The 20th Khon Kaen Veterinary Annual International Conference (KVAC) 2019

"Global Challenge on Veterinary Profession and Education"

March 21 - 22, 2019

AVANI Khon Kaen Hotel & Convention Centre
Khon Kaen, THAILAND
The 20th Khon Kaen Veterinary Annual International Conference (KVAC) 2019

March 21-22, 2019

AVANI Khon Kaen Hotel & Convention Centre
Khon Kaen, Thailand

Organized by

Faculty of Veterinary Medicine, Khon Kaen, Thailand

with the support of

Khon Kaen University

KKU Vet Alumni

Department of Livestock Development

Thai Swine Veterinary Association

Research Group for Animal Health Technology

Research Group on Toxic Substances in Livestock and Aquatic Animals
Abstracts and full-text papers of all oral and poster presentation published in The 20th Khon Kaen Veterinary Annual International Conference (KVAC) 2019: “Global Challenge on Veterinary Profession and Education” proceeding passed a peer review process by the scientific committee of the conference. Proceedings did not require all authors of a research paper to sign the letter of submission, nor do they impose an order on the list of authors. Submission to the conference is obtained by scientific committee meaning that all the listed authors have agreed all of the contents. The corresponding (submitting) author is responsible for having ensured that this agreement has been reached, and for managing all communication between the committee and all co-authors, before and after publication. Each author is responsible for the content and accuracy of the entire manuscript.

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Dear Conference participants,

My name is Assoc. Prof. Dr. Charnchai Panthongviriyaakul, the President Khon Kaen University. On behalf of Khon Kaen University, it is my great honor to organize “The 20th Khon Kaen Veterinary Annual International Conference or KVAC 2019” in this year and welcome all the honored guests to our beloved Khon Kaen Province.

At present, Khon Kaen University is going on the 56th year Anniversary of the establishment. We have been doing our best benefits in all appointed missions for 55 years. The missions include producing nearly 200,000 high-quality scholars to serve the society in all levels - local, regional, national, or international, and producing a good number of useful researches for solving issues in broad and various aspects, providing academic services for maintaining the well-being of individuals; especially in the north-eastern region and neighbor countries, which corresponds with the initial intention for establishing of the University by previous King Rama IX of Thailand. Recently on January 30, 2019, Khon Kaen University is one of eleven organizations in Thailand who received the Thailand Quality Class: TQC.

Khon Kaen University also plays an important role in up keeping the local art and culture and continually publicizing it. KKU considers the academic service for the society as an indispensable mission and has been operating in this matter continually through its internal units for a very long time, which earns the University a great deal of compliment from both external parties and people in general. KKU’s slogan, “Social Devotion”, also reflects our determined resolution to fulfill this mission seriously. Finally, on behalf of KKU, I would like to pay a high gratitude to the Faculty of Veterinary Medicine for organizing this wonderful international-level academic conference, the guest speakers, the event organizers, and all conference attendees, for all your kind support, cooperation, and collaboration to this event, Thank you.

May I once again welcome all of you to Khon Kaen province and declare the 20th Khon Kaen Veterinary Annual International Conference open and wish this meeting to meet all objectives. Thank you.

Assoc. Prof. Dr. Charnchai Panthongviriyaakul
President of Khon Kaen University
March 21st, 2019
Dear all Guest Speakers, Lecturers, Researchers, and Conference Attendees,

On behalf of faculty of Veterinary Medicine, Khon Kaen University, it is my great honor for us again to organize “The 20th Khon Kaen Veterinary Annual International Conference or KVAC 2019” in this year at AVANI Hotel and Conventional Centre during 21-22 March 2019 and welcome all the honored guests to our beloved Khon Kaen Province.

Faculty of Veterinary Medicine, Khon Kaen University is going on the 32nd year Anniversary of the establishment with devoting our knowledge and experience in academic services, researches and social responsibility especially in the north-eastern region and neighbor countries. The 20th Khon Kaen Veterinary Annual International Conference or KVAC2019 is one the most significant outcome that we have a responsible for our social responsibility. The KVAC2019 includes update on One-health, veterinary education and state-of-the-art in diagnostic and treatment in veterinary medicine and also allow scholars and graduate students to present their outstanding work and researches in the field of veterinary sciences.

Finally, on behalf of faculty of Veterinary Medicine, KKU, I would like to say thank you all Guest Speakers, Lecturers, Researchers, Conference Attendees, Co-organizers and all sponsors for all your kind support, cooperation, and collaboration to this event, Thank you.

Associate Professor Dr. Chuchart Kamollert
Dean, Faculty of Veterinary Medicine, KKU
March 21st, 2019
Welcome Speech from Chairperson

Dear All participants KVAC2019

It gives us immense pleasure to inform you that the faculty of veterinary medicine, Khon Kaen University together with the co-organizers the department of Livestock Development (DLD), the Veterinary Medicine KKU Alumni Association, the Swine Veterinary Association, the Animal Health Technology, KKU and the Research group on Toxic Substances in Livestock and Aquatic Animals, KKU is organizing “the 20th Khon Kaen Veterinary Annual International Conference or KVAC 2019” this year. This conference is held in AVANI hotel & Conventional center, Khon Kaen from 21st -22nd March, 2019

For this year, we have about 550 participants with a conference theme of “Global Challenge on Veterinary Profession and Education” meaning that veterinary profession is affected by the global issues in both animals and human health. Therefore, the conference and scientific seminar will focus on the improvement of the role of veterinary profession on One-health, veterinary education and recent developments, trends and technologies available for disease prevention, diagnosis and treatment including productivity and quality of animals and animal products.

The main objective of this conference is to bridging government-academicians-scholars-business sector and veterinary practitioner’s participation to achieve the goal for better quality of service of veterinary profession by presenting and sharing information during the conference and scientific seminar.

On behalf of the KVAC2019 organizing committee, faculty of Veterinary Medicine, Khon Kaen University, co-organizers, the key note speakers, speakers and all participants in this conference, we are very appreciating the kindness of your participations and looking forward to welcome all of you at the KVAC2019.

Thank you very much

Assistant Professor Dr. Prawit Butudom
Chair person, KVAC2019
March 21st, 2019
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7. Miss Nathapop Sechang
8. Mrs. Aoythip Subso
## Keynote Topics

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<td><strong>Veterinary Curriculum Modernization (Curriculum and Outcomes Assessments, Comprehensive Written Exam/Osce) An Experience from the US Veterinary Schools</strong></td>
<td><strong>Jonathan H Foreman (DVM, MS, Dipl. ACVIM)</strong></td>
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<td>Associate Dean for Academic &amp; Student Affairs</td>
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<td></td>
<td>Professor, Veterinary Clinical Medicine</td>
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<td></td>
<td>Professor, Veterinary Teaching Hospital</td>
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<td></td>
<td>Department of Veterinary Clinical Medicine, College of Veterinary Medicine,</td>
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<td></td>
<td>University of Illinois at Urbana-Champaign, Urbana, IL, USA</td>
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<tr>
<td><strong>Problem-Based Learning (Pbl) to Improve Day-One Competencies of Veterinary Graduates</strong></td>
<td><strong>Md. Ahasanul Hoque (DVM, MS, PhD)</strong></td>
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<tr>
<td></td>
<td>Professor, Department Of Medicine And Surgery</td>
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<tr>
<td></td>
<td>Former Dean, Faculty Of Veterinary Medicine, Chittagong Veterinary And Animal</td>
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<td>Sciences University, Chittagong-4225, Bangladesh</td>
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Problem Based Learning is a key to attaining Day One competencies for veterinary graduates

Md. Ahasanul Hoque\textsuperscript{1*}, Abdul Ahad\textsuperscript{1} and M Sawkat Anwer\textsuperscript{2}

\textsuperscript{1}Chittagong Veterinary and Animal Sciences University, Bangladesh and \textsuperscript{2}Tufts University Cummings School of Veterinary Medicine, USA

\textsuperscript{*}E-mail (Presenting author): md.hoque@my.jcu.edu.au

Problem-Based Learning (PBL) is a student-centered cooperative and active learning approach. In this process students accomplish their learning goals through problem solving, self-directed learning, self-motivation and collaboration. It has been incorporated for the first time in Doctor of Veterinary Medicine (DVM) curriculum at Chittagong Veterinary and Animal Sciences University (CVASU) in the session of 2013-14, Bangladesh with the support of OIE Veterinary Education Twinning Project between CVASU and Tufts Cummings School of Veterinary Medicine (TCSVM), USA. CVASU faculty provided PBL training to four other veterinary schools in Bangladesh and two of those schools have recently incorporated PBL in their Doctor of Veterinary Medicine Curricula. PBL helps enhance student learning ability and knowledge base by solving real-life problems using self-learning and team-work approaches. Our experiences in PBL case development, implementation, assessment, monitoring and feedback (both from students and facilitators) will be presented at the \textsuperscript{20}\textsuperscript{th} Khon Kaen Veterinary Annual International Conference (KVAC) (Theme: “Global Challenge on Veterinary Profession and Education”) to be held in 21-22 March 2019. Our analysis showed that PBL had a significant positive impact in pave way OIE-recommended Day One Competencies for veterinary graduates.

\textbf{Keywords:} Problem based learning, Day One Competencies, Veterinary Graduates, Bangladesh
## Panel Discussion: An Update on Rabies in Thailand and Clinical Practice Guideline (CPG)

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<tr>
<td><strong>Professor Dr. Sanipa Suradhat</strong></td>
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<tr>
<td>Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand</td>
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<td><strong>Dr. Issara Ponyawon</strong></td>
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<tr>
<td>Director of Animal Health, Office of Regional Livestock 4, Department of Livestock Development (DLD), Khon Kaen province, Thailand</td>
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<tr>
<td><strong>Assistant Professor Ru-japorn Macotpet</strong></td>
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<td>Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand</td>
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## Exotic Pets and Wildlife

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<td>Dr. Warut Wiboonkullaphan</td>
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<td>How to Approach, Handle and What to Do with Sick Birds?</td>
<td>Dr. Chayanee Veerakul</td>
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<td>Common Diseases in Pet Birds</td>
<td>Dr. Pathavee Nimsakul</td>
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# Thai Swine Veterinarian Association & Research Group of Animal Health Technology

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Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, Il, USA |
| Nutritional Associated with Colic           | **Prof. Dr. Jonathan H Foreman**  
Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, Il, USA |
| Colic: Medical or Surgical Treatments       | **Prof. Dr. Jonathan H Foreman**  
Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, Il, USA |
| Clinical Problems Associated with Equestrains (Eventing & Reining) | **Prof. Dr. Jonathan H Foreman**  
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<td>Dr. Trasida Ployngam&lt;br&gt;Faculty of Veterinary Medicine, KKU</td>
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Faculty of Veterinary Science, CU |
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Faculty of Veterinary Medicine, KKU |
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Faculty of Veterinary Medicine, KU |
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Faculty of Veterinary Medicine, KU |
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Faculty of Veterinary Medicine, KU |
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Faculty of Veterinary Science, CU |
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Faculty of Veterinary Medicine, KU |
| Clinical Practice of Reproductive Problems in Cattle: Retained Placenta, Metritis, Cystic Ovary, Repeat Breeder | Dr. Panupan Pongpeng  
DLD |
| Clinical Practice of Reproductive Problems in Cattle: Retained Placenta, Metritis, Cystic Ovary, Repeat Breeder | Assist. Prof. Dr. Chaiyapas Thamrongyoswittayakul  
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<td>Dr. Sakchai Jarernsiripornkul&lt;br&gt;MBA, KKU</td>
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## Vet Hospital Management & Wildlife Clinic

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Abstracts and Notes from Invited Speakers

Preparation of African Swine Fever Contingency Plan, Thailand

Chanin Nachom

Veterinary Professional, Region Livestock Office, Region 4, Khon Kaen, Thailand

Abstract

In August 2018, an African Swine Fever (ASF) outbreak was reported in China, FAO predicted that countries in Asia were in high risks situation. After that, the virus has spread throughout the country of China. Recently, the outbreak of ASF in Vietnam, first detected on February 2019, is accelerating and will almost certainly emerge in other countries in Asia. The recent occurrence and spread of ASF in China and Vietnam is perceived as a serious threat for the swine industry, small commercial and backyard swine holdings as well as the value chains in Thailand. According to ASF virus can survive long periods in contaminated environment, high mortality rate, no effective vaccine has yet been approved and cannot treat when infection.

Therefore, to prevent the economic loss from ASF in pig production in Thailand, Department of Livestock Development (DLD) has prepared a national ASF contingency plan to control and prevention ASF in the country: This plan was separated into three periods (Before, responding and after ASF outbreak occurred) It composed of (A) Driving plan in term of man materials and budgets (B) Early detection of the disease and rapid laboratory diagnosis, (C) Disease surveillance and notification system, (D) public-private participation and collaboration, (E) Strengthening biosecurities activities to prevent introduction and spread of ASF, (F) Emergency response plan. (G) Conducting advocacy and capacity building to provide knowledge and disease information as well as disease preparedness and (H) Rehabilitation of farmers. Due to a spread of ASF outbreak is closed to Thailand, to prevent the introduction of ASF into Thailand was the most important measure by assessing the risks pathway of ASF entry, prohibition of imports from affected countries and restriction of live animals and pork products movement. ASF is one of the more difficult transboundary animal diseases to control as the virus and still be infectious in cured pork products, which promotes disease transmission through swill feeding. In summary, effective prevent measures are essential to control and avoid the introduction of ASF into Thailand. At high-alert we have learnt from the Chinese and Vietnamese experience in order to maximize ASF preparedness and emergency management, improve early detection and rapid response capacity.

Keywords: African swine fever, Control, Contingency plan, Thailand
Infectious Bursal Disease: Review and Update

Jiroj Sasipreeyajan

Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

Abstract

Infectious bursal disease (IBD) or Gumboro is an important disease of chickens and it is one of the important immunosuppressive diseases. The disease is caused by infectious bursal disease virus (IBDV), an Avibirnavirus. It has been classified as part of the Birnaviridae family. The virus has been divided into 3 strains, according to their virulence; 1) the classical strain, 2) the variant strain and 3) the very virulent strain. In Thailand, only the classical and the very virulent strains have been identified. Infected chickens of less than 3 weeks old will show no clinical signs but severe immunosuppressive conditions will have occurred. Clinical signs can be seen when chickens are infected at the age of older than 3 weeks old. Infected chickens show clinical signs of depression, ruffled feathers, anorexia, white watery diarrhea, dehydration and death. Layer-type chickens display severe clinical signs and a higher mortality rate than broiler chickens. Necropsy findings show dehydrated carcasses, hemorrhaging at the pectoral and thigh muscles and the junction of proventriculus and gizzard and swollen kidneys with urate retention in the ureters. Around 3-4 days after infection, hemorrhage or necrosis of swollen bursas and gelatinous, yellowish exudate covering the bursas are found. Atrophy of the bursas is found by day 7-8 of infection. Immunosuppressive conditions will be the consequences of the infection due to B-lymphocytes in the bursa have been damaged by the IBDV. Therefore, the recovered chickens will not respond well to any vaccines and are more susceptible to any kind of infections. IBD can be prevented mainly by 2 parts. The first part is by management procedures which are including biosecurity, effective cleaning and disinfection, controlling all vectors, carriers and reservoirs, longer downtime and practicing all-in, all-out system. The second part is vaccination. Maternally-derived antibodies against IBDV, virulence of modified live vaccine or type of vaccines, date of vaccination and basic biosecurity of the farm play the key role on successful of the vaccination programs. Choice of vaccines and vaccination programs will be presented.
Update on Neoplastic Diseases in Chickens

Aunyaratana Thontiravong

Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
Email address: Aunyaratana.T@chula.ac.th

Abstract

Unlike human neoplastic diseases which mainly occur from non-infectious causes, neoplastic diseases in chickens are primarily caused by virus infection. Three important viruses inducing neoplastic diseases in chickens include herpesvirus associated with Marek’s disease and retroviruses associated with avian leukosis and reticuloendotheliosis. Although, most of the virus-induced neoplastic diseases in chickens are caused by Gallid herpesvirus 2 (GaHV-2), commonly known as serotype I Marek’s disease virus (MDV-1), neoplastic diseases induced by avian leukosis virus (ALV) and reticuloendotheliosis virus (REV) can sporadically be found. Generally, MDV induces rapid onset T cell lymphomas in chickens through action of MDV genes associated with oncogenicity, while avian retroviruses induce late onset lymphomas by proviral insertional activation of the cellular oncogene. Besides causing neoplastic diseases, these viruses can also cause immunosuppression in chickens, leading to increased susceptibility to secondary infection and the reduction of the protective efficacy of vaccine. In addition to single infection, co-infection of these 3 viruses has been commonly detected in chickens in several countries, contributing to increased disease severity and virus transmissibility in infected chickens. MDV is transmitted horizontally through infected dust and dander and can be prevented by vaccination. Meanwhile, ALV and REV spread both vertically and horizontally and are mainly controlled by virus eradication from breeder stock. However, despite widespread MDV vaccination and effective eradication of retroviruses from breeder stock, MDV and retrovirus infected cases have continuously detected worldwide, resulting in significant economic losses in the poultry industry. In Thailand, our recent studies showed that virulent MDV-1 and REV have been continuously detected in chickens showing runting-stunting symptom and tumor-like lesions. Our findings raise the awareness of MDV and REV as causative agents of running and neoplastic diseases in chickens in Thailand and highlight the necessity of the effective measures for disease control and prevention.

Keywords: Avian leukosis virus; Chickens; Marek’s disease; Neoplastic diseases; Reticuloendotheliosis virus
Clinical abnormalities of the 33 newly diagnosed diabetic dogs presented at Small Animal Veterinary Teaching Hospital, Khon Kaen University during January 2016-December 2018 had examined (unpublished data). Clinical signs of diabetic dogs included PU/PD (69.70%), weight loss (63.64%), cataract (45.45%), vomiting (30.30%), and polyphagia (18.18%). The clinical abnormalities, apart from hyperglycemia and glucosuria were:

1. Leukocytosis (20 of 33 dogs; 60.6%)
2. Fasting blood glucose > 600 mg/dL (5 of 3 dogs; 15.15%)
3. Increased ALP activity (22 of 26 dogs; 84.62%)
4. Increased ALT activity (25 of 33 dogs; 75.75%)
5. Metabolic acidosis (4 of 5 dogs; 80%), mean ± SEM of blood pH was 7.236 ± 0.05 and mean ± SEM of serum bicarbonate concentration was 12.66 ± 1.91 mEq/L.
6. Hypokalemia (5 of 5 dogs; 100%)
7. Hypochloremia (3 of 5 dogs; 60%)
8. Hyponatremia (4 of 5 dogs; 80%)
9. Abnormal level of serum cPL activity (16 of 21 dogs; 77.27%)
10. Changes in the ultrasonographic findings (echotexture, size and/or shape) of pancreas (4 of 5 diabetic dogs with abnormal serum cPL activity; 80%)
11. Ketonuria (23 of 33 dogs; 69.7%) and non-ketonuria (10 of 33 dogs; 30.30%)

These abnormalities or complications have related to pathological processes of diabetic dogs. Mostly, acute life-threatening complications of diabetic dogs are diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar state (or syndrome; HHS).

DKA is identified as systemic acidemia due to extreme alterations of metabolic state leading to ketonemia, or ketonuria, metabolic acidosis, dehydration and electrolyte disturbances. A relative lack of insulin results in inability of glucose utilization for energy production in most cells, thus promotes gluconeogenesis and glycogenolysis. In addition, glucagon is responsible for stimulation of lipolysis. Free fatty acids released from adipocytes are metabolized into triglycerides by the liver, or formed into ketone bodies (b-hydroxybutyrate, acetoacetate and acetone). Large amounts of ketone bodies production develop DKA. b-hydroxybutyrate is predominant ketone body produced during DKA. Lack of insulin and the increased counterregulatory (or stress) hormones, including glucagon, epinephrine, cortisol and growth hormone secreted in response to a secondary to stressor can cause the progressive DKA. It had been reported that diabetic keto(acido)sis was associated with a concurrent disease such as infections (24%), neoplasia (24%), pancreatitis (22.2%), renal failure (13%), hypercortisolism (7.4%) or heart failure (7.4%) (De Causmaecker et al., 2009).

DKA is diagnosed by the presence of hyperglycemia, glucosuria, ketonemia or ketonuria, metabolic acidosis, and clinical signs (i.e. lethargy, mental depression, vomiting and weight loss) while dog with diabetic ketosis (DK) does not have acidosis and systemic clinical signs. The cut-off values of serum bicarbonate concentration (≥15 mEq/L) and anion gap (< 20 mEq/L) had utilized for the discrimination of DK from DKA in 72 dogs with diabetic mellitus and it was found that 47 (68.24%) dogs had DK and 25 (34.72%) dogs had DKA.
Tommaso et al., 2009). Diagnosis of DKA should be confirmed by measurement of blood β-hydroxybutyrate level (ketonemia). Measurement of ketonemia is accurate and more effective than measurement of ketonuria (Tommaso et al., 2009). Sensitivity and specificity of ketonemia and ketonuria to diagnose DKA are shown (Table 1).

Only ketonuria or presence of ketone bodies in urine is not straightforward for the diagnosis of DKA. Urine strip test provides a semiquantitative estimate of acetoacetate and acetone in the urine, does not detect the presence of β-hydroxybutyrate. The assessment indirect ketonemia by urine strip test can be underestimated or undetected (Duarte et al., 2002). Ketonuria may be undetected in some dogs with severe DKA. Furthermore, ketonuria measured by urine strip (equal to 3+) may be detected in dog with diabetic ketosis. Therefore, the absence of ketonuria does not exclude dogs with DKA and the presence of ketonuria may lead to false positive diagnose in dog without DKA (Tommaso et al., 2009).

Table 1. Sensitivity and specificity of ketonemia and ketonuria to diagnose DKA in dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut-off value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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</thead>
<tbody>
<tr>
<td>Ketonemia (β-hydroxybutyrate level)</td>
<td>2.3 mmol/L</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ketonemia (β-hydroxybutyrate level)</td>
<td>3.8 mmol/L</td>
<td>72&lt;sup&gt;a&lt;/sup&gt;-76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95&lt;sup&gt;a&lt;/sup&gt;-95.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ketonemia (β-hydroxybutyrate level)</td>
<td>4.3 mmol/L</td>
<td>64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ketonuria&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1+</td>
<td>92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ketonuria&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3+</td>
<td>44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> Urine strip test  
<sup>b</sup> Duarte et al., 2002  
<sup>a</sup> Tommaso et al., 2009

HHS is characterized by hyperglycemia and hyperosmolality leading to hypovolemia or severe dehydration by which osmotic diuresis promotes the progression of this condition. HHS in dog can be identified by plasma osmolality ≥ 325 mOsm/kg (Trotman et al., 2013). Insulin deficiency and the inhibition of lipolysis resulted from hepatic glucagon resistance are involved in the pathogenesis of HHS.

Management of complicated diabetic mellitus

1. Fluid administration is an essential therapy for complicated diabetic dog. The objectives are to correct dehydration deficit and maintain vascular volume, improve renal perfusion, reduce blood glucose and ketone concentration, and correct acidemia by increase in urinary glucose and ketone excretion.
   1) Dog with hypovolemic state, crystalloid intravenous shock fluid therapy should be initiated, then the total needs for dehydration deficit, maintenance requirement and ongoing loss.
   2) A crystalloid fluid should be given for several hours before initiating insulin therapy to prevent a rapid decrease in blood glucose.
   3) Rehydration in dog with HHS and concurrent chronic heart failure must be over 12-24 hours.

2. Managing electrolyte abnormalities
   1) Correction of potassium deficiency should be considered before initiating insulin administration. Basal serum potassium concentration before insulin therapy should be at least 3.5 mEq/L. Potassium level may decrease rapidly with insulin therapy and should be monitored frequently every 6-8 hours (Koenig 2013).
   2) Decrease in serum sodium concentration should not exceed 0.5 mEq/L per hour in hypernatremic dogs.
   3) Bicarbonate therapy is not generally reserved to treat diabetic dogs with DKA. Sodium bicarbon-
ate administration should be considered in diabetic dogs with severe acidemia (blood pH<7.1, bicarbonate concentration < 8 mEq/L) and signs consistent with severe metabolic acidosis such as refractory hypotension, arrhythmias and stupor or coma (Koenig 2013).

3. Insulin therapy

1) Insulin therapy should be considered after the correction of hypovolemia as well as severe dehydration and electrolyte disturbances. The purpose of insulin administration is to slowly lower blood glucose concentration less than 50-75 mg/dL/hour. Insulin promotes glucose uptake to the cells and inhibits further lipolysis and ketogenesis. However, insulin therapy is less critical for reversal of HHS, thus the initial dose is lower than the insulin dose for DKA therapy (Koenig 2013).

2) Short acting insulin (regular, lispro) is recommended in dogs with diabetic crisis.

3) DKA dogs, the dosage of regular insulin is 2.2 U/kg in 250 ml 0.9% NaCl intravenous constant rate infusion (CRI) at 10 ml/hour (0.05-0.1 U/kg/hour). Blood glucose concentration should be measured every 2 hours. Outline blood glucose monitoring and insulin dose adjustments are shown (Table 2). Intermittent intramuscular protocol of regular administration, initial dose for DKA is 0.2-0.25 U/kg, then 0.1 U/kg every 2-4 hours. Subsequent insulin doses are increased or decreased by 25%. If blood glucose level belows 250 mg/dL, then dextrose should be added to fluid.

4) HHS dogs, the dosage of regular insulin is 1.0 U/kg in 250 ml 0.9% NaCl intravenous constant rate infusion (CRI) at 10 ml/hour. Blood glucose level is checked every 2 hours. Insulin CRI is adjusted as necessary (Table 2). Protocol for IM administration is 0.1 U/kg of regular insulin, then 0.05 U/kg every 2-4 hours. Subsequent insulin doses are increased or decreased by 25%. If blood glucose belows 250 mg/dL, then dextrose should be added to fluid.

Table 2. Blood glucose monitoring and insulin dose CRI adjustment

<table>
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<tr>
<th>Blood glucose (mg/dL)</th>
<th>Insulin CRI (ml/hr)</th>
<th>Dextrose administration</th>
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<tbody>
<tr>
<td>&gt;250</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>200-250</td>
<td>7</td>
<td>Plus 2.5% dextrose</td>
</tr>
<tr>
<td>150-199</td>
<td>5</td>
<td>Plus 2.5% dextrose</td>
</tr>
<tr>
<td>100-149</td>
<td>5</td>
<td>Plus 5% dextrose</td>
</tr>
<tr>
<td>&lt;100</td>
<td>0</td>
<td>Plus 5% dextrose</td>
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References


Renal Failure Management in Dogs and Cats

Chalermpol Lekcharoensuk

Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University

Abstract

Basic knowledge for diagnosis, treatment, and management of renal failure is consisted of applied anatomy, applied physiology, clinical pathology, applied pharmacology, image interpretation, and internal medicine. The clinical pathology is emphasized on urinalysis, blood gas analysis, complete blood count, and blood biochemistry interpretation. The internal medicine including fluid therapy, acid-base balance correction, and clinical nutrition should be introduced.

The diagnosis of renal failure is followed the International Renal Interest Society (IRIS) guideline. Stage I is explained at non-azotemic stage. The early detection should be the symmetrical di-methyl arginine (SDMA) > 14 µg/dl, when the glomerular filtration rate (GFR) decreased > 40%, and loss of ability of urine concentration, GFR decreased >67% in dogs. Stage II is identified as mild renal azotemia with upper bound creatinine =2 mg/dl in dogs and to 2.8 mg/dl in cats. Stage III is classified as moderate renal azotemia with creatinine = 2.1-5.0 mg/dl in dogs and = 2.9-5.0 mg/dl in cats. Stage IV is the last stage with creatinine > 5.0 mg/dl and prone to have uremic crises.

The treatments are comprised of specific, symptomatic, supportive, and palliative treatment. The symptomatic and supportive treatment will be introduced the most. The concept of treatment is in titled “WECARE”. The “W” is defined as water or fluid therapy. The “E” comes from electrolyte management. The “C” is calorie management. The “A” is acid-base imbalance correction. The “R” is retention of waste handle. And the “E” is endocrin management.
Metabolic Disorders in Small Dairy Herds: Research and Practical Approaches

Theera Rukkwamsuk* and Supawit Triwutanon

Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen, Nakhon Pathom 73140, Thailand
*Corresponding Author E-mail: theera.r@ku.ac.th

Abstract

Metabolic diseases are of great economic impact in dairy production. Affected dairy cows produce suboptimal milk yield, perform low reproductive efficiency, and possibly increase vulnerability of other diseases or disorders during periparturient period. The majority of dairy production in Thailand belongs to small-farm holders, in which less than 20 lactating dairy cows are kept for milk production. In general, small-scale farmers have limited knowledge on nutrition and health management; therefore, these cows are prone to suffer from any mismanagement related to metabolic disturbances. In western countries where dairy production is far advanced, metabolic disorders are related to high milk production. Small-scale dairy farms in Thailand typically produce relatively lower milk yield; however, dairy cows remain confronting metabolic disorders. Important metabolic diseases in dairy herds include fatty liver, ketosis, rumen acidosis and milk fever. Prevention and control of these disorders require understanding of the mechanisms of these pathophysiological changes as well as the management practices of the dairy farms in different situations. Therefore, approaches to tackle these problems depend largely on the production management, and require information from both research and practical observation.

The achievement of production cycle in dairy cows could be determined by the milk production level, the postpartum reproductive performance, and the absence of periparturient pathological disorders. In comparison with the dairy industry in western countries, Thai dairy farming, though established over 50 years, remains in the process of development and improvement. Most dairy farms in Thailand are small to medium scale, and the production management depends on the farmers’ experiences, particularly on feed and feeding management. In general, most small to medium scale dairy farms provide feed concentrates after milking period followed by roughages twice a day. The amount of concentrates offered per day is approximately calculated as a half of the amount of daily milk produced by the cows. However, as the roughage quality is relatively low, the farmers have to add more concentrates in order to meet the requirement of the cows. This practice, therefore, may relate to increased metabolic disorders during periparturient period.

Transition period is known as the period between 3 weeks before and 3 weeks after parturition. This period is challenging, because there are a number of physiological changes that cows have to cope with. Cows that could go smoothly through this transition period could have less periparturient problems, and as a consequence they could satisfactorily produce milk, return to fertility, and become healthy. Therefore, good management during transition period of dairy cows are recommended in practice. In term of prevention of metabolic disorders, i.e. fatty liver, ketosis, rumen acidosis and milk fever, management of transition period aims to adapt rumen health, reduce the negative energy balance, and stimulate calcium homeostasis pathway.

Transition management has been intensively studied and results have been published worldwide. However, applying those published results, especially from western countries, may not be always appropriate unless the conditions of the farms are similar to those published reports. It is necessary to integrate results from western and tropical (Thailand) countries in order to understand the underlying mechanism of the pathophysiology of metabolic disorders during the transition period, hence practically providing prevention and control strategies to the farmers.

Keywords: Dairy cow, Fatty liver, Ketosis, Milk fever, Rumen acidosis
Implementation of Dairy Herd Health and Production Management Program for Decreasing of Days Open in Dairy Herds

Chaiwat Jarassaeng1*, Awirut Wichaiwong2, Weerapol Taweenan3, Saksiri Sirisathein1

1Theriogenology Unit, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
2Animal Hospital, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
3Pathobiology Unit, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
*Corresponding author Email: chajar@kku.ac.th

Abstract

Dairy herd health and production management program (DHH&PM) is the key success in dairy production. The program has been used in many countries for more than a century and widely accepted to provide benefits to dairy farms. In Thailand, although DHH&PM has been introduced to dairy farms for more than 30 years there has been no successful result. The dairy herd health unit (DHHU) was established by the department of livestock development (DLD) to be operated with 37 dairy co-operatives all over the country, however, the outcome has not achieved satisfaction to farmer mainly because of the demand on data collection and investigation. The project of “implementation of dairy herd health and production management program for decreasing of days opened in dairy herds” was funded by the National research council of Thailand (NRCT) to employ the DHH&PM-based approach to Khon Kaen dairy co-operative for solving the problem of long period of days open after an outbreak of foot and mount diseases (FMD) in that area. The activity of project included providing new knowledge on reproductive management and hormonal program application. The results showed that the days open was decreased from 236, 236, and 230 days to 156, 187, and 170 days in large, medium, and small holder farms, respectively. Moreover, the incidences of metritis were reduced from 1.32 cases to 0.72 cases and mastitis were reduced from 2.68 cases to 1.80 cases per farm. The DHH&PM was showed to produce an overall benefit of the project for more than 13 million baht. The successful results of this project suggested that the protocol for introducing the DHH&PM to the farmers should be based on the willing of the farmers to solve their problems rather than data collection and investigation.

Keywords: Herd health management program, Dairy farms
Clinical Practice of Reproductive Problems in Cattle

Suneerat Aiumlamai

Faculty of Veterinary Medicine, Khon Kaen University

The reproductive performance of dairy cattle in Thailand was generally lower than the target. A recent DLD report in 2016 on reproductive performance of dairy cows showed that calving to first service, calving to conceive, calving interval, and age at first calving were 133, 199, 466 days and 33 months, respectively. Factors affecting fertility and production of dairy cattle in Thailand are nutrition deficiency, improper feeding, reproductive diseases, reproductive disorders, and heat stress, as well as improper artificial insemination and health services (1). Several studies showed that the conception rate was significantly decreased during hot and humid climate at which THI (temperature and humidity index) was higher than 75. Studies on metabolic profiles revealed the presence of subclinical ketosis/negative energy balance and rumen acidosis (laminitis). The prevalence of IBR, BVD, neosporosis, ureaplasmosis, brucellosis, leptospirosis, and FMD, as well as blood parasites in dairy cattle were reported in several studies in Thailand. Premature culling of cows due to reproductive problems is a severe economics loss to the farms.

When investigating the reproductive problems in cattle, both bull and cow must be taken into considered. For bull, problems could be poor breeding soundness or lack of libido. For cow, problems involving nutrition, environment, diseases, and genetics are concerned. However, this article will put an emphasis mainly on the cow problems. Nutrition tends to be the most common causes among other problems. A good body condition score (BCS) prior to calving and post parturition are the vital key to allow cows to breeding back in time. Studies in Thailand clearly showed that cows with BCS less than 2.5 (in 5-scale score) were commonly found during early lactation. Heat stress are known to adversely affect reproduction by reducing embryo viability and sperm quality. A variety of diseases can cause reproductive failure and increase percentage of open cows. Herd-health veterinarians are urged to get involve as early as possible in identifying the cause of problem.

Reproductive disorders in cows such as repeat breeding, anestrus, retention of fetal membrane, metritis, uterine prolapse, cystic ovaries, dysostocia and abortion were commonly found in Thailand (2). These problems could adversely affect the fertility of cattle resulting in poor reproductive performance, particularly when those cases have not been early diagnosed and treated to achieve and maintain good herd fertility. From the recorded service of cattle from the ruminant unit of KKU Veterinary Teaching Hospital in 2018, reproductive disorder was found at 15.8% of all cases, mostly were the open cow problems caused by repeat breeder, anestrus, and cystic ovary (mainly luteal cyst). Others disorders were dystocia (receiving C-section), retained placenta, anestrus, metritis, vaginal/cervical prolapse, abortion, and uterine torsion. Freemartins, mummify fetus and mucometra of uterus were rarely found.

Repeat breeder, retained placenta, metritis, and cystic ovary seem to be mostly found in dairy and also beef cows in Thailand. Therefore, clinical practice of those cases should be discussed on diagnosis, treatment, post care, and prevention. The proper practice is crucial for helping those cows in returning to normal cycle and being fertile soon.

Repeat Breeding (RB) is defined as cows requiring three or more services without conception. Pathological conditions causing RB could be subclinical endometritis, delayed ovulation, and insufficient corpus luteum function. Treatment with intrauterine antibiotic therapy after insemination could be used for subclinical endometritis problem. Administration of GnRH at time of insemination showed better pregnancy rate (3).

For retained placenta, it is recommended not to do manual removal of placenta, be better to allow placenta to separate on its own. Drugs administered directly into uterus after parturition or drugs that increase uterine motility can be used (2, 4).

The treatment for metritis mainly consists of intra-uterine antibiotics. The additional systemic treatment is
needed in cases of toxaemia. Irritating agents such as lugol/iodine solution is not recommended (5). Use disposable uterine catheter for intrauterine infusion (IU), do not use AI gun/AI sheath for IU. Recently, only systemic treatment with an effective antibiotic whose spectrum of activity cover the bacteria causing metritis is suggested. However, information of organisms causing metritis in Thailand is limited (6).

Cystic ovaries show irregular estrus interval, however, 70% of cystic cows are anestrus. The treatment of choice is administration of GnRH followed by administration of prostaglandin at 9-10 days later. Prostaglandin therapy is used to treat cows with luteal-type cysts. However, the response and cure rate depends on luteal tissue and accuracy of diagnosis. If cows are examined early after calving, cystic cows be found then treatment could apply in time and result in good cure rate (7).

In conclusion, reproductive problems are needed to concern the causes and how to do prevention. Herd health and production management program is a tool to reduce the loss.

**Keywords:** Cattle, Reproductive disorders, Reproductive performance, Thailand

**References**


Wildlife: Experiences in Clinical Case Management

Wacharin Hin-on

Thailand Wildlife Services (TWS), Pak Chong Animal Hospital

Abstract

Wildlife cases in veterinary practice include free-living animals and captive wildlife. The most common casualties of wildlife case are wild birds. Therefore, principles of avian and wildlife medicine and surgery are very important to the treatments.

Most of the free-ranging wildlife cases presented to veterinary practitioners have been found with several traumatic conditions, especially car accident. Apart from those, some visit the wildlife clinic with gunshot wound, electrical injury, shell/bone fracture, foreign body, fish hook in legs, horns, and gastrointestinal tract, plastic bag ingestion, cat and dog bite wounds. All of these required the appropriate rescue protocols, such as hand rearing for orphaned wildlife, wildlife capture and trap, wildlife translocation with or without field anesthesia and sedation.

As for captive wildlife, what they require is quite different from those of free-ranging ones. Basic practices, including preventive treatments, such as vaccination program, animal ID and microchip, parasitic controls, sexing (endoscopy), pregnancy detection (ultrasonography, hormone analyses), annual health check, and blood profile to monitor infectious diseases (e.g., Tuberculosis, Brucellosis, Melioidosis, Trypanosomiasis) are in need.

The cares of wild animals are complicated by many factors, especially stress factors. Moreover, they include adverse effects of close contact with humans, as well as a confinement in the captivity; these contribute to the different managements of cases between captive and free-living animals. Consequently, the hospitals for wild animals (mammals, bird, reptile, amphibians) should be well prepared for wild animals; besides, the patient cages must be designed proportionately to the animals’ size (large, medium, small). Furthermore, brooders and Incubators (warmth, quiet, darkness) are also necessary to install. Barriers for living are important to lower stress from the environment and to help separate them from dogs, cats, and other predators.

The veterinarians should minimize stress factors for them during the treatment. In addition, they should be liberated at the original site where they are found (hard or soft releases); besides, the practitioners should monitor them after the liberation.

Finally, treatment and rehabilitation of wildlife should be performed with ethical and legal aspects. In addition, the veterinarian should advise the right ways of care to the clients appropriately.
Oral Presentation
Opisthorchis viverrini Metacercariae in Cyprinoid Fish in Khammouane Province, Lao PDR

Benjamabhorn Pumhirunroj1,2,3,4, Ratchadawan Aukkanimart2,5, Thidarut Boonmars1,2,3*, Praneet Sriraj2,5, Parichart Boueryou1,2, Atcharat Aratchayasawat1,2, Jiraporn Laummaunwai1,2, Panupan Sripan1,2,3, Kanpicha Chomphumee3, Panna Ratana Suwan4, Pornthip Changelkaew5, *Corresponding author: boonmars@yahoo.com

Abstract

Objective To determine the prevalence and distribution of Opisthorchis viverrini metacercariae of Hampala dispar from Khammouane Province - Lao PDR.

Materials and Methods Hampala dispar were collected from Khammouane Province, Lao PDR during January 2012 to November 2018. Fish were digested by artificial digestion methods and O. viverrini metacercariae were identified and collected under a stereomicroscope. A total of 36 fish of Hampala dispar were dissected, separated into various parts of the body. The distribution of O. viverrini metacercariae was examined by compression and artificial digestion methods to know the infection status in various parts of the body.

Results During 2012-2018, we found 100% O. viverrini metacercarial infection in Khammouane Province, Lao PDR. The highest intensity of O. viverrini metacercariae was found in 2016 (835.5 Mc/kg). The infection of O. viverrini metacercariae in the fish from Khammouane Province was mostly found in muscle body (55%), followed by tail fin (16%), and pectoral fin (12%), and rarely in dorsal fin (5%), ventral fin (9%), and anal fin (3%).

Conclusion The results suggested that O. viverrini metacercariae were found 100% in Khammouane Province, Lao PDR and in throughout the entire body of fish. The muscle body was the preferential site of O. viverrini infection.

Keywords: Fish-borne trematode, O. viverrini, metacercariae, cyprinoid fish, distribution, prevalence
Introduction

The human infection with fish-borne trematodes has been estimated about >18 million people [1]. The important food-borne trematodes include liver flukes, intestinal flukes, and lung flukes [2,3]. Fish-borne trematodes especially liver and intestinal flukes are the important parasites of human pathogens in Asia [1,4]. Humans acquire infection by eating raw freshwater fish contaminated with metacercariae [3]. In Lao PDR, Opisthorchis viverrini is a serious public health problem and highly prevalent in this region [1,5] and more than 2 million people are estimated to be infected with this parasite [1]. In Lao PDR, Saravane, Savannakhet and Khammouane Provinces along the Mekong River in the south were highly prevalent areas of O. viverrini infection in humans with the infection rate of 32.2, 25.9 and 21.5%, respectively [6]. The previous survey reported that the prevalence of O. viverrini in the southern province of Champasak was 58.5%, among 814 persons sampled from 13 villages [7]. O. viverrini is one of the most common found in the Greater Mekong sub-region of Southeast Asia [8,9]. The important factors for geographical distribution of O. viverrini infection in humans are associated to the distribution of the first (freshwater snails) and second intermediate hosts (cyprinoid fish) and human habits of eating raw or undercooked fish [10]. Traditional dishes of cyprinoid fish such as koi-pla, lab-pla, pla-som, and pla-raare the common sources of O. viverrini infection in northeastern Thailand and the Lao PDR [8,9,11,12]. The previous study reported that young fish have particular susceptibility to infection from food borne trematode due to their skin and immune status [13]. The salinity, pH, and water temperature affect the infectivity of metacercariae to the hamster. The optimal conditions suitable for maintaining metacercarial infectivity are the temperature at 20°C or lower, the salinity of 0.85%, and pH 7.4. The infection rate in the hamster is decreased after exposure of metacercariae to higher temperature and salinity and higher or lower pH. In 13.6% sodium chloride condition, worm recovery was not found in an animal model [14]. The present study is aimed to determine the prevalence and distribution of O. viverrini metacercariae in the cyprinoid fish collected from Khammouane Province, Lao PDR.

Materials and Methods

A total of 1,803 Hampala dispar fish were collected from Khammouane Province, Lao PDR during the rainy and winter season (August-February) of the years 2012-2018 (Figure 1). Fish were digested by artificial digestion methods (0.25% pepsin and 0.15% HCl) and incubated at 37°C for 1 h and then filtered and precipitated with normal saline in a sedimentation jar [15]. O. viverrini metacercariae were identified and collected under a stereomicroscope. A total of 36 fish of Hampala dispar were weighed and the distribution of metacercariae was examined. The fish bodies were dissected and separated into the pectoral fin, dorsal fin, ventral fin, anal fin, tail fin, and body muscle (Figure 2). Each part of the body components was individually determined and identified fluke metacercariae under a stereomicroscope. Residual fish bodies were individually digested in artificial digestion fluid and identified under a stereomicroscope.

Results

From 2012 to 2018, we examined 1,803 H. dispar fish collected in Khammouane Province, Lao PDR and found that 100% of them were infected with O. viverrini metacercariae (Table 1). In the present study, O. viverrini metacercariae were widely distributed in various part of fish body (Figure 3) with the predominance in the body muscles (55%) followed by tail fin (16%), pectoral fin (12%) and ventral fin (9%), dorsal fin (5%), anal fin (3%) (Figure 4).

Discussion

The O. viverrini infection in H. dispar fish in Khammouane Province, Lao PDR was extremely high (100%) during the study period. Many factors can affect the distribution of O. viverrini metacercarial infection in fish including season, setting, number of parasite and cyprinoid fish species. In this study, fish were collected in late rainy and winter season (August-February) because the high infection of O. viverrini metacercariae in fish are mainly found in the late rainy season and winter season while low infection
Table 1. Infection rate and the density of *Hampala dispar* fish with *O. viverrini* metacercariae

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of fish</th>
<th>Total weight of fish (kg)</th>
<th>No. of MC</th>
<th>Mc/kg fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>300</td>
<td>15</td>
<td>6,500</td>
<td>433.3</td>
</tr>
<tr>
<td>2013</td>
<td>306</td>
<td>15.3</td>
<td>9,000</td>
<td>588.2</td>
</tr>
<tr>
<td>2014</td>
<td>120</td>
<td>6</td>
<td>2,100</td>
<td>350.0</td>
</tr>
<tr>
<td>2015</td>
<td>250</td>
<td>12.5</td>
<td>7,000</td>
<td>560.0</td>
</tr>
<tr>
<td>2016</td>
<td>304</td>
<td>15.2</td>
<td>12,700</td>
<td>835.5</td>
</tr>
<tr>
<td>2017</td>
<td>360</td>
<td>18</td>
<td>11,000</td>
<td>611.1</td>
</tr>
<tr>
<td>2018</td>
<td>163</td>
<td>5</td>
<td>2,004</td>
<td>400.8</td>
</tr>
</tbody>
</table>

MC = metacercariae

Figure 1. Location of Khammouane Province - Lao PDR, the study area for *O. viverrini* metacercariae infection.
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O. viverrini metacercariae were widely distributed in various part of fish body with the predominance in the body muscles. Previously Vichasri et al. reported that O. viverrini metacercariae were mainly found in the body muscles (90%) of the cyprinoid fish collected in the Northeast Thailand [17]. Similar to our present results, Manivong et al. reported that O. viverrini metacercariae were mainly found in the body muscles (58%) of the cyprinoid fish from three rivers (the Namdone, Nampakane, and Mekong rivers) in Khammouane Province, Lao PDR [5]. In contrast, Harinasuta and Vajrasthira reported that O. viverrini metacercariae were mostly found in the base muscles of the pectoral fins (90-100%) [18]. Touch et al. reported that the distribution of O. viverrini metacercariae in freshwater fish in southern Cambodia was preferentially found in the body muscles (>90%), but not found in ventral fin and anal fin [19]. As an overall agreement, the body muscles of cyprinoid fish are the preferential

Figure 2. The body components of Hampala dispar.

Figure 3. O. viverrini metacercariae in various parts of fish body (magnification, x10).
site of *O. viverrini* metacercariae infection. The prevalence of *O. viverrini* metacercariae is extremely high in cyprinoid fish (90-95%), but low in snail hosts (<1%) [9,17,20]. A variety of freshwater fish species are reported to be the hosts of *O. viverrini* metacercariae, including *Cyclocheilichthys armatus*, *Puntius orphoi-des*, *Hampala dispar*, *Henicorhynchus siamensis*, *Osteochilus hasseltii*, and *Puntus proctozysron* [21]. *Ham-pala dispar* is one of the important hosts for *O. viverrini* metacercariae infection. Previously, reported that the infection rate of *Hampala dispar* with *O. viverrini* metacercariae was 44% in Khammouane Province, Lao PDR [5]. Since our results showed 100% infection rate of *H. dispar* with *O. viverrini* metacercariae with high density, careful analysis is required for this drastic increase of infection rate in *H. dispar* in the study area. Since consumption of fluke-free fish is the most important factor for liver fluke control, campaigns and education are necessary to teach safe cooking fish or killing *O. viverrini* metacercariae by heating and freezing process so avoid liver fluke infection [22].

**Figure 4.** Percentages of *O. viverrini* metacercariae in various parts of fish body. PF: pectoral fin, DF: dorsal fin, VF: ventral fin, AF: anal fin, TF: tail fin.

**Acknowledgements**

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**References**


A Retrospective Study Canine Parvovirus in Thailand Found CPV-2c Circulation in 2016

Natnaree Inthong¹,²,⁴,⁵, Sarawan Kaewmongkol¹, Kaitkanoke Sirinarumitr², Theerapol Sirinarunitr³,⁴,⁵*

¹Department of Veterinary Technology, Faculty of Veterinary Technology, Kasetsart University, 50 Ngamwongwan Road, Chatuchak, 10900 Thailand
²Department of Companion Animal Clinical Sciences, Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom, Thailand
³Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, 50 Ngamwongwan Road, Chatuchak, 10900 Thailand
⁴Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Sean Campus, Nakhon Pathom, Thailand
⁵Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok, Thailand
*Corresponding author Email: fvettps@yahoo.com

Abstract

Objective To retrospective study the molecular characterization of canine parvoviruses collected in 2016.

Materials and Methods Ten fecal samples positive for CPV were collected in 2016 from acute diarrhea or hemorrhagic diarrhea dogs. The full-length VP2 of the CPV positive samples using specific primers were cloned, sequenced and compared with those of CPV strains reported in GenBank.

Results The amino acid sequences analysis of these isolates revealed that 7 samples were CPV type 2c, 2 samples were CPV-2a and 1 sample was CPV-2. CPV-2a in this study is classified as new CPV-2a due to amino acids substitution at position 297 (Ser-Ala) and 555 (Ile-Val). The phylogenetic analysis revealed that most of the CPV-2c isolates in this study were closely related to CPV-2c isolates in China, Indonesia, Taiwan and Singapore. CPV-2a isolates in this study were closely related to CPV-2a isolates from Thailand, Singapore and Uruguay.

Conclusion This paper might be the first study to show the existence of CPV-2c in Thailand. The CPV-2a in Thailand has been evolved to be new CPV-2a since 2016.

Keywords: Canine parvoviruses, Diversity, VP2 gene

Introduction

Canine parvovirus (CPV) is highly infectious in young dogs. CPV belongs to the family Paroviridae, subfamily Parovirinae and genus Parovirus. It is a non-enveloped, linearized single-stranded DNA virus. The genome of CPV is approximately 5.2 kb in length. The virus encodes two nonstructural proteins (NS1 and NS2) and three structural proteins (VP1, VP2 and VP3). The VP2 capsid protein is the main capsid protein and plays an important role in the determination of antigenicity and host range of CPV [1] Canine parvovirus type 2 (CPV-2) was first identified in the USA in 1978 [2]. Currently, there are three genotypic variants (CPV-2a, CPV-2b, and CPV-2c) which differs at the amino acid position 426. In addition, the differences between CPV-2 and CPV-2a are the substitution of five amino acids in VP2 protein (Met87Leu, Ile101Thr, Ala300Gly, Asp305Tyr and Val555Ile). The differences between CPV-2a and CPV-2b are the substitution of two amino acids in the VP2 capsid protein that is Asn-426 in 2a (Asp-426 in 2b) and Val-555 (Ile-Val). The differences between CPV-2a and CPV-2b are the substitution of two amino acids in the VP2 capsid protein that is Asn-426 in 2a (Asp-426 in 2b) and Val-555 in 2a (Val-555 in 2b). Recently, CPV-2a and CPV-2b having a mutation at the amino acid position 297 from serine to alanine have been named as the new CPV-2a and new CPV-2b. Moreover, new CPV-2a has a mutation at amino acid position 555 from isoleucine to valine [3-10]. CPV-2c is a new CPV mutant that has a glutamate substitution at the 426 residue of the VP2 protein [11-13]. Canine parvovirus type 2c (CPV-2c) was first detected in Italy in 2000 [13]. Nowadays, CPV-2c has been detected in around the world such as in Argentina, Australia, Germany, Italy, Laos, Spain, Brazil and Uruguay [4,11,14-16]. Therefore, it is interest-
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ing to retrospective study the current genotypes of CPV that have been circulated in Thailand and to compare with the genotype of CPV that had been reported previously.

Materials and Methods

Samples: Ten fecal samples positive for CPV were collected in 2016 from acute diarrhea or hemorrhagic diarrhea dogs at the Veterinary Teaching Hospital, Kasetsart University, Bangkok, Thailand. These dogs had aged from 2 months to 2.5 years. These fecal samples were stored at -80°C until used for DNA extraction.

Virus DNA extraction and polymerase chain reaction (PCR): Viral DNA was extracted from feces using an E.Z.N.A® Tissue DNA kit (OMEGA Bio-tek, Norcross, GA, USA) according to manufacturer’s instructions. The extracted DNA samples were used as a template for performing PCR technique. A set of primers was designed for the full-length VP2 gene: F (5’-ATG AGT GAT GGA GCA GTT CA) and R (5’-TTA ATA TAA TTT TCT AGG TGC TAG TTG). The PCR mixture (25 µl) was composed of 20mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM dNTPs, 2.5 mM MgCl₂, 100 pmol of each forward and reverse primers, 1 units Taq DNA polymerase (Invitrogen™) and 2.5 µl of DNA template to give the total volume of 25 µl. After an initial denaturing at 94°C for 5 min, the amplification was performed by 35 cycles at 94°C for 40 s, annealing at 50°C for 40 s, and extension at 72°C for 90 s followed by a final extension at 72°C 10 min. The expected PCR products were 1,755 bps in size. The PCR products were analyzed by 1 % agarose gel electrophoresis, at 100 V for 30 min and visualized under ultraviolet illumination. The PCR products were purified using UltraClean® 15 DNA purification kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) and cloned into plasmid pGEM-T easy (Promega Corporation, Madison, WI, USA). The sequences of the plasmids were determined at First BASE Laboratories Sdn Bhd, Selangor, Malaysia.

Analysis of VP2 gene and phylogenetic study: The sequencing results were analyzed using the Bioedit biological sequence alignment editor computer package (version 7.1.3; Ibis Biosciences; Carlsbad, CA, USA). The nucleotide phylogenetic analysis was constructed from nucleotide sequence of the CPV-2 in this study and other sequences obtained from Genbank database (Table 1) with MEGA program (version 6.0, The Biodesign Institute, Tempe, AZ, USA) by using neighbor-joining method and running 1,000 replicates in the bootstrap.

Results

The amino acid sequences analysis of 10 CPV-positive samples revealed that 7 samples were CPV-2c, 2 samples were CPV-2a and 1 sample was CPV-2. Interestingly, CPV-2c in this study different from CPV-2c that isolated in European and South America at the amino acid positions 267 (Phe-Tyr), 370 (Gln-Arg), 324(Tyr-Ile) (Table 2 and Figure 1). Moreover, the amino acid position 370 (Gln-Arg) has been only found exclusively in Asian CPV2-c. Thus, CPV-2c isolates in this study were similar to Asian CPV-2c. The CPV-2a isolates in this study had amino acids substitution at position 297 (Ser-Ala) and 555 (Ile-Val). According to this result, the CPV-2a isolates in this study were new CPV-2a. A phylogenetic tree was constructed using the 10 full-length VP2 nucleotide sequences obtained in this study and an additional 26 sequences retrieved from the GenBank database was shown in Table 1. The phylogenetic analysis of the CPV-2a isolates in this study were closely related to CPV-2a isolates from Thailand, Singapore and Uruguay and the CPV-2c isolates in this study were closely related to CPV-2c isolates in Asia such as in China, Indonesia, Taiwan and Singapore (Figure 2).

Discussion

Interestingly, the majority of positive samples were CPV-2c. This study showed that CPV-2c has been circulated and might be the predominant genotype in Thailand since 2016. For CPV-2c in this study, there was single base transition at nucleotide 1278 from T-to-A which causes amino acid substitution from Asp to Glu. According to this finding, the origin of CPV-2c in Thailand might be mutated from CPV-2b because amino acid position 555 is Valine. Currently, CPV-2c has been circulated in Asia such as in China [17], Taiwan [18,19], Laos [16] and all of these isolates had a characteristic mutation at amino acid position...
### Table 1. Canine paroviruses used in phylogenetic tree construction

<table>
<thead>
<tr>
<th>No.</th>
<th>Order</th>
<th>Origin</th>
<th>Year</th>
<th>GenBank accession no.</th>
<th>Genetic Type</th>
</tr>
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<td>2014</td>
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### Table 2. Amino acids at important positions for genotyping of canine parovirus isolates in this study

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<th>No.</th>
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<td>R L N T A I Y A G Y I R E T V</td>
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<td>2.5 yr</td>
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Feline panleukopenia virus
Figure 1. Amino acid alignment of VP2 gene of CPV-2 (CPV-2020.59), CPV-2a (CPV-2097.59) and CPV-2c (CPV-2091.59) in this study with FPV and other CPV isolates from Genbank database.
370 (Gln370Arg) [16-19]. We also found this amino acid substitution in all Thai isolates in this study. The CPV-2a isolates in this study were new CPV-2a due to amino acids substitution at position 297 (Ser-Ala) and 555 (Ile-Val). Moreover, these new CPV-2a had amino acid substitution at residues 324 (Tyr-Ile) and 440 (Thr-Ala) which were similar to those reported previously [20]. The amino acid substitution at position 324 (Tyr-Ile) substitutions is due to a T-to-A transition at nucleotide 970 and an A-to-T transition at nucleotide 971 and the amino acid substitution at position 440 (Thr-Ala) is due to an A-to-G transition at nucleotide 1318. The amino acid substitution at residue 324 (Tyr-Ile) was also reported in Brazil [21], China [22], Hungary [23], India [24,25], Italy [26], Nigeria [27], South Korea [28], Taiwan [19,29] and Uruguay [30]. The amino acid substitution at position 440 (Thr-Ala) was also reported in the previous studies in China [22], India [24,25], Nigeria [27,31,32], Pakistan [33], South Africa [31], South Korea [28]. A CPV-2 in this study might be collected from a dog recently vaccinated with CPV-2 genotype vaccine [34]. This study indicated that CPV-2c and new CPV-2a have been existed in Thailand since 2016. For further study, the study of evolving canine parvovirus is considered important for the prediction of disease severity and

Figure 2. Phylogenetic tree constructed using the nucleotide sequences of the VP2 gene of CPV isolates in this study and other sequences obtained from GenBank database using the neighbor-joining method and bootstrap analysis performed with 1,000 trials, MEGA version
may be important to develop the more effective vaccine in the future.

Acknowledgements

This research was supported by grants from the Center for Agricultural Biotechnology (CAB), Kasetsart University, Kamphaeng Saen campus, Thailand, the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education (AG-BIO/PERDO-CHE), Bangkok, Thailand.

References


Efficacy Study of *Artemisia* spp. on Inhibition of *Eimeriatenella* Infection in Broiler Chicken

Chayanad Poomdonket, Somboon Sangmaneedet, Weerapol Taweenan, Peerapol Sukon

1Master of Veterinary Science Program, Faculty of Veterinary Medicine, KhonKaen University.  
2Department of Pathobiology, Faculty of Veterinary Medicine, KhonKaen University  
3Department of Anatomy, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen 40002, Thailand  
*Corresponding author Email: sombn_sa@kku.ac.th

Abstract

**Objective** This study aims to evaluate the anticoccidial efficacy of *Artemisia* spp. on *Eimeriatenella* infection in broiler chicken.  

**Materials and Methods** One hundred and twenty of 1-day-old broilers (Ross 308) were randomly set into 5 groups (n = 24/group): negative control; positive control; and 3 treatment groups with *Artemisia* spp. at different concentration levels; 1%, 5% and 10%. Broilers in positive control and 3 treatment groups were orally inoculated with 3,000 sporulated oocysts of *E. tenella* at 14 days old. The anticoccidial efficacy of *Artemisia* spp. was evaluated from clinical signs, mortality rate, fecal oocyst output, caecal lesion score, weight gain and feed conversion ratio.  

**Results** The results showed that the *Artemisia* spp. at 1%, 5% and 10% could reduce the number of oocysts released in the feces. *Artemisia* spp. mixed in feed at 5% decreased the severity in caecum, while 1% concentration increased a weight gain of broilers and yielded the best feed conversion ratio after an infection.  

**Conclusion** *Artemisia* spp. leaf powder mixed in feed at levels 1%, 5% and 10% could reduce the severity in caeca of broilers infected with *Eimeria tenella*; however, using this medicinal herbs should consider on palatability of feed and its effect on the animal’s growth.

**Keywords:** Caecal coccidiosis, *Eimeria tenella*, *Artemisia* spp., broilers
Detection of Antibody Titers against Caprine Arthritis Encephalitis Virus Infection using Milk Samples of Dairy Goat by cELISA

Supachart Panneum¹*, Dusita Plodkratoke², Phannipa Phungsombun², Penporn Nanan², Pakawat Savettalake², Pantira Wongkor², Theera Rukkwamsuk¹

¹Faculty of Veterinary Medicine, Kasetsart University, KamphaengSaen, NakhonPathom
²6th year veterinary student, Faculty of Veterinary Medicine, Kasetsart University
*Corresponding author Email: fvetshp@ku.ac.th

Abstract

Objective Serological detection of Caprine Arthritis Encephalitis Virus (CAEV) infection in goat by ELISA technique was usually performed using serum sample; however, the aim was to evaluate the performance of the cELISA when goat milk samples were applied.

Materials and Methods Milk and blood samples were collected from 10 dairy goat farms located in Ratchaburi province. In each farm, 10 milk samples and 10 serum samples were collected from 10 goats. Two pool milk samples (5 milk samples per 1 pool) per 1 farm were prepared. Then 20 pool milk samples, 100 individual milk samples and 100 serum samples were tested for antibody titers against CAEV infection using commercial cELISA. Reviewing the test results of antibody against CAEV infection from difference samples using descriptive statistical analysis and the agreement of test between individual milk samples and serum samples was performed using Cohan’s kappa coefficient.

Results Two pool milk samples from 2 difference farms were tested positive, in which 1 positive individual milk sample was detected in one pool sample while the other had 4 positive individual milk samples. Eighteen pool milk samples were tested negative, 93.33% (84/90 of individual milk samples) were found negative while 6.66% (6/90) were found positive. Agreement of test using cELISA between 2 difference types of sample (individual milk vs serum sample) revealed Cohan’s kappa coefficient which was 0.756 indicating good or substantial. cELISA testing result of individual milk samples and serum samples from 100 goats were compared. Seven goats were positive for both milk and serum testings, while 3 goats were positive from milk testing but negative from serum testing. And 89 goats were negative from both milk and serum testing while only 1 goat was negative from milk testing but positive from serum testing.

Conclusion For herd detection of CAEV infection using pool milk sample could be beneficial due to reducing cost when eradication or control measure is considered, individual sample testing is necessary. In comparison with the result of cELISA using serum samples, cELISA were beneficial in application for screening test due to positive predictive value at 70% (7/10) even though high false positive result was evaluated and for the confirmatory test due to high negative predictive value at 98.88% (89/90).

Keywords: Caprine Arthritis Encephalitis Virus, cELISA, goat milk
Efficacy of Condensed Tannin Extract on Fecal Gastrointestinal Nematode Egg Count in Dairy Goats

Supachart Panneum†, Niorn Ratanapob‡, Theera Rukkwamsuk‡

†Kasetsart University Veterinary Teaching Hospital Nong Pho, Thailand, 70120
‡Department of Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Thailand, 73140
*Corresponding author Email: fvetshp@ku.ac.th

Abstract

Objective Widespread anthelmintic resistance in small ruminants challenges the use of alternative organic substances, including condensed tannin. This study evaluated efficacy of condensed tannin extract on reducing fecal gastrointestinal nematode egg count in dairy goats.

Materials and Methods Thirty-nine goats in a dairy farm were randomly assigned into three groups. Group 1 received a single subcutaneous injection of ivermectin and dietary condensed tannin supplement for 30 days, group 2 received dietary condensed tannin and group 3 received ivermectin. Fecal samples were collected before deworming and at 2, 4, 6 and 8 weeks after deworming. Fecal egg counts (FEC) were measured by the modified McMaster technique. FECs were transformed with natural logarithm and repeated-measures ANOVA were used for comparing the results among groups and times of sampling.

Results No significant effect of condensed tannin on FECs were observed among 3 groups at all times of sampling. FECs significantly differed among sampling times (p = 0.002). The means FECs at week 0, 2, 4, 6 and 8 after deworming were 1,196 (±1,249), 670 (±730), 610 (±662), 972 (±1,160) and 1,250 (±1,724) eggs/gram of feces, respectively. However, only FECs at week 2 tended to be lower than FECs at week 0 when multiple comparison with the Bonferroni adjustment was performed (p = 0.06).

Conclusion Condensed tannin extract can be an alternative anthelmintic for reducing gastrointestinal nematode egg excretion in dairy goats.

Keywords: Tannin, Gastrointestinal nematode, Fecal egg count, Goat
The Prevalence of Periodontal Disease in Dogs Underwent Neutering

Supasun Klinmalee1*, Pichet Boonchan1, Ronnakrit Bunchan1, Thanikul Srithunyarat1

1Division of Surgery, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand, 40002.
*Corresponding author

Abstract

Objective Periodontal disease is a major oral disease in dogs. The prevalence in dogs under 4 years was 40.8%1. Daily tooth brushing is an effective preventive treatment for periodontal disease in dogs2. However, the prevalence of periodontal disease in Thailand has not yet been reported. This study was conducted to determine the prevalence of periodontal disease and owner awareness of oral homecare in dogs underwent neutering.

Materials and Methods Dogs, aged between 1-3 years, underwent neutering, with the owner consent, in a month period were included. Owner awareness of oral homecare had been interviewed. After surgery, dental charting and radiography were performed and stage of periodontal disease was classified3.

Results Fourteen dogs, 7 males and 9 females, were included. 64.3% (9/14) of the owners perceived a toothbrushing homecare, and only 14.3% (2/14) brushed their dogs’ teeth infrequently. None of the dogs had professional oral examination and cleaning. Owner awareness of oral care was low for 42.9% (6/14), moderate for 42.9% (6/14), and high for 14.3% (2/14). The prevalence of periodontal disease in this study was 100% with periodontal disease stages 1-3. Most of the dogs experienced stage 2 of periodontal disease. Canine teeth for both upper (89.3%) and lower (85.7%) jaw had the most prevalence of periodontal disease. Periodontal disease stage 3 had been found in lower incisor teeth (35.7%) and stage 2 in upper incisor teeth (84.5%).

Conclusion This is the first study reporting the prevalence of periodontal disease in dogs with the prevalence of 100%. Most affected teeth are both upper and lower canine and incisor teeth which required intensive care. Client education and daily home brushing is warranted.

Keywords: Periodontal disease, Dogs, Prevalence, Neutering
3D Printed Bone Models from CT Scan

Karn Yongvanit

1Veterinary Teaching Hospital, Faculty of Veterinary Medicine, KhonKaen University
Corresponding author Email: karnyo@kku.ac.th

Abstract

Objective 3D printing is any of various processes in which material is joined or solidified under computer control to create a 3D object with material being added together typically layer by layer. 3D models can be generated from personal computer or scanned from 3D scanner. Up to date, CT scan is able to reconstructing and rendering high quality bone model from Dicom files which suitable for 3D printing. This study was conducted to create 3D plastic bone model from CT scan.

Materials and Methods A dog was anesthetized and scan with CT scan to obtain desired bone part. In this study, canine skull was selected. Dicom files from CT scan were converted into STL files with free open source program, InVesalius or 3Dslicer. The STL file can adjust size and smoothen the texture surface by using Meshmixer program. The adjusted file had to be converted again for each 3D printer. Ideamaker is used to create gcode files for Raise3d N2 Plus 3D printing machine. The files were uploaded and printed. In this study, PLA plastic is used. The canine skull size is 10 cm long, 7 cm wide and 6 cm high, the printing time is 17 hours.

Results The 3D plastic bone model was successfully constructed with high accuracy for anatomical structure and size of the dog skull.

Conclusion 3D printing is a new technology that could change the way of education. Not only anatomical study but also clinical study such as bone fracture repair planning or bone with disease lesion. For ethics reason, fewer animals would have to be euthanized for bone study. The cost of material is cheap but the 3D printer and CT scan is expensive. Varieties of material can be chosen depending on objective of the model such as PLA plastic, ABS plastic, Wood filament, etc. The printing size depended on printer size, a large printer able to print a full size giant breed dog’s femur.

Keywords: 3D printing, Dogs, Bone, CT scan
Prevalence of *Batonella* spp. in Domestic Dog in Kalasin province, Thailand

Wilasinee Srisanyong\(^1\)*, Aphinya Sabangban\(^1\), Chirasak Srimekarat\(^1\), Fanan Suksawat\(^2\)

\(^1\)Faculty of Veterinary Technology, Kalasin University, Muang, Kalasin, Thailand, 46000

\(^2\)Faculty of Veterinary Medicine, KhonKaen University, Muang, KhonKaen, Thailand, 40002

*Corresponding author Email: am_bios@hotmail.com

Abstract

**Objective** To study the prevalence of *Batonella* spp. in Domestic Dog in Kalasin province, Thailand

**Materials and Methods** Molecular detection of *Bartonella* spp. in domestic dogs was employed during October 2018 and January 2019. 80 domestic dog blood samples were collected from Animal hospital in Kalasin University and Kalasin Animal Clinic in Kalasin province. PCR was used to detect *Bartonella* spp. DNA.

**Results** PCR results of eighty samples were five positive for *Bartonella* spp. DNA.

**Conclusion** The study is useful and necessary for surveillance of bartonellosis, the zoonotic disease in dogs.

**Keywords:** *Bartonella* spp., Domestic dogs, PCR, Kalasin
Poster Presentation
Objective To determine the ability of California mastitis test (CMT) for identifying intramammary infection at quarters level.

Materials and Methods A total of 308 dairy cows from 20 farms were used in the study. Quarter milk samples of all lactating cows were collected. CMT were performed as cow-side test, the CMT results were recorded as 0 (negative), 1 (trace or 1), 2 and 3. Quarters milk sample were collected aseptically for bacteriological examination. Sensitivity and specificity of CMT for identifying infected quarters were analyzed at the threshold of CMT score ≥ 1 and infected quarters.

Results Thirty-two quarters were nonfunctional, resulting in a total of 1200 quarters used in the analyses. Overall 40.7% of quarters had an IMI; the most common pathogens isolated were coagulase-negative staphylococci (CNS), Staphylococcus aureus and Streptococcus agalactiae. Most infected quarters were found in CMT score 3 (77.5%). All CMT positive samples (799 quarters), 47.2% (377 out of 799 quarters) found no infections and CMT negative samples, 88.5% (335 out of 401 quarters) found no infections. The sensitivity and specificity of CMT for identify the infected quarters were 87% and 47%, respectively. Positive predictive value and negative predictive value were 52% and 83%, respectively.

Conclusion In conclusion, with the sensitivity at 87% on detecting IMI and negative predictive value was 83%. CMT has a very useful role in dairy herd monitoring program as a convenient and cost-effective tool.

Keywords: California mastitis test, Intramammary infection, Dairy cow, Sensitivity, Specificity
Single-agent L-asparagenase for the Treatment of Vincristine and Doxorubicin-resistant Canine Transmissible Venereal Tumor

Arayaporn Macotpet1*, Ekkachai Pattarapanwichien2, Nuttha Pothiard3

1Division of Small Animal Medicine, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen, 40002, Thailand
2Division of Pathobiology, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen, 40002, Thailand
3Veterinary Teaching Hospital, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen, 40002, Thailand

*Corresponding author Email: arayama7972@gmail.com

Abstract

Case Description A six year old, spayed female, mixed breed, 18.2 kg dog was referred from a private clinic with transmissible venereal tumor (TVT) at the genitalia which was resistant to vincristine.

Clinical Findings Physical examination revealed a cauliflower like, 7 x 10x 2 cm³ mass located in the vestibule and vagina area. Cytological diagnosis confirmed TVT. Hematology and biochemistry indicated that the dog was anemic with low levels of albumin in the circulation. No abnormalities were seen from thoracic radiography and abdominal ultrasonography.

Treatment and Outcome As the TVT was found to be resistant to vincristine treatment, the treatment plan initially was to use doxorubicin chemotherapy once every three weeks. However, after having given it twice the dog did not respond to the treatment and L-asparagenase, once every two weeks, for four treatments was given as an alternative, leading to a successful treatment with no adverse effects.

Clinical Relevance Genital TVT can be transmitted between dogs during mating where the neoplastic cell is transmitted through the damaged or abraded mucosa. Currently, vincristine chemotherapy is the treatment of choice for this type of tumor and most dogs respond fully to the treatment. However, studies have shown that the tumor can develop drug resistance. Resistance to chemotherapy can occur by several mechanisms. In cases of dogs that are resistant to vincristine, chemotherapy with doxorubicin is primarily chosen as an alternative treatment. L-asparagenase is another chemotherapy used in dogs resistant to vincristine and doxorubicin. L-asparagenase has been used in combination with vincristine or L-asparagenase in combination with prednisolone and surgical removal of the tumor. There has been no report on the use of L-asparagenase alone for treatment of TVT resistant to vincristine in dogs. In this study, the dog was treated only with L-asparagenase chemotherapy and responded fully to the drug with no adverse effects seen. This shows that L-asparagenase can be used in treatment of TVT resistant to vincristine and doxorubicin which should cause less adverse effects when compared to using a combination of drugs for treatment.

Keywords: Transmissible venereal tumor, TVT, Dog
Prevalence of Genotypic Fimbrial Antigens of Enterotoxigenic E. Coli Isolated in Thai Pig Herds

Ruangurai Kitchodok¹*, Sittikorn Triyarach², Kitti Sutheerakul³, Chinda. Serod⁴, Dittapong Chompupun⁵

¹Technical service Swine, HIPRA Thailand, Thailand, 10310
²Regional technical and marketing specialist Swine Asia and Oceania, HIPRA Thailand, Thailand, 10310
³Area sales manager, HIPRA Thailand, Thailand, 10310
⁴Diagnos manager, HIPRA Thailand, Thailand, 10310
⁵Laboratory assistant, HIPRA Thailand, Thailand, 10310

Corresponding author Email: ruangurai.kitchodok@hipra.com

Abstract

Objective The field study was to estimate the prevalence of fimbrial antigens (F4, F5, F6) and heat-labile (LT) ETEC which cause neonatal diarrhea in pig farms in Thailand.

Materials and Methods Prevalence of fimbrial antigens of E. coli were identified and determined in 94 pooled swab samples collected from 1-14 days old suckling pigs in 25 swine husbandries in several parts of Thailand during 2017-2018. Three rectal swab samples from pre-weaning piglets in the same litter were pooled on ELUTE cards (FTA-like) as transport used (Enterocheck®, HIPRA SPAIN). DNA extraction process, the one-step real-time multiplex PCR reaction procedure for the identification of the portion of virulence genes (F4, F5, F6 and LT) and β toxin for the detection of ETEC and Clostridium perfringens (C. perfringens) type C using specific probes was carried out in accordance with laboratory guidelines of Diagnos HIPRA laboratories (HIPRA SPAIN). The results were determined as positive or negative based on the Cycle threshold (Ct) values below 38.5 being considered as positive sample.

Results The present of field study exhibited the existence of multiple genes for adhesins and enterotoxin representing the virulence factors and their associations in the E. coli isolated from piglets with diarrhea. The overall prevalence of enterotoxigenic E. coli (ETEC) in twenty swine farms was proved by real-time PCR diagnostic assay manifesting 80% (20/25), whereas C. perfringens type C (β toxin) was undetectable (0%). Furthermore, the fimbrial genes for F4, F5, F6 and enterotoxin (LT) of ETEC isolated from 2 weeks old piglets were identified in 51.06% (48/94), 2.13% (2/94), 0% (0/94) and 10.64% (10/94). Some pooled samples could carry virulence gene for adhesions also harbored genes for enterotoxin (F4+LT) 14.89% (14/94), (F4+F5) 1.06% (1/94), (F4+F6) 1.06% (1/94) and (F4+F5+LT) 2.13% (2/94). The remaining samples showed all ETEC-negative up to 17.02% (16/94).

Conclusion To our knowledge, it is clearly accepted that the presence of E.coli is an underlying problem across many herds and is likely a substantial cause of diarrhea and death in suckling pigs. Enterocheck® and real-time PCR assay are a good diagnostic tool and the fundamental importance to prove the disease that makes it possible to interpret a prove prevalence of ETEC for further determining the significant economic impact on pig production costs and outbreaks. Current study may provide a key database for field surveillance in Thailand which in turn would have an impact on vaccine selection and application of specific antibodies against ETEC after vaccination.

Keywords: Enterocheck®, Enterotoxigenic E. coli, Real-time PCR assay, Neonatal diarrhea, Swine
Effects of Single Dose Mypravac® suis on Pig Naturally Infected with Mycoplasma hyopneumoniae

Ruangurai Kitchodok¹*, Kriangkrai Lohaprom², Mongkol Lumyai³, Sittikorn Traiyarach⁴

¹Technical service Swine, HIPRA Thailand, Thailand, 10310
²Sales representative, HIPRA Thailand, Thailand, 10310
³Area sales manager, HIPRA Thailand, Thailand, 10310
⁴Regional technical and marketing specialist Swine Asia and Oceania, HIPRA Thailand, Thailand, 10310

*Corresponding author Email: ruangurai.kitchodok@hipra.com

Abstract

Objective The purpose of this study was emphasis on compare the field efficacy and the safety of two commercial one dose program vaccines and to access the growth performance as a cause of mycoplasma infection in pigs under farm with low infection pressure from Mycoplasma hyopneumoniae and porcine reproductive and respiratory syndrome virus (PRRSV).

Materials and Methods A total of 2,125 piglets were randomly allocated into two groups. Group 1 weaning pigs (n = 1,100) were intramuscularly vaccinated with 2 ml of Mypravac® suis at 5 weeks. The remaining group (n = 1,025) was administered a one dose schedule by 2 ml of vaccine A. Monitoring of adverse reactions were critical to the safety had been recorded daily until 21 days post vaccination. Field efficacy on one shot Mycoplasma vaccines were evaluated primarily through the results of grower finisher pig’s production with respect to growth performance parameters and pathological lesions. Overall experimental pigs were analyzed the pig production, % coughing index, safety test and lung score lesion using statistical program for comparison on a group basis by SPSS statistic base 22.0. Statistical results were considered significant when the P-values were <0.05.

Results Here we briefly described the monitoring results displayed the absence of systemic reaction such as increased breathing in vaccinated pigs in Group 1. Mycoplasma hyopneumoniae vaccination (Mypravac® suis) significantly improved average daily gain up to 9.29% (Group 1: 776.6 g/day, Group 2: 704.46 g/day) and significantly reduced medication cost up to 53.28% (Group 1: 5.99 EUR, Group 2: 12.82) and % coughing index up to 72.57% (Group 1: 0.455, Group 2: 1.659) more than Group 2 (Vaccine A). Mortality rate and culling rate influence by Mypravac® suis were more decreased 3.47% and 1.70%, respectively than the remaining group (mortality rate: 4.01% and culling rate: 1.94%).

Conclusion To the extent of our knowledge it is acceded that Mypravac® suis appears to be a relatively safe vaccine which can improve growth performance, reflecting a general enhancement in farm status. Our findings indicate that Mypravac® suis efficacious in an extent of lung lesions in a farrow-to-finish in Thai swine farm.

Keywords: Enzootic pneumonia, Mypravac® suis, Mycoplasma hyopneumoniae, Swine
Antimicrobial Activity of Oxystelma esculentum R. Br., Paedaria linearis Hook. f. and Azadirachta indica var. siamensis valeton Extracts against Bacteria and Yeasts

Arinee Chatchawanchonteera¹*, Jinda Wangboonskul², Jareerat Aimsa-ard¹, Pimchanok Suwannathada¹, Arany Sirigraiwan¹, Woraphanit Sonbanpai¹, Areeya Pomeok¹, Supatsorn Panyalert¹

¹Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand. 40002
²Faculty of Pharmaceutical Sciences, Thammasart University, Bangkok, Thailand.
*Corresponding author; E-mail: arinee@kku.ac.th

Abstract

Objective To study antimicrobial activities of Oxystelma esculentum R. Br., Paedaria linearis Hook. f. and Azadirachta indica var. siamensis valeton extracts against some yeasts and some pathogenic bacteria isolated from animals.

Materials and Methods Oxystelma esculentum R. Br., Paedaria linearis Hook. f. and Azadirachta indica var. siamensis valeton were collected and extracted by ethanol. Antimicrobial activities of the crude extracts were tested using broth microdilution method. The isolated bacteria were Staphylococcus aureus, Staphylococcus intermedius, Streptococcus agalactiae, and Gram-negative bacteria were Klebsiella spp., Pseudomonas aeruginosa, Escherichia coli, and Aeromonas hydrophila. Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, together with gentamycin and ketoconazole were also tested as the standard controls. The minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) of all bacteria and MIC, MFC of yeasts, Cryptococcus neoformans and Candida albicans were determined.

Results The result showed that Oxystelma esculentum R. Br. and Paedaria linearis Hook. f. extract had antibacterial activities against all bacteria isolated from animals and also antifungal activities against the yeasts with different MIC, MBC, MFC values. The minimal bactericidal concentration (MBC) values of Oxystelma esculentum R. Br. and Paedaria linearis Hook. f. extracts against Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus hyicus, Streptococcus agalactiae, Pseudomonas aeruginosa, Aeromonas hydrophila, E. coli, Klebsiella spp. were 0.38, 24.25, 97, 34.29, 48.49, 24.25, 68.59, 68.59 mg/ml and 7.08, 10.01, 1.76, 28.31, 35.67, 14.15, 56.62, 56.62 mg/ml respectively. For the yeasts, The minimal fungicidal concentration (MFC) values of Oxystelma esculentum R. Br., Paedaria linearis Hook. f. and Azadirachta indica var. siamensis valeton extracts against Cryptococcus neoformans and Candida albicans were 61.11, 122.21 mg/ml; 113.24, 452.95 mg/ml and 2000, 2000 mg/ml, respectively.

Conclusion Ethanol extract of Oxystelma esculentum R. Br. and Paedaria linearis Hook. f. had antibacterial activities against various types of pathogenic bacteria that were isolated from animals, and antifungal activities against some yeasts. In addition, MIC and MBC value of both extracts against Gram positive bacteria groups were lower than Gram negative bacteria groups. For antifungal activities, Oxystelma esculentum R. Br. showed lower MIC and MFC values against Cryptococcus neoformans than Paedaria linearis Hook. f. In comparison, between Cryptococcus neoformans and Candida albicans, Oxystelma esculentum R. Br. showed lower MIC and MFC values against Cryptococcus neoformans than Candida albicans. And Azadirachta indica var. siamensis valeton could also inhibit Cryptococcus neoformans and Candida albicans.

Keywords: Oxystelma esculentum R. Br., Paedaria linearis Hook. f., Azadirachta indica var. siamensis valeton, extract, antimicrobial activity, animals, pathogenic bacteria, yeasts
Isolation of Pathogenic Fungi from Pigeon Feces in Populated Area in Mueang Nakhon Ratchasima, Thailand

Praphat Deunkrathok¹, Nattida Prasnagsit¹, Krittana Saokaew¹, Catthareeya Sukwan¹*

¹Program of Veterinary Technology, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand
Corresponding author email: Catthareeya.s@nrru.ac.th

Abstract

Objectives The purpose of this study was to isolate and identify molds on pigeon feces samples as a possible source and reservoir of human infections.

Material and Methods Pigeon feces samples were collected from 4 different densely populated areas in Mueang Nakhon Ratchasima by means of purposive sampling method. Multiple samples, with the total of 32 samples per each selected site, were collected between October-November 2018. The samples were then processed to isolate fungi with standard isolation method before purified by re-culturing on appropriate media. The method to identify filamentous fungi was by observing the presence of their colonies that include color, texture and pigment production. Gram stained smears, technical germ tube, microculture, and standard biochemical tests were performed for yeasts identification.

Results There were 355 isolates of filamentous fungi and yeasts found, to include Candida spp. (90 isolates), Aspergillus spp. (88 isolates), Penicillium spp. (60 isolates), Rhizopus spp. (50 isolates), Pseudallesheria spp. (33 isolates), Thricodera spp. (26 isolates) and Trichophyton spp. (8 isolates). High prevalence of potential organism (Candida spp. and Aspergillus spp.) was observed on samples collected from Nakhon Ratchasima Rajabhat University, Salaloi Temple and Monument of Tao Suranaree, respectively.

Conclusion There are clinical-significant fungi present in the samples from the selected sites. This finding indicates that pigeon feces pose a substantial risk on public health. Therefore, proper maintenance and periodic inspection of urbanized residential areas with high population of pigeons are vital to ensure a sustainable social well-being.

Keywords: fungi, pigeon feces, populated area

Introduction

Fungal infection around the globe has risen in recent decades due to massive growth in population, urban development, and climate change. These microorganisms are pervasive because of their simultaneous reproduction. They also pose a serious threat to public health because of their ability to contaminate the environment in many areas of human territory. Nowadays, there is a substantial increase of pigeons inhabiting in urbanized cities, and their waste could stand as a reservoir for potential pathogenic molds (1-2). The major infection route occurs through inhalation of any fungi or yeasts from contaminated pigeon feces, which lead to many clinical diseases that can include asthma, primarily via fungal toxins and allergens (3). More importantly, there is an extensive risk in places where potential pathogens are abundant, which could jeopardize their chance of invading, colonizing and infecting humans (4-5).

Mueang Nakhon Ratchasima, Thailand is a densely populated city with many tourist attractions. The city faces an overwhelming population increase of both human residents and city-pigeons. As such, transmittable diseases associated with pigeon feces on humans are becoming a public concern. This study was designed to evaluate the prevalence of fungi in pigeon’s feces, especially in densely human-populated area. The outcome of this study may provide valuable data to establish a standard for public health surveillance in this subject area or other similar communities.
Materials and Methods

Pigeon feces were collected from 4 different sites in Mueang Nakhon Ratchasima including Bung-phyaprab Elementary School, Nakhon Ratchasima Rajabhat University (NRRU), Salaloi Temple and Tao Suranaree Monument. The sites were selected on the basis of high population count and substantial number of pigeon present. A total of 32 samples per site were collected between October-November 2018. The fungi were isolated by using the modified method of Machado et al., (1993) (6). In brief, 1 g of feces was homogenized by mortar and pestle before the sample was transferred to a sterile falcon tube with 10 mL of sterile distilled water. A vortex mixture then homogenized each sample for 5 minutes and kept them at rest for 30 minutes until decantation of the supernatant was obtained. The serial dilution suspensions was performed and seeded in duplicate by scattering technique on Sabouraud’s dextrose agar with chloramphenicol. The inoculated plates were incubated at 25°C for 7-14 days with daily observation of fungal growth. Isolated fungal strains were purified by re-culturing on appropriate media before identification by macroscopic and microscopic studies. Filamentous fungi were identified by visual observation of colonies based on color, texture and pigment production. Gram stained smears, technical germ tube, microculture, and standard biochemical tests were conducted for yeasts identification. The number of isolation and species identified from each site were individually recorded and calculated. The following formulas were used to make the calculation per each site.

Different types of colony in each sample were recorded based on their physical appearance. The number of types found was recorded and calculated using the following formula in order to categorize the severity of the co-infection based on the average of different types of colony from the selected sites.

The pathogenic strains were also identified as the factor to determine potential clinical risk in each site. The prevalence of pathogenic strains per site was calculated using the following formula.

Results

Out of 128 samples examined, 355 isolates of filamentous fungi and yeasts were recovered. The samples from the Tao Suranaree Monument exhibited the highest number of positive sample (93.75%), hence the highest of prevalence of pathogenic fungal strain of 74.42% was found. Although NRRU exhibited the lowest number of positive sample (56.25%), its prevalence of pathogenic fungal strain of 65.26%is still noteworthy. This study found three pathogenic strains that include Candida spp., Aspergillus spp., and Penicillium spp. All data are shown on Table 1.

<table>
<thead>
<tr>
<th>Place of Sampling</th>
<th>No. of Positive Sample</th>
<th>Total No. Isolates</th>
<th>Average No. of colony/sample</th>
<th>Average No. of differences colony/sample</th>
<th>No. of pathogenic strains</th>
<th>Prevalence of pathogenic strains/site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Bungphyaprab Elementary School</td>
<td>20/32 (62.50%)</td>
<td>86</td>
<td>4.3</td>
<td>2.55</td>
<td>3</td>
<td>61/86 (70.93%)</td>
</tr>
<tr>
<td>2. NRRU</td>
<td>18/32 (56.25%)</td>
<td>95</td>
<td>5.2</td>
<td>2.56</td>
<td>3</td>
<td>62/95 (65.26%)</td>
</tr>
<tr>
<td>3. Salaloi Temple</td>
<td>26/32 (81.25%)</td>
<td>88</td>
<td>3.4</td>
<td>3.38</td>
<td>3</td>
<td>51/88 (57.95%)</td>
</tr>
<tr>
<td>4. Tao Suranaree Monument</td>
<td>30/32 (93.75%)</td>
<td>86</td>
<td>2.9</td>
<td>1.97</td>
<td>3</td>
<td>64/86 (74.42%)</td>
</tr>
</tbody>
</table>

Table 1. Fungal growth isolated from pigeon feces samples collected from 4 sites in Mueang Nakhon Ratchasima, Thailand.
Regarding the distribution of various species of mold, the most frequently isolated were *Candida* spp. (90 isolates). Filamentous fungi included *Aspergillus* spp. (88 isolates), *Penicillium* spp. (60 isolates), *Rhizopus* spp. (50 isolates), *Pseudallesheria* spp. (33 isolates), *Thricoderma* spp. (26 isolates) and *Trichophyton* spp. (8 isolates).

There was a constant observation of *Candida* spp. and *Aspergillus* spp. in samples obtained from...
Discussion

The purpose of this study was to isolate and identify molds on pigeon feces samples as a possible source and reservoir of human infections. This study showed the prevalence of yeast and filamentous fungi in densely populated area. The rise of this concern correlates with the overwhelming increase in pigeon population in Mueang Nakhon Ratchasima, which might be due to additional pigeon reservations designated by the city. Although these feeding sites are well maintained by the city government, the congested population, poor sanitation, warm temperature and high humidity might facilitate the growth and distribution of significant pathogenic microorganisms (7). Bird feces, particularly pigeons, provide good nutrients for the growth of some pathogenic molds (8-9). The result of fungal culture in this study corresponds to results found in many literatures (1, 10). The finding showed a high prevalence of Candida spp., Aspergillus spp. and Penicillium spp. Previous reports demonstrated that Candida spp. and Aspergillus spp. were common pathogenic fungi isolates from wild birds and city pigeons (10). In addition, a recent study illustrated that the prevalence rates of fungi found outdoor were comparable regardless of the seasons (11). Therefore, this suggestion supports the prevalence rate found in this study, as the samples collected in this experimental period could substitute as prototypical samples for other potential samples from any other time of the year.

Candida genus can widely disperse in the environment as common saprophytic constituents of normal microflora of humans. They are eukaryotic opportunistic microorganism, and some of these species could become opportunistic pathogens that can induce critical risk depending on altering health status of the host (12-13). The predominance of Candida spp. identified in nearly all observed areas indicates these sites may harmful, especially for immunocompromised patients. Aspergillus spp., fast-growing fungi, are categorized as hazardous since they can produce mycotoxin that cause allergy and severe invasive infections to susceptible patients. Moreover, they are opportunistic pathogens isolated from the wild and city bird feces that include pigeons (14). By comparing each observed area (Bungphyaprab Elementary School, NRRU, Salaloi Temple and Tao Suranaree Monument);our finding demonstrated that the prevalence for Aspergillus spp. was not significantly different. The culturing result indicates that the prevalence of yeast was higher than filamentous fungi, but the collected samples had mixed yeast and molds infestations. The prevalence of co-infection between Aspergillus spp. and Penicillium spp. was 54%. Previous reports suggested that they had ability to produce mycotoxin under favorable conditions (15) which lead to severe progression of chronic respiratory granulomatous diseases (16).Our result also showed that the isolation of Rhizopus spp., Pseudallesheria spp., Tricoderma spp. and Trichophyton spp.could be isolated from pigeon feces. Although these fungi are trivial opportunistic pathogens, they have the ability to cause mild or acute infections to immunocompromised patients (17-19). Furthermore, they could resist against some antifungal drugs (20). Therefore, the co-infection with these classes of fungi may be substantial during the medical treatment of patients.

Conclusion

This study primarily exhibits the occurrence of yeast and filamentous fungi in pigeon feces collected from 4 different sites in Mueang Nakhon Ratchasima. The observed areas include academic campuses and eminent public places in the center of the city. The detection of Candida spp., Aspergillus spp. and Penicillium spp. contaminated in pigeon feces is clinically significant and their invasive nature poses medical concerns to human health. Moreover, there were both yeast and fungi isolate from almost all samples tested. This evidence strongly indicates that pigeon feces area prominent source of substantial risk on the public health.

Acknowledgements

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References

Effect of Live Yeast *Saccharomyces cerevisiae* 
Supplementation on Milk Productive Performance 
in Dairy Cows

Natdaw Klangsaeng¹, Teeraporn Saksaringkan¹, Paramintra Vinitchaikul²*

¹6th year student, Faculty of Veterinary Medicine, Chiang Mai University, Thailand, 50100  
²Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Thailand, 50100  
*Corresponding author Email: pvinitchaikul@gmail.com

Abstract

**Objective** The objective of this study was to determine the effects of live yeast *Saccharomyces cerevisiae* (SC) supplementation on milk yield and milk compositions during lactation period in dairy cows.

**Materials and Methods** Twenty-four crossbred Holstein-Friesian cows matched with parity and lactation stage in a small holder dairy farm were randomly into two groups, (control; n=12 and SC yeast; n=12). The experimental period lasted in 11 weeks with cross-over design, 4 weeks in each treatment period and 3 weeks for washout period between each treatment period. Both groups were fed by the same basal diet throughout the study. SC yeast at $5 \times 10^{10}$ cfu/day was on-top fed over commercial concentrate diet in a morning meal of treatment period. Milk yields and milk samples were collected once a week. Dry matter intake (DMI), body weight (BW) and body condition score (BCS) were comparing observed between the first week and the last week. Data were analyzed by using the MIXED model and repeated measurement analysis packages in R program.

**Results** No significant difference ($P>0.05$) was observed on milk yield, milk compositions, DMI, BW and BCS.

**Conclusion** Live yeast SC supplementation have no negative impact on dairy productive performance. Further studies may necessary in order to elucidate the obvious effects of SC yeast supplementation in dairy cows.

**Keywords:** *Saccharomyces cerevisiae*, Rumen, Milk, Dairy cows
Comparison of Tyrosine Phosphorylated Protein Expression in Seminal Vesicle between Types I and II DM Mice

Sitthichai Iamsaard¹,²*, Supataechasit Yannasithinon¹, Supatcharee Arun, Tarinee Sawatpanich, Amnart Chaichun, Nongnut Uabandit, Pipatpong Kanla

¹Department of Anatomy, Faculty of Medicine, Khon Kaen University, Thailand, 40002
²Mekong Health Science Research Institute, Khon Kaen University, Thailand, 40002
*Corresponding author Email: sittia@kku.ac.th

Abstract

Objective This study was attempted to investigate the expression of tyrosine phosphorylated proteins, important for sperm capacitation and acrosome reaction, compared between type I and II diabetes (DMs) using mice as animal models.

Materials and Methods Twenty-four mice were divided into 3 groups (n = 8); control, DM1, and DM2, respectively. The DM1 mice were induced with multiple low-dose of streptozotocin (MLD-STZ; 40 mg/KgBW) for 5 consecutive days. In DM2 induction, the mice received the commercially high-fat diet (HFD) for 14 consecutive days before injection with a single dose of STZ. All DM animals were experimented for 72 days. The blood glucose levels from tail prick were considered to be DM when greater than 250 mg/dl. The seminal vesicle of both groups were homogenized and applied into SDS-PAGE. Equal total proteins were then detected fortyrosine phosphorylated proteins by using immuno-western blot.

Results It was found that only a 72 kDa protein was a tyrosine-phosphorylated protein present in seminal lysates in control, DM1 and DM2 groups. Significantly, the intensity analysis revealed that this seminal phosphotyrosine protein was increased both DM mice as compared to that of the controls (P<0.05). Moreover, the increase of 72 kDa-phosphorylated protein intensity in DM1 mice was significantly higher than that of DM2 mice (P<0.05).

Conclusion This finding has demonstrated the presence of a 72 kDa-phosphorylated protein in mouse seminal vesicle for the first time and its expression was increased in DM1 animals. This evidence may provide some explanations especially seminal dysfunction associated with the infertility of DM males.

Keywords: Seminal vesicle, Tyrosine phosphorylated protein, Diabetes, Mice
Effect of Type 2 DM on Morphology and Secretions of Seminal Vesicle in Mice Induced by HFD-STZ

Supatcharee Arun 1,* , Supataechasit Yannasithinon 1,2, Porntip Boonruangsri1, Kowit Chaisiwamongkol 1, Sitthichai Iamsaard 1,2,*

1Department of Anatomy, Faculty of Medicine, Khon Kaen University, Thailand, 40002
2Mekong Health Science Research Institute, Khon Kaen University, Thailand, 40002
*Corresponding authors Email: supatar@kku.ac.th and sittia@kku.ac.th

Abstract

Objective This study was to investigate the histopathological changes of seminal vesicles and secretion levels of seminal fluids in mice after high fat diet plus streptozotocin induction.

Materials and Methods Sixteen male ICR mice were divided into two groups (n=8); control and DM2 groups. The control mice were received normal diet for 14 consecutive days before injection with 0.1 M citrate buffer (pH 4.5). In the type 2 DM induction (DM2), mice were received the commercially high-fat diet (HFD) for 14 consecutive days before injection with a single dose of STZ (85 mg/kgBW). The animals were experimented for 72 days. The blood glucose levels from tail prick were considered to be DM when greater than 250 mg/dl. The seminal vesicles were dissected and squeezed to gain the seminal fluid for magnesium and fructosamine assays. In addition, the morphology and histology of seminal vesicle were observed.

Results The size of seminal vesicle in DM2 mice was obviously smaller than that of control. Corroborate with the gross structure, the absolute and relative weights of seminal vesicles in DM2 mice were significantly decreased as compared to control (P<0.05). In histological observation, DM2 mice showed small vacuolization and hyperchromatic nuclei of epithelial seminal tissue as compared to the control. In addition, the epithelial height of seminal vesicles in DM2 was significantly as compared to control group (P<0.05). Moreover, the levels of magnesium and fructosamine were significantly lower than that of control mice (P<0.05).

Conclusion DM2 affected the seminal vesicle tissue and decreased the secretion levels of seminal fluid. This evidence could be some explanation of seminal dysfunction associated with infertility of DM males.

Keywords: Seminal vesicle, Morphology, Secretion levels, Diabetes, Mice
Case study: Grazing Management and Deworming Program Affect to Risk of Multisystemic Infection in Meat Goat Agriculture

Phuncharat Nilsuwan*, Chutikarn kaewaim†, Suttirak petcharat‡, Yanyong pongsatha‡, Nattida mallitong‡, Chunyanuch wichaidit†

1Animal health unit, Regional Bureau of Animal Health and Sanitary 9, Songkhla 90000, Thailand
2Animal health unit, Songkhla Provincial Livestock Office, Songkhla 90000, Thailand
3Veterinary diagnostic Laboratory, Veterinary Research and Development Center (lower southern region), Songkhla 90110, Thailand
*Corresponding author Email: phucharat@hotmail.com

Abstract

Case Description In 9th livestock region area present, one herd meat goat has problems clinical illness present in month ago. Feeding system is included with mainly high concentrate feed and communal grazing with other animal herd, deworming program in every 6 months. Clinical problems have present in late rainy season on December and January.

Clinical Findings 10 in 34 Goats are present health trouble included with low body condition score, present yellowish-brownish watery diarrhea, lethargy, shudder, low appetite and pot-belly sign. 3 goats of them often show nonproductive cough, respiratory efforts. 5 goats had icteric sign and pale membranes. We decided to collect blood, serum and fecalsample for laboratory diagnostic process. Fecal simple floatation test reported gastrointestinal nematodes and Coccidian. Theileria spp. was detected in blood samples. In immunoserology test, one sample had positive to caprine arthritis encephalitis with competitive enzyme-linked immunosorbet assay (cELISA) technique. Indirect hemagglutination test showed positive to melioidosis. Leptospirosis infection was confirmed by latex agglutination.

Treatment and Outcome All ill goats were treated by oral albendazole suspension dosage 10 milligrams per kilogram (mg/kg) and piperazine powder in sterile water 200 mg/kg for deworming. Antibiotic for infection control, selected with enrofloxacin dosage 10 mg/kg for 7 days continuously. Most of them response to treatment and improved to well status, but some goat still show new case and deceased later. After we got reports, antibiotic was changed into oxytetracycline administration dosage 10 mg/kg for blood parasite control in 14 days with adjusts grazing management, feed process, household disinfecting treatment, and separate them from healthy goats.

Clinical Relevance Normally, goats behavior are communal grazing due to character of weather in southern is higher than other region of Thailand, which may cause to gastrointestinal parasites infection. Deworming program is the most important management to reduce risk of infection. However, farmer should be change types of drug for prevent anthelminthic tolerances. Risk of infection was reduced by avoid communal grazing in goat production and replace by forage management with grass chop method. Treatment process not only use with single type antiparasitic drugs but also must to use another drugs following to any clinical sign. In addition, housing management is also important to do in conjunction with drug treatment.

Keywords: Goat, Livestock, Grazing management, Deworming program, Multisystemic infection,
Molecular Characterization of S2-3a/3b-E-M-4b/4c-5a/5b-N Gene of QX-like and Variant Genotype Infectious Bronchitis Virus Isolated in Thailand Reveals a Distinct E Gene

Tawatchai Pohuang\textsuperscript{1,2*}, Sucheeva Junnu\textsuperscript{1,2}

\textsuperscript{1}Faculty of Veterinary Medicine, KhonKaen University, KhonKaen 40002, Thailand. \\
\textsuperscript{2}Research Group of Preventive Technology in Livestock, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen 40002, Thailand. \\
*Corresponding author Email: ptawat@kku.ac.th

Abstract

Objective This study investigated the genetic diversity of infectious bronchitis virus isolated in Thailand by analysis of the nucleotide sequences covering S2-3a/3b-E-M-4b/4c-5a/5b-N gene.

Materials and Methods Two isolates of infectious bronchitis virus (IBV) were used in this study including THA80151 (Thai QX-like) and THA50151 (Thai variant genotype). The S2-3a/3b-E-M-4b/4c-5a/5b-N gene was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR products were sequenced in both the forward and reverse directions. The nucleotide sequences were assembled, aligned and compared with published IBV sequences deposited in the GenBank database. Phylogenetic analysis of the nucleotide sequences was performed with the neighbor-joining method in MEGA7.

Results They had nucleotide identity of 96.3% with each other. They showed the highest similarity (92.7-93.8%) with KM91 isolated in South Korea. Phylogenetic analysis of the nucleotide covering S2-3a/3b-E-M-4b/4c-5a/5b-N gene showed that they had a relationship with KM91. Interestingly, Thai IBVs were clustered into a novel group when the phylogenetic analysis was performed by using E gene.

Conclusion The QX-like and variant genotype IBV circulating at the present time in Thailand were originated from the similar lineage. They have a relationship with KM91 isolated in South Korea with some of mutation in their genome.

Keywords: infectious bronchitis virus, nucleotide sequence, phylogenetic analysis
Antibacterial Effect of *Eleutherine americana* Merr.
Extract and It’s Combination with Cephalexin
against Clinical Isolates of *Staphylococcus intermedius*

Jareerat Aiemsard¹*, Korawuth Punareewattana²

¹Faculty of Veterinary Medicine and Biofilm Research Group, Khon Kaen University, Muang, Khon Kaen, Thailand, 40002
²Faculty of Veterinary Medicine, Khon Kaen University, Muang, Khon Kaen, Thailand, 40002
*Corresponding author: Email: Jaraim@kku.ac.th

**Abstract**

**Objective** To determine antibacterial effect of *Eleutherine Americana* Merr. extract and it’s combination with cephalexin against clinical isolates of *Staphylococcus intermedius*.

**Materials and Methods** *Eleutherine Americana* Merr. was extracted from freshly collected plant using 70% ethanol. The organisms used in this study were 34 samples of *Staphyllococcus intermedius* isolated from dogs in Khon Kaen province. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of *E. americana* and cephalexin toward the tested organisms were evaluated by broth microdilution method. The synergistic effect between *E. americana* and cephalexin against *S. intermedius* was evaluated using checkerboard titration method.

**Results** The study revealed that the MICs/MBCs ranges of *E. americana* and cephalexin were 0.488-7.812/0.488-7.812 mg/ml and 0.002-0.064/0.002-0.064 mg/ml, respectively. Synergistic effects of *E. americana* and cephalexin was found to be indifferent interaction in low sensitivity group and partial synergistic interaction in high sensitivity group.

**Conclusions** The present study has demonstrated the antibacterial activity of the *E. americana* extract on *S. intermedius* isolated from dogs suffering from skin pyoderma. However, an attempt on synergistic study with a group of antibiotics found unfavorable outcomes. Nevertheless, *E. americana* as a local herb is still worthwhile for further investigations for its potential use for bacterial skin infections.

**Keywords:** *Eleutherine Americana* Merr. extract, Anti-bacterial effect, *Staphylococcus intermedius*

**Introduction**

Pyoderma is one of the most common diseases of the dog. The predominant pathogen is *Staphylococcus intermedius*, a commensal bacterium that resides on the mucosal and skin surface of dogs [1]. Pyoderma can often become a chronic and/or recurrent condition if the primary underlying cause is not identified and adequately resolved or controlled. Beta-lactam antibiotics have been recommended for use in treating canine pyoderma because of their relevant antimicrobial spectrum [2]. With a growing incidence of antibiotic resistance [3], natural products from plants were increasingly advocated as alternatives for antimicrobial purposes. *Eleutherineamericana*Merr., a native Thai plant, has been demonstrated for its various medicinal properties including antibacterial, antifungal and antioxidant activities [4,5,6]. However, up to date there is no reports demonstrating the antibacterial activity of *E. americana* on *S. intermedius*. In the present study, we investigated primarily the antibacterial activity of *E. americana* on clinical isolates of *S. intermedius* and secondarily the possibility of synergistic effect of *E. americana* in combination with cephalexin.
Materials and Methods

**Plant and organisms:** *Staphylococcus intermedius* were obtained from dogs with pyoderma. *S. intermedius* DMST 3482 was used as a reference strain. *Eleutherine americana* Merr. was extracted from freshly collected plant using 70% ethanol. The filtrates were concentrated on a rotary evaporator at 45°C and freeze-dried before being used.

**Determination of MIC by Microdilution Method:** Thirty four samples of *S. intermedius* were used to evaluate the sensitivity profile of the extract and cephalexin. The plant extract and antibiotic were serial diluted in Mueller Hinton broth, then bacterial inoculum size of 106 CFU /ml was added to each well. Controls without plant extract, without bacterial inoculums were also included in the experiment. Each experiment was performed in duplicate. The test plates were incubated at 37°C for 18 h. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the tested microorganism [7].

**Synergy testing:** The organisms tested were divided into 2 groups, low sensitivity (n=6) and high sensitivity (n=4), according to their response to cephalexin. The checkerboard method was used for determining synergistic effect. The fractional inhibitory concentration (FIC) index was then calculated for each bacterial samples to demonstrate its type of drug interaction. The Fractional Inhibitory Concentration (FIC) was derived from the lowest concentration of cephalexin and *E. americana* combination permitting no visible growth of the test organisms on the plates. FIC value for each agent was calculated using the formula: FIC (cephalexin) = MIC of cephalexin in combination / MIC of cephalexin alone. FIC (E. americana) = MIC of E. americana combination / MIC E. americana alone. Combinations were classified as synergistic, if the FIC indices were ≤0.5, partial synergy at >0.5 but <1.0, indifferent if the FIC indices were >1 and antagonistic if the FIC indices were ≥4. FIC index = FIC of E.americana + FIC of cephalexin [8].

Results and Discussion

The antibacterial of *E.americana* extract and cephalexin on *S. intermedius* clinical isolates were investigated. The MIC50 and MIC90 values and MIC range were shown in Table 1. The range of MIC values of cephalexin were 0.002 to 0.064 mg/ml and the MIC50 and MIC90 for *S. intermedius* were 0.008 and 0.016 mg/ml. The result indicated that bacterial samples tested in this study were in the range of sensitive to intermediately sensitive to cephalexin, according to the general criteria of antibiotic sensitivity testing. The MIC values of *E. americana* extract were in the range from 0.488 to 7.812 mg/ml, the MIC50 and MIC90 for *S. intermedius* were 3.906 mg/ml. These results could be considered as low concentration, and could interpret as that *E. americana* extract possesses strong antibacterial effect on *S. intermedius*. Our findings were in agreement with previous study that demonstrated antibacterial activity of *E. americana* extract against *Staphylococcus aureus* [9]. In light of the possible antibacterial mechanism of the extract from *E. americana*, previous reports had chemically analyzed and hypothesized that naphthoquinone derivatives may play a major role in damaging bacterial cell membrane [10].

The results of checkerboard titration expressed as FIC index were presented in Table 2. For low sensitivity organisms, interaction was indifferent. However,*E. americana* was lowered from 3.906 mg/ml to 0.122 mg/ml (1/32MIC) in combination with 0.016 mg/ml (1MIC) of cephalexin. The partial synergy was observed in high sensitivity of tested bacteria, the MIC of cephalexin was lowered from 0.002-0.008 mg/ml to 0.001-0.002 mg/ml in combination with 0.122 mg/ml (1/8 MIC). It was found that the presence of sub-inhibitory concentrations (1/8 MIC) of the extracts modulated the activity of cephalexin by reducing the concentration of antibiotic 1-4–fold in inhibiting the growth of bacteria. There was no evidence of synergistic effect of drugs on *S. intermedius*.

| Table 1. In vitro antibacterial susceptibility of *Staphylococcus intermedius* by broth microdilution |
|---|---|---|---|
| **S. intermedius strains** | **Agents** | **Boroth microdilution (mg/ml)** |
| | | MIC range | MIC50 | MIC90 |
| Clinical isolates (n=34) | *E. americana* | 0.488-7.812 | 3.906 | 3.906 |
| Cephalaxin | 0.002-0.064 | 0.008 | 0.016 |
Acknowledgements

This study was supported by Faculty of Veterinary Medicine, KhonKaen University.

References


**Table 2. Synergistic effects of *E.americana* with cephalaxin against *Staphylococcus intermedius*.**

<table>
<thead>
<tr>
<th><em>S. intermedius</em></th>
<th>Agents</th>
<th>MIC (mg/ml)</th>
<th>FIC</th>
<th>FIC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alone</td>
<td>Combination</td>
<td>(mg/ml)</td>
<td>Index</td>
</tr>
<tr>
<td>Low sensitivity (n=6)</td>
<td>Cephalexin</td>
<td>0.016</td>
<td>0.016</td>
<td>1.00</td>
<td>1.03</td>
</tr>
<tr>
<td>High sensitivity (n=4)</td>
<td><em>E. americana</em></td>
<td>3.906</td>
<td>0.122</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td><em>S. intermedius DMST 3482</em></td>
<td>Cephalexin</td>
<td>0.002-0.008</td>
<td>0.001-0.002</td>
<td>0.50</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td><em>E. americana</em></td>
<td>0.976</td>
<td>0.122</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> FIC index was interpreted as synergy at ≤0.5, partial synergy at >0.5 but <1.0, indifferent at >1.0 and <4.0, and antagonistic when values were ≥4.0.
Beta-hydroxybutyrate, Fat to Protein Ratio, Ovarian Activity and Subclinical Ketosis in Early Postpartum Dairy Cows in Khon Kaen Province

Panupong Romsi¹, Worawee Sapprasert¹, Korapat Sriwallapanonth¹, Peerapat Deesuk², Avirut Wichaiwong², Suvaluk Seesupa¹, Kanawit Patano¹, Suneerat Aiumlamai²*

¹Fifth year student, Faculty of Veterinary Medicine, Khon Kaen University
²Faculty of Veterinary Medicine, Khon Kaen University
³Dairy Farming Promotion Organization of Thailand (Northeast)
*Corresponding author Email: suneerat@kku.ac.th

Abstract

Objective To study a correlation of subclinical ketosis (SCK) and ovarian activity in early postpartum cows and evaluated fat to protein ratio (FPR) in bulk milk for SCK diagnosis.

Materials and Methods A total of 78 crossbred Holstein dairy cows from 24 farms in Khon Kaen province were used in the study. Reproductive examination of all cows was done and used to classify those cows as cycling (found corpus luteum) and noncycling cows (found no corpus luteum). Blood samples were collected individually at the same time of reproductive examination for Beta-hydroxybutyrate (BHBA) analysis. Based on the BHBA level, ketosis status of each cow be classified as clinical ketosis (BHBA > 2.9 mmol/l), subclinical ketosis (BHBA ranges from 1.2-2.9 mmol/l), normal (BHBA < 1.2mmol/l) groups. FPR data of bulk milk analysis (BM) in the same month of blood sampling was calculated. Herds were classified as high-risk of having SCK cows once the FPR greater than 1.3. Descriptive analysis, ANOVA test and Chi-square test were used to analyze data.

Results The prevalence of SCK and clinical ketosis was 20.15% and 3.85%, respectively. Effect of the ketosis status of the cows on their ovarian activity on 40-50 days postpartum were not different (X² = 0.0939, df=2, p-value > 0.05). For herd-level detection of SCK by using BM, there were six out of 24 farms (25%) had FPR greater than 1.3. The proportion of herds that had FPR greater than 1.3 in groups of clinical ketosis (2/3; 66.6%), SCK (6/13; 46.15%) and normal (6/53; 11.32%) were significantly different (X² = 12.0028, df=2, p value < 0.005).

Conclusion The prevalence of SCK in early post calving of dairy cows in smallholder farms in Thailand was high. Effect of the ketosis status of the cows on their ovarian activity on 40-50 days postpartum were not different. The FPR at herd level which greater than 1.3 was high and could be used to monitor SCK of cows in those farms.

Keywords: Beta-hydroxybutyrate, Fat to protein ratio, Ovarian activity, Subclinical ketosis, Dairy cows

Introduction

Subclinical ketosis (SCK) is an important and common condition of early-lactation dairy cattle. After calving, dairy cows lose her body condition score (BCS) rapidly, increasing risk for negative energy balance (NEB) which could develop an excess of ketone bodies; ketosis. The ketosis cow would have several health disorders consequences from metritis, mastitis, displaced abomasum to increase days to conception. Furthermore, several studies suggest that low energy balance in early lactation negatively affects oocyte quality and decrease conception rate at first service (1). Diagnosis of subclinical ketosis (SCK) by measuring beta-hydroxybutyrate (BHBA) concentrations in the blood is a practice clinical used tool. A new parameter, ratio of fat to protein percentage (FPR) in milk has been proposed to diagnosis SCK in dairy cows, as a cow with a high FPR have a risk of SCK (2). Aims of this study were to investigate a correlation of SCK and ovarian activity in early postpartum cows and evaluated FPR in bulk milk for SCK diagnosis.
Materials and Methods

A total of 78 crossbred Holstein dairy cows from 24 farms in KhonKaen province were used in the study under criteria as follow; within 40-50 days postpartum period, >2.5/5.0 BCS and in the 2nd-4th lactation. Reproductive examination of all cows was done and used to classify those cows as cycling (found corpus luteum) and noncycling cows (found no corpus luteum). Blood samples were collected individually at the same time of reproductive examination for BHBA analysis. Based on the BHBA level, ketosis status of each cow be classified as clinical ketosis (BHBA > 2.9 mmol/l), subclinical ketosis (BHBA ranges from 1.2-2.9 mmol/l), normal (BHBA <1.2mmol/l) groups (3). FPR data of bulk milk analysis (BM) in the same month of blood sampling was calculated. Herds were classified as high-risk of having SCK cows once the FPR greater than 1.3 (4). Descriptive analysis, ANOVA test and Chi-square test were used to analyze data.

Results and discussion

The average BCS, lactation number and days in milk (DIM) were not different among the three groups (p-value > 0.05). Among the cows having our criteria as within 40-50 days postpartum period, >2.5/5.0 BCS and in the 2nd-4th lactation, the prevalence of SCK and clinical ketosis was 20.15% and 3.85%, respectively (Table 1). The prevalence of SCK in smallholder dairy herds in our study and other regions of Thailand were similar; 20.4 % - 21.1% (5, 6).

Table 1. BHBA, non-cycling cows, FPR, BCS, lactation number (Lac no.) and DIM of 78 cows in three groups. Different superscripts show that the values in the same column are significantly different (P<0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>BHBA mmol/l Mean±SD (%;cow number)</th>
<th>Non-cycling cows / total cows (%)</th>
<th>FPR &gt; 1.3 Mean±SD (% of cows FPR&gt;1.3;cow number)</th>
<th>BCS Mean±SD</th>
<th>Lac no. Mean±SD</th>
<th>DIM Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical ketosis (&gt; =3 mmol/l)</td>
<td>3.72 ±0.35a</td>
<td>1/3</td>
<td>1.34±0.02ab</td>
<td>2.75 ±0.25</td>
<td>3.67</td>
<td>46.67 ±5.77</td>
</tr>
<tr>
<td>(2.5-2.9 mmol/l)</td>
<td>(3.85%;3/78)</td>
<td>(33.33%)</td>
<td>(66.67%;2/3)</td>
<td></td>
<td>±0.58</td>
<td></td>
</tr>
<tr>
<td>Subclinical ketosis (1.2-2.9 mmol/l)</td>
<td>1.69±0.29a</td>
<td>4/16</td>
<td>1.33±0.01a</td>
<td>2.66 ±0.20</td>
<td>2.69</td>
<td>44.44±2.87</td>
</tr>
<tr>
<td>Normal cow (&lt;1.2 mmol/l)</td>
<td>0.81±0.20a</td>
<td>16/59</td>
<td>1.32±0.03b</td>
<td>2.66 ±0.24</td>
<td>2.68</td>
<td>44.73±2.94</td>
</tr>
<tr>
<td>(&lt;1.2 mmol/l)</td>
<td>(75.64%;59/78)</td>
<td>(27.11%)</td>
<td>(11.32%;6/53)</td>
<td></td>
<td>±0.73</td>
<td></td>
</tr>
</tbody>
</table>

Effect of the ketosis status of the cows on their ovarian activity on 40-50 days postpartum were not different (X² = 0.0939, df=2, p-value > 0.05). The finding probably affected the day of serum sampling and inadequate reproductive examination. As indicated by Jenkins et al. (2015), most cases of SCK cows occur on 14-21 days and 21-42 days after calving in group-fed and component-fed, respectively. Thus, at the sampling time of 40-50 days postpartum, we probably missed some SCK cow that lowered their BHBA level at the time to be normal. Determining the incidence of SCK required repeated testing of cows throughout the risk period. Testing must occur twice or more weekly in order to accurately access the incidence of SCK (2). Furthermore, the reproductive examination should be done repeatedly for an accurate reproductive status.

For herd-level detection of SCK by using BM, there were six out of 24 farms (25%) had FPR greater than 1.3. Previous study reported that FPR > 1.3 was only11.8% (7). This might be most cows in this study were in early lactation. Herd in SCK group had a higher average FPR than the normal (Table 1). The propor-
tion of herds that had FPR greater than 1.3 in groups of clinical ketosis (2/3; 66.6%), SCK (6/13; 46.15%) and normal (6/53; 11.32%) were significantly different ($X^2 = 12.0028$, df=2, p value < 0.005). This study showed that the FPR of BM could be used to monitor SCK. However, FPR of BM is not a proper tool to define SCK cows because all cows are not in the same stage of lactation. FPR of individual cows is wildly used to monitor SCK in dairy cows and could assign the proper treatment in those cows and improve nutrition in farm (1, 2). However, determining the incidence of SCK required repeated testing of cows throughout the risk period. Thus, herd health and production management program combine with BHBA and FPR could be a tool for early detection of SCK and improve fertility and productivity in dairy farms (8).

Conclusions

The prevalence of SCK in early post calving of dairy cows in smallholder farms in Thailand was high. Effect of the ketosis status of the cows on their ovarian activity on 40-50 days postpartum were not different. The FPR at herd level which greater than 1.3 was high and could be used to monitor SCK of cows in those farms. Therefore, BHBA and FPR in the early postpartum period could be used as a monitoring tool to diagnosis SCK for early treatment and improve nutrition in a dairy farm.

Acknowledgements

This study was supported by Faculty of Veterinary Medicine, KhonKaen University. Thanks to Dairy Farming Promotion Organization of Thailand (Northeast region) for farms information.

References

Multidrug-resistant Enterobacteriaceae Isolated from Dogs and Cats at the Veterinary Teaching Hospital, Khon Kaen University, Thailand during 2011 to 2018

Jeerasak Khlongkhlaeo, Pattara-anong Buppata, Manassanan Borisutpeth, Pithai Kanbutra

Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
Corresponding author Email: kpitha@kku.ac.th

Abstract

Objective The aim was to determine the occurrence of antimicrobial resistant and multidrug-resistant Enterobacteriaceae isolated from clinical specimens collected from dogs and cats.

Materials and Methods This study reviewed retrospectively the bacterial culture and antimicrobial susceptibility records of 299 clinical specimens collected from 260 dogs and 39 cats examined at the veterinary diagnostic laboratory between 2011 and 2018. The bacterial identification using RapID systems and ERIC® software and antimicrobial susceptibility testing (AST) by disk diffusion method were carried out. Antimicrobial categories/agents discs used to determine the antimicrobial resistant (AMR) bacteria were aminoglycosides/amikacin (AK), fluoroquinolones/enrofloxacin (ENR), folate pathway inhibitors/trimethoprim-sulphamethoxazole (SXT), tetracyclines/doxycycline (DO), and penicillins + β-lactamase inhibitors/amoxicillin-clavulanic acid (AMC). The AMR included multidrug-resistant (MDR), not MDR, and MDR possible extensively drug-resistant (XDR) bacteria were defined as per criteria created by ECDC and CDC.

Results There were 299 clinical specimens examined, including 125 urine (41.8%), 107 pus (35.8%), 17 surgical specimen (5.7%), 16 ear swab (5.4%), 10 nasal swab (3.3%), and 24 miscellaneous (8.0%). A total of 350 Enterobacteriaceae strains were recovered, consisting of 154 Escherichia coli, 97 Proteus spp. (P. mirabilis, P. vulgaris, and P. penneri), 47 Klebsiella spp. (K. pneumoniae and K. oxytoca), 30 Enterobacter spp. (E. cloacae and E. aerogenes), 11 Morganella morganii, and 11 others. There was 74.3% of the isolated Enterobacteriaceae defined as AMR bacteria, whereby 40.0, 23.4, and 10.9% were defined as not-MDR, MDR, and MDR possible XDR, respectively. Among the AMR Enterobacteriaceae, MDR combined with MDR possible XDR was counted to be 20.0, 5.5, 4.6, 2.6, 0.6, and 1.2% for E. coli, Klebsiella spp., Proteus spp., Enterobacter spp., M. morganii, and others, respectively. In descending order, the AMR Enterobacteriaceae showed resistance to SXT, DO, ENR, AMC, and AK to be 54.3, 51.0, 50.0, 23.8, and 4.6%.

Conclusion In this study, E. coli, P. mirabilis, K. pneumoniae, and E. cloacae were the majorities Enterobacteriaceae isolated from dogs and cats. The AMR Enterobacteriaceae emerged in extremely high prevalence (74.3%), moreover, the MDR combined with possible XDR Enterbacteriaceae were also occurring in high frequency (34.3%). The use of antimicrobials that widely administrated in veterinary practice, including trimethoprim-sulphamethoxazole, doxycycline, and enrofloxacin should be concerned because of the high occurrence of the resistant strains. However, aminoglycosides (AK) appeared to possess the most efficient and could be considered as an antimicrobial of choice for Enterbacteriaceae infections treatment.

Keywords: Antimicrobial susceptibility, Multidrug-resistant bacteria, Antibiotic resistance
Clinical Study of Diabetes Mellitus in 33 Dogs

Sarocha Permsab, Nittiwadee Lertittikul, Narichsara Lertchaisathaporn, Numfa Fungbun*

Small Animal Veterinary Teaching Hospital, KhonKaen University, Thailand, 40002
Corresponding author Email: numfa@kku.ac.th

Abstract

Objective To describe the clinical findings of the newly diagnosed diabetic dogs presented at Small Animal Veterinary Teaching Hospital, Khon Kaen University during January 2016-December 2018.

Materials and Methods Clinical diagnosis of diabetic mellitus (DM) consisted of the presence of clinical signs (e.g. polyuria/polydipsia; PU/PD, polyphagia, weight loss) with fasting hyperglycemia and glucosuria. Signalment and clinical signs of the newly diagnosed diabetic dogs were recorded. Routine hematology, serum biochemistry as well as urinalysis were assessed. Venous blood gases of 5 diabetic dogs were examined. Serum canine pancreatic lipase (cPL) activity was measured by a semiquantitative cPL immunoassay (Snap cPL). Abdominal ultrasound examination of some diabetic dogs was done.

Results The diabetic dogs were mostly middle to old age of small breed. Common signs of the newly diagnosed diabetic dogs were PU/PD (69.70%), weight loss (63.64%), cataract (45.45%), vomiting (30.30%) and polyphagia (18.18%). Diabetic dogs with leukocytosis (60.6%) were noticed. Only 5 of 33 diabetic dogs (15.15%) had fasting blood glucose level more than 600 mg/dl. Clinical abnormalities, apart from hyperglycemia and glucosuria were increased activities of ALP (84.62%), and ALT (75.75%), ketonuria (69.7%) and non-ketonuria (30.30%). Decrease in blood pH (mean±SEM; 7.236±0.05) was found (4 of 5 diabetic dogs; 80%). Mean±SEM of serum HCO₃⁻ concentration was 12.66±1.91mEq/L. Mild to moderate hypokalemia (5 of 5 dogs; 100%) and hypochloremia (3 of 5 dogs; 60%) were observed. Serum sodium concentration corrected for hyperglycemia was performed and hyponatremia was found 80% (4 of 5 diabetic dogs). Abnormal level of serum cPL activity of 16 from 21 diabetic dogs (77.27%) was detected. The newly diagnosed diabetic dogs with abnormal level of serum cPL activity had no vomiting (52.38%) and vomiting (23.81%). Finally, ultrasonographic findings of pancreatic change (eg. echotexture, size and shape) with abnormal serum cPL activity (4 of 5 diabetic dogs; 80%) were found. Other diagnosed concurrent diseases were blood parasite infection (39.39%), urinary tract infection (33.33%), kidney disease, tumor, skin disease (each 12.12%), cystic calculi (6.06%), pyometra (3.03%), hyperadrenocorticism (3.03%) and hypothyroidism (3.03%).

Conclusion Classical signs of the newly diagnosed diabetic dog were PU/PD and weight loss. Cataract as well as vomiting were also observed in some dogs. Complicated DM were diabetic keto(acido)sis. However, hyperglycemic hyperosmolar state might present in some dogs. It was suggested that pancreatitis might be one of concurrent diseases in some diabetic dogs.

Keywords: Diabetes mellitus, Dogs
Prevalence of Gastrointestinal Parasitic Infections of Goats in Non Thai, Nakhon Ratchasima, Thailand

Sompong Wongma, Pimchanok Lohtongkam*, Ratchaneekorn Moonpa, Jantima Krisakun, Jirada Kamma

1,2,4,5 Veterinary Technology Program, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, THAILAND
3 Agriculture Program, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, THAILAND
*Corresponding author Email: pimchanok.l@nrru.ac.th

Abstract

Objective To assess the prevalence of gastrointestinal parasites in goats of Non Thai district of Nakhon Ratchasima, Thailand

Materials and Methods In this study, the prevalence of parasitic infections was recorded from different 13 farms of Non Thai District, Nakhon Ratchasima. Total of 224 goats including 15 goat kids, 128 yearlings and 81 adult goats were sampled once between February and December 2018 in three different seasons for assessing seasonal fluctuations. Fecal samples were analyzed using standard parasitological screening techniques for intestinal parasites, namely, simple salt floatation technique followed by Formalin-ether centrifugation sediment technique, direct saline and iodine smear observations.

Results The present study revealed that, out of the total 224 fecal samples collected, 224(100%) were positive for gastrointestinal parasites, predominated by Strongyloides spp. (90.62%) and least predominant gastrointestinal parasites was Fasciola spp. (2.67%).

Conclusion The current study is useful in the development of appropriate control strategies for the different areas. This has a potential to reduce production losses and improve rural livelihoods.

Keywords: Gastrointestinal parasites, Goat, Non Thai district

Introduction

Domestic goat is among the earliest animals domesticated by man. They are distributed worldwide with higher concentration in tropical areas and in dry zones, since they are extremely hardy animals that can survive and reproduce under extremely high temperature and low humidity with minimum available feed[1]. Environmental factors and vector abundance have been incriminated in the distribution of most parasitic diseases. Gastrointestinal parasites are common in both temperate and tropical countries, but more prevalent in warm countries where sanitation is poor and standard of living is low [2]. Gastrointestinal parasitism is associated with lowered productivity, reduced animal performance [3], mortality and morbidity [4]. Gastrointestinal nematode infections are the main prevalent parasitic diseases affecting small ruminant productivity worldwide, especially in tropics and sub-tropics [5]. Due to parasitism, the animals become susceptible to other health problems which can lead to death. Globally the most common nematode species known to affect small ruminants are Haemonchus contortus, Trichostrongylus colubriformis, Teladorsagia circumcincta and some species such as Nematodirus spp. [6]. In general, severe gastrointestinal nematode infections has been attributed to the migration of the infective larvae after ingestion rather than the adult worms in the gut [7]. Goats infected with internal parasites show a rough dull-coat, weakness, diarrhea, tail rubbing, signs of hypo-proteinaemia, submandibular oedema (bottle jaw), loss of appetite and weight loss. Additionally gastrointestinal nematodes have also been known to infect livestock, having moderate to high pathogenic effects [8]. Considering the importance of gastrointestinal parasite infections in goats and their implication for public health, limited area-specific studies conducted in Thailand have generated limited information on gastrointestinal parasite prevalence in the different agro-ecological regions and associated risk factors to parasite infection. Information on the prevalence can be used to control parasite infections. The present study was undertaken to assess the prevalence of gastrointestinal parasites in goats of Non Thai...
Materials and Methods

Study Area and Sampling Technique: In this study, the prevalence of parasitic infections was recorded from different 13 farms of Non Thai District, Nakhon Ratchasima. Total of 224 goats including 15 goat kids, 128 yearlings and 81 adult goats were sampled once between February and December 2018 in three different seasons for assessing seasonal fluctuations. The animals were categorized as (a) kids: age below 12 months, (b) yearlings: 12 to 24 months old, (c) adults: above 24 months old. Fecal samples were appropriately collected from the rectums of goats using protective disposable gloves, kept in clean and dry universal bottles and taken to laboratory section of the Veterinary Technology Program, Nakhon Ratchasima Rajabhat University.

Laboratory Techniques: Fecal samples were analyzed using standard parasitological screening techniques for intestinal parasites, namely, simple salt flotation technique followed by Formalin-ether centrifugation sediment technique [9], direct saline and iodine smear observations. Fecal smears were prepared from fresh faecal samples on glass slides using saturated salt solution and covering with cover slips. The slides were examined microscopically for helminth eggs, oocysts and larvae using 10x and 40x objectives. The parasite eggs/oocysts, larvae, and cysts were examined and identified to the generic level of the parasite by microscopy based on the morphological identification keys described by Anne and Conboy[10].

Results

The present study revealed that out of the total 224 fecal samples examined, all(100%) were positive for gastrointestinal parasites. As summarized in Table 1, the highest prevalence of gastrointestinal parasites infection was observed in yearlings 57.14%, (128/224) followed by the adults 36.16% (81/224) and kids 6.70% (15/224). In rainy season the prevalence was 35.71% (80/224), while in winter season 33.04% (74/224) and in summer 31.25% (70/224). Female goat had the prevalence of 93.31% (209/224) while male 6.69% (15/224). The preference of various gastrointestinal parasites was displayed in Figure 1. The highest prevalence of gastrointestinal parasites was Strongyle type eggs 98.21% (220/224) followed by Strongyloides spp. 90.62% (203/224), Trichuris spp. 13.83% (31/224), Moniezia spp. 22.32% (50/224), fluke worms 7.14% (16/224) and the oocysts of coccidia 8.93% (20/224). The mixed gastrointestinal parasitic infection was also detected in 82.59% (185/224).

Table 1. Prevalence of gastrointestinal parasites in relation to age, season and sex.

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. (%) Examined</th>
<th>No. (%) Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kid</td>
<td>15</td>
<td>6.70</td>
</tr>
<tr>
<td>Yearling</td>
<td>128</td>
<td>57.14</td>
</tr>
<tr>
<td>Adults</td>
<td>81</td>
<td>36.16</td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
<td>100.00</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rainy</td>
<td>80</td>
<td>35.71</td>
</tr>
<tr>
<td>Winter</td>
<td>74</td>
<td>33.04</td>
</tr>
<tr>
<td>Summer</td>
<td>70</td>
<td>31.25</td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
<td>100.00</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>209</td>
<td>93.31</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>6.69</td>
</tr>
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<td>Total</td>
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</table>
Discussion

In Thailand, the detection of gastrointestinal parasites in cattle and sheep have been widely reported. However, there is a conspicuous lack of relevant data focusing on gastrointestinal nematodiasis isolated from goats in Thailand. Current finding showed that 100% of studied animals were positive with gastrointestinal parasites, predominated by strongyle nematodes (98.21%), following by *Strongyloides* spp. (90.62%) and the least predominant gastrointestinal parasites were fluke worms (7.14%). The findings were closely related to the results reported by Hassan and co-authors [11] in Bangladesh who found 51.74% of goats were infected with *Strongyloides* spp. However, the results from studies done in Penang Island [12] and Peninsular Malaysia [13] revealed that the most prevalent nematode species infecting the goats was *Haemonchus* spp., one of strongyle nematodes, with 45.7% and 46.3%, respectively. Likewise, the predominance of *Haemonchus* spp. in goats has also been reported in Nigeria (87.3%) [14] and South Africa (54%) [15]. The most prevalence was high in the rainy season. In our study, high infections with *Strongyloides* spp. could be explained by the environment in which the goats being reared, and also by poor animal management. The only cestode observed in the study area was *Moniezia* spp. The occurrence of this parasite in the tropics is associated with the ingestion of oribatid mites infected with larvocysts of *Moniezia* spp. [16]. Results for fluke worm were as low as those reported by Khanjari and co-authors [17]. According to these authors, for the development of the intermediate host, temperature (>9.5°C), rainfall and soil moisture are also important factors influencing the development of the parasite from egg to miracidium. However, infections may have been low in goats due to their foraging behavior, which minimizes chances of ingesting the metacercaria which are found on plants closer to the ground. Factors affecting in this study, age-wise prevalence with young animals was more susceptible and higher than the adult animals. The protective effect in older animals is therefore attributed to acquired immunity through frequent exposure. We observed that females were more susceptible than males (Table 1). The results from this study were similar to the findings of Emiru and co-authors [18] and Vieira and co-authors [19] in Ethiopia and Brazil, where females were more susceptible to parasitic infection than males. This was attributed to lowered resistance of female animals due to their reproductive events and insufficient diet against higher needs.

In conclusion, the results from the current study indicated that the prevalence of gastrointestinal nematodes was high in goats and strongyle nematodes were the most common parasites identified. The study
identified season, sex and age as the most relevant risk factors for the development of gastrointestinal parasites. Knowledge on these gastrointestinal parasite species is important in the development of appropriate control strategies for the different areas. This has a potential to reduce production losses and improve rural livelihoods.

Acknowledgments

The authors would like to express our gratitude to the owners of the farms and Nakhon Ratchasima Provincial Livestock Office. The study was funded by the Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University.

References


Transcriptome Analysis of Infected CRFK Cells by Canine Parvovirus Type 2c from Laotian Isolates Analyzing via RNA-Sequencing

Soulasack Vannamahaxay¹, Benjaporn Sornpet², Kidsadagon Pringproa¹, Prapas Patchanee³, Phongsakorn Chuammitri¹*

¹Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand, 50100
²Central Laboratory, Faculty of Veterinary Medicine, Chiang Mai University, Thailand, 50100
³Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Thailand, 50100
*Corresponding author Email: phongsakorn@gmail.com, phongsakorn.c@cmu.ac.th

Abstract

Objective The objective of the current study was to determine the changes in RNA expression in infected Crandell Rees feline kidney (CRFK) cells with canine parvovirus type 2c (CPV-2c) isolated from Vientiane, Lao PDR by performing RNA sequencing (RNA-seq).

Materials and Methods Two isolates of potent CPV-2c from diarrheic dogs were in vitro challenged in CRFK cells or left uninfected (n = 2 each), and later confirmed virus infectivity by mouse anti-CPV monoclonal antibody via Immunocytochemistry (ICC) and IFA assay. Subsequently, cells were subjected to RNA extraction, library preparation and RNA sequencing via HiSeq2000 Illumina technology. Data analysis was performed, including novel gene prediction, differential expression analysis (DEG), GO/KEGG enrichment, and protein-protein interaction (PPI) analysis. Data availability: GEO database under accession code GSE124753.

Results We show for the first time the unique gene expression profiles induced by the in vitro infection of CPV-2c. Our results revealed a number of unique DEG; upregulation (789 genes) and downregulation (814 genes) in infected cells. The overall top upregulated or downregulated genes in infected cells are MMP9, IL11, Novel00973 and Novel00507, GSTA2, ENPP1, PIK3R3, respectively. Notably, we have identified a total of 1,762 novel genes in this study. The weighted gene co-expression network analysis (WGCNA) identified gene module with 297 genes in 15 enriched gene ontology (GO) pathways that corresponding to immune response/defense response to virus, with FDR < 0.05. For KEGG analysis, we identified 38 downregulated genes in three distinct pathways; Rap1 signaling, Complement, and NOD-like receptor signaling pathway. According to WGCNA results, we have selected 5 representative genes (DDIT4, ISG20, NPC2, SERINC5, and TARBP2) in defense response to virus to further investigate. The DEG results showed that TARBP2 and ISG20 genes were significant upregulated whereas DDIT4, NPC2, SERINC5 genes had the opposite results in infected cells. The Cytoscape gene network generated from defense response to virus gene dataset revealed the exclusive of some putative immune response genes to the virus (i.e., MX2, IRF9, RSAD2) in our findings.

Conclusion The transcriptomic results of current study provided the molecular insight into the pathogenesis of CPV-2c as well as how host cell response to the virus. This information could contribute to more understanding of the capacity of CPV in infection of different hosts.

Keywords: Canine parvovirus, CRFK cells, Differentially Expressed Gene, RNAsequencing, Response to virus
Malondialdehyde Levels in Cats with Feline Leukemia Virus Infection

Ekkachai Pattarapanwichien¹*, Arayaporn Macotpet², Nitiwadee Lertittikul³, Pattaraanong Bupata³, Jeerasak Khlongkhlaeo³, Thitirat Chaimee⁴

¹Division of Pathobiology, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen, 40002, Thailand
²Division of Small Animal Medicine, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen, 40002, Thailand
³Veterinary Teaching Hospital, Faculty of Veterinary Medicine, KhonKaen University, KhonKaen, 40002, Thailand
⁴Zoetis (Thailand) Limited, Silom, Bangrak, Bangkok 10500, Thailand

*Corresponding author Email: ekkpat@kku.ac.th

Abstract

Objective To compare the level of malondialdehyde (MDA), an indicator of oxidative stress, in cats with feline leukemia virus and healthy cats.

Materials and Methods Blood samples were collected at the Veterinary Teaching Hospital, KhonKaen University from 11 healthy cats and 11 FeLV positive cats, confirmed by FeLV antigen test kit (WITNESS®FeLV-FIV test, Zoetis) twice, on day 0 and day 28. The serum was then separated from the blood of the healthy cats and the FeLV positive cats, collected on day 0, and was used to determine MDA levels using the thiobarbituric acid reactive substance assay, by measuring light absorption at wavelength 532 nm with a spectrophotometer, and comparing with a malondialdehyde control solution. The data were statistically analyzed using the independent-samples T test. P values of less than 0.05 were considered significant. The data were presented as mean ± standard deviation.

Results The MDA levels of cats infected with FeLV were significantly higher when compared to healthy cats (p<0.05). The average MDA level of cats with FeLV was 13.19±3.87 µmol/L, whereas the level in healthy cats was 7.32±1.05 µmol/L.

Conclusion Results from this study support that cats infected with FeLV may have impairment of the oxidative balance of lipid peroxidation. Further studies should evaluate other parameters to confirm the oxidative balance status of cats with this disease.

Keywords: Malondialdehyde, Feline leukemia virus, Cat
Antibacterial Effect of *Piper betle* Extract on Antibiotic-resistant *Staphylococcus aureus*

Jareerat Aiemsaard¹, Korawuth Punareewattana²*

¹Faculty of Veterinary Medicine and Biofilm Research Group, Khon Kaen University, Muang, Khon Kaen, Thailand, 40002
²Faculty of Veterinary Medicine, Khon Kaen University, Muang, Khon Kaen, Thailand, 40002
*Corresponding author Email: korawut@kku.ac.th

**Abstract**

**Objective** To study antimicrobial activity of the ethanolic extract of *Piper betle* leaves on *Staphylococcus aureus* from bovine mastitis.

**Materials and Methods** *Piper betle* leaves were extracted by 90% ethanol and freeze-dried. Bacterial samples were 28 isolated of *Staphylococcus aureus* from mastitis cows. Antimicrobial activity was evaluated by broth microdilution test to determine minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The test was performed on all bacterial isolates with two antibiotics (cephalexin and gentamicin) and *P. betle* extract. Both descriptive and inferential statistics were used to describe the experimental results.

**Results** Bacterial samples were classified into antibiotic-sensitive and antibiotic-resistant based on their MIC values of cephalexin or gentamicin. The MIC₉₀ and MBC₉₀ of *P. betle* extract were 1.0 and 4.0 mg/ml respectively.

**Conclusion** The study demonstrated strong antibacterial activity of *P. betle* extract on *Staphylococcus aureus* that commonly cause bovine mastitis.

**Keywords:** Antibacterial, Broth microdilution, *Staphylococcus aureus*

**Introduction**

Bovine mastitis is the most concerned-infectious condition in dairy industries. The disease is defined as an inflammation of the mammary gland which is a complex disease involving many factors. Major mastitis pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and the coliforms [1]. Bovine mastitis is considered to be one of the most economically important in most agricultural countries. The economic losses caused by this disease come from both direct and indirect losses. The direct losses include treatment cost, discarded milk, labour cost, and fatality. The indirect losses include decrease in milk yield, milk quality changes, and replacement cost [2].

Mastitis management was relied centrally on prevention and focusing on hygienic manipulation before and after milking routines. However when the infection is erupted, the use of antibiotic drugs is crucial. Even though current therapeutic agents are effective, long-term use of any chemicals may eventually cause development of resistance. The emergence of antibacterial resistance among pathogens that impact animal health has been a growing concern in veterinary medicine. Additionally, antibacterial-resistant pathogens in animals have also been incriminated as a potential health risk for humans from possible transmission as foodborne pathogens [3].

Antibacterial agents from herbal sources used as an alternative or a supplement to current agents can be an alternative approach to reduce the amount of antimicrobial agents used in dairy farms, and then reduce the rate of drug resistance. A variety of scientific papers have revealed that a variety of plants contained antimicrobial substances and were able to actively affect pathogenic microorganisms [4].

Among various herbal plant extracts, *Piper betle* extract has been studied extensively in Asia. The
extract contains various substances such as essential oil, tannins, flavonoids, alkaloids, which among them hydroxychavicol and eugenol are the well-known components [5]. It has antimicrobial activities against a variety of microorganisms including bacteria and fungi, and it also exhibits antioxidative activity [6]. In this study, P. betle extract was evaluated for its antibacterial activity against Staphylococcus aureus isolated from cows with bovine mastitis in order to determine the potential of the extract for using as an alternative antiseptics.

Materials and Methods

Experimental designs: Twenty-eight clinical isolates of Staphylococcus aureus were assessed for their sensitivity to antibiotics by means of microdilution methods. The MIC values were then used to classify these isolates into 3 groups (sensitive, intermediate-sensitive, and resistant). The isolates were then determined again for their sensitivity profile to Piper betle extract.

Extraction of P. betle: The betel leaves were purchased from a local market. The leaves were washed, dried, and ground into powder. Ethanol extract was prepared by mixing the powder with 95% ethanol at a ratio of 1:3 (w/v) for 3 days with constant shaking. Then the mixtures were filtered, concentrated by evaporation, and freeze-dried.

Microorganisms: The bacteria used in this study were 28 samples of Staphylococcus aureus isolated from tissues of cows with mastitis. They were maintained in Mueller-Hinton Agar (MHA; Difco) at 4 °C and cultured in Mueller-Hinton Broth (MHB; Difco) before each experiment.

MIC and MBC determination: Antibacterial test was performed using the microdilution technique in sterile flat-bottom microplates according to the Clinical and Laboratory Standards Institute. Each well contained appropriate test samples, and approximately 10^3 cfu/ml of bacteria in Mueller-Hinton Broth (MHB). Serial two-fold dilutions of the extract was performed in a 96-well microdilution plate that contained 50 μl of culture medium. The 50 μl of inoculum was then added to each well. The microplates were incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited visible growth. The minimal bactericidal concentrations (MBC) were determined by sub-culturing 10 μl of the culture from each well without visible growth and from the control on Mueller-Hinton Agar (MHA). Plates were incubated at 37 °C during 24 h. The lowest concentration without bacterial colonies was considered the MBC [7].

Data analysis: The MIC and MBC values were described by descriptive statistics. Student T-test was used to compare the MIC of the extract used between antibiotic-susceptible or resistant bacteria.

Results

Susceptibility profile of bacterial samples to antibiotics: The detail of the profile was shown in Table 1. The susceptibilities were heterogeneous and different among antibiotics. Based on the MIC values, 3.5% of bacterial samples were susceptible to cephalaxin, 3.5% were moderately susceptible, and 93% were resistant. For gentamicin, none of bacterial samples were susceptible, 7% were moderately susceptible, and 93% were resistant.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>MIC of Cephalexin (µg/ml)</th>
<th>MIC of Gentamicin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>8-256</td>
<td>4-256</td>
</tr>
<tr>
<td>Mean ± std</td>
<td>135.7 ± 87.2</td>
<td>91.7 ± 62.4</td>
</tr>
<tr>
<td>MIC_{50}</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>MIC_{90}</td>
<td>256</td>
<td>128</td>
</tr>
<tr>
<td>No. of Susceptible samples</td>
<td>1 (3.5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>No. of Moderately susceptible samples</td>
<td>1 (3.5%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>No. of Resistant samples</td>
<td>26 (93%)</td>
<td>26 (93%)</td>
</tr>
</tbody>
</table>
Susceptibility profile of bacterial samples to *P. betle* extract: The detail of profile was shown in Table 2. The MIC range was 0.5-1.0 mg/ml with the MIC$_{90}$ of 1.0 mg/ml. The MBC range was 0.5-8.0 mg/ml with the MBC$_{90}$ of 4.0 mg/ml. The ratio of MBC$_{90}$/MIC$_{90}$ was about 4.

### Table 2. MIC and MBC values of *P. betle* extract on *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Statistics</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
<th>MBC/MIC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>0.5-1</td>
<td>0.5-8</td>
<td>-</td>
</tr>
<tr>
<td>Mean ± std</td>
<td>0.82 ± 0.24</td>
<td>1.95 ± 1.98</td>
<td>2.38</td>
</tr>
<tr>
<td>MIC$<em>{50}$ or MBC$</em>{50}$</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>MIC$<em>{90}$ or MBC$</em>{90}$</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Discussion**

Staphylococcal bacteria used in this study were clinical isolates, therefore, they could reflect real clinical situation in dairy farms. The susceptibility profiles confirmed the variability of bacterial samples. They contained mostly antibiotic-resistant bacteria. Bacterial samples were resistant to both cephalin (93%) and gentamicin (93%). Previous reports have demonstrated that *S. aureus* was the major mastitis bacteria and commonly showed higher rate of antibiotic resistance [8, 9]. Based on these information, we may consider these bacteria as highly resistant and it may need strong antibacterial agent for the treatment.

Antibacterial activity of *P. betle* extract was shown in this study as expected. Both MIC and MBC values were quite low as MIC$_{90}$ and MBC$_{90}$ were below 8 mg/ml. According to the MBC/MIC ratio shown in Table 2, the activity of the extract could be classified as bactericidal. Several previous reports have demonstrated similar antibacterial effects of *P. betle* extract [10, 11].

The mechanism of antimicrobial action of *Piper betle* extract has not been yet clearly elucidated. In general, plant-derived antimicrobial substances are less active, however synergistic effect of various molecules makes them more effective [12]. *Piper betle* leaf contains essential oil and different kind of small molecules as mention earlier, and hypothetically they could produce synergistic effect as well. Phytochemical analysis revealed that *Piper betle* extract contains various compounds in which the major component is hydroxychavicol [5, 13]. This phenolic compound is known to have a variety of useful pharmacological effects and proposed to play a major role in antimicrobial effect [6].

In conclusion, the ethanol extract of *P. betle* leaves has a potential for developing to a preparation for bovine mastitis treatment. It was able to inhibit antibiotic-resistant bacteria isolated from mastitis lesion at low concentrations. Other properties of the extract, such as bactericidal activity and anti-inflammatory effect would also support its use as antimicrobial agent.

**References**

6. Dwivedi V, Tripathi S. Review study on potential activity of *Piper betle*. Journal of Pharmacognosy and


**Risk factors Related to Liver Damage in Rabbits by Using Aspartate Transaminase Enzyme**

Kamolak Jirajesda¹*, Patinya Malaithong¹, Suphanut Junthong¹, Sompoth Weerakhun¹, Peerapol Sukon¹

¹Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand  
*Corresponding author Email: kamonlakjirajesda@gmail.com

**Abstract**

**Objective** To study the risk factors associated with liver damage in the rabbits using the liver enzyme Aspartate transaminase; AST as indicator of liver damage.

**Materials and Methods** The liver damage in rabbits was studied on the retrospective; the risk factors data were collected on the out-patient form at Kwuncum Animal Hospital from January to December, 2017. The data such as gender, age, body temperature, hydration status, body condition score, plasma protein, heat stroke, dental diseases, respiratory diseases, gastrointestinal diseases, urogenital diseases, neuronal disease, and other infectious diseases were analyzed by SPSS version 17.

**The Results** Using AST elevation which was main indicator of the liver damage in rabbits. The results from 271 rabbits who were hospitalized at Kwuncum Animal Hospital showed that they were 53 male rabbits, mostly Holland lop, with the age of 3.9 years old (43.3%). The factors were significantly associated with the incidence of hepatic damage in rabbits at 95% confidence interval (p<0.05). Including gender (male 13.67%), body temperature (high temperature 49.30%), hydration status (dehydration 12.63%), body condition score (emaciation 27.21%), and gastrointestinal disease (45.97%).

**Conclusion** The risk factors including male, high body temperature, dehydration, emaciation, and gastrointestinal diseases which may be the cause or consequence of liver damage. Although, some serious disease was not risk in this study, the prospective observation need to progressive research and experiment.

**Keywords:** Aspartate transaminase, Risk factors, Liver, Rabbit
Prevalence of Bacterial Intramammary Infection in Clinically Healthy Cows before Drying off from Small Dairy Farms, Chiang Mai, Thailand

Chya Vannkovida1, *, Kannika Na Lumpang1, Khwanchai Kreausukon2, Raktham Mektrirat2

1 Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand
2 Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand

*Corresponding author Email: raktham.m@cmu.ac.th

Abstract

Objective The objective of this study was evaluated to the prevalence of bacterial intramammary infection in clinical healthy cows prior entering dry period and the antimicrobial resistant patterns of mastitis-causing bacteria.

Materials and Methods A cross-sectional study was conducted between July and August 2018 from Mae-On and Mae-Wang dairy cooperative, Chiang Mai province, Thailand. A total of 140 milk samples from 35 dairy cows were collected at the last milking before drying off. All of samples were determined microbiologically according to the National Mastitis Council. Antimicrobial susceptibility test was examined using Kirby Bauer disk diffusion test according to CLSI guidelines 2014. The epidemiological data and the antimicrobial resistant patterns of mastitis pathogens were evaluated by descriptive statistical analysis.

Results The quarter-level prevalence of bacterial intramammary infection was 47.86%. Overall prevalence of environmental Streptococcus spp. (11.43%) and contagious S. agalactiae (8.57%) clearly predominated followed by coagulase-negative staphylococci (CNS) (6.43%). The highest frequency antimicrobial resistant patterns of Streptococcus spp., Streptococcus agalactiae, and CNS were CN-K-S (56.25%), CN-K-S (41.67%), and K-P-S-SXT-TE (22.23%), respectively.

Conclusion The present study concluded that high prevalence of mastitis pathogens prior enter during dry period was occurred in smallholder dairy farms, Chiang Mai, Thailand. Alarmingly, the dry period of a dairy cow should be considered an important phase. Therefore, efforts should be directed to the decreased by improving dry cow managements, proper hygienic practice, dry cow therapy and teat sealant infusion.

Keywords: Prevalence, Mastitis, Dairy cows, Dry period, Antimicrobial resistance
Feedback Flipped Classroom of 5th Veterinary Students Chiang Mai University to Topic: Equine Dental Care and Floating

Porrahkote Rungsri¹ Kannika Na Lampang ²

¹Equine Clinic, Department of Companion Animal and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Thailand.

²Division of Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand.

*Corresponding author Email: p_kulchaiwat@hotmail.com, porrahkote.rungsri@cmu.ac.th

Abstract

Objectives This study aimed to survey the satisfaction feedback on self-active learning, activity in classroom and media online learning about a topic “equine dental care and floating” in five-year veterinary students.

Materials and Methods The 5th year Veterinary student participants included 66 students who attended the course Equine Clinical Rotation, Topic: Equine Dental care and Floating during December 2014 to October 2015. The students were separated into 9 small groups, 6-8 persons for each. Then, they turn to equine clinic one by one group for 10 days. The data collection methods were: interviews with the participants about satisfaction of self-active learning by using media and material of this topic which preparing and put on https://elearning.cmu.ac.th, no lecture but teacher action as a facilitator. Students participated on theory section class for 2 hours. The activity divided to 3 stations included: Anatomy of horse teeth, how to practice dental floating with horse’ skull and Aging using animation. Then they moved to lab clinical practice for 3 hours to get hand skill. The interviews about the satisfaction of knowledge and hand skill before and after clinical practicing; interviews with cooperating teachers and video recorded during theory and clinical lab practicing. The data was analyzed by using a descriptive analysis. Wilcoxon signed-rank test was used in this study.

Results and Conclusion The score of knowledge higher than before participate in this class (Development score; after- before average 6.98±4.01). The students used media of e-learning 95.32%. They prepare themselves active learning before class. The veterinary students got more confidence to do equine dental care and floating practice after class. They satisfied with flipped classroom in small group discussion and efficient to clinical practicing. Teacher action as facilitator can also encourage learning atmosphere.

Keywords: Flipped classroom, Active learning, Veterinary student, horse, Dental care
Hormone Profiles during Ovulation Induction in Postpartum Anestrus Beef Cattle

Chaiwat Jarassaeng, Adisak Sangkaew, Phankamol Tromgvanichnam, Tanutcha Kudeerak, Watcharapong Thongwarothai

1Theriogenology Units, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
25th-year student of Veterinary Medicine, Khon Kaen University, Thailand, 40002
*Corresponding author Email: chajar@kku.ac.th

Abstract

Objective The present study was designed to assess hormone profiles during ovulation induction program to improve reproductive problem of postpartum anestrus beef cattle.

Materials and Methods Ten postpartum anestrus beef cattle were divided into 2 experimental groups. All cows were inserted intravaginally with progesterone (CIDR) (EAZI-breed CIDR™) for 11 days and were injected with 300 IU of equine chorionic gonadotropin (Folligon®) on the CIDR removal day. The subjects were performed fixed time artificial insemination at 48 hours later with injection 10 µg of Buserelin acetate (Receptal®) in the first group and 1500 IU of human chorionic gonadotropin (Chorulon®) in the second group. Blood samples were collected at the days 1, 11, 13, 15 and 45 after ovulation program. The progesterone and estradiol were measured by ELISA. Pregnancy diagnosis was used by progesterone level at days 23, ultrasound sonography at days 35 and rectal palpation at days 60 after Timed-AI.

Results The progesterone profiles indicates the successful induction by 80% and 100% of the GnRH groups and hCG group at the day of Timed-AI, respectively. The pregnancy rate was greater in hCG group than GnRH group (60%:40%) at 35 days post Timed-AI but no significant difference at 23 days after Timed-AI by progesterone level.

Conclusion Both of hormone profiles during ovulation program show the improvement of reproductive performance which was determined by responsive result of hormone profiles in both group but there was no different of pregnancy rate at the 22 days after Timed-AI. Therefore, the postpartum anestrus cow with injection of hCG after using CIDR show higher pregnancy rate than the GnRH group.

Keywords: Ovulation induction, Postpartum anestrus, Beef cattle
Histological Description and Histometric Assessment of the Peripheral Blood Cells in Wild Indochinese Water Dragon (*Physignathus cocincinus* Cuvier, 1829) from Nakhon Ratchasima, Thailand

Prayuth Kusolrat¹, Xin Huo¹, Panich Kumropthanasarn¹ and Piyathida Kusolrat²*

¹Veterinary Technology Program, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand, 30000
²Biology Program, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima, Thailand, 30000
*Corresponding author Email: piyathida.k@nrru.ac.th

Abstract

**Objective** To study the hematological characteristics of wild Indochinese water dragon at Nakhon Ratchasima province, Thailand

**Materials and Methods** Hematology of the circulating blood of *Physignathus cocincinus* were studied during 2015 to 2016. Blood samples were taken from caudal tail vein, and different blood parameters were measured for a population sample of the species, considering the sex of the lizards. Blood samplings were collecting from total 8 males and 5 females dragons from population Khanong Phra subdistrict, Pakchong district, Nakhon Ratchasima province, and used for the complete blood count analysis (CBC).

**Results** According to the blood hematology study, cell blood type were divided into 3 major groups including, thrombocytes, erythrocytes and leukocytes (lymphocytes, heterophils, monocytes, eosinophils, basophils). The lymphocyte was the most common cell found on the CBC. Hematological values showed that levels of total red blood cell (RBC) erythrocyte, haemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not significantly different between males and females.

**Conclusion** The data obtained from this study can be used as a baseline in veterinary medicine, zoology and related fields, including also as a guideline for future conservation.

**Keywords:** Indochinese water dragon, *Physignathus cocincinus*, Hematology, Nakhon Ratchasima

Introduction

The Indochinese water dragon, *Physignathus cocincinus*, is one of the more popular lizards encountered in the pet trade like Iguana. The natural history of the Indochinese water dragon is known, and detailed information regarding care in captivity has been published. [1,2]. There are currently two different recognized species of *Physignathus*, the Indochinese water dragon and the Australian water dragon, *Physignathus lesuerii*. The Indochinese water dragon, *P. cocincinus* is one of the four native species of reptile in Thailand. It is also found in China, Japan, and northern and central Vietnam. However, present populations are decreasing may also as a result of habitat loss and collection for the Chinese herbal medicine and pet markets. Nowadays, the International Union for Conservation of Nature considers *P. cocincinus* to be an endangered species [3].

Blood analyses are useful, widely used tools that aid in the diagnosis and monitoring of animal health and disease, and in the differentiation of physiologic processes. These techniques have been used with several wildlife species, especially with threatened or endangered populations, and may aid in evaluating ecosystem health [4]. Animals have very complicated and delicate responses to stress that protect against environmental perturbations and which may be disadvantageous to their physiology, psychology, growth, and breeding [5].
Hematological studies and the establishment of baseline ranges for *P. cocincinus* would be useful for understanding general aspects of this species ecology and may give us information on the health status of individual dragons or entire populations in the wild. Various publications have reported different aspects of herpetiles hematological information, but baseline hematological parameters of wild *P. cocincinus* in Thailand have not yet been published. The data presented should be beneficial to the conservative medicine of this endangered species. Therefore, the present study aimed to evaluate the selective hematological parameters of water dragon specimens at Khanong Phra subdistrict, Pakchong district, Thailand. These results will serve as baseline reference data for future health assessment studies of the water dragon, as well as for the epidemiologic, conservation and captive-breeding studies, among other applications.

**Materials and Methods**

**Animals:** Thirteen dragons (*P. cocincinus*), including 8 males and 5 females were collected from the village wild near Lam Takhong watershed in Khanong Phra subdistrict, Pakchong district, Nakhon Ratchasima province (14.6102° N, 101.4818° E), Thailand during August to December 2015. Before blood collection, specimens were weighted and their total length was measured.

**Blood collection:** The dragons were manually restrained and blood was collected from the coccygeal tail vein into heparinized syringes for hematological analysis. Briefly, the venipuncture site was cleaned and aseptically prepared prior to blood collection. A needle (22- to 25-gauge) was inserted at an angle of 45-60° between the scales on ventral midline and, once blood appeared in the needle hub, held steady while continuing to apply gentle negative pressure [6]. Later, the dragons were released back to the closest forest areas.

**Hematologic assay:** Whole blood smears were obtained using a push slide technique, air-dried, fixed with methanol and stained with Wright’s-Giemsa. Five blood smears were prepared per individual. The proportions of heterophils including potential eosinophils, which could not be definitively identified by morphology alone, lymphocytes, basophils, and combined monocytes were classified through manual counts of blood smears as previously published by Stacy et al., 2011 [7, 8].

**Interpretation of data:** Hematological data resulting from our study were transformed into means and standard deviation (SD) via the software SPSS 17 for Windows. Significant differences between means were determined using an independent sample t-test model. Results were considered significant at \( P < 0.05 \).

**Results**

**Blood cell morphology and hematologic values**

The mature erythrocytes were oval, with slightly acidophilic cytoplasm and a strongly basophilic centrally located ellipsoidal nucleus, which was more elongated than the erythrocyte itself. The immature erythrocytes were smaller than the mature cells, with more rounded basophilic cytoplasm and a more rounded, centrally located nucleus (Figure 1A).

In the present work, leukocytes were categorized into 5 groups; lymphocyte, monocytes, eosinophils, basophils, and heterophils. Mostly monocytes were found to be oval or rounded in shape with eccentric, kidney-shaped, and deeply stained nucleus (Fig. 1B). The heterophils were circular with four to five lobes nucleus, and it appeared dark pink while the cytoplasm was lighter in color (Fig. 1C). The basophils were oval in shape having a lobed nucleus with granules over the nucleus as well as the entire cell (Fig. 1D). The eosinophils were identified by lightly stained cytoplasm and darkly stained two-lobed nucleus with a large granular appearance (Fig. 1E). Lymphocytes were rounded or spherical in shape with rounded nuclei in both the lymphocytes occupying at the entire cell (Fig. 1F). Thrombocytes were ellipsoidal cells containing slightly basophilic bluishgray cytoplasm and a strongly basophilic centrally located nucleus; however, these could be oval, with a similar nucleus surrounded by irregularly formed cytoplasm (Figure 1F).

Ranges for RBC parameters are given in Table 1. All hematological parameters were not influenced by sex. Females and males showed non-significantly values for the mature erythrocyte count.
Figure 1. Photomicrograph of the morphological observations of blood cells of *Physignathuscocincinus*. Mature erythrocyte (A), monocyte (B), heterophil (C), basophil (D), eosinophil (E), lymphocyte (F, arrow) and thrombocytes (F, circle) stained with Wright stain. Bars = 10 μm.

### Table 1. Red blood cell (RBC) range parameters of *P. cocincinus* blood.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=8)</th>
<th>Female (n=5)</th>
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<tbody>
<tr>
<td>Total erythrocyte count (10^6 cells/ Cu. mm)</td>
<td>Range: 3.04 - 4.68</td>
<td>Mean ± SD: 3.86 ± 0.64</td>
</tr>
<tr>
<td>Haemoglobin; HGB (g/dl)</td>
<td>Range: 6.16 - 9.46</td>
<td>Mean ± SD: 7.81 ± 1.30</td>
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<tr>
<td>Haematocrit; HCT(%)</td>
<td>Range: 39 - 60</td>
<td>Mean ± SD: 49.50 ± 8.19</td>
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<tr>
<td>MCV(fl)</td>
<td>Range: 128.03 - 128.41</td>
<td>Mean ± SD: 128.16 ± 0.13</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>Range: 20.18 - 20.30</td>
<td>Mean ± SD: 20.22 ± 0.04</td>
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<tr>
<td>MCHC(g/dl)</td>
<td>Range: 15.75 - 15.86</td>
<td>Mean ± SD: 15.78 ± 0.04</td>
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</tbody>
</table>
The documentation and the description of the different white cell types will aid the clinician in the future analysis of the blood smear prepared from the water dragon [3]. This is the first study to determine hematological reference range values for the wild Indochinese water dragon in Thailand. The data indicated that there was no significant difference in all selective hematological between gender. The hematological values found in this study were similar to results of a more recent iguana study [9].

The blood of reptiles contains nucleated erythrocytes, heterophils, eosinophils, basophils, lymphocytes, monocytes and thrombocytes. Hematology is used to detect conditions related to these cells, such as anemia, inflammatory diseases, parasitemias, hematopoietic disorders, and hemostatic alterations [10]. Factors that affect the hematologic values of reptiles include environmental conditions, age, gender, nutrition, season, use of anesthetics, and the physiologic status of the reptile such as dehydration and estrus [10,11]. Quite a few publications reported the different components and biochemical composition of blood vary with season, age, molting, pathological conditions, reproductive state, and with ecological factors in different reptiles. The identification of the cells in smear from the circulating blood of reptiles is often difficult since the animals have the cells of all ages and show intermediate stages between the different cell types [8].

Among all the hematological parameters, RBCs are becoming well known in reptiles, similarly, our present report described oval or elliptical RBCs in *P. cocincinus* with rounded nucleus. The size of RBCs was almost similar in the male and female lizards of this species. In an earlier report, Saint Girons (1970)
demonstrated that the shape and size of RBCs are variable for different vertebrates and morphologically similar among various species of reptiles. Our findings in *P. cocincinus* found that there is no major difference in leukocyte count both in male and female lizards, but the lymphocytes are higher in males than the females. The lymphocytes count was higher in female lizards, but the percentage of eosinophils, basophils, monocytes, and heterophils in male lizards is higher than the female lizards. Similar to Wojtaszwk and his coworkers (1991) reported that lymphocytes are the most numerous fractions of the leukocytes in reptile blood. In addition, the number of circulating eosinophils in normal reptiles varies with species and season changes, i.e., during hibernation, the eosinophils counts are usually highest. The shape of different leukocytes of *P. cocincinus* is almost rounded, circular, or disk-shaped, and WBC count closely coincides with the findings of other lizards [8,12]. The hemoglobin concentration of *P. cocincinus* is within the range of values published for other lizards [8,12], but there is no difference between the male and female lizards which coincides with the findings of *Liolaemus multiformis* [13].

This study has established a baseline data regarding the selected hematological and of *P. cocincinus* which are similar to the reference ranges for other lizards. Establishment of hematometry is highly dependent on physiological processes and the understanding of different internal as well as external factors that govern reference ranges for lizards that may be useful in many fields, ranging from exotic animal practice to wildlife rehabilitation. Moreover, monitoring lizard blood parameters can be a way to manage and evaluate the physiological and health status of their populations which may be a useful indicator of the environmental status. Most importantly, the small number in any specific lizard populations limits the interpretation of the results, and further validation is required.

The literature suggests that gender can affect RBC mass, although there appears to be some conflicting information about this effect. Harr, et al, (2001) found that female iguanas had higher PCV’s and hemoglobin levels than males, while others report higher RBC numbers in male reptiles [2,9]. This study found no gender based distinction in RBC mass but might be less sensitive to effects of sex given the small size of the population studied. The hematologic data from this study provide useful ranges for evaluating the health status of *P. cocincinus*. Reference values for *P. cocincinus* obtained in this study should be of benefit to future clinical and conservation work on the endangered Chinese water dragon.

In conclusion, hematometry are highly dependent on physiological processes and the understanding of different factors that govern reference ranges for reptiles. More importantly, although hematometry is less accurate for reptiles than mammals, it is still a useful guideline in the evaluation of health status. However, the small numbers in any specific dragon populations limit the interpretation of the results so that further validation is required. As the reproductive programs influence the number of snake species, more research will be necessary to determine the effects of climate, microhabitat, environmental conditions, ambient temperature, and possible seasonal fluctuations on the dragon’s hematometry and plasma chemistry parameters.

Acknowledgements

We would like to thank Nakhon Ratchasima Rajabhat University for providing the grant to carry out this work. We are extremely grateful to Mr. Sa-ard Mutchapramarnkul and the villager in Khanong Phra subdistrict, Pakchong district, Nakhon Ratchasima province for their kindness cooperation.

References


A Survey of the Canine Raw Food Compositions in Thailand

Sathita Areerat¹,², Chalermpol Lekcharoensuk²*, Attawit Kovitvadhi³*

¹Veterinary Clinical Studies, Faculty of Graduate School, Kasetsart University. Thailand
²Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Bang Khen Campus, Bangkok. Thailand 10900
³Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bang Khen Campus, Bangkok. Thailand 10900
*Corresponding author Email: fvetcpl@ku.ac.th andfvetawk@ku.ac.th

Abstract

Objective To evaluate the composition of raw food ingredients of dogs using in Thailand.

Materials and Methods A nonprobability sampling (snowball sampling) was used to receive information on raw food formulas. The initiation of data was collected by finding diet formulas in Thailand via the internet and inquiry in the group of people in the information media. The data was collected in all part of Thailand based on the geographic area between September and December 2018. Ingredients of BARF were translated into five groups (raw meaty bone, offal, vegetables, fruits and additives) following Dr. Ian theory. All formulas were grouped by Hierarchical cluster analysis with Euclidean distance to establish the average of each group. After that, the comparison between the groups and Dr. Ian recommendation were illustrated by non-metric multidimensional scaling of dissimilarity (nMDS) with Euclidean distance.

Results One hundred and ten formulas were collected in this study. After cluster analysis, there were 5 groups which contain 9, 47, 6, 46 and 2 formulas, respectively. There was a large variation on the dietary proportion between cluster groups. Based on nMDS results, group 1 was the highest similar ratio to BARF theory, following by group 2, 3, 4 and 5, respectively.

Conclusion The large varieties on the composition of raw dog foods in Thailand were presented. Most formulas had no precise on the ingredients and quantities respecting to the recommendation. Therefore, the risk of malnutrition and/or metabolic disease from imbalance nutrients should be considered.

Keywords: BARF, Canine, Diet compositions, Nutrition, Raw food

Introduction

Nowadays, dog owners are increasingly interested in raw food calling “Biologically Appropriate Raw Food” or “BARF” which is uncooked diets. BARF was proposed in 1993 by Australian veterinarian “Ian Billinghurst” based on the principles of Evolutionary Nutrition[1]. This theory believed in the evolution of dogs today has a wild dog ancestor which consumes a high proportion of meat[1]. It is intended for domestic dogs, the natural diet to be imitated by wolves. Bones or raw food was served as the main part up to 16.2% of BARF formulation for dogs by most the owners, whereas less than 3% of owners fed exclusively home-prepared diets[2]. Feeders of home-prepared or raw food diets cite various benefits, including control over ingredients used, avoidance of artificial preservatives, food coloring or bad-quality ingredients in extruded diets[3,4]. The survey of BARF in the United States of America and Australia reported that 98.7% of dog owners deemed their pet healthy[2,5]. However, none of the published data are available to support health benefit to pets fed such diets.

The composition of BARF is consisting of raw meaty bone, offal, vegetables, fruits, and additives which the amount of giving can be modified depending on the dog’s status and their activity based on owner’s perception, experience and knowledge[1]. Therefore, the large variation of the compositions and ingredients were observed from the survey in the United States of America and Australia[4]. The theory of the BARF that has been referred to Thailand and several dog’s owners start to formulate BARF for their...
dogs. As we were known, the consumption of diets with inappropriate to nutrient requirements promotes the deterioration of animal health. Therefore, the objective of this survey is the evaluation of the composition of raw food ingredients of dogs using in Thailand to understand the current situation and compare to other countries.

Materials and Methods

The sample collection: A nonprobability sampling by snowball sampling was used to receive information on BARF formulas. The initiation of data was collected by finding recipes in Thailand via the internet and inquiry in the group of people in the information media. The data was collected in all part of Thailand based on the geographic area between September and December 2018.

Statistical analysis: All of the ingredients were transformed into different proportion based on BARF theory which was raw meaty bone, meat, offal, vegetables, fruit or additives [1]. A Hierarchical cluster analysis was used to group the data then using Euclidean distance to establish the symmetrical matrix of all group distances. Then, two dimensions were chosen for the ordination. The distance matrix on dissimilarity was used for nonmetric multidimensional scaling (nMDS) plots with Euclidean distance and Kruskal’s S-Stress was calculated. On nMDS plots, the near point shows higher similarity than the far point. R-statistic with Rcmdr Package was used for statistical analysis in this study (R Development Core Team).

Results

Sample characterization: One hundred and ten formulas were collected in this study. After cluster analysis, there were 5 groups which contain 9, 47, 6, 46 and 2 formulas, respectively. Mean of each group was illustrated in Figure 1.

Analysis results: In relation to compositions, there was a large variation on the proportion between raw meaty bone, meat, offal, vegetables, fruits and additives between groups. The bar chart with 100% stacked column (Figure 1) was presented differences between the percentages of mean for each diet composition of each group. Most of the formulas using in Thailand were concluded in groups 2 (42.7%) and 4 (41.8% of the total formula in this study). The composition of BARF was a difference from Dr.Ian recommendations [1]. The proportion of raw meaty bone ratio in group 2, 3, 4 and 5 (58.9, 53.3, 39.8 and 7.5%, respectively), all groups of offal (0, 5.1, 8.3, 5.1 and 6.9%, respectively), vegetable in group 1, 2 and 4 (10, 11.4 and 10.1%, respectively), fruits in group 3 and 5 (.8 and 3.5%, respectively) and additives in group 1, 3 and 5 (6.1, 1.7 and 7.2%, respectively) were lower than BARF theory which raw meaty bone, offal, vegetable, fruit and additive were 60, 10, 15, 5 and 10%, respectively [1]. The groups with higher mean percentage over the BARF theory, all groups of meat composition (.6, 5.1, 11.7, 21.5 and 54.6%, respectively), group 3 and 5 of vegetable composition (24.2 and 20.5%, respectively), group 1, 2 and 4 of fruit composition (23.3, 5.8 and 10.1%, respectively) and group 2 and 4 of additive composition (13.7 and 13.2%, respectively). Based on nMDS results, group 1 was the highest similar ratio to BARF theory, following by group 2, 3, 4 and 5, respectively (Figure 2).

Discussion

A survey of the mean percentage of each composition was verified in the formulas investigated in the present study, each group has a variety of composition quantities that differ from Dr.Ian’s theory. Furthermore, raw meat, offal and meaty bones at 70% with 30% of vegetables and fruits were recommended by Schäfer and Messika [6] which meaty bones comprised between meat and bones at 30 and 70%, respectively. The higher proportion of meat, offal and meaty bones at 75-90% with less of vegetables at 10-25% was suggested by another researcher which around 10% is bone [7]. Therefore, the recommendation of BARF generally contains a higher proportion of proteins the veterinary nutritionist z. B. recommendation which the highest nutrients in diets are carbohydrates (50%) following by 40% proteins, 5% fats, 2-5% crude fiber and vitamin and mineral supplements [8]. Moreover, at least 20-25% of carbohydrates were suggested in dog’s feed formulation [9], whereas BARF diets are none or low of carbohydrate sources. Thus, protein
Figure 1. Comparison between mean of cluster groups and BARF theory.

Figure 2. The non-metric multidimensional scaling plots of the mean of cluster groups and BARF theory.
sources should be the major source of daily energy consumption of dogs fed BARF comparing to dogs fed commercial complete diets which using carbohydrate and fat. Unfortunately, a few studied has investigated on the long-term consequences of BARF in dogs, therefore further study should be confirmed. Schmidt et al. reported the BARF dogs were fed a significantly higher amount of protein (mean 44.4 ± 5.8 Standard Deviation (SD) in % DM) caused by a high content of animal products like meat, offal, and bones in the diet. Furthermore, fat in the form of marbled meat, animal fat and fish- or vegetable oil played an important role as an energy source (28.4 ± 6.7 in % DM) in BARF diets, whereas carbohydrates were commonly only used in small amounts or infrequently as energy source (15.8 ± 8 in % DM). The fiber intake was significantly lower in BARF dogs (2.7 ± 1.9 in % DM) and some dogs were fed hay cobs which resulted in a higher intake of crude fiber compared to the other dogs [10]. Same as Thailand compositions, the BARF diets were fed a significantly higher amount of protein and fat, a lower amount of NFE and fiber and calcium-phosphorus ratio imbalance.

Ingredients of BARF rations were meat (e.g. horse, lamb, chicken, beef, duck, veal and venison) and offal (e.g. heart, liver and rumen), bones, fish, dairy products, vegetables and fruits and plant oils. Supplements included egg yolk, cod liver oil, seaweed, nuts, linseed, sesame, coconut flakes, eggshell, bone meal, herbs and/or some commercial mineral mixtures [11]. The difference of proportion was adjusted based on the owners depending on the size and condition of the dog as well as the individual compatibility. The ration also contains different amounts and animal species including lamb ribs, bovine breast or chicken necks [12]. The offals are considered as mineral sources, therefore the difference in offal sources influences on mineral composition in diets and reflects animal health. From the results of this survey, the rations of raw meaty bone range from 13.6-78.0% and up to 20% of the offal rations. It was implied that the diet contained the required level of calcium-phosphorus imbalance that developed to nutritional secondary hyperparathyroidism following intake of a nutritionally incomplete and unbalanced diet [13,14]. And thiamine deficiency has been reported in dogs are often fed species of raw fish which contain thiaminase [15]. Moreover, if the material of this diet including the body parts of ruminants (beef, sheep, goat, deer etc.) neck with trachea and adherent thyroid gland. It is possible that a large amount of raw thyroid gland tissue ends up in the diet can cause dietary hyperthyroidism [16].

These ingredients are supplemented with a variety of fruits and vegetables that are either minced or mashed. Most owners did not feed any carbohydrates in their dogs’ main diet and the product-based was combined with vegetables, different kinds of oil, and sometimes eggs and dairy products [17]. Dog’s owners prefer to use herbs as additives, seaweed, vegetable and fish oil as same as various commercially available BARF adds in different composition and quantity [9,18]. These common forms of rationing, often named after the respective founders and other recommends feed the dog with raw food. On the other hand, fruit, vegetables and dairy products are not needed in BARF following the method of Prey which focuses on the prey contained components as accurately as possible because ancestor Wolf ate only animal [17]. Fruits and vegetables were sources of fiber and vitamins. Dietary fibers play an important role in modulating bowel movement, influencing immune function and gut microbiota profile, diluting caloric density, contributing to weight loss, affecting laxative and stool quality [19]. In addition, the appropriate additive amount may be sufficient for vitamins. Every formula in this study has enough supplements.

The amount daily consumption of BARF for adult dogs is often recommended the scientific literature, as a total amount of feed (fresh substance) 2-3% of body weight [1]. However, 2% of the body weight does not take into the energy density of the food and the individuals often vary to energy needs of the individual dog that provided by veterinary nutritionist according to the National Research Council (NRC) [9]. Most formulas had no precise determination of the ingredients and their quantities based on recommendation because owners may search for diet formulas in inappropriate resources such as websites, books or magazines [18]. According to a study by Becker et al. [20] 77% of owners (n = 68, multiple answers possible) got recipes from books, 34% from the internet, 27% from their own experiences, 19% from friends and 10% from the breeder and Handl et al. reported 45.9% of owners referred their information of BARF from the internet, popular science Books and other dog owners, respectively. Moreover, 90.2% of owners formulated BARF by themselves without consultation to veterinarians or animal nutritionists [21]. Basic information of appropriate BARF formulas must be based on nutritional requirements for a dog. The source of the proteins and fats is less important than the quality and digestibility of these essential components of the dog’s diet. Dogs can thrive if they are fed a properly balanced vegetarian diet. However, an all-meat diet would be unbalanced and would not meet all of a dog’s nutritional requirements [22]. A balanced diet
must include an appropriate amount of essential amino acids, essential fatty acids, carbohydrate, minerals, vitamins and water. These components are needed to build and maintain tissue and biological reactions, and the necessary amounts vary with the dog’s stage of life. Feeding your dog an appropriate amount of a well-balanced diet is vital to its overall health and well-being. Thus, to make sure these diets are complete and balanced for the dogs, we recommend that the owners should be cooperating to share a sample to our laboratory for a complete nutrient profile.

**Acknowledgements**

We would like to thanks the Department of physiology and all staffs of nutrition laboratory to help us provide facilitation, assist and sampling collection of diets in this research.

**References**


Long Axis Brightness Mode (B-mode) Echocardiography in Ratus Snake

I Putu Gede Yudhi Arjentinia *, I Gede Soma 1, Puveanthan Nagappan Govendan 1, I Gusti Made Krisna Erawan 1, I Wayan Batan 1, Putu Ayu Sisyawati Putriningsih 1

1 Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, Indonesia, 80034
*Corresponding author Email: yudhiarjentinia@umud.ac.id

Abstract

Objective To determine the normal brightness mode echocardiography in healthy Python reticulatus, in Bali, Indonesia. As in others, echocardiography should provide cardiac anatomy and function in snakes.

Materials and Methods Six healthy Python reticulates were quarantined and acclimatized for 1 year prior to ultrasonography. Ultrasonography was performed on the short axis and long axis of the heart by one operator using a 8 MHz micro-convex probe. Views only obtained from the long axis include left atrioventricular long-axis section, right atrioventricular long-axis section, transarterial long-axis section, transarterial long-axis section, and transcaval long-axis section. Data obtained were then compared to the heart illustration.

Results Ultrasonography was performed from the ventral of the snake to avoid shadowing from the ribs. Sectional imaging was taken from the long axis (5 views) of the heart. The left atrioventricular long axis section, right atrioventricular long axis section, transarterial long-axis section, transarterial long- axis section, and transcaval long-axis section. Echocardiography should be performed following clinical symptoms related to cardiovascular diseases. Proper restraint is necessary to obtain a good sonogram.

Conclusion Normal echoanatomy of the unsedated Reticulated Python’s heart can be obtained through echocardiography with manual restraint.

Keywords: Cardiovascular, Echocardiography, Long axis, Phyton reticulatus

Introduction

Python reticulatus gained its name from the zigzag pattern on the dorsal of its back. It is one of the longest snake species in the world, reaching up to 9-10 meters in body length [7]. The snake can be infected with various disease, including cardiovascular disease. Compared to mammals, the ophidian heart has a unique anatomical, because it only has three chambers of the heart; the right atrium, left atrium, and ventricles that are not insulated by septum. Most of cardiovascular disease in snake are detected post mortem [6, 9]. Ultrasonography is the method of choice for imaging organs structure in animals or human, especially located in thorax or abdominal and cannot detected using radiology [1, 10].

Ultrasonography is non-invasive technique, including echocardiography is a part of cardiac evaluation, does not require general anesthesia, and one of the modern diagnostic tool and highly beneficial aid in the diagnosis of cardiac evaluation [1, 9]. Normal echocardiographic measurements are important to detect abnormalities [3, 8]. Brightness mode (B-mode) echocardiography, uses the principle that each returning echo is displayed on the screen as a dot; the brighter dot the higher the intensity of the returning echoes [5].

There are few reports of echocardiography in snake, thus the echocardiographical limitations of the snake heart examination, which is why the purpose of this research was to determine the normal brightness mode echocardiography in healthy Python reticulatus. As in others, echocardiography should provide cardiac anatomy and function in snakes.

Materials and Methods
This research was conducted at the Udayana Animal Hospital, Faculty of Veterinary Medicine, Udayana University. Six healthy Python reticulates weighing between 1.5 – 4.5 kg (snout-vent length between 1.7–2.7 meters) were kept under controlled conditions in Denpasar, Bali. The snakes were quarantined and acclimatized for 1 year prior to ultrasonography. They were kept in separate cages (temperature between 25–35°C and humidity between 80–92%) and given water ad libitum. Physical examination performed on the snake include general appearance, activeness, body mass, presence of ectoparasites, exudate, tumors, wounds, ulcers, and appetite [4, 8]. Only animals that presented normal on physical examination are used in this research. Animals in this research were not sedated but gently restrained by two assistants on dorsal recumbence. The heart was located by the presence of the ventral precordial tap [3,12].

A thick layer of acoustic gel was then applied on the ventral scales where the heart was located [2, 11]. To ensure better visualization, there should be no air between the scale and the probe. The probe was then moved cranially and caudally to visualize all parts of the heart [9]. Ultrasonography was performed on the short axis and long axis of the heart by one operator using a 8 MHz micro-convex probe. Views only obtained from the long axis include left atrioventricular long-axis section, right atrioventricular long-axis section, transarterial long-axis section, transarterial long-axis section, and transcaval long-axis section. Data obtained were then compared to the heart illustration [9].

Results and Discussion

Snakes used in this research were not sedated but manually restrained to dorsal recumbence by one or two assistants. Ultrasonography was performed from the ventral of the snake to avoid shadowing from the ribs. Sectional imaging was taken from the long axis (5 views) of the heart.

Long axis views were obtained by rotating the probe by 90 degrees from the short axis view. The left atrioventricular long axis section (Figure 1) was obtained by placing the probe slightly to the left. It showed the aortic arch, pulmonary trunk, left atrium, cavum pulmonale, cavum venosum, cavum arteriosum, muscular ridge and the ventricular septum. The right atrioventricular long axis section (Figure 2) was obtained by placing the probe slightly to the right. It shows the aortic arch, pulmonary trunk, right atrium and cavum venosum. The transarterial long-axis section (Figure 3) was obtained by placing the probe between the atriums showing the right aortic arch and the pulmonary trunk. The transarterial long-axis section

Figure 1. Left atrioventricular long-axis section: AA, aortic arch; CP, cavum pulmonale, CV, cavum venosum; VS, ventrical septum; CA, cavum arteriosum; MR, muscular ridge; PT, pulmonary trunk; LA, left atrium. (A) Probe position [9]; (B) Brightness mode illustration [9]; (C) Brightness mode of the reticulated python.
(Figure 4) was obtained by moving the probe a little ventrally. It shows the pulmonary trunk and the cavum pulmonale. The transcaval long-axis section (Figure 5) was obtained by moving the probe towards the right showing the pulmonary vein and the caudal vena cava.

**Figure 2.** Right atrioventricular long-axis section: AA, aortic arch; PT, pulmonary trunk; RA, right atrium; CV, cavum venosum. (A) Probe position [9]; (B) Brightness mode illustration [9]; (C) Brightness mode of the reticulated python.

**Figure 3.** Transarterial long axis section (a): RAA, right aortic arch; PT, pulmonary trunk. (A) Probe position [9]; (B) Brightness mode illustration [9]; (C) Brightness mode of the reticulated python.
Echocardiography should be performed following clinical symptoms related to cardiovascular diseases. Proper restraint is necessary to obtain a good sonogram. Table 1 summarizes the heart sections that can be obtained from each view. This data can be used as a reference for veterinary practitioners to detect and diagnose diseases related to the cardiovascular system.

**Figure 4.** Transarterial long axis section (b): PT, pulmonary trunk; CP, cavum pulmonum. (A) Probe position [9]; (B) Brightness mode illustration [9]; (C) Brightness mode of the reticulated python.

**Figure 5.** Transcaval long axis section: CVC, caudal vena cava; PV, pulmonary vein. (A) Probe position [9]; (B) Brightness mode illustration [9]; (C) Brightness mode of the reticulated python.
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**View**

<table>
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<tr>
<th>Left atrioventricular long-axis section</th>
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| RA, right atrium; LA, left atrium; CP, cavum pulmonum; CV, cavum venosum; CA, cavum arteriosum; PT, pulmonary trunk; AA, aortic arch; MR, muscular ridge; VS, ventricular septum; CVC, caudal vena cava; PV, pulmonary vein; √, yes; -, no.

**Acknowledgements**

The authors would like to thank to Udayana Animal Hospital, Faculty of Veterinary Medicine, Udayana University for help us to provide facilitation, assist and sampling collection in this research.

**References**

Abstract

Objective To develop an immunoperoxidase monolayer assay (IPMA) for detecting Fowl adenovirus (FAdV) antigen in cell culture using recombinant hexon protein.

Materials and Methods Chicken embryo liver cells were prepared and FAdV inoculated onto monolayer CEL cells. The infected CEL cells were observed for the CPEs affected cells within 24 h. Mouse anti recombinant hexon protein were standardized for the IPMA assay.

Results A 1:500 dilution of mouse anti hexon protein serum proved to be the optimum for the test. A higher concentration of antiserum (1:100) produced an intense staining reaction with infected cells and a non-specific background. The color intensity of the infected cells was reduced with 1:1000 dilution of antiserum.

Conclusion The recombinant hexon protein can be used for developing the IPMA which is sensitive for detecting FAdV in cell culture.

Keywords: Fowl adenovirus, Immunoperoxidase monolayer assay, Recombinant hexon protein

Introduction

Inclusion body hepatitis (IBH) was initially described in the 1960s in the United State [1] and was distributed widely throughout the world [2]. IBH is caused by group 1 Fowl adenovirus (FAdV) consisting of 12 serotypes [3]. At present, in Thailand, IBH is caused by FAdv serotype 2 and has caused economic loss to the poultry industry[4,5]. Infected flocks revealed low hatchability, poor chick quality, and high mortality [4]. Viral isolation in cell culture is an important diagnostic aid [6]. FAdV produce the swelling and rounding up cytopathic effect (CPE) in cell culture [7]. Viral isolation in cell culture has several limitations. Frequently several blind passages are needed before the CPE are observed [8,9,10]. The development of CPE is slow and visual assessment is often equivocal. The detection to FAdV antigen in infected tissues has also been reported using immunoperoxidase staining [11]. Reports are also available for the detection of non-cytopathogenic strains of viruses by immunostaining [12,13]. Immunoperoxidase assays have been used to identify both specific antibody and antigen. The immunoperoxidase monolayer assays (IPMA) have been widely used to detect the specific antigen in cell cultures. These techniques are sensitive and rapid and withappropriate reagents they can be very specific. In this study, the development of IPMA assay for detecting FAdV antigen in cell cultures. This test will have application in diagnosing FAdV infection in chicken.

Materials and Methods

Cell cultures and virus isolation: Chicken embryo liver (CEL) cells were prepared from 12- to 14-day-old chicken embryos. The cells were dispersed in 0.25% Trypsin, grown in growth media: 1XMinimum Es-
essential Medium with L-glutamine, 10% Fetal Bovine Serum, 10% Tryptose Phosphate Broth and Penicillin-Streptomycin and further incubated at 37 ºC with 5% CO₂. The FAdV were inoculated onto monolayer CEL cells. The inoculated CEL cells were observed for a few or more CPE affected cells within 24 hours.

**Recombinant hexon protein preparation for mouse immunization:** The recombinant hexon protein were used to immunized mouse and antiserum were used perform IPMA as previous described [14,15].

**Immuno-peroxidase monolayer assay procedure:** The infected cells were washed with 1xPBS for 3 times and air-dried completely in the safety cabinet. The infected CEL cells were then fixed with 4% paraformaldehyde at RT for 10 minutes, after 4% paraformaldehyde was rinsed, the cells were washed with 1xPBS for 3 times and incubated with 0.5% saponin at room temperature (RT) for 10 minutes. Then, saponin was rinsed and infected CEL cells were washed 3 times again. Mouse anti recombinant hexon protein serum [15] were standardized for the assay from 1:100, 1:500 and 1:1000 dilution’s in 1xPBS dilution buffer. Then were added onto monolayer CEL cells and incubated at RT for 1 hour. The infected CEL cells were washed with 1xPBS for 3 times and incubated with goat anti-mouse IgG (H+L) which was diluted with 1xPBS at 1:500 for 1 hour. The color was developed using DAB, as a chromogen.

**Results**

**Cell cultures and virus isolation**

The CEL cells were infected with FAdV, CPEs were observed 24 hours post-infection. Those were characteristics of FAdV infected CEL are in the form of cell swelling, rounding up, clumping and cells detachment (Figure 1).

![Image](image1.png)

**Figure 1.** A: The CEL cells in a monolayer after 48 hours of growth. B: Cytopathic effects (CPEs) with rounding, ballooning and detachment observed in CEL cells after 24 hours of infection. x400

**Immuno-peroxidase monolayer assay**

A 1:500 dilution of mouse anti hexon protein serum proved to be the optimum for the test. A higher concentration of antiserum (1:100) produced an intense staining reaction with infected cells and a non-specific background. Although the background reaction was reduced with a 1:1000 dilution of antiserum. However, intensity of color of the infected cells was also reduced. Goat anti-mouse IgG (H+L) conjugate at 1:500 dilution stained equally well. The infected cells had an intense brown color and uninfected cells stained without the counter stain (Figure 2)
CEL cultures observations are in agreement with Philippe et al. (2005) who found CEL to be superior to chicken liver cells. IPMA was successfully applied for the detection of antigen and used to diagnose FAdV infections in chickens. In standardizing the test with mouse anti hexon protein at 1:500 was appropriate to use less than previous study [17] that use hyper-immune chicken antiserum 1:200. The recombinant hexon protein further made it possible to use IPMA for confirming the endpoint neutralization and viral titration [18]. Moreover, IPMA can be stored for a long time, and are considered to be easier to interpret [19]. These observations support the hypothesis made that the assay has the ability to detect viruses isolated directly from infected tissues without the need of blind passages where the CPE are not visible by light microscope. The use of highly sensitive IPMA for demonstration of viral antigen aids to diagnosis and can be used routine field diagnosis [20]. The results indicated that the recombinant hexon protein can be used for developing the IPMA which is sensitive for detecting FAdV in cell culture.

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References

18. Ahmad MD. Studies on Avian Adenoviruses (Group 1) associated with inclusion body hepatitis. Ph.D. Thesis. Department of Biomedical and Tropical Veterinary Sciences, James Cook University of North Queensland, Australia. 1996.
Prevalence of Feline Coronavirus isolate from clinical samples submitted to Animal Hospital, Faculty of Veterinary Medicine, KhonKaen University

Suphattra Jittimanee¹, Parinya Sroithongkham¹, Pornpithai Suwanaklom¹, Mari Ishida¹NiitiwadeeLertitthikul²*

¹Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
²Animal Hospital, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
*Corresponding author Email: niti_lertitthikul@hotmail.com

Abstract

Objective The study purposed to investigate the prevalence of Feline Coronavirus (FCoV) and Feline Infectious peritonitis virus (FIPV) isolates from clinical samples submitted to Animal Hospital, faculty of veterinary medicine, Khon Kaen University using a real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) technique.

Materials and Methods Sixty-four clinical samples submitted to Animal Hospital, faculty of veterinary medicine, Khon Kaen University during July 2018 to January 2019 were included in this study. All clinical samples were collected from 51 cats, which classified into 2 groups according to their health status. Group 1 was referred to FIP clinically suspected cats (N=29) and group 2 was collected from healthy cats (N=22) consist of10 healthy cats living individually (group 2A) and 12 healthy cats living in groups (group 2B). Three types of clinical samples collect from both group were extracted and identified the FCoV genome targeting on 3'- untranslated region gene (3'UTR) using real-time RT-PCR technique.

Results The percentage of FCoV positive cats from group 1, group 2A andgroup 2B were 58.62% (17/29), 90.00% (9/10) and 91.66% (11/12)respectively. The highest percentage of FCoV positive from FIP clinically suspected group was found in fecal sample 100%follow by body effusions 41.67% and serum41.18%. Moreover, group 2 was also revealed major positive results in feces(2A=91.66% and 2B=90.00%), while minor FCoV positive was presented in serum(2B=85.71%).

Conclusion The prevalence of FCoV positive cats from all groups was 70.59%. On the other hand, the prevalence was ranged from 58.62% to 91.66% when separated by health status group. Interestingly, we detected FCoV in serum from healthy cats living in groups. Thus, these cats have potential to develop FIP in future. Hence, serum samples is suitable specimen for investigate subclinical FIPV infection in healthy cats living in groups or shelters.

Keywords: Feline coronavirus (FCoV); Feline infectious peritonitis (FIP); Prevalence, real-time Reverse transcription polymerase chain reaction (real-time RT-PCR)

Introduction

Feline infection peritonitis (FIP) is an immune-mediated disease caused by feline infectious peritonitis virus (FIPV), a mutated of feline coronavirus (FCoV) in cat. FCoV is a large, enveloped RNA virus belonging to the Coronaviridae family that exists in two biological type: feline enteric coronavirus (FECV) and Feline infectious peritonitis virus (FIPV). FECV is virtually non-pathogenic or mild enteric infection. Whereas, FIPV is a pathogenic virus can affect cats of any age [1], but is most prevalent among cats under 3 years and older than 13 years of age [2].FECV is normally shed in the feces of most healthy cats living in groups or nearby large multi-cat environments. FECV can spread via ingestion of contaminated feces/liter or direct contact with other fomites [3]. Interval time from initial FECV infection to develop a clinical sign
are differs in cats, can be as short as 2 weeks or as long as a couple month to several years. This subclinical infection could be a valuable circumstance for mutant FIPV to evolve. Recently, three different genes associated with the FECV-to-FIPV mutation have been report consist of ORF 3c accessory gene, regions within the C terminus of S gene and FIPV virulence in ORF 7b [4]. Subclinical FIPV infections are normally restricted within the mesenteric lymph nodes and can resolve or disease progress [5]. However, only 5% of FECV infections could develop into FIP [1]. Clinical signs of FIP are involve to various organs including kidneys, liver, pancreas, eyes and nervous system. All clinical findings that occur in FIP are a direct consequence of vasculitis leading to organs failure. FIP have been classified into 2 forms; 1) effusive form (wet form) characterized by polyserositis, thoracic and/or pericardia, abdominal effusion; 2) non-effusive form (dry form) categorized by granulomatous inflammation, glomerulonephritis, chronic diarrhea, lymph node enlargement, ocular signs (uveitis, chorioretinitis) and neurological signs (ataxia, seizures and cranial nerve defect). Due to clinical signs of FIP are varies and non-specific, several laboratory have been used for FIP diagnosis. Indirect tests such as albumin/globulin ratio, effusions analysis and Rivalta test were performed to help differentiate FIP from other diseases. However, these tests are not suitable for cat affected with dry form of FIP. Furthermore, direct detection of the conserved 3′-untranslated region (3′ UTR) of FCoV genome in clinical sample using a reverse transcription polymerase chain reaction (RT-PCR) was developed [6]. Up to date, FCoV and FIPV infection in cats has been reported worldwide [7, 8]. The percentages of FCoV serological positive were reneged from 25% of individual living cats and up to 80 to 90% of multi-cat households [9]. In Thailand, the incidence of FCoV infection among cat populations during 2003 was 30.97% (FIPV = 1.63%, n=184) [10] and during 2010-2011 was found 46% (n= 103)[11]. Due to the sample from previous studies were limited to the central and eastern parts of Thailand. In this study, we modified the previous nRT-PCR into real-time RT-PCR technique and use for investigate the prevalence of FCoV and FIPV isolates from clinical samples submitted to Animal Hospital, faculty of veterinary medicine, KhonKaen University.

## Materials and Methods

### Animals and samples: During July 2018 to January 2019, 64-clinical samples collected from 51 cats submitted to Animal Hospital, faculty of veterinary medicine, Khon Kaen University were included in this study. Cats in this study were classified into 2 groups according to their health status. Group 1: FIP clinically suspected cats (N =29) referred to ill cats that show one of various clinical signs including respiratory distress, the presence of pleural/abdominal effusion, ascites, jaundice, nephritis, chronic diarrhea, nervous signs and ocular sign. Group 2: healthy cats (N=22) were divided into 2-subgroup; 2A was referred to healthy cats living alone in household (N=10) and 2B was mentioned to multi-healthy cats living within one household (N=12). Three types of clinical samples consist of serum, effusions and feces were collected both group (Table 1). At least 200 μl of serum and body effusions were collected, centrifuged, and then the supernatant were harvested and kept at -80°C until used. Approximately 1-gram of feces sample from fecal swab preserved in sterile phosphate buffer saline were collected, centrifuged, and then the supernatants were kept at -80°C until used.

### Table 1. Number of samples separated by group and type of samples.

<table>
<thead>
<tr>
<th>Groups of study</th>
<th>Serum (n)</th>
<th>Effusions (n)</th>
<th>Feces (n)</th>
<th>Total cats/ group (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (N=29)</td>
<td>17</td>
<td>12</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>Group 2A (N=10)</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Group 2B (N=12)</td>
<td>7</td>
<td>-</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Total sample (n)</td>
<td>24</td>
<td>12</td>
<td>28</td>
<td>64</td>
</tr>
</tbody>
</table>

### RNA extraction: Viral RNA was extracted from clinical sample using NucleoSpin® RNA Virus extraction kit (Macherey-Nagel, DÜren, Germany) according to the manufacturer’s procedure. Briefly, 150 μl of fluid
sample was mixed with lysis buffer and heated at 70°C. After 5 minutes absolute ethanol was added then total lysate fluid was load into the column and spin down. After several washing steps and dry the silica membrane the RNA was eluted with RNase-free water and kept at -80°C until RT-PCR was performed.

**Reverse transcription polymerase chain reaction (RT-PCR) for 3’ UTR detection:** Reverse transcriptase reaction was performed from extracted RNA using 5x ReverTra Ace® qPCR RT Master Mix (Toyobo Co., Ltd., Japan) according to the manufacturer’s instruction. After cDNA synthesis was done, the cDNA was kept at -20°C until PCR was performed. Subsequently, PCR assay to identified 233 nucleotides of FCoV-3’UTR was performed using forward primer 3’UTR (5’→3’) GGCAACCCGATGTTTAAAACTGG and reverse primer (5’→3’) CACTAGATCCAGCTAGCTC following previous publication [6]. The real-time PCR components consist of 3 μl of template cDNA, 10 μl of 2x THUNDERBIRD® SYBER® qPCR Mix (Toyobo Co., Ltd., Japan) and 7 μl of DNase/RNase-free water. The real-time PCR mixture was run under conditions; hot start at 95°C for 2 min, followed by 39 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and elongation at 72°C for 45 sec. The positive control was the positive FCoV RNA confirmed with sequencing, while the negative control was without genomic RNA. Feline GAPDH primers were also run parallel with selected sample as an internal control.

**Results**

In this study we were performed a real-time RT-PCR assay targeting on FCoV - 3’UTR. The positive results indicated by the amplification curve similar to the positive control with a Cq value less than or equal to 35 and the 233 product size was observed by melting curve peak at 79.5°C similar to the positive control. Negative results are absence of any amplification curve or Cq value greater than 35. The percentage of FCoV positive cats from group 1, group 2A and group 2B were 58.62% (17/29), 90.00% (9/10) and 91.66% (11/12) respectively. The highest percentage of FCoV positive from group 1 was found in fecal sample 100% (6/6) follow by body fluid 41.67% (5/12) and serum 41.18% (7/17). Similarly, FCoV positive result was major found in feces of group 2A (91.66%) and 2B (90.00%) respectively. While, less percentage of FCoV positive results was observed in serum sample of group 2B (85.71%). It’s should be note that, FIP was diagnosed 17.24% (5/29) from group 1 cats due to FCoV positive results was found in body fluids (Table 2). The prevalence of FCoV positive cats from all groups in this study was 70.59% (36/51).

**Table 2.** The percentage of FCoV positive results separated by group and type of samples.

<table>
<thead>
<tr>
<th>Groups of study</th>
<th>% FCoV positive (no. of positive sample/ total sample)</th>
<th>% FCoV positive cats*/ group</th>
<th>% FIPV positive cats** / group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Effusions</td>
<td>Feces</td>
</tr>
<tr>
<td>Group 1 (N=29)</td>
<td>41.18 (7/17)</td>
<td>41.67 (5/12)</td>
<td>100 (6/6)</td>
</tr>
<tr>
<td>Group 2A (N=10)</td>
<td>-</td>
<td>-</td>
<td>90 (9/10)</td>
</tr>
<tr>
<td>Group 2B (N=12)</td>
<td>85.71 (6/7)</td>
<td>-</td>
<td>91.66(11/12)</td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Remark: *FCoV positive cat = at least 1 sample/ cat was positive, **FIPV positive cat = FCoV positive in effusions, NA = not analyzed.

**Discussion**

FIP is one of the most fatal disease in cat of any aged due to it’s an immune-associated disease resulting in varies and non-specific clinical signs and without a successful vaccination program. Generally, FECV infected cats were limited detected within intestine only but FIPVs might be found in blood circulation [4]. In this study, we aim to modify the previous nRT-PCR into real-time RT-PCR technique to reduce the contamination and enhance the specificity of assay. Then, using this developed assay for investigate the prevalence of FCoV and FIPV isolates from clinical samples submitted to Animal Hospital, faculty of veterinary medicine, Khon Kaen University. Our study reveals the prevalence of FCoV within FIP clinically sus-
The prevalence of FCoV in serum from cats was 58.62% (n=29) higher than the previous reports in Thailand 46% (n=103) [11]. This higher prevalence of FCoV results are most likely due to less number of total cats in the study. It should be noted that, the FCoV positive in serum from this study could not distinguish between FCoV or FIPV. Interestingly, we detected FCoVs in serum from healthy cats living in groups 85.71% (n=7). Since FIPV is a mutated FCoV, these cats have potential to develop FIP in future and should be routinely monitoring the FCoV status. As the results, serum samples should be an appropriate specimen for investigates sub-clinical FIPV infection in healthy multi-cats in household or shelters. Furthermore, the advantage of using a real-time RT-PCR assay was useful for investigate the FCoV carriers cat before entering to FCoV free catteries. At present, S gene subtyping from all positive samples is in process.

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References

Feline Herpesvirus-1 in Cats with Ocular and Upper Respiratory Tract Diseases in Khon Kaen, Thailand

Nitiwadee Lertitthikul1,2, Naroepon1, Nopwinyoowong1, Kanyarat Manopinives1, Sitanan Manitnark1, Preenun Jitasombuti1, Patchara Phuektes1,2

1Faculty of Veterinary Medicine, KhonKaen University, Muang, KhonKaen Thailand, 40002
2Research Group for Animal Health Technology, Faculty of Veterinary Medicine, KhonKaen University, Muang, KhonKaen Thailand, 40002

*Corresponding author Email niti_lertitthikul@hotmail.com

Abstract

Objective To investigate the prevalence of feline herpesvirus type 1 (FHV-1) in cats with clinical evidence of ocular and upper respiratory tract diseases (URTD) and to determine whether the presence of FHV-1 is associated with FHV-1 related clinical manifestations.

Materials and Methods Conjunctival and corneal swab samples were collected from 38 cats showing signs of ocular disease and/or URTD and 14 clinically normal cats enrolled at two animal hospitals located in KhonKaen, Thailand. Samples were analyzed for the presence of FHV-1 by conventional PCR assay.

Results Of 38 diseased cats, 5 cats presented with URTD, 21 cats had ocular symptoms, and 12 cats presented with both URTD and ocular symptoms. Overall prevalence rate of FHV-1 as assessed by conventional PCR assay was 98%. Prevalence in the group of diseased cats and the control group were at 100% and 92.8%, respectively. There was no significant difference for the presence of FHV-1 among the 2 groups of cats (P=0.60).

Conclusion This study described, for the first time, prevalence for FHV-1 in privately owned cats in KhonKaen, Thailand. FHV-1 was present in a high percentage of the population in both diseased cats and clinically normal cats. No significant difference for the presence of FHV-1 in both groups suggested that conventional PCR assay may not be suitable for the diagnosis of FHV-1 related clinical manifestations.

Keywords: Feline herpes virus-1, PCR

Introduction

Feline herpesvirus type 1 (FHV-1) is one of the leading causes of upper respiratory tract and ocular infections in cats and kittens worldwide [1]. FHV-1 can replicate within the epithelia of conjunctiva and upper respiratory tract, and sensory ganglia [2]. A long-term sequelae of exposure to this virus includes damage to the ocular and periocular tissue [2]. After primary infection, approximately 80% of cats become latent infected, with the virus residing primarily in the trigeminal ganglion [3]. About 50% of latently infected cats will shed virus following spontaneous and stress-induced reactivation, and some of these cats will develop FHV-1 associated clinical diseases [3].

During primary FHV-1 infection, cats may develop ocular symptoms including blepharospasm, epiphora, conjunctival hyperaemia, serous ocular discharge, mild to moderate conjunctival swelling, and conjunctival ulcer [4]. Some cats develop keratitis and corneal ulcer characterized by transiently dendritic prior to quickly coalesce to become geographic ulcer. In chronic or severe infection, cats with keratitis may develop corneal sequestration or chronic dry eyes (keratoconjunctivitis sicca or KCS). The ocular signs can be seen in association with typical signs of upper respiratory infection such as acute rhinitis, sneezing and purulent nasal discharge [5]. Clinical signs are particularly severe in young kittens [4].

Presumptive diagnosis for FHV-1 infection is based on clinical signs. Although FHV-1 usually causes more severe upper respiratory tract and conjunctival and corneal signs than other feline respiratory pathogens [5], specific diagnosis requires laboratory tests. Diagnostic tests to detect FHV-1 include virus iso-
lation, immunofluorescence antibody (IFA) assays and PCR assays. IFA can be useful but has fallen out of favor due to the superiority of virus isolation and PCR assays (6). Virus isolation has been accepted as a reasonable test for diagnosis of active infection, but it is time-consuming and has a lower sensitivity than PCR (6). PCR analysis is the most rapid and sensitive test to identify infected cats, but it may be of limited use in a clinical setting, particularly in the area where there is a high prevalence of FHV-1 infection in the population of clinically normal cats. Positive PCR results should be interpreted with caution since they may be due to low level shedding or viral latency. Quantitative PCR assays may be useful in which high viral loads are indicative of active infection.

Prevalence of FHV-1 infections in different studies are varied, ranging from 2.28 to 99.7% (7-13). Discrepancy results have also been reported concerning the prevalence of FHV-1 in cats with ocular diseases and/or URTD and normal cats. Some previous studies have shown no significant differences in the prevalence of FHV-1 in diseased and clinically normal cats (6, 8). Conversely, other studies reported markedly higher prevalence rates of FHV-1 infections in groups of diseased cat compared to normal cats (14, 15). Currently, there is no information on the prevalence of FHV-1 infection in Thailand. In order to evaluate the usefulness of PCR assay as a tool for diagnosis of FHV-1 infection, it is necessary to determine the presence of FHV-1 in diseased and normal cats. This study aimed to investigate the prevalence of FHV-1 in cats with clinical evidence of ocular diseases and URTD and to determine whether the presence of FHV-1 is associated with FHV-1 related clinical manifestations.

Materials and Methods

Animals: A cross sectional study was conducted at 2 animal hospitals located in Khon Kaen province, Thailand. Inclusion criteria were cats presenting with clinical signs of URTD including sneezing, coughing and nasal discharge, and/or ocular diseases including conjunctivitis, corneal ulcer, blepharospasm, epiphora, chemosis and ocular discharge. A control group consisted of cats with none of the clinical signs mentioned above. A total of 52 cats were enrolled in this study. Of these, 38 cats had clinical signs of URTD and or ocular diseases and 14 cats were used as a control group.

Sample collection: From each cat, conjunctival and corneal swab were obtained by gently rolling a sterile, cotton-tipped swab along the ventral conjunctiva and corneal surface. In cats with corneal ulcerations, samples were collected after the application of 0.5% tetracaine hydrochloride (Alcon). Each sample was placed into a sterile tube containing 1 mL of phosphate buffered saline. Samples were stored at 4°C before being sent to the laboratory and were kept at 20°C until analyzed.

PCR assay: Samples were thawed and equilibrated at 37°C for about 10 min. DNA was then extracted from 200 µL of sample using QIAamp DNA mini kit (Qiagen) according to the manufacturer's instruction. PCR for FHV-1 detection was performed with primers targeting FHV-1 terminase gene (11). The primer sequences were as follows: FHV1f (CGG GAA AAT CCAGTA CGA GT) and FHV1r (AGG AAG AGT TCG GCGGTA TT), which amplified a 200-base pair DNA fragment. The reaction was performed in a 25 µL reaction mixture composed of 5 µL of DNA template, 1.25 units Taq DNA polymerase (Qiagen), 200 µM of each dNTP, 0.2 µM of each primer, and 1xPCR buffer containing 1.5 mM MgCl₂. Amplifications was performed on a thermal cycler for the following conditions: 94°C for 3 min, and then 40 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min, followed by a final extension at 72°C for 10 min. PCR for amplification of housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was also carried out to verify the presence of DNA in the sample. The primers used for GAPDH detection were designed by Sandmeyer et al. (2010) and the sequences were as follows: GAPDHF (AGC CTT CTC CAT GGTGGT GAA GAC) and GAPDHR (CGG AGT CAACGG ATT TGG TCG), which produced an amplicon of 320-base pair. PCR reactions and amplification cycles for detection of GAPDH were performed as described above for FHV-1 detection. Positive control containing DNA of FHV-1 or GAPDH and negative control containing no DNA template were routinely included. The PCR products were analyzed on 1.5% agarose gel. Selected PCR products of FHV-1 were verified through direct DNA sequencing.

Statistical analysis: Descriptive statistics was used to determine the prevalence rate of FHV-1 infections. Chi-square test was used to compare FHV-1 infections between a group of diseased cats and a control group. Significance was defined at values of P<0.05.
Results

PCR amplifications of FHV-1 and GADPH yielded PCR products of expected sizes at 200 and 320-base pair, respectively as shown in figure 1. Selected PCR amplicons of FHV-1 was confirmed through direct DNA sequencing and the nucleotide sequences compared against other FHV-1 sequences in GenBank database (data not shown).

Figure 1. PCR amplifications from some of the conjunctival and corneal swab samples. Lane 1 and 2, samples positive for GADPH; 3 positive control for GADPH; 4 negative control for GADPH; 5 and 6 samples positive for FHV-1, 7 positive control for FHV-1, 8 negative control for FHV-1. The molecular weight markers are the vc100bp plus DNA ladder (Vivantis).

Conventional PCR assay for FHV-1 was performed on 52 conjunctival and corneal swabs sampled. Samples were collected from 14 cats without ocular disease and/or URTD, and from 38 cats showing clinical evidence of ocular disease and/or URTD (6 cats with URTD, 21 cats with ocular symptoms, and 12 cats with both URTD and ocular symptoms). The overall prevalence rate of FHV-1 was 98% (Table 1). Both diseased and control groups had high prevalence rates at 100% and 92.8%, respectively (Table 1). There was no significant difference for the presence of FHV-1 infections among the 2 groups of cats (P value = 0.6).
Table 1. Prevalence of FHV-1 as determined by conventional PCR assays

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number of cats</th>
<th>FHV-1 positive</th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage(%)</td>
</tr>
<tr>
<td>Clinically normal cats</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Cats with ocular diseases and/or URTD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-URTD</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>-Ocular diseases</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>-URTD and ocular diseases</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>51</td>
</tr>
</tbody>
</table>

Discussion

This study described here was conducted to determine the prevalence of FHV-1 for 52 privately-owned cats presented at 2 animal hospitals in Khon Kaen, Thailand by using conventional PCR assay. Overall, FHV-1 was present in a high percentage of the population in both diseased and clinically normal cats. The detection rate of FHV-1 in this study is higher than previous publications, particularly in clinically normal cats (7-15). Prevalence of FHV-1 infections in FHV-1 associated ocular diseases and/or URTD was found to be diverse, ranging from 2.28 in a study in Canada to 99.7% in California, USA (7-15). The detection rates of FHV-1 in clinically normal cats were also reported to vary from 3 to 49%. The possible reasons for different detection rates may be related to the source of cats enrolled for each study, for example privately-owned cats or cats in shelters, sample sites, sample collection and storage, and sensitivity of the PCR assays. Our study found a higher detection rate in clinically normal cats than previous studies (8,14-15). This could be due to widespread of FHV-1 infections among population of cats in Khon Kaen, Thailand. However, larger numbers of samples from normal cats will needed to be included in further study for more accurate determination of prevalence rate.

Results from this study indicated that the use of conventional PCR assay as a tool for diagnosis of FHV-1 associated diseases in Thailand may be complicated due to the high prevalence of FHV-1 in both diseased and clinically normal cats. Positive PCR results could be due to a low level of shedding of FHV-1 that is not associated with clinical symptoms. It has been shown that clinically normal cats may shed virus following recovery from experimental infection (3). In addition, FHV-1 vaccine strains can also be detected by PCR (16). Thus, history of vaccination with modified live FHV-1 is unnecessary for interpretation of PCR assay. In clinical cases, positive PCR results may indicate that FHV-1 is the cause of primary disease or may be a result of reactivation secondary to a primary disease. It is also possible that the presence of FHV-1 in clinical cases may be unrelated to the primary disease. Further studies of quantitative PCR assay and correlation of viral load to FHV-1 clinical manifestations will be further investigated for the indicative of active FHV-1 infections.

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References


Pneumatosis Coli in a Dog: a Case Report

Nitaya Boonbal*, Chalermkwan Nonthakotr1, Nichanan Maneeganondh1

1Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Khon Kaen University, KhonKaen 40002, Thailand
*Corresponding author Email: nit-ying-da@hotmail.com

Abstract

Case Description A 12-year old intact male poodle was presented to the KKU Veterinary Teaching Hospital with a history of an acute episode of tenesmus and passage of ribbon-shape stools. There was no history of trauma or pulmonary disease.

Clinical Finding Digital rectal palpation revealed mild crepitus of soft tissue at the anal sphincter. The fecal floatation and wet mount preparation were negative for parasite. Leukocytosis and elevated Alkaline Phosphatase (ALP) level were found. Canine-pancreatic lipase (CPL) is normal. Survey abdominal radiographs revealed extensive intramural emphysema of colon and rectum. Ultrasonography of the abdomen revealed bright echoes within the layers of the colon wall, confirming the accumulation of intramural gas. Colonoscopic examination revealed erosion and ulceration of the mucosa can be seen through the colon without intramural mass or obstruction of the colon.

Diagnosis and Treatment Based on the radiographic, ultrasonographic and colonoscopic examination the present case was diagnosed as pneumatosis coli with an underlying cause of bacterial overgrowth. The patient was treated with antibiotic for twenty-five days. Clinical sign were resolved after seven days of treatment. Decreased intramural gas accumulation was evident during radiography of the abdomen performed at sixteen days after the initial evaluation.

Clinical Relevance Pneumatosis coli is a rare disease in dogs, but should be considered when the patient having clinical signs of colitis. Pneumatosis colic was characterized by radiographic finding as intramural emphysema of the colon was seen. The etiology should be pursued by fecal culture. Antibiotic may be indicated to decrease the anaerobic bacteria in the colon.

Keywords: Colon, Dog, Emphysema
Effect of Rice Bran and Germ Oil on Blood Glucose, Insulin, Triglyceride and Cholesterol Levels in Induced Hyperglycemic Cats

Arisara Taechavichein¹, Monthicha Chaiyarak¹, Runchida Phanphat¹, Ranee Singh²*  

¹Fourth years student of Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002  
²Pharmacology and Toxicology Unit, Faculty of Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002  
*Corresponding author Email: sranee@kku.ac.th

Abstract

Objective The objective was to examine the efficacy of rice bran and germ oil to decrease blood glucose level in cats that were induced to have hyperglycemia conditions alike that occur in type II DM.

Materials and Methods Eleven healthy mixed-breed cats were separated into 2 groups, they were all raise in same environment. First 4 weeks of the experiment they were feed with control feline adult diet (carbohydrate 31% Metabolic energy). In the last day of feeding, blood samples from all the cats were collected to analyze blood glucose level and Intravenous Glucose Tolerance test were performed. Then all cats were fed with high carbohydrate (carbohydrate 51% ME) in order to induce them to have hyperglycemia condition. On week 8th, began treatment of group one with rice bran and germ oil (500 mg/cat, orally) and group two with Acarbose® (25 mg/cat/day, PO). Blood sample were collected after four weeks to analyze blood glucose, triglyceride and cholesterol levels. Intravenous Glucose Tolerance test were performed again on the last day of the experiment. Acarbose was used as reference hypoglycemic drug.

Results Cats fed with high carbohydrate diet for 4 weeks showed significant levels in blood glucose as compare to controlled diet fed cats (86.6 ± 1.8and 82.3 ± 2.6 mg/dL respectively). Rice bran oil and germ oil significantly reduced blood glucose level(p<0.05), as the average blood glucose level reduced from 86.6 ± 1.8 to 76.0 ± 1.6 mg/dL after treated with Rice bran oil and germ oil for 4 weeks. For the result of Area Under the Curve (AUC) of intravenous glucose tolerance test (IVGTT), 120 mins blood glucose levels of cats receiving high carbohydrate diet (146.4 ± 4.1 mg/dL) was significantly higher than those receiving control diet (144.1 ± 5.8 mg/dL) or receiving Rice bran oil and germ oil 500 mg for 4 weeks(125.8 ± 6.2 mg/dL). In conclusion, Rice bran oil and germ oil has the efficacy to reduce blood glucose level in hyperglycemia cats. Rice bran oil and germ oil is an alternative food supplement to combine with hypoglycemic drug(s) for controlling blood glucose level in hyperglycemia cats or diabetic (TypeII) cats.

Conclusion Results from this experiment provided preliminary evidence that Rice bran oil and germ oil is an alternative food supplement for controlling blood glucose level in hyperglycemia cats or diabetic (TypeII) cats.

Keywords: diabetes mellitus, cat, feline, Type II DM
Abstract

Objective This study was designed to determine the effects of trehalose at different concentrations (12.5, 25, and 50 mM) on post-thawed quality of buffalo sperm.

Materials and Methods Two bulls aged between 4-5 years were used in this experiment. Ejaculates were collected via artificial vagina twice a week. Semen samples were divided into 3 groups for dilution with Tris-egg yolk glycerol extenders (8% glycerol and 20% egg yolk) those containing different concentrations of trehalose (12.5, 25, and 50 mM). The diluted semen was loaded into 0.25 ml straws, cooled to 5 °C slowly and equilibrated for 3 h. After the equilibration, the samples were frozen in liquid nitrogen vapor, and plunged into liquid nitrogen (−196 °C) for storage. Frozen straws were thawed at 37 °C for 30 s in a water bath for evaluation. The sperm viability and sperm membrane integrity (HOST) were evaluated by microscopic examination and the sperm motility was assessed by using computer assisted semen analyzer (CASA).

Results On sperm viability, membrane integrity, motility, progressive motility, straight-line velocity and average path velocity there were no superiority on the protective capacity of the different concentrations of trehalose in post-thaw sperm analysis. However, the straightness and linearity of sperm in 12.5 and 25 mM trehalose were better than in 50 mM trehalose group (P<0.05).

Conclusion The usage of trehalose at 50 mM was reduced in the straightness and linearity of sperm motility compared to 12.5 and 25 mM group.

Keywords: Buffalo, Trehalose, Semen, Frozen
Prevalence of Surra in Dogs near Slaughterhouse of Ban Dongmafai, Khamin Subdistrict, Mueang District, Sakon Nakhon Province

Kanokwan Bootyothee¹, Hanchai Umpapol¹, Tharadol Jitjak¹, Jakkapat Prachachit¹, Sermwich Bootyothee²

¹Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Thailand, 47000
²Faculty of Humanities and Social Sciences, Sakon Nakhon Rajabhat University, Thailand, 47000

*Corresponding author Email: aj.kanokwan@hotmail.com

Abstract

Objective The aim of this study is to examine the prevalence of *Trypanosoma evansi* in dogs lived near slaughterhouse of Ban Dongmafai, Khamin, Mueang, Sakon Nakhon.

Materials and Methods 50 blood samples were collected from 50 dogs near the slaughterhouse of Ban Dongmafai, Khamin Subdistrict, Mueang District, Sakon Nakhon Province. All blood samples were tested within 24 hours after collected, the hematocrit centrifugation technique (Woo’s technique) and thin blood smear were used.

Results The blood result found prevalence of *T. evansi* infection in 2 dogs from 50 dogs (4%) which developed clinical sign such as corneal opacity and edema, *T. evansi* was found in thin blood smear. Other blood parasites were found such as *Ehrlichia canis* (*E.canis*), *Anaplasma* spp. The co-infection between *Babesia* spp. and *Anaplasma* spp., *E.canis* and *Anaplasma* spp. were observed.

Conclusion The prevalence of *T. evansi* infected dog found 4%, the risk factor which leads to infected this parasite was the raising type especially the free-living or outdoor which found in most dogs (population). The study area near the slaughterhouse can cause infected because the dog population can eat blood or by-product from slaughterhouse. In our study, the *T. evansi* infected dog was found in young dogs (<1-year-old). The depression, leg edema and severe ocular inflammation were observed, similarly, with the previous study.

Keywords: *Trypanosoma evansi*, Surra, Dogs, Slaughterhouse
Preliminary Study of Hematological Parameters in Native Goats Rearing in KhonKaen Province, Northeastern Region, Thailand

Thanakarn Nasri¹, Nusara Suwannachot¹, Suttisak Nopwingyoowong¹

¹Division of Pathobiology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen

Corresponding author: Email: tanakarn@kku.ac.th

Abstract

Objective The objectives of this present study was to investigate the hematological parameters of Thai native goat rearing in Khon Kaen province, Northeastern Thailand

Materials and Methods The study was performed on 125 apparently healthy Thai native goats (120 female and 5 male) of ages from 6 month to 6 years, rearing in Khon Kaen province in 2018. Blood samples (2 ml) were obtained by jugular vein puncture in the vacuum tubes with EDTA. Hematological parameters were determined using an automated cell count (CELLDYN 3700). Parameters were analyzed by SPSS® for Window version 10.0

Results The mean value of hematocrit was 0.325 L/L, hemoglobin concentration 100.4 g/L, red blood cell count 10.37 x 10¹²/L, mean corpuscular volume (MCV) 31.68fL, mean corpuscular hemoglobin concentration (MCHC) 30.85 g/dL, Total white blood cell count 10.90 x 10⁹/L, Segmented neutrophil 5.680 x 10⁹/L and lymphocyte 4.145 x 10⁹/L. Platelets count 516.73 x 10⁹/L

Conclusion We expect these data to be applied to the further studies.

Keywords: Hematology, Thai nativegoat, Reference values
Results in Supplementation of Bioplex® HighFive at Prepartum for Decreasing Incidence of Retained Fetal Membrane in Dairy Cow

Chaiwat Jarassaeng1*, Saksiri Sirisathein1, Papawee Maneechot2, Puttida Krittaphadol2, Suweeraya Nachamroen2

1Theriogenology Units, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
2 Fifth years student, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
*Corresponding author Email: chajar@kku.ac.th

Abstract

Objective The objective of this study was to determine the effects of Bioplex® HighFive at prepartum for decreasing incidence of retained fetal membrane in dairy cow.

Materials and Methods The questionnaire was interviewed of the postpartum problem in 20 dairy farms in Khon Kaen dairy cooperative. All pregnant cows were supplemented with trace minerals (Bioplex® HighFive) by 5 mg/head/days about 20 days before calving date. The incident of retained fetal membrane was investigated between July 2017 to June 2018.

Results The questionnaire were found retained fetal membrane and mastitis mostly problem in postpartum periods. Retained fetal membrane was found in 16 farms, only 4 farms no incidence of retained placenta in 2017-2018. The incident of retained fetal membrane was decreased from 43 dairy cow in 2017 to 17 dairy cows in 2018 in about 361 calving cows.

Conclusion Supplementation of trace minerals at prepartum period has advantage results in reproduction in dairy cows.

Keywords: Retained fetal membrane, Trace minerals, Dairy cow
Effect of Rice Bran Hydrolysate on Blood Glucose, Insulin, Adiponectin, Triglyceride and Cholesterol Levels in Induced Hyperglycemic Cats

Ranee Singh¹*, Supawan Thawornchinsombat², Patchareewan Pannangpetch³

¹Pharmacology and Toxicology Unit, Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
²Department of Food Science, Faculty of Technology, Khon Kaen University, Thailand 40002
³Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Thailand 40002

Corresponding author Email: ranees@kku.ac.th

Abstract

Objective The objective of this study was to determine whether rice bran hydrolysate could modulate insulin resistance in high carbohydrate diet fed cats.

Materials and Methods Eleven non-obese, mixed-breed, adults, of clinically healthy cats were used in this study. Following consumption of a controlled diet for 4 weeks, venous blood was collected to determine the baseline of blood glucose. Then the cats were received high carbohydrate diets (HC) for 4 weeks to induce insulin resistance. After that, cats were randomly divided into 2 groups, first groups were received a HC diet with rice bran hydrolysate at doses of 0.5g or 500 mg/day and group 2 received acarbose (alpha glucosidase inhibitor) of 25 mg/cat orally once daily. An intravenous glucose tolerance test (IVGTT) was performed after the 4th week of treatments in these groups and fasting blood were collected to measure insulin, glucose, triglycerides, cholesterol and adiponectin. The glucose and insulin values were used to calculate HOMA-IR.

Results the IVGTT showed the AUC glucose of HC-diet control group was significantly higher than that of cats that received control diets (p<0.05). Interestingly, the AUC of glucose levels of HC diet group treated with rice bran hydrolysate was significantly lower than that of HC group (125.3±3.5, and 135.9±5.6 respectively). In addition, the HC diet group had a significant high HOMA-IR score as compared to normal diet group. This high HOMA-IR was returned to normal level in the group that received rice bran hydrolysate.

Conclusion The results indicated that the rice bran hydrolysate can reduce blood glucose and by reducing insulin resistance in HC diet fed cats. Rice bran hydrolysate may be used as alternative medicine in type 2 diabetic cats.

Keywords: Diabetes mellitus, Cat, Feline, Type II DM, Rice bran hydrolysate
The Efficiency of Clove Essential Oil on *Staphylococcus intermedius* Biofilm from Canine Pyoderma

Yanisa Teranuchapon¹, Puangphet Viriyasuebphong¹, Pimpakarn Rungsitumpanya¹, Wannasiri Siriprachya¹, Jareerat Aiemsard²*

¹ Faculty of Veterinary Medicine, Khon Kaen University, Thailand, 40002
² Faculty of Veterinary Medicine and Biofilm Research Group, Khon Kaen University, Thailand, 40002
*Corresponding author Email: Jaraim@kku.ac.th

Abstract

**Objective** The objective was to study the efficiency of clove essential oil on *Staphylococcus intermedius* biofilm from canine pyoderma.

**Materials and Methods** This study used clove (*Syzygium aromatium*), which obtained from steam distillation. The organisms tested is *Staphylococcus intermedius* (n=7) isolated from canine pyoderma. To determine the efficiency of clove essential oil on *S. intermedius* planktonic and biofilm using crystal violet at the concentration range of 0.0048-0.078 µg/ml

**Results** The results showed dose showed dose dependent, which was found that the best of % inhibition of planktonic cell was 93-99% whereas biofilm cell was 50-95% at a concentration of 0.078 µg/ml.

**Conclusion** Clove essential oil can inhibited *S. intermedius* in biofilm less than planktonic form

**Keywords:** Superovulation, Native cattle, Ovulation
The Effect of Meditation on Metacognitive, Working Memory Ability and Stress of Khon Kaen University Students, Thailand

Suwit Uopasai¹, Chuleeporn Saksangawong¹*, and Patr Thongwisong²

¹Faculty of Veterinary Medicine, Khon Kaen University, Muang, Khon Kaen, Thailand, 40002
²Faculty of Economics, Khon Kaen University, Muang, Khon Kaen, Thailand, 40002
*Corresponding author Email: suwuop@kku.ac.th

Abstract

Objective To study the effect of meditation on metacognitive, working memory ability and stress of Khon Kaen University Students, Thailand

Materials and Methods This quasi-experimental research was employed in 2nd semester 2018. The sample was KKU students, a public university in Khon Kaen province, Thailand. The total participants were 60 and equally distributed to 30 students in treatment and control groups. The experimental group was enrolled meditation program, while the control group was not received this program. Researchers employed experimental research pre-test and post-test control group design. The metacognitive ability was measured using the metacognitive awareness inventory (MAI), the working memory used the Thai working memory computerized battery test (TWMBT), and the stress used SPST-20. Analysis was done using MANOVA statistic

Results Results of this study showed that there was no significant difference between groups on the dependent variables before the intervention. However, all the dependent variables namely metacognitive ability, working memory ability and stress had significant differences between groups after the intervention.

Conclusion As a result, the meditation program can raise their metacognitive ability, promote their working memory ability and reduced stress.

Keywords: Meditation program, Metacognitive ability, Working memory ability, Stress

Introduction

Meditation is a set of attentional practices leading to an altered state or trait of consciousness characterized by expanded awareness, greater presence, and a more integrated sense of self[1]. There are two general types of meditation, the first is concentrative meditation, and the second is mindfulness meditation [2]. Focusing on awareness, from which a detached observation of the contents of consciousness develop, may represent a powerful cognitive behavioral coping strategy for transforming the way a person responds to lifeevents[3]. Breathing is a fundamental strategy for both types of meditation. Relaxation, de-automaticization of consciousness, ascendance, descendence, and transcendence are explained for cognitive and emotional changes achieved through meditation [4]. It is easy for even beginners to practice without much preliminary knowledge. Metacognitive ability refers to the knowledge, awareness of students’ ability to control and assess their own thinking processes to their intellectual thought processes and strategies[5].

Metacognitive ability comprises knowledge about cognition and regulation of cognition. Knowledge of cognition covers declarative, procedural, and conditional knowledge while regulation of cognition refers to a set of activities that help students to control their learning such as planning, monitoring, evaluating, information management, and debugging [6].

Working memory is defined as our ability to process and remember information which is interconnected to a range of cognitive activities from cerebral tasks to verbal communication [7]. In short, working memory is an active system of storing information and information processing, and is essential for correct functioning of other complex cognitive functions [8].
Piyavhatkul et al reported that the use of Consciousness Transformation Program for Stress Management, comprising multimodal meditative components for first-year medical students, yielded some significant decreases in Symptom Checklist-90 scores and increased of self-awareness/acceptance. However, the study score did not change significantly (19). So, the aim of this study was to investigate whether meditation program will influence on metacognitive ability working memory ability and can reduced stress of undergraduate students or not.

Materials and Methods

A total of 60 healthy, right-handed undergraduate students age ranged within 19 to 22 years old with corrected-to-normal vision and no history of neurological or psychiatric conditions from a public university located at Khon Kaen province, Thailand were selected as participants. These 60 participants equally distributed into experimental and control groups respectively. Each group consisted of 10 males and 10 females. A 2 (meditation vs no) x2 (time of measure: pretest vs posttest) design was utilized in this study. On top of that, the experiment group was assigned to attend to meditation program training while the control group was not. At the initial stage, all the participants were get pretest (MAI, TWMBT and SPST-20). The students in the experimental group followed breathing meditation program every week for 15 weeks. After the introductory session, the participants in the meditation group meditated in a sitting position, in a quiet, air-conditioned lecture room every weekday from 5.00 to 8.00 p.m. for 45 days. They used breathing meditation 30 minute/time that was based on the Buddhist Anapanasati Meditation that emphasizes mindful awareness of the breath during inhaling and exhaling.

The metacognitive awareness inventory (MAI) is a rating scale used to measure two components, metacognitive knowledge (declarative, procedural, and conditional knowledge) and metacognitive regulation (planning, monitoring, evaluating, information management, and debugging) which consisted of 52 items. This instrument was adapted from Schraw & Dennison [6] and translated from English to the Thai language to ensure that the participants were clear about the statements. The reliability (KR20) was 0.95. The working memory battery test was orally in Thai version and adopted from Bunterm et al. [10] which comprised 7 tasks. The 7 tasks covered Odd – even, Vowel – consonant, Switch – Thai letter number, Thai stroop, 0 back, 1 back and 2 back. This working memory battery test allowed the researchers to measure working memory accuracy and reactiontime. Students were given 10 trials for each task, adding up to a total of 100 trials. Thereaction time below 200 milliseconds were excluded, and data was analyzed in the range of X±3S.D.

After intervention, all the participants were get posttest (MAI, TWMBT and SPST-20).

Results

Results are presented according to the purposes of the study as indicated above, we can report in 4 parts.

Part I Basic data

The age and BMI (body mass index) of participants in both groups did not have significant difference, \( T^2 = 0.3 ; F^2 = 0.860 ; p = 0.429 \). Thus, the experimental and control groups were identified as the same sampling distribution and appropriate to follow up with the intervention. The mean and standard deviation of these variables were shown in table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Age (year)</td>
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<tr>
<td>BMI (Kg/m²)</td>
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</tr>
</tbody>
</table>
Part II Working memory ability

Before intervention, there is no mean difference of accuracy (7 tasks of TWMBT) between groups, ($T^2 = 0.12; F_{(7,52)} = 0.90; p = 0.51$, Partial Eta Squared= 0.11). After intervention, the experimental group had possessed significantly higher accuracy in all TWMBT than the control group ($T^2= 1.31; F_{(7,52)} = 9.76; p <0.01$, Partial Eta Squared= 0.57). Then, we compare both groups with univariate analysis, the mean and standard deviation of these variable and univariate analysis results were shown in table 2.

### Table 2. The accuracy of TWMBT of participants

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th></th>
<th>After</th>
<th></th>
<th>univariate analysis</th>
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<tr>
<td></td>
<td>Experimental group</td>
<td>Control group</td>
<td>Experimental group</td>
<td>Control group</td>
<td></td>
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<tr>
<td>Odd – even</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
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<td>15.16</td>
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<td>0 back</td>
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<td>1.54</td>
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<td>8.43</td>
</tr>
<tr>
<td>1 back</td>
<td>5.63</td>
<td>1.40</td>
<td>5.23</td>
<td>1.30</td>
<td>7.47</td>
</tr>
<tr>
<td>2 back</td>
<td>4.10</td>
<td>1.16</td>
<td>4.00</td>
<td>1.70</td>
<td>5.83</td>
</tr>
</tbody>
</table>

**p<0.01

Before intervention, there is no mean difference of reaction time (7 tasks of TWMBT) between groups, ($T^2= 0.11; F_{(7,52)} = 0.85; p = 0.56$, Partial Eta Squared= 0.10) After intervention, the experimental group had possessed significantly shorter reaction time in all TWMBT than the control group ($T^2= 4.77; F_{(7,52)} = 35.42; p <0.01$, Partial Eta Squared= 0.83). Then, we compare both groups with univariate analysis, the mean and standard deviation of these variable and univariate analysis results were shown in table 3.
Table 3. The reaction time of TWMBT of participants

<table>
<thead>
<tr>
<th>Metacognition Variables</th>
<th>Before</th>
<th></th>
<th>After</th>
<th></th>
<th>Univariate analysis</th>
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</thead>
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<tr>
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</tr>
<tr>
<td>Odd – even</td>
<td>Experimental group Mean</td>
<td>SD</td>
<td>Control group Mean</td>
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<td>Experimental group Mean</td>
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<td></td>
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<td>41.67</td>
<td>587.63</td>
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<tr>
<td>Switch –Thai letter number</td>
<td>703.78</td>
<td>45.68</td>
<td>725.16</td>
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</tr>
<tr>
<td>Thai stroop</td>
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<td>65.01</td>
<td>737.60</td>
<td>65.79</td>
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<td>0 back</td>
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<td>533.50</td>
<td>70.20</td>
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<tr>
<td>1 back</td>
<td>591.96</td>
<td>98.43</td>
<td>581.59</td>
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<td>704.30</td>
<td>76.28</td>
<td>689.44</td>
<td>61.19</td>
<td>534.76</td>
</tr>
</tbody>
</table>

** p<0.01

Part III Working memory ability

Before intervention, there is no mean difference of metacognitive ability (8 variables of MAI) between groups, \( T^2 = 0.06; F_{(8,51)} = 0.365; p = 0.934, \) Partial Eta Squared= 0.05). After intervention, the experimental group had possessed significantly higher metacognitive ability than the control group \( (T^2 = 4.18; F_{(8,51)} = 26.68; p <0.01, \) Partial Eta Squared= 0.81) Then, we compare both groups with univariate analysis, the mean and standard deviation of these variable and univariate analysis results were shown in table 4.

Table 4. The metacognitive ability of participants

<table>
<thead>
<tr>
<th>Metacognition Variables</th>
<th>Before</th>
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<th>After</th>
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<th>univariate analysis</th>
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<td></td>
<td></td>
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<tr>
<td>declarative knowledge</td>
<td>29.70</td>
<td>3.82</td>
<td>29.13</td>
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<td>14.37</td>
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<tr>
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<td>2.93</td>
<td>18.50</td>
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<td>monitoring strategies</td>
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<td>debugging strategies</td>
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<td>2.78</td>
<td>19.33</td>
<td>2.51</td>
<td>23.03</td>
</tr>
</tbody>
</table>

* p<0.05 , ** p<0.01
Part IV stress

Before intervention, the stress score of student of experimental group was 46.10±14.43. While, the control group was 50.50±9.25. that not significant between groups (t=1.40, p=0.17). After intervention, the stress score of student of experimental group was 37.13±13.56 and the control group was 45.57±16.31. The experimental group was significant less stress than the other one (t=2.18, p<0.05).

Discussion

The results of this study show that there is no significant difference between groups on the dependent variables before the intervention. However, all the dependent variables namely metacognitive ability, and working memory accuracy and reaction time have significant differences between groups after the intervention. Innes [12] reveal that suggest that a meditation program is feasible to implement in adults with cognitive impairment and their caregivers, and may offer a cost-effective intervention for improving perceived stress, mood, sleep, and blood pressure in this population. They suggest that an 8-week meditation program may offer an acceptable and effective intervention for reducing perceived stress and improving certain domains of sleep, mood, and memory in adults with cognitive impairment and their caregivers. Conversely, mindfulness practice aims at accurate metacognition, including of intentions, and may lead to the development of finer grained higher order representations of intending. Thus, the long-term practice of mindfulness may produce an earlier judgement of the time of an intention [13].But in this study, indicated that the experimental group elucidate higher score in metacognitive inventory score. Meditators also gained efficiency in long-term memory retrieval not just short term working memory, as measured by more fluid word association. According to the lead researcher Fadel Zeidan,[14] “…this seems to be strong evidence for the idea that we may be able to modify our own minds to improve our cognitive processing – most importantly in the ability to sustain attention and vigilance – within a week’s time. The meditation group did particularly well on all the cognitive tests that were timed – where participants had to process information under time pressure causing stress. So, in this study, reveal that the students of experimental group had more working memory ability.

Conclusion

As a result, the meditation program can raise their metacognitive ability, promote their working memory ability and reduced the stress.

References

Effects of Bacterial Contaminations and Somatic Cell Count in Milk on Resazurin Reduction Test and Methylene Blue Reduction Test

Sarawanee Phansuwan¹, Wipapohn Thagudrua¹, Ratsamee Donto¹, Patchara Phuektes¹, Aran Chanlun¹*

¹Faculty of Veterinary Medicine, KhonKaen University, Muang, KhonKaen, Thailand, 40002
*Corresponding author Email: aran_jan@kku.ac.th

Abstract

Objective To determine the application of the dye reduction tests test for detection of bacterial contamination and somatic cell count in milk.

Materials and Methods A total of 52 milk samples were aseptically collected at the smallholder dairy farms in Khon Kaen province, Thailand. All milk samples were screening tested by using the California Mastitis Test (CMT) and the somatic cell count (SCC) in all were further determined by using DeLaval TM cell counter (DeLaval, Sweden). In addition, the dye reduction tests, i.e. the resazurin reduction and methylene blue, were carried out. Duration of color changes of both of the dye tests was recorded. In addition, total bacteria count (TBC) was counted by using the 3M petrifilm® Aerobic Count Plate (AC) and Coliforms were also counted by using the 3M Petrifilm® E. coli/Coliform Count Plate. SCC was categorized into 3 groups, i.e. high SCC (HSCC), medium (MSCC) and low (LSCC) whereas TBC was classified into 2 groups as high (HTBC) and low (LTBC).

Results HTBC milk samples changed the color of the resazurin reduction test with a mean of 53 min, ranging from 15 min to 135 min whereas LTBC changed the color of resazurin dye with the mean of 106, ranging from 20 min to 260 min. Changes of methylene blue reduction test of LTBC and HTBC were 5.5 h (range: 2-6 h) and 3.6 h (range: 2.5-4.5 h), respectively. Both in HTBC had the shorter duration of a color change than that of LTBC group. However, there was no difference in duration of color changes the two dye tests between LSCC and MSCC. Interestingly, HSCC milk but was grouped in LTBC had the duration of the color change of resazurin and methylene blue test differed

Conclusion Bacterial contamination in milk had higher potential on color changes of both the dye tests compared to somatic cell counts.

Keywords: SCC, Dye reduction test, bulk milk, milking cow, resazurin, MRBT