<td>Y1</td>
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<tr>
<td>CSSM022</td>
<td>FAM</td>
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<td>160-200</td>
<td>B10</td>
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<tr>
<td>CSSM038</td>
<td>A565</td>
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<td>160-190</td>
<td>R2</td>
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<td>A550</td>
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<td>130-150</td>
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<tr>
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<td>A565</td>
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<td>130-150</td>
<td>B3</td>
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<td>ITLST033</td>
<td>YY</td>
<td>green</td>
<td>120-160</td>
<td>G6</td>
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</tr>
<tr>
<td>ITLST006</td>
<td>YY</td>
<td>green</td>
<td>270-310</td>
<td>G7</td>
<td></td>
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</tbody>
</table>

Table 2: The representative of 5 family of parentage testing by multiplex microsatellite data and comparison of allele size of sire, dam and calf according to Mendelian fashion in the present DNA typing.

<table>
<thead>
<tr>
<th>Sample</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M8</th>
<th>M9</th>
<th>M10</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>sire 70B</td>
<td>143/147</td>
<td>161/177</td>
<td>133/135</td>
<td>225/253</td>
<td>207/207</td>
<td>121/121</td>
<td>183/185</td>
<td>151/151</td>
<td>sire 70B</td>
</tr>
<tr>
<td>Fam 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>מצב השאר</td>
</tr>
<tr>
<td>Dam 32</td>
<td>141/147</td>
<td>165/179</td>
<td>137/139</td>
<td>245/245</td>
<td>207/207</td>
<td>111/111</td>
<td>181/187</td>
<td>147/147</td>
<td>sire 70B</td>
</tr>
<tr>
<td>calf 85p</td>
<td>131/147</td>
<td>160/165</td>
<td>137/157</td>
<td>245/245</td>
<td>207/207</td>
<td>111/111</td>
<td>181/187</td>
<td>151/151</td>
<td>sire 70B</td>
</tr>
<tr>
<td>sire 70B</td>
<td>143/147</td>
<td>161/177</td>
<td>133/135</td>
<td>225/253</td>
<td>207/207</td>
<td>121/121</td>
<td>183/185</td>
<td>151/151</td>
<td>sire 70B</td>
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<td>Fam 1</td>
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<td>מצב השאר</td>
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<tr>
<td>Dam 32</td>
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<td>137/137</td>
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<td>181/187</td>
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<td>137/137</td>
<td>245/245</td>
<td>207/207</td>
<td>111/111</td>
<td>181/187</td>
<td>147/147</td>
<td>sire 70B</td>
</tr>
<tr>
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<td>165/171</td>
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<td>sire M1</td>
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* Exclusion = no match loci  ** Inclusion = match loci  Fam: Family

Figure 1: This chart of representative for the genotype of family pedigree of swamp buffalo.

In this family, shown that a genotype of a calf1 (#85p) loci no match with sire (#70B) genotype.

Table 1: Eleven microsatellite markers of primer was labeled with fluorescent dye

Table 2: The representative of 5 family of parentage testing by multiplex microsatellite data and comparison of allele size of sire, dam and calf according to Mendelian fashion in the present DNA typing.
Assessment of boar sperm motility characteristics by Sperm Class Analyzer (SCA®) diluted in new semen extender “Power-Pit” compared to a commercial Optim-I.A

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Keywords: Boar semen, SCA, progressive motility, Optim-I.A, Power-Pit

Introduction
Using computer assisted semen analyzer is a useful tool to evaluate kinetics properties of individual spermatozoa that cannot be identified by conventional light microscope [1, 2]. The objective of the study was to determine the sperm motility characteristics by Sperm Class Analyzer (SCA®) after dilution in a new semen extender (Power-Pit) formulated by our department compared to a commercial Mid-term (Optim-I.A).

Materials and Methods
Animals and management: Eight ejaculates from 6 mature boars (2 Large white, 4 Duroc) were collected by using a glove hand method at Nakhon Ratchasima Livestock Breeding and Research Center, Department of Livestock Development (DLD) and the Large Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University at Nakhon Pathom province from December 2017-January 2018. The semen were diluted at a concentration of 3x10⁹ spermatozoa/100 ml in two different semen extenders, Mid-term boar semen extender (Optim-I.A., Magapor, Spain) or Power-Pit (a new extender formulated by J. Suwimonteerabutr). Ejaculates at least 70% subjective motility and 90% morphologically normal were included. The extended fresh semen were kept in controlled temperature refrigerator (LIEBHERR, Germany) which was set at 16°C during preservation. The sperm motility characteristics were evaluated after 3, 5 and 7 days of storage, using a computer assisted sperm analysis system (SCA®, Proiser S.L., Valencia, Spain) [3].

The average speed index of the spermatozoa including velocity curved line (VCL), velocity straight line (VSL), velocity average path (VAP), linearity (LIN), straightness (STR), wobble (WOB), rapid velocity and progressive motility on Day 3, Day 5 and Day 7 of storage were investigated. The statistical analyses were performed by using multiple ANOVA using PRO GLM of SAS. P<0.05 was regarded to be statistically significant.

Results and Discussion

![Graphs showing sperm motility characteristics](image)

* statistical difference (P < 0.05)

Figure 1. Frequency distribution on percentage of various parameters of sperm motility characteristics (Mean+S.D) on Day 3, 5 and 7 between Optim-I.A and Power-Pit.
The characteristics of sperm motility for semen stored in Optim-I.A and Power-Pit as evaluated on days 3 and 5 post-collection (Figure 1). All parameters were not significant different. On Day 7, the VSL, LIN, STR and WOB in Power-pit were significant higher than in Optim-I.A (p<0.05), but percentages of rapid velocity and progressive motility are not different in each other.

Figure 2: (A) The sperm were clumping in Optim-I.A on Day 5 of storage. (B) The sperm were scattered (no clumping) in Power-Pit on Day 5 of storage. Scale bars = 50 um (A, B)

Sperm motility is important parameter for evaluating boar semen quality, because it indicates active metabolism and integrity of membranes, and also important for fertilizing capacity [4, 5]. As previously mentioned, semen containing boar spermatozoa exhibiting increased VSL and LIN are associated with larger litter sizes after insemination [6, 7] And, we noticed that the sperm in Optim-I.A had clumping phenomena on Day 5 of storage (shown in Figure 2), so it can affect to those speed indexes.

Our result indicated that boar sperm possessed a satisfied motility diluted in Power-Pit extender which was developed at the Faculty of Veterinary Science, Chulalongkorn University for at least 5 days, similar to those diluted in a commercial extender “Optim-I.A”.

Our formulated extender “Power-Pit” was upplemented with amino acids which possess antioxidant property to removing of free radicals (i.e reactive oxygen species, ROS) which help to reduce sperm membrane damage and protection against oxidative stress, including against cold shock. Power-Pit can maintain sperm motility until at least Day 7 of storage.

In conclusion, Power-Pit boar semen extender was provably standardized to test all sperm motility characteristics as a good extender in vitro. The further study should be tested in fertility and litter size.

Acknowledgments
This study was supported by Pornchai Intertrade Limited Partnership. The authors would like to thank staffs at Nakhon Ratchasima Livestock Breeding and Research Center, DLD for technical assistance on semen collection. I would like to also thank Prof. Dr. Padet Tummarak for helping statistical analysis in this study.

References
Boar sperm viability, acrosome integrity, JC-1 and sHOST after dilution in Mid-term (Optim-I.A) and Power-pitboar semen extender

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Keywords: Boar semen, Sperm Viability, Acrosome Integrity, JC-1, sHOST, Optim-I.A, Power-Pit

Introduction
Artificial Insemination for using liquid-stored boar semen have been increasing dramatically all over the world. The cryopreserved semen are still limitedin successfully fertility [1], and all of them are imported. The objective of the current study was to evaluate the sperm viability, acrosome integrity, JC-1 and sHOST in boar semen after dilution with Mid-term extender (Optim-I.A.) or a new semen extender (Power-Pit) which formulated locally by our department.

Materials and Methods
Animals and managements: Eight ejaculates from 6 mature boars (2 Largewhite, 4 Duroc) were included. The semen were collected by using gloved hand method. The semen were diluted to be a concentration of 3x10⁹ spermatozoa/ 100 ml in 2 semen extenders, Mid-term boar semen extender(Optim-I.A.) or Power-Pit (a new extender formulated by J. Suwimonteerabutr) were kept in semen tube. Ejaculates at least 70% subjective motility and 90% morphologically normal were used. The extended fresh semen were kept in controlled temperature refrigerator (LIEBHERR, Germany) which was set at 16 °C during preservation. The semen were observed after 3, 5 and 7 days of storage with parameters as followings:

Sperm Viability: The diluted semen were assessed using SYBR-14/Ethidiumhomodimer-1 staining technique (Fertilight®, Sperm Viability Kit, Molecular Probes Europe, Leiden, The Netherlands), which have some modification [2] (Fig. 1A).

Acrosome Integrity: The diluted semen were assessed using fluorescein isothiocyanate–labeled peanut agglutinin (FITC-PNA) staining. The positive results were presented as the percentage of spermatozoa with intact acrosome [3] (Fig. 1B).

JC-1: Sperm mitochondrial membrane potential was determined by using the fluorochrome 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1; Molecular Probe Inc.) [4] (Fig. 1C).

sHOST: The short hypoosmotic swelling test (sHOST) was assessed by using 75 mOsm kg⁻¹ of hypoosmotic solution [5] (Fig. 1D).

Statistical analyses: The statistical analyses were performed by using SAS (SAS version 9.0, Cary, NC., USA.). All sperm parameters were analyzed by using multiple ANOVA using PRO GLM of SAS. P<0.05 was regarded to be statistically significant.
Results and Discussion

Our result indicated that boar sperm are able to survive in vitro Power-pit extender which was developed at the Faculty of Veterinary Science, Chulalongkorn University for at least 5 days, similar to those diluted in a commercial extender “Optim-I.A”. Our formulated extender “Power-Pit” was supplemented with amino acids which possess antioxidant property to removing of free radicals and protection against oxidative stress, so it can maintain sperm quality of all parameters until Day 7 of storage. In conclusions, Power-pit extender provide a better result than Optim-I.A, but it is not significantly different. This will be a good candidate to commercial boar extender in market.

Acknowledgments

This study was supported by Pornchai Intertrade Limited Partnership. The authors would like to thank staffs at Nakhon Ratchasima Livestock Breeding and Research Center, DLD for technical assistance on semen collection, and also thank Prof. Dr. Padet Tummarak for helping statistical analysis in this study.

References

Bovine Endometrial Cells Secrete Interleukin-8 in Response to Chitosan Oligosaccharide

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Keywords: Bovine, endometrial cell, interleukin-8, chitosan oligosaccharide

Introduction
Cattle are remarkable among domestic animal as bacterial contamination of the uterus is ubiquitous after parturition (1). Uterine diseases are highly prevalent in cows shortly after calving, with over 20% of cows suffering persistent endometritis (2). Bovine endometritis may also occur after artificial insemination besides normal or abnormal parturition (3). Endometritis is associated with lower conception rates, increased intervals from calving to first service or conception and more culls for failure to conceive. More recently, biomaterials originate from natural substances and are widely used in medicine. Chitosan is a natural non-toxic biopolymer produced by the deacetylation of chitin, a major component of the shells of crustaceans such as crab, shrimp, and crawfish (4). Chitosan oligosaccharides exhibit anti-inflammatory activities both in vitro (5) and in vivo (6). Chitosan oligosaccharides protect against colitis by inhibiting NF-κB activation and preventing TNF-α and oxidative stress-induced apoptosis of intestinal epithelial cells (7). Nevertheless, until now, no literature report about the effects of chitosan oligosaccharide in bovine uterus. Therefore, the study of anti-inflammatory activities of chitosan oligosaccharide in bovine endometrium may leads to the novel therapeutic development of bovine endometritis or uterine infection.

Materials and Methods
Primary cultures of mixed endometrial epithelial and stromal cells were challenged with LPS (100 ng/ml) after treated with and without chitosan oligosaccharide (500 µg/ml). Samples without chitosan oligosaccharide and LPS treatment served as control. Secretory protein of IL-8 was measured by using enzyme-linked immunosorbent assay (ELISA). Univariate analysis of variance was used to evaluate the effect of chitosan oligosaccharide and LPS. Bonferroni post hoc tests were carried out to identify sources of differences. Significance levels were set as P<0.05.

Results and Discussion
The secretion of IL-8 was not significantly different in LPS challenged bovine endometrial cells pre-treated with chitosan oligosaccharide compared to LPS treatment group as expected. While, LPS up-regulated the secretion of IL-8 (P<0.05) (Fig. 1). These finding indicated that interleukin-8 secretion in bovine endometrial cells may not be influenced by anti-inflammatory activity of chitosan oligosaccharide directly. Other signaling pathways or other gene expressions that may involve with the link between immune response and chitosan oligosaccharide should be further investigated.

Figure 1
The concentrations of IL8 were measured, using the human IL8 ELISA in different groups (control, LPS, chitosan oligosaccharide-LPS). Values are means ± SEM. Differences between chitosan oligosaccharide-LPS, LPS treatments and control are indicated by *p < 0.05

Acknowledgements
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References
The effect of melatonin on cardiac differentiation of rabbit induced pluripotent stem cells (iPSCs) under different oxygen tensions

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Keywords: cardiac differentiation, hypoxia, induced pluripotent stem cells, melatonin, rabbit

Introduction
Melatonin, a molecule regulates circadian rhythms, has protective effects against myocardial injury (1). It has been reported that melatonin helped in the maturation of cardiac progenitor cells under low oxygen condition (hypoxia)(2). Hypoxia increased ROS (3), the secondary messenger has been demonstrated to promote cardiac differentiation, especially H2O2 (4). However, the effect of melatonin under normal atmosphere oxygen (normaxia) is unknown and there is no report of melatonin effect in persuasion pluripotent stem cells into cardiac progenitor cells. In this study we determined whether melatonin could induce rabbit induce pluripotent cells (iPSCs) to cardiac progenitor cells under hypoxia and normaxia. We also investigated H2O2 level under melatonin treatment and hypoxia condition.

Materials and Methods
Rabbit iPSC were maintained in DMEM/F12 supplemented with 20% knockout serum replacement (KSR) containing 10 ng/ml, bFGF and 1000IU LIF at 37°C 5% CO2. For cardiac differentiation, rabbit iPSCs were induced into three dimensional structure so-called embryoid bodies (EBs) using a hanging drop technique (day 0) in the medium containing FBS (HyClone™, Utah, USA) at 37°C and 5% CO2. Two oxygen tensions including atmosphere O2 (normaxia) and 5%O2 (hypoxia) were tested in the study. EBs(day 3) were plated on dishes coated with 0.1% gelatin. On day 6-8, the cells were treated with or without 100 nmol/L melatonin. To study H2O2 level, the cells after melatonin treatment 24 h were measured for H2O2 level, the cells after melatonin treatment and hypoxia treated cells under normoxia may cause by the lack of hypoxia-inducible factors α (HIFα) which normally presents under limited oxygen availability(5). HIFα involved in ROS production and in vitro cardiomyocytes maturation (2,6). Further investigation will be performed.

Results and Discussion
On day 6 after EB induction without melatonin treatment, the levels of H2O2, secondary messenger promotes cardiac differentiation significantly upregulated in differentiated cells under hypoxia (HC)(P<0.05). In hypoxia and melatonin treatment group (HM), H2O2 levels were decreased comparing to HC but there was not significant among HM, normoxia melatonin treatment group (NM) and normoxia control group (NC). The differentiated cells under low O2 condition with melatonin treatment (HM) for 48 h showed significantly upregulated of cardiac progenitor marker, NKX2.5 when compared to other groups (Figure 1). The results supported that the combination of melatonin and hypoxia promoted iPSCs into cardiac progenitors. However, different results of melatonin treated cells under normoxiamay cause by the lack of hypoxia-inducible factors α (HIFα) which normally presents under limited oxygen availability(5). HIFα involved in ROS production.

![Image](imagej.nih.gov/ij/) was applied to measure the mean gray scale which referred to ROS level. To determine cardiac progenitor stage among different conditions, the levels of mRNA expression of cardiac progenitor marker NKX2.5 were investigated on day 8 using relative real-time polymerase chain reaction.

![Figure 1](imagej.nih.gov/ij/)

Figure 1 The levels of H2O2 production(left panel) in different conditions after 24 h of melatonin treatment were measured using mean gray scales. Up-regulation of cardiac progenitor NKX2.5 in melatonin differentiated cells under low oxygen condition (right panel). HM: hypoxia melatonin treatment group, HC: hypoxia control group, NM: normoxia melatonin treatment group and NC: normoxia control group.

Acknowledgements
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References
Effect of Zona Pellucida on Survivability of Bisected Bovine Embryos:  
A Preliminary Study

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Keywords: bisection, bovine, zona pellucida, embryo

Introduction

In farm animals, embryo splitting has successfully been established for several livestock species (1). The absence of zona pellucida has been reported to have several advantages instead of being detrimental. In human, transfer of zona-free embryos is an effective method to increase implantation of poor-quality blastocyst (2). Here, we describe the results of a modified procedure (3, 4, 5) for bisection of Day7-8 bovine embryos using a fine microsurgical blade. This study aimed at examining the possibility to bisect bovine embryos. The presence of zona pellucida during culture of bisected embryos was also study.

Materials and Methods

Blastocyst: Bovine blastocysts were obtained by in vitro fertilization of oocytes matured in vitro (6). In brief, bovine ovaries were collected from a local abattoir and transported in normal saline solution to the laboratory within 4 h. The immature oocytes were collected via follicle aspiration using a 21 G needle attached to a 10 ml syringe. The oocytes were then examined under a stereomicroscope. The oocytes with more than 5 layers of cumulus cells were selected for in vitro maturation. The in vitro maturation of oocytes was performed at 38.5 ºC for 22 h. The immature oocytes were collected via follicle aspiration using a 21 G needle attached to a 10 ml syringe. The oocytes were then examined under a stereomicroscope. The oocytes with more than 5 layers of cumulus cells were selected for in vitro maturation. The in vitro maturation of oocytes was performed at 38.5 ºC for 22 h. In vitro fertilization was performed using frozen-thawed semen. The semen was thawed and counted. The mature oocytes were finally inseminated with bovine sperm at a final concentration of 1x10⁶ sperm per ml. The cleavage was determined on day 2 and further cultured until blastocyst stage (day 7-8).

Bisection of bovine blastocysts: The microsurgical bisection was performed at room temperature using an inverted microscope equipped with a mechanical micromanipulator (Zeiss) which was placed on the right side of the microscope. Only morphologically normal blastocysts were selected for bisection. Bisection was done in a microdrop of medium (Dulbecco’s phosphate buffered saline plus 10 % fetal bovine serum (FBS; Gibco). All embryos were bisected using a sterile microsurgical blade (Bio-cut blade, No. 730, Feather safety razor, Osaka, Japan) attached to the micromanipulator. The blastocysts were held with a holding micro-pipette, and inner cell mass was oriented toward the microsurgical blade so that the embryo could be bisected symmetrically. A total of 60 embryos were bisected to give rise 120 bisected/demi-embryos. The bisected embryos were then allocated into experiments. In experiment 1, the 40 bisected embryos were cultured without their zona pellucida (ZP free culture) in order to examine embryonic survival after bisection. Experiment 2 was performed as similar as experiment 1 except that the bisected embryos (n=40) were left intact with their original ZP. The development of ZP-intact embryos after bisection was then compared with ZP free bisected embryos (n=20) and with ZP surrogate embryos (n=20). The surrogate ZP was obtained from oocytes following completely ooplasm removal. The bisected embryos were transferred into surrogate ZP using a micropipette (20 micron diameter).

Culture of bisected embryo and survival assessment: The bisected embryos were transferred and cultured in a 50 µl droplet of synthetic oviductal fluid (SOF) supplemented with 10 (v/v) FBS. The culture condition was set at 38.5 ºC in a humidified condition with 5% CO₂. The survivability of the bisected embryos was determined by means of re-expansion of blastocoelic cavity and intactness of trophectodermal cells. The examination was daily performed for 48 h after bisection. Chi-Square was used to test the differences of embryonic survival after bisection among the experimental groups. P value less than 0.05 was considered statistically different.

Results and Discussion

In experiment 1, we demonstrated the possibility of embryo bisection using modified technique. Although we successfully bisected the embryos, the efficiency of this technique remained poor. Of 40 bisected embryos, only 7 ZP-free embryos (17.5%) were re-expanded. It is likely that extensive manipulation of embryos by means of bisection and micromanipulation may inevitably damage to an embryonic proper, in particular when the bisected embryos were cultured without their ZP. In experiment 2, our results indicated that ZP significantly affected on embryo survival (P<0.05), only when the demi-embryos were left in the original ZP after bisection. A total of 12 demi-embryos that remained within their original ZP (30%, 12 of 40 embryos) further developed after bisection. This survival rate was...
significantly higher than those obtained from ZP-free (15%, 3 of 20 embryos) and ZP-surrogate embryos (20%, 4 of 20 embryos). Although the reason of this is unclear, it is possible that minimization of micromanipulation with microenvironment of the bisected embryos within the ZP may promote cell repair and proliferation. Further study is required to elucidate this hypothesis. Interestingly, it has been previously reported that the bisected embryos with intact ZP could be transferred successfully to recipients without compromising pregnancy rates (7). It is therefore advisable to keep the bisected embryos in their original ZP in order to improve survival rate of the bisected embryos. Further studies to improve development of embryos following bisection and fertility testing after transfer of bisected embryos to recipients are essentially required.

Acknowledgements
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References
Effects of Mulberry Leaves Supplementation on Goat Performance

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Keywords: Goat performance, Mulberry leaves, Supplementary feeds

Introduction
Mulberry leaf (Morus alba) belongs to the Moraceae family and have long been the single feed for silkworm (Bombyx mori). Mulberry trees grow under varied climatic conditions, ranging from temperate to tropical, all over the world (1). The biomass yield of fresh leaves is often in the order of 25-30 tonnes/ha/yr with a cutting interval of about 9-10 wk, while leaves have high protein content (15.0-27.6 % in dry matter) and high (75-85 %) in vivo dry matter digestibility (2). Therefore, mulberry leaves have a high potential as a protein-rich forage supplement for animal production. The mulberry leaves can be used as the main feed for sheep and goats (3). Moreover, they have been used to replace concentrates in dairy cattle, goat, sheep, and swamp buffalo diets (1, 4, 5). Therefore mulberry leaves have a high potential as a protein-rich forage supplement for animal production. The mulberry leaves can be used as the main feed for monogastrics, ruminants and rabbits (6). Since mulberry leaves are rich in nitrogen, sulphur and minerals (7), they have the potential to be used as a supplementary feed for improving livestock productivity. The broilers, which were supplemented with 15 percent mulberry leaves, showed the lowest cavity deposited fat (8). The present study was conducted to evaluate the effect of supplementation with fresh mulberry leaves on body weight, growth rate and carcass quality of goat. And the mulberry leaves will be harvested all year round for study the seasonal effect on the nutritive value.

Materials and Methods
Nutrient composition: Green mulberry leaves (Burirum 60 variety) were obtained from a plantation kept at Chulalongkorn University farm at Saraburi province. They were analyzed for nutrient composition using standard methods of AOAC (9). The radioimmunoassay (RIA) technique was used to analyze the estrogen like compound in mulberry leave (10).

Animals and design: Twelve, male crossbred Boer goat (mean initial body weight of 18.92 kilogram, 3 months old) were received dietary treatment according to completely randomize design. All animals were kept in individual pen.

Experimental feeds: The fresh mulberry leaves were chopped and were fed at 0, 2.5, 5, and 10 % (kg of BW), once per day at 07.00 h and weight fortnightly prior to feeding and watering to determine average daily gain (ADG). The individual weights were recorded. Feeding systems mainly consist pangola dried grass was fed ad libitum, together with some commercial concentrate. The protein content of concentrate was 14 % and fed with 3 % /BW. Each treatment had 3 goat. The experiment was conducted for 120 days. At the end of the 120 days 1 goat from each group were randomly selected and slaughtered. The animals were slaughtered by Halal method for carcass evaluation. Analysis of the data was done with Duncan’s new multiple range test (11,12) and SAS (13).

Results and Discussion
The chemical composition of the mulberry leaf in Burirum 60 variety is presented in Table1. Our results showed that cool season was the suitable time for harvesting the high nutritive value of mulberry leaves as shown in Table 1. As shown in Table 2, the lowest of feed conversion ratio (FCR) was obtained for goat fed mulberry at 5%. The highest of final live weight and of average daily gain (ADG) and the highest carcass yield and the lowest abdominal fat were obtained for goat fed mulberry at 10%. These results were also agreed with Nualchuen et al., reported previously (14). In conclusion, fresh mulberry leaves supplementation up to 5% did affect body weight, growth rate and carcass quality.

Acknowledgements
This research was supported by The Project of The Use of Nuclear Technology to Improve Artificial Insemination in Dairy Cattle and Swamp Buffalo under Thai Government budget.
Table 1 Nutrient composition of mulberry leaf in Burirum 60 variety samples harvested all year round (three seasons).

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<td>Crude protein</td>
<td>17.87±0.13</td>
<td>17.74±0.19</td>
<td>19.95±1.31</td>
<td>10.32</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>45.50±0.23</td>
<td>44.67±0.25</td>
<td>47.83±0.29</td>
<td>17.56</td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>4.32±0.25</td>
<td>3.56±0.26</td>
<td>4.78±0.32</td>
<td>13.93</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>6.82±0.22</td>
<td>7.46±0.78</td>
<td>6.89±0.22</td>
<td>2.16</td>
<td></td>
</tr>
<tr>
<td>Crude fibre</td>
<td>14.99±0.21</td>
<td>13.73±0.36</td>
<td>12.55±0.25</td>
<td>5.96</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>12.98±0.22</td>
<td>14.78±0.25</td>
<td>11.55±0.26</td>
<td>10.49</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>1.74±0.20</td>
<td>1.89±0.17</td>
<td>1.88±0.13</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.26±0.03</td>
<td>0.29±0.02</td>
<td>0.37±0.03</td>
<td>19.40</td>
<td></td>
</tr>
<tr>
<td>Gross energy (cal/g)</td>
<td>3,912.70±0.25</td>
<td>3,901.22±0.17</td>
<td>4,007.70±1.72</td>
<td>20.20</td>
<td></td>
</tr>
<tr>
<td>Estrogen (pg/g)</td>
<td>80.50±0.26</td>
<td>81.76±0.20</td>
<td>83.60±0.26</td>
<td>9.72</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Effects of mulberry leaf supplementation on goat performance (Mean±SD).

<table>
<thead>
<tr>
<th>Items</th>
<th>0</th>
<th>2.5</th>
<th>5.0</th>
<th>10</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals (head)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0.83</td>
</tr>
<tr>
<td>Initial weight (kg)</td>
<td>19.83±1.04</td>
<td>18.16±2.46</td>
<td>18.83±1.26</td>
<td>18.84±1.25</td>
<td>0.83</td>
</tr>
<tr>
<td>Final live weight (kg)</td>
<td>31.16±4.36</td>
<td>29.00±1.73</td>
<td>38.00±1.00</td>
<td>32.16±2.36</td>
<td>6.19</td>
</tr>
<tr>
<td>Average weight gain (kg)</td>
<td>11.34±3.32</td>
<td>11.83±1.60</td>
<td>19.03±1.78</td>
<td>13.34±3.01</td>
<td>5.78</td>
</tr>
<tr>
<td>Average Daily Gain (g/day)</td>
<td>94.44±6.42</td>
<td>98.62±6.29</td>
<td>158.61±5.98</td>
<td>111.12±5.11</td>
<td>5.02</td>
</tr>
<tr>
<td>FCR (kg DM/kg gain)</td>
<td>13.52±2.32</td>
<td>14.53±2.14</td>
<td>12.14±2.88</td>
<td>15.64±3.54</td>
<td>0.87</td>
</tr>
<tr>
<td>Estrogen in plasma (pg/ml)</td>
<td>1.53±0.36</td>
<td>1.54±0.40</td>
<td>1.66±0.32</td>
<td>1.70±0.51</td>
<td>2.41</td>
</tr>
<tr>
<td>Carcass (%)</td>
<td>46.67</td>
<td>48.33</td>
<td>49.18</td>
<td>48.48</td>
<td>-</td>
</tr>
<tr>
<td>Abdominal fat (g)</td>
<td>514</td>
<td>316</td>
<td>252</td>
<td>220</td>
<td>-</td>
</tr>
<tr>
<td>Feed cost per kilogram gain (Bath)</td>
<td>86.40±4.8b</td>
<td>87.04±4.93b</td>
<td>98.56±8.74b</td>
<td>112.00±9.89b</td>
<td>6.63</td>
</tr>
</tbody>
</table>

*Different letters (a,b,c) within the same column represent significant differences (p<0.05).
Effects of supplementation of green tea waste on productive performances in crossbred Saanen lactating goats

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Keywords: Crossbred dairy goat, Green tea waste (GTW), Tannin

**Introduction**
Protein-rich commercial feedstuffs such as soybean meal and alfalfa hay are often used in livestock production, but they are generally expensive. Protein-rich forage became a renewed interest after there were BSE incidence and some cost variations of soy product due to climate change and other factors. The green tea waste (GTW) was a valuable protein source (22-35% of crude protein (CP)) but it comprised high tannin content (Yang et al., 2003). GTW has been a tentative source of protein diet in livestock production. However, animals fed tannin-rich diets decreased feed intake (Silanikove et al., 1994), rumen degradability and feed digestibility (Kondo et al., 2007). The objective of this study aimed to investigate the effects of different levels of GTW addition (0, 5 and 10%) in diets on productive performances (body weight change, feed intake and milk yield) in crossbred Saanen lactating goats.

**Materials and Methods**
The experiment used nine crossbred Saanen lactating goats, with body weight approximately 30 ± 3.50 kilograms. Animals were multiparous and in early lactation period. They were fed three diets in 3×3 crossover designed experiment. Goats were raised in metabolic cages providing feed and water. Basal diet contained corn silage mixed with concentrate in the ratio of 40:60 (DM basis) in a form of total mixed ration (TMR). Animals were fed twice a day at 0700 and 1600 hr. The first treatment (T1) is a control group with a basal diet. Treatment groups (T2 and T3) were fed diets containing GTW at 5% and 10% (DM basis) respectively. All diets were calculated based on isonitrogenous (16 % CP) and isocaloric (1.6 Mcal/Kg DM, NE₃) (NRC, 1981). Each period lasted for 21 days including 14 days for adaptation period and 7 days for collection period. The goats were weighed at the beginning of adaptation and collection periods as well as the end of collection period to calculate body weight change (BWC) and average daily growth (ADG). Daily food intake was determined using the different amount provided feed and remaining feed. Feed samples were randomly collected to determine dry matter intake (DMI). Goats were collected milk by hands at 0700 and 1600 hr. Ruminal fluid was collected by oral stomach tube (OST) method before feeding and after feeding at 2 and 4 hours to measure pH using pH-meter (MettlerToldo, S220 SevenCompact™, Thailand).

**Results and Discussion**

<table>
<thead>
<tr>
<th>Table 1 Effects of treatments on body weight change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Items</strong></td>
</tr>
<tr>
<td>Initial weight (kg)</td>
</tr>
<tr>
<td>Final weight (kg)</td>
</tr>
<tr>
<td>Body weight change (%BW)</td>
</tr>
<tr>
<td>ADG (kg/day)</td>
</tr>
</tbody>
</table>

Per = Period  
Trt = Treatment  
T1 = Treatment 1 (0% of GTW)  
T2 = Treatment 2 (5% of GTW)  
T3 = Treatment 3 (10% of GTW)

Table 1 shows the performance of dairy goat fed with and without GTW inclusion. There were not differences among treatments on BWC and ADG. GTW diets had no effect on BWC and ADG.

<table>
<thead>
<tr>
<th>Table 2 Effect of treatments on feed intake and milk yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Items</strong></td>
</tr>
<tr>
<td>Dry matter intake (g/day)</td>
</tr>
<tr>
<td>Dry matter intake (%BW)</td>
</tr>
<tr>
<td>Milk yield (g/d)</td>
</tr>
</tbody>
</table>

Table 2 shows the DMI and milk yield of dairy goats fed with different levels of GTW diets at 0, 5 and 10% of DM. Animals in the control group consumed less than those in the group fed with 10% GTW diet (P<0.05). No differences in DMI were found between control diet and 5% GTW diet. However, it is likely that animals with 10% GTW diet consumed higher in terms of DMI (%BW) but no differences were found among treatments. This study was similar to that of Kondo et al. (2004a) who found that goats receiving GTW at 3.1-11.4 % in diets consumed nearly the same amount as the control diet. The same findings were also found in dairy cattle when the same amounts of GTW were included (Theeraphaksirinont et al., 2008; Kondo et al., 2004b). The bitter taste of tannin in GTW could decrease the palatability of diets. It was postulated that...
effect of phenolic compound varied depending on type of diets and nature level of tannin (Kumar et al., 2017). Naturally, goats prefer the feed sources containing phytochemical secondary compounds including tannins and other alkaloids (Min et al., 2015). In addition, animals previously exposed to plant secondary compounds could eat much more secondary compounds-containing feeds than inexperienced animals (Villalba et al., 2004). Milk yields are not different among treatments. It means that the GTW inclusion in diets 5% and 10% inclusion had no deleterious effect on milk production.

Table 3 Effect of treatments on ruminal pH

<table>
<thead>
<tr>
<th>Items</th>
<th>Time (hour)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0</td>
<td>6.71±0.30</td>
<td>6.75±0.23</td>
<td>6.70±0.19</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.07±0.19</td>
<td>5.98±0.22</td>
<td>6.11±0.17</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.28±0.27</td>
<td>6.24±0.21</td>
<td>6.24±0.15</td>
<td>0.087</td>
</tr>
<tr>
<td>pH (means)</td>
<td></td>
<td>6.35±0.37</td>
<td>6.32±0.39</td>
<td>6.35±0.31</td>
<td>0.095</td>
</tr>
</tbody>
</table>

Table 3 shows ruminal pH of dairy goat fed with and without GTW. There were no differences among treatments. The experimental results were similar to those of Kondo et al. (2004b) and Xu et al. (2007) when 5% and 10% of GTW were included in diets fed to dairy cattle and sheep respectively.

Conclusion

It can be concluded from this study that inclusion of Green tea waste (GTW) at 5-10% in diets fed to crossbred Saanen goat did not have any deleterious effect on the productive performances. Further study need to be investigated whether the higher level of GTW can be used to replace the expensive source of protein.

References

Exploration of the geographic information and proportion of dairy cattle from using the farm management application program

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*Corresponding author: manakant.iti@msu.ac.th

Keywords: Dairy cows, farm management program, information

Introduction

Muang and Borabue districts are important areas for dairy farming of Mahasarakham province. There are many dairy farms, but veterinary service providers have no information about the farming conditions of each area and the problems of dairy herd management. Geographic information system (GIS) can be applied to various businesses including informatics of dairy farms (1, 2). This exploration aimed to present the geographic coordinates of each farm on the map and the farmers can record all daily activities that they had done on the dairy farming. The exploration was conducted at the Khok-kho dairy farmers cooperative limited. A total of 71 farms located in different sites, 11 of which were in Muang district, and another 60 were in Borabue district of Mahasarakham province. The GPS datasets were collected by Garmin etrex® GPS receiver. The number of dairy cows was collected monthly from December 2016 until November 2017 and was classified into 7 different categories such as dry dairy cows, heifers, young heifers, female calves, pregnant heifers, dry cows, and bulls. Descriptive statistics were used to describe the information included: the density of farms in the area, herd size and the proportion of the herd producing milk of both research sites.

Results and Discussion

As proposed, the eleven farms in Muang district had a total of dairy cattle about 378 heads and occupied approximately 0.27 square kilometers (66 acres) of land located. In Borabue district, the sixty farms held 1.10 square kilometers (271 acres) and had a total of dairy cattle about 2,310 head. According to the set of coordinates, farms located in each area are not far apart (Fig.1). The density of farms revealed the duties of the veterinarians to address the health problems in the area. The veterinarian is the one that can detect diseases that could affect the entire herd of dairy cattle and had responsibilities in public health to reduce any pathogens transmitted from animals to humans.

Figure 1: The map showing the position of the geographic coordinates of each farm.

This study found that the dairy farm herd size was around 35 heads per farms. The proportion of the cows producing milk was interesting because it was less than the nonproductive stock (dry cows, calves, heifers, and bulls). The data of Muang and Borabue districts demonstrated 39.94± 0.13 and 42.65±0.10 percent of milking cows per herd, respectively (Table 1, Table 2). Consequently, the high proportion of nonlactating cows in farms absolutely affected on total herd productivity. On average, milking cows in both districts produced low milk production, about 10.98± 4.33 kg/head/day for Muang district, while the dairy cows of Borabue district gave 12.72±3.73kg/head/day (Table 1, Table 2). The results of the monthly monitoring in Borabue district also showed that the proportion of the milking cows was noticeably decreased for the period from July to November, while the proportion of the dry cows was increased for the same period. Therefore, this was evidence that the dairy farmers were faced with herd...
composition and milk production were also not uniform throughout the year.

Table 1 The monthly report of average of milk production, the proportion of the milking cows, dry cows of 11 farms in Muang district of Mahasarakham province. (n=11)

<table>
<thead>
<tr>
<th>Month</th>
<th>Milkproduction (kg/head/day)</th>
<th>Milkingcows (%)</th>
<th>Dry cows (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2016</td>
<td>10.87±4.06</td>
<td>39.10±0.10</td>
<td>12.61±0.14</td>
</tr>
<tr>
<td>Jan. 2017</td>
<td>12.52±3.79</td>
<td>37.16±0.17</td>
<td>15.12±0.23</td>
</tr>
<tr>
<td>Feb. 2017</td>
<td>11.12±3.99</td>
<td>40.66±0.15</td>
<td>13.80±0.20</td>
</tr>
<tr>
<td>Mar. 2017</td>
<td>9.91±5.59</td>
<td>43.04±0.20</td>
<td>8.54±0.13</td>
</tr>
<tr>
<td>Apr. 2017</td>
<td>10.55±3.39</td>
<td>42.63±0.17</td>
<td>11.02±0.14</td>
</tr>
<tr>
<td>May 2017</td>
<td>10.98±4.07</td>
<td>40.52±0.11</td>
<td>13.90±0.16</td>
</tr>
<tr>
<td>Jun. 2017</td>
<td>10.64±4.35</td>
<td>41.35±0.12</td>
<td>14.86±0.15</td>
</tr>
<tr>
<td>Jul. 2017</td>
<td>9.78±4.52</td>
<td>42.41±0.11</td>
<td>10.23±0.12</td>
</tr>
<tr>
<td>Aug. 2017</td>
<td>12.28±5.66</td>
<td>38.16±0.15</td>
<td>17.89±0.12</td>
</tr>
<tr>
<td>Sep. 2017</td>
<td>11.72±6.22</td>
<td>34.09±0.12</td>
<td>19.88±0.09</td>
</tr>
<tr>
<td>Oct. 2017</td>
<td>10.01±2.46</td>
<td>38.64±0.12</td>
<td>11.94±0.03</td>
</tr>
<tr>
<td>Nov. 2017</td>
<td>11.62±3.54</td>
<td>37.86±0.08</td>
<td>12.09±0.06</td>
</tr>
<tr>
<td>Average</td>
<td>10.98±4.33</td>
<td>39.94±0.13</td>
<td>13.17±0.14</td>
</tr>
</tbody>
</table>

Table 2 The monthly report of average of milk production, the proportion of the milking cows, dry cows of 60 farms in Borabue district of Mahasarakham province. (n=60)

<table>
<thead>
<tr>
<th>Month</th>
<th>Milkproduction (kg/head/day)</th>
<th>Milkingcows (%)</th>
<th>Dry cows (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2016</td>
<td>13.94±3.18</td>
<td>44.50±0.11</td>
<td>5.26±0.05</td>
</tr>
<tr>
<td>Jan. 2017</td>
<td>14.40±3.27</td>
<td>45.03±0.09</td>
<td>5.41±0.06</td>
</tr>
<tr>
<td>Feb. 2017</td>
<td>14.12±3.84</td>
<td>43.44±0.12</td>
<td>5.25±0.06</td>
</tr>
<tr>
<td>Mar. 2017</td>
<td>15.17±4.14</td>
<td>44.17±0.10</td>
<td>5.55±0.05</td>
</tr>
<tr>
<td>Apr. 2017</td>
<td>13.55±4.14</td>
<td>44.53±0.11</td>
<td>5.92±0.06</td>
</tr>
<tr>
<td>May. 2017</td>
<td>13.51±3.20</td>
<td>46.49±0.08</td>
<td>5.32±0.05</td>
</tr>
<tr>
<td>Jun. 2017</td>
<td>12.53±3.50</td>
<td>46.49±0.09</td>
<td>6.84±0.06</td>
</tr>
<tr>
<td>Jul. 2017</td>
<td>13.03±3.30</td>
<td>42.31±0.08</td>
<td>10.71±0.07</td>
</tr>
<tr>
<td>Aug. 2017</td>
<td>12.39±3.22</td>
<td>39.57±0.12</td>
<td>14.23±0.13</td>
</tr>
<tr>
<td>Sep. 2017</td>
<td>11.29±2.37</td>
<td>37.62±0.10</td>
<td>12.56±0.09</td>
</tr>
<tr>
<td>Oct. 2017</td>
<td>11.37±2.82</td>
<td>39.45±0.09</td>
<td>11.14±0.07</td>
</tr>
<tr>
<td>Nov. 2017</td>
<td>11.74±2.70</td>
<td>39.98±0.09</td>
<td>8.42±0.07</td>
</tr>
<tr>
<td>Average</td>
<td>12.72±3.73</td>
<td>42.65±0.10</td>
<td>8.05±0.08</td>
</tr>
</tbody>
</table>

Dairy herd composition found in this study might be the result of many interrelated management actions of farmers, such as culling policy, replacement heifers management, ability to identify cows in heat and the chances of success at insemination (3). The problematic in the proportion of milking cows is an important factor lead to the emphasis on veterinarians in the area to play

Acknowledgements
The research was financially supported by the Clinic Technology, Ministry of Science and Technology; and by Faculty of Veterinary Science, Mahasarakham University. In developing the ideas for dairy farming community, I have received helpful input from the Khok-kho dairy farmers co-operative limited and all kindness of dairy farmers in Mahasarakham province.

References
Growth-inhibitory effect of pyroligneous acid on Bacillus cereus

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*Corresponding author: following_sunday@hotmail.com

Keywords: Bacillus cereus, Pyroligneous acid, spread plate technique

Introduction

Bacillus cereus—a rod-shaped gram-positive spore-forming facultative anaerobic bacterium was well-recognized for their growth and tolerance to wide-range temperatures (4–55 °C), pH (4.9–9.3), and water activities values (0.92–1.0) (1, 2). B. cereus are surplus in environment, by which their spores and cells are common in soil and dust causing contamination in foods including milk and several milk products. (2, 3). B. cereus’ endospores and their emetic toxin are well recognized for their extreme environment resistance making it one of the most important food poisoning pathogen in public health field (4, 5).

Only few disinfectants with violent oxidation property and toxicity to consumer and environment—such as peroxide or chlorine were proved effective against B. cereus endospores (6). According to this reason, the disinfectant replacement natural substance for B. cereus should be of great benefits to both public health and environment. Interestingly, the pyroligneous acid—commonly known as the wood vinegar has been proved for its significant antibacterial activity against several clinical antibiotic-resistant pathogens suggesting it as a good candidate for B. cereus disinfectant replacement (7). In this study, the growth inhibitory effect of pyroligneous acid on B. cereus was thus preliminarily determined. Of note, our preliminary result implied pyroligneous acid as an effective natural replacement for conventional B. cereus disinfectants.

Materials and Methods

Chemical substances: Eight-fold dilution of crude bamboo pyroligneous acid, crude mixed pyroligneous acid, crude eucalyptus pyroligneous acid, 99.95%ethyl alcohol, 4.8% chloroxylenol B.P. (Dettol®) and 30% hydrogen peroxide were prepared with sterile distilled water.

Grow-inhibitor test: The procedure was performed modify from Tantipaibulvut et al. (8). 500 µl B. cereus in dissension (0.5 McFarland turbidity) was evenly mixed with 500 µl volume of each dilution of the prepared chemical substance in microcentrifuge tubes for 5, 10, 20, and 30 minutes. At the end of each time period, the incubated dispense was spread on Muller Hinton agar plate (2 replications per experiment group), and incubated at 35 °C for 18-24 hours. The colony number of B. cereus acquired from each experiment group was then counted and recorded. The colonies count less than 300 colonies indicate the significant grow-inhibitory effect of the substance (8).

Results and Discussion

All substances used in this study including all types of pyroligneous acid could potentially inhibit the growth of B.cereus. Sorting by mean colony count, the order would rank from lowest to highest as following: hydrogen peroxide, bamboo pyroligneous acid, Dettol, ethyl alcohol, mixed pyroligneous acid and eucalyptus pyroligneous acid. As expected, hydrogen peroxide was likely to be the most potential growth-inhibitor. Interestingly, bamboo pyroligneous acid tended to possess greater growth-inhibitory effect than those of Dettol and ethyl alcohol—implying it as the effective disinfectant (at suitable concentration) comparable to conventional disinfectants like 4.8% chloroxylenol B.P. and ethyl alcohol. Since our preliminary study rendered us the promising results, we are now studying the growth-inhibitory effect of bamboo pyroligneous acid in more details using more experiment replicates with various acid concentrations and contact times.

Acknowledgments

This study was supported by Faculty of Veterinary Medicine, Rajamangala University of Technology Tawan-Ok.

References

6. Srisukontarat W., 2015. FDA MOPH TH.
Key Factors Associated with Sustainability of Small Scale Beef Farms in Thailand

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Keywords: Herd health management, Reproduction, Career sustainability, Beef Farms, Thailand

Introduction

Over last few decades, beef farms in Thailand have been developing in order to support continually increasing demands for meat consumption. However, the Thai Department of Livestock Development (DLD) reported overall trends in beef cattle number in the last 10 years that show the total number falling ~4 million (44.9%) from ~8.9 million in 2007 to ~4.9 million in 2017 along with a decline of beef farmer number, which have fallen 42.9% from a high of 1.4 million to 0.8 million. Therefore, the present study aimed to investigate the key factors that related to the intention of farmers’ descendant to continue careers in small scale beef farms.

Materials and Methods

Fieldworks were conducted during December, 2014 to December, 2016. A comprehensive questionnaire survey were carried out with 1,175 farmers who registered as a member of Mobile Development Unit (MUD), Armed Forces Development Command (AFDC) to gather the information of socio-economic profiles of farmers, farm size, herd management, reproductive management and cattle health management, and intention of farmers’ descendant to continue careers. In addition, a survey of 54 experts in the field of cattle was carried out. The survey contained 28 questions regarding the important of factors influencing beef herd production, based on a 5-point Likert scale from "strongly agree" to "strongly disagree".

The potential score of herd, health and reproductive management were calculated in each farm followed by classified their potential into 3 groups, i.e. low, medium and high potential. Statistical analyses were performed using general linear model (GLM, IBM SPSS Statistics version22). A logistic regression were used to identify associations between the intention to continue careers and profiles of farm management potential, farmer socio-economic and farm size. In addition, correlations between herd management score, reproductive management score and health management score were examined by Spearman’s rho correlation coefficient. Data are presented as sample mean ± SEM. Charts were created using GraphPad PRISM software (version 5.00; GraphPad Software Inc., San Diego, CA).

Results and Discussion

Our findings show tight correlations among herd management score, reproductive management score and health management score (Table 1). In addition, important factors links with sustainability were identified at farmer and farm levels. These include farm potential according to the herd, health and reproductive management, farmer gender and career (Table 2 and Figure 1). Whilst farmer age, education and duration of farming as well as farm size show no significant association.

In conclusion, the present study provided information of the key management in the small-holder beef farms in Thailand that influenced the intention of farmers’ descendant to continue careers which is useful for further establishment of strategies for sustainable improving beef cattle production.

Table 1 Correlations (Spearman’s rho correlation coefficient) between management score, reproductive management score and health management score. All results are significant at less than 1% level (P < 0.01).

<table>
<thead>
<tr>
<th>Management</th>
<th>Herd</th>
<th>Reproduction</th>
<th>Health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproduction</td>
<td>.476</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Health</td>
<td>.447</td>
<td>.529</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Table 2 Factors influencing the intention of farmers’ descendant to continue careers in small scale beef farms.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Beta</th>
<th>P-value</th>
<th>OR</th>
<th>95% confidence interval Lower-Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Farm potential</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Herd management score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Low</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2 Medium</td>
<td>1.06</td>
<td>&lt;0.01</td>
<td>2.89</td>
<td>2.07-4.03</td>
</tr>
<tr>
<td>3 High</td>
<td>1.74</td>
<td>&lt;0.01</td>
<td>5.68</td>
<td>3.63-8.90</td>
</tr>
<tr>
<td><strong>Reproductive management score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Low</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2 Medium</td>
<td>1.63</td>
<td>&lt;0.01</td>
<td>8.02</td>
<td>3.65-7.07</td>
</tr>
<tr>
<td>3 High</td>
<td>2.08</td>
<td>&lt;0.01</td>
<td>5.08</td>
<td>5.08-12.67</td>
</tr>
<tr>
<td><strong>Health management score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Low</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2 Medium</td>
<td>0.38</td>
<td>0.03</td>
<td>1.46</td>
<td>1.05-2.03</td>
</tr>
<tr>
<td>3 High</td>
<td>1.63</td>
<td>&lt;0.01</td>
<td>5.09</td>
<td>3.24-7.98</td>
</tr>
<tr>
<td><strong>Farmer socio-economic profiles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>-0.41</td>
<td>0.02</td>
<td>0.66</td>
<td>0.47-0.98</td>
</tr>
<tr>
<td>Female</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Age (Year)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 45</td>
<td>0.25</td>
<td>NS</td>
<td>1.28</td>
<td>0.86-1.91</td>
</tr>
<tr>
<td>46-60</td>
<td>-0.02</td>
<td>NS</td>
<td>0.98</td>
<td>0.71-1.36</td>
</tr>
<tr>
<td>&gt;=61</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Education (School)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>-0.24</td>
<td>NS</td>
<td>0.79</td>
<td>0.46-1.36</td>
</tr>
<tr>
<td>Secondary</td>
<td>-0.33</td>
<td>NS</td>
<td>0.72</td>
<td>0.39-1.32</td>
</tr>
<tr>
<td>Others</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Career</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Farmer</td>
<td>1.76</td>
<td>&lt;0.01</td>
<td>5.84</td>
<td>2.26-15.08</td>
</tr>
<tr>
<td>2 Government employee</td>
<td>0.61</td>
<td>0.33</td>
<td>1.83</td>
<td>0.55-6.16</td>
</tr>
<tr>
<td>3 Subdistrict administration</td>
<td>2.01</td>
<td>0.004</td>
<td>7.47</td>
<td>1.87-29.88</td>
</tr>
<tr>
<td>4 Others</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Duration of farming (Year)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 6</td>
<td>0.13</td>
<td>NS</td>
<td>1.14</td>
<td>0.69-1.88</td>
</tr>
<tr>
<td>7-20</td>
<td>0.42</td>
<td>NS</td>
<td>1.52</td>
<td>0.97-2.36</td>
</tr>
<tr>
<td>&gt;=21</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Farm size (Total animal)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 5</td>
<td>-0.34</td>
<td>NS</td>
<td>1.29</td>
<td>1.13-1.33</td>
</tr>
<tr>
<td>6-20</td>
<td>-0.04</td>
<td>NS</td>
<td>1.80</td>
<td>0.59-1.90</td>
</tr>
<tr>
<td>&gt;=21</td>
<td>Ref.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Chart displays estimated probability (%) of the intention of farmers’ descendant to continue careers in small scale beef farms that significantly associated with farm potential and farmer profile. Data are presented as sample mean ± SEM.

Acknowledgements
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Laparoscopic artificial insemination in goat in smallholder farms, using PMSG/hCG for synchronization program

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Keywords: goat, PMSG/hCG, smallholder farms

Introduction
Goat farming in the Northeast of Thailand is increasing. Inbreeding is a major problem of the production with ratio male:female of 1:30-40. Using artificial insemination has advantages by preventing inbreeding and diseases transmission from infected male. Progesterone impregnated vaginal device accompanied by an intramuscular injection of pregnant mare serum gonadotropin (PMSG) is a classical oestrous synchronization program in goat (1). While a combination of PMSG and human chorionic gonadotropin (hCG) like natural FSH and LH have been commonly used in sow for estrus induction. A few of extra-label usages by veterinarian for induction of follicle development in ewes were done (2, 3) while no report in goat. This study was to evaluate the effect of PMSG plus hCG (PMSG/hCG) for oestrous synchronization by fixed time laparoscopic artificial insemination (LAI) in goats raising in smallholder farms.

Materials and Methods
Thirty-six crossbred nannies, aged between 1-3 years, weigh between 25-37 kg (average 30.8 kg) selected from 3 smallholder farms (farm A (n=7), farm B (n=12), and farm C (n=17)) in Northeast of Thailand were included in the experiment. Non pregnant animals were inserted with intravaginal with Controlled Internal Drug Release (CIDR-G® Zoetis; containing progesterone 0.3g) for 13 days. At CIDR-G removal, a 2.5 mL of PMSG/hCG (P.G.600 Merck & Co., Inc.; containing 200 IU of PMSG and 100 IU of hCG) was given intramuscularly. Oestrous behavior included wagging of their tail, vaginal discharge, mount other does, redden vulva was detected twice a day (am/pm) for 30 min during 24-36 hrs after PMSG/hCG injection. In each farms, laparoscopic artificial insemination (LAI) were performed within 48-53hrs after PMSG/hCG treatment under sedation with xylazine and local anesthesia. The pregnancy diagnosis was performed at 35 days after LAI, usingtranzsectal B-mode ultrasonography (DP-2200vet, Mindray Co. LTD, Shenzhen, China) and the data was recorded.

Results and Discussion
The present experiment was the first report of the oestrous synchronization using PMSG/hCG in goat. The oestrous behavior was 50% (18/36) of does exhibited tail wagging this sign was frequently found, it was 36.1% (13/36) of nannies showed the redden vulva, and 16.7% (6/36) of nannies mounted others, and 13.9% (5/36) of nannies presented the vaginal discharge. The percentage of pregnancy in animals after fixed time AI in farm A, B, and C are 57, 50, 41, respectively and the average percentage is 49.3.

Table 1 Percentage of pregnancy in smallholder farms

<table>
<thead>
<tr>
<th>Farms</th>
<th>Does (N)</th>
<th>Pregnancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>57</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>C</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>49.3</td>
</tr>
</tbody>
</table>

Figure 1 Laparoscopic artificial insemination in goat.

The fertility rate in this study is similar to previous study in ewes. The fertilization rate in ewes synchronized by fluorogestone acetate intravaginal sponge (FGA), PGF2, eCG, and GnRH equalled 60%, it was 50% in FGA and PGF2α program, and 40% in FGA, PGF2α and GnRH program (3). The treatment of
PMSG/hCG with melengestrol acetate in ewes cause variation of the interval between progesterone impregnated vaginal device removal and oestrous (4). hCG have long half-life compared with pituitary gonadotropin (Luteinizing Hormone; LH), then premature ovulation may occur before oestrous (5). The conclusion of this experiment is PMSG/hCG synchronization program can be used for oestrous synchronization in goats. More investigation on ovulation time and proper time of insemination after PMSG/hCG treatment were planned in order to get a higher pregnancy.

Acknowledgements
This study is a joint research project between the Faculty of Veterinary Science, Chulalongkorn, KhonKaen and Mahasarakarn Universities and the KhonKaen Artificial Insemination and Biotechnology Research Center, KhonKaen, Department of Livestock Development. We thank for farms at Roi-et, Udon-thani (Bantung Farm, Kitchapat Farm, Rabeab Farm, Sriwarom Farm, and Thanachat Farm) for research support. The project was financially supported by The Agricultural Research Development Agency (Public Organization).

References
Ovarian responses on Boer goats with Split-single or Conventional multiple FSH treatment

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Keywords: Does, follicle stimulating hormone, hyaluronic acid, superovulation

Introduction

Superovulation as controlled ovarian hyperstimulation is a technique used in assisted reproduction involving the use of fertility medications to induce ovulation by multiple ovarian follicles. A series of hormone injections are given to donor females, causing the release of multiple ova at a controlled time. Resulting in superovulation which is the ovulation of a larger-than-normal number of ova, generally in the sense of at least two. Follicle stimulating hormone (FSH) is commonly used to superovulate in small ruminants, it’s commonly administered twice daily in a series of decreasing doses administered over 3 days (1). The several intramuscular (IM) administration of FSH is take time in the superovulation protocol for animals in which handling stress and increasing labor. Nowadays, hyaluronic acid (HA) is used to induce superovulation with a single injection of FSH that results in a sustainable slow release over several days (2). The present study aimed to compare the efficacy of the superovulation protocols between a conventional multiple administration method and a split-single intramuscular (IM) administration of Folltropin-V® in HA given to Boer goats.

Materials and Methods

Purebred Boer goats (n=7) with a body condition score of 3 to 3.5 and 40-45 kilograms (kg) of body weight, all does received the 2 treatments; at intervals of 1 year. Prior to treatment, all does were inserted an intravaginal device impregnated with Eazi-Breed® CIDR® contains 1.38 grams of progesterone in silicone molded over a nylon spine during the breeding season and leave in place for 13 days. Two treatments were designed to evaluate the superovulatory response of does following two methods of IM administrations. Treatment 1: does received six doses Folltropin-V® (50, 50, 30, 30 10 and 10 mg), given 12 h apart, on day 11, 12 and 13 of synchronization respectively. Treatment 2: does received 150 mg Folltropin-V on day 11 and 30 mg 48 h later in a 13 mg/mL HA solution (MAP-5, Bioniche Animal Health, Inc.). In the morning, and evening of day 13, all does received 150 µg cloprostenol (PGF2α, MERCK, Germany) and CIDR were removed in the morning of day 13 after PGF2α injection. In day 13, does received 200 IU hCG (Chorulon, MSD Animal Health, Inc.) after standing heat and; were inseminated by laparoscopic artificial insemination (LAI) and semen was deposited in the uterine horn within 22-24 h later. The numbers of corpus luteum (CL) was observed. The numbers of CL between two treatments were compared using paired T-test via IBM SPSS Statistics version 22.

Results and Discussion

All of donors does responded to superovulation program at 100%. Ovarian responses and ova/embryo (mean±SEM) collection are summarized in Table 1.

Table 1 Comparison of the number of CL and ova/embryo of does between a conventional multiple administration method and a split single intramuscular administration of Folltropin-V® in HA given to Boer goats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Administration (n=7)</td>
<td>13.5±5.5</td>
</tr>
<tr>
<td>Split-single MAP-5 (10 mg/mL HA) (n=7)</td>
<td>4.7±3.1</td>
</tr>
</tbody>
</table>

In the present study, there were significantly different in the mean of ovulation rate between multiple (13.5±5.5) and split-single (4.7±3.1) administration of Folltropin-V® (p<0.05) (Fig 1A, 1B). This result is different from previous study, stress and labor intensive to donor, which results in a reduced superovulatory response (3) and inhibited luteinizing hormone (LH) surge for ovulation (4). Distribution of FSH in diluent is one of factors that affect the success of superovulation. Because FSH was easier to dilute in diluent than HA. A solution of 10 mg/ml HA was quite viscous, it was not possible to extract all the mixture from the vial. This resulted in a numerically lower superovulatory response following a split-single administration, which may have been because of a lower amount of Folltropin-V actually being injected. Therefore, superovulation requires attention to detail and increase the possibility of failure because of mishandling and errors in giving treatment (1). When compared to split-single IM administration protocol, the conventional multiple administration method of Folltropin-V was very efficacious.
In conclusion, our findings suggested that a conventional multiple administration of Folltropin-V resulted in the acceptable superovulatory response in Boer goats.

**Acknowledgements**

This work was supported by the National Research Council of Thailand and the Industries (RRI) Ph.D. program, the Thailand Research Fund and the Agricultural Research Development Agency (Public Organization), ARDA, 2015, Thailand.

**References**


**Figure 1** Superovulation response, the ovary of does, which responded to conventional multiple injection (A) and split-single IM injection with Folltropin-V (B).
Rain tree pod (Samanea saman), the miracle feed for ruminants

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Keywords: Buffalo, di-hydroxypyridine, mimosine, purine derivatives, rain tree pod

Introduction
The tree legume rain tree is a multipurpose tree, with special properties of its can make and use in many aspects, such as environment improvement (agroforestry, carbon dioxide absorption, shade and shelter, soil improvement), fuels (charcoal and fuel wood), shellac (rain tree is used for cultivating the lac insect (Laccifer lacca)), timber, craft woods/canoe/boat/raft and animal feed etc. The rain tree pods (RTPs) have been used in animal feed for a long times, during shipment from Venezuela when growth of the cattle industry (1). Ways to use rain tree pods as a feed for ruminants, can be classified into 6 patterns; 1) directly giving ripen RTPs to animals, nutritive value of RTPs are considered as molasses, 2) oven-dried rain tree pods, RTPs were dried in a hot oven at 75 ºC for 72 h after collecting, then immediately ground (<4 mm Ø) prior to using or keeping in air-tight storages, nutritive value of oven-dried RTPs are considered as sources of energy and protein (3), 3) sun-dried rain tree pod pellets were dried by sun for 12-18 h, and keeping in air-tight storages before using them, nutritive value of sun-dried rain tree pod pellets considered as sources of energy and protein (3), 4) phytoneutrual additives, rain tree pod powder is mixed with 4 Thai herbs, nutritive value of RTP phytonutrient additives are not only use to reduce stress in animals, particular in hot season, but also it is used for manipulating rumen fermentation to reduce methane releasing to atmosphere (Research in progress), 5) it can be used for substituting molasses, being produced a bio-extracted solution, which uses to treat rice straw in order to increase nutritive value of rice straw (4), and 6) fermented rain tree pods, this is a superior of animal feed, due to sugar containing in rain tree pod is changed to microbial protein with a bio-extracted solution, therefore sugar is not influence on digestibility of fiber. The nutritive value of fermented rain tree pods increase proteins and minerals contents instead (Research in progress).

The objectives of the experiments reported in this paper were to determine and compare the effects of different proportions of sun-dried rain tree pod pellets(RTPP) and fixed amount of chopped fresh leucaena leaves (LL) in mixed diet as a supplemental diet, in Thai swamp buffalos, on the amounts of purine derivatives (PDs), mimosine+di-hydroxypyridine (DHP) in the urine and the ratios of purine derivatives to digestible organic matter intakes (PDs/DOMI).

Table 1 Dietary supplements (kg/animal/day) (g/kg on fed basis)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Supplement (kg on fed basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Time (600RTPP)</td>
</tr>
<tr>
<td>Sun-dried RTPP</td>
<td>0.60</td>
</tr>
<tr>
<td>Chopped fresh LL</td>
<td>6.00</td>
</tr>
</tbody>
</table>

Materials and Methods
Animals and experimental design and diets
Four male Thai, swamp buffaloes weighing 442±8.6 kg (36-40 month old) were fed ad libitum with ammoniated rice straw (1% NH₃) as a basal diet and allocated to one of four supplemental diets consisting of (kg on fresh weight/animal/day): i) 600 g sun-dried RTPP + 6 kg LL used as the control (RTPP = 1 Time), ii) 1,200 g sun-dried RTPP + 6 kg LL (RTPP = 2 Time), iii) 2,200 g sun-dried RTPP + 6 kg LL (RTPP = 4 Time) and iv) 3,200 g sun-dried RTPP + 6 kg LL (RTPP = 6 Time) (Table 1). The experimental design was 4 × 4 Latin square with arrangement of rice straw treatments. The effects different proportions of RTPP in supplemental diets on the purine derivatives (PDs), mimosine+di-hydroxypyridine (DHP) in the urine and the ratios of purine derivatives to digestible organic matter intakes (PDs/DOMI) in Thai, swamp buffalos had studied with 4 experimental periods. Each period 21 day consisted of 14 days for dietary adaptation and 7 days for experimentation. During the experimentation period, the animals were placed in individual crates fitted with containers for urine and faecal collections.

Determinations purine derivatives excretion and mimosine+di-hydroxypyridine in the urine
The amounts of feed intake, faeces and urine outputs were recorded daily for 7 days for urinary purine derivatives and mimosine+di-hydroxypyridine DHP studies. Ten percentage representatives of supplements and ammoniated rice straw offered, and representative of ammoniated rice straws refusal, faecal outputs and urine excretions were collected and stored at -20°C, until used for chemical analyses. The allantoin in the urine was determined according to the colorimeter method (5).

Determination of uric acids in the urine was carried out by its reaction with uricase using a commercial kits. The mimosine and DHP in the urine were determined by using HPLC (6).

Results and Discussion

Chemical composition and nutritional value of rain tree pods

Characteristics of the mature rain tree pods (Samanea saman) are black-brown, oblong, lumpy, 10-20 cm long, 15-19 mm wide, ca. 6 mm thick, straight or slightly curved, not dehiscing but eventually cracking irregularly, and they are filled with a sticky, brownish pulp that is sweet and edible. Chemical analysis of the rain tree pods contained 760 g dry matter (DM)/kg as fresh-basis, 87.3 g ash, 22.0-32.0 g nitrogen, 330-460 g total nonstructural carbohydrates (TNC), 180-400 g total sugar (TS; sucrose 68.5%, glucose 5.5%, xylose 9.0%, galactose 4.2, fructose 8.9% and other sugar 4.0%), 15-70 g starch, 140-150 g reducing sugar (RS) and 170-250 g non reducing sugar (NRS) and 10-50 g total phenolic compounds. Metabolizable energy for ruminants is 10 mega joules/kg DM and organic matter digestibility is 840-870 g/kg DM.

Table 2

Urinary purine derivatives in urine, the ratios of PD to DOMI, mimosine and DHP in urine of swamp buffalo fed ad libitum of ammoniated rice straw and supplemented with the different proportions of rain tree pod added into leucaena leaves

<table>
<thead>
<tr>
<th>Items</th>
<th>Proportions of sun-dried rain tree pod pellets</th>
<th>S.E. D</th>
<th>Difference (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>linear</td>
</tr>
<tr>
<td>Number of animals</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Purine derivatives in urine (mmol kg BW$^{0.75}$ d$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allantoin</td>
<td>$1.45^{bc}$</td>
<td>$1.41^c$</td>
<td>$1.65^b$</td>
</tr>
<tr>
<td>Uric acids</td>
<td>0.07$^a$</td>
<td>0.06$^b$</td>
<td>0.07$^{ab}$</td>
</tr>
<tr>
<td>Total PDs</td>
<td>$1.52^{bc}$</td>
<td>$1.48^c$</td>
<td>$1.72^b$</td>
</tr>
<tr>
<td>PD/DOMI (mmol/kg DOMI)</td>
<td>39.1</td>
<td>38.4</td>
<td>43.8</td>
</tr>
<tr>
<td>Mimosine and DHP in urine (mg/kg BW$^{0.75}$ d$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mimosine</td>
<td>4.65$^b$</td>
<td>9.42$^c$</td>
<td>1.37$^c$</td>
</tr>
<tr>
<td>3,4-DHP</td>
<td>18.2$b$</td>
<td>20.9$b$</td>
<td>28.7$^a$</td>
</tr>
<tr>
<td>2,4-DHP</td>
<td>52.6</td>
<td>57.6</td>
<td>61.0$^a$</td>
</tr>
<tr>
<td>Mimosine+DHP</td>
<td>75.5$^b$</td>
<td>87.9$^{ab}$</td>
<td>91.1$^a$</td>
</tr>
</tbody>
</table>

1 S.E.D: Standard error of mean averaged throughout experiments
2 $^{abc}$: Values within the same column with different superscripts are significantly (P <0.05) different.
3 $^{abc}$: Values within the same column without different superscripts are not significantly (P<0.05) different.

Table 2 shows that the increased proportions of sun-dried RTPP in dietary supplements linearly increased (P<0.001) the purine derivatives in urine, and the efficiency microbial production (PD/DOMI) in the rumen in buffaloes linearly increased (P<0.05) when proportions of sun-dried RTPP in mixed supplement diet increased. The urinary mimosine+DHP excretions linearly increased (P<0.01) in animals when the proportions of sun-dried RTPP in leucaena of dietary supplements increased. The values of mimosine+DHP in the urine was nearly closed to 100 mg mimosine+DHP/kg BW$^{0.75}$ in the urine, but the average excretion rate of mimosine+3,4-DHP did not reach to 100 mg mimosine+DHP/kg BW$^{0.75}$ in the urine. Therefore, leucaena toxicity considered not to be anticipated to the animals. This study demonstrated rain tree pod pellets, one of six processing of rain tree pods was used as a good ingredient feed when combination with leaves of leucaena for enhancing microbial production in the rumen. RTPP was produced from a simple modified meat grinder, it is suitable to both commercial farm and small holding farm. Rain tree pod there fore is justified to be a miracle feed for ruminants, one of nature nutritious feed, which supply energy, protein and minerals sources. Studies proved the benefit of rain tree pods as a feed in several ruminant species (1-4 and 7-9) thus rain tree is one of the most potent multipurpose legume tree in the tropical area.
Acknowledgements
The funds were provided by a Thai government budget under the increasing efficiency of food and under the increasing efficiency of food and agricultural production by a nuclear technology project. (Project Code EFF 06/58) are acknowledged.

References
Targeted gene functional enrichment analysis of the milk miRNome acquired from dairy cow and nursing mother during 2-6 months of lactation

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Keywords: Functional enrichment analysis, miRNome, milk, dairy cow, nursing mother

Introduction
Milk contains variety of essential nutrients and bioactive molecules crucial for newborn growth and development. MicroRNA (miRNA)—the non-coding RNA of 18-22 nucleotides responsible for gene post-transcriptional regulations is among bioregulatory molecules in lipid phrase of milk with cryptic significance (1,2). Since miRNAs in milk lipid are considered as the unique extra-chromosomal genetic materials passively transferred from mother to newborn, milk miRNome has thus become an exciting research field in human and other mammals (1,2). While regulatory roles of milk miRNAs were already implied by their proved/predicted targeted genes in several mammals, the direct functional comparison among the species is still rarely illustrated. Since such knowledge could contribute to insight into conserved role of milk miRNA among mammals, we hereby performed such demonstration using the full spectrum data of miRNAs expressed in genome—the miRNome of milk lipid acquired from diary cow (Bos taurus) and nursing mother (Homo sapiens).

Materials and Methods
Data source: The miRNA-seq raw datasets of nursing mother milk lipid during day 60 (D60), 120 (D120) and 180 (D180) of lactation and dairy cow milk lipid during day 70 (D70), 130 (D130) and 170 (D170) were obtained from bioprojects PRJNA338273 and PRJNA305166 in the Sequence Read Archive (SRA) format (www.ncbi.nlm.nih.gov). Proved and predicted targeted genes of both human and cow miRNA were acquired from TargetScan database (http://www.targetscan.org/vert_71).

Data pre-processing and alignment: Adapter trimming (5’ and 3’ primes) and quality trimming (phred score ≥ 20) were performed by ‘cutadapt’ (3). Only reads ranging from 18-30 nucleotides with lesser than 2 ambiguous bases were selected. Data quality was determined by ‘FastQC’. STAR and ‘HTSeq’ softwares were applied for genome alignment and counting, accordingly (4).

Functional enrichment analysis: Presented miRNAs of each sample group were adopted for their proved/predicted targeted genes. Gene Ontology (GO) enrichment analyses using hypergeometric model was performed with all targeted gene clusters and compared among each of sample groups using ‘clusterProfiler’ package (5).

Data visualization: Heatmaps of miRNA read counts in regularized logarithm of each sample group were drawn and observed (4). Enriched functional categories of each gene clusters acquired among sample groups were visualized and compared for their gene ratios and adjusted p-values together by dotplot (5).

Results and Discussion
Read count results revealed 129 and 149 mature miRNAs in dairy cow and nursing mother milk samples—randomly distributed among lactating days (Fig 1). This hereby implied cognate miRNA profiles during 2-6 months of lactation in both species. When determining targeted genes of such miRNAs, much larger genes (7,955 genes) were rendered by dairy cow miRNAs, than those of nursing mother (2,096 genes) (Fig 2). This was likely due to the fact that most bovine miRNA targeted genes were merely predicted rendering exaggerated numbers of targeted genes.

Figure 1 Heatmap showing miRNA read count number in regularized logarithm (Exps) and scaled regularized logarithm (Scaled Exps) acquired from dairy cow (D60, D120 and D180) and nursing mother (D70, D130 and D170), accordingly.
Gene Ontology (GO) enrichment analysis revealed several GO terms related among dairy cow and nursing mother miRNAs’ targeted genes (adjusted p-value ≤ 0.001). These GO terms involved varieties of body system developments and gene transcription control. Top 6 most significant annotated terms from each of 3 major GO categories—Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) suggested nervous system as the most significant body system targeted by miRNAs. In conclusion, the acquired results not only implied the conserved role of milk miRNAs for development of both calf’s and baby’s body systems, but also possibly several other mammals. With the acquired promising results, we plan to decipher into annotated terms and core genes targeted by the miRNAs of interest in the future study.

Acknowledgments
This research was technically supported by the Thailand Research Fund (TRF) through New Research Scholar Programme (Grant No. TRG5880003) and The TAGC laboratory (Marseille, France)

References
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5. Yu et al., 2012. OMICS. 16(5): 284-287
Use of Plasma Progesterone Levels for Monitoring Ovarian Activity in Recipients Beef Cows for Cloned Gaur Embryo Transfer and Early Pregnancy Diagnosis

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Keywords: Progesterone, recipient, cloned gaur embryo, ovarian activity

Introduction
More than 30 yrs progesterone (P4) has been used to monitor ovarian activity, estrus detection accuracy, and pregnancy in cattle and buffalo. One factor of the success of cloned bovine embryo transfer is reproductive status of recipients. Suitability of recipients is dependent on the timing of estrous and the presence of a functional corpus luteum (CL). Ovarian activity and pregnancies derived from recipients for cloned gaur embryo transfer and early pregnancy related to peripheral plasma progesterone (P4) have not been reported. Therefore, the objective this study was the use of plasma P4 for assessing cyclic ovarian activity and early pregnancy diagnosis in crossbreeds beef cows (Brahman X Native) recipients by using radioimmunoassay (RIA) technique.

Materials and Methods
The study was conducted at a private farm located at Saraburi province in the central part of Thailand during March 2016- Feb 2017. Six healthy crossbred beef cows (Brahman X Thai Native) were first or second calving non-lactating. They had been rectally palpated and found to have no abnormality of the reproductive tract. The ovary containing a palpable functional CL of recipients was transferred nonsurgically with 2-3 cloned gaur embryos. All cows were fed with 19% protein concentrate, mineral salts, rice straw and fresh water ad libitum. Blood sampling were collected from all recipients 10 days interval for monitoring ovarian activity and early pregnancy diagnosis (5) and at the day of transferring embryos. Plasma was separated by centrifugation (15mins, 1,500g) and stored at -20°C until assayed. Plasma P4 levels were determined by using RIA technique as described by Kamonpatana et al (6).

Validation of RIA: Plasma P4, the reliability of this method was tested in two pools of low and high standard P4 added in the blank plasma pools. The coefficient of variation (CV) of the two pools of internal control of the assay were 10.01 (0.1 ng/ml) and 8.70 (1 ng/ml), respectively. The CV of inter assay were 16.62% and 10.3 % respectively. The sensitivity of the assay was 0.05 ng/ml. Data presented as: basal level of P4 were 0.05 ng/ml. The obtained P4 values of ten days interval (day 0 and day 10) were interpreted as follows:
1. High P4 levels on day 0, but low on day 10 or low on day 0, but high on day 10 were interpreted as an ovulation with normal cycle.
2. High P4 levels on both day were interpreted the presence of CL, persistence CL or pregnant
3. Low P4 on both day were interpreted as ovarian dysfunction.

Results and Discussion
Plasma P4 level and ovarian activity: Ten days interval of plasma P4 levels in six recipients (Figure 1) were showed normal estrous cycle which had an ovulation and CL formation. Levels P4 at luteal phase were 3.5 ±1.4, 2.8 ±1.8, 3.4 ±0.9, 3.8 ± 0.4, 1.7 ±0.4 and 3.0±1.2 ng/ml in animal no.2, 6, 7, 16, MD006 and MD008, respectively. And P4 on day of ovulation were 0.2±0.1, 0.2± 0.1, 0.2 ±0.1, 0.4±0.3 and 0.2 ±0.1 ng/ml in animals no.2, 6, 7, 16, MD006 and MD008, respectively.

Figure 1 Ten days interval plasma P4 in six recipients.
At the beginning of our study, all recipients showed irregular estrous cycle for 52, 56, 122, 185 and 91 days, respectively as was reported in post partum cows (7). This phenomenal through plasma P4 can classify estrous cycles in recipients into 3 types: 1) anestrous cycle which P4 was less than 0.4 ng/ml for 91 days following with normal cycle (no.MD008). 2) discontinuous luteal phase which low P4 ranging from 0.5-1.0 ng/ml were found in 4 animals(no. 2, 6, 7 and 16). And 3) normal cycle following with prolonged luteal activity which P4 levels >1 ng/ml for more than 20 days in absence of cloned embryo was transferred was found in one animal (no.MD006). In nine times of embryo transfer, each recipient was transferred 1-2 times while their ovary containing a palpable functional CL(8). Five recipients were in luteal phase on the day of clone gaur embryo transfer and the mean ± SD of plasma P4 (Figure 1) were 2.0 ± 1.3 ng/ml (0.8 - 4.0 ng/ml). And there were four recipients were in follicular phase, which mean ± SD plasma P4 were 0.5 ± 0.2 ng/ml (0.3 - 7.4 ng/ml). The results obtained could not relate the phase of the cycle indicated by palpation to the plasma P level. These data indicated 40% of recipients were not transferred on the right timing of estrous cycle. Obviously the rectal palpation technique depends on the farm technician expertise.

**Early pregnancy diagnosis:** The pregnancy periods after embryo transfer at follicular phase and at luteal phase were range between 53 and 42-77 days, respectively (Table 1). There were six out of nine transferring had successfully become pregnant with the gaur clone blastocysts (both frozen and fresh blastocyst) 42-77 days after transfer. Our results were similar to previous report use ultrasound to validate, eight out of thirty-two were pregnant 40 days (9).

In conclusion, Plasma P4 on the day of cloned embryo transfer suggested that cloned gaur embryos were transferred while recipients were in follicular phase (44.4%) and luteal phase (55.6%). Which plasma P4 mean ± SD in follicular phase were 0.5 ± 0.2 ng/ml and in luteal phase were 2.0 ± 1.3 ng/ml. There were 66.7% of recipients had successfully become pregnant range between 42-77 days after transfer.

**Acknowledgements**
The authors gratefully acknowledge Prof. Maneewan Kamonpatana, founder and former leader of the Research and Development Center for Livestock Production Technology, Faculty of Veterinary Science, Chulalongkorn University, for her advice and providing all the facilities. This research was supported by the project of The Used of Nuclear Technology to Improve Artificial Insemination in Dairy Cattle and Swamp buffalo.

**References**

**Table 1** Level of plasma P4 of recipients on the day of cloned gaur embryo were transferred and period of pregnant.

<table>
<thead>
<tr>
<th>No. Recipients</th>
<th>Stage of embryos (n)</th>
<th>P4 on day of embryo transfer (ng/ml)</th>
<th>Stage of estrous cycle</th>
<th>Early pregnancy diagnosis (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Fresh early BL (2)</td>
<td>0.5</td>
<td>Follicular phase</td>
<td>Confirm</td>
</tr>
<tr>
<td>16</td>
<td>Fresh early BL (2)</td>
<td>0.7</td>
<td>Follicular phase</td>
<td>Confirm</td>
</tr>
<tr>
<td>16</td>
<td>Fresh BL (2)</td>
<td>0.3</td>
<td>Follicular phase</td>
<td>53</td>
</tr>
<tr>
<td>MD008</td>
<td>Fresh BL (2)</td>
<td>0.6</td>
<td>Follicular phase</td>
<td>Non pregnant</td>
</tr>
<tr>
<td>Mean± SD</td>
<td></td>
<td>0.5 ± 0.2</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>Fresh early BL (2)</td>
<td>2.6</td>
<td>Luteal phase</td>
<td>77</td>
</tr>
<tr>
<td>6</td>
<td>Fresh early BL (2)</td>
<td>1.6</td>
<td>Luteal phase</td>
<td>43</td>
</tr>
<tr>
<td>7</td>
<td>Fresh morula (2)</td>
<td>0.8</td>
<td>Luteal phase</td>
<td>Non pregnant</td>
</tr>
<tr>
<td>MD006</td>
<td>Fresh BL (2)</td>
<td>1.1</td>
<td>Luteal phase</td>
<td>54</td>
</tr>
<tr>
<td>MD008</td>
<td>Frozen BL (3)</td>
<td>4.0</td>
<td>Luteal phase</td>
<td>42</td>
</tr>
<tr>
<td>Mean± SD</td>
<td></td>
<td>2.0 ± 1.3</td>
<td></td>
<td>48 ± 19</td>
</tr>
</tbody>
</table>

BL= Blastocyst
Administration of non-steroidal anti-inflammatory drugs prior to artificial insemination increases litter size in gilts and sows

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Keywords: Artificial insemination; Litter size; NSAIDs; Pig; Stress

Introduction
Non-steroidal anti-inflammatory drugs (NSAIDs) is normally used for anti-pyretic, anti-inflammation, reduced pain and inhibit endotoxin in postpartum sows [1-2]. In general, NSAIDs is safer for a long-term use compared to steroid drug (e.g., dexamethasone). NSAIDs reduce inflammatory reaction by inhibiting prostaglandin F2alpha (PGF$_{2\alpha}$) synthesis and can also be combined with PGF$_{2\alpha}$ receptor. A previous study has demonstrated that the plasma concentration of PGF$_{2\alpha}$ is increased after artificial insemination (AI) in sows but this is not observed when the sows are mated naturally by boar [3]. The raising of PGF$_{2\alpha}$ may interfere sperm transportation, which may compromise the efficacy of AI in some sows. Therefore, if the raising of PGF$_{2\alpha}$ after AI can be inhibited, conception rate and litter size at birth of the sows maybe increased. The objective of the present study is to determine the effect of NSAIDs administration before AI in gilts and sows on PGF$_{2\alpha}$ concentrations and their subsequent litter size at farrowing.

Materials and Methods
The experiment was carried out in a commercial swine herd in the middle part of Thailand from March to June 2017. In total, 48 gilts and 218 sows (parity numbers 2-6) were included. The gilts and sows were randomly allocated into two groups: control (n = 172) and treatment (n = 94). In the control group, the gilts and sows were inseminated 2-3 times during standing estrus. In gilts, the insemination was carried out once the standing estrus was observed and again at 12 and 24 h later. In the sows, inseminations were carried out at 12 h after the onset of standing estrus and again at 12 and 24 h later. In the treatment group, both gilts and sows received 2 mg/ kg of NSAIDs intramuscularly (1 mL/20 kg body weight, Tolfedine® CS, Vétoquinol, Québec, Canada) at 11.4±9.7 min before AI. After AI, the gilts and sows were housed in an evaporative cooling system during gestation. The pregnant gilts and sows were sent to the farrowing house at about one week before farrowing.

In general, the replacement gilts were mated at 8 months of age at the second or later observed estrus. Estrus synchronization was performed in the gilts by using an oral supplementation of altenenogest 20 mg/ day for 18 days (Virbagem®, Virbac, Carros Cedex, France). Feed was provided twice a day (1.5–3.5 kg per day) during gestation and were fed 2-4 times a day during lactation (5–6 kg per day) with a corn-soybean-chicken ration. The feed was formulated according to the nutritional requirements of gestating and lactating sows [4]. The gestating feed contained 16.0% CP, 2,800 kcal/kg ME, and 1.0% lysine. The lactation feed contained 18.0% CP, 3,200 kcal/kg ME, and 1.1% lysine. Water was provided ad libitum by water nipples. The health management of the animals was carried out by the herd veterinarians. Both gilts and sows were recommended to be vaccinated against classical swine fever virus, foot and mouth disease virus, pseudorabies virus, porcine parvovirus, porcine reproductive and respiratory syndrome virus, porcine circovirus type 2 and a combined vaccine of Actinobacillus pleuropneumoniae and Mycoplasmosis. After farrowing, the reproductive performance of sows including farrowing rate (%), total number of piglets born per litter (TB), number of piglets born alive per litter (BA), number of stillborn piglets (SB) and number of mummified fetuses per litter (MM) were recorded. Also the reasons of reproductive failure including abortion, return to oestrus, not being pregnant and culling were also recorded.

Blood samples were randomly collected from 18 gilts from both control (n = 9) and treatment (n = 9) groups. The samples were collected at 30 min before and after the first insemination. Serum was extracted and was kept at -20 °C before analyses. Prostaglandins F$_{2\alpha}$ were determined by using a competitive immunoassay kit (Abnova GmbH, Heidelberg, Germany).

The statistical analyses were carried out by using SAS version 9.0 (SAS Inst. Inc., Cary, NC, USA). Descriptive statistics including number of non-missing value, mean, standard deviation (SD) and range of the data were analyzed. Continuous data were presented as mean ± SD and proportional data were presented as percentage. Farrowing rate was compared between control and treatment groups by using Chi-square test. Frequency distribution TB in control and treatment groups were also analyzed by using frequency analyses (PROC FREQ). Pearson’s correlation was used to determine the linear association between serum concentration of PGF$_{2\alpha}$ and all litter traits (i.e., TB, BA,
SB and MM). The effect of NSAIDs treatment on litter traits were analyses by using multiple ANOVA. The statistical models included the fixed effects of group (control and treatment), type of animal (gilt and sow) and two-ways interaction. Least-square means were obtained from each class of the factors and were compared by using least-significant difference (LSD) test. P<0.05 were regarded to be statistically significant.

Results and Discussion
On average, gestation length, TB, BA, SB and MM were 115.2 ± 1.8 days, 14.6 ± 4.0 piglets/litter, 12.1 ± 4.2 piglets/litter, 1.3 ± 1.8 piglets/litter and 1.2 ± 2.8 mummified fetuses/litter, respectively. Farrowing rate was 85.3%. Reproductive failure consisted of returned to estrus (7.1%), not being pregnant (3.0%), abortion (2.3%) and culling (2.2%). Reproductive performances of sows in control and treatment groups are presented in Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total born</td>
<td>14.2</td>
<td>15.0</td>
<td>0.345</td>
</tr>
<tr>
<td>Born alive</td>
<td>11.6</td>
<td>11.0</td>
<td>0.427</td>
</tr>
<tr>
<td>Stillborn (%)</td>
<td>6.1</td>
<td>11.2</td>
<td>0.016</td>
</tr>
<tr>
<td>Mummy (%)</td>
<td>10.4</td>
<td>12.1</td>
<td>0.878</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>83.7</td>
<td>88.3</td>
<td>0.313</td>
</tr>
</tbody>
</table>

Figure 1 demonstrated TB in gilts and sows in control and treatment groups. It was found that TB in the NSAIDs treated gilts was 1.1 piglets higher than the control gilts. Likewise, TB of sows in the treatment group was 0.3 piglets higher than control group (Figure 1).

Figure 2 demonstrated the frequency distribution of TB classes in control and treatment groups. As can be seen, the proportion of the litter with ≥16 TB was 7.3% higher in the NSAIDs treated pigs than control pigs.

Table 2Concentration of serum PGF$_{2\alpha}$ (pmol/l) in gilts before and after AI in control and treatment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before</th>
<th>After</th>
<th>Difference</th>
<th><strong>P</strong>&lt; 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>556.3</td>
<td>640.1</td>
<td>+ 83.8</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1335.5</td>
<td>1012.9</td>
<td>- 322.6</td>
<td><strong>&lt; 0.01</strong></td>
</tr>
</tbody>
</table>

Table 3Pearson’s correlation between serum PGF$_{2\alpha}$ (pmol/l) in gilts before and after AI and litter traits (n=17)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum PGF$_{2\alpha}$</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total born</td>
<td>r = -0.22 (P=0.46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Born alive</td>
<td>r = -0.40 (P=0.017)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillborn</td>
<td>r = 0.20 (P=0.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mummy</td>
<td>r = 0.09 (P=0.77)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the previous study, the basal level of PGF$_{2\alpha}$ in inseminated sows was around 400 pmol/l and maximum values was around 1000 pmol/l [3]. Norrby et al. [3] found that mating of sows with boar (i.e., natural mating) results in a greater increase of cortisol than AI without an elevation of plasma PGF$_{2\alpha}$ levels. Furthermore, it was also found that either conventional AI or intra-uterine insemination could induce a dramatic increase of the concentration of PGF$_{2\alpha}$ which is not seen in the naturally mated sows [3]. Kotwica et al. [5] demonstrated that mating of gilts resulted in an
increased in plasma cortisol levels for 30 min. These hormonal changes around insemination period may compromise fertilization rate and litter size in gilts and sows. Thus the control of hormonal changes during insemination may help to optimize fertilization and litter size in pig.

In conclusions, treatment with NSAIDs before AI reduced the serum concentration of PGF$_{2\alpha}$ in gilts. Both gilts (+1.1 piglet/litter) and sows (+0.3 piglet/litter) treated with NSAIDs before AI tended to have a larger litter size at farrowing compared to the control animals.

Acknowledgements
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References
Association between meconium staining of the skin and incidence of stillborn piglets

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Keywords: Meconium, Piglet, Skin, Stillborn, Sow

Introduction

Stillborn pigs remain a major problem in intensive managed pig farms and account for 5% to 10% mortality in most commercial herds worldwide[1,2]. Factors associated with stillborn piglets includes umbilical status, sow parity number and skin color (meconium staining). Meconium staining of the skin and aspiration of meconium are clinical indicators of prolonged or severe intrauterine hypoxia in aborted fetuses and stillborn [3]. It has been demonstrated that all intrapartum stillbirths (100%) were stained with meconium, and 60% had meconium visible grossly in the oropharynx and 40% in trachea and bronchi [4]. The objective of the present study was to determine the association between skin color (meconium staining) and the incidence of stillborn piglets.

Materials and Methods

The experiment was carried out in a 2400-sow commercial swine herd in Thailand in June 2017. The sows were housed in an evaporative cooling system. Gestating sows were moved from breeding to farrowing unit within 1 week before parturition. Skin color (meconium stained) was collected from 992 newborn piglets from 80 Landrace x Yorkshire crossbred sows. Meconium staining was divided into 3 scores: 1, 2 and 3 (Figure 1). Score 1 was defined as the skin without meconium staining. Score 2 was defined as the skin having a small amount of meconium staining (light yellow). Score 3 was defined as the skin having a lot of meconium staining (dark yellow). Meconium staining was determined immediately after the piglets was born.

SAS (SAS version 9.0 Cary, NC, USA) was used to analyze descriptive statistics (mean, standard deviation and range). Chi-square was used in the comparison between stillbirth (binomial trait) and meconium stainingscore. P < 0.05 was regarded to be statistically significant.

Results and Discussion

Descriptive statistics on reproductive performances of sows included in the study are presented in Table 1. On average, the incidence of stillborn piglets was 6.6%. Descriptive data on piglets with meconium staining is presented in Table 2. The association between skin color (meconium staining) and incidence of stillborn piglets was significant (P = 0.004). It was found that the piglets born with the score 3 of meconium staining had a higher risk of being stillborn (Figure 2).

Table 1 Descriptive statistics on reproductive performances of sows (n=80)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Means ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity number</td>
<td>2.8 ± 1.88</td>
<td>1 – 6</td>
</tr>
<tr>
<td>Gestation length (days)</td>
<td>115.6 ± 1.52</td>
<td>113 – 121</td>
</tr>
<tr>
<td>Total born</td>
<td>15.3 ± 3.78</td>
<td>3 – 26</td>
</tr>
<tr>
<td>Born alive</td>
<td>15.3 ± 3.71</td>
<td>1 – 21</td>
</tr>
<tr>
<td>Stillborn piglets</td>
<td>1.3 ± 1.72</td>
<td>0 – 10</td>
</tr>
<tr>
<td>Mummified fetuses</td>
<td>1.0 ± 1.97</td>
<td>0 – 15</td>
</tr>
<tr>
<td>Stillborn (%)</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Mummified (%)</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Farrowing duration (min)</td>
<td>331 ± 314</td>
<td>58 – 1,818</td>
</tr>
<tr>
<td>Body condition score</td>
<td>3.0 ± 0.12</td>
<td>2.7 – 3.4</td>
</tr>
<tr>
<td>Backfat thickness (mm)</td>
<td>14.1 ± 2.62</td>
<td>8.0 – 24.0</td>
</tr>
</tbody>
</table>

Figure 1 Grades of meconium stained on piglet’s skin
In conclusion, increasing of score of meconium staining on the skin of the newborn piglets was significantly associated with the incidence of stillborn piglets.

Acknowledgements

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References

Concentrations of total immunoglobulin G in colostrum of sows

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Keywords: Colostrum, Concentration, IgG, Parity, Time

Introduction
Colostrum is a rich source of immunoglobulin and nutrient for newborn piglets. The concentration of total immunoglobulin G (IgG) in sow colostrum is associated with many factors (i.e., breed, parity and environment) [1]. Moreover, the concentration of IgG in colostrum is dramatically decreased after farrowing [2]. Therefore, the quantity of sow colostrum and time elapse from farrowing to first suckling are important for piglet survival and growth [3]. Knowledge concerning factors influencing the concentration of IgG in sow colostrum is important to reduce the proportion of piglet pre-weaning mortality and improve growth performance under field conditions. The present study was performed to investigate the concentration of IgG in colostrum and to determine the association between the time interval from farrowing to sample collection, sow parity number and IgG concentration in colostrum.

Materials and Methods
The present study was performed in a commercial swine herd in the western part of Thailand in February 2017. A total of 161 colostrum samples from 81 sows were investigated. The sows were classified into two groups, i.e., primiparous (n = 16) and multiparous sows (parity number 2-6, n = 65). The sows were kept in an evaporating cooling-housing system. The sows were moved to the farrowing pens about one week before the expected farrowing date. The colostrum samples were collected manually at 1 and 6 h after the onset of parturition. Colostrum samples were collected by hand from all functional mammary glands and were pooled. The samples kept in a clean bottle (30 ml) and were stored at 4 ºC on ice in a foam box during collection process. The samples were cryopreserved at –20 ºC within 24 h after collection until analyzes. Colostrum were thawed at room temperature for 2 h and were centrifuged at 15,000 ×g for 20 min at 4 ºC (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany). Supernatant was collected and was used for IgG assay. The fat was discarded and the remaining liquid was collected. Thereafter, the liquid part was diluted 1: 500,000 with sample conjugate diluent (50 mM Tris buffer, 0.14 M NaCl, 1%BSA and 0.05% Tween 20). Immunoglobulin G was determined by using ELISA. The ELISA plate was coated with polyclonal antibody of Pig-IgG (Bethyl Laboratories Inc., Texas, USA). The absorbance was recorded at 450 nm using ELISA plate reader (Tecan Sunrise™, Männedorf, Switzerland). The IgG concentration in the colostrum samples were quantified by interpolating their absorbance from the standard curve generated in parallel with the colostrum samples. The concentration of IgG in colostrum at 1 and 6 h after farrowing were analyzed by using the general linear models (GLM) procedure of SAS. The statistical model included the effect of sow parity number, time interval from the onset of farrowing to sample collection and interaction. Least square means were obtained from each class of the factor and were compared by using Student’s t test. P < 0.05 was regarded to be statistically significant.

Results and Discussion
On average, the concentration of total IgG was 61.1 ± 28.8 mg/ml (range 20.8–201.7 mg/ml). Frequency distribution of the concentration of total IgG in the sow colostrum is demonstrated in Figure 1.

![Figure 1 Frequency distribution of the concentration of total IgG in sow colostrum](image)

Both sow parity number and collection time influenced the concentration of IgG (P < 0.05) but no effect of interaction between sow parity number and collecting time was found (P > 0.05). The concentration of IgG at 1 and 6 h after the onset of parturition were 66.1 ± 3.8 and 48.8 ± 3.8 mg/ml, respectively (P < 0.001). The concentration of IgG in sow colostrum in primiparous and multiparous sows are presented in Figure 2. The figure illustrated that multiparous sows (63.2 ± 2.4 mg/ml) had a higher concentration of IgG in colostrum than primiparous sows (51.8 ± 4.8 mg/ml, P = 0.03).
In conclusion, the concentration of IgG in colostrum was decreased 26% from 1 to 6 h after onset of parturition. Multiparous sows had a higher concentration of IgG in colostrum than primiparous sows under field conditions in Thailand. Therefore, piglet should receive colostrum as soon as possible after birth especially in late-born piglets. In addition, the supplementation of colostrum in piglets born from primiparous sows is recommended.

Acknowledgements
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References
Concerning chitooligosacchride levels and term applications as dietary additive in weaned pigs’ weight gain and small intestinal morphology

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Keywords: chitooligosaccharide, small intestine, weaned pig, weight gain

Introduction
There is continued need for novel agents with term application to improve intestinal function and growth in weaned pigs. Previously, some studies suggested that dietary chitooligosaccharide (COS) supplementation could be affecting pigs’ intestinal function after weaning such as small intestinal morphology and nutrient digestibility (1, 2). Moreover, findings in some studies suggest that the effect of COS might be dependent to the supplementation level and trial application in starter diets (1, 2, 3). COS; a D-glucosamine oligomer produced via hydrolysis of polymeric chitosan, may influence some digestive enzymes and their activity thus improving apparent digestion of most nutrients and intestinal health due to its low molecular weight. However, COS mechanism of action is not fully understood and COS-mediated changes in cellular function and cellular markers of the small intestine remain to be studied. Therefore, the main objective of this study was to determine the level effect of COS with different trial applications on weight gain, and small intestinal morphology and crypt cell proliferation in weaned pigs.

Materials and Methods
The animal and experimental design of this present study was approved as partially protocol described (3). In brief, fifty-six female piglets (Large White x Landrace x Duroc) weaned at 18 days of age (5.68 ± 0.07 kg) were randomly allocated into one of the four dietary treatment groups with 14 animals per treatment and fed for 56 days. Treatment groups differed only in their COS supplementation level: 1) Control diet, no COS supplementation; 2) 75 mg/kg of COS supplementation; 3) 150 mg/kg of COS supplementation; and 4) 225 mg/kg of COS supplementation. During the 56 days of the study, the pigs were weighed at the beginning, 28 and 56 days of the experiment. Three pigs randomly selected pigs from each group at 28 and 56 days of experiment were euthanized to collect small intestinal tissue. Intestinal tissues sampled from the duodenum, jejunum and ileum were collected to perform histomorphological and immunohistochemistry analysis. Data for weight gain, small intestinal morphology and crypt cell proliferation were analyzed by ANOVA by using the GLM procedure of SAS. Differences in means among treatment groups were separated by Tukey’s test. Orthogonal polynomial contrasts were performed to find a linear or quadratic response by inclusion level effect of COS. Results are presented as least squares means with SEM. Statistical significance was taken at $P < 0.05$.

Results and Discussion
For weight gain: During day 1 to 28 of the experiment, pigs supplemented with 150 mg/kg of COS tended to be higher weight gain than the other treatment groups ($P < 0.10$). During day 1 to 56 of the experiment, no difference in weight gain was observed among treatment groups. Our result suggests that these COS levels as dietary additive have no adverse effects on weaned pigs’ weight gain throughout the study.

For small intestinal histomorphology: On day 28 of the experiment, dietary supplementation of COS at 150 mg/kg increased ($P < 0.01$) the villus height and ratio villus height to crypt depth at the duodenum compared to the other experimental groups. In addition, dietary supplementation of COS at 150 mg/kg increased the villus height and ratio villus height to crypt depth at the jejunum and the ileum compared to control group ($P < 0.01$, respectively). With increasing COS supplementation, linear and/or quadratic response ($P < 0.05$) for the villus height, the crypt depth and ratio villus height to crypt depth at the duodenum, jejunum and ileum were observed at day 28 of the experiment. The results from the present study together with the results in the bibliography indicate that COS effect is dose dependent. Thus, the increase of villus height after dietary supplementation with COS or other oligosaccharides may lead to enhanced nutrient digestibility and absorption in weaned pigs (4).
(P < 0.05) the ratio villus height to crypt depth and decreased (P < 0.01) the crypt depth at the jejunum compared with the control group. On this day of the experiment, linear and quadratic increase (P < 0.01, P < 0.05, respectively) for the villus height, linear decrease (P < 0.01) for the crypt depth, and linear increase (P < 0.05) for the ratio villus height to crypt depth at the jejunum were observed with increasing COS supplementation.

For active cell division as indicated by Ki-67 immuno-positive staining; On day 28 of the experiment, pigs fed a diet supplemented with COS at 150 mg/kg had greater (P < 0.05) Ki-67 positive cell counts at the duodenum and ileum than pigs in 75 and 225 mg/kg COS inclusions. With increasing COS supplementation, Ki-67 positive crypt cells at the ileum improved linearly (P < 0.05) on this day of the experiment.

On day 56 of the experiment, a linear increase (P<0.05) for Ki-67 positive cell counts at the duodenum, jejunum and ileum was observed with increasing COS supplementation. The treatment group containing 150 mg/kg of COS significantly enhanced (P<0.01) the Ki-67 positive duodenal crypt cell numbers compared to the other treatment groups. The positive crypt cell numbers at the jejunum was greater (P < 0.01) than those in the basal control diet and diet supplementation of COS at 75 mg/kg group. In our previous studies (5, 6), we observed that ontogenetic adaptation of histomorphology, crypt cell proliferation and turnover rate in each region of the small intestine was important to affect the growth and the respond to diet transition in post-weaners. The manipulation of intestinal mucosa architecture and function has been recognized as an important way to improve growth of weaning pigs (4). Furthermore, it may be hypothesized that the 150 mg/kg of COS supplementation used in the present experiment had the potential to promote intestinal morphology through cell proliferation.

**Conclusion and Implication**

Pigs supplemented with 150 mg/kg COS showed: (i) a trend to higher weight gain than the other treatment groups during the one month trial and no difference in weight gain during two month trials, (ii) increased absorption capacity (e.g. increased villus height and the villus height/crypt depth ratio for three intestinal segments) on day 28 of the experiment and (iii) more active cell division (as indicated by Ki-67 marker of duodenal and jejunal crypt cells) on day 56 of the experiment. These physiological adaptations might play an important role to support optimal intestinal status of weaned pigs. Therefore, a direct benefit to the swine industry during the post-weaning period will move forward to solve problems or more cost competitive in farm.

**Acknowledgements**

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**References**

Correlation and agreement between serum and plasma progesterone concentrations in pigs as determine by an enzyme-linked immunosorbent assay

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Keywords: Correlation, Pig, Plasma, Progesterone, Serum

Introduction

Blood progesterone concentration indicates luteal function and the reproductive status of pigs [1]. The level of progesterone is used to determine puberty attainment and pregnancy in pigs [2, 3]. Knowledge concerning puberty status of gilts is necessary for developing appropriated pig breeding program under field conditions. Likewise, effective pregnancy detection helps the farmer to reduce non-productive days in swine breeding herd and hence increase the sow productivity. It is therefore important to establish an accurate and practical progesterone measurement assay to determine the reproductive status of the female pigs. However, under filed conditions, the type of blood samples appropriated for progesterone measurement is sometime doubtful. The present study was performed to compare the concentrations of progesterone in plasma (heparinized tube) and serum samples (plain tube) collected from nursery and pregnant pigs by using an enzyme-linked immunosorbent assay (ELISA) recently developed at the Faculty of Veterinary Science, Chulalongkorn University.

Materials and Methods

Blood samples were collected from jugular vein of 5 nursery pigs (age 8 weeks) and 10 pregnant sows (64.9 ± 4.1 days of pregnancy) in a commercial swine herd in Thailand. A 10 mL of blood was transferred into either heparinized tube (the tube was coated with LH lithium heparin, 6 mL VACUATTE® TUBE, Greiner Bio-One GmbH, Kremsmunster, Austria) or plain tube without any anti-agglutination agent (Z Serum Clot Activator 6 mL, VACUATTE® TUBE). The blood samples were withdrawn from jugular vein by using 18 g x 1.5-inch needle connected with a 10-mL syringe. The blood samples in the heparinized tube were centrifuged at 2000 xg for 10 min to obtain plasma sample. Likewise, blood samples in the plain tube were allowed to clot at room temperature for 1-2 hour before centrifuged at 2000 xg for 10 min to obtain serum sample. Both serum and plasma samples were kept at -20 °C before progesterone assay.

An enzyme-linked immunosorbent assay (ELISA) was used to determine the progesterone concentrations in the plasma and serum samples. The ELISA materials consisted of reagent and a solid phase high-binding clear microplates plate. The microplate was coated with goat anti-mouse IgG 0.01 mg/ml (cat. #A008 10 mg, ARBOR assays Inc., Michigan, USA). The standard progesterone hormone (4.0 ng/ml) was added into the well. Two-fold dilution was performed to obtain standard progesterone concentrations of 200.0, 100.0, 50.0, 25.0, 12.5, 6.25, 3.12, 1.56 and 0.78 pg/well. A 50 µl of standard and samples (dilute 1:30) were pipetted into the microplate, in duplicate. Progesterone-HRP (25 µl) and progesterone antibody (25 µl) (kindly provided by J.L. Brown, Smithsonian Conservation Biology Institute, VA, USA.) were added in all tube. The progesterone antibody was added in all tube except blank well. The plate was cover with plastic sheet and shake for 2 h at room temperature (25 °C). The plate was washed 5 times before TMB peroxidase substrate (3,3´,5,5´-tetramethylbenzidine, 100 µl) was added. After 15 min, 50 µl stop solution (1N HCl) was added. Optical density (OD) was determined by using ELISA reader with 450 nm. The OD was transformed to percentage of binding by comparing with standard curve. The concentration of progesterone (ng/ml) = [(progesterone (pg/well) x dilution factor) x 20]/1000. The sensitivity of the assay was 0.06 ng/ml. The intra-assay coefficient of variation was 2.05%.

Figure 1 Pearson’s correlation between serum and plasma progesterone concentrations in pigs (n = 14).

The statistical analyses were carried by using SAS. Descriptive statistics were conducted. The concentrations of progesterone in plasma and serum samples were compared by paired t test. Pearson’s correlation was used to determine the association between plasma and serum progesterone concentration.

Agreement between methods was determined by plotting the difference between two methods and the
mean value of progesterone concentrations [4]. Values with \( P < 0.05 \) were regarded as statistically significant.

**Results and Discussion**

On average, the plasma and serum progesterone concentrations in pregnant sows were 50.5 ± 58.3 and 48.4 ± 56.5 ng/mL, respectively (Table 1). The concentrations of progesterone in plasma and serum was not significantly difference (the difference was 0.05 ng/mL, \( P = 0.948 \)).

**Table 1** Descriptive statistics on reproductive data and progesterone concentrations (P\(_4\)) in pregnant sows (n = 10)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Means ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity number</td>
<td>3.8 ± 2.2</td>
<td>1 – 8</td>
</tr>
<tr>
<td>Gestation (days)</td>
<td>64.9 ± 4.1</td>
<td>60 – 72</td>
</tr>
<tr>
<td>Plasma P(_4) (ng/ml)</td>
<td>50.5 ± 58.3</td>
<td>14.5 – 202.7</td>
</tr>
<tr>
<td>Serum P(_4) (ng/ml)</td>
<td>48.4 ± 56.5</td>
<td>15.2 – 206.5</td>
</tr>
</tbody>
</table>

In the nursery pigs, the means plasma and serum progesterone concentration were 0.06 and 0.06 ng/mL, respectively.

Agreement between progesterone concentrations in serum and plasma was illustrated in Figure 2. As can be seen, the difference was observed within means ± 2SD [4]. Furthermore, the difference was not changed when the mean value of progesterone concentration was increased. This indicated that both serum and plasma samples can be used for the ELISA assay.

**Figure 2** Determination of agreement between plasma and serum progesterone concentrations (ng/mL) measurement.

In conclusions, progesterone concentrations in either serum or plasma in pig were highly correlated. The novel ELISA that was developed was able to measure the concentrations of progesterone precisely in both plasma and serum of pigs. This method enables a possibility for distinguish between pre-pubertal and pubertal gilts. Thus, proper treatment and/or management could be applied for gilts with delayed puberty and silent heat.

**Acknowledgements**

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**References**

The development of enzyme-linked immunosorbent assay for determining plasma progesterone concentration in pre-pubertal and pregnant pigs

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Keywords: ELISA, Ovulation, Pig, Progesterone, Reproduction

Introduction

The concentration of progesterone in blood provides a measurement of luteal function and can indicate the reproductive status of pigs [1]. Progesterone profiles have also been used as a useful diagnostic tool for measuring the age at puberty and anestrus in pigs [2, 3]. Tummaruk et al. [4] demonstrated that level of progesterone was 0.03 ng/ml in plasma of pre-pubertal gilts and it is significantly increased after puberty attainment.

Under field conditions, the most common reasons for culling pig from swine breeding herds are reproductive failures, e.g., anestrus, repeat service, conception failure and not being pregnant [5]. Engblom et al. [6] demonstrated that sows removed because of old age had the highest piglet production, while sows removed because of reproductive disorders had the highest number of non-productive days. Gilts removed from breeding herds took 97–120 non-productive days [7,8]. These studies indicate that the management of gilts under field conditions is an important area of research to be investigated. In Thailand, it has been demonstrated that delayed puberty and anestrus are major problems among gilts [5,8]. An increase in the culling rate and the replacement rate in the modern pig industry has led to the requirement to increase the size of the gilt pools and optimize the management of replacement stock. Therefore, information concerning the puberty status of gilts (i.e., low vs high progesterone levels) under field conditions is necessary for developing proper pig breeding management. It is therefore important to develop a simple, accurate and practical progesterone measurement tool to monitor the reproductive status of pig. The present study was performed to determine the concentrations of plasma progesterone in pre-pubertal and pregnant pigs by using an innovative enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

Plasma samples were obtained from 12 pre-pubertal pigs (age 8 weeks) and 10 pregnant sows (9 weeks of gestation) in a commercial swine herd in Thailand. Blood samples were collected from jugular vein by using 18 g x 1.5-inch needle connected with 10 ml syringe. The blood was transferred to EDTA tube and was centrifuged at 2000 xg for 10 min. Plasma samples were obtained and were kept at -20 °C before analyses.

An enzyme-linked immunosorbent assay was used to determine the progesterone concentrations in the plasma samples. The ELISA tools included reagent and an ELISA plate coated with antibodies to progesterone. A solid phase high-binding clear microplates was coated with goat anti-mouse IgG 0.01 mg/ml (cat. #A008 10 mg, ARBOR assays Inc., Michigan, USA). The standard progesterone hormone (4.0 ng/ml) was added into the well. Two-fold dilution was performed to obtain standard progesterone concentrations of 200.0, 100.0, 50.0, 25.0, 12.5, 6.25, 3.12, 1.56 and 0.78 pg/well. A 50 µl of standard and samples (dilute 1:30) were pipetted into the microplate, in duplicate. Progesterone-HRP (25 µl) was added in all tube. Progesterone antibody (25 µl) was added in all tube except blank well. The plate was cover with plastic sheet and shake for 2 h at room temperature (25 °C). The plate was washed 5 times before TMB peroxidase substrate (3,3´,5,5´-tetramethylbenzidine, 100 µl) was added. After 20 min, 50 µl stop solution (1N HCl) was added. Optical density (OD) was determined by using ELISA reader with 450 nm. The OD was transformed to percentage of binding by comparing with standard curve. The concentration of progesterone (ng/ml) = [(progesterone (pg/well) x dilution factor) x 20]/1000. The sensitivity of the assay was 0.06 ng/ml. The intra-assay coefficient of variation was 1.67%.

The statistical analyses were carried by using SAS. Descriptive statistics and frequency analysis were conducted. The concentrations of progesterone in pre-pubertal and in pregnant pigs were compared by Student’s t test using PROC TTEST of SAS. Equality of variances was tested and the method of t-test
analyses (pooled vs Satterthwaite methods) were selected based on the equality test. Values with $P < 0.05$ were regarded as statistically significant.

**Results and Discussion**

Plasma progesterone concentration (means ± SD) in pre-pubertal pig and pregnant sows are presented in Figure 2. As can be seen, the concentrations of plasma progesterone in pregnant sows was significantly higher than pre-pubertal pigs (35.68 ± 0.015 ng/ml, $P<0.001$). This indicated that the novel developed ELISA for measuring the concentrations of progesterone in plasma of pigs is able to distinguish between pre-pubertal and pregnant pigs. In addition, this innovation can be used as a useful tool for discriminating delayed-puberty gilts from normal-puberty gilts. Thus, proper treatment and/or management could be applied.

Figure 3 illustrated the frequency distribution of the plasma progesterone concentration in pregnant sows. It was found that the concentration of plasma progesterone in pregnant sows varied among individual sow from 27.8 to 47.0 ng/ml.

In conclusions, plasma progesterone concentration was clearly different between pre-pubertal and pregnant pigs. The novel developed ELISA for measuring the concentrations of progesterone in plasma of pigs was able to distinguish between pre-pubertal and pregnant pigs. This innovation can be used as a tool for discriminating delayed-puberty from normal-puberty gilts. Thus, proper treatment and/or management could be applied. Nevertheless, during pregnancy, the concentration of plasma progesterone varied among individual sow from 27.8 to 47.0 ng/ml.

**Acknowledgements**

Financial support was provided by a grant for International Research Integration: Chula Research Scholar, Ratchadaphiseksomphot Endowment Fund.

**References**

Dual Infection between a Thai Isolate HP-PRRSV and the Pandemic H1N1 SIV in Pigs

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Keywords: HP-PRRSV, H1N12009, pathogenesis, SIV

Introduction

Porcine respiratory disease complex (PRDC) is one of the most common respiratory problems in the swine industry worldwide. Porcine reproductive and respiratory syndrome virus (PRRSV) is the most common pathogen which usually associated with PRDC (1). Moreover, the virus causes negative immunomodulatory effects leading to secondary infection (2). In Thailand, swine respiratory problem usually associates with viral infection especially for a highly pathogenic PRRSV (HP-PRRSV) (3), which cause more severe clinical appearance along with high morbidity and mortality rates. Interestingly, the pandemic H1N1 2009 (pdmH1N1) SIV outbreak occurred in humans in Mexico and the United States before spreading worldwide (4) including Thailand (5). In humans, the pdmH1N1 infection showed fever, dry cough, muscle pain and dead due to the respiratory failure (6). However, the clinical signs in infected pigs showed only mild clinical signs (7). Furthermore, previous surveillance data showed pdmH1N1 and reassortant viruses of pdmH1N1 origin have been circulating in the Thai swine farms after the introduction of pdmH1N1 (8). Notably, pigs infected alone with a Thai isolate pdmH1N1 showed mild respiratory signs and minimal gross lesions (9). Since, the PRRSV could enhance disease severity and has been circulating in the Thai swine farms, a study of the co-infection between these two viruses could provide more information about a role of pdmH1N1 in PRDC cases.

The co-infection between PRRSV and SIV previously showed an increase in post-weaning mortality (10), decreased SIV vaccine efficacy, increased clinical appearance and viral shedding during an acute phase of SIV infection (11). Thus, the goal of this study is to investigate the clinical appearance, pathological and virological studies of the co-infection between the Thai isolate HP-PRRSV and the pdmH1N1 SIV in weaning pigs.

Materials and Methods

Viruses and cells: A Thai isolate pdmH1N1 SIV (A/swine/Thailand/CU-PL65/2010(H1N1)) and a Thai isolate HP-PRRSV (HP-PRRSV/10PL01) were provided by Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL, Bangkok, Thailand). SIV was propagated in Madin Darby Canine Kidney (MDCK) cells (ATCC, USA) and titrated for 10^5.5 tissue culture infectious dose (TCID50/ml). HP-PRRSV was propagated in MARC-145 cells and titrated for 10^2 TCID50/ml.

Animals: Thirty two, three-week old, crossbred pigs from a commercial farm in the central part of Thailand with negative results against PRRSV, SIV, PCV-2 and Mycoplasma hyopneumoniae antigens and antibodies were used

Experimental design:

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>PRRSV (Intranasal)</th>
<th>SIV (Intratracheal)</th>
<th>Nasal Swab</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>Day -2, 6-10</td>
<td>Day 8 and 10</td>
</tr>
<tr>
<td>PRRSV</td>
<td>9</td>
<td>Day 0</td>
<td>-</td>
<td>Day -2, 6-10</td>
<td>Day 8 and 10</td>
</tr>
<tr>
<td>SIV</td>
<td>9</td>
<td>-</td>
<td>Day 6</td>
<td>Day -2, 6-10</td>
<td>Day 8 and 10</td>
</tr>
<tr>
<td>Co-infection</td>
<td>9</td>
<td>Day 0</td>
<td>Day 6</td>
<td>Day -2, 6-10</td>
<td>Day 8 and 10</td>
</tr>
</tbody>
</table>

Results and Discussion

After the HP-PRRSV challenge, the pigs in the PRRSV (5/9) and Co-infection groups (4/9) showed depression and respiratory distress at 2 days post inoculation (dpi) and all pigs in both HP-PRRSV-infected groups showed clinical signs at 5 dpi. Then, one day after the SIV challenge (dsc), the pigs in SIV (5/9) and Co-infection (4/9) showed mild sneezing with nasal discharge (6/9 and 9/9, respectively). All pigs in the PRRSV and Co-infection groups showed respiratory distress, while only SIV-infected pigs showed normal respiration throughout the experiment. Lung lesions score was conducted at 2 dsc and 4 dsc. All gross lung lesions from the Negative and only SIV-infected groups showed normal lung lesions score, whereas, lungs from the PRRSV and Co-infection groups showed firm, fail to collapse with no significant difference (p>0.05) at 2 dsc. However, lung lesions score was more severe in the Co-infection group than that of the PRRSV group (p<0.05) at 4 dsc (Fig. 1).
Figure 1. Lung lesions score after SIV challenge (2 and 4 dsc) (* indicates significant difference between groups (p<0.05))

For SIV lung loading, at 2 dsc, fresh lung tissues from the SIV (4/5) and Co-infection (3/5) groups showed no significant difference (p>0.05), whereas, at 4 dsc, all remaining lung tissues had SIV loading with higher detection in the Co-infection group than that of the SIV group with significant difference (p<0.05) (Fig. 2).

Figure 2. SIV lung loading after SIV challenge (2 and 4 dsc) (* indicate significant difference between groups (p<0.05))

In summary, a comparison of the lung lesions score between the PRRSV and Co-infection groups at 2 dsc showed no statistical difference, but at 4 dsc, the lung lesions score of the Co-infection group had higher average score than that of the PRRSV group. Also, the SIV lung loading of the Co-infection group showed higher viral load at 4 dsc comparing to the SIV group. This showed the potentiation of the co-infection of the HP-PRRSV and pdmH1N1 SIV.

Acknowledgements
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References
Effect of backfat loss during late gestation and lactation on milk yield in sows

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Keywords: Backfat, Milk yield, Sow, Piglet

Introduction
Nowadays, the size of swine commercial herd in Thailand has dramatically increased and become more industrialize due to a high competition. One of the most important components for increasing the sow productivity is the number of piglets at weaning per litter [1]. Utilizing superior reproductive performances is an important strategy to enhance the number of piglet at weaning.

Reproductive performances of sows are determined by age, body weight, body condition score, estrus expression and backfat thickness [2,3]. Backfat consists of water, collagen and lipid. Triacylglycerol is the main composition of lipid that reserves in sow body [3]. During farrowing, some sow lose part of their backfat for body maintenance, body growth (especially in gilt), the development of fetus and, the most important for piglet survival, the synthesis of colostrum and milk. Therefore, milk yield is one of the important factor that might be associated with backfat thickness in sow. Milk yield depends on genotype, feeding, stage of lactation, health status and age of the sow. There is a lacking of study that determines the influence of backfat thickness before farrowing and backfat loss after farrowing on milk yield in sows. The aim of the present study was to determine the influence of backfat thickness before farrowing and backfat loss after farrowing on milk yield in lactating sows.

Materials and Methods
The experiment was performed in June 2017 in a commercial swine herd in Thailand. Sows are reared in a continuous weekly system. In total, 80 Landrace x Yorkshire sows (parities number 1 to 6) were included in the study. Sows were kept in individual crates during gestation in an evaporative housing system equipped with individual water sprinklers. During gestation, sows were fed a commercial gestation diet twice a day and during lactation the sows received a lactation diet 4 times a day.

After farrowing, a daily amount of feed was offered to the sows until ad libitum feed was reached after one week of lactation. The induction of parturition was not applied, and farrowing intervention was kept to a minimum. Manual extraction was not performed. Cross-fostering was not performed in the present study. The pens were fully slatted with concrete at the center for sows and with steel slats at both sides of the farrowing crate for piglets. Each pen was provided with a creep area for piglets (0.60 m²) placed on the floor on one side, covered by a plastic plate and heating lamp during the first week after farrowing. The heating lamp was turn on during the night time.

Backfat thickness of the sows was determined at P2 position (approximately 6-8 cm away from dorsal midline at the last rib curve) by using Renco® lean-meter [2]. An average value from both sides was used. Milk yield of the sows was determined by weighing the body weight of the piglets at Days 0, 1, 3, 10 and 17 of lactation. The milk yield was predicted following a previous study in Denmark [4]. Milk sample (20 ml) was collected from each sow on day 3, 10, 17 of lactation to evaluate the milk compositions. Samples were collected by administrating 10 IU oxytocin and manually milking. The milk samples were immediately frozen at -20°C and analyzed by using an infrared milk analyzer (Milko Scan 133B, Analyser; Foss Electric, Hillerød, Denmark).

Statistical analyses were performed using SAS 9.0 (SAS Inst. Inc., Cary, NC, USA). Descriptive statistics (mean, SD, median and range) were calculated. Pearson’s correlation was used to determine correlation between milk yield and backfat thickness. The effect of backfat loss and backfat thickness before farrowing on milk yield of sows were analyzed by regression analyses.

Results and Discussion
On average, backfat thickness of sows before farrowing was 14.0 ± 2.6 mm. After farrowing, the backfat thickness of sows at days 1, 3, 10, 17 and 21 were 13.7 ± 2.5, 13.7 ± 2.8, 14.3 ± 3.1, 13.8 ± 2.5 and 13.4 ± 2.4 mm, respectively. The backfat loss and the relative backfat loss from day 0 to 17 of lactation were 0.34 mm and 1.4%, respectively. Of all the sows, 12.8% loss backfat from 0-17 days of lactation more than 20%.

The estimated milk yield of sows from days 3 to 10 of lactation was 10.4 ± 2.2 kg (3.9 to 15.1) and from days 10 to 17 of lactation was 12.8 ± 2.1 kg (6.2 to 17.2) per sow.
Pearson’s correlation between milk yield of sows and backfat thickness (BF) before farrowing and at 17 days of lactation, and backfat loss during lactation

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Milk yield (kg)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3-10</td>
<td>Day 10-17</td>
<td></td>
</tr>
<tr>
<td>BF before farrowing</td>
<td>$r = 0.316^{**}$</td>
<td>$r = 0.183^{NS}$</td>
<td></td>
</tr>
<tr>
<td>BF at day 17</td>
<td>$r = 0.007^{NS}$</td>
<td>$r = 0.054^{NS}$</td>
<td></td>
</tr>
<tr>
<td>BF loss</td>
<td>$r = -0.381^{**}$</td>
<td>$r = -0.186^{NS}$</td>
<td></td>
</tr>
</tbody>
</table>

The correlation between milk yield of sows and backfat thickness variables are presented in Table 1.

Regression analyses on the effect of backfat loss during 0-17 days of lactation and BF before farrowing on milk yield of sows 3-10 days of lactation. Backfat loss was negative associated with milk yield but backfat before farrowing was positive associated with milk yield. The sow with an increased 1 mm of backfat before farrowing was associated with an increase milk yield of sows between 3 and 10 days of lactation ($P= 0.008$). In addition, for every 1 mm of backfat loss from farrowing to day 17 of lactation, the milk yield from days 3 to 10 was decreased 403 grams ($P=0.002$).

Table 2 Regression analyses on the effect of backfat(BF) loss during 0-17 days of lactation and BF before farrowing on milk yield of sows 3-10 days of lactation

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Intercept</th>
<th>Slope</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF loss</td>
<td>10,273</td>
<td>-403.7</td>
<td>0.002</td>
</tr>
<tr>
<td>BF before farrowing</td>
<td>6,640</td>
<td>271.2</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Backfat loss in sows is occurred due to body maintenance, fetal development and milk synthesis. A negative association between milk yield and changes in backfat during days 85-109 of gestation could be seen because of a high energy demand of sows for mammary glands development and mammogenesis [5]. Besides, weight loss due to fat and protein mobilization is common in lactating sows. Sows are unable to consume sufficient energy and protein to meet their requirements for maintenance and milk production, resulting in a severe negative energy and nitrogen balance. In conclusions, decreasing of backfat from 0 to 17 days after farrowing affect milk yield of sows. Thus, appropriate management, e.g., nutrition, environment and stress, play an important role on backfat thickness of postpartum sow.

Acknowledgements

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References

Effect of partial supplementation of Everwell™ in nursery pigs performance

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Keywords: yeast β-glucan, individual body weight, performance, pig

Introduction
Multifactorial stressors related with the weaning of young pigs are associated with substantial reductions in growth rates and heightened disease susceptibility (1). Everwell is a proprietary baker’s yeast 1,3/1,6-β-glucan of high purity and potency, specifically recommended to promote the young animal inherent defense mechanisms during this critical period. The objective of this study was to evaluate the effect of Everwell partial supplementation over the initial 2 weeks post-weaning on the performance during the entire nursery period.

Materials and Methods
A total of 163 pigs weaned at 28 days were used in this study. Pigs were allocated to 11 experimental pens, on the basis of weaning weight and gender. All animals received a common pre-starter diet to an average body weight (BW) of 7 kg. At this point (D0), pens were randomly allocated to 1 of 2 experimental treatments, in which animals received a control feeding program (N=6) composed of 2 phases [phase 1: D0-14; phase 2: D14-35] or were supplemented with Everwell (45 g/MT) during phase 1 only (N=5). All feeding phases were inclusive of a customary antibiotic program. Individual BW and pen feed intakes (FI) were determined coinciding with the change of feeding phase. BW uniformity and feed conversion ratio (FCR) were determined for each feeding phase and overall period. Data was statistically assessed by analysis of variance (ANOVA) using treatment as main effect. Data are presented as means and least significant differences (l.s.d.), which illustrates the critical value required to see statistical differences between treatment means.

Results and Discussion
Everwell supplementation had a significant impact on individual BW on day 14 (P<0.05; Table 1), which was supported by increased growth rates and feed efficiency during Phase 1 (P<0.05; Table 2). These results suggest that Everwell may have offered a protective role during the initial post-weaning period, as it has been reported previously in other controlled studies using yeast β-glucans (2). Yeast β-glucans have been known to promote both arms of the immune system, namely innate and acquired immunity (3), and their effects on mucosal immunity have also been reported when orally administered in pigs (4). No significant treatment differences were observed during Phase 2 on average daily gain (ADG) or FCR (Table 2; P>0.1). It is possible that the lack of supplementation during this phase precluded any difference to become of statistical significance. However, the positive influence of Everwell was carried through to day 35, as indicated by a tendency for higher individual BW in the yeast β-glucan supplemented group (P<0.1; Table 1). This was also reflected in ~60% of pigs in the Everwell group with final BW above the average, compared to ~45% in the control group (data not shown). This boosting effect on pig BW will have a positive influence in the management of the heard, as well as reducing possible loss of income due to variability amongst animals.

It can be concluded that Everwell worked alongside antibiotic-based strategies in partial supplementation post weaning, to deliver significant improvements in overall productivity of nursery pigs.

Table 1. Effect of treatment (Control or Everwell) on individual BW at various ages post weaning

<table>
<thead>
<tr>
<th>BW</th>
<th>Control (N=89)</th>
<th>Everwell (N=74)</th>
<th>l.s.d.</th>
<th>P&lt;</th>
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<tbody>
<tr>
<td>Day 0</td>
<td>7.39</td>
<td>7.39</td>
<td>0.182</td>
<td>0.9854</td>
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<tr>
<td>Day 14</td>
<td>13.63</td>
<td>14.25</td>
<td>0.0408</td>
<td>0.0030</td>
</tr>
<tr>
<td>Day 35</td>
<td>25.58°</td>
<td>26.32°</td>
<td>0.843</td>
<td>0.0877</td>
</tr>
</tbody>
</table>

Table 2. Effect of treatment (Control or Everwell) on pen ADFI, ADG and FCR at various ages post weaning

<table>
<thead>
<tr>
<th>ADFI, g/day</th>
<th>Control (N=6)</th>
<th>Everwell (N=5)</th>
<th>l.s.d.</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>579</td>
<td>542</td>
<td>66.9</td>
<td>0.2340</td>
</tr>
<tr>
<td>Phase 2</td>
<td>1,039</td>
<td>1,077</td>
<td>58.3</td>
<td>0.1763</td>
</tr>
<tr>
<td>Overall</td>
<td>855</td>
<td>863</td>
<td>28.6</td>
<td>0.5615</td>
</tr>
<tr>
<td>FCR, g/g</td>
<td>Control (N=6)</td>
<td>Everwell (N=5)</td>
<td>l.s.d.</td>
<td>P&lt;</td>
</tr>
<tr>
<td>Phase 1</td>
<td>446°</td>
<td>489°</td>
<td>38.2</td>
<td>0.0304</td>
</tr>
<tr>
<td>Phase 2</td>
<td>570</td>
<td>575</td>
<td>43.1</td>
<td>0.7948</td>
</tr>
<tr>
<td>Overall</td>
<td>520</td>
<td>540</td>
<td>39.2</td>
<td>0.2647</td>
</tr>
<tr>
<td>References</td>
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</table>
Effect of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Type 1 and 2 on the Viability of Porcine Endometrial Epithelial Cell Culture

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Keywords: PRRSV, viability, apoptosis, porcine endometrial epithelial cell

Introduction
Porcine reproductive and respiratory syndrome (PRRS) is an important disease in swine industry caused by PRRS virus (PRRSV). The recirculation of late term abortion has not been elucidated in PRRSV-positive herds leading to world-wide economic loss. The fetal-maternal interface organs comprising fetal membrane and maternal endometrium is deserved responsiveness (1). Feto-maternal junction of placenta has been indicated as the PRRSV replication site and associated with the pathogenesis of PRRSV-induced still birth and abortion (2). In addition, the feto-maternal membrane of sow with inactivated vaccine revealed the PRRSV replication at this site (3). Changing of cellular mechanism relevant to viral replication, macrophages and surrounding cell viability in particular in response to PRRSV has been suggested in vivo, but remains unclear. Moreover, the comparison between type 1 and type 2 PRRSV directly on the uterine epithelium has not been examined. Therefore, we hypothesized that two strains of PRRSV could alter the viability of porcine endometrium. The apoptotic cell and viable cells were assessed concurrently using primary culture of porcine endometrial epithelial cells (4).

Materials and Methods
The uterus of 4-6 months old gilts was collected from the Bangkok slaughter house, Minburi province, Bangkok, Thailand (n=3 pigs). The glandular endometrium was isolated and cultured following our previous methods (4). After the porcine endometrial epithelial cells (PE cells) culturing in DMEM with 5% fetal bovine serum revealed 70% confluence, the cells were infected with either PRRSV type 1 (EU) or type 2 (US) isolated from the lung of infected-pigs at the concentration of TCID₉₀/2 ml. After 1 h of incubation, the inocula were removed and replaced with fresh culture medium. Based on the cytopathic detection, at 0 and 4 days-post-infection (dpi), the viability and apoptosis of the cells were performed by MTT assay and annexin V/PI assay, respectively. Briefly, following the manufacturer’s protocol, triazolyl blue tetrazolium bromide (MS655, Sigma, St. Louis, US) was incubated in PE cells, then dimethyl sulfoxide (DMSO, VWR, Ohio, US) was used to dissolve the formazan dye. The absorbance of dissolved solution was measured at a wavelength of 570 nm, and 620 nm as a non-specific background by microplate reader (Epoch, BioTek, Vermont, US). Apoptosis assay was determined by annexin V/PI (annexin V FITC-conjugated, ImmunoTools, Friesoythe, Germany) and analyzed by flow cytometer (Guava EasyCyte™ System, Merk, Darmstadt, Germany). All results were statistically analyzed using the Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) was performed to determine significant difference among groups. Post-hoc test was followed with Newman-Keuls to compare the differences between two groups. The significant differences were considered at the p value less than 0.05.

Results and Discussion
The result showed that PRSSV type 1 and type 2 increased the viability of PE cell significantly (p<0.05) at 4 dpi as compared to mock (Fig. 1).

Figure 1 Cell viability detected by MTT assay. Histogram demonstrated the mean ± SEM (n=3 experiments in each group) of the absorbance 570-620 nm of formazan dissolved from PE cells infected with mock, type 1-(US) or type 2-(EU) PRRSV at 0 or 4 dpi. Bars with different letters (a, b) mean significantly different at the p<0.05.

In the annexin V/PI assay, PRSSV type 2 group demonstrated the early apoptosis percentage (11.95%) higher than PRSSV type 1 (8.93%) and mock (8.74%) at 4 dpi. In contrast, the highest percentages of late apoptosis were presented in PE-cells infected with PRRSV type 1 infection (18.76%) (Fig. 2).
Figure 2 Annexin V/PI assay represents the percentage of cell population staining with Annexin V and/or PI as detected by flow-cytometry technique. The early apoptosis was represented in the lower right quadrant, a single positive annexin V stained population. The upper right quadrant showed the late apoptosis which was double-positive annexin V and PI stained cells. The result was performed in PE cells (n=1 experiment) infected with mock, type 1-(US) or type 2-(EU) PRRSV at 0 or 4 dpi.

The present study is the first time to demonstrate the viability of primary PE cell culture directly affected by type 1- and type 2-PRRSV. Both strains were found to improve the viability of PE cells. However, the PRRSV seems to stimulate early and late apoptosis in our model. Correspondingly, in vivo study, PRRSV induced apoptosis in porcine endometrial tissue of pregnant sows. (5). Since the pathogenesis caused by viruses appears to conflict between cell death and proliferation, the mechanism of the proliferation and/or apoptosis of PE cells stimulated by PRRSV in the recent study remains to be clarified. Various endometrial abnormalities including dysynchrony in the maturation of the glandular epithelium and the stroma have been suggested in the failure of pregnancy in humans (6). In conclusion, PRRSV type-1 and type-2 can directly infect reproductive epithelial cells and promote either cell proliferation or apoptosis. The evidence of PRRSV-induced apoptosis of PE may associate with an impairment of maternal endometrium function leading to still born or late-term abortion.

Acknowledgements
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References
Efficacy and safety of Suiseng® in prevention of neonatal diarrhea according to enterotoxigenic E. coli under a mixed infection with PRRSV involved from the field

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Keywords: Enterotoxigenic E. coli, Neonatal diarrhea, Return on investment (ROI), Suiseng®, Swine

Introduction
Enterotoxigenic E. coli (ETEC) strains are important causes of diarrhea problems in suckling piglets namely F4-positive ETEC (1) and can be more devastating for economic growth in pig production which is currently estimated to be worth over 5,000 baht per sow per year (2). Nowadays, one major issue at the various events displays the current state of restricted and prohibited antibiotics in agricultural economy and its influences on swine industries worldwide (3). For these reasons, E. coli has been less inclined to decrease in farm and contributes to fluctuated herd health status. More than half of all swine producers in the United States (4) and Europe (5) vaccinate sows leading to provide passive lacteal immunity against E. coli infection to pre-weaning piglets. It is accepted that the investment in a sow E. coli vaccination program is expected to result in a >120% return on investment (ROI) (6). Herein, aim of this study was emphasis on compare the clinical effect and interpret a ROI of Suiseng® against neonatal diarrhea under a mixed infection with porcine reproductive and respiratory syndrome virus (PRRSV) in commercial swine herd.

Materials and Methods
Farm condition and selection: This study was conducted in 400-sow farrow to finish breeding and production farm located in Nakhon Pathom province, Thailand during 2017. This farm had a history considered typical of the symptoms of colibacillosis and porcine reproductive and respiratory syndrome (PRRS) in suckling pigs up to 15% and 65% of the litters affected. Additionally, only F4-positive ETEC was scrutinized by Enterocheck® diagnostic kit provided by HIPRA SPAIN, using multiplex PCR assay able to investigate the multiple genes encoding for different ETEC and β toxin produced by Clostridium perfringens type C. Losses of these episodes appeared to be more severe whenever the herd was subjected to increase stress factor especially E. coli contaminated water more than 200 coliforms per ml following by the evidence-based history taking.

Field trial design and statistical analysis: The study farm is among the pioneer users of Suiseng® in its first introduction at the end of the year 2016. Twenty-four fourth parity sows were randomly allocated into two groups. Briefly, Group 1 sows (n = 12) were received 2 doses administered at 6 and 3 weeks before farrowing in accordance with the manufacturer’s recommendations. The remaining group was intramuscularly inoculated by 2 ml of normal saline solution (NSS; 0.9% NaCl) as control group. Safety test of two doses vaccination schedules in sows was monitored daily for local and systemic reactions until 48 hours post vaccination. Efficacy of Suiseng® in piglets could determine by comparing the data among groups including piglets with diarrhea (%) as previously described (7). Furthermore, ROI was calculated based on this solution (8). Some data of this trial was statistically analyzed using the SAS statistical program (version 9.0).

Results and Discussion
The results of safety monitoring using Suiseng® were summarized in Table 1. No adverse reaction and no negative effects on clinical manifestations were observed in all vaccinated sows (Group 1).

Table 1 Analysis of the safety of the Suiseng® two doses vaccination under field condition
Clinical features | Suiseng* | Control
--- | --- | ---
Numbers of sows ($n$) | 12 | 12
Local reactions (%) | 0 | 0
Pyrexia (%) | 0 | 0
Abdominal breathing (%) | 0 | 0
Vomiting (%) | 0 | 0
Mortality rate (%) | 0 | 0
Avg. feed intake during the clinical inspection (kg) | 2.16±0.24<sup>a</sup> | 2.13±0.26<sup>a</sup>

* Different letters (a, b) within the same column represented significant differences ($p<0.05$).

### Table 2 Comparison of piglet production parameters between Suiseng* – sow vaccination and control

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Suiseng*</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. pig born alive/litter</td>
<td>11.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.92&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Avg. pigs weaned/litter</td>
<td>9.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Avg. piglets birth weight (kg)</td>
<td>1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Avg. weight gain at 21d (kg)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>4.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Avg. ADG (g per day)</td>
<td>214.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>183.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mortality linked to enteric signs ($E. coli$) at &lt; 11d (%)&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0</td>
<td>2.52</td>
</tr>
<tr>
<td>Increased weaning weight (kg/pig)</td>
<td>0.822</td>
<td></td>
</tr>
<tr>
<td>ROI (%)&lt;sup&gt;***&lt;/sup&gt;</td>
<td>294.4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>†</sup> Pig weaned at 21 days of age
<sup>**</sup> All piglets were medicated each day after 11 days old
<sup>***</sup> Cost of vaccination and the estimate average cost of piglet production should be recorded to access the ROI. **a, b values in a row for a parameter with different superscripts differ significantly ($p<0.05$).

Moreover, this field study exhibited the performance improvement was significant as noted in Table 2, with especially a reduction of mortality associated with $E. coli$ infection up to 2.52%. On top of that weaned pigs from vaccinated sows tended to have a higher weight gain and average daily gain (ADG) up to 650g and 30.96 g per day, respectively. Vaccination with Suiseng* improved farm performance and results in ROI of 294.4% for herds. To the extent of our knowledge it is acceded that Suiseng* is safe and provides protection against $E. coli$ infection in piglets with 294.4% ROI under this field surroundings ($E. coli$ and PRRS involved). Our findings indicated not only $E. coli$ was one of the key issues of concern for this farm, but PRRSV infection and fluctuated thermal environment also increased pig mortality. Hence, the stressors reduction should be done through maximize the herd health immunity and strictly biosecurity. Undeniable, there is concern over the augmented phenomenon of antibiotic resistance rates in $E. coli$ (3). Effective vaccination and farm biosecurity are guaranteed to prevent colibacillosis before it occurs. Future study should be done to prove the efficacy of Suiseng* in piglets with long term diarrhea effects from $E. coli$ and porcine epidemic diarrhea virus (PEDV) in field under unsuccessful outcome combination treatment with antibiotics and PED vaccine (IPVS 2018, China).

### Acknowledgements

The authors would like to thank to commercial swine farm at Nakhon Pathom province, Thailand co-operatives for providing feces samples and encouragement.

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Evaluation of pig performance after vaccination of weaned pigs with PCV2 vaccine in Southeast Asia pig farms

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Keywords: Pig performance, PCV2 vaccine, weaned pigs

Introduction
Porcine Circovirus type 2 (PCV2) caused the clinical and subclinical diseases in the infected pigs in many regions including Asia. There are several studies confirmed that vaccination with PCV2 vaccine can reduce viral shedding, viremia, mortality, clinical signs, gross and microscopic lesions (1, 2). Many studies of vaccine efficacy have included the serology, histopathology, viral isolation, and qPCR for the evaluation (3). However, there are pig farms in the countries with no laboratory facility to identify the virus or antibody but the clinical signs of PCV2 diseases such as PCV2-SD or PDNS with high mortality and poor performance of fattening pigs were observed. This study aims to report the performance of growing and fattening pigs after vaccination with PCV2 whole virus vaccine (Circovac®, Ceva) in a farm in Southeast Asia with the suspected PCV2 infection.

Materials and Methods
Two contracted wean-to-finish pig farms, were selected according to the criteria of the same location and size, only 20 m distance, the same source of sows, the same feed and feeding program, and showing poor performance and clinical signs of PCV2 infection in the history. Both farms introduced piglets at 3 WOA, on average of 5 kg BW, from breeder farms, and piglets were raised from weaning to slaughter at the market weight and age. On farm Farm A two batches were evaluated, 1) no vaccination of PCV2 vaccine so-called Farm A-NVac, as a history control, and 2) after harvesting Farm A and vaccinating the new piglets with Circovac® at 3 WOA, IM, 0.5 ml. so-called Farm A-Vac. Farm B, not vaccinated against PCV2, was observed at the same time as Farm A-Vac, as the control group. Mothers of these piglets were PRRSV free and not vaccinated against PCV2.

At slaughter, all pigs were individually weighed, the data of weight (kg) and days to slaughter were collected individually. Individual weight gain and ADG were calculated and statistically analyzed by t-test at P<0.05 to compare the performance difference among groups. Data of mortality was collected as average per batch. The slaughtered weight distribution and variation of slaughtered weight were compared among farms.

Results and Discussion
The result illustrated the statistical difference of performance between Farm A-NVac, Farm A-Vac, and Farm B as showed in Table 1. Pigs from Farm A-Vac showed higher weight gain and ADG comparing to the non-vaccinated control farm, Farm A-NVac and Farm B, as well as the lower mortality. ADG of Farm A-Vac improved 6.02% from the previous batch in Farm A-NVac and 4.83% comparing to Farm B. Moreover, mortality of Farm A-Vac improved by 78.53% and 21.21%, respectively, comparing to Farm A-NVac and Farm B.

Table 1 The performance of fattening pigs from three groups, with or without PCV2 Vaccination

<table>
<thead>
<tr>
<th>Group</th>
<th>No. pigs slaughtered</th>
<th>Wt-In (kg)</th>
<th>Wt-Out (kg)</th>
<th>Wt-Gain (kg)</th>
<th>ADG (g/day)</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A-Vac</td>
<td>100</td>
<td>5</td>
<td>109.89 a</td>
<td>104.89 a</td>
<td>627.84 a</td>
<td>3.5</td>
</tr>
<tr>
<td>Farm A-NVac</td>
<td>104</td>
<td>5</td>
<td>105.67 b</td>
<td>100.67 b</td>
<td>592.19 b</td>
<td>16.3</td>
</tr>
<tr>
<td>Farm B</td>
<td>79</td>
<td>5</td>
<td>105.62 b</td>
<td>100.62 b</td>
<td>598.94 b</td>
<td>4.68</td>
</tr>
</tbody>
</table>

*Different letter (a, b) within the same column represent significant difference (P<0.05).

The distribution and the variation of slaughtered weight were illustrated in Figure 2 and Figure 3. The distribution of slaughtered weight in Farm A-Vac is less in the lower weight and more in the higher weight. The coefficient variation (CV) in Farm A-Vac is lower than other farms as well.

Figure 1 Comparing the distribution of slaughtered weight among Farm A-Vac, Farm A-NVac, and Farm B
In many studies, the serological test and histopathology will help for diagnosis and the disease status in farm and provide more scientific data (1, 2, 3). However, in the countries with limited laboratory facility, the details of performance data can help to assess the health status. By the good data collection and data management, this study can provide the evaluation of the improvement of performance of pigs vaccinated with Circovac® at weaning.

References
Evaluation the use of oral fluid PRRSV antibody ELISA under the field condition

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Keywords: PRRSV, antibody, oral fluid, ELISA

Introduction

In contemporary commercial swine populations, porcine reproductive and respiratory syndrome virus (PRRSV) is a cause of major economic loss to the pig industry worldwide (1,2). Oral fluid antibody ELISA can provide efficient, cost-effective PRRSV monitoring in commercial herds and PRRSV surveillance in elimination programs (3,4). Currently, PRRSV oral fluid antibody based-assays are commercially available and practically detect PRRSV antibodies from individual and pen-based oral fluid samples. Under the field condition, sample-to-positive (S/P) ratios of PRRSV oral fluid antibody by indirect ELISA demonstrated some false positive results detected in PRRSV negative animals, that can cause more expense and frustration to confirm the results. Analysis of the antibody responses from PRRSV negative and positive pigs can provide more information on effectively use of oral fluid testing, in term of its application and interpretation. This study aimed to evaluate the diagnostic performances of “PRRSV oral fluid antibody ELISA” using samples of known PRRSV status under the field condition.

Materials and Methods

A total of oral fluid (n =669) and serum (n = 669) samples collected from pigs with known PRRSV status were used to evaluate the diagnostic performances of PRRS antibody ELISA. Negative samples (240 oral fluids and 240 serum) were collected from replacement gilts at 18 weeks of age from four PRRSV-negative farms. Positive samples (429 oral fluids and 429 serum) were collected from replacement gilts at 32 weeks of age from a PRRSV-positive farm. In the positive farm, pigs were vaccinated twice with PRRSV modified live vaccine (Ingelvac PRRS® MLV, Boehringer Ingelheim,) at 22 and 25 weeks of age. Oral fluid and serum samples were assayed by indirect PRRSV oral fluid antibody ELISA (IDEXX PRRS Oral fluid Ab Test, IDEXX Laboratories, Inc., Westbrook, ME USA) and indirect PRRSV serum antibody ELISA (IDEXX PRRS X3 Ab Test, IDEXX Laboratories, Inc.) respectively. Procedures for performing the oral fluid and serum PRRSV antibody ELISAs and the S/P value calculation were followed the manufacturer’s instructions. Summary statistics were used to decribe oral fluid and serum S/P responses detected in negative and positive animals. Correlation between oral fluid and serum S/P responses from the same animal was evaluated using Pearson’s correlation coefficient (r). Diagnostic sensitivity, specificity, and associated 95% confidence intervals of the PRRSV oral fluid ELISA were estimated by receiver operating characteristic (ROC) analysis using the S/P results from 669 oral fluid samples. Statistical analyses were performed using MedCalc® version 17.8.6 (MedCalc Software, bvba, Ostend, Belgium). The p value less than 0.05 were considered statistically significant.

Results and Discussion

The distribution and variation of the S/P ratios detected in oral fluid and serum from PRRSV negative and positive animals were shown in Fig 1. Within negative and positive groups, PRRSV antibody responses detected in oral fluids showed wider rages and higher ratios of the S/P responses than serum. The S/P ratios (mean±SD) of negative and positive oral fluid samples were 0.08±0.10 and 4.03±2.13 respectively. In serum, the S/P ratios detected in negative and positive samples were 0.01±0.03 and 1.16±0.51 respectively. By using the same cut-off value as serum at 0.4, three false positive results (out of 240) were detected from negative oral fluid samples. No false positive results were obtained from negative serum testing.

Figure 1

Summary of PRRSV antibody results using oral fluid and serum collected from PRRSV-negative and PRRSV-positive pigs.
An assessment of the association between oral fluid and serum S/P ratios from the same animals showed a correlation of 0.535 (Pearson’s correlation coefficient (r), \( p < 0.0001 \)) (Fig 2).

**Figure 2** Correlation of ELISA sample-to-positive (S/P) ratios between oral fluid and serum samples.

The ROC analysis estimated the area under the curve (AUC) at 0.999 (95% CI: 0.992, 1.000). In this analysis, diagnostic sensitivity and specificity of different S/P cut-off values were shown in Table 1.

**Table 1** Sample-to-positive (S/P) cut-off and associated diagnostic sensitivity and specificity (95% confidence interval) from commercial PRRS antibody ELISA performed using oral fluids collected from known PRRSV negative and positive pigs.

<table>
<thead>
<tr>
<th>S/P</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.27</td>
<td>100.0 (99.1, 100.0)</td>
<td>97.1 (94.1, 98.8)</td>
</tr>
<tr>
<td>≥0.29</td>
<td>99.3 (98.0, 99.9)</td>
<td>97.5 (94.6, 99.1)</td>
</tr>
<tr>
<td>≥0.31</td>
<td>99.1 (97.6, 99.7)</td>
<td>97.9 (95.2, 99.3)</td>
</tr>
<tr>
<td>≥0.33</td>
<td>98.8 (97.3, 99.6)</td>
<td>98.8 (96.4, 99.7)</td>
</tr>
<tr>
<td>≥0.56</td>
<td>96.7 (94.6, 98.2)</td>
<td>98.8 (96.4, 99.7)</td>
</tr>
<tr>
<td>≥0.61</td>
<td>96.7 (94.6, 98.2)</td>
<td>99.6 (97.7, 100.0)</td>
</tr>
<tr>
<td>≥0.78</td>
<td>94.4 (91.8, 96.4)</td>
<td>100.0 (98.5, 100.0)</td>
</tr>
</tbody>
</table>

*IDEXX PRRS Oral fluid Ab Test, IDEXX Laboratories, Inc., Westbrook, Maine USA.

Cumulatively, the data suggested that selection of an optimal cut-off value for the use of PRRSV oral fluid antibody detection should be considered, e.g., for monitoring the disease in negative herd, or surveillance in positive herd. Within PRRSV negative population, the S/P ratio at ~0.8 was considered to be used to avoid false positive results. Of course, collected large sample size from a real disease setting, i.e., field condition, could provide more information on the multiplicity of the antibody responses detected by oral fluid ELISA. This evidence can enhance precision of the PRRSV diagnosis and serological interpretation using oral fluid samples.

**Acknowledgements**

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**References**

Factors associated with colostrum consumption of neonatal piglets

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Keywords: Sow, High prolificacy, Litter size, Reproduction, Tropical climate

Introduction
Colostrum is first milk secreted by the mammary gland that piglets can absorb to gut only within the first 24 h of age. Insufficient colostrum consumption is one of the major problem causing an increased neonatal piglet mortality. Colostrum provides neonatal piglets with both energy and immunoglobulins, thereby playing an essential role on piglet survival. Adequate colostrum consumption of neonatal piglets is therefore ensuring optimal passive immunity for piglets [1]. In addition, colostrum also provides the newborn piglets with high metabolisable energy, i.e., fat and lactose. These energies are efficiently used by the newborn piglets to cope with cold stress and maintaining its homeothermic balance during the first day of life. Quesnel et al. [2] estimated that 250 grams of colostrum consumption per piglet should ensure an optimal growth and passive immunity to the animals. A previous study found that colostrum consumption stimulates the development of the hippocampus structure by the stimulation of brain protein synthesis and brain development during the early postnatal period [3]. The aim of the present study was to investigate management factors associated with colostrum consumption of the neonatal piglets.

Materials and Methods
The study included 1,140 neonatal piglets from 80 Landrace x Yorkshire crossbred sows. The experiment was conducted in a commercial swine herd in the eastern part of Thailand in June 2017. Factors associated with piglet colostrum consumption determined included body weight at birth of the piglet, birth order, birth interval, heart rate, blood oxygen saturation, rectal temperature at 24 h, gestation length, total number of piglets born per litter (TB), number of piglets born alive per litter (BA), sow body conditions score and sow parity number.

The sows were moved to the farrowing house before their expected farrowing date about one week. The feed was provided twice a day (2-5 kg per day) during gestation. During lactation, the sows were fed 3 times a day (about 3-6 kg of feed per day). The sows were vaccinated against CSFV, ADV, PPV, PRRS and PCV2. All gilts and sows was performed FMD vaccine every 4 months. The herd was a PRRS seropositive herd.

Rectal temperature 24 h after birth measured using a digital thermometer (Verridian Healthcare Co. Ltd., IL, USA). Piglets were weighed immediately after birth and again at 24 h using a digital scale (SDS® Digital Scale Co. Ltd., Yangzhou, China). All piglets were individually identified by an ear tattoo performed at birth. Individual colostrum intake of the piglets was estimated by an equation published by Theil et al. [4]:

$$\text{Colostrum consumption (g) } = -106 + 2.26\text{WG} + 200\text{BWB} + 0.111\text{D} - 1414\text{WG/D} + 0.0182\text{WG/BWB},$$

where WG is piglet weight gain (g), BWB is birth weight (kg) and D is the duration of colostrum suckling (min). The colostrum yield of the sows was defined as the sum of individual colostrum consumption of all piglets in the litter.

Statistical analyses were carried by using SAS. Descriptive statistics and frequency analysis were conducted. The associations among these factors and colostrum consumption of the piglets were analyzed by using Pearson’s correlation. The effect of sow parity number on the colostrum consumption by piglets was analyzed by using general linear model procedure (PROC GLM) of SAS. Values with $P < 0.05$ were regarded as statistically significant.

Results and Discussion
The results revealed that the colostrum consumption averaged 405 ± 183 grams. Frequency distribution of colostrum consumption by piglets are presented in Figure 1. As can be seen, 20.1% of the piglets received colostrum below optimal level [2]. Thus, these piglets might have had a high risk of being death or had a poor growth rate.

Figure 1 Frequency distribution on colostrum consumption of piglets in a commercial swine herd in Thailand (n = 1,140)
Body weight at birth of the piglet, birth order, TB, BA, body conditions score, heart rate and rectal temperature were significantly correlated with colostrum consumption of the neonatal piglets (Table 1). On the other hand, gestation length, birth interval, blood oxygen saturation and sow parity number were not correlated with colostrum consumption.

In the present study, the piglet rectal temperature is associated with colostrum consumption. Likewise, Tuchscherer et al. [5] found that rectal temperature in piglets is associated with colostrum consumption. These findings indicate that piglets with low rectal temperature at 24 h might have low thermoregulation abilities. Thermoregulation is a crucial physiological event for all newborn piglets. The piglets that die during the first day of life are not able to maintain optimal rectal temperature during the first 24 h of life. Nuntapaitoon et al. [6] found that rectal temperature at 24 h of life was associated with piglet pre-weaning mortality rate. Therefore, increasing colostrum intake of neonatal piglet during first day after birth is very important in swine herd.

Table 1 Correlation between colostrum consumption (mean ± SD = 405 ± 183 grams) and litters and piglet characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Colostrum consumption</th>
<th>n</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation length (days)</td>
<td>1,140</td>
<td>0.01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Total born</td>
<td>1,140</td>
<td>-0.21</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Born alive</td>
<td>1,140</td>
<td>-0.19</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Body conditions score</td>
<td>1,140</td>
<td>0.06</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>1,140</td>
<td>0.29</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Birth order</td>
<td>1,140</td>
<td>-0.07</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Birth interval (min)</td>
<td>1,140</td>
<td>-0.02</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>872</td>
<td>0.11</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Blood oxygen saturation (%)</td>
<td>872</td>
<td>0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Rectal temperature(°C)</td>
<td>862</td>
<td>0.30</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05, ** 0.05<P<0.01, *** P<0.001, NS = P > 0.05

Figure 2 demonstrates colostrum consumption of piglets by parity number of sow. It was found that colostrum consumption of piglets was highest in sow parity number 3. The piglets reared by primiparous sow had a lower colostrum consumption than the piglets reared by sow parity number 3 (P<0.05). Likewise, piglets reared by sow parity number 5 also had a lower colostrum consumption than piglets reared by sow parity number 3.

In conclusion, the body weight at birth of the piglet, birth order, total born, born alive, body conditions score, heart rate and rectal temperature at 24 h of life were significantly associated with piglet colostrum consumption in the swine herd.

Acknowledgements
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References
Factors influencing litter size in a modern Landrace x Yorkshire hyper-prolific sows in a swine commercial herd in Thailand

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Keywords: Sow, High prolificacy, Litter size, Reproduction, Tropical climate

Introduction

The modern hyper-prolific sow is a new population of sows in swine breeding herd, in which their genotype has been selected for an extremely large litter size at farrowing. As a result, the number of piglets born alive per litter (BA) has increased up to 16-18 piglets per litter. Under field condition, litter size at farrowing comprises a variety of measurements, i.e., the total number of piglets born per litter (TB), BA, stillborn piglets (SB) and mummified fetuses (MM) per litter. Of these variables, BA is the parameter most correlated with the number of piglets weaned per litter. Litter size at farrowing in pig is affected by ovulation rate, fertilization rate, and embryonic/fetal survival. The fetal survival of the piglets is highly correlated with size of the sow uterus (r = 0.9) [1]. Litter size depends on both genetic and environmental factors. The genetic impact on most reproductive traits is relatively small [2], while environmental factors, e.g., management, parity, climate, lactation length and nutrition, are known to have a relatively high impact on litter size [3]. The present study aims to determine the variation of litter size and to analyses potential factors influencing litter size in a modern Landrace x Yorkshire hyper-prolific sows under field conditions.

Materials and Methods

Data were collected from a 12,000-sow commercial swine herd in Thailand. The data included sow identities, farrowing date, parity number, BA, SB, MM, litter birth weight and number of piglets at weaning per litter. The total number of piglets born per litter (TB) was defined as the sum of BA, SB and MM. The data were collected from sows that farrowed from January to December 2017. The raw data were carefully scrutinized for accuracy. The analyses data included 23,517 litters from 11,961 Landrace x Yorkshire sows. The replacement gilts were produced within the herds using their own grandparent stock, imported from Denmark.

The gilts and sows were kept in individual crates during gestation. Pregnant sows were moved to the farrowing pens about 1 week before farrowing. In general, the gilts were mated at 8 months of age with a body weight of ≥135 kg at the second or later observed estrus. Artificial insemination was used for all gilts and sows. Feed was provided twice a day (1.5–3.5 kg per day) during gestation. The gestating feed contained 16% CP, 2,800 kcal/kg ME, and 0.9% lysine. The sows were fed 2-4 times a day during lactation (5-6 kg per day) with a corn-soybean-chicken ration. The lactation feed contained 18% CP, 3,250 kcal/kg ME, and 1.1% lysine. Water was provided ad libitum by water nipples. The health management was carried out by veterinarians. Both gilts and sows were vaccinated against CSF, AD, PPV, FMD and PRRS.

Statistical analyses were carried by using SAS. Descriptive statistics and frequency analysis were conducted. General linear models (GLM) were used to analyze factors associated with TB and BA. The models included parity number (1, 2, 3, 4, 5, 6, 7 and ≥8), farrowing month and unit (1 and 2) as independent variables. Least square means were obtained from each class of the factor and were compared by using least significant difference test. Values with P < 0.05 were regarded as statistically significant.

Results and Discussion

Descriptive statistics on the reproductive performances data of sows are presented in Table 1. Frequency distribution of TB are presented in Figure 1. As can be seen, 24.1% of the litter had TB ≥16 piglets/litter (Figure 1). Likewise, the proportion of the litter with BA ≥14 and ≥ 16 piglets/litter was 34.7% and 9.9%, respectively.

Figure 1 Frequency distribution on the total number of piglets born per litter in sows in a commercial swine herd in Thailand (24.1% of the sows produced ≥16 piglets/litter) (n = 23,517 litters)
Factors influencing both TB and BA of sows included parity number \((P<0.001)\) and farrowing month \((P=0.001)\). The unit of farrowing (i.e., housing and management) significantly influenced TB \((P<0.001)\) but not BA \((P=0.813)\). Low TB was observed in September (12.9 TB) and October (12.9 TB), while high TB was observed in March (13.4 TB, \(P<0.001\)). Likewise, the same tendency was also observed for BA \((12.3 \text{ vs } 11.8 \text{ BA, } P < 0.001)\). This indicated that a 0.5 piglet/ litter reduction of BA was observed during the farrowing that occur in late rainy season. These sows had been inseminated since late hot season (i.e., May). The reason might be due to that heat stress are able to directly induce autophagy in the porcine ovaries during follicular development [4]. An increased in ambient temperature during hot season are potentially compromise oocyte integrity and reduce developmental competence of embryo. Hence, the litter size at farrowing was reduced. A recent study demonstrated that a steadily increasing of room temperature from 20.0 °C to 31 °C for 5 days during follicular phase are able to alter some ovarian proteins (e.g., Beclin 1, microtubule associated protein, ATG5 complex and BCL2L1) that play a role on autophagy and apoptosis of oocyte and granulosa cells [4]. Furthermore, an increase of room temperature during hot season may reduce feed intake of sows during lactation and subsequently compromise oocyte quality. Therefore, sow body weight loss or backfat loss during lactation should be carefully monitored during hot season.

### Table 1 Descriptive statistics (n=23,517 litters)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity number</td>
<td>3.82 ± 2.20</td>
<td>1 – 14</td>
</tr>
<tr>
<td>Total born/ litter</td>
<td>13.2 ± 3.3</td>
<td>1 – 29</td>
</tr>
<tr>
<td>Born alive/ litter</td>
<td>12.1 ± 3.1</td>
<td>0 – 25</td>
</tr>
<tr>
<td>Stillborn (%)</td>
<td>6.0</td>
<td>0 – 100</td>
</tr>
<tr>
<td>Mummy (%)</td>
<td>2.2</td>
<td>0 – 100</td>
</tr>
<tr>
<td>Weaned piglet/ litter</td>
<td>11.7 ± 1.6</td>
<td>0 – 18</td>
</tr>
</tbody>
</table>

Both TB and BA reached a plateau in sow parity numbers 3 and 4 and significantly declined after parity number 5 (Figure 2). Interestingly, primiparous sow had a larger TB than sow parity numbers 7 and ≥8 and had a larger BA than sow parity numbers 6, 7 and ≥8 (Figure 2).

Figure 2 Litter size at farrowing in Landrace x Yorkshire sows by parity numbers

In conclusions, the modern hyper-prolific sows in Thailand produced up to 29 TB and 25 BA. Both TB and BA reach a plateau in sow parity numbers 3 – 4 and significantly declined after parity number 5. Interestingly, primiparous sow had a larger TB than sow parity numbers above 7 and had a larger BA than sow parity numbers above 6. Season significantly compromise litter size at farrowing in the hyper-prolific sows in Thailand.

### Acknowledgements

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### References

Immunoassay precision of S27 PEDV IgG/IgA ELISA kit for antibody detection against porcine epidemic diarrhea virus in colostrum and milk

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Keywords: antibody, colostrum, ELISA, milk, porcine epidemic diarrhea virus

Introduction
Porcine epidemic diarrhea (PED) is caused by porcine epidemic diarrhea virus (PEDV), genus Alphacoronavirus in the family of Coronaviridae (1). Disease of porcine epidemic diarrhea has been found high mortality and morbidity rate up to 100%, especially piglet lower than 1 week of age. In contrast to pig more than 4-5 weeks of age showed a low mortality rate and severe diarrhea that usually occurred in all ages of pig (2). Currently, many swine industrial countries have remain a trouble economic losses because PED became endemic disease and causes sporadic outbreaks throughout the country including Asian countries (3), the North America and several countries continent that the PEDV responsible for outbreaks were associated with the 2013 disease outbreak among swine farms in the United States(4-7). Therefore, outbreak protection of PED in herd have to plan a strategy for decrease mortality rate in piglet. Subsequently, lactogenic immunity was employed to provide protection.

Standard method for antibody detection of PEDV usually used viral neutralization (VN) assay, however this method was inconvenient and time consuming. In this study, we developed new ELISA test kit for IgG and IgA investigation in colostrum and milk samples. In the development of assay, the precision test is the one main part of validation process, which demonstrate agreement between test results obtained under stipulated condition. The Precision test has three types including, repeatability, intermediate precision and reproducibility (8). Therefore, the objectives of the study were to investigate precision of S27 IgG/IgA ELISA kit for antibody detection against porcine epidemic diarrhea virus in colostrum and milk including, repeatability, within plate repeatability (intra-assay) and between plate repeatability (inter assay) and reproducibility or interlaboratory test.

Materials and Methods
Colostrum and milk samples were collected from PEDV-positive farm and PEDV-negative farm. All of samples will be confirmed positive and negative neutralizing antibody by viral neutralization assay (VN). Colostrum samples were collected from sows within 3 h post parturition. Colostrum and milk samples were centrifuged at 10,000×g for 20 min at 4°C for lipid layer separation and the middle layer were collected. Samples were heat-inactivated at 56°C for 30 min before VN test.

The samples were confirmed positive and negative antibody against PEDVby the VN test. In brief, the samples were two-fold serially diluted in maintenance medium (MM). Each dilution of samples was mixed with an equal volume of PEDV (10⁵ TCID₅₀/0.05 ml). The samples/virus mixtures were incubated at 37°C for 1 h and transferred to new 96-well microtiter plate containing confluent Vero cell monolayer, 2 days old and incubated at 37°C for 3 days. The cell monolayers were fixed with a cold acetone–methanol solution and the presence of virus was detected by indirect fluorescent microscopy using monoclonal antibody (Medgene labs, Brookings, SD, USA). The titers were read VN titer under fluorescent microscope.

Enzyme linkage immunosorbent assay (ELISA) was performed, using recombinant S12 (rS12) protein as an antigen. An ELISA plate (Thermo Fisher Scientific, Roskilde, Denmark) was coated with recombinant spike protein in 0.1 M Na₂CO₃/NaHCO₃ buffer for IgG ELISA or IgA ELISA and hold overnight at 4°C. The plate was blocked with 3% bovine albumin serum (BSA) and incubated on horizontal for 3 h at room temperature. The colostrum and milk samples were diluted with sample diluent 1:250 and then there were pipetted 100 µl/sample to ELISA plate and incubated at room temperature for 1 h. Following five times washing with washing buffer (0.05% PBS-T), prior to incubate with anti-pig IgG-HRP or anti-pig IgA-HRP (Bio-Rad AbDSerotec, Kidlington, UK) for at room temperature for 1 h. Subsequently, the ELISA plates were washed 4 times with washing buffer prior to incubation with 3,3′,5,5′-tetramethylbenzidine (TMB) liquid substrate system for ELISA (Sigma-Aldrich, St. Louis, Missouri USA) at 37°C for 15 min. The reaction was stopped using 1 N H₂SO₄, and the optical density at 450 nm (OD₄₅₀) value was measured by using an ELISA plate reader (AccuReader, Meter tech, Taipei, Taiwan). The OD results were converted to sample-to-positive (S/P) ratio using the equation as follow.

In trial of within plate repeatability, 1 plate ELISA containing triplicates ten of VN positive samples and ten of VN negative samples were used. As for between
plate repeatability was evaluated in 5 different plates of triplicate ten colostrum or milk samples in different days. An inter-laboratory test of the study were conducted by testing ten samples of the colostrum or milk samples which known VN titer were used for IgG and IgA investigation by ELISA from 2 operators in difference laboratory. The ELISA test kit and the samples were tested in laboratory of Veterinary Microbiology (lab A), the Faculty of Veterinary Science, Chulalongkorn University and compared to the results from Veterinary of Microbiology laboratory (lab B), Miyazaki University. The sample was tested in replicates test both IgG and IgA ELISA. After repeatability and reproducibility test, measured OD value of IgG and IgA. Thereafter, mean, standard deviation (SD), and percentage of coefficient of variation (%CV)of OD and S/P ratio were calculated as follow equations.

\[
S/P \text{ ratio} = \frac{\text{OD of sample} - \text{OD of Neg control}}{\text{OD of Pos control} - \text{OD of Neg control}}
\]

\[
\% \text{ CV} = \frac{\text{SD} \times 100}{\text{Mean}}
\]

**Results and Discussion**

Within plate repeatability test of ELISA showed low level of the percentage of coefficient of variation (%CV), which less than 10% in both OD and S/P ratio of positive and negative sample observation (Table 1). In contrast to the repeatability test in different plate presented low level of %CV of OD, while in S/P ratio showed more wide range of %CV than in OD value in both IgG and IgA (Table 2). However, normally the accepted value of %CV for precision test should not exceed 10% in intra-assay and not exceed 15% in inter-assay, however 20%-30% was acceptable (9). Therefore, we can conclude that the results of this ELISA kit are acceptable of repeatability in both intra-assay and inter-assay.

**Table 1** Range of mean, range of SD and range of CV (%) from triplicate test of 10 positive and 10 negative samples in same plate (within plate repeatability) by IgG ELISA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IgG ELISA</th>
<th>IgA ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td>S/P ratio</td>
</tr>
<tr>
<td>Mean</td>
<td>0.060-1.654</td>
<td>0.070-1.557</td>
</tr>
<tr>
<td>SD</td>
<td>0.005-0.089</td>
<td>0.022-0.123</td>
</tr>
<tr>
<td>CV (%)</td>
<td>2.36 - 9.75</td>
<td>-31.54-12.82</td>
</tr>
</tbody>
</table>

The interlaboratory test of this ELISA kit presented perfected agreement between two laboratories that showed in plotted graph in Fig.1. The results of IgG and IgA of ELISA test were presented R-squared (R²) value higher than 0.9 that is demonstrated ELISA kit had highly reproducibility results.

Although, OD value from lab B lower than OD value of lab A, all samples from ELISA test in both laboratories correlated with the result from VN (data not show).

**Table 2** Range of mean, range of SD and range of CV (%) from 10 milk samples tested in 5 different plates at different days (between plate repeatability).

**Acknowledgements**

This study was supported by the Research and Researcher for industry (RRi) under the Thailand Research Fund (TRF). Also I would like to thank Dr. Tamaki Okabayashi and Mr. Keigo Ikeda from Miyazaki University for interlaboratory test and accommodated us in this experiment.
References
Molecular characterization, etiology, and phylogenetic analysis of complete genome sequences of Porcine Deltacoronavirus

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Keywords: Porcine Deltacoronavirus, pig, virus, phylogenetic.

Introduction
Porcine deltacoronavirus (PDCoV) was first reported in Hong Kong in 2009 (Woo et al., 2012) and subsequently reported in many countries including of USA, South Korea, China, Thailand, Lao PDR, Vietnam, and, Japan, respectively (Janetanakit et al., 2016; Lee and Lee, 2014; Lorsirigoool et al., 2016; Marthaler et al., 2014; Saeng-chuto et al., 2017; Song et al., 2015; Suzuki et al., 2017; Woo et al., 2012). The clinical signs of PDCoV affected pigs is similar to that of porcine epidemic diarrhea virus (PEDV) which causing watery diarrhea, villous atrophy could be observed by histopathological examination (Chen et al., 2015).

PDCoV is an enveloped, single-stranded, positive-sense RNA virus in the genus Deltacoronavirus, family Coronaviridae. The genome arrangements are in the order of: 5’ untranslated region (UTR), open reading frame 1a/1b (ORF 1a/1b), spike (S), envelope (E), membrane (M), nonstructural protein 6 (Nsp6), nucleocapsid (N), nonstructural protein 7 (Nsp7), 3’ UTR.

In south east asia (SEA) countries, there were reports of the complete genomes in Thailand, Vietnam and Laos PDR, which isolated in February 2013 (Saeng-chuto et al., 2017), December 2015 and January 2016 (Lorsirigoool et al., 2016), respectively. These agents decreasing productivity of pig industry, especially in Vietnam which the largest scale of pork production in SEA. Due to the previous studies, there still no phylodynamic study which including of the isolates of these SEA countries. In this study, we did performed analysis of the complete genomes using molecular clock concept with Bayesian probability and Markov Chain Monte Carlo (BMCMC) to describe their molecular characterization, etiology and phylogenetics. The estimated substitution rate of complete genome and estimated divergences were also described.

Materials and methods
All 70 complete genome sequences of PDCoV in Genbank from previous studies were used in this study, all of the genomes were displayed the accession numbers and isolate names in Figure 1. The sequences were aligned using MAFFT (Katoh et al., 2002) and phylogenetic analyses were performed using BEAST 1.8.4, GTR+G+I model with 600 million states with

loged every 10,000 states were performed for an exponential relaxed clock using Bayesian skyline tree prior (Drummond et al., 2006; Drummond et al., 2002; Drummond and Rambaut, 2007; Drummond et al., 2005; Drummond et al., 2012), the result will be accepted with ESS > 200. The results were interpreted using Tracer 1.6.0 (Rambaut and Drummond, 2013). Maximum clade credibility (MCC) trees were annotated using TreeAnnotator, and, chronogram, estimated divergences, posterior probability and 95% HPD displays were generated using FigTree 1.4.2 (Rambaut and Drummond, 2014).

Results and Discussion
According to the molecular characterization, we can characterize the PDCoV complete genomes into 3 geographic distinct, South East Asia (SEA), China and Hong Kong (CHN), and, USA, South Korea and Japan. The number displaying at nodes were the years of estimated divergences, branches displaying posterior probabilities, and, node bars displaying 95% HPD of heights.
and the most recently common ancestor of PDCoV was estimated to be diverged in 1989 into 2 main lineages.

The first lineage was estimated to be diverged in 2003 to the concestor of Vung Tau isolate (P29-15-VN-1215), and, the rest of SEA isolates. The rest of SEA isolates concestor was diverged into Hanoi and Binh isolates of Vietnam, and, Thai and Laos isolates, in 2006. Laos isolate shared the same monophyletic clade as Thai concestor whereas the Vietnamese isolates were not the same monophyletic clade as Thai isolates, but still the same lineage. Due to the information described above, the origin of Laos isolate shall be introduced from the isolates in Thailand. The 95% HPD of Vietnamese isolates (Hanoi6 and Binh2) seem to be lack of range while the Vung Tau isolate was the paraphyletic clade with those two, this shall indicate that might lose some clue of the strain in Vietnam or which originated from other area.

The second lineage was estimated to be diverged since 1998-2009 into various of Chinese and Hong Kong strains. US concestor was estimated to be diverged from those of Chinese isolates in 2009, and, the concestor of DH1 and DH2 of South Korea was estimated to be diverged from US isolates in 2012, and, close to some US isolates of Minesota, Arkansas, and, Illinois. The Japanese isolate and KNU1404 of South Korea shared the same clade and close to the isolates of USA of Iowa, Ohio, Miami, and, Illinois. In addition, KNU16-11 of South Korea was closest to KY4813 of Kentucky, USA, and, KNU16-07 was located in the same clade, together with the isolates from Illinois and Nebraska of USA. This information suggests that Japanese and South Korean isolates shared the same clade and might be the same originated as US isolates.

In conclusion, up to the present, we found more information on the PDCoV complete genomes, especially, the import and export of pigs and their materials were the important factor of the outbreaks of the virus, however, there is lack of some details especially how the virus introduce to Vietnam or USA. According to the previous study, the sequences of deltacoronavirus in Asian Leopard Cats and Chinese Ferret Badgers (Fang et al., 2016) were very close to the isolates of SEA. The further study on some other species deltacoronavirus or active surveillances of swine herds shall provide a further information and strongly support the hypothesis of their etiology.

Acknowledgements
This research was financially supported by the Thailand Research Fund (Grant Number MRG5080323), National Research Council of Thailand (Grant Number 5080001) and Agricultural Research Development Agency (Public organization; Grant number PRP5805021650). Partial funding was provided by Special Task Force for Activating Research (STAR), Swine Viral Evolution and Vaccine Research (SVEVR), Chulalongkorn University (Grant number GSTAR 59-013-31-007).

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13. Rambaut and Drummond, 2013. Tracer 1.6 [computer program].
14. Rambaut and Drummond, 2014. FigTree 1.4.2 [computer program].
Novel Thai unique insertion of Porcine epidemic diarrhea spike gene from the outbreak during 2014

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Keywords: Thailand, mutation, porcine epidemic diarrhea virus

Introduction
Porcine epidemic diarrhea virus (PEDV) is causing sporadic outbreaks of PED in Thailand since 2007. At the present, there were 6 genogroups (TH1-6) in Thailand (Stott et al., 2017). In 2014-2015, several herds had experienced severe PED outbreaks and the reason of the re-current outbreaks was unknown. This study was aimed to find some possible causes to describe this event by sequence-based analysis of S1 gene.

Materials and Methods
154 PEDV isolates from passive surveillance of the outbreak between 2008-2016 were sequenced for complete S1 gene and compared together with reference sequence of PEDV (NC_003436) and 15 previous isolates provided by GenBank® for any substitution changes. All were aligned using MAFFT software then BEAST was performed using GTR+G+I model with exponential relaxed clock and Bayesian skyline tree prior. 400 million states with 5,000 logged were applied (Drummond et al., 2006; Drummond et al., 2002; Drummond and Rambaut, 2007; Drummond et al., 2005; Drummond et al., 2012). The result will be accepted if qualified ESS>200. The results were interpreted using Tracer 1.6.0 (Rambaut and Drummond, 2013). Maximum clade credibility (MCC) trees were annotated using TreeAnnotator, and, chronogram was generated using FigTree 1.4.2 (Rambaut and Drummond, 2014)

Results and Discussion
Based on retrospective study of PEDV spike gene, we found that there were 9 isolates from Chonburi in 2014 that have the insertion of 12 nucleotides ATA CAA CAG GTC between 688th – 689th, and substitution at position 689th from A to G which caused the amino acid insertion of T T G R. This finding is unique for only Thai isolates when compare with all available sequence in GenBank®, and, were different from our 29 isolates of our previous report (TH-S-INS) that have 9 nucleotides unique insertion of CAA GGG AAT between the same position which resulted in amino acid change from N to T at position 229th and insertion of R E Y between 229th - 330th.

Figure 1 Chronogram of S1 gene of Thai PEDV. The novel 12 nucleotides insertion strains are indicated by TH7 bracket whereas the other brackets are indicating previously TH1 and TH2 9 nucleotides insertion.

In conclusion, we found 9 nucleotides unique insertion of PEDV spike which the novel isolates during 2014-2015 that causes outbreak in farms located in Nakornpathom, Ratchaburi, Saraburi, Lopburi, Chonburi, Buriram, and Nakornpanom, and, the 12 nucleotides unique insertion of PEDV spike that caused the outbreak in Chonburi in 2014. The 9 nucleotides unique insertion were the predominant group of isolates during that time that might be one of the important factor that causing the outbreak of PEDV during 2014-2015, however, the 12 nucleotides unique insertion
might be another cause of re-current outbreak in Chonburi with shorter in the interval of times during 2014.

Acknowledgements
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2. Drummond et al., 2006. PLOS Biol. 4: e88.
7. Rambaut and Drummond, 2013. Tracer 1.6 [computer program].
8. Rambaut and Drummond, 2014. FigTree 1.4.2 [computer program].
Ovulation time in weaned sows did not differ between Landrace and Yorkshire breeds but differ between primiparous and multiparous sows

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Keywords: Breed; Ovulation; Parity; Sows; Ultrasonography

Introduction
After farrowing, the sows enter into an anestrus period. The secretion of gonadotropin releasing hormone (GnRH) from hypothalamus is decreased by sucking reflexes which is induced by the piglets during lactation [1]. This mechanism inhibits the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) during lactation which suppress the follicular growth. After weaning, the sucking reflex disappear and the sows normally return to estrus cycle within 3-5 days[2]. In general, the sows ovulate within 24 to 68 h after the onset of standing estrus [3]. Ovulation time in sowsis influenced by parity number [4]. In Thailand, no study on the influence of parity and breed of sows on ovulation time has been done. To obtain more knowledge on optimal timing for artificial insemination in pig, factors associated with ovulation time in sows should be carefully investigated. The present study aims to investigate the effects of breed and parity group on ovulation time in weaned sows in Thailand.

Materials and Methods
The experiment was performed in a commercial swine herd in the middle part of Thailand in June 2016. In total, 35 weaned sows (16 Landrace and 19 Yorkshire) were included. The sows were weaned at 22.3 ± 2.1 days (18 to 27 days) after farrowing. Parity number of sows averaged 2.7±1.9 (1 – 7). The sows were kept in 0.5 x 2.0 m 2 stall in a concrete-slatted floor after weaning. The temperature and humidity in the barn were controlled by evaporative cooling system. All of the sows received 2.4-2.7 kg of a commercial feed twice a day. The feed contained 16.0% CP, 2,800 kcal/kg ME and 1.0% lysine. The water was provided ad libitum. After weaning, body condition score (BCS) of the sows was determined.

The sows were detected for standing estrus twice a day at 0800-0900 and 1500-1600. The estrus detection was started from the day after weaning onwards. Estrus detection was performed by back-pressure test in the presence of mature boar in front of the stalls. The standing estrus of the sows was defined when the sows show ear popping sign, arching back and immobilizing legs when back pressure test was applied.

Ovulation was determined by using trans-rectal ultrasonography after the onset estrus. The trans-rectal ultrasonography was performed by using realtime B-mode ultrasonography (HS-2000; Honda electronics, co., ltd. Japan) equipped with 5.0 and 7.5 MHz linear probe transducers. The ultrasonography was performed every 8 h interval from the onset of estrus until ovulation took place. The pre-ovulatory follicles on left and right ovaries were monitored and the diameter of the largest follicles was measured. The pre-ovulatory follicle was defined as the follicles with >5.0 mm diameter. Ovulation was defined when most of the pre-ovulatory follicles disappeared. The follicle with >15 mm in diameter was considered as cystic ovaries.

The data were analyzed by using Statistical Analysis System (SAS version 9.0, Cary, NC, USA.). The statistical models included breed (Landrace vs Yorkshire) and parity group (primiparous vs multiparous). The weaning-to-estrus interval (WEI), estrus-to-ovulation interval (EOI) and weaning-to-ovulation interval (WOI) were calculated. Continuous data were presented as mean ± SD. The categorical data was presented as percentage. Least square means of continuous data were compared by using GLM procedure. The percentage of cystic ovaries was compared by using Chi-square test. Differences with P<0.05 were regarded to be statistically significance.

Results and Discussion
On average, BCS of Landrace was lower than Yorkshire sows (2.9 vs 3.3, P = 0.030). The interval from estrus-to-ovulation ranged from 49.7 to 123.2 h in Landrace and 42.8 to 108.9 hin Yorkshire breeds. Both EOI and WOI were not different between Landrace and Yorkshire breeds (P> 0.05). To our knowledge, no study has compared ovulation time between Landrace and Yorkshire sows. Tummaruk et al. (2000) found that the weaning-to-service interval of Landrace and Yorkshire were not different. This is in agreement with the present study.
Table 1 Reproductive data of sows by breed

<table>
<thead>
<tr>
<th>Variables</th>
<th>Landrace</th>
<th>Yorkshire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sows</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Parity number</td>
<td>2.7±0.5</td>
<td>2.7±0.4</td>
</tr>
<tr>
<td>Body condition score</td>
<td>2.9±0.5a</td>
<td>3.3±0.6b</td>
</tr>
<tr>
<td>Lactation length (d)</td>
<td>22.6±2.1</td>
<td>21.9±2.0</td>
</tr>
<tr>
<td>Weaning-to-estrus (d)</td>
<td>4.0±0.7</td>
<td>4.0±0.8</td>
</tr>
<tr>
<td>Estrus-to-ovulation (h)</td>
<td>77.0±23.3</td>
<td>70.4±20.3</td>
</tr>
<tr>
<td>Weaning-to-ovulation (h)</td>
<td>175±39</td>
<td>166±26</td>
</tr>
<tr>
<td>Follicle diameter (mm)</td>
<td>9.5±1.6</td>
<td>10.0±2.0</td>
</tr>
<tr>
<td>Ovarian cyst</td>
<td>12.5%</td>
<td>15.8%</td>
</tr>
</tbody>
</table>

Different superscript differed significantly (P<0.05)

Table 2 illustrates ovulation time in primiparous and multiparous sows. On average, primiparous sows had lower BCS than multiparous sows (P = 0.048). After weaning, multiparous sows exhibited standing estrus earlier than primiparous sows (3.6 vs 4.8 days, respectively, P<0.001). Likewise, multiparous sow ovulated earlier than primiparous sows (Table 2). On average, the diameter of follicle and the incidence of cystic ovaries were not different between breeds and parity.

Table 2 Reproductive data of primiparous and multiparous sows

<table>
<thead>
<tr>
<th>Variables</th>
<th>Primiparous</th>
<th>Multiparous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sows</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>Body condition score</td>
<td>2.9±0.4a</td>
<td>3.3±0.6b</td>
</tr>
<tr>
<td>Lactation length (d)</td>
<td>21.6±2.1</td>
<td>22.6±1.9</td>
</tr>
<tr>
<td>Weaning-to-estrus (d)</td>
<td>4.8±0.7a</td>
<td>3.6±0.3b</td>
</tr>
<tr>
<td>Estrus-to-ovulation (h)</td>
<td>87.4±26.1a</td>
<td>66.5±15.6b</td>
</tr>
<tr>
<td>Weaning-to-ovulation (h)</td>
<td>203±29a</td>
<td>154±20b</td>
</tr>
<tr>
<td>Follicle diameter (mm)</td>
<td>9.5±1.9</td>
<td>9.9±1.8</td>
</tr>
<tr>
<td>Ovarian cyst</td>
<td>23.1%</td>
<td>9.1%</td>
</tr>
</tbody>
</table>

Different superscript differed significantly (P< 0.05)

Parity numbers of sow influence WEI [5]. This is in agreement with the present study. This indicates that primiparous sows require longer time to recover from postpartum disorders i.e., fever, in-appetite and vaginal discharge [6]. These may affect the estrus expression after weaning. Furthermore, primiparous sow had a higher backfat loss during lactation than multiparous sow [6]. This impairs the follicle development due to negative energy balance [4].

In conclusion, no effect of breed on the estrus expression, ovulation time and the incidence of cystic ovaries in Landrace and Yorkshire sows. The ovulation time after weaning differed significantly between primiparous and multiparous sows.

Acknowledgements

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References
PEDV monitoring after the PEDV outbreak using oral fluid, fecal and surface swab samples

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Keywords: PEDV, monitoring, oral fluid, feces, surface swab

Introduction
Porcine epidemic diarrhea (PED) is an endemic disease after the late 2007 outbreak in Thailand. PED is a highly contagious disease causing 80-100% morbidity and mortality in newborn piglets. After 12-24 hours of PEDV infection, the infected pig shown clinical signs and shed virus in the fecal - oral mode of transmission. The virus spread to susceptible pigs and contaminates farm personnel and vehicles, equipment and formites to other farms. In order to know the PEDV status after the farm outbreak, a monitoring using various sample types to investigate the remaining viral residues in pigs and the environment formites is of importance for a better risk control and prevention of the re-outbreak in the endemic farms.

Materials and Methods
This study was conducted in a participated commercial swine farm recently reported of the PED outbreak. This farm is a farrow to wean system with internal replacement. Oral fluid samples were collected from gilts, fecal samples were collected from sows in every unit and surface swab samples were collected from the floor and formites that presumably contaminated with PEDV. Those samples were collected 1 and 2 months after the outbreak (MAO). All samples were conducted for 5 duplications. RT-PCR assay was done by animal health diagnostic center, (AHDC) CPF Thailand. Briefly, oral fluid samples were centrifuged before tested. PBS was added (30 ml) in each fecal and surface swab samples and centrifuged before used. A volume of 200-μl of each sample was used for the extraction with the Geneaid Viral nucleic acid extraction kit II (Geneaid Biotech. Ltd., Taipei, Taiwan). S gene and ORF gene primers [1] were used for RT-PCR (15 min at 95°C, followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 50°C and extension for 45 sec at 72°C).

Results and Discussion
In both studied periods of sampling, neonatal pigs still had diarrhea but no clinical signs were found in growing pigs. All oral fluid samples were tested from both periods. However, 4 fecal samples were found positive (primiparous and multiparous sows after 3 weeks farrowing, 1 MAO and gestated sows and multiparous sows after 1 week farrowing, 2 MAO). All surface swab samples were found negative at 1 MAO and 16 surface swab samples were found positive at 2 MAO (Table1). It should be noted that the surface swab samples were done only at the clean area (1 MAO) but after receiving the results from the 1 MAO, we decided to collect the surface swab samples in both clean and dirty areas for the 2 MAO sampling. The results indicated that after the outbreak, both gilts and sows still intermittently shed the virus via the feces and susceptible piglets were exposed to the contaminated virus from the environment similar to the previously study [2]. It should be noted that none of oral fluid samples were found positive. Evidently, the PEDV shedding route is via the feces and the collected saliva might not be exposed with the contaminated feces or the virus load in the oral fluid samples was not enough for the detection. The results of the fecal samples indicated that PEDV still be found in the feces and the contaminated surface areas after the outbreak at least 2 months. Those gilts and sows had no clinical signs but the susceptible piglets having insufficient lactogenic immunity would show the clinical signs. The results obtained from this study suggested that fecal and surface swab samples could be used for PEDV subpopulation detection after the outbreak. Various management strategies implemented after the PEDV outbreak could be monitored via fecal and surface swab samples. Level of biosecurity and quality of gut feedback may affect the herd immunity reflecting the remaining of the viral residues in the environment.

In summary, after the outbreak, the period of PEDV eradication depends on the management strategy. Fecal and surface swab samples were samples of choice for
PEDV monitoring after the outbreak. Together with the performance index, the results from chronological, cross-sectional samplings of fecal and surface swab areas are suggested for PEDV monitoring and evaluating the farm status.

**Table1:** Number of porcine epidemic diarrhea virus positive samples after the outbreak in both studied periods.

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>Group of animals</th>
<th>RT-PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 month after the outbreak*</td>
</tr>
<tr>
<td>Oral fluid</td>
<td>Gestated Gilts</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Gestated Sows</td>
<td>(0/5)</td>
</tr>
<tr>
<td>Feces</td>
<td>Gestated Gilts</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Gestated Sows</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>primiparous sows after 1 week farrowing</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>multiparous sows after 1 week farrowing</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>primiparous sows after 3 week farrowing</td>
<td>(1/5)</td>
</tr>
<tr>
<td></td>
<td>multiparous sows after 3 week farrowing</td>
<td>(1/5)</td>
</tr>
<tr>
<td>Surface Swab</td>
<td>Empty Gilt house</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Empty Farrowed house</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>primiparous sow pen after 1 week farrowing</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>multiparous sow pen after 1 week farrowing</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>primiparous sow pen after 3 week farrowing</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>multiparous sow pen after 3 week farrowing</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Cleaned boots</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Cleaned beddings</td>
<td>(0/5)</td>
</tr>
</tbody>
</table>

* The surface swab was done in the clean area
** The surface swab was done in both clean and dirty areas

**Acknowledgements**

This study was financially supported by CPF Thailand Co., Ltd. for Mrs. Busayamas Jantrasakul Master Program in Veterinary Science and Technology, Chulalongkorn University.

**References**

Phenotypic and genotypic characteristics of ESBL-producing and colistin resistance in Salmonella enterica and Escherichia coli isolated from pigs in the border provinces between Thailand and Cambodia, Lao PDR and Myanmar

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**Keywords**: colistin, ESBL, Escherichia coli, Pig, Salmonella enterica

**Introduction**

Thailand, Cambodia, Lao PDR and Myanmar are neighbouring countries that are located in the Mekong region and share a common border. At the crossing points, there is routine movement of livestock, in particular swine and bovine. There are approximately millions of people travelling across the borders per year. These include also tourists, villagers and merchants. Such movements are significant factors contributing to emergence and spread of antimicrobial resistance (AMR) bacteria, including extended-spectrum beta-lactamase (ESBL) producing and colistin resistant *Salmonella* and *Escherichia coli*. The bacterial species may contaminate food and transfer into food chain. This could cause a significant effect on public health. However, the knowledge of ESBL production and colistin resistance in *Salmonella* and *E. coli* is still limited. Therefore, the aim of this study was to determine the prevalence of ESBL producing and colistin resistance of *Salmonella* and *E. coli* isolated from pigs and their meat products.

**Materials and Methods**

A total of 809 samples obtained from rectal swab from pig slaughterhouses (n=441) and carcass swab from retail markets (n=368) from the crossing provinces between Thailand and Cambodia (Sa Kaeo-Banteay Meanchey province), Thailand and Lao PDR (Nong Khai-Vientiane), and Thailand-Myanmar (Chiang Rai-Tachileik) during October 2016 and March 2017. The *Salmonella* strains were isolated according to ISO 6579:2002(E) and were serotyped by using slide agglutination method. One isolate of each serovars was collected from each positive sample. The *E. coli* strains were isolated by using the standard method and biochemically confirmed in order to previously published (1). One *E. coli* isolate was collected from each positive sample. ESBL production was initially screened by disk diffusion method and confirmed by a combination disk method used as described by CLSI (2013). The *blaCTXM* and *blaTEM* genes were detected by PCR in all ESBL producing isolates (2). Colistin susceptibility was examined by determination of MICs according to EUCAST (2013). The presence of colistin resistance encoding genes (i.e. *mcr-1* and *mcr-2*) were investigated by PCR in all the isolates.

**Results and Discussion**

Among 809 samples, the prevalence of *Salmonella* (Table 1) and *E. coli* (Table 2) varied between locations and sample sources. A total of 463 *Salmonella* isolates were collected from 403 *Salmonella* positive samples. The prevalence of *Salmonella* was much higher in carcass samples from markets (61.04%) than those in rectal swab from slaughterhouses (13.6%) in all countries. These findings may be related to the evisceration process and potential cross-contamination between carcasses and markets. A total of 767 *E. coli* isolates were obtained, isolates from rectal swab and isolates from carcass swab. High contamination of *E. coli* was found in carcass samples from retail markets (44.72%). The results suggest cross-contamination during transportation and in the retail store.

Nine *Salmonella* isolates (1.9%) that were resistant to at least one cephalosporin tested and eight were found to be ESBL producers. These included 7 isolates from Thailand and 1 isolate from Lao PDR. Forty-eight *E. coli* isolates (6.3%) were also resistance to at least one cephalosporin tested, of which 47 isolates were confirmed to be ESBL producers. These included 20 isolates from Thailand, 3 isolates from Cambodia, 6 isolates from Lao PDR and 18 isolates from Myanmar. Of all ESBL positive isolates (9 *Salmonella* and 48 *E. coli*), the prevalence of *blaCTXM* was found in *Salmonella* (1.7%) and *E. coli* (6.1%) and the prevalence of *blaTEM* was detected in *Salmonella* (1.5%) and *E. coli* (3.4%) respectively.
Of the 12 colistin resistant *Salmonella* isolates (2.6%), all the isolates were positive to *mcr-1*, these included 6 isolates from Thailand, 4 isolates from Cambodia, 1 isolate from Lao PDR and 1 isolate from Myanmar. Eighty isolates of *E. coli* (10.4%) were resistant to colistin. Forty-three isolates (5.6%) were positive to *mcr-1*, these included 6 isolates from Thailand, 9 isolates from Cambodia, 26 isolates from Lao PDR and 2 isolates from Myanmar.

The *mcr-2* gene was not found in any *Salmonella* and *E. coli* isolates. The percentage of *mcr-1, mcr-2, bla*\(_{CTXM}\), *bla*\(_{TEM}\) in *Salmonella* and *E. coli* were shown in Figure 1 and Figure 2.

**Table 1** Prevalence of *Salmonella* in the border provinces of Thailand, Cambodia, Lao PDR and Myanmar (n=809).

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample type</th>
<th>Positive samples</th>
<th>Total No.</th>
<th>Total (%)</th>
<th>Total No.</th>
<th>Total (%)</th>
<th>Total No.</th>
<th>Total (%)</th>
<th>Total No.</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thailand</td>
<td>Cambodia</td>
<td>Lao PDR</td>
<td>Myanmar</td>
<td>Thailand</td>
<td>Cambodia</td>
<td>Lao PDR</td>
<td>Myanmar</td>
</tr>
<tr>
<td>Slaughter house</td>
<td>Rectal swab</td>
<td>200</td>
<td>184</td>
<td>93</td>
<td>82</td>
<td>75</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Carcass swab</td>
<td>180</td>
<td>152</td>
<td>89</td>
<td>76</td>
<td>66</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Market</td>
<td>Carcass swab</td>
<td>180</td>
<td>163</td>
<td>91</td>
<td>76</td>
<td>64</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>560</td>
<td>485</td>
<td>424</td>
<td>404</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Grand total</td>
<td></td>
<td></td>
<td>343</td>
<td>322</td>
<td>296</td>
<td>302</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Prevalence of *E. coli* in the border provinces of Thailand, Cambodia, Lao PDR and Myanmar (n=809).

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample type</th>
<th>Positive samples</th>
<th>Total No.</th>
<th>Total (%)</th>
<th>Total No.</th>
<th>Total (%)</th>
<th>Total No.</th>
<th>Total (%)</th>
<th>Total No.</th>
<th>Total (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thailand</td>
<td>Cambodia</td>
<td>Lao PDR</td>
<td>Myanmar</td>
<td>Thailand</td>
<td>Cambodia</td>
<td>Lao PDR</td>
<td>Myanmar</td>
</tr>
<tr>
<td>Slaughter house</td>
<td>Rectal swab</td>
<td>200</td>
<td>184</td>
<td>93</td>
<td>82</td>
<td>75</td>
<td>1</td>
<td>10</td>
<td>1</td>
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<tr>
<td></td>
<td>Carcass swab</td>
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<td>152</td>
<td>89</td>
<td>76</td>
<td>66</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Market</td>
<td>Carcass swab</td>
<td>180</td>
<td>163</td>
<td>91</td>
<td>76</td>
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<td>424</td>
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<td>296</td>
<td>302</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1** Percentage of *mcr-1, mcr-2, bla*\(_{CTXM}\), *bla*\(_{TEM}\) in *Salmonella* (n=463).

**Figure 2** Percentage of *mcr-1, mcr-2, bla*\(_{CTXM}\), *bla*\(_{TEM}\) in *E. coli* (n=767).

**Conclusions**

ESBL producing and colistin resistant *Salmonella* and *E. coli* are common among pigs and their carcass in the border provinces among Thailand, Cambodia, Lao PDR and Myanmar. The results highlight the role of pigs and pig products as reservoirs of resistance to last line drugs in these areas.

**Acknowledgements**

This work was supported by National Research University Project, Office of Higher Education Commission NRU59-015-HER. Kyaw Phyoe Sunn is a recipient of the Scholarship Program for ASEAN Countries, Chulalongkorn University.

**References**

Piglet Brooder System in Thailand: Problem Identification and Conceptual Solution Development

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Keywords: Piglet Brooder, Pig, Conceptual Development, Problem Identification

Introduction
Piglet brooder plays a major role in piglet survival rate, weaning weight and piglet health status. Proper temperature should be provided to the piglet all the time according to thermoneutral zone recommendation publish by Dong, H. [1] in 2001. Environment temperature from 36°C to 32°C should be controlled and adjusted by the stockman to meet piglet requirement all the time.

In Thailand, there are two majority types of farrowing pen, Natural air with or without fan and Evaporative Cooling System(EVAP) Housing. Both types of the housing have the same problem in controlling the temperature for piglet due to the cooling system was design for sow requirement which differ from piglet, especially for new born piglet. Farrowing pen in Thailand were conditioned from 25°C to 30°C [2]depending to environmental temperature and efficacy of EVAP system equipped in daytime and ranging from 25°C to 20°C in night time while EVAP system is turned off manually and operated only with ventilation fan. This thermal difference and improper environment effect on piglet behavior spotted by huddling in group, burial in bedding and less milk consumption.

To provide the suitable environmental condition for piglet external heat source were introduced to farrowing pen. Electric infrared lamp was widely used to provide heat directly to piglet in Thailand with the consumption of 175 Watts to 250 Watt of electricity per hour. Average usage of external heat source is 8 hours per day in summer and raining season and 12 hours per day in winter. that lead to operational cost of 6 Thai Baht to 9 Thai Baht per day. The efficacy of heating of electric infrared heating lamp is low due to the narrow distribution of radiation which varied by distance from the infrared heating lamp.

Another type of heating is hot-water heating system using a heat plate located underneath the piglet brooder, heated by the hot water from the boiler, which was energized by electricity, propane gas, electric generator engine or solar collector panel. This type of heating is widely use in European and cold climate country due to the need of heating at all time. For hot climate country like Thailand this system is rarely utilized by Thai swine farmer due to the economic reason, very high equipment and installation cost, high maintenance cost and high operating cost. Even most of the Thai swine farmer already have free energy source such as biogas system and electric generator or a long daytime for solar collector this system still not being adopt in to farm.

Figure 1. Huddling in group behavior of piglet
Figure 2. Infrared Heating Lamp
Wind is also the issue of piglet husbandry, strong wind of 2 meter per second is recommended for sow in farrowing pen to provide good ventilation, reduce sow temperature and humidity control. This effect on the stress of piglet in the same house. Piglet affected by wind chill will show the sign of coldness and reduction in milk consumption.

Providing a suitable environment to the piglet will increase productivity and health status to piglet, which result in production in nursery pig and finishing pig in a result of average daily gain (ADG) and feed conversion ratio (FCR).

Plastic farrowing pen with plastic piglet brooder has been introduce to Thailand market for many years with very few adopter and negative response from users.
Materials and Methods

The qualitative study was conducted to investigate the unmet need for piglet brooder usage by structural in-depth interview from 8 swine farmers in 5 provinces of Thailand. The structural information was collected and analyzed to generate the piglet brooder unmet needs.

The obtained needs were developed into conceptual solution using innovation process front end concept of Booz, Allen, Hamilton Model [5]. Starting from product Strategies followed by the idea generation stage, idea screening stage and concept development and testing stage.

Morphological Chart and PUGH Matrix were used to develop conceptual solution. The most viable prototype (MVP.) in 3D Computer Aided Design (3D CAD) is presented to Thai swine farmer to validate the target market.

Finding

In-depth interview was conducted to investigate the unmet need for piglet brooder usage from 8 swine farm owners in 5 provinces of Thailand, Ratchaburi, Nakhon Pathom, Singburi, Chonburi and Suphanburi.

All samples were collected from Thai swine farmer and have their own self-made piglet brooder. All were informed about plastic piglet brooder, water system heater and electric heater before from magazine, exhibition and internet. Social network service such as Facebook and Line are also the importance source of information they choose to receive. Thus, the existing product were not met the requirement of their usage, some of the sample admit that they were once bought the plastic pen with plastic brooder in the past, and face the issue of using such as cleaning, corrosion, melting from heating source, improper wind circulation, unergonomic design and low return on investment (ROI).

Custom made piglet brooder from plastic sac also utilized by some of the sample. This design help farm owner to save the cost of construction and cleaning, replace with new sac is cleaning method can reduce the cleaning time for worker and ensure that there is no pathogen in brooder. Hemp sac and infrared heating bulb were utilized in this design to provide heat but due to the worker skill, wind and proper alignment of the lamp were important to proper heating.

Custom made brooder from metallic sheet or partition board was majority design of this in-depth interview. To provide proper internal wind circulation, heat insulation to the piglet, infrared bulb also utilized in this design to provide heat and hemp sac also used to block the wind underneath the pen. Farmer with this design concern about welfare of the animal the importance of stress free raising with environmental control, the negative effect of coldness and benefit of thermoneutral zone on colostrum consumption and growth.

All samples have the same issue from infrared heating lamp in term of unevenly distributed irradiate infrared ray, which is common in specification of infrared bulb.

Finding

None of the samples use water heating system due to high installation cost, high maintenance cost and high operating cost, free energy source was considered as low return on investment compared to former explanation.

The need of sample is evenly heating in thermoneutral zone, power by electricity, anticorrosive, durability, easy to clean, no skilled worker usage and high return in investment.
The obtained needs were developed into conceptual solution using innovation process front end concept of Booz, Allen, Hamilton Model [5]. Starting from product Strategies followed by the idea generation stage, idea screening stage and concept development and testing stage.

**Figure 9.** Heat distribution of floor type heating.[7]

### Idea Generation Phase

All possible design and technology were analyzed and written in morphological chart table. Grouping by material, heating technique and accessory.

Metal, wood, plastic and fabric are 4 option of material that is available in the first morphological chart for idea generation. Electric power supply, solar-electric or solar-heat collector, propane gas coal and wood are option in source of heat in the chart.

Medium for heat transfer can be selected from water, air, metal and direct heat transfer from the surface along with controller which can be thermostat, digital thermostat, dimmer and none.

There are 4 potential ideas from morphological chart,

Option 1 – Plastic brooder with metallic frame heated with solar-water floor heater, controlled by digital thermostat in forming in one piece.

Option 2 – Plastic brooder with plastic frame, Heated by electric floor heater with no controller in knock-down design.

Option 3 – Fabric Brooder framed with plastic, cover by fabric with plastic floor, Heat by radiation from led bead, Knock-down design.

Option 4 – Metallic heating floor with electric heater and digital thermostat, design in one piece.

All 4 designs were analyzed by polymer specialist and Industrial design specialist to evaluate the possibilities of production, production technique and cost.

### Idea Screening Phase

PUGH Matrix and Concept Assessment were used to develop conceptual solution.

**Table 1.** Morphological chart in piglet brooder idea generation.

<table>
<thead>
<tr>
<th>Material</th>
<th>Option 1</th>
<th>Option 2</th>
<th>Option 3</th>
<th>Option 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal</td>
<td>Metal</td>
<td>Metal</td>
<td>Metal</td>
<td>Fabric</td>
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<tr>
<td>Wood</td>
<td>Wood</td>
<td>Wood</td>
<td>Wood</td>
<td>Plastic</td>
</tr>
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<td>Plastic</td>
<td>Plastic</td>
<td>Plastic</td>
<td>Fabric</td>
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<tr>
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<td>Fabric</td>
<td>Fabric</td>
<td>Fabric</td>
<td>Plastic</td>
</tr>
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<td>None</td>
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<td>None</td>
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<td>None</td>
<td>Thermostat</td>
<td>None</td>
<td>Digital Controller</td>
</tr>
<tr>
<td>Accessory</td>
<td>None</td>
<td>Thermostat</td>
<td>None</td>
<td>Digital Controller</td>
</tr>
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<td>Lighting</td>
<td>LED</td>
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<td>LED</td>
<td>Incandescent</td>
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<tr>
<td>Type</td>
<td>One Piece</td>
<td>Knock Down</td>
<td>One Piece</td>
<td>One Piece</td>
</tr>
</tbody>
</table>

**Table 2.** PUGH Matrix of Piglet Brooder

The top score alternative solution is “Option 2” with 9 Positive and 1 Negative criteria, the selected option will be named as “DATUM” (Current Solution) for further PUGH Matrix analysis.

Combination of Option 2 and Option 4 was suggested by both specialist to reduce the negative criteria of “DATUM” Option.
Table 3. 2nd Morphological chart from combination idea.

The 2nd morphological chart of idea generation shown 4 new innovative potential idea of design

Option 5 is a combination of Option 2 and Option 4 from first morphological chart which replace plastic floor in to metallic floor for better heat dissipation.

Option 6 is a combination of Option 1 and Option 2 from first morphological chart, which individual water heating system in each brooder for better distribution of heat and convenient of installation and maintenance.

Option 7 is a new innovative idea of heat conductive plastic in the floor piece of the brooder. To aid the distribution of heat from the heater to all area of the heating floor instead the use of expensive metallic material. LED Light was introduced to the brooder for piglet attraction.

Option 8 is a new innovative idea of utilization of rubber, the common raw material in Thailand. Rubber can be used to make a heating floor with electric heater embedded inside. Heat conductive property of rubber is discussed for this utilization.

Table 4. PUGH Matrix of 2nd Morphological chart of piglet brooder

Option 7 is selected among other ideas, knock-down design with plastic, floor heating by electric heater and heat dissipation additive in plastic material seem to be the best idea for this study.

Concept Development

Most viable prototype (MVP) with 3D Computer Aided Design (3D CAD) is created to simulate the look and function of this Option 7 innovative brooder.

Figure 10. 3D Computer Aided Design of Option 7 Piglet Brooder

The 3D CAD was design by Sketchup Pro 2017 Software with the size of 120cm x 60cm 60cm dimension for the size of piglet area.

The function of non-porous surface with antibacterial, evenly distributed heating with self-regulated circuit controller, wind circulation control, embedded LED Light for piglet attraction, were explained to the customer to simulate the use of innovation.

Market Validation

Market validation was determined by customer acceptance model from storytelling, prototyping, interview and survey.

10 pig farms in Ratchaburi, Kanchanaburi, Nakhon Pathom, Suphanburi, Chonburi, Singburi and Petchaburi were presented with 3D CAD Prototype and storytelling to validate the market.

All farm owner willing to buy the innovative piglet brooder on condition of 10 years warranty, less than 100 watts per hour electrical consumption and 2,000-2,500 Thai Baht Price.
Durability property of plastic material was main concern for farm owner. Due to the practice of cleaning in farrowing pen with metal brush, corrosive agent and flame.

Positive feedbacks from farm owner were cleaning process reduction, evenly distributed heat, fast installation, and economy of operation.

The benefit in swine production is major concern for decision making to buy this innovation as a replacement part of the pen, average daily litter weight gain (ADLWG) and pre-weaning mortality (PWM) are key indicator to prove the benefit of technology.

**Discussion**

The business feasibility of this innovation is positive due to the positive feedback and the result of buy decision in market validation.

Further development is important to estimate the real benefit of this innovation in production improvement, most of the farm owner in this validation willing to test on the real prototype of innovation 20-30 set of this innovative piglet brooder is requested to trial in the existing pen on performance, durability and piglet behavior. Weaning weight and time spending inside the brooder will be record and measure to evaluate the acceptance of animal.

Surface swab after routinely cleaning process must be done to measure the antibacterial feature of the plastic, polishing with plastic brush, spray with disinfectant and others corrosive agent must be done also.

**References**

Prevalence of *Streptococcus suis* isolated from pigs in Northern Thailand

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*Corresponding author: rchuanchuen@yahoo.com

**Keywords:** *Streptococcus suis*, pig, slaughterhouse, northern Thailand

**Introduction**

*Streptococcus suis* is an important pathogen in pigs causing meningitis, septicaemia, endocarditis, bronchopneumonia, polyarthritis and polyserositis leading to high mortality (1). This pathogenic organism can cause serious economic losses in pig industry worldwide (2). *S. suis* is also emerging zoonotic pathogens causing septicemia and meningitis in humans(3). *S. suis* can be transmitted from pigs to humans by consumption of raw pork products and exposure to sick or carrier pigs(2, 4). The naturally inhabits of *S. suis* are the nasal cavities and tonsils (5). To date, there are 33 classified serotypes of *S. suis* based on capsular polysaccharides. Serotype 2 is the most prevalent and virulent serotype associated with disease in pigs and humans(3). In Thailand, there have been reported that *S. suis* remains major public health problem, especially in Northern region (6). Most infections in humans in this region is due to consumption traditional homemade raw pig’s blood soup. In 2010, the Ministry of Public Health reported that there was 171 human cases from an outbreak of *S. suis* in the Northern part of Thailand (7). However, the situation of *S. suis* from pigs in this region is poorly known and needed to be addressed. The actual rate of infection is might be higher than that reported by the Bureau of Epidemiology. Therefore, the aim of this study was to determine the prevalence of *S. suis* from pigs in Northern Thailand.

**Materials and Methods**

A total of 768 nasal swab samples were collected from slaughterhouse pigs in Chiang Mai (n = 130), Chiang Rai (n = 180), Mae Hong Son (n = 66), Nan (n = 190) and Phayao (n = 202) provinces in northern part of Thailand between April and October 2017. The nasal swabs were transported in sterile tubes containing Stuart transport medium and processed within 24-48 h after collection. Samples were directly cultured on 5% sheep blood agar(Oxoid, Hampshire, U.K.) containing polymyxin B (15 U/mL) and nalidixic acid (15 µg/mL) (Sigma-Aldrich, St. Louis, MO, USA)(4), and incubated at 37 °C with 5% carbon dioxide for 24 h. Four colonies, showing α-hemolytic and typical *S. suis* growth habit were selected and identified by using Gram-stain, catalase test, optochin susceptibility testing (Oxoid, Hampshire, England). All α-hemolytic isolates that were gram-positive, catalase negative and resistant to optochin were further identified using conventional biochemical tests as previously described(2, 8). The isolates were tested for growth in 6.5% NaCl, hydrolysis of esculin, amylase test (starch hydrolysis), acid production from arabinose, inulin, lactose, mannitol, salicin, sorbitol, sucrose, raffinose and trehalose (Sigma Aldrich, USA).

**Results and Discussion**

In this study, 82 samples (10.7%) collected were positive for *S. suis* using conventional biochemical tests. All isolates were gram positive, catalase negative, resistant to optochin, and no growth in 6.5% NaCl. Most of *S. suis* isolates were positive for lactose, salicin, sucrose and trehalose reaction, while negative for arabinose, mannitol, and sorbitol reaction. The occurrence of *S. suis* varied between samples from different provinces (Fig. 1).

![Figure 1](chart.png)

**Provinces**

Overall, the total prevalence of *S. suis* was found 125 (16.3%) isolates. The prevalence of *S. suis* in different provinces range from 10.0% to 32.7%. The *S. suis* isolates was most frequently found in Chiang Rai province 49 (32.7%) isolates, while 19 (10%) isolates were found in Nan as shown in Table 1. These findings are in agreement with several studies (4, 9, 10) had reported a high prevalence of *S. suis* strains from clinically healthy pigs, indicating that pigs are important reservoir of *S. suis*.}

---

199
Table 1: Prevalence of *S. suis* isolates from pigs in different provinces in Northern Thailand.

<table>
<thead>
<tr>
<th>Provinces</th>
<th>No. of samples</th>
<th>No. of positive isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiang Mai</td>
<td>130</td>
<td>16 (12.3%)</td>
</tr>
<tr>
<td>Chiang Rai</td>
<td>180</td>
<td>49 (32.7%)</td>
</tr>
<tr>
<td>Mae Hong Son</td>
<td>66</td>
<td>12 (18.2%)</td>
</tr>
<tr>
<td>Nan</td>
<td>190</td>
<td>19 (10.0%)</td>
</tr>
<tr>
<td>Phayao</td>
<td>202</td>
<td>29 (14.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>768</td>
<td>125 (16.3%)</td>
</tr>
</tbody>
</table>

In conclusion, the prevalence of *S. suis* isolated from pigs were found in different provinces in Northern Thailand. Slaughterhouse pigs are important carriers of *S. suis*. The result provides the situation of *S. suis* from pigs in this region that will be useful for monitoring and controlling the disease from this bacteria. However, confirmation and detection of serotypes of *S. suis* is required, therefore we will conduct further studies.

Acknowledgements

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References

Protective Efficacy of Modified-live Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Vaccines Against PRRSV challenge in experimental pigs

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Keywords: Protective efficacy, porcine reproductive and respiratory syndrome virus, modified-live vaccines, challenge, pig

Introduction

Two genotypes of porcine reproductive and respiratory syndrome virus (PRRSV) including genotype 1 and 2 are recognized and several countries, especially in Asia continent, reported the co-infection of both genotypes [1]. Recently, commercial of modified-live PRRSV vaccines are available in Thailand and the use of vaccines of corresponding genotype is suggested. However, in the presence of both genotypes, and questions remain as to what genotypes of PRRSV MLV should be used in herds concurrently infected with both genotypes. The objectives of the study were to investigate the protective efficacy of genotypes 1 and 2 MLVs against dual challenge with genotype 1 and 2 PRRSV isolates in experimental pigs.

Materials and Methods

Sixty, PRRSV-free pigs were allocated into 6 groups (n=10), including the NonVac, Porcilis, Amervac, Fostera, Ingelvac MLV and PrimePac groups, respectively. The Neg group was not vaccinated. The Por and Am groups were intramuscularly vaccinated with genotype 1 MLVs, including Porcilis® PRRS (MSD Animal Health, The Netherlands) and Amervac® PRRS (Hipra, Spain), respectively. The Fos, Ing and Prigroups were intramuscularly vaccinated with genotype 2 MLVs, including Fostera™ PRRS (Zoetis, USA), Ingelvac® PRRS MLV (BoehringerIngelheim, USA) and PrimePac™ PRRS+ (MSD Animal Health, The Netherlands), respectively. At 35 days post-vaccination (DPV), all pigs were inoculated intranasally with tissue culture supernatant containing Thai field genotype 1 (SB_EU02, 10⁴ TCID₅₀/mL) and genotype 2 (ST_US021, HP-PRRSV, 10⁵ TCID₅₀/mL) PRRSV isolates. Serum samples were collected at 3, 5 and 7 days post-challenge (DPC) and quantitatively assayed for either genotype 1 or 2 PRRSV RNA using RT-qPCR as previously described [2]. At 7 DPC, all pigs were euthanized and necropsied. Macroscopic and microscopic lung lesions were evaluated as previously described [3]. Lung tissues were collected and PRRSV antigens were analyzed using immunohistochemistry as previously described[4]. All of the method were reviewed and approved by the Chulalongkorn University Animal Care and Use Committee (protocol number 1731047)

Results and Discussion

Following challenge, PRRSV RNA in serum were summarized in Table 1. The results of this study showed that PRRSV RNA in serum of all vaccinated groups were lower than in the NonVac group (p<0.05). The Amervac group had the highest genotype 1 PRRSV RNA. Meanwhile, the Fostera and Ingelvac MLV groups had the highest genotype 2 PRRSV RNA compared with the other vaccinated groups. In contrast, the PrimePac group had the lowest of both genotype 1 and 2 PRRSV RNA compared with the other vaccinated groups.

Table 1: Mean PRRSV genomic copies in serum following challenge

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype 1 (1,000 copies)</th>
<th>Genotype 2 (1,000 copies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 DPC</td>
<td>5 DPC</td>
</tr>
<tr>
<td>NonVac</td>
<td>2.3±0.2a</td>
<td>2.7±0.3a</td>
</tr>
<tr>
<td>Porcilis</td>
<td>1.4±0.3b</td>
<td>0.4±0.1c</td>
</tr>
<tr>
<td>Amervac</td>
<td>1.3±0.2b</td>
<td>0.8±0.2b</td>
</tr>
<tr>
<td>Ingelvac MLV</td>
<td>0.2±0.6b</td>
<td>0.9±0.1b</td>
</tr>
<tr>
<td>PrimePac</td>
<td>0.6±0.1c</td>
<td>1.1±0.2b</td>
</tr>
<tr>
<td>Groups</td>
<td>Genotype 2 (1,000 copies)</td>
<td>Genotype 2 (1,000 copies)</td>
</tr>
<tr>
<td></td>
<td>3 DPC</td>
<td>5 DPC</td>
</tr>
<tr>
<td>NonVac</td>
<td>2.3±0.2c</td>
<td>2.7±0.2e</td>
</tr>
<tr>
<td>Porcilis</td>
<td>1.4±0.2b</td>
<td>1.5±0.3c</td>
</tr>
<tr>
<td>Amervac</td>
<td>1.7±0.2c</td>
<td>0.8±0.3c</td>
</tr>
<tr>
<td>Ingelvac MLV</td>
<td>1.5±0.2c</td>
<td>1.3±0.3c</td>
</tr>
<tr>
<td>PrimePac</td>
<td>1.2±0.2c</td>
<td>1.8±0.3c</td>
</tr>
</tbody>
</table>

*DPC; days post-challenge. Values are reported as mean ± SEM. Different lowercase letters (a, b, c, d) within the same column represent significant differences (p<0.05).

Additionally, lung lesion scores and PRRSV antigen in lung tissues were summarized in Table 2. All vaccinated groups had lower lung lesion scores and...
PRRSV antigens compared with the NonVac group \((p<0.05)\). However, the Porcilis and Fostera groups had higher microscopic- and macroscopic lung lesion scores compared with the other vaccinated groups, respectively. Pigs in the Porcilis group had the highest genotype 1 PRRSV antigens in lung tissues. Meanwhile, pigs in the Fostera and PrimePac groups had the lowest genotype 2 PRRSV antigens in lung tissues compared with in the other vaccinated groups.

Table 2 Macroscopic and microscopic lung lesion scores and PRRSV antigens in lung tissues at 7 days post-challenge (DPC).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lung lesion scores</th>
<th>PRRSV antigens in lung tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macroscopic</td>
<td>Microscopic</td>
</tr>
<tr>
<td>NonVac</td>
<td>72.7±8.8(^a)</td>
<td>1.40±0.08(^a)</td>
</tr>
<tr>
<td>Porcilis</td>
<td>54.7±1.7(^b)</td>
<td>1.24±0.06(^a)</td>
</tr>
<tr>
<td>Amervac</td>
<td>45.0±5.7(^b)</td>
<td>0.92±0.08(^b)</td>
</tr>
<tr>
<td>Fostera</td>
<td>59.0±4.4(^a)</td>
<td>0.82±0.08(^b)</td>
</tr>
<tr>
<td>Ingelvac MLV</td>
<td>55.3±5.5(^b)</td>
<td>0.83±0.08(^b)</td>
</tr>
<tr>
<td>PrimePac</td>
<td>42.7±4.6(^b)</td>
<td>0.87±0.08(^b)</td>
</tr>
</tbody>
</table>

* Values are reported as mean ± SEM. Different lowercase letters \((a, b, c)\) within the same column represent significant differences \((p<0.05)\).

Our results were corresponded with the previous investigation which the genotype 2 PRRSV vaccines vaccination can reduce both genotype 1 and genotype 2 PRRSV viremia. In contrast, the reduction of both genotypes viremia in genotype 1 PRRSV vaccines vaccinated groups had been observed in the present study \([5]\). Additionally, vaccination with either genotype 1 or genotype 2 PRRSV vaccines exhibit reduced lung lesion and PRRS-positive cells. However, the difference in reduction of lung lesions may be due to the difference efficacy between genotype 1 and genotype 2 PRRSV vaccines against dual challenge and difference in pathogenicity of the challenged PRRSV isolates. In conclusion, modified-live PRRSV vaccines can reduce viremia and lung lesions after PRRSV challenge regardless of vaccine genotypes.

Acknowledgement

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References

Reproductive performance of sows after single fixed-time artificial insemination in a commercial swine herd in Thailand

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Keywords: Buserelin; Farrowing rate; Litter size, Ovulation; Sows

Introduction
In pigs, it is well documented that ovulation usually occurs at about 70% of the way through estrus [1]. However, the duration of estrus in sows is highly variable, producing the difficulty to perform artificial insemination (AI) in sows at the optimal time [2]. In practice, sows are usually inseminated for 2 to 4 times at 12 to 24 h interval during estrus, in an attempt to deposit sperm at the optimal time prior to ovulation. Due to these reasons, the ovulation time should be controlled. Gonadotropin releasing hormone (GnRH) has been used in sow after weaning to induce the pre-ovulatory LH surge and subsequently induce ovulation [3]. To achieve an advance AI management, single-dose insemination or “single fixed-time AI” in combination with ovulation induction should be investigated. The fixed-time AI in pig has been investigated in other countries during the past 5 years [4-6]. The reproductive performances of sows after single fixed-time AI is rather promising [4-6]. However, factors such as breed, parity and season may also affect reproductive performance of sows under field conditions [7]. Up to date, no study on practical application of fixed-time AI and ovulation induction in swine has been conducted in commercial herd in Thailand. The present study was performed to determine reproductive performance of sows after single fixed-time AI under field conditions.

Materials and Methods
The experiment followed the guidelines documented in The Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand, and was approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with the university regulations and policies governing the care and use of experimental animals. (approval no. 1731011). The experiment was conducted in a commercial swine herd in the southern part of Thailand from October 2015 to January 2016.

In total, 269 Landrace x Yorkshire crossbred weaned sows were included in the experiment. After weaning, the sows were allocated into 2 groups: control and fixed-time groups. The ovulation of fixed-time group was induced by administration of buserelin intramuscularly (10 µg, Receptal®, MSD, USA). The sows were inseminated at 32 h after the hormonal administration. In the control group, the sows were inseminated twice within 24 h interval after standing estrus. Reproductive performance data of each group were analyzed.

The sows were weaned at 22.4 ± 3.0 days after farrowing. Parity number of sows averaged 2.9 ± 1.6 (1 – 5). The sows were kept in 0.5 x 2.0 m2 stall in a concrete-slatted floor after weaning. The temperature and humidity in the barn were controlled by evaporative cooling system. Feed was provided twice a day (1.5–3.5 kg/ day) during gestation and were fed 2-4 times a day during lactation (5–6 kg/ day) with a corn-soybean-chicken ration. The gestation feed contained 16.0% CP, 2,800 kcal/kg ME and 1.0% lysine. The lactation feed contained 18.0% CP, 3,200 kcal/kg ME, and 1.1% lysine. Water was provided ad libitum by water nipples. The health management was carried out by veterinarians.

Estrus detection was conducted twice a day (0800 and 1600) using a fence-line boar contacted and back-pressure test. The sows that exhibited a standing reflex were considered to be in estrus. Sows in the treatment group were inseminated at 32 h after buserelin administration without estrus detection, while sows in the control group were inseminated after estrus symptom was observed. The sows in control and fixed-time AI groups were inseminated using either conventional AI (n = 88) or intra-uterine insemination (IUI, n = 148). The number of sperm per doses used for AI and IUI were 3 x 10⁹ sperm (100 ml) and 1.5 x 10⁹ sperm (50 ml), respectively.

Table 1 Reproductive performance of sows after single fixed-time AI compared to control group (mean ± SD)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Single fixed-time AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sows inseminated</td>
<td>137</td>
<td>132</td>
</tr>
<tr>
<td>Sows farrowed</td>
<td>120</td>
<td>116</td>
</tr>
<tr>
<td>Farrowing rate (%)</td>
<td>87.6</td>
<td>87.9</td>
</tr>
</tbody>
</table>
The data were analyzed by using Statistical Analysis System (SAS version 9.0, Cary, NC, USA.). The data included total number of piglets born per litter (TB), number of piglets born alive per litter (BA), number of stillborn piglets/litter, number of mummified fetuses per litter and body weight of the piglets at birth. All the data were analyzed by using multiple ANOVA under PROC GLM of SAS. The statistical models included the effect of group (control and treatment), catheter type (AI and IUI), parity number (1, 2, 3, 4 and 5) and interaction between group and catheter type. Least square means were obtained from each class of the factors and were compared by using least-significant different test. Farrowing rate were compared between control and treatment groups by using Chi-square test. The values with \( P<0.05 \) were regarded to be statistically significant.

### Results and Discussion

Reproductive performance of sows in control and single fixed-time AI group did not differ significantly (Table 1). Total number of piglet born per litter in sows inseminated using a single fixed-time AI compared with control group by insemination catheter type are presented in Figure 1. As can be seen, TB did not differ significantly between control and fixed-time AI in either conventional AI and IUI.

Additionally, another technology that have been developed previously to reduce the number of sperm per insemination is the “intra-uterine insemination or IUI” [8]. The combination of these technologies, i.e., IUI, single fixed-time AI and ovulation induction offers opportunity to enhance the use of semen from superior boars to aid in global distribution of economically important traits [5]. The present study has proved that the implementation of IUI, single fixed-time AI and induction of ovulation in pig altogether is possible under field condition with a promising fertility outcome.

In conclusion, a single fixed-time AI in sows resulted in a promising reproductive performance (i.e., 87.9% farrowing rate and 12.2 TB) under field conditions. Inseminations with either conventional AI catheter or IUI catheter did not influence the reproductive performances of sows after a single fixed-time AI. Therefore, the single fixed-time AI is a possible AI innovation method that could be implemented in a higher scale of swine industry to reduce the cost of pig production.

### Acknowledgements

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### References

Reproductive performance of Berkshire sows in Thailand

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Keywords: Berkshire, Litter size, Pig, Reproduction, Season

Introduction

During recent years, Thai consumers have shown a higher preference in meat quality of pig. Berkshire pig has become one of the most popular alternative product for high quality pork. To our knowledge, only few studies on the reproductive performance of the Berkshire have been reported [1,2], and no such study have been made in Thailand. The present study was performed to retrospectively analyze reproductive performance of Berkshire sows in Thailand with respect to parity influence and seasonal variation.

Materials and Methods

The herd in this study was located in the eastern region of Thailand at latitude 13.1 °N and longitude 101.3 °E. Day length varies from 11 h in June to 13 h in December. Data were collected from data bank of the herd. The data included farrowing records during a 3-year periods from January 2014 to December 2016. The farrowing records included 1,251 litters from Berkshire sows. The records consisted of sow identities, parity number, farrowing date, number of total piglets born per litter (TB), number of piglets born alive per litter (BA), number of stillborn piglets per litter (SB), number of mummified fetuses per litter (MM) and litter birth weight.

The sows were housed in an evaporative cooling system both during gestation and lactation. Gestating sows were moved to the farrowing pens at one week before the expected farrowing date. The replacement gilts were mated at 8 months of age at the second or later observed estrus. Artificial insemination was used. Feed was provided twice a day (1.5–3.5 kg per day) during gestation and were fed 2-4 times a day during lactation (5–6 kg per day) with a corn-soybean-chicken ration. The feed was formulated according to the nutritional requirements of gestating and lactating sows [3]. The gestating feed contained 16.0% CP, 2,800 kcal/kg ME, and 1.0% lysine. The lactation feed contained 18.0% CP, 3,200 kcal/kg ME, and 1.1% lysine. Water was provided ad libitum by water nipples. The health management was carried out by veterinarians. Both gilts and sows were vaccinated against CSF, AD, PPV and FMD.

The statistical analyses were carried by using SAS. Descriptive statistics were conducted. Frequency analysis was carried out to determine frequency distribution of TB, BA, SB and MM. Multiple analysis of variance was conducted to determine the effect of parity number (1, 2, 3, 4, 5, 6 and ≥7) and farrowing month on litter traits. Least square means were obtained from each class of the factors and were compared by using least significant difference (LSD) test. Values with \( P < 0.05 \) were regarded as statistically significant.

Results and Discussion

Reproductive performance of Berkshire sows in a commercial swine herd in Thailand are presented in Table 1. Frequency distribution of TB and BA in Berkshire sows are presented in Figure 1. As can be seen, only 23.3% and 22.2% of Berkshire sows had TB ≥11.0 and BA ≥10.0 piglets per litter. Thus, most of Berkshire sows (almost 80%) had poor litter size at farrowing. On average, TB and BA of Berkshire sows in Thailand were 8.9 and 7.6 piglets/ litter, respectively.
In Japan, TB of purebred Berkshire sows varied from 7.7 piglets/ litter in primiparous sows to 9.3 piglet/ litter in sow parity numbers ≥6 [2]. In a similar sire-line breed, purebred Hampshire sows produced 9.8 TB and 9.0 BA [4].

Table 1 Reproductive performance of Berkshire sows in a commercial swine herd in Thailand (n = 1,251 litters)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity number</td>
<td>3.4 ± 1.6</td>
<td>1 – 11</td>
</tr>
<tr>
<td>Total number of piglet</td>
<td>8.9 ± 2.5</td>
<td>1 – 16</td>
</tr>
<tr>
<td>born/ litter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of piglet born</td>
<td>7.6 ± 2.4</td>
<td>0 – 15</td>
</tr>
<tr>
<td>alive/ litter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillborn (%)</td>
<td>4.3</td>
<td>-</td>
</tr>
<tr>
<td>Mummified fetuses (%)</td>
<td>5.5</td>
<td>-</td>
</tr>
<tr>
<td>Birth weight of piglets (kg)</td>
<td>1.62 ± 0.27</td>
<td>0.5 – 2.60</td>
</tr>
</tbody>
</table>

Table 1 shows the reproductive performance of Berkshire sows in a commercial swine herd in Thailand (n = 1,251 litters). The parity number was 3.4 ± 1.6, with a range of 1 – 11. The total number of piglets born/litter was 8.9 ± 2.5, ranging from 1 to 16. The number of piglets born alive/litter was 7.6 ± 2.4, ranging from 0 to 15. Stillborn percentage was 4.3%, while mummified fetuses percentage was 5.5%. The birth weight of piglets was 1.62 ± 0.27 kg, ranging from 0.5 to 2.60 kg.

Farrowing month did not influence both TB and BA (P>0.05). Litter size at farrowing was relatively stable among the farrowing month (Figure 3). Park and Ho [1] suggested that Berkshire breed might be the type of pig breed that is best fitting to the outdoor system. In Thailand, the seasonal influence on litter size at farrowing is common in LY sows [5]. Therefore, the present study revealed that Berkshire sows are relatively tolerant to seasonal influence.

Parity number of sows significantly influenced TB (P<0.001), BA (P=0.009) and SB (P=0.005) but not MM (P=0.407). Both TB and BA were highest in parity 1 and lowest in parity 7-11. Interestingly, TB was significantly decreased from parity 1 to 2 (9.4 vs 8.4, P<0.001). This indicated a severe second parity drop on litter size (i.e., 1.0 piglet/litter) in Berkshire sows. Thus, intensive care of primiparous sows during postpartum period and during lactation should be emphasized.

In conclusions, Berkshire sows in Thailand produced 8.9 TB and 7.6 BA. The percentage of MM (5.5%) was relatively high causing poor BA. Season did not influence litter size at farrowing in Berkshire sows. However, a second parity drop on litter size was clearly observed, indicating that care of postpartum primiparous sow should be emphasized.

Acknowledgements

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References

Reprogramming porcine somatic cells into transgene-free induced pluripotent stem cells using episomal plasmid vectors

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Keywords: porcine, induced pluripotent stem cells, episomal vectors, transcription factors

Introduction
The induced pluripotent stem cells (iPSCs) are pluripotent stem cells generated from reprogramming of adult somatic cells (1). The techniques for production of iPSCs can be divided in two gene delivery approaches including integration and non-integration methods. The integration method will permanently integrate transcription factors into host genome, for example, by using retrovirus or lentivirus. By these methods, reasonable efficiency is usually obtained. However, it is difficult to silencing the ectopic genes and the risk of insertional mutation may be found (2). The non-integration method is a better method to produce iPSCs for therapeutic application, but its efficiency of reprogramming is currently poor (0.001%) (3). These non-integration methods include adenovirus, sendai virus and episomal plasmids (4). Pig has been considered as a beneficially research model for human medicine because anatomical and physiological are similar to humans (5). In this experiment, Sertoli cells were used as a novel somatic cells origin. In addition, the Sertoli cells were reprogramming into iPSCs via non-integration method by using episomal plasmid vectors. We compared reprogramming efficiency between using plasmid DNA of 3 (OCT4, SOX2 and KLF4; 3F) and 5 (OCT4, SOX2, KLF4, L-MYC and LIN28; 5F) transcription factors.

Materials and Methods
Neonatal testes were collected from 1-week old piglets. The testes were digested by two-step enzymatic method in order to isolate the Sertoli cells. Anti-Müllerian hormone (AMH) expression was detected in Sertoli cells by immunofluorescence staining. Sertoli cells were transfected with pmaxGFP for optimization protocol (EH138, CM137, EN150 and CB150) of Nucleofector 4DX Device (Lonza, Germany). After electroporation for 24 hours, we observed green fluorescence expression under fluorescent microscope. The EH138 protocol was demonstrated 70% positive pmaxGFP expression (C and D). The primary colonies of 3F and 5F were appeared 5 days after nucleofection (E and F). Seven days after transfection of 3F, we observed 0.038% of primary colonies formation but no colony showed positive to alkaline phosphatase staining. However, 5F could generate 0.229% of primary colonies formation and 0.149% of the primary colonies were positive to alkaline phosphatase staining (G). Seven primary colonies from 5F were picked up on day 11-21 after nucleofection (H).

Results and Discussion
Primary Sertoli cells demonstrated polygonal-shaped morphology (A). For immunofluorescence staining, AMH protein was stained in cytoplasm of porcine Sertoli cells (B). According to optimization the 4DX Lonza protocol, EH138 protocol was demonstrated 70% positive pmaxGFP expression (C and D). The primary colonies of 3F and 5F were appeared 5 days after nucleofection (E and F). Seven days after transfection of 3F, we observed 0.038% of primary colonies formation but no colony showed positive to alkaline phosphatase staining. However, 5F could generate 0.229% of primary colonies formation and 0.149% of the primary colonies were positive to alkaline phosphatase staining (G). Seven primary colonies from 5F were picked up on day 11-21 after nucleofection (H).
cells after electroplated with pmaxGFP showed 70% positive green fluorescence expression. Scale bar 50 μm (C; bright field and D; GFP expression).

Figure 2 Primary iPSCs cloned of 3F (E) and 5F (F) reprogramming on day 5 after transfection. Scale bar 30 μm. Colonies of 5F condition were expressed alkaline phosphatase staining. Scale bar 30 μm (G). Morphology of iPSCs-like cells derived from 5F condition at passage 1. The colonies exhibited a high nuclear per cytoplasm ratio with prominent nucleoli Scale bar 50 μm (H).

The 5F condition was better than 3F in aspects of reprogramming efficiency and alkaline phosphatase screening. From the results, 3F nucleofection could not contribute to pluripotent iPSCs-like colony. Three transcription factors (OCT4, SOX2 and KLF4) were not sufficient to induce somatic cell reprogramming.

Unfortunately, most of primary iPSCs-liked colonies via using episomal plasmid vectors could not maintain undifferentiated state. The main obstacles of episomal plasmids method are only transiently express transgenes and extremely low efficiency (6). However, episomal reprogramming method has not been able to generate transgene-free pig iPSCs. According to persistent expression of reprogramming, transcription factors is important to maintain undifferentiation state of pig iPSCs (7). Further study by increase plasmid DNA transcription factors should be considered for improve reprogramming efficiency and long term iPSCs maintenance.

Acknowledgements
This study was financial supported from the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0143/2556), Biology of Embryo and Stem cell Research in Veterinary Science Group (59-007-31-005).

References
Quantitative Microbial Risk Assessment of *Klebsiella pneumoniae* to Ciprofloxacin from Pork in Bangkok

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**Keywords:** Bangkok, ciprofloxacin, *Klebsiella pneumoniae*, market, risk assessment

**Introduction**

*Klebsiella* spp. is a Gram-negative, rod-shape, non-motile bacterium which belongs to Family *Enterobacteriaceae* (1). *Klebsiella* spp. was isolated from human gastrointestinal tract (2). *K. pneumoniae* has been recognized as a foodborne pathogen (3, 4, 5) besides various infections of respiratory tract, gastrointestinal tract, urinary tract and septicemia (5, 6, 7, 8, 9, 10). *K. pneumoniae* has been isolated from the animal intestinal tract, foods and environment (2, 5, 11, 12, 13, 14). Then, *K. pneumoniae* was generally transmitted among human, animal and environment (15). Therefore, foods e.g. retail meats and vegetables could serve as a vehicle of such transmission (15). The foodborne disease caused by *K. pneumoniae* could be aggravated by antimicrobial resistant *K. pneumoniae* (2, 6, 15). The adverse health consequences of antimicrobial resistant *K. pneumoniae* could range from an antimicrobial treatment failure, a longer hospitalization to a higher mortality rate (16). *K. pneumoniae* has been isolated from pork (6) as well as its environment (17). Even though, the prevalence of the *Klebsiella* spp. in pork was low (2), the pork consumption of Bangkok population was as high as 20.70 gram/person/day. Consumption of contaminated pork with antimicrobial resistant *K. pneumoniae* could pose a serious illness. Information regarding likelihood and magnitude of adverse health effect of antimicrobial resistant *K. pneumoniae* from pork consumption is still limited. Moreover, ciprofloxacin is the antibiotic of choice for *K. pneumoniae* infection in the hospital yet rate of successful treatment was not always predictable (18). Therefore, it is crucial to determine the contamination level and risk estimate of both *K. pneumoniae* and ciprofloxacin-resistant *K. pneumoniae* derived from retail pork sold in fresh markets.

**Materials and Methods**

Pork were collected from retail fresh markets in Bangkok including central Bangkok markets (*n* = 60), eastern Bangkok markets (*n* = 66), northern Bangkok markets (*n* = 64), southern Bangkok markets (*n* = 68), upper Thonburi markets (*n* = 60) and lower Thonburi markets (*n* = 60). Twenty-five grams of pork samples were 10-fold serially diluted by buffer peptone water and 100 microliters were spread onto MacConkey agar. After incubated for 24 hours at 37 °C, the plates containing typical colonies were counted and further confirmed by biochemical tests (19). Then confirmed *K. pneumoniae* isolates were kept in 10% glycerol at -30°C (19, 20). All isolates were subjected to antimicrobial susceptibility test to determine minimum inhibitory concentration (MIC) using agar dilution method according to Clinical and Laboratory Standard Institute (21). The antimicrobial agent was ciprofloxacin (18). *Escherichia coli* ATCC 25922 was used as quality control strain (21). All data were analyzed by descriptive statistics, risk assessment and Monte Carlo simulation.
Quantitative risk assessment has 4 steps (22, 23)

1) Hazard Identification is the step to identify *K. pneumoniae* as foodborne pathogen causing adverse health effect.

2) Hazard characterization is the step to evaluate the relationship between adverse health effect of foodborne pathogen as a result of pathogen intake usually through the dose-response model. The output of this step is the probability of foodborne illness depending on the dose (number of cells of pathogen).

3) Exposure assessment is the step to evaluate the likelihood of consumer to expose against foodborne pathogen. The output of this step is the probability of exposure. The model for exposure assessment essentially requires the prevalence, concentration of pathogen in food including food consumption.

4) Risk characterization is the integration step of hazard characterization and exposure assessment. The output is so-called risk estimate. This risk estimate could be reported in the form of foodborne illness cased per population at risk.

**Results and Discussion**

The prevalences of *K. pneumoniae* and ciprofloxacin-resistant *K. pneumoniae* in pork collected from 6 Bangkok areas were shown in Table1. The prevalences of *K. pneumoniae* in pork ranged approximately from 83 to 96% with average prevalence of 89.21% while those of ciprofloxacin-resistant *K. pneumoniae* in pork ranged approximately from 2 to 1% with average prevalence of 8.43% (Table 1). However a previous study found that the prevalences of *K. pneumoniae* and *K. pneumoniae* resistant to ciprofloxacin were as low as 60% and 0%, respectively (2). There was no study regarding risk assessment of *K. pneumoniae* so this study employed dose-response model of *E. coli* as a surrogate model. The model predicted *K. pneumoniae* illness and mortality of *K. pneumoniae* resistant to ciprofloxacin of 12.4 and 0.134 cases per year per million of Bangkok population, respectively. The estimated illness and death from both *K. pneumoniae* and *K. pneumoniae* resistant to ciprofloxacin was much lower than those of *E. coli* as Hemolytic Uremic Syndrome of 65.24 and 3.35 cases per year per million people. The accurate risk estimate of *K. pneumoniae* and *K. pneumoniae* resistant to ciprofloxacin will be performed when the models of *K. pneumoniae* will be developed in the further study.

**Table1.** Prevalences of *K. pneumoniae* and *K. pneumoniae* resistant to ciprofloxacin isolated from pork in 6 Bangkok areas

<table>
<thead>
<tr>
<th>Bangkok metropolitan</th>
<th><em>K. pneumoniae</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Bangkok</td>
<td>58/60(96.67)</td>
</tr>
<tr>
<td>Eastern Bangkok</td>
<td>55/66(83.33)</td>
</tr>
<tr>
<td>Northern Bangkok</td>
<td>62/64(96.87)</td>
</tr>
<tr>
<td>Southern Bangkok</td>
<td>59/68(86.76)</td>
</tr>
<tr>
<td>Lower Thonburi</td>
<td>53/60(88.33)</td>
</tr>
<tr>
<td>Upper Thonburi</td>
<td>53/60(88.33)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>340/378(89.95)</strong></td>
</tr>
</tbody>
</table>

**References**

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Quantitative risk assessment of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* from pork in Bangkok

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**Keywords:** *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, Pork, QMRA, Bangkok

**Introduction**

*Staphylococcus aureus* (*S. aureus*) is Gram-positive, facultative anaerobic, cocal bacterium that can be found on respiratory tract and skin of human as a normal flora and occasionally as an opportunistic pathogen. This bacterium causes foodborne disease. *S. aureus* is considered as world top three foodborne illnesses. (1) Upon nosocomial infections, some strains of *S. aureus* carrying antimicrobial resistant genes are called methicillin-resistant *S. aureus* (MRSA). *S. aureus* can proliferate in various types of food including milk, cheese, raw meat, cooked meat and pork. (2) It can also contaminate environment during food preparation procedure. Moreover, *S. aureus* is able to produce a variety of heat-resistance staphylococcal enterotoxins (SE) e.g. SEA, SEB, SEC, SED and SEE. The enterotoxins remain in food after cooking process and attributable to typical foodborne intoxication symptoms such as abdominal pain, diarrhea and mainly vomiting. Although the foodborne illness symptoms are self-limited within 24 hours (10), their impacts range from economy, international trade to the reliability of exporting country. Presence of *S. aureus* in foods indicated the improper handling and storage of foods. The objectives of this study were to evaluate the risk estimates upon consuming pork contaminated with *S. aureus* and MRSA.

**Materials and Methods**

Pork samples were obtained from 17 local markets across 6 Bangkok areas. All samples was stored on ice during the transportation to the laboratory. The pork samples were chopped and weighed exactly 25 grams/sample. Then samples were mixed with buffered peptone water (BPW) 225 ml. Ten-fold serial dilution was performed three times with BPW. Then pipetting 0.1 ml of the suspension was spread onto Baird-Parker agar supplemented with 10% egg yolk and 1% potassium tellurite. The plates were incubated in 37°C for 48 hours. For each sample, selecting up to 5 colonies with metallic sheen was transferred to Manitol salt agar and incubated at 37°C for 24 hours. Then, selecting the positive isolation culture on blood agar was to confirm for beta hemolysis. The characteristic colonies were tested for catalase activity with 3% hydrogen peroxide and tested for coagulase activity with rabbit serum. All isolates were tested for antimicrobial susceptibility test using agar dilution method. The antimicrobials used in the test were oxacillin and ceftoxitin. Additional, mecA gene was also detected by PCR method for MRSA confirmation.

The prevalence and concentration of *S. aureus* and MRSA were used in quantitative microbial risk assessment (QMRA) that consists of 4 steps.

1) Hazard identification: the information about biological hazard in food were addressed

2) Exposure assessment: Probability of exposure (P_E) was calculated from prevalence (P), concentration (C) and amount of pork consumption (M).

\[
P_E = P \times (1 - e^{-PD})
\]

\[
D = C \times M
\]

3) Hazard characterization: Probability of illness (P_I) is calculated by the dose-response model of *S. aureus* along with Probability of AMR consequence (P_M) for MRSA model with hospitalization rate (h), prescription rate (p) and mortality rate (m).

\[
P_I = 1 - e^{(-7.64 \times 10^{-8} \times D)}
\]

\[
P_M = hpm
\]

4) Risk characterization: Risk estimation (P_S) was the integration step of P_I and P_E

For *S. aureus* risk characterization

\[
P_S = P_E \times P_I
\]

For MRSA risk characterization

\[
P_S = P_E \times P_I \times P_M
\]

All Monte-Carlo simulation used Simulación 4.0 software (José Ricardo Varela®) for 20,000 iterations

**Result and discussion**

Prevalence and concentration: Among total 378 pork samples, the prevalence of *S. aureus* was 46.29% while concentration of *S. aureus* were varied from 1x10^3 to 2.9x10^6 cfu/g. The mean concentration was 5.596x10^4 ±
2,707×10^3 cfu/g. Previous studies in Italy and US. indicated that prevalence of \textit{S. aureus} in pork was 15.15%and 64.8% respectively. (3,4) Only 1.05% of total samples were found positive for MRSA with the mean concentration of 6.1×10^4 ± 9.3×10^3 cfu/g. Similar to the result from China and US., prevalence of MRSA in various raw meats were 1.7% and 1.2% respectively. (5,6) The potential risk factors of \textit{S. aureus} contamination could be swine farm, slaughter house, transportation time, storage process, climate and sanitation at the retail shops.

2) MRSA risk assessment. Probability of illness (P_I) from consuming pork contaminated with MRSA was 5.1794×10^-6. While the probability of exposure (P_E) was 0.02. Probability of AMR consequence (P_M) was simulated from hospitalization rate (h) which was 0.1894 (9) and mortality rate cause by MRSA in the hospital (m) which was 0.936 (8). Due to the prescription rate (p) was not reported, 1.0 was used as the maximum value for prescription rate in the model to represent the worst case scenario. In the risk characterization step, the result of risk estimation when people consumed pork contaminated with MRSA and were hospitalized and died because of antimicrobial resistance was 2.307×10^-9. So, the annual cases of MRSA foodborne illness caused by pork consumption per year was 8.62 cases per 10 million people. The risk factors influencing on risk estimation were concentration of MRSA in pork, prevalence, hospitalization rate, prescription rate, mortality rate and amount of pork consumption. The concentration of MRSA in pork is the top one risk factor. The lack of information regarding prescription rate and mortality rate should have been available in order to more accurately evaluate risk of consuming pork contaminated with MRSA.

### Table1 Prevalence of \textit{S. aureus} and MRSA in pork samples at the retail level in Bangkok

<table>
<thead>
<tr>
<th>Areas</th>
<th>Total samples</th>
<th>Prevalence of \textit{S. aureus}</th>
<th>Prevalence of MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Bangkok</td>
<td>60</td>
<td>43.33</td>
<td>0</td>
</tr>
<tr>
<td>East Bangkok</td>
<td>66</td>
<td>50.00</td>
<td>1.51</td>
</tr>
<tr>
<td>North Bangkok</td>
<td>64</td>
<td>51.56</td>
<td>0</td>
</tr>
<tr>
<td>South Bangkok</td>
<td>68</td>
<td>55.88</td>
<td>1.47</td>
</tr>
<tr>
<td>Lower Thonburi</td>
<td>60</td>
<td>41.67</td>
<td>1.67</td>
</tr>
<tr>
<td>Upper Thonburi</td>
<td>60</td>
<td>33.33</td>
<td>1.67</td>
</tr>
<tr>
<td>Total</td>
<td>378</td>
<td>46.29</td>
<td>1.05</td>
</tr>
</tbody>
</table>

**Risk assessment:**

1) \textit{S. aureus} risk assessment

After simulated, probability of illness (P_I) was 8.95×10^-6. While the probability of exposure (P_E) was 0.46. The risk estimation (P_S) which was the product of P_I and P_E was 4.12×10^-6. So, the annual cases of \textit{S. aureus} foodborne illness caused by pork consumption per year were 150.3 cases per 100,000 people. Prevalence and concentration of \textit{S. aureus} in pork as well as the amount of pork consumption were the major risk factors. From the annual epidemiological report for staphylococcal food poisoning indicated that these were 13.6 cases per 100,000 people (7). The lower foodborne disease rate may be attributable to the under annual epidemiological report since the symptoms of staphylococcal food poisoning can be self-limited. So, these patients were not included in the report. Additionally, the overestimated cases by the model as a result of surrogate dose-response model by \textit{S. aureus} skin infection instead of \textit{S. aureus} intoxication.

### Reference

Surveillance of porcine epidemic diarrhea virus (PEDV) in Thailand, 2014-2017

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Keywords: Genetic characterization, porcine epidemic diarrhea virus, Surveillance, Thailand

Introduction
Porcine epidemic diarrhea virus (PEDV) causes global economic losses in swine industries. The clinical signs, gross lesions and histopathological lesions are watery diarrhea, vomiting and thin intestinal wall (1). Genome organization of PEDV consists of ORF1ab, Spike (S), ORF3, Envelop (E), Membrane (M) and Nucleocapsid (N) genes. M gene is used for PEDV detection while S gene is noted as PEDV differentiation (2). At present, PEDVs could be classified into 4 major genotypes including G1a circulating in China, G1b circulating in China and USA, G2a circulating in China and Asia, and G2b circulating in China and USA (3). This study aimed to reveal the occurrence of PEDV in swine diarrheal outbreak prone farms and point out the major circulating strains of PEDV in Thailand.

Materials and Methods
Sample collection: During November 2014 to October 2017, a total of 688 samples of feces and small intestinal tissues from 63 diarrheal outbreak prone farms were collected in 4 livestock regions (Table 1).

Virus identification: Viral RNA was extracted from 10% suspension of fecal samples and 10% homogenization of small intestinal tissue samples using the QIAmp viral RNA minikit (Qiagen, Valencia, CA). PEDV M gene detection was performed using real-time RT-PCR assay.

Virus characterization: Representative PEDVs (n=15) were selected for sequencing and genetic characterization. PEDVs were sequenced on their S gene and ORF3 gene with specific primer sets (4).

Phylogenetic analysis: Thirty-four reference PEDV sequences were retrieved from GenBank database including 1a, 1b, 2a and 2b genotypes. Thai PEDVs were aligned with reference sequences using Muscle program. Phylogenetic tree of PEDVs was constructed with Neighbor-joining method by MEGA7 software (5).

Results and Discussion
The occurrence of PEDV in 63 farms in Thailand was 53.97% (Table 1). Fifteen representative Thai PEDVs were isolated from Chonburi (n=2), Khon Kaen (n=1), Nakhon Pathom (n=2), Nakhon Ratashaima (n=5), Prachinburi (n=2) and Ratchaburi (n=3). Phylogenetic analysis showed that all of the 15 Thai-PEDVs were grouped into genotype 2. Eight of those viruses were G2b while other subgroups could not be classified.

Table 1 Occurrence of PEDV in swine diarrheal outbreak prone farms in Thailand.

<table>
<thead>
<tr>
<th>Livestock regions</th>
<th>PED positive farms*</th>
<th>Positive**</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>63.16%(12/19)</td>
<td>44.30%(70/158)</td>
</tr>
<tr>
<td>3</td>
<td>58.33%(7/12)</td>
<td>48.17%(197/409)</td>
</tr>
<tr>
<td>4</td>
<td>25.00%(1/4)</td>
<td>71.43%(10/14)</td>
</tr>
<tr>
<td>7</td>
<td>46.67%(14/30)</td>
<td>49.53%(53/107)</td>
</tr>
<tr>
<td>Total</td>
<td>53.97%(34/63)</td>
<td>46.66%(321/688)</td>
</tr>
</tbody>
</table>

* blanket ( #Positive farm / #farm )
** blanket ( #Positive / #Samples )

Acknowledgements
We would like to acknowledge Chulalongkorn University for its financial support to the Center of Excellence for Emerging and Re-emerging Infectious Diseases in Animals. This work was supported by grants from the 16th Royal Golden Jubilee Ph.D. Program (PHD/0026/2556). We would also like to thank the Thailand Research Fund for its financial support to the TRF Senior Scholar to AA (RTA6080012).

References
Synergistic Energy Providing by Lecithin and L-carnitine Supplemented to Pigs in Lactating Period

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Keywords : Synerlac, Lactating period, Growth Performance, %Pre-weaning mortality

Introduction

The most common measure of productivity for the sow herd in pig farm is pigs weaned/sow/year (PSY). The period after farrowing is very important because of reducing feed intake in each sows. Many factors effects to level of nutrients and energy for body maintenance and moreover, not enough for milk productions supplied to suckling piglets. Its effects easily produce negative energy balance. Feed intake in sows is very important to their piglets especially milk. Normally, each farms have solutions to solve their problems by increasing feed concentration especially ratio of fat in feed formulations. That formulations is increased in level of energy. Therefore, fat utilization is important to improve maximize sow performance related with decreasing feed intake in sows.

Synerlac, which is lactating sow additive effects to feed intake stimulate, increase quality of colostrum and milk. Following increase energy utilizations to reduce negative energy balance for lactating sow. Losing weight after weaning is decreased by reducing fat mobilization. The study Overland et al., 1993 reported lecithin has showed to be emulsifier for lipid source. That effects to fat emulsify and digest from large molecule to micelle, which will be absorbed to intestinal cells. Those micelles are accessed to next process to produce energy for sows. Additionally, many study report that l-carnitine supplementation to sows improve fat utilization performance by mechanism in body cells. L-carnitine effects triglyceride uptake from cytosol to mitochondria to produce energy source for cells. Eder et al., 2002 reported l-carnitine supplementation to lactating sows reduced losing of pre-weaned piglet and moreover, increased weaning weight. Therefore Synerlac acts to support energy source utilization to be completely process. That will be supplied enough for lactating period and next breeding circulation. Synerlac also composed by natural extract for stimulated milk production by induce mammary gland. The aim of this study is to investigate the effect of Lecithin and L-carnitine supplementation (Synerlac®) on improving lactating sow performance (Average daily litter weight gain: ADLWG) and %Pre Weaning Mortality (%PWM) daily litter weight gain (ADLWG).

Materials and Methods

A total of 24 Danish sows were included in the study and average parity of 2.5. All sows were allocated equally into two treatment groups. Twelve sows were assigned to two treatment groups by complete randomized design equally: i) Control group(Normal feed) ; ii) Treatment group(lecithin and l-carnitine mixed with normal feed). Lecithin and l-carnitine (Synerlac) were administered by mixed with feed in ration 1.5 kg/ton of feed from farrowing day until weaning. Data were analyzed by T-Test using the SAS program to detect outliers and to determine SEM and p-value. Significance was always defined as p < 0.05 and highly significance was defined as p < 0.01.

Results and Discussion

The result (Table 1) demonstrated that ADLWG 2,443 g/piglet/day of Synerlac group had no significantly difference with 2,228 g/piglet/day of control group (P>0.05). Synerlac group had no difference of %pre-weaning mortality from control group 10% and 12% (P>0.05) . Feed intake also had no difference from control group 4.92 and 5 (P>0.05). But found lactating period had significantly difference between 2 groups (P<0.05).

Table 1 Litter performance between control and SynerLac® supplemented group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Besow Plus®</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sow</td>
<td>12</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Litter size</td>
<td>12.75</td>
<td>12.42</td>
<td>0.59</td>
</tr>
<tr>
<td>Born alive litter size</td>
<td>11.33</td>
<td>11.50</td>
<td>0.59</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.67</td>
<td>1.72</td>
<td>0.43</td>
</tr>
<tr>
<td>Pre-weaning mortality (%)</td>
<td>10.29</td>
<td>12.31</td>
<td>0.29</td>
</tr>
<tr>
<td>Pig weaned per litter</td>
<td>10.08</td>
<td>10.00</td>
<td>0.85</td>
</tr>
<tr>
<td>Weaning weight (Kg)</td>
<td>7.94</td>
<td>8.12</td>
<td>0.44</td>
</tr>
<tr>
<td>Days in lactation period</td>
<td>28.42</td>
<td>26.61</td>
<td>0.02*</td>
</tr>
<tr>
<td>Average daily weight gain (g/d)</td>
<td>2,228</td>
<td>2,443</td>
<td>0.19</td>
</tr>
</tbody>
</table>
* Different from the control group by significantly difference (P < 0.05)

Effects of increasing of ADLWG is mainly indicator in lactating sow production cause it can indicate farm performance so that from this study indicate improving of sows performance from the result ADLWG in treatment group was higher than control group by no significantly difference while lactating period had lower by significantly difference.

Due to ADLWG was calculated from number of pig weaned per litter, weaning weight, birth weight and lactating period. In this study treatment group had lactating period less than control group by significantly difference(P<0.05). This result indicate to save time for lactation. Sow after weaned can be faster move to next production cycle. Therefore, effect of Synerlac is important to increase growth performance then shows in no difference in ADLWG parameter.

Synerlac effects are increasing energy utilizations to reduce negative energy balance for lactating sow. The study Overland et al., 1993 reported lecithin has showed to be emulsifier for lipid source. That effects to fat emulsify and digest from large molecule to micelle, which will be absorbed to intestinal cells. Those micelles are accessed to next process to produce energy for sows. So that, it shows effect from litter performance, weaning weight. Additionally, many study report that l-carnitine supplementation to sows improve fat utilization performance by mechanism in body cells. L-carnitine effects fatty acid uptake from cytosol to mitochondria to produce energy source for cells. Eder et al., 2002 reported l-carnitine supplementation to lactating sows reduced losing of pre-weaned piglet as this study shows reducing of %PWM and moreover, increased weaning weight. Therefore, Synerlac acts to support energy source utilization to be completely process.

**Conclusion**

In conclusion, this study demonstrated that lecithin and l-carnitine supplementation (SynerLac®) in lactating sows can improve growth performance resulting in reducing lactating period to get same growth rate. Therefore, lecithin and L-carnitine supplementation (SynerLac®) will be benefit for farms production.

**Acknowledgements**

1. The authors are thankful Vet products Research and Innovation Center, Vet Products Group, Bangkok, Thailand
2. Agintel Co.,Ltd. for providing all the facilities and funds to carry out this study.
3. Mr.Chanon Chalothorn, farm owner in Thailand

**References**

Variations of Morphology and Immune Cell Infiltration in the Endometrium of Culling Gilts with Ovarian Cysts

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Keywords: Pig, reproductive failure, ovarian cyst, uterus, immune cells

Introduction
The most important problem for eliminating replacement gilts is the reproductive disorders composed of repeat breeding, anestrus, not pregnant and abortion and this problem is considered as the cost-effective losses in the pig industry (1, 2). It is known that the reproductive tracts of culling pigs from the abattoirs are the useful source for inspecting the abnormalities (3). However, at least in sows, several studies suggested that the lesions of these reproductive organs could not be commonly observed by macroscopic study (4, 5). Consequently, the microscopic analysis has been obligatory to scrutinize the reproductive tracts and the abnormalities of female reproductive organs appeared in different manners in pigs (6, 7). The cystic ovarian disease is an endocrinologic abnormal that associated with ovarian dysfunction affecting the reproductive disturbance (8). In general, the ovarian cysts were regularly considered into single and multiple cysts (9) and the incidence of this disease in pigs culled due to infertility varies between 2 to 24% (10, 11). Remarkably, in replacement gilts, the cystic ovaries were noticed approximately 14% and roughly 60% of these gilts were multiple ovarian cysts (12). At present, the variations of physiology and behavior in gilts depend on the sorts of ovarian cysts and multiple ovarian cysts are proposed to be the severe cause for breeding in pigs since the gross pathological signs were not noticed in reproductive tracts (13). Therefore, the aim of this research was to investigate the effect of the ovarian cysts on the morphological changes and the infiltration of immune cells in the endometrium of gilts culled due to fertility complications.

Materials and Methods
The reproductive tracts of the crossbred Landrace × Yorkshire replacement gilts (n=40) were collected at the abattoirs. Their historical data were recorded particularly the culling reasons and blood samples were kept prior to slaughter for analyzing serum progesterone. The genital organs were taken to the research laboratory in a cool container about 6 to 8 hours. The ovaries and uterine horns were removed out of the tracts and culling gilt ovaries were classified into 3 groups: normal ovaries at follicular stage (n=9), single cyst (n=13) and multiple cysts (n=18) as previously described (13). The uterine horns were dissected and fixed in 10% neutral buffer formalin for routine histological procedure. All sections were stained using hematoxylin and eosin (H&E). The histological parameters composed of the characteristics of epithelial surface, the epithelial cell height, the numbers of blood vessels (arteriole, vein and capillary), the numbers of superficial endometrial glands and the edematous score were evaluated under light microscopy (BX50, Olympus, Tokyo, Japan) with a program of Image Pro® Plus version 6 (Media Cybernatics Inc., MD, USA). The numbers of immune cells (lymphocytes, neutrophils and macrophages) in epithelial lining were quantified under light microscope with ocular micrometer as formerly described (14). The tissue micrographs were taken by a digital camera Micro-publisher 5.0 (Qimage, Surrey, Canada). Data were analyzed using SAS version 9.0 (SAS Inst., Cary, NC, USA). A value of $p<0.05$ was regarded as statistically significant.

Results and Discussion
The culling reasons of reproductive problems related to the ovarian cysts and the progesterone levels in the culling gilts were displayed in figures 1 and 2, respectively. Approximately 50% of the replacement gilts with multiple cysts were eliminated due to anestrus and the progesterone level in the group of multiple cysts was significantly higher ($p<0.05$) than the other groups.

Figure 1 Culling reasons of reproductive abnormalities related to single cyst and multiple cysts on the ovaries in the replacement gilts.
Figure 2 Progesterone levels in serum collected in normal follicular, single cyst and multiples cysts groups (mean±SEM). *Different letters (a, b) differ significantly (p<0.05).

The histological parameters, i.e. the characteristics of epithelial surface, the epithelial cell height, the numbers of blood vessels, the numbers of superficial uterine glands and the edematous score in each group of culling gilts were represented in Table 1. The number of the blood vessels and also the subepithelial edematous scores were significantly higher (p<0.05) in the multiple cysts group.

Table 1 Histological parameters in the endometrium of the normal follicular group compared to the single cyst and multiple cysts group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Single</th>
<th>Multiple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial lining type</td>
<td>high cuboidal/</td>
<td>high cuboidal/</td>
<td>high cuboidal/</td>
</tr>
<tr>
<td></td>
<td>Columnar/</td>
<td>Columnar/</td>
<td>Columnar/</td>
</tr>
<tr>
<td></td>
<td>Pseudostratified</td>
<td>Pseudostratified</td>
<td>Pseudostratified</td>
</tr>
<tr>
<td>Epithelial cell height</td>
<td>36.3±2.1^a</td>
<td>34.6±3.2^a</td>
<td>33.5±2.6^a</td>
</tr>
<tr>
<td>Numbers of blood vessels</td>
<td>5.6±0.5^a</td>
<td>4.8±0.6^a</td>
<td>8.6±0.6^b</td>
</tr>
<tr>
<td>Numbers of uterine gland</td>
<td>4.1±2.5^a</td>
<td>3.6±2.7^a</td>
<td>2.5±2.1^a</td>
</tr>
<tr>
<td>Edematous score</td>
<td>0.9±0.1^a</td>
<td>1.0±0.1^a</td>
<td>1.4±0.2^b</td>
</tr>
</tbody>
</table>

*Different superscripts (a, b) differ significantly (p<0.05)

The infiltration of immune cells within the epithelial surface of the culling gilts was depicted in figure 3 and 4. The lymphocytes were the predominant immune cells in the epithelial surface which were seen in all groups. The numbers of lymphocytes, neutrophils and macrophages in the epithelial lining of endometrium in multiple cysts group were significantly (P<0.05) increased compared to other groups.

Figure 3 Number of immune cells infiltrated in the epithelial surface of endometrium in normal follicular, single cyst and multiple cysts groups (mean±SEM). *Different letters (a, b) differ significantly (p<0.05).

In this study, about 50% of the replacement gilts with multiple ovarian cysts were removed because of anestrus. Form previous study, the replacement gilts in Thailand were culled due to various reproductive reasons and more than 44% were anestrus and approximately 51% of these gilts were not seen the pathological lesions in the reproductive organs (12). Additionally, the high incidence of return to estrus after artificial insemination in sows was found with the ovarian cysts (15). Consequently, these research findings supported the influence of ovarian cysts on the functions of female reproductive tracts of pigs.

Although, the major cause of the ovarian cysts was not comprehensively studied yet, this study tried to describe the correlation between the categories of ovarian cysts and the culling reasons. The current results designated the replacement gilts removed due to anestrus related to the multiple ovarian cysts. Considering to progesterone level, the level of progesterone is significantly highest in the gilts with multiple ovarian cysts. This finding might be the essential source that approved the high progesterone level could be able to interrupt the regular estrous cycle and could be the vital clarification for the gilts culled by anestrus (16). As mentioned earlier, the pathological lesions of reproductive organs were not frequently remarked in the culled pigs (4, 5) and more than 50% of the gilts culled due to reproductive problem were detected normal reproductive tracts (12). Definitely, the additional investigations, i.e. the microscopically analysis, have required to determine the abnormalities of these gilts. In the present results, the numbers of blood vessels, the edematous score and also the numbers of intraepithelial immune cells were significantly observed in the gilts with multiple ovarian cysts. Up to date, the etiology of ovarian cysts in pigs
has not been revealed but it was documented that the regulations of adrenergic nerves and noradrenalin induced the multiple ovarian cysts in rat and pig (17) and the increase of ACTH and cortisol can also stimuli the ovarian cysts (9). Absolutely, the hormonal changes influenced on the uterine functions and the infiltration of immune cells (6, 14) corresponded to the findings in this study. In conclusion, the data in this research indicated that most gilts culled due to reproductive problem with multiple cysts commonly had anestrus and the uterine horns of these gilts can be detected the abnormalities by microscopically evaluations.

Acknowledgements
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References
CDC25A Expression on the Immunolocalization of Asian Elephant
(Elephas maximus) Testis

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Keywords: cell cycle, regulation, spermatogenesis

Introduction
Histological structure of testis is classified by two types of testicular cells in seminiferous tubules, including germ cells and Sertoli cells. Spermatogenesis is a complex process that normally occurs within the seminiferous tubules. This process includes mitosis of spermatogonia, meiosis of spermatocytes and also spermiogenesis, resulting of haploid sperm. Spermatogenesis is highly ordered and requires the precise co-ordination of cell cycle events. Cell division cycle 25 A (Cdc25A), a specificity protein phosphatase, mainly activates cyclin-dependent kinases 2 and thereby induces progression from G1 to S phase. Therefore, Cdc25A is one of the most important cell cycle regulators for mitosis in the developing embryo and meiosis in the spermatogenesis (1). Asian elephant (Elephas maximus) has become a symbol of Thailand. They were originally living in the wild and captured for training as domesticate animals. In general, the Asian elephant population has been declined due to several threats such as habitat loss, degradation and fragmentation (2). The elephant bulls have strikingly intra-abdominal testes that may involve the subfertility of elephant bulls. Indeed, only small populations of captive elephant bull produce semen containing sufficient sperm quality for their fertility. To date, little is known about the role of cell cycle regulators in elephant spermatogenesis. To investigate the Cdc25A expressions in elephant spermatogenesis, we immunohistochemically examined Cdc25A.

Materials and Methods
Three Asian elephant testes were obtained from post mortem and divided in normal, abnormal spermatogenesis and neonatal. The normal spermatogenesis was classified by the presence of spermatozoa. The sections were deparaffinized by xylene and ethanol. Some sections were stained with hematoxylin-eosin (H&E) for observations of general histology. The sections were then subjected to antigen retrieval by 0.01M citric acid buffer (pH 6.0) in a microwave and incubated with goat IgG in phosphate buffered saline to reduce background staining caused by secondary antibody (Dako REAL™ EnVision™/HRP, Rabbit/Mouse). The primary antibody was rabbit polyclonal Cdc25A (1:200). The primary antibody was replaced with mouse IgG as for negative control. The sections were immersed in AEC and counterstained with Hematoxylin.

Results and Discussion
Cdc25A was positive in the cytoplasm of spermatocytes and round spermatids in normal spermatogenesis. The abnormal spermatogenesis sample was expressed only in the cytoplasm of spermatocytes. However, Cdc25A expression was decreased in abnormal spermatogenesis. This result suggests that Cdc25A might active during meiosis cell cycle in Asian elephant spermatogenesis. Our result was similar to human testis. The Cdc25A expressions decreased in spermatogenic failure and reduced spermatogenic cycles within the seminiferous epithelium. These results in human were in agreement with the biological role of Cdc25A as an essential regulator of cell cycle progression or cell proliferation (3, 4). From the literature, many molecular markers have been shown to associate with the sperm production and cell cycle regulation (5). Further studies are necessary to explore the functional roles of Cdc25A and tumor suppressor protein p53 as these proteins are highly conserved in elephant and act as cell cycle regulator.
Figure 1 Immunohistochemical staining of Cdc25A (arrows head) in the cytoplasm of spermatocytes and round spermatids. Normal spermatogenesis (A) compared with abnormal spermatogenesis (B) and neonatal Asian elephant testes (C). Negative controls for normal, abnormal spermatogenesis and neonatal testis, respectively. Scale bars represent 10 μm.

Acknowledgements
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References
Histomorphology of reproductive organs and placenta in wild gaur (Bos gaurus)

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Keywords: gaur, histomorphology, placenta, reproductive organs

Introduction
The essential information on reproductive organs especially during pregnancy in wildlife may help to manage the conservative program for these species. Since, reproductive morphology was remarkable different in wildlife compared to those in domestic animals, the specific data on the reproductive organ should be documented. Though, the bovine placenta has been described in several studies [1,2], none have done in the reproductive organ of the gaur (Bos gaurus) especially during pregnancy. Therefore, the present study aimed to study the anatomy and histology of reproductive organs in gaur with special reference to during pregnancy.

Material and Methods
The pregnant uterus was obtained from the gaur which accidentally died from the Zoo. The organs were transported to anatomical laboratory after necropsy. The gaur reproductive tract and placenta were dissected and studied for anatomy. In addition, some of the organs were fixed in 10% formaldehyde for histological study. In addition, the main whole part of the reproductive organs together with the placenta and fetus was subjected to organ preservation by plastination.

Results and Discussion
The female gaur reproductive organs and placenta after fixation was shown in figure 1. In general, the reproductive organs resembled those in the bovidae family. The vagina has long tubular structure with slightly longitudinal fold inside. The cervix was resembling to those found in goat than in cattle that the cervix was narrow with finger-like projection to the cervical canal. This may make the assisted reproductive program such as AI more difficult in this species compared to other cattle. The ovary was similar to other domestic cattle but with less follicle found in the gaur. Moreover, a dense connective tissue capsule was found over the ovarian surface. In the pregnant uterus, the fetus was found in the left uterine horn together with the corpus luteum of pregnancy on the left ovary. This may be suggested that the embryonic transuterine migration is not common in this species [3] compared to ewe [4] and goat [5]. Caruncles were observed in sessile convex shape and were distributed both in the uterine horn and uterine body. The large caruncle was always found in the middle of the horn where the embryonic attachment occurred. Similarly, the most develop placentome was found near the embryo as observed in cattle [6]. The number of caruncles in pregnant horn was 63 which corresponded to the number of cotyledon on the chorioallantoic surface. This may imply that the number of cotyledon/placentome is determined since the early of pregnancy. As pregnancy progressed, the placenta may compensate for higher demand of the fetus by expansion of the cotyledonary surface rather than increase in the number of cotyledon.

Conclusion
The present study is the first morphological description of the uterus with special reference to pregnancy in the gaur (Bos gaurus). Although, most of the reproductive morphology in gaur was similar to other species in bovidae family, some descriptions of reproductive organs as well as of the placenta were obviously different. Therefore, these data on the anatomy and physiology of the gaur may be valued for the assisted reproductive program for these endanger species.

Figure 1 Female reproductive organ and placenta from the gaur. VU= vulva, Va= vagina, C= cervix, L= left pregnant uterine horn, R= right uterine horn, F= Fetus. Arrow indicate cotyledon on the chorioallantoic surface. CAS= chorioallantoic sac
Figure 2 the caruncles in the pregnant uterine horn. C= caruncle, I= intercaruncle area. Noted the crypt surface on the sessile shape caruncle.

Figure 3 the cotyledon (co) on the chorioallantoic sac. Noted the villous type of the cotyledon.

Acknowledgements
The authors would like to thank the staffs of The Zoological Park Organization for specimen collection and transportation. This study was supported by the grant from National Research Council of Thailand (grant no.27/2559)

References
Metastatic Squamous Cell Carcinoma in a Richardson’s Ground Squirrel (Spermophilus richardsonii): A Case Report

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Keywords: Squamous cell carcinoma, Richardson’s ground squirrel, metastasis, pancytokeratin, vimentin

Introduction
The Richardson’s ground squirrel (S. richardsonii), a North American medium-sized rodent, had been used as an experimental animal and recently destined as an exotic pets (1, 2). Squamous cell carcinoma (SCC) is a malignant tumor of epidermal cells with varying degrees of keratinocyte (squamous cell) differentiation (3). Ultraviolet radiation, injured or chronically diseased skin, carcinogenic stimulus, and Papilloma viral-induced epithelial proliferation had been documented as causes of SCC.

In exotic pets, SCC was reported in the non-glandular stomach, the subungual, eyelid, and extremities of rabbits (4), skin of African pygmy hedgehogs (5), and the anterior right leg of the California ground squirrel (6). A limitation of spontaneous tumor in this species had been documented (1, 2). However, SCC had not been reported in Richardson’s ground squirrel. The objective of this study is to describe SCC with pulmonary metastasis in a Richardson’s ground squirrel.

Materials and Methods
Case history: An approximately 2-year-old, intact female, Richardson’s ground squirrel was presented to the animal hospital due to swelling of left cheek for 2 weeks. The squirrel had a 1.5 cm in diameter, soft tissue mass on the left cheek. The root tooth abscess was preliminary diagnosed. The animal was treated with 5 mg/kg marbofloxacin and 0.5 mg/kg meloxicam in conjunction with laser therapy. The mass significantly decreased in size within the first 2 weeks of treatment. However, the mass rapidly grew and revealed a fistula tract. Wound dressing and supportive treatment were performed for 3 weeks. Then, the squirrel deteriorated and transferred to the critical care unit. Unfortunately, the squirrel died within 3 days after admission.

Pathological examinations: A complete necropsy was performed. Tissue samples were collected and fixed in 10% neutral buffered formaldehyde, embedded in paraffin wax for histopathological examination.

Immunohistochemistry: The section of the mass using autoimmunohistochemistry stainer (Bond™ Polymer Refine Detection, Leica Biosystems, United Kingdom). Anti-pancytokeratin (AE1/AE3) and vimentin were used as primary antibodies.

Results and Discussion
Gross findings: A 3.5×2.5×2 cm, round mass was located at the left cheek. The mass was centrally ulcerated (Fig.1). On the cut surface, the mass was whitish with multiple hemorrhage and had central necrosis. The mass also compressed the tongue (Fig. 1, inset). In addition, multiple white firm masses (up to 2 mm) were seen throughout the lung. The remaining organs were normal grossly.

Microscopic findings: An unencapsulated mass was located in the dermis. The mass invaded and induced necrosis of the adjacent bone. The mass composed of nests of polygonal cells surrounded by fibrous connective tissue. Neoplastic cells had distinct cell borders, prominent intercellular bridging, abundant of eosinophilic cytoplasm and round to oval nucleus with hyperchromatic and prominent nucleoli. There were obviously cellular pleomorphism and high mitotic activity (2-3 cell/high power field). Moreover, there were multineucleated giant cells within the stroma of the neoplasm. The lung parenchyma was replaced by the clusters of neoplastic cells that showed the same features as the primary origin.

Immunohistochemistry: The neoplastic cells showed immunoreactivity to both pancytokeratin (AE1/AE3) and vimentin in their cytoplasm in the primary origin.
and metastatic site (Fig. 4), similar to a previous study (7).

This present study described the first case of SCC in the Richardson’s ground squirrel (1, 2). Unlike the domestic animals, SCC in exotic pets, especially squirrels, are quite rare (3-7). SCC in this case occurred in a young animal as previously described cases of mast cell tumor (2) and salivary adenocarcinoma (1) in this species when compared to SCC in dogs (3). SCC predominately affected the limbs in dogs (3) and rabbits (4). However, SCC in the present case was located on the head similar to a case of an African pygmy hedgehog (6) and differentiated from salivary gland adenocarcinoma (2) using the neoplastic cell features.

Rapid growth of the neoplastic cells, local invasion and distant metastasis indicated the malignancy behavior of the mass (5-7). Interestingly, the neoplastic cells revealed co-expression of cytokeratin and vimentin that suggested a potential origin of the tumor from epithelial cells with epithelial to mesenchymal transition (7).

In conclusion, this present study described SCC at the left cheek of Richardson’s ground squirrel with pulmonary metastasis based on pathological features and immunohistochemistry results. SCC should be considered as a differential diagnosis for a mass on the head of Richardson’s ground squirrels.

![Figure 1](image1.png)

**Figure 1.** The mass located on the left cheek with fistula tract. Inset, a well-circumscribed mass with multiple hemorrhage, central necrosis and tongue compression (arrow).

![Figure 2](image2.png)

**Figure 2.** Microscopically, the mass composed of nests of epithelial tumor and necrosis (N), HE, 4x. Inset, the neoplastic cells showed typical characteristics of squamous cell with high pleomorphism and occasional giant cells.

![Figure 3](image3.png)

**Figure 3.** The neoplastic cells in the lung had the similar features as the primary mass.

![Figure 4](image4.png)

**Figure 4.** The neoplastic cells had immunoreactivity to pancytokeratin (4a, 4c) and vimentin (4b, 4d) in both primary site and lung.

**Acknowledgements**

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**References**

Plastinated specimen from wildlife: a new approach for anatomical study in giraffe reproductive organs

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Keywords: plastination, giraffe, reproductive organs

Abstract
The present study aimed to preserve the organs from wildlife after death for further study in the far future. The organs used for plastination procedure in this present study was obtained from giraffe which died from general respiratory failure. The reproductive organs, consisted of vagina, cervix, uterus and ovary, were dissected and processed for plastination. The results showed that the reproductive organs of giraffe were successfully preserved with plastination procedure. However, some disadvantages such as the slightly shrinkage of the organs could be observed from the present study. In summary, plastinated organs provides an ideal tool for long-term preservation using in anatomical study especially in wildlife.

Introduction
Preserved specimens are needed for study of morphology and anatomy especially in wildlife. The essential information on reproductive organs may help to manage the conservative program for these species. Plastination is the method to persevere long-term organs or tissue. The principle of plastination is to remove water and lipid from biological organs and replace with a curable polymer. The plastinated organs from appropriate protocol will be dry, odorless with flexible texture. In human, plastinated specimens were widely used in medical teaching since these kind of specimens can be kept for a long period without special care. However, in veterinary study, the plastinated organs are rare which may due to the difficulties for plastination process and the cost of substance used for high quality plastination. Therefore, we aimed to apply the plastination technique to animal tissues especially in wildlife species in order to preserve the organs from these species for the long term veterinary study.

Materials and Methods
The organ used in this study was the female reproductive organs from giraffe which is died from other problems besides reproductive failure. The organs were transported to anatomical laboratory within 24 hours after necropsy. After dissection for anatomical study, the giraffe reproductive tract was fixed in 10% formaldehyde for 2 weeks and it was checked for everyday after 72 h. of fixation. When the fixation is ready, the giraffe reproductive tract will be plastinated by the standard protocol. Briefly, the specimen was freeze substitution dehydration with acetone for 2 weeks. Thereafter, the dehydrated specimen was force impregnated with a polymer mixture of commercial Biodur®S10 and Biodur®S3 at -20°C until the polymer was completely done. Thereafter, the specimen was hardened by using commercial Biodur®S6 [1]. Finally, the specimen was applicable for veterinary teaching procedure.

Results and Discussion
The female giraffe reproductive organs after plastination was shown in figure 1. In general, the reproductive organs resemble those with ungulate family. The vagina has long tubular structure with the cervical rings in the cervix. The uterine body was long and prominent with symmetrical uterine horns. Caruncles were observed in convex shape in the uterine wall. The ovary was oval and large compared to other ungulate animals and no follicles were observed at the time of necropsy. Regarding plastination, the preserved giraffe reproductive tract was dry, odorless, flexible and have the same color as fresh specimen. However, the organ was slightly shrinkage and slightly rigid compared to fresh specimen. This suggested that different tissues type even in the same organ have different level of shrinkage [2] and they may need specific different plastination protocols. Nevertheless, this disadvantage was overcome with the ideal long term preservation of the rare specimen.

Conclusion
Plastinated organs or tissue provides an ideal tool for long-term preservation. The well dissected, rare to find specimen should be processed with this kind of preservation technique. However, different tissue or organs allowed different protocol of plastination and that further study should be considered to develop the technique in order to obtain higher qualification of plastinated specimens for long-term veterinary study especially in wildlife.
Figure 1 Plastinated reproductive tract from the dead giraffe. The morphology of the specimen was well preserved though there was slightly degree of shrinkage in different tissues even in the same organ.

Acknowledgements
The authors would like to thank the staffs of The Zoological Park Organization for specimen collection and transportation. This study was supported by the grant from National Research Council of Thailand (grant no.27/2559)

References
Protective Effect of Hydroxyxanthone on the Leakiness of Intestinal Epithelia Induced by TNF-α

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Keywords: Caco-2 cell, hydroxyxanthone, leaky, protection, TNF-α

Introduction
Gram negative bacteria especially Escherichia coli (E.Coli) in gastrointestinal tract causes diarrhea and death in animal (1). Leaky gut which have the damage and loss of tight junction barrier has the characteristics of decreased transepithelial resistance (TER) and increased tissue permeability, allowing the pathogen invading to the bloodstream and causing septicemia (2). The endotoxin lipopolysaccharide (LPS) comprising in the cell wall of gram negative bacteria is one of the major causes mediated by pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) via LPS-toll-like receptor-4 (TLR4) signaling pathway (3). Hydroxyxanthone (HDX) is the major phenolic compounds found in many plants including mangosteen (Garcinia mangostana). It has many beneficial effects, including anti-oxidant and anti-inflammation (4). The different forms of HDXs have been demonstrated to inhibit cAMP-activated Cl− secretion in intestinal epithelial cells. This evidence has indicated the advantage of HDXs for treatment of gastrointestinal disorders (5). In the present study, we aimed to investigate the preventive effect of HDXs on leaky gut induced by TNF-α using the human intestinal epithelial cell line Caco-2.

Materials and Methods
Cell Culture: Caco-2 cells (passage no. 40-50) obtained from the American Type Culture Collection (Manassas, USA) were cultured and maintained in culture medium supplemented with 10% fetal bovine serum for 7 days. The cells were stripped and plated on 96-well plate or transwell microporous filters (12 mm, 0.4 μm pore size) (Corning-Costar, USA).

Drug and TNF-α treatment: Cells were incubated with 10 or 100 μM of 1-monoHDX, 1,3-diHDX, 1,3,6-triHDX, 1,3,6,8-tetraHDX or vehicle DMSO for 4 or 24 h. In the TNF-α study, HDXs were added 24 h before challenged with TNF-α 100 ng/ml for 4 or 24 h.

Cytotoxic analysis: To determine whether the concentration of HDXs used in the present study was non-cytotoxic, MTT assay was performed in 96-well plate cells. Following the manufacturer’s protocol, MTT (triazolyl blue tetrazolium bromide; MS655, Sigma, St. Louis, USA) 5 mg/ml was incubated for 3 h. DMSO was then added to dissolve the formazan dye, and the optical density (OD) of specific dye and non-specific background was detected by spectrophotometer at 570 and 620 nm, respectively.

Measurement of epithelial leakiness: TER and subsequently paracellular permeability of FD-4 (FITC-Dextran; MW=4 kDa) were measured in Caco-2 cells culturing on transwell microporous filters. TER was measured by volt-ohmmeter (Millipore, USA), and paracellular permeability was performed by applying FD-4 (1 mg/ml; Sigma, Singapore) into the apical side. After 4 h incubation, intensity of FD-4 fluorescence traces was measured by fluorescence 96-well plate reader (Synergy™, Biotek, USA) at excitation/emission wavelength of 485/530 nm. The apical-to-basolateral transport of FD-4 was reported as $P_{app}$ (hr⁻¹.mm⁻²), which was calculated according to the following equation:

$$P_{app} \text{(hr}^{-1}\text{.mm}^{-2}) = \frac{\text{Conc at basolateral side } / \text{hrs of incubation}}{\text{Area of filter } \times \text{Conc at apical side}}$$

Statistical analysis: Data was reported as means ± SEM of 3-5 experiments. Statistical test was performed by analysis of variance (ANOVA) followed by Dunnett’s post hoc test. A $p$ value <0.05 was indicated significant differences from DMSO group.

Results and Discussion
Cytotoxic test of HDXs at 10 μM or 100 μM for 24 h was demonstrated in Fig. 1. The results indicated that treatment with these two concentrations of all HDXs, except 1,3,6,8-tetraHDX 100 μM, produced non-cytotoxic to Caco-2 cells.

Figure 1 Cytotoxicity effects of HDXs showed the optical density at 570-620 nm of formazan dissolved from Caco-2 cells treated for 24 h with 10 or 100 μM of
different forms of HDXs or vehicle (DMSO). * is significantly different at \( p<0.05 \).

The cells treated with TNF-\( \alpha \) for 4 and 24 h produced a marked decrease in TER. Moreover, all HDXs studied were found to decrease, to a lesser extent, the TER especially at 24 h as compared to DMSO. However, incubation of 1-monoHDX and 1,3,6,8-tetraHDX for 4 h did not affect the TER (Fig. 2). The cells challenged with 1-monoHDX or 1,3,6,8-tetraHDX treatment prior to TNF-\( \alpha \) challenge prevented the decreased TER induced by TNF-\( \alpha \) at 24 h (Fig. 3). The decreased TER could be due to stimulation of transcellular ion transport and/or intestinal barrier disruption allowing macromolecules passing through the paracellular route (6). TNF-\( \alpha \) caused tight junction barrier disruption in a variety of cultured epithelia (7). The present results of cytotoxic assay and TER study suggest that all HDXs did not have cytotoxicity on the intestinal epithelial cell, and that 1-monoHDX maintained barrier function under normal and prevented the TNF-\( \alpha \) induced barrier disruption.

In conclusion, among HDXs being studied, only 1-monoHDX shows prominent effects in promoting intestinal barrier and protecting against bacterial endotoxin consequence. The mechanism of action of HDXs remains to be studied for a candidate drug use for treatment of gastrointestinal disorders.

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**References**

The effects of Hydroxyethyl starch (HES) on quality of equine sperm following cryopreservation: a preliminary study

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Keywords: horse, frozen semen, Hydroxyethyl starch (HES)

Introduction
Cryopreservation of equine semen is an important tool for artificial insemination. However, equine sperm are intolerant to cryopreservation due to several reasons such as individual variation with different membrane properties (1, 2, 3). Although a number of protocols have been used to cryopreserve equine semen, overall post-thawed quality of equine sperm is currently poor (4). There are two types of cryoprotective agent (CPA) including penetrating and non-penetrating CPAs. These CPAs protect sperm against cryoinjuries during cryopreservation process (5). Hydroxyethyl starch (HES) is a non-penetrating CPA which has been used for freezing several types of cells, especially red blood cells (6). HES is a synthetic modified polymer based on purified starch of corn or potatoes. The first synthetic of HES was used to replace human albumin or used for a blood expander. The HES will absorb water molecules up to 0.5g water per 1 g and reduce the intracellular ice crystal formation when cooling (7). This study aimed at examining the effect of different concentrations of HES on quality of frozen-thawed equine sperm following cryopreservation and thawing.

Materials and Methods
The semen was collected from 6 proven-fertility stallions (18 replicates) at The First Livestock and Agriculture Division, Veterinary and Remount Department, Thai army, Kanchanaburi province and subsequently cryopreserved using freezing extender containing different concentrations of HES (2.5%, 5%, 10%, 15% and 20%). Cryopreservation in an absence of HES (0% HES) served as a control group. The freezing extender was formulated using glucose EDTA based medium supplemented with 20% (w/v) egg yolk and 4% (v/v) glycerol. After thawing, sperm were examined for motility, viability, membrane functionality (hypoosmotic swelling test, HOST), DNA integrity, and acrosome integrity.

Results and Discussion
Overall, HES improved quality of frozen-thawed equine sperm. However, the protective effect of HES to protect sperm against cryoinjury was in a manner of concentration dependence. The sperm frozen with 2.5% HES significantly had higher motility compared with the control (41.5±2.5 % vs. 33.8±2.7, p<0.05). This post-thaw sperm motility is acceptable for artificial insemination (8). Similarly, the 2.5% and 5% HES based extenders also significantly improved post-thaw viability and acrosomal integrity over the control (P<0.05). However, the membrane functionality and DNA integrity were not significantly improved (P>0.05).

It is possible that the HES at optimal concentration could protect the sperm membrane by attenuation of ice formation around sperm membrane. However, this strategy is insufficient to completely eliminate the intracellular ice formation that occur during cryopreservation. This hypothesis is in an agreement with the findings that the HES did not significantly reduce DNA damage of the frozen-thawed equine sperm (6).

In all cases, high concentration of HES (10%, 15% and 20%) adversely affected to quality of froze-thawed equine sperm. The HES at 2.5% and 5% supplemented into freezing extender improved post-thawed quality of equine sperm. However, longevity and fertility tests will need to be performed.

Acknowledgements
The author would like to thank the Livestock and Agriculture division, veterinary and remount department, Kanchanaburi province for semen samples collection. The students and staff at the Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University. This work was support by The 90th Anniversary of Chulalongkorn University (Ratchadaphiseksomphot Endowment Fund).

References
Effect of lactic acid bacteria strains on reduction of plasmid mediated colistin resistant gene (mcr-1) conjugation in *Escherichia coli*

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**Keywords**: lactic acid bacteria, cell-free supernatants, Colistin resistance, mcr-1

**Introduction**
Colistin or polymyxin E is the cationic polypeptide antibiotics which is a broad-spectrum antibiotic against Gram-negative bacteria especially *Enterobacteriaceae* (1,2). Colistin is chosen as the last-resort antibiotic against the bacteria resistant to carbapenem and extended spectrum beta-lactamase producing ESBL *Enterobacteriaceae* in life-threatening clinical case (1). In the recent years, the incidence of *mcr*-1 associated nosocomial infection and its contamination into livestock environment (3) have been reported in several countries i.e. Japan, China, Germany, Switzerland, Algeria, including Thailand (4), that directly impacted to national policies. In general, the *mcr*-1 gene locates on an extrachromosomal gene called plasmid that is transferable via microbial sex phenomenon (2). To the best of our knowledge, lactic acid bacteria (LAB) is one of the probiotic members which confer several advantages including enhance nutrient utilization, elevate immune activities, antagonism to intestinal pathogens (5). Besides, live and supernatant of probiotic could limit the spread of antibiotic resistance genes, for example, thermostable metabolites produce of *Bifidobacterium* spp. significantly reduced among antibiotic resistant gene transmission (beta-lactam, kanamycin, and tetracycline) amongst *Enterobacteriaceae* (6). The objective of this study was to evaluate the effect of cell-free supernatant (CFS) of LAB strains; *L. plantarum* 22F, 25F, 31F, *Pediococcus acidilactici* 72N and *P. pentosaceus* 77F obtained from our previous study (7) to the plasmid conjugative rate in colistin resistant *E. coli* positive to *mcr*-1 gene. The effect of CSF was also compared between acidified and neutral conditions.

**Materials and Methods**

*Cell free supernatant (CFS) preparation;* Thai LAB strains comprising *L. plantarum* 22F, 25F, 31F, *Pediococcus acidilactici* 72N and *P. pentosaceus* 77F and the commercial strains *P. acidilactici* DSM 20284 and *L. plantarum* subsp. *plantarum* JCM 1149 were grown on MRS (de Mann Rogosa Sharpe) agar (Becton, Dickinson and Company, Sparks, USA) at 37°C, 5% CO₂ for 48 hr. then the colonies were transferred into MRS broth and incubated for overnight. After incubation, the suspensions were centrifuged at 4500 rpm, at 4°C for 15 min and cell debris were discarded. The suspension was adjusted the pH to 5.7 by 1M NaOH before filtration by surfactant-free cellulose acetate (Corning, NY, USA).

**Donor strain;** *E. coli* E5 isolated from Thai healthy fattening pigs since 2014 was selected by the criteria including its plasmid replicon profile, *mcr*-1 gene positive and phenotypic colistin resistance (MIC value at 8 μg/ml).

**Recipient strain;** *E. coli* J53 strain was used which exhibited sodium azide resistance at over 512 μg/ml. It also was susceptible to colistin at lesser than 2 μg/ml.

**In vitro conjugation;** Donor and recipient strains were grown on LB (Luria-Bertani) agar at 37°C for 16-18 hr. The donor and recipient strains were mixed in LB broth at 10⁻⁷ final concentration. At 1:16 dilution of CFS in each LAB with neutralized pH was confirmed the lack of bactericidal effect to *E. coli* that being ready to use for the conjugation assay. In each LAB strains, 0.5 microliter of the CFS suspension was mixed and incubated at 37°C for 24 hours, while LB broth was used as a placebo in control. Transconjugants were selected on LB agar supplemented with sodium azide (200 μg/ml) and colistin (2 μg/ml). Transfer frequencies were determined by using efficacy of conjugation (number of transconjugants on selective media/ number of donor) (8).

**Data analysis:** The transfer frequencies were statistically evaluated using SPSS version 22 for one-way ANOVA (SPSS, Chicago, IL, USA), and the significance was defined by *P* < 0.05.

**Results and Discussion**
CFS from LAB strains were determined for decreasing of conjugative rate of plasmid contained *mcr*-1gene in *E. coli*. At 1:16 diluted suspensions (pH 5.70-5.92), the conjugative rates were significantly decreased by all strains (Table 1). In this study, the CFS of *L. plantarum* 22F demonstrated the highest reduction on conjugative rate (approximately 100 times whereas the others gave about 10 times of reduction of gene transfer.)
Table 1  Transferability of mcr-1 gene from E. coli E5 to E. coli J53 under CFS suspensions derived from the seven LAB strains.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.79x10^{-4} ± 0.0003</td>
</tr>
<tr>
<td>L. plantarum 31F</td>
<td>2.75x10^{-4} ± 0.00003*</td>
</tr>
<tr>
<td>L. plantarum 25F</td>
<td>1.91x10^{-4} ± 0.00002*</td>
</tr>
<tr>
<td>L. plantarum 22F</td>
<td>8.89x10^{-6} ± 0.000007*</td>
</tr>
<tr>
<td>P. pentosaceus 77F</td>
<td>1.1x10^{-5} ± 0.00001*</td>
</tr>
<tr>
<td>P. acidilactici 72N</td>
<td>1.08x10^{-5} ± 0.000001*</td>
</tr>
<tr>
<td>L. plantarum JCM1149</td>
<td>1.8x10^{-4} ± 0.000009*</td>
</tr>
<tr>
<td>P. acidilactici DSM 20284</td>
<td>1.8x10^{-5} ± 0.000003*</td>
</tr>
</tbody>
</table>

* Significant difference at P < 0.05

Interestingly, the conjugative rate between control and treatment groups was similar and became lack of the ability once the CSF were adjusted pH to 6.5 ± 0.1 (Table 2) (P < 0.05).

Table 2  Transferability of mcr-1 gene from E. coli E5 to E. coli J53 in neutralized condition.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.79x10^{-4} ± 0.0003</td>
</tr>
<tr>
<td>L. plantarum 31F</td>
<td>4.84x10^{-4} ± 0.00002</td>
</tr>
<tr>
<td>L. plantarum 25F</td>
<td>2.8x10^{-4} ± 0.00007</td>
</tr>
<tr>
<td>L. plantarum 22F</td>
<td>3.67x10^{-4} ± 0.00002</td>
</tr>
<tr>
<td>P. pentosaceus 77F</td>
<td>3.6x10^{-4} ± 0.00005</td>
</tr>
<tr>
<td>P. acidilactici 72N</td>
<td>2.07x10^{-4} ± 0.0001</td>
</tr>
<tr>
<td>L. plantarum JCM1149</td>
<td>3.76x10^{-4} ± 0.00005</td>
</tr>
<tr>
<td>P. acidilactici DSM 20284</td>
<td>3.09x10^{-4} ± 0.00001</td>
</tr>
</tbody>
</table>

From our findings, it was hypothesized that some chemical substances might be well-functional in slight acid condition (pH 5.70-5.92) or acidity in CSF directly retarded the conjugative process, itself. Previously, the acid pH was an important parameter to transconjugant number, however its mechanism is still unclear (9). In addition, certain substances including organic solvents or probiotic metabolites could be influential the conjugation components such as the formation of donor sex pili or cell surface of the recipient strain (6,9).

Further work requires to be done with more numbers or variety sources of bacterial strains containing gene associated colistin resistance for insight the properties of our LAB strains. In summary, the CFS of all our LAB strains could reduce the conjugative rate for plasmids mediated colistin resistance (mcr-1 gene) in E.coli. L. plantarum strain 22F showed the most efficiency to reduce risk of mcr-1 gene transfer.

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