

The 19<sup>th</sup> Khon Kaen Veterinary Annual International Conference (KVAC) 2018

**“Indigenous Knowledge for  
Global Animal Health”**

**April 4 - 5, 2018**



**AVANI Khon Kaen Hotel &  
Convention Centre  
Khon Kaen, THAILAND**

**The 19<sup>th</sup> KVAC 2018: Indigenous Knowledge for Global Animal Health**



**The 19<sup>th</sup> Khon Kaen Veterinary Annual  
International Conference (KVAC) 2018**

**April 4-5, 2018**

**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**

**Department of Livestock Development**

**Thai Swine Veterinary Association**

**Research Group for Animal Health Technology**

**KKU Vet Alumni**



# **PROCEEDINGS**

## **The 19<sup>th</sup> Khon Kaen Veterinary Annual International Conference (KVAC) 2018**

### **“Indigenous Knowledge for Global Animal Health”**

**April 4-5, 2018**

**AVANI Khon Kaen Hotel & Convention Centre  
Khon Kaen, Thailand**

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*A publication of:*

Faculty of Veterinary Medicine, Khon Kaen, Thailand

Abstracts and full-text papers of all oral and poster presentation published in The 19th Khon Kaen Veterinary Annual International Conference (KVAC) 2018: “Indigenous Knowledge for Global Animal Health” 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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## **Welcome & Opening Speech**



Good morning, all Guest Speakers, Lecturers, Researchers, and Conference Attendees, My name is Assoc. Prof. Dr. Chuchat Kamollert, Dean of Faculty of Veterinary Medicine, Khon Kaen University. I preside over this event on behalf of Assoc. Prof. Dr. Kittichai Trairattanasirichai, President of Khon Kaen University, as the President has already been engaged in other missions. On behalf of Khon Kaen University, it is my great honor to organize “The 19th Khon Kaen Veterinary Annual International Conference or KVAC 2018” in this year and welcome all the honored guests to our beloved Khon Kaen Province.

At present, Khon Kaen University is going on the 55th year Anniversary of the establishment. We have been doing our best benefits in all appointed missions for 54 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.

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 19th Khon Kaen Veterinary Annual International Conference open and wish this meeting to meet all objectives. Thank you.

**Associate Professor Dr. Chuchat Kamollert**

Dean of Faculty of Veterinary Medicine, Khon Kaen University

## Welcome speech from Chairperson



Dear KVAC delegates, KKU alumni and sponsors;

Welcome to Khon Kaen city and the 19th Khon Kaen Veterinary Annual International Conference (KVAC). I am very proud to help organize this conference. It is a meeting place where researchers, veterinary practitioners, animal producers, and business companies are all together. On this occasion, KVAC will build up research network and provide continuing education for veterinary practices and animal productions, covering various species. Along the meeting, it is also our tradition to welcome home all Vet KKU alumni.

Current animal situations in Thailand are Rabies outbreaks in many provinces, increased antimicrobial resistance in livestock, over demand of pig production, and circulation of avian influenza in ASEAN countries. In this meeting, we hope to strengthen our local practice and knowledge for the good sake of global animal health.

We hope all of you enjoy this special meeting and I wish to see you all in the next year symposium.

**Asst. Prof. Dr. Kochakorn Direksin**

Chairperson, the 19th KVAC

4-5th April, 2018

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## Keynote Topics

Kenote Topics	Invited Speakers	
<p><b>Antimicrobial Resistance in Livestock in Thailand</b></p>		<p><b>Dr. Julaporn Srinha (DVM)</b> Senior Professional Veterinarian Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives, Thailand</p>
<p><b>PODD: A Digital Tool for Community-based Surveillance</b></p>		<p><b>Assist. Prof. Dr. Kwankate Kani- stanon (DVM, MS, PhD)</b> Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand</p>
<p><b>Rabies Control and Prevention in Field Practice</b></p>		<p><b>Dr. Khanittha Thitidilokrat (DVD)</b> Petchaburi Provincial Livestock Officer Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives, Thailand</p>

## Lecture Note

# PODD: A Digital Tool for Community-Based Surveillance



Dr. KwanKate Kanistanon

Faculty of Veterinary Medicine, Khon Kaen University, Thailand

## Introduction

Participatory One Health Disease Detection project, PODD, started in 2014 in Chiang Mai. It was initiated by Assoc.Prof.Dr. Lertrak Srikitjakarn, member and former dean of Faculty of Veterinary Medicine, Chiang Mai University. The project is funded by SKOLL Global Threats Fund from the US. The main purpose is to promote self-monitoring of one health problems in the community using digital tools in order to prevent and control epidemics in humans and animals. Later in 2017, PODD has expanded to Khon Kaen, and the college of veterinary medicine, Khon Kaen University is the main collaborator.

## Initiation

The current outbreak monitoring system in rural Thailand depends mainly on cooperation of local volunteers, which is consisting of village health volunteers for human diseases and livestock volunteers for animal diseases. They have improved the efficacy of reporting suspected events by applying the most popular mobile application, LINE. It is free, easy to use, photo attachment is feasible, and you can even create a closed group for this. However, since LINE is not designed to be a surveillance tool, the lack of important features needed for epidemic surveillance leads epidemiologists, scientists in related fields and mobile application experts to develop a mobile app that is more effective. The result of this combined effort is PODD, an application for village volunteers to report any unusual events that occur in their village. The events can be any human or animal health problems and environmental hazards. The data submitted from village volunteers will be sent to central processor and distributed directly to community head administrators, for example, sub-district mayor, director of public health unit, and district livestock officers, as well as the PODD team.



## Khon Kaen Model

Khon Kaen is consisting of 225 sub-districts in 26 districts. The goal is to install PODD surveillance system in 110 sub-districts in all 26 districts of Khon Kaen within the year 2018. Among these 110 sub-districts, 30 will be entirely funded by PODD, and 80 will be partially funded by Provincial Administrative Organization of Khon Kaen. PODD started hiring 5 full-time staff since mid-2017 and recruited 5 faculty members for the project administrative team. The installation of the system can be explained in process order as follows;

1. PODD seeking cooperation from 110 sub-district mayors
2. Sub-district office recruits 20-40 volunteers who are locals in the area. Volunteers must be observative and willing use their own mobile phone to report via the application
3. One-day training for the volunteers by PODD staff on application use and characteristics of suspected events
4. Volunteers start to report via the application in case suspected event occurs, or report normal situation once a week if nothing happens
5. Sub-district staff adds mobile numbers of key persons at the sub-district level to the control panel
6. The system automatically alerts sub-district key persons via mobile SMS when reports of suspected events are submitted and confirmed by the system
7. The problems will be discussed and solved by decision of the sub-district mayor with the advice and supportive action from associated governmental offices when needed



# Mobile Application and Control Panel

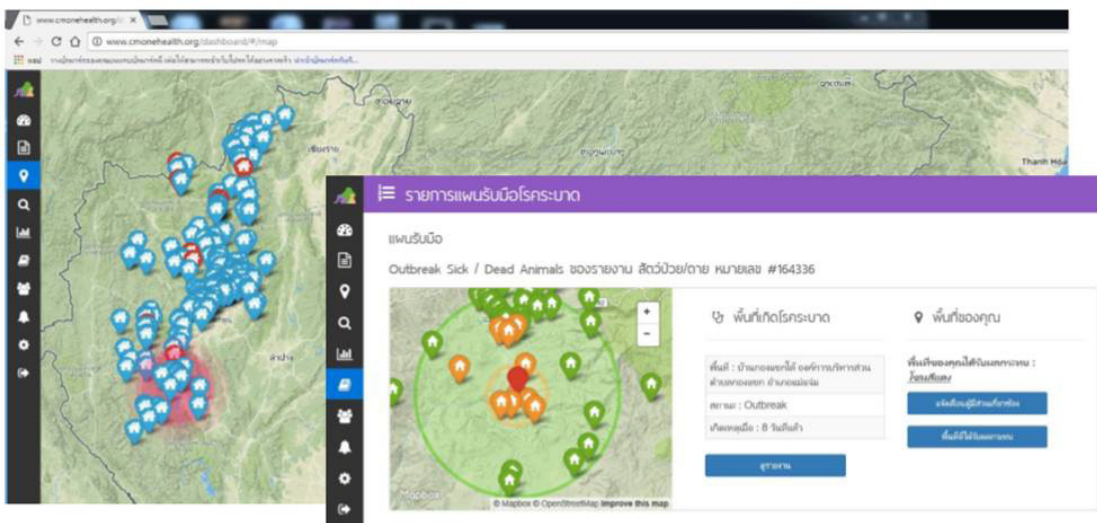


ภาพรวมการทำงานของระบบมือถือ



แผงตรวจติดตามระบบงานมือถือ (Dashboard)

[www.cmonehealth.org/dashboard](http://www.cmonehealth.org/dashboard)



## Expected outcome

Community at the sub-district level has an effective surveillance system to monitor their own one-health problems. The system is operated by the local volunteers and sub-district staff, with the technical support from PODD and associated governmental organizations.

## Collaborating organizations



Major contributor: SKOLL Global Threats Fund

### *Main Collaborator*

- Khon Kaen Provincial Administrative Office
- Khon Kaen University

### *Associated Governmental Organizations*

- Khon Kaen Provincial and District Livestock Development Office
- Khon Kaen Provincial and District Health Office
- Office of Disease Prevention and Control 7
- Khon Kaen Provincial Office of Disaster Prevention and Mitigation

### *Digital Technology Support*

- Opendream

## Opportunity and Obstacles

PODD has always received enthusiastic response from the sub-district offices and local volunteers. We expect to expand the system installation to all 225 sub-districts of Khon Kaen and perhaps other NE provinces when opportunity allows. There are suggestions from local administrators on application features that should be developed or added to enhance the performance, for example, rabies watchdog and air pollution application. Free and accessible wifi signal in the village for volunteers would have greatly facilitated the data submission.

Application and Control Panel Figures: Courtesy of Opendream (2017). Bangkok, Thailand.










## Reproductive Biotechnology in Ruminants

Topics		Invited Speakers
Update on Production and Cryopreservation of Bovine embryos		<b>Dr.Saksiri Sirisathien</b> Faculty of Veterinary Medicine, KKU
Embryo Transfer in Ruminants		<b>Dr. Anone Thuangsanthia</b> Veterinarian Officer, DLD
Bovine Seminal Plasma and Fertility		<b>Dr.Thanapol Nongbua</b> Faculty of Veterinary Sciences, MSU

## Poultry Group

Topics		Invited Speakers
<p><b>Current Avian Influenza Situation in Thailand and Neighboring Countries</b></p>		<p><b>Prof. Dr. Taweesak Songserm</b> Faculty of Veterinary Medicine, Kasetsart University</p>
<p><b>Infectious Bronchitis</b></p>		<p><b>Asst. Prof. Dr. Tawatchai Pohuang</b> Faculty of Veterinary Medicine, KKU</p>
<p><b>Standard for Antibiotic-Free Chicken Farms</b></p>		<p><b>Dr. Sompiss Jullabutradee</b> Veterinarian &amp; Livestock Production Auditor</p>

## Exotic Pets and Wildlife

Topics		Invited Speakers
<b>Ocular Examination in Exotic Pets and Wildlife</b>		<b>Dr. Anon Chumkamlue</b> Anon Animal Hospital
<b>Ocular Pharmacology</b>		<b>Dr. Anon Chumkamlue</b> Anon Animal Hospital
<b>Kick off the Tip of Ophthalmology in Exotic Pets and Wildlife</b>		<b>Dr. Anon Chumkamlue</b> Anon Animal Hospital

## Thai Swine Veterinarian Association & Research Group of Animal Health Technology

Topics		Invited Speakers
Round table Discussion; Standard for Beta-agonists-Free Farms		<b>Dr. Adun Phoemphon</b> Animal Feed and Veterinary Products Control, DLD  <b>Mr. Chaliao Sachamanee</b> SC Farm, Petchaburi Province, Pig Producer's Representative
Round table Discussion; Pig Markets in Vietnam, Cambodia, Myanmar, China, and the Philippines		<b>Asst. Prof. Dr. Khampee Korth-eerakul,</b> Veterinarian & Business owner  <b>Dr. Termsitthi Paphavasit</b> Key Account Manager & Technical Support,Boehringer Ingelheim Animal Health (Vietnam) Ltd.


## Topics by Zoetis (Thailand) Co., Ltd

Topics		Invited Speakers
<b>Update on Feline Infectious Diseases and Management</b>		<b>Asst. Prof. Dr. Tassanee Jar-ernsong</b> Faculty of Veterinary Medicine, KU
<b>TSOFP Vaccination Program</b>		<b>Asst. Prof. Dr. Tassanee Jar-ernsong</b> Faculty of Veterinary Medicine, KU

## Small Animals

Topics		Invited Speakers
<b>General consideration in Small animal Vaccine</b>	 A portrait of Prof. Dr. Sanipa Suradhat, a woman with short dark hair and glasses, wearing a dark jacket over a blue shirt. She is standing outdoors in front of a tree with pink blossoms.	<b>Prof.Dr. Sanipa Suradhat</b> Faculty of Veterinary Science, Chulalongkorn University

## Thai Dairy Productivity: A Future

Topics		Invited Speakers
<p><b>Policy and Practice to Improve milk quality : TS &amp; SCC</b></p>		<p><b>Assoc. Prof. Dr. Suneerat Ai-umlamai</b> Faculty of Veterinary Medicine, KKU</p>
<p><b>How to improve milk production in Thai dairy farms</b></p>		<p><b>Dr. Grit Podjana-a-ree</b> Veterinarian</p>
<p><b>How to improve milk quality in small holder dairy farms</b></p>		<p><b>Asst.Prof.Dr. Aran Chanrun</b> Faculty of Veterinary Medicine, KKU</p>

# Lecture Note from Thai Dairy Productivity: A Future

## Policy and practice improve milk quality: SCC&TS

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### Abstract

Dairy farming plays an important role in agricultural sector in Thailand at which Thai dairy products have been growing well in ASEAN markets. Dairy products have increased due to expansion of domestic markets and exportation to ASIAN countries. However, numerous difficulties are affecting dairy productivity particularly farm and feeding management. The effects of environment on milk production, milk quality and fertility have been studied. The current knowledge and experiences of dairy farmers and improvement in genetics and milk processing are acceptable considering the small and medium scale dairy farms are operated under tropical climate conditions. During the last three years, the governmental policy (Milk Board of Thailand; MB) of dairy farming, school milk program and dairy industry put down a great impact on productivity and milk quality. In 2015, MB announced that raw milk using for school milk program need to have somatic cell count (SCC) lower than 500,000 cells/ml and total solids (TS) has to be higher than 12.5 within 3 years as well as GMP (Good Manufacturing Practice) of all dairy cooperative need to be certified. In 2017, an average somatic cells in bulk tank milk was dramatically decreased to be at 464,000 cells/ml. However, %TS did not reach 12.5% because of improper feed and feeding to dairy cows. Several supports from government and also education on feeding management and dairy herd health and production management program have been intensively applied all over Thailand to improve %TS of milk. In 2018, %TS for school milk was allowed to be higher than 12.25% and need to improve to reach 12.5% in near future. Furthermore, end of 2017, MB requested dairy products using for school milk program need to have TS higher than 11.85%. Meanwhile, days open (calving to conception) was long (199.75days) and milk production was still low (4,310 kg/lactation). Thai milk quality is improving by strong policy but Thai dairy production and days open remain to be improved. Several studies showed that dairy herd health and production management program (DHH&PM) is a key success to improve dairy productivities. Therefore, DHH&PM need to apply to every dairy farm in Thailand in a near future.

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**Keywords:** Dairy productivity, Milk quality, Somatic cell count, Total solids



# How to improve milk Quality in smallholder dairy farms

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## Abstract

The quality of milk is the most important issue that potentially affects all of the stakeholders in the milk chain, particularly the dairy farmers, milk processor and milk consumers. Quality milk is commonly produced from the healthy milking cows with the nutritiously sufficient feeding, suitably environmental management and properly milking management. Udder health has the most potent effects on both milk production and milk quality. Somatic cell counts in milk is a good parameter that indicates whether the mammary tissue is presently inflamed and has a risk of bacterial infection. Rumen health is the vital indicator that influences the overall health of cows and especially milk components. Changes in milk compositions totally depend on feeding management. Daily dry matter intake, physical effective fiber and particle size of the forage need to be concerned in order to sustain the proper rumen ecology. Milking management is the important risk factors that contribute to both udder health and the bacterial contamination in raw milk. The environmental factor is the crucial risk factors mentioned above that are udder health, rumen health, milking management and especially the hygienic aspects of milk. In order to improve milk quality in the smallholder dairy farms, all stakeholder of the milk chain, particularly the dairy farmers, need to better understand and changes their attitudes for the sustainability of the dairy farming.

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**Keywords:** milk, quality, composition, cow, dairy farms

## Importance of milk quality

Currently, milk has played an important contribution to human diet in many countries worldwide. Over many years considerable attention has been paid to improve the milk quality, especially its composition, hygiene, and contaminants. Quality milk can be defined as (1) complete composition; (2) free from off-flavors and odors; (3) free from contaminants (i.e. detectable antibiotic or antiseptic residues, added water, or other sediments); (4) having low total bacteria counts; and (5) having low SCC. Milk composition is economically important to the milk producer, processors, and nutrients essential to milk consumer. Most consumers are more interested in a pleasant tasted of milk than other aspects of its composition or packing methods. Raw milk is a complete food which contains water, protein, fat, sugars (lactose), vitamins and minerals. These components influence the milk composition. Milk composition and component yields can be affected by several factors including breed, genetic variation within the breed, health, level of milk production, stage of lactation, and age of cow, season, environment, management practices, and feeding (Nasrollahi et al., 2015, 2016; Palmonari et al., 2010; Rico et al.; Zebeli et al., 2006).

## Biosynthesis of milk components

Cow's milk is produced in the mammary gland, i.e. alveoli, of the udder. The udder is primarily composed of the millions of milk-producing alveoli. Each alveolus is fed by an artery carrying all the raw elements needed. The alveolar epithelium is the site of accumulation and transformation of these materials into milk components. The majority of cow's milk (60%) is usually stored in the alveoli and the rest is kept in the milk ducts and the gland cistern at the equal proportions (20%)(Nickerson, 1995). Therefore most of the milk cannot be collected without the collaboration of the cow. As the milk production increased, the cow's udder becomes increasing stress. The inflammation of the udder can permanently damage milk-secreting tissues and the milk production can be persistently diminished. The quantity and quality of milk vary from one quarter to another because the udder's four quarters are independent of one another. Completely understand the biosynthesis of milk components is essential in order to improve both its quality of and quantity on the dairy farm.

Milk fat is synthesized in the mammary gland, which concentrates the free fatty acids from blood circulation. Most milk fat is neutral triglyceride containing fatty acids of short-(C4-C10), intermediate-(C12-C16) or long-chain (C18) length. The short-chain fatty acids (SCFA) are synthesized within the mammary gland using acetate and beta-hydroxybutyrate as the precursors. The long-chain fatty acids are derived from plasma fatty acids of the dietary origin whereas intermediate-chain fatty acids are taken up from both sources. The triacylglycerol component is synthesized in the endoplasmic reticulum via the glucose metabolism of the cells. Milk fat is the most variable milk component within a milking and reflects the dynamic nature of milk fat secretion from the mammary gland.

Caseins,  $\beta$ -lactoglobulin, and  $\alpha$ -lactalbumin are the major milk proteins synthesized in the mammary epithelial cells and only produced by the mammary gland. The synthesis of milk proteins requires both essential and nonessential amino acids. The immunoglobulins and serum albumin in milk are absorbed from the blood instead of the synthesis by the epithelial cells. A limited amount of immunoglobulin is originated from plasma cells, which reside in the mammary tissue providing local immunity in the mammary gland.

Lactose is formed in the alveolar cells become the predominant carbohydrate in milk. The principal biological function of lactose in milk is to regulate water content and osmotic pressure in the alveoli. The epithelium of alveolus is highly water permeable, water plus lactose move into the alveoli to maintain osmotic equilibrium with surrounding fluid. Water can also move back across the epithelial membrane in case of the cow is dehydrated. Lactose content is the most constant components of milk. Thus, the rate of lactose synthesis regulates water secretion and milk yield.

The mineral content of milk is derived from minerals found in circulating fluids. Minerals of milk contribute to various parts such as the buffer capacity, milk pH, the ionic strength, and osmotic pressure. Calcium and phosphorous are the major minerals found in milk although other minerals such as zinc, magnesium, iron, copper, manganese, and molybdenum, are also detected.

## Udder health

Mastitis is an inflammation of the mammary gland that affects changes in milk-secreting tissue, milk production, and milk compositions. Mostly, bovine mastitis is infected by the pathogenic microorganisms, i.e. bacteria, but aseptic inflammation, e.g. a trauma, can also contribute to the inflammation of the udder. Somatic cell count (SCC) in milk, mainly leukocytes, is a good parameter indicating the udder health status and its milk quality (Nyman et al., 2014). SCC is high immediately after calving but decreases (<200,000 cells/ml) in healthy cows together with the decrease of colostrum in milk within 4-5 days. Generally, a healthy udder has SCC less than 100,000 cells/ml. In healthy udder, the milk yield, the stage of lactation and age of the cow do not influence SCC in milk, but an interval between milking and sampling and the breed of the cow has a significant effect on the SCC.

Mostly, an increased SCC mostly indicates the inflammation of the udder tissues but it is absolutely not a sign of an udder infection. SCC only indicates that the udder has responded to the irritant. A cow suffering from subclinical mastitis has higher SCC after calving than that of a healthy one, and increase significantly towards the end of lactation. In subclinical *Staphylococcus aureus* infections, the SCC may vary considerably from day to day depending on the number of bacteria in milk. Therefore, a single milk sample gives the unreliable information about the causal bacteria and the dynamics of the cow's response to infection. The daily variations of SCC are higher in an infected quarter than in a healthy one. The increased SCC of an infected quarter may occur if the milking cow is under the stressed condition.

Udder infections cause a decrease in fat and casein but an increase in whey, water, the salt content of milk. These changes alter manufacturing properties, have the impact on the shelf life of dairy products and increase the risk of foodborne illness. Milk from cows with elevated SCC (greater than 500,000 /ml) has longer coagulation time and forms weaker curds than milk from cows with lower SCC.

SCC in the milk sample can be used to assess the health status at different levels such as quarter, cow, and herd. California Mastitis Test (CMT) is a quick, cheap and commonly used method to determine milk SCC, especially at the quarter level (Dufour et al., 2011; Lima et al., 2016). Importantly, the CMT result is scored as quality data, only an experienced person can reliably classify whether the quarter is healthy or unhealthy. Therefore, the CMT results can easily be wrong or unreliable misclassification if the tester has limited experience. Currently, CMT can be used to detect inflammation, to aid decision before submitting the milk sample for a bacteriological test, to monitor mastitis treatment results and to help for making a decision to reject cow's milk having high somatic cell count. Practically, the CMT is considered to be a very useful cow-side test. In addition, SCC in milk can be assessed with a particle or cell counter (e.g. Fossomatic® equipment or Delaval® Cell Counter) and its results are given as numeric SCC. However, SCC results from either CMT or the cell counter can be applied as a potential tool to monitor the udder health status of both the cow and the herd.

In Thailand, a bulk tank milk samples are collected monthly at the milk collection centers and then submitted for assessing at the Veterinary Research and Development centers at different parts of the country. To date, the bulk milk sample result has been certainly used for the milk-pricing system. As the result contain the useful information indicating of milk quality and the mastitis status of the herd. Therefore, the reports from the bulk milk sample should be critically analyzed and used in both mastitis control and milk quality improvement in smallholder farms. To accomplish the target, all the stakeholder, especially the farmers, must understand and reduce all the risk factors associated with mastitis. Although the risk factors are not a direct cause of mastitis they promote its development. The reduction of the SCC on the farms can give the potential benefits, including the improvement of milk quality and receive quality bonuses, improvement of the health and productivity at both the cow and farm levels.

## Rumen health

Rumen pH is a function of acid production and removal that is determined by the balance between the production of fermentation acid and buffer secretion in the rumen. Ruminant pH varies considerably during the course of a day and is mainly influenced by the amount of fermentable carbohydrate in each meal (Geishauser et al., 2012; Zebeli et al., 2006). Shifts of 0.5 to 1.0 pH units within a 24-hour period are common (Palmonari et al., 2010). Ruminant pH drops below physiological levels when ruminants consume excessive amounts of rapidly fermentable carbohydrates (Zebeli et al., 2006). Lactic acid and volatile fatty acids (VFAs- acetate, propionate, and butyrate) are ruminally produced by the rumen microbes. As ruminant pH drops below such a level, VFA absorption can be disrupted by an increase of lactate production. Most of the butyric acid is converted in the rumen wall into hydroxy-butyric acid whereas all of the propionic acids are transformed into glucose in the liver. VFAs are the main energy source of the cows, only acetic acid reaches the peripheral circulation.

Subacute ruminal acidosis (SARA) occurs when ruminal pH drops below about 5.5 for long-lasting 5 to 6 hour (Rustomo et al., 2006). SARA can potentially affect dry matter intake (DMI), fiber digestibility, and microbial population and milk production. Thus, the clinical signs of SARA vary such as intermittent anorexia or diarrhea, poor body condition, liver abscesses, impaired rumen motility, laminitis, and decreased milk

production and milk composition. The low rumen pH leads to increase the osmolality of the ruminal contents inducing an inflammation of the ruminal epithelium. The cows in early lactation should be particularly susceptible to SARA if they are fed with the poorly prepared diet. Therefore, ruminal acidosis is critically concerned in aspects of both economics and animal welfare.

Nutrition or dietary is a key factor influencing milk production and milk composition in dairy cows. The milk components vary greatly depending on the manipulation of cow's diet (Zebeli et al., 2006). Milk fat is the most sensitive to dietary changes and can vary over a range of nearly 3.0 percentage units whereas milk protein concentration can vary approximately 0.60 percentage units (Nickerson, 1995). The concentrations of lactose, minerals, and the other solids constituents of milk do not respond to diet manipulation. Roughage in the diets is critically essential to maximize production and to maintain health by sustaining a stable environment in the rumen. Physically effective fiber is the important fraction of feed that stimulates chewing activity and saliva secretion and rumination. Neutral detergent fiber (NDF) and particle size (PS) of the forage are increasingly attended as an indicator to assess the adequacy of the fiber in the cow's diet (Mertens, 1997; Nasrollahi et al., 2015; Yang and Beauchemin, 2009). Feeding diets with low level of physically effective NDF and of short particle size will decrease chewing activity and salivary buffer secretion (Mertens, 1997). Endogenous buffers, i.e. bicarbonate and phosphate, in saliva can neutralize organic acids produced by fermentation of rapidly degradable (non-fiber) carbohydrates in the rumen. However, saliva production is only triggered by the amount of physical fiber present in the diet but is not determined by declining ruminal pH.

Daily intake of rapidly fermentable carbohydrate and physically effective fiber should be considered carefully when diets for dairy cattle are evaluated and formulated. In addition, the feed particle size has significant effects on feed intake behavior, chewing, rumination, rates of the digesta passage, ruminal fermentation, and ruminal acid-base balance (Mertens, 1997; Rustomo et al., 2006; Yang and Beauchemin, 2009; Zebeli et al., 2006).

Generally, DMI of the high-producing cows should be 3.5 to 4.0 % of their body weight. To maximize potential DMI and milk yield, the cows should be fed ad libitum with a 5% daily feed refusal. The minimum roughage to concentrate ratio needed to maintain milk composition content, especially milk fat, is about 40:60 on a dry matter basis. The average forage length should be at least 0.65 cm. At least, the fiber requirements for dairy cattle in terms of acid detergent fiber (ADF) and NDF should be carefully defined. Feeding excessive grain (> 65% of total DM) should be avoided. The diet should be comprised of 17 to 19% ADF and 25 to 35% NDF in order to reduce rumen disorders. However, the diet with high NDF concentrations limits ruminal DMI and, the diet with low NDF concentrations, energy intake feedback inhibitors limit DMI (NRC, 2001).

It is critical to maximize feed intake of the cow in early lactation in order to minimize negative energy balance. Cows should intake more energy than used to regain body weight, to minimize losses in body condition and to produce milk of normal compositions. Feeding long forage particle size increases the content of physically effective NDF in the diet with positive effects on rumen function (Zebeli et al., 2012; Zebeli et al., 2006), i.e. rumination and rumen buffering, results in decreasing the risk of SARA. However, increasing particle size causes lower passage rate of the rumen content and may decrease net fiber degradation in the rumen, due to a lower availability of surface area for microbial attack resulting in decreasing feed intake and nutrient uptake. Endogenous buffering can be estimated by observing the number of cows ruminating. Commonly, it is acceptable if at least 40% of cows is ruminating at any given time.

Ascertaining the ration actually consumed by the cows requires a careful investigation of how feed is delivered to the cows, accurate weights of the feed delivered, and updated nutrient analyses of the feeds delivered, particularly the dry matter content of the fermented feed ingredients.

The formulation of feed rations and feed management is essential to solve rumen acidosis because the need to allow for proper adaptation of the ruminal mucosa and the ruminal microflora in the transitional period of calving. Physically effective fiber may be a potential tool to stimulate chewing, saliva production and rumen buffering and regulate rumen pH (Mertens, 1997; Yang and Beauchemin, 2009).

Sodium bicarbonate ( $\text{NaHCO}_3$ ) are regularly added to feed rations. It has been shown to be beneficial in the prevention of acidosis in dairy cows where the fiber content in the feed rations is too low. The addition of 150 g of  $\text{NaHCO}_3$  to the lactation feed per day may give a positive effect on the milk yield, feed intake and

milk fat percentage.

## Milking management

In healthy udder, raw milk is relative free from bacteria, however, contamination of milk by microorganisms can occur during milk handling, storage and other pre-processing activities. Commonly, contamination with bacteria from environment and milking equipment is predictable. The dairy farmers are responsible to avoid and to control the source of contamination such as milking equipment, milking practice, mastitis cow, on-farm storage and collection of milk.

In Thailand, machine milking tends to be common on smallholder dairy farms. Milking equipment has potential effects on both cow's udder health and milk quality because milk is sucked out of the teats by vacuum. All types of milking machines have the same basic components and functions. The basic components of milking machine are a system for vacuum production and control, pulsation components, and milk clusters. However, the installation, maintenance and operation as the manufacture's instruction are the greatly important issues in order to achieve a healthy udder and good milk quality at farm level (Dufour et al., 2011; Ouweltjes et al., 2007). The vacuum pump of the milking system should be able to create a vacuum of 42 to 50 kPa (13 to 15 in. Hg). In addition, the vacuum pump should be able to take out a certain amount of air. An adequate air flow is needed to operate the milking unit, to compensate air leaks, and to serve as a reserve in case of accidental air admission. The pulsation rate should be set at approximately of 60 cycles/min with the pulsation ratio of 64:40. The milking claw should has a volume of 250 to 350 mL. The milking liner has a significant impact on milking efficiency, hygiene and cow comfort - more than most other milking machine components. The optimum liner should have a collapse force of 37 to 40 kPa.

Milking practices is critically influence to milk quality, especially, in aspect of bacterial contamination. Good quality milk have no coliform in milk and the reduction time if methylene blue reduction test (MBRT) would be longer than 5 hours. The contamination can occur before, during and after machine milking. Pre-milking udder preparation involves sanitation of the teat, forestripping, drying, and timing of milking-unit attachment. Such efforts aim at improving overall milk quality, proper milk letdown, mammary health, and milking-time efficiency.

Cleaning udder and teats the udder wash solution containing effective disinfectant is the most common practice in order to keep milk hygiene. However, a premilking teat dip is also recommended in order to improve the disinfectant power for reduction of bacterial the rate of contamination by environmental bacteria. Chlorine solution at the concentration of 50-200 ppm have been recommended for application of such purposes. Importantly, teats needed are clean before applying a teat dip and the contact time of premilking teat dip should be at least 20 to 30 seconds, there is no use in using a teat dip on dirty teats. To date, the chlorine powder of calcium hypochlorite (65%  $\text{Ca}(\text{ClO})_2$ ) is commercially available and easy to access. In addition, chlorine tablet containing sodium dichloroisocyanurate or sodium troclosene is a chemical compound widely used as a cleansing agent and disinfectant in the dairy farm.

Duration of the time between first tactile stimulation and attachment of milking unit, has potential effect on the amount of milk harvested from the udder. A study has revealed that the greatest amount of milk is accomplish if the milking unit is attached on the cow's teat in the first 2 min of milking.

A bimodal milk curve, which indicating attachment of milking unit before milk letdown, should be avoided. The mean average of milk flow rate for the dairy cows is approximately 4 kg/min.

To keep the healthy udder overmilking should be avoid. Overmilking is the most important cause of an inflammation of udder tissues that can occur at both the beginning and the end of machine milking. It leads to cause of congestion and edema of the teat tissue. A long term of overmilking cause changes in teat-end tissue to a callous ring around the teat orifice that leads to easier penetration of microorganisms. The rougher the callous ring the easier it is for bacterial to reside and reproduce as the predisposing cause of mastitis. Moreover, overmilking has the potential effects not only on the udder health but also the bacterial contamination.

Thus, the appearance of an callous ring around the teat orifice indicate that use of improper use of milking machine (prolonged milking), impaired function of milking machine (too high a vacuum level, faulty pulsation) or its components (inadequate liner). In addition, the type and concentration of udder wash and teat dip

can also contribute to teat end damage.

## **Environmental management**

The cow's environment is the most potential risk factors associated with the general health of the cows, udder health, and milk quality. Variation of milk components (e.g. fat and protein) is related to changes in both the types of available feed, climatic conditions and cow's health. Hot weather and high humidity decrease dry matter intake and increase feed sorting, resulting in lower forage and fiber intake. The shortage of roughage (both quantity and quality) in most of the small dairy farms leads to lower level of milk production and its component.

The structure and environment of the barn have a tremendously direct influence on nutrition, cow's defense mechanism, and the cow's welfare, especially, in rainy or wet season. Careful attention must be paid to the feeding and watering areas as well as the alleys leading to the milking parlor. In Thailand, most of cow's barn in the smallholder dairy farms do not have the roof's gutter for water draining during raining. Therefore, rainfall is usually kept inside the barn resulting in wetting the floor, no dry and comfortable area for the cows to lie down. In the rainy season, heat and humidity encourage the proliferation of the number of environmental microorganism living in the cow's environment.

Manure is well-known as the common source of bacterial contamination of milk and source of the environmental microorganisms. Manure handling in the most smallholder dairy farms is commonly poor. With the more manure in the living area of the cows, there will be more manure on the teats when the cow lies down and the higher risk of new intramammary infection.

As several factors, such as the climate, nutrition, and housing, have an influence on a cow's immunity. Thus, good cow's environment, i.e. dry and comfortable, can improve not only the cow's comfort but also encourage the cows to lie down for the longer period. Furthermore, as an extra benefit, clean udders at milking time improve milker attitude and encouragement to do the better job and get the higher quality of milk.

## **Conclusion**


Improvement of milk quality is critical issue shared by all milk producers, processors, and consumers in order to increase the productivity, profitability, competitiveness and especially sustainability of the smallholder dairy farms. Several factors can potentially influence the quality of milk including cow's health, environmental management, and milking procedures. Cow should be monitored regularly to ensure good health. Only healthy cows produce optimal amounts of milk in the most economical way. Feeding management is the most important factors contribute to the cow's health and the level of milk components, particularly fat and protein. Environmental management and milking procedures play mainly an important role in bacterial contamination of milk and probable risk of inflammation of the mammary tissues. Therefore, extensive milk quality checks and testing unique to the milk industry ensure that milk consumers can always consume high quality, safe and nutritious products. Attitude and perspectives of all stakeholders in the milk chain may become the most important factors contributing to the improvement of milk quality.

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## Dairy Health and Production in Thailand




Topics		Invited Speakers
<b>Consequences of feeding on Hoof health in Dairy cows</b>		<b>Dr. Suvaluk Seesupa</b> Faculty of Veterinary Medicine, KKU
<b>Bovine Pestivirus in Thailand; what we've learned from 15 years' study</b>	 A portrait of a woman with dark hair, wearing a dark blue top, smiling slightly. She is positioned in front of a textured, light-colored wall.	<b>Asst. Prof. Dr. Jaruwat Kampa</b> Faculty of Veterinary Medicine, KKU



## Veterinary Business

Topics		Invited Speakers
<b>Marketing 4.0 for Animal Hospitals and Clinics</b>		<b>Dr. Pinyo Rattanapahan</b> Faculty of MBA, KKU
<b>Tax Management for Veterinary Business</b>		<b>Miss Rattana Arnupassanan</b> Independent Director, Chairperson of the GH Bank's Audit Committees

## Small Animals

Topics		Invited Speakers
Prudent use of Antimicrobial drugs in Small animals		<b>Dr. Nipattra Suanpirin</b> Department of Pharmacology, Faculty of Veterinary Science, Chulalongkorn University
Antimicrobial Choices for Treatment of Urinary Tract Infection in Small animals		<b>Assistant Prof. Dr. Supranee Jitpean</b> Department of Veterinary Sur- gery, KCU
Pain Assessment in Small Animal Practice		<b>Dr. Thanikul Srithunyarat</b> Department of Veterinary Sur- gery, KCU

<p><b>SIRS, Sepsis and Septic Shock Criteria and Management</b></p>	 A portrait of Assistant Prof. Dr. Siram Suwanwipat, a man with short dark hair, wearing a white dress shirt and a red tie, against a blue background.	<p><b>Assistant Prof. Dr. Siram Suwanwipat</b> Department Of Veterinary Medicine, Chulalongkorn University</p>
<p><b>Laser Therapy in Small Animal Practice</b></p>	 A photograph of Dr. Somphong Hoisang, a man in a light blue lab coat, smiling and holding a ginger and white cat in a clinical setting.	<p><b>Dr. Somphong Hoisang</b> Animal Teaching Hospital, KCU</p>

## Lecture Note from Small Animals

# Laser Therapy in Small Animal Practice

**Somphong Hoisang (DVM)**

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## Introduction

*LASER* is an acronym for “light amplification of stimulated emission of radiation” (Riegel and Godbold, 2017) that named by A.L. Schawlow in 1958 (Godbold, 2008). The main properties of laser are monochromatic, coherence, collimation and can be intensity (Sattayut, 2003).

Lasers devices are classified based on power level and their relative risk for causing biological damage to eyes or skin devices (Riegel and Godbold, 2017). Class 1 is safe under all conditions of normal use and the mean output power less than 0.4 mW, including laser pointer, CD player. Class 1M is safe for all conditions of use except when passed through magnifying optical instrument. Examples are some medical devices. Class 2/2M is safe because the blink reflex will limit the exposure to no more than 0.25 second and this laser classes are limited the power 1 mW. Many laser pointers and bar code scanners are class 2. Class 3R laser is considered safe if handled carefully, with restricted beam viewing less than 0.25 second. The mean output power can be up to 5 mW, including laser pointer. Class 3B can be have a mean output power up to 500 mW. There is a visual hazard when there is direct or reflected viewing of the light beam. Laser protection eyewear or goggles must be worn when operating these devices. Class 3B emit power output between 5 and 500 mW are Class 3B. Class 4 is the highest and most dangerous class of laser. Class 4 laser has a power greater than 500 mW and can cause fire or burn the skin or cause devastating and permanent eye damage as a result of direct, diffuse, or indirect beam viewing. Any therapeutic laser that has an average output power greater than 500 mW is within class 4. A goggles must be worn when operating these devices. Most laser therapy or therapeutic lasers available for use in the veterinary practice are classified as 1M, 3B and 4 which intended to distinguish them from surgical lasers.

Historically, laser therapy has been described in the literature by any of the following terms: low-intensity laser therapy (LILT), low-level laser therapy (LLLT), low-power laser therapy, phototherapy, Class 3 laser therapy, Class 4 laser therapy, and high-power laser therapy (HPLT) (Riegel and Godbold, 2017). In recent years, therapeutic laser has been referred to photobiomodulation (PBM) (Anders et al., 2015, Hamblin, 2016) that describes for a form of light therapy that uses non-ionizing forms of light sources, including lasers, LEDs, and broadband light, invisible and infrared spectrum.

The mechanisms of laser are obtained through the mitochondria (Karu, 1989, Zecha et al., 2016), particularly cytochrome C oxidase which absorbs the photons, resulting in the transfer of electrons within the electron transport chain and releasing nitric oxide (NO), reactive oxygen species (ROS), energy in the form of adenosine triphosphate (ATP), cyclic AMP and others. The resulting of energy is then used power metabolic processes, including synthesize DNA, RNA, proteins, collagens, enzymes and other products (Karu, 1989, Karu et al., 1995, Karu, 1999, Enwemeka, 2004, Karu et al., 2005, Ayuk et al., 2016). This process results in beneficial therapeutic outcomes including pain relieve (Bjordal et al., 2006) or inflammation, immunomodulation, and promotion of wound healing and tissue regeneration (Chung et al., 2012, Anders et al., 2015) but does not significant increase in tissue temperature (Ebrahimi et al., 2012, Feitosa et al. 2015, Mizutani et al., 2016) and has no remarkable side effects (Marinho et al., 2013).

## Benefits of therapeutic laser in small animal practice

Most responses of cells and tissue to therapeutic laser have been proved in cell cultures, experiment animals and conducted in human. However, extrapolation to veterinary medicine is reasonable. The therapeutic laser is uses for three main purposes (Chung et al., 2012, Farivar et al., 2014);

- to promote wound healing, tissue repair and the prevention of tissue death
- to relieve inflammation and edema
- to induce analgesia and treatment for other neurological problems

## Therapeutic dosage and applying to animals:

The World Association of Laser Therapy (WALT) has been suggested a dosage of 1-4 joule and an intensity of 12-60 mW/cm<sup>2</sup> per point for infrared GaAs 904 nm pulse laser and 6-24 joule per session and intensity of 30-210 mW/cm<sup>2</sup> for GaAAs 820-830 nm laser (WALT, 2010). Although, in small animal practice have been used therapeutic laser follow by manufacturers recommend. In fact, the veterinary patients have different hair thickness, skin pigment, body type and skin thickness, and each will have an individual response to therapy (Riegel and Godbold, 2017). Unfortunately, the optimal wavelengths, intensities, and dosages for laser therapy in dogs and cats have not been adequately determined. There are no standard dosage and protocol, the patients can be treated with doses reported as being successful in clinical research publications. Reported doses are safe starting points and can be adjusted as needed based on the patient response. Moreover, applying therapeutic laser in animals should be clipped their hair and cleaned the skin to maximize laser energy penetration (Ryan and Smith, 2007). Recently, *Laser therapy in veterinary medicine* book has been presented a dosage guideline for various conditions and applications (Table 1). These guidelines will vary depending on the species, condition, and body area being treated.

Laser therapy guideline	Dose recommendation
<b>Small animals</b>	
- Superficial tissue conditions	1-5 J/cm <sup>2</sup>
- Deep tissue conditions	8-10 J/cm <sup>2</sup>
- Chronic complex conditions	15-25 J/cm <sup>2</sup>
<b>Equine, large animals and large zoo species</b>	
- Superficial tissue conditions	1-5 J/cm <sup>2</sup>
- Superficial musculoskeletal conditions	8-20 J/cm <sup>2</sup>
- Deep musculoskeletal conditions	15-35 J/cm <sup>2</sup>
<b>Exotic animals</b>	
- Small mammals	0.5-8 J/cm <sup>2</sup>
- Avian	0.5-5 J/cm <sup>2</sup>
- Reptiles	2-8 J/cm <sup>2</sup>

Table 1: Laser therapy dose recommendations for veterinary practitioners (Adapted from: Riegel and Godbold, 2017. Fundamental Information: The theory and science of laser therapy)

## Laser safety

Laser safety is everyone's concern, including doctors, staff, students, patients, and observers. The standards indicate procedures for maintaining a controlled access room (Smalley, 2011). Some of the important point are:

1. Regulation warning signs on the entryway and removed when use of laser is finished.
2. Appropriate goggles for laser in use (class 3B and class 4 laser), is placed with the signs at each entryway.
3. Note: class 1M is safe for viewing without optical aids, but potentially hazardous with magnification aids (including microscopes, loupes, binocular, etc.)
4. Windows are covered with blinds to prevention of laser beam.
5. Doors are kept closed.

Contraindications to direct laser therapy include tumor, thyroid gland, active hemorrhage, and autonomic nerve centers. Laser therapy should be avoided in patients in which immune stimulation is not desired, including those with lymphoma or on immunosuppressant medications. In immature patients, higher powered laser therapy devices may stimulate premature closure of epiphyses. Thus, caution is warranted over long bones in young animals (Robinson, 2016).

## Summary

Laser therapy can be delivered physical energy to produce therapeutic biological effects. There have been shown to have significant promise in stimulating wound healing, tissue repair, bone healing, relieve of pain, reduce inflammation and edema. There is various size of devices that are friendly used, non-invasive and low cost. In addition, in patients with negative treatment outcomes, laser has no harmful side effects. However, laser therapy has a large number of treatment parameters relating to variety of laser class, wavelength diversity, average power and energy, total power density and the number of treatment sessions. Information or knowledge of using laser therapy in small animals are limited and no standardized ideal dosage for small animal practice. The user can be adjusted as needed based on the patient response.

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# Oral Presentation



# Natural Coinfection by *Francisella* sp. and Tilapia Lake Virus in Farmed Red Tilapia (*Oreochromis* spp.) in Chiang Mai, Thailand

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## Abstract

**Objective** To report the natural coinfection by *Francisella* sp. and Tilapia Lake Virus (TiLV) in farmed red tilapia in Chiang Mai, Thailand.

**Materials and Methods** In November 2017, a tilapia farm in Ping River reported a massive mortalities in red tilapia juveniles that introduced into cage farm from hatcheries after one month. Infected fish showed haemorrhage at skin, necropsy found granulomas in spleen and kidney. Spleen, kidney, liver, gill, heart and brain were collected for bacteria isolation, molecular identification and histological examination. Polymerase chain reaction (PCR) and reverse transcription quantitative PCR (RT-qPCR) were performed to confirm *Francisella* sp. and TiLV, respectively.

**Results** Histological examination showed the focal necrosis in brain and syncytial hepatocytes in liver. PCR and RT-qPCR results showed the presence of *Francisella* sp. and TiLV.

**Conclusion** It can be concluded that the red tilapia were naturally concurrent infected by *Francisella* sp. and TiLV. It could be a significant threat to tilapia industry.

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**Keywords:** Natural coinfection, *Francisella* sp, Tilapia Lake Virus, Tilapia

## Introduction

Tilapia is a commercially important freshwater fish, which is also one of the most important farmed fish species in the world. The world production of tilapia was 6.4 million tons, and the estimated value was 9.8 billion dollar. The world tilapia production is increasing year by year. The main tilapia producers are China, Indonesia, Egypt, Brazil, Philippines, and Thailand [1]. Tilapia is the main farmed freshwater fish species in Thailand. The red tilapia was normally cultured in cages or pond. As the extensive cultivation and exposed to poor environment, these fish were much more susceptibility to multiple pathogens including bacteria, virus, fungi and parasites [2].

Francisellosis is a chronic granulomatous disease with high morbidity, which can result in high mortality [3]. The bacterial *Francisella* was considered as aerobic, facultative, intracellular, nonmotile, gram-negative coccobacilli [4]. *F. noatunensis* is the causative agent of piscine francisellosis, which consists of two subspecies [5]. *F. noatunensis* subsp. *orientalis* (*Fno*) is the causative pathogen of warm-water fish, such as tilapia (*Oreochromis niloticus*), while *F. noatunensis* subsp. *noatunensis* (*Fnn*) is the causative pathogen of cold-water fish, such as cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) [6-8]. During the winter season in recent years, Thailand was suffered to *Francisella* infection among Nile tilapia and red tilapia [4]. Tilapia lake virus (TiLV) is an orthomyxo-like virus. It is an emerging disease that cause massive mortality of cultured tilapia in Israel, Ecuador, Colombia, Egypt, Thailand and Malaysia [2, 9-13]. Recent years, the outbreaks of TiLV have caused cumulative mortality rate of 20-90%, which result in significant socio economic losses and impact on food security [2].

Outbreaks of fish disease caused by single pathogen had significant influence on tilapia industry. However, reports revealed that the disease outbreak in farmed tilapia occurred as multiple pathogen infections [14, 15]. Coinfection among bacteria, virus and parasite in farmed tilapia may aggravate the problem. Although the outbreak of *Francisella* sp. and TiLV has been reported in Thailand. However, the coinfection of *Francisella* sp. and TiLV has not been reported so far. The aim of this research is to report the outbreak of natural coinfection by *Francisella* sp. and TiLV in red tilapia in Chiang Mai, Thailand.

## Material and methods

### Sample collection:

A mass mortality among juveniles of red hybrid tilapia cultured in cage farm was occurred in Chiang Mai, Thailand in 2017. Over 200 dead fish were recorded daily and lasted for 2 weeks. Ten naturally moribund fish were collected from two farms located in Ping River of Chiang Mai province. Fish showed haemorrhages at the skin and operculum area and base of dorsal, caudal and anal fin. Kidney, liver, spleen and brain of each diseased fish was collected for bacteria isolation, molecular detection and histology analysis. Cysteine heart agar with 10% sheep blood and 100unit/mL polymyxinB (CHA) was used for isolating *Francisella* sp. Tissue homogenate was smeared on CHA and incubated at 28 °C for 5 days.

### DNA extraction and PCR detection of *Francisella*:

Genomic DNA of spleen was extracted using a kit (MACHEREY-NAGEL), following the manufacturer's instructions. The concentration of DNA was measured by spectrophotometer. Polymerase chain reaction (PCR) based on 16S rDNA gene was performed using primer F11 and F5 [16]. The PCR amplification was performed in 25µL reaction mixture containing 12.5 µL master mix (TOYOBO), 0.5µL forward primer, 0.5µL reversal primer, 6.5 µL water and 5µL sample. A thermocycler was used to perform PCR reactions including initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s each for 35 cycles, annealing at 55 °C for 60 s, extension at 72 °C for 1 min and a finalextension at 72 °C for 10 min. PCR products were analyzed by electrophoresis in 1.5% agarose gels and stained with RedSafe.

### RNA extraction and RT-qPCR detection of TiLV:

Total RNA of liver and brain was extracted using TRIzol™ reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized using the RNA reverse transcription kit (*Vivantis*). The RT reaction contained 65°C for 5 min, 42°C for 60 min and 85°C for 5 min. RT-qPCR was performed in a 20µL reaction mixture containing 10 µL of 2 x iTaq™ universal SYBR green supermix (Bio-Rad) 0.3µM of forward or reverse primer, 4µL of cDNA template. The cycling conditions consisted of denaturation at 95°C for 3 min followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. At the end of the qPCR cycle, melting curve analysis was performed at a temperature ranging from 65°C to 95°C with 0.5°C per 5s increments [17].

### Histological examination:

Kidney, liver, spleen and brain were preserved in neutral buffer formalin (10%) for 24h. Samples were embedded in paraffin, sectioned at 4 µm and stained with haematoxylin and eosin (H&E) and acid fast. The specimens were examined under a light microscope.

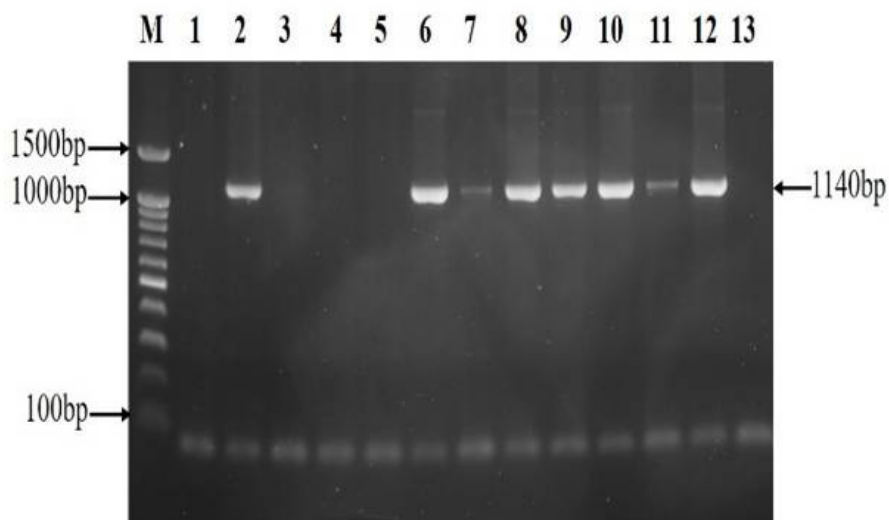
## Results

### Bacteria isolation and identification:

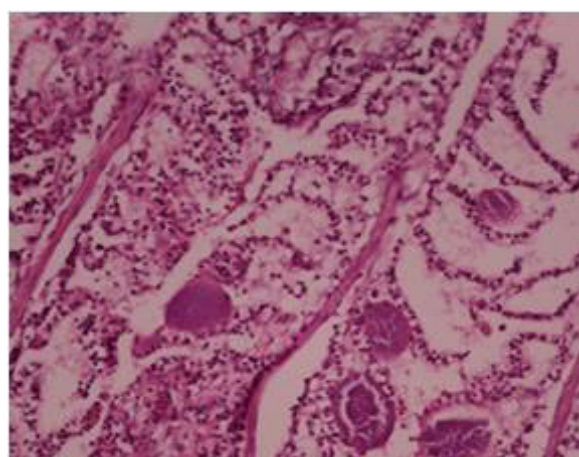
*F. noatunensis* was not successfully isolated, while PCR identification of tissue successfully amplified the band based on the 16S rDNA gene of *Francisella* genus (Fig 1).

### TiLV identification:

TiLV was successfully detected from liver and brain of the sampled redtilapia juveniles.



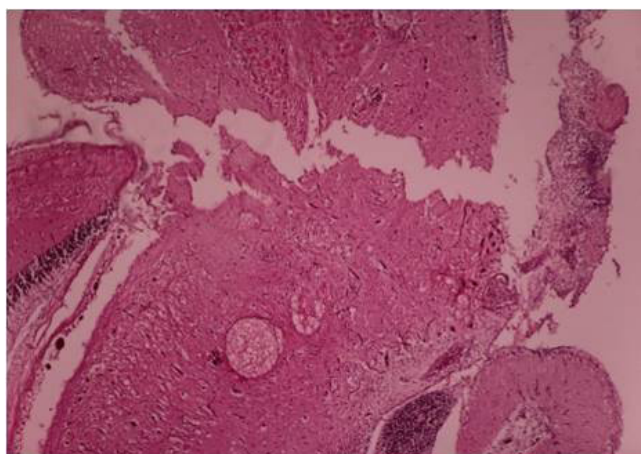
**Figure 1.** The PCR product of *Francisella* sp. showing 1140bp band. Lane M: DNA marker (1500bp DNA ladder), Lane1: negative control, Lane2: positive control. Lane3-13: sample



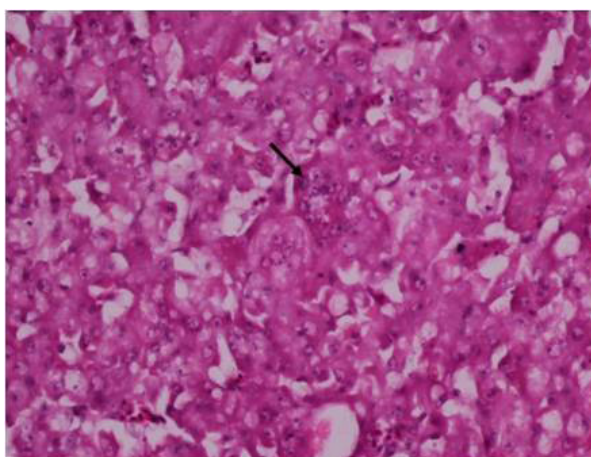
**Figure 2.** Histopathology of gill showing granulomas (HEX200)

**Histological results:**

Histological examination showed moderate multifocal hemorrhagic myocarditis in heart. Multifocal necrotic encephalomalacia and focal necrosis were found in brain (Fig 3). Syncytial hepatocytes, multifocal necrotic and degenerative hepatitis were observed in the liver (Fig4). The acid fast stain of organs with granulomas was negative.



**Figure 3.** Histopathology of brain showing multifocal necrotic encephalomalacia and focal necrosis (HEEx200)



**Figure 4.** Histopathology of liver showing Syncytial hepatocytes (HEX400)

## Discussion

Tilapia has been considered one of the most important fish in the world. However, the disease infection and transmission are the main problem that decrease the production of tilapia [15].

In cage culture system, fish are exposed to environment with different pathogens, it is susceptible to multiple pathogens. The coinfection by bacteria and virus in tilapia has been reported. The identified concurrent pathogens in tilapia included *Streptococcus agalactiae* and *Fno* [15], *Flavobacterium columnare*, *Aeromonas veronii*, *S. agalactiae*, *Plesiomonas shigelloides*, *Vibrio cholera* and *Iridovirus* [14]. In this study, the results showed the coinfection of TiLV and *Francisellasp.* in red tilapia in Chiang Mai, Thailand. The PCR confirmed the presence of TiLV and *Francisellasp.* The massive mortality rate, clinical signs, symptoms, histological examinations suggested the coinfection of TiLV and *Francisellasp.* A case report showed the co-infection of TiLV and *A. veronii* in red tilapia in Malaysian [13]. Other researches also showed that multiple bacteria including *Flavobacterium*, *Streptococcus* and *Aeromonas* were found in TiLV infected tilapia [12, 18]. However, *Fno* was not identified in these TiLV infected tilapia. Francisellosis is chronic granulomatous disease. Water temperature had a significant influence on *Fno*. The immersion challenges

of tilapia with *Fno* at water temperatures of 25 and 30°C were conducted. The results demonstrated that tilapia maintained at 25°C had higher mortality and bacterial concentrations than fish maintained at 30°C. Increasing the water temperature prevented the development of clinical signs [19]. TiLV is an emerging tilapia orthomyxo-like virus causing syncytial hepatitis [20]. Reports suggested that the incidence of TiLV is increasing year by year after first report in farm-raised and wild tilapia in Israel [2]. Similarly, previous study also showed that the outbreak of TiLV was occurred when water temperature rose to 25°C [21]. *In vitro* growth of virus at different temperature also showed that the optimal temperature for maximal growth of TiLV was 25°C [22]. During October to February, the water temperature of Chiang Mai was about 24°C. It may be a reason for the natural coinfection by *Francisella* sp. and TiLV in red tilapia. However, future *in vivo* study should be conducted to understand the relationship of both pathogens.

Most researches showed that the tilapia infected by bacteria or virus in horizontal transmission. There was also a good understanding of horizontal transmission of varieties pathogens. Therefore, the measures for controlling disease infections had been performed. Previous studies reported the presence of vertical transmission of pathogens including TiLV, *Fno* [23, 24]. In this study, the disease tilapia infected by *Francisella* sp. and TiLV was collected from Ping River. However, some farms in the same river were TiLV free as the different source of fry. The results demonstrated the development of tilapia stocks that resistant to pathogens should be promoted.

In conclusion, both *Francisella* sp. and TiLV are significant threat to tilapia production, while the coinfection of these two pathogen can aggravate the problem. Therefore, the disease control should be improved in the future.

## Acknowledgements

This work was financial supported by the National Research Council of Thailand and the community outreach project “Development of monitoring system in infectious diseases and sustainable net-Tilapia culture in Chiang Mai and Lamphun provinces”, Faculty of Veterinary Medicine, Chiang Mai University.

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# Distribution of *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*) after Oral Inoculation

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## Abstract

**Objective** This study was to determine the distribution of *Streptococcus agalactiae* in fish organs at various intervals after oral inoculation.

**Materials and Methods** Twenty-seven Nile tilapia (*Oreochromis niloticus*) were orally infected with 0.3 ml of bacterial suspension containing  $5.92 \times 10^8$  CFU/ml and maintaining the fish out of water for 5 min. After inoculation, three fish were randomly selected at 30 min, 1, 3, 6, 12, 18, 24, 36 and 48 h. Then the stomach, intestine, spleen, liver, kidney, heart, eye and brain were sampled for bacterial recovery and PCR to evaluate bacterial infection.

**Results** *S. agalactiae* was recovered from stomach, intestine, spleen, liver, kidney and eye at 30 min but the heart and brain at 1 h. All tissue samples were consistently recovered *S. agalactiae* by the end of the experiment, excepting intestine and eye at 48 h. In addition, *S. agalactiae* could be positively detected from all tissue samples by PCR from 30 min to 48 h. Although no clinical signs of the disease were consistently observed during the time period of this experiment, splenomegaly was found in a fish sampled at 1 h and remained intensely by the end of the experiment.

**Conclusion** From this study suggests that gastrointestinal tract was successfully produced *S. agalactiae* infection and differently disseminated to important organs in Nile tilapia. The present study would be advantage to further understanding about pathogenesis of *S. agalactiae* infection in Nile tilapia by oral inoculation.

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**Keywords:** Distribution, *Streptococcus agalactiae*, Nile tilapia, Oral inoculation

# Elongated Soft Palate in a Labrador Retriever Dog: Case Report

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## Abstract

**Case Description** A twelve-year-old intact male Labrador retriever weighting 26.0 kg was referred to Small Animals Teaching Hospital, Mahanakorn University of Technology for the cardiology clinic with the primary diagnosis of chronic ulcer enteritis and heart diseases from the private vet hospital. The clinical signs were periodic vomiting, exercise intolerance and inspiratory difficulty.

**Clinical Findings** The dog was alerted mental status and good response. The general physical examination founded increase in laryngeal sound, loud inspiratory dyspnea. Other vital signs were normal. Since the patient had been sent for ruled out heart disease, the thoracic radiography, echocardiography, and electrocardiography were initially performed. First, thoracic radiography revealed normal cardiac size and normal aging lung. The echocardiogram found mild mitral regurgitation with no other abnormalities. The electrocardiography was also normal. So the next plan was laryngoscopy due to increased laryngeal sound from general physical examination. The blood samples were collected before anesthesia for laryngoscopy. The results showed normocytic normochromic mild anemia with normal biochemical profile (BUN, creatinine, ALT, ALP). Intravenous induction of propofol (4 mg/kg) for light anesthesia was given to perform the laryngoscopy and the result were soft palate elongation and the partial paresis of larynx (unilateral paralysis).

**Treatment and Outcome** Staphylectomy was performed to remove excessive soft palate. The dog was placed in sternal recumbency. The head was restrained with the mouth open. Tracheal intubation was performed and prepared the oral cavity for surgery by pack the deep portion of the pharynx with swabs and grasped the caudal border of the soft palate with two stay sutured. Then use the electrocautery to remove the excessive soft palate by incised the thickness of soft palate approximately by half the width of the soft palate. Then, simple continued pattern was sutured with PDS 3/0. The dog was recovered with better inspiration pattern and no sign of dyspnea present after the surgical was performed. However, this operation just for correction the elongated soft palate alone. For unilateral laryngeal paralysis, in this case, the conservative management including anti-inflammatory drugs to decrease laryngeal swelling, a weight control and changes in the patient's routine and environment would be considered in older patients with minimal to moderate clinical signs.

**Clinical Relevance** Elongated soft palate is the thickened and abnormally that mostly involved in the pathogenesis of both nasopharyngeal and oropharyngeal narrowing, affecting the respiratory activity usually found 85-100% in brachycephalic dogs. Laryngeal paralysis is the effect of an inability to abduct the arytenoid cartilages during inspiration, resulting in respiratory signs consistent with partial airway obstruction. The etiology of the disease can be congenital (hereditary laryngeal paralysis or congenital polyneuropathy), or acquired (trauma, neoplasia, polyneuropathy, endocrinopathy)

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**Keywords:** Elongated soft palate, Laryngeal paralysis, Staphylectomy, Labrador retriever

# Bacterial Isolations and Antimicrobial Susceptibility Test in Dogs diagnosed with Urinary Tract Infections: A Retrospective Study During 2013-2017 at Veterinary Teaching Hospital, Khon Kaen University, Thailand

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## Abstract

**Objective** Urinary tract infections (UTIs) are required antimicrobial prescription in small animal practice. The proper antimicrobial drug use is crucial for successfully treating UTIs in dogs. Several studies have reported the use of antimicrobials in dogs with UTIs, however, the report in tropical countries are limited. The objectives of the present study were to identify bacterial pathogens and to investigate antimicrobial drug susceptibility patterns on dogs diagnosed with UTIs at Teaching Animal Hospital, Khon Kaen University, Thailand during 2013-2017

**Methods** Of 197 urine samples from dogs diagnosed with UTIs were collected and later immediately cultured on blood agar and MacConkey agar. Bacterial isolations and identifications were performed using commercially biochemical tests and antimicrobial susceptibilities were tested by standard disk diffusion method

**Results** Of total 137 culture-positive urine specimens, the most common bacterial isolations were 26.55% *Staphylococcus* spp., 20.90% *Proteus mirabilis* and 15.81% *Escherichia coli*, respectively. Additionally, 26.27% of culture positive urine samples had more than one bacterial species isolated. Amoxicillin-clavulanic acid 82.61% and tetracycline 73.91% were the most antimicrobial susceptibilities in dogs diagnosed with UTIs. Meanwhile, sulfa-trimethoprim and enrofloxacin were the most drug resistance accounting for 45.70% and 41.30%, respectively. Additionally, *E. Coli* found the most common drug resistance to sulfa-trimethoprim 88.90%, enrofloxacin 77.80% and doxycycline 66.70%, respectively.

**Conclusion** The most common bacterial isolation from suspected UTIs in dogs of this study was *Staphylococcus* spp., and *Proteus mirabilis*. Enrofloxacin has been widely used in dogs with UTIs in Thailand. Regarding to the present study, the use of enrofloxacin should be used depending on drug sensitivity testing. Importantly, emerging multi-drug resistance is of concern; therefore, continuous monitoring is important for sensible use of antimicrobial drugs

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**Keywords:** Bacteria urinary tract infection, Bacterial identification, Antimicrobial susceptibility, Canine

# Incidence and Classification of Bone Fracture in Dogs and Cats: A Retrospective Study at Veterinary Teaching Hospital, Khon Kaen University, Thailand (2013-2016)

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## Abstract

**Objective** To determine the incidence and classification of bone fracture that relating to risk factors in dogs and cats at Veterinary Teaching Hospital, Khon Kaen University, Thailand during 2013-2016.

**Material and Methods** A total of 1,780 dogs and 255 cats diagnosed as bone fracture cases were pulled out from all referral animals, 106,286 dogs and 22,258 cats during four years (2013 -2016) at Veterinary Teaching Hospital. The data collection were composed of the individual information of each animals such as: breeds, genders, ages, body weight including the detail of bone fracture, part of body, affected bone, cause of fracture, type of fracture and fracture management techniques. The data was analyzed and presented as percentage by descriptive statistics.

**Results** The incidence of bone fracture in dogs and cats were 1.67% and 1.15%, respectively. Regarding to breeds in both dogs and cats, mixed breeds were the most affected at 40.56% and 66.27% respectively. Male dogs (58.43%) were more affected than female (41.57%) whereas in cats were similar in male and female 49.61% and 50.39% respectively. Approximately, 55% and 65% of bone fracture were presented in dog and cats less than one-year-old. Medium size dogs weighting 10-25 kg were the most affected (35.22%). Road traffic accident was the highest cause of bone fracture in both dogs (79.43 %) and cats (56.47 %). Pelvic limb was the most affected part at 85.20%. The highest incidence of affected bone in dogs and cats was femur at 29.58% and 35.70% respectively. Intramedullary pin fixation technique was used mostly both in dogs (22.49 %) and in cats (28.44%).

**Conclusion** The principle cause of bone fracture in dogs and cats was the road traffic accident and mostly occurred at age less than one-year-old. Pelvic limb especially femur was the most affected bone in dogs and cats. Fracture management depended on affected part and types of fracture, in this study, intramedullary pin was mostly used in dogs and cats.

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**Keywords:** Bone fracture, Incidence, Classification, Canine, Feline

# Insulin Resistance and Metabolic Diseases in Dairy Cows under Thai Feeding Conditions

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## Abstract

**Objective** The objective was to investigate the relationship between insulin resistance and metabolic diseases in dairy cows under Thai feeding conditions.

**Materials and Methods** Thirty dairy cows on five smallholder farms were enrolled using a cohort study design. During the seven day before their expected calving dates to 30 days after calving, the incidence rates of metabolic diseases were calculated. On the 7 day before their expected calving date and 14 and 28 days after calving, blood samples were taken at before afternoon feeding. Plasma insulin, glucose, triglyceride, beta hydroxyl butyrate (BHBA) and non-esterified fatty acid (NEFA) were measured. Insulin resistance index was calculated as Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) =  $1 / [\log \text{glucose (mg/dL)} + \log \text{insulin } (\mu\text{U/mL)} + \log \text{NEFA (mmol/L)}]$ . The relationship between insulin resistance, blood metabolites and metabolic diseases in dairy cows was estimated using multiple regression analysis.

**Results** The highest incidence of metabolic disease was ketosis, which was observed 14 days after calving. The incidence of ketosis in early dairy cows was 16.7%. Cows with ketosis had a lower ( $P < 0.01$ ) insulin resistance index than healthy cows. Blood metabolites, concentration of plasma NEFA and BHBA were significantly higher in cows with ketosis than those of healthy cows ( $P < 0.05$ ). Moreover, cows with ketosis had a tendency to have a higher plasma insulin ( $P = 0.051$ ), while plasma glucose ( $P = 0.051$ ) and triglyceride values ( $P = 0.052$ ) tended to be lower compared with the healthy cows. The results demonstrated that the values of NEFA and insulin were found to be associated with the insulin resistance index ( $R^2 = 0.407$  and  $0.300$ , respectively).

**Conclusion** Under small holder feeding conditions, ketosis was a high incidence rate observed in early lactation cows. In the present study, insulin resistance index was negatively correlated with the occurrence of ketosis in early lactating dairy cows.

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**Keywords:** Insulin resistance, Metabolic diseases, Dairy cow

# Shortening of Days Open by Modified Cosynch Protocol in Early Postpartum Dairy Cows: A Preliminary Report

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## Abstract

**Objective** The objective was to compare effect of two hormonal treatment programs between eight day-Cosynch (Cos) and eight day-Cosynch+CIDR (CosCD) on days open of postpartum dairy cows.

**Materials and Methods** Forty two cross bred Holstein cows were selected into the study under criteria as follow, 40 -50 days postpartum, lactation number 2 to 4, body condition score between 2.5 -3.5 (scale of 1 - 5), and never been inseminated before. Cows were randomly divided into three groups. Cows in the control group (n = 14) were inseminated after detection of natural estrus by a.m.-p.m. rule. The Cosynch group (n = 14) was synchronized by injecting intramuscularly (i.m.) of 10 µg GnRH (buserelin, Receptal®; Merck animal health, USA), then on day eight 500µg of PGF<sub>2α</sub> (cloprostenal, Estrumate®; Merck animal health, USA) was injection (i.m.). The second GnRH (10 µg) injection was done between 60 and 66 h post PGF<sub>2α</sub> with fixed time insemination (TAI). The cows in Cosynch+CIDR (n =14) received the Cosynch protocol plus a CIDR (1.38 g of progesterone, CIDR®; Zoetis, USA) insert intravagina for 8 days, beginning at the first GnRH injection. The CIDR was removed at PGF<sub>2α</sub> administration time. The second GnRH injection was done with TAI, between 60 and 66 h post PGF<sub>2α</sub>. The reproductive organ of every cows were examined on day 0 by transrectal ultrasonography and were examined again on day 8 in the treatment groups (Cos and CosCD). Pregnancy status was determined by ultrasonography on day 30 after TAI and on day 60 by transrectal palpation. Non-pregnant cows with return-to-estrus before 30 days after first AI were re-inseminated (second AI) by a.m.-p.m. rule.

**Results** The days open of cows in treatment groups (Cos = 93±4.87 days, CosCD = 109±27.59 days) was lower than that in cows in control group (P= 0.050) analyzed by survival analysis. Overall pregnancy rate within 200 days in Cos (64.29%) and CosCD groups (78.57%) were significantly higher than that in control group (28.57%) (P< 0.05).

**Conclusion** This study suggested that the use of hormone in early postpartum dairy cows could decrease the days open and increase pregnancy rate. The use of modified Cosynch protocols in early postpartum dairy cows was resulted in shorter days open compare to control cows.

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**Keywords:** Days open, Dairy cow, Cosynch, CIDR, Fixed-time AI

# Prevalence and Antimicrobial Resistance of *Salmonella* spp. Isolated from Pigs in Thailand

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## Abstract

**Objective** The objective of this study was to determine prevalence and antimicrobial resistance patterns of *Salmonella* spp. isolated from pigs at slaughterhouses in Thailand.

**Materials and Methods** A total of 562 samples from rectal swab of pigs were collected from November 2015 to June 2016. The samplings were isolated and identified for *Salmonella* spp. according to the ISO 6579:2002. In addition, the antimicrobial resistance patterns were examined through agar disk diffusion technique using 9 antimicrobial drugs.

**Results** The finding revealed that 211 (37.54%) out of 526 samples were *Salmonella* positive with 19 different serovars. The high prevalent serovars were *Salmonella* Rissen (36.97%), *S. Typhimurium* (21.33%), *S. Weltevreden* (14.70%) and *S. Stanley* (6.64%), respectively. In this study, 76.30% of the isolates were resistant at least one antimicrobial drug, 38.39% were multidrug resistant and 9 different antimicrobial resistance patterns were found. The most antimicrobial resistances were found in Ampicillin (69.20%), Tetracycline (66.35%), Sulfamethoxazole/trimethoprim (35.55%) and Chloramphenicol (9.00%), respectively.

**Conclusion** The results showed the high prevalence of *Salmonella* spp. in pigs and high antimicrobial resistance among the isolates. Based on the findings, a monitoring program would be necessary to control *Salmonella* contamination, and use of antimicrobial drugs should be more concern in order to reduce the dissemination of antimicrobial resistance in pig supply chain.

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**Key words:** Antimicrobial resistance, *Salmonella* spp., Slaughterhouse

# Antimicrobial Resistance Patterns of *Salmonella* spp. Isolate from Pig at Slaughterhouses in Thailand and China

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## Abstract

**Objective** *Salmonella* spp. is the most important food-borne pathogenic bacteria causing gastroenteritis in humans worldwide, especially in Asia. The aim of this study was to determine serotypes and antimicrobial resistant patterns of *Salmonella* spp. isolated from pig slaughterhouses at some areas of Thailand and China.

**Materials and Methods** A total of 109 isolates were collected from pig slaughterhouses in KhonKaen, Thailand (n=70) during February to August 2017 and Chongqing, China (n=39) during March to October 2015. All samples were isolated and identified by ISO 6579:2002. The serotyping of isolates was determined using the commercially available antiserum by slide agglutination method following Kaufman-White scheme. The antimicrobial susceptibility test used disk diffusion method follow by the Clinical and Laboratory Standards Institute (CLSI) with 9 antimicrobials.

**Results** A total 17 serotypes were found from Thailand and China including 3 common serotypes: *S. Typhimurium*, *S. Give* and *S. Rissen*. In Thailand, 6 serotypes from 70 isolates were relatively concentrated in *S. Rissen* (51%) and *S. Weltevreden* (24%). In China, 14 serotypes from 39 isolates were found such as *S. Derby* (21%) and *S. London* (18%). The majority of Thailand and China isolates resistant to ampicillin was 69% and 70% whereas tetracycline was 79% and 50%, respectively. The common epidemic serotypes (*S. Rissen* and *S. Typhimurium*) are 100% resistant to tetracycline in China and also high resistant (50% and 86%) in Thailand.

**Conclusion** The same *Salmonella* serotypes were isolated both in Thailand and in China. All isolates have similar antimicrobial resistant patterns. This indicated that it may have some relatedness about trade, tourism, import and export during the *Salmonella* spreading between Thailand and China.

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**Keywords:** *Salmonella* spp., Antimicrobial Resistance, Thailand, China



# Comparisons of Body Weight Gain in Broiler Chickens Resided in Different Parts of Modern Broiler Houses

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## Abstract

**Objective** To compare body weight gain in broiler chickens resided in different parts of modern poultry houses.

**Materials and Methods** A total number of 6,000 broiler chickens (n = 2,000 in each breed (1,000 for male and 1,000 for female)) involving three broiler breeds (Cobb 500, Ross 308, and Arbor Acres) and 6 broiler houses were used in this study. Each breed was raised under identical conditions of the commercial housing and feeding system and in the same rearing period. For study eligibility, chickens must have their body weight within  $\pm 2.5\%$  of their standard body weight for each breed. Each broiler house (typically with a total length of 120 m) was divided equally into 10 parts called room 1 to room 10 starting from the front to the back (at a 12 m interval). Measurements were made on live body weight at 28 and 37 days of age. In addition, microenvironments including ambient temperature, relative humidity, air velocity, and ammonia level were measured from each part of the house. Average daily gain (ADG) was also calculated from 28 to 37 days of age. One way analysis of variance (ANOVA) with Tukey's HSD test was used to compare ADG of broiler chickens resided in different parts of the house at 28 and 37 days of age.

**Results** Unaware of breeds and sex, chickens resided in room 3 to 6 (in the middle of the house) tended to have greater ADG than those resided in other parts of the house. At 37 days of age, means  $\pm$  standard deviations (in grams) of the live body weight for Cob 500, Ross 308, and Arbor Acres were  $2,621 \pm 187$ ,  $2,564 \pm 196$ , and  $2,684 \pm 188$  in male chicken broilers, and  $2,131 \pm 177$ ,  $2,266 \pm 164$ , and  $2,203 \pm 167$  in the female, respectively.

**Conclusion** Results from this study indicated that chickens resided in the middle of the house tended to have greater weight gain than those resided in other parts of the house.

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**Keywords:** Broiler chicken, Cobb, Ross 308, Arbor Acres, Weight gain

# Survey of Antibiotic Residues in Chicken Sold at Fresh Markets in Khon Kaen Province

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## Abstract

**Objective** The aim of this study was to detect antimicrobial drug residues in chicken meats and livers sold at fresh markets in Khon Kaen province.

**Materials and Methods** During November to December 2017, we collected fifty samples of chicken breasts (n = 25) and chicken livers (n = 25) from 5 fresh markets in Amphoe Muang district, Khon Kaen province. Each one gram a minced sample was either mixed with 2 different reagents of Rojanarak-antibiotic test kits to indirectly detect for A) Tetracycline or B) Macrolide-aminoglycoside-sulfonamide groups. The extracted samples, antibiotic positive and negative controls were inoculated in a ready-to-use *Bacillus* agar micro-tube, one-to-one tube. Growth of *Bacillus* spp. was indicated by color change from purple to yellow after incubation at 64 °C for 2 hours.

**Results** There were 15 (30%) positive samples. Two chicken breasts and one liver were positive for Tetracycline. Twelve chicken livers and one breast were positive for Macrolide-aminoglycoside-sulfonamide. One liver was positive for both groups of antibiotics. Positive antibiotic was found in chicken samples sold at every market in this study.

**Conclusion** The result of this survey indicates relatively high rates of antibiotic residues in chicken. The chicken livers have higher rates of antibiotic positive than chicken breast (24% vs. 6%). From this study, further analysis for specific antibiotics by HPLC method is valuable.

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**Keywords:** Antibiotic, Residue, Chicken, Liver, Khon Kaen

# Detection of *Salmonella* spp. in Acidic Fermented Pork Sausage (Nham-mooh) in Khon Kaen Province

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## Abstract

**Objective** The study targeted to identify *Salmonella* spp. in fermented pork sausages (Nham-mooh) with acidic pH.

**Materials and Methods** Nham-mooh is a homemade product, prepared by a traditional fermentation method. We randomly collected Nham-mooh from small retail markets in Khon Kaen province between April-May 2017. In our previous study, we found vast subtypes of *Salmonella* spp. in freshly prepared-not yet fermented Nham-mooh. In this study, only Nham-mooh samples with pH below 4.6 were selected. Bacterial culture was performed at the Regional Medical Sciences Center 7<sup>th</sup>, Bureau of Laboratory Quality Standards, accreditation number 4023/49 ISO 17025.

**Results** From a total of 20 acidic Nham-mooh samples, 16 isolates of *Salmonella* spp. were detected. Serotyping results showed 9 different serovars of *Salmonella* spp. From the most to least order of prevalence were *Salmonella* Rissen (5 isolates), *Salmonella* Virchow (3 isolates), *Salmonella* Typhimurium (2 isolates), and one isolate of *Salmonella* Agona, *Salmonella* Brandenburg, *Salmonella* Derby, *Salmonella* Hvitittingfoss, *Salmonella* Idiken, and *Salmonella* Senftenberg.

**Conclusion** *Salmonella* spp. can grow well at pH ranges from 5 to 9. It is unexpected that *Salmonella* spp. could survive in Nham-mooh with pH below 5. The contamination could either from pork meat or the processing of Nham-mooh. According to the guide of Standard Microbiological Limits for Food, Ministry of Public Health, Thailand (2010), *Salmonella* spp. must not be present in 25 grams of a food sample. In this study, 80% of acidic Nham-mooh samples were not passed the food safety standard. The result of this study antagonizes our traditional belief on sour taste of Nham-mooh is safe for consumption.

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**Keywords:** Fermented pork sausage, Nham-mooh, *Salmonella*

# Hair Cortisol Levels in Dogs with Calm and Energetic Temperaments

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## Abstract

**Objective** The aim of study was to compare hair cortisol levels of dogs with calm or energetic temperaments. We hypothesized that dog temperaments may influence levels of cortisol hormone.

**Materials and Methods** Hair samples from a total of 4 dogs with calm and energetic characters (2 males and 2 females in each group) were collected. The dogs were mixed breed, average age of 8 years, adopted from stray dogs, and all living together in one house. The owner gives well-care and attention equally to all of them. The dogs were in a good health status by physical and blood examinations. The dog hair was longitudinally collected from the dorsal neck at Days 1, 3, 5, 7, 14, 28, and 35, and kept in the -20 C freezer before being used. The hair samples were washed with absolute ethanol twice, and then subjected to ethanol extraction. The extracted solutions were measured for cortisol levels by method of radioimmuno assay (CORT- CT2Cisbio Bioassays - Model 24) at the Srinagarind hospital.

**Results** Average hair cortisol of dog A (male/alert), dog B (female/alert), dog C (male/calm), dog D (male/calm) were 471, 461, 617, and 523 pg./ml, respectively.

**Conclusion** Hair cortisol is a non-invasive and promising method for revealing the activity of the hypothalamic pituitary axis over a longer period of time. Hair cortisol was not different between male and female dogs. Dogs with calm temperament tend to have higher levels of hair cortisol (617 & 523 vs. 471 & 461 pg./ml). Due to small sample size, however, we need a further study to draw a conclusive result.

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**Keywords:** hair cortisol, dog, temperament, calm, energetic

# Poster Presentation



# Antimicrobial Activity of *Azadirachta indica* var. *siamensis* Valeton Leaf Extract against Pathogenic Bacteria Isolated from Animals

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## Abstract

**Objective** To determine antimicrobial activity of *Azadirachta indica* var. *siamensis* Valeton leaf extract against pathogenic bacteria isolated from animals.

**Materials and Methods** *Azadirachta indica* var. *siamensis* Valeton leaf was collected and extracted by ethanol. Antibacterial activities of the crude extract were tested by using a broth microdilution method with 5 replications against 27 samples of bacteria isolated from animals. The isolated bacteria were 3 Gram-positive bacteria: *Staphylococcus* spp., *S. intermedius*, *Streptococcus agalactiae*, and 4 Gram-negative bacteria: *Klebsiella* spp., *Pseudomonas aeruginosa*, *Escherichia coli*, and *Aeromonas hydrophila*. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 were also tested as the standard controls. The minimal bactericidal concentrations (MBC) of all bacteria were determined.

**Results** The result showed that *Azadirachta indica* var. *siamensis* Valeton leaf extract had antibacterial activity against all bacteria isolated from animals. The minimal bactericidal concentration (MBC) values of *Azadirachta indica* var. *siamensis* Valeton leaf extract against *Streptococcus agalactiae*, *Staphylococcus aureus*, *Staphylococcus intermedius*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *E. coli*, and *Klebsiella* spp. were 7.81, 31.25-62.5, 500, 125, 500, 1000, 1000mg./ml, respectively.

**Conclusion** The ethanol extract of *Azadirachta indica* var. *siamensis* Valeton leaf had antibacterial activity against various types of pathogenic bacteria isolated from animals. In comparison, MBC values of the Gram positive bacterial groups were lower than those of the Gram negative bacterial groups.

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**Keywords:** *Azadirachta indica* var. *siamensis* Valeton leaf extract, Antimicrobial activity, Animals, Pathogenic bacteria

# Antibacterial Efficacy of *Clausena excavate* Burm. f. and *Clausena harmandiana* Pierre against *Staphylococcus aureus* Isolated from Bovine Mastitis

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## Abstract

**Objective** To evaluate antibacterial activity of *Clausena excavate* Burm. f. and *Clausena harmandiana* Pierre against *Staphylococcus aureus* isolated from bovine mastitis.

**Materials and Methods** *Clausena Excavata* Burm. f. leaf, stem, leaf stalk, root and *Clausena harmandiana* Pierre root were extracted by ethanol and some of *Clausena harmandiana* Pierre root were extracted by ethyl acetate. All crude extracts was tested *in vitro* for antibacterial activity against 10 isolates of *Staphylococcus aureus* isolated from bovine mastitis by microdilution broth method. *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and gentamycin were also tested as experimental controls. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) from 4 parts of *Clausena Excavata* Burm. f, extracted by ethanol, and 2 *Clausena harmandiana* Pierre root extracted by ethanol and ethyl acetate were determined.

**Results** *Clausena Excavata* Burm. f. leaf, stem, leaf stalk, root and *Clausena harmandiana* Pierre root both extracted by ethanol and ethyl acetate showed antibacterial activity against *Staphylococcus aureus* isolated from bovine mastitis; with median of MIC values of 1.3932, 0.2370, 0.5898, 0.0019, 7.8125, 15.6250 mg./ml and median of MBC values of 1.3932, 0.3555, 0.7865, 0.0025, 7.8125, 15.6250 mg./ml, respectively.

**Conclusion** *Clausena Excavata* Burm. f. root extract had significantly higher efficacy of inhibitory activity against *Staphylococcus aureus* isolated from bovine mastitis than *Clausena harmandiana* Pierre root extract. The root of *Clausena Excavata* Burm. f. extract showed significantly higher inhibitory effect than the leaf, the stem and the leaf stalk. In addition, the ethanol extract of *Clausena harmandiana* Pierre root showed significantly higher inhibitory effect than its ethyl acetate extract.

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**Keywords:** Bovine mastitis, *Clausena excavata*, *Clausena harmandiana*, *Staphylococcus aureus*, Antimicrobial efficacy, MIC, MBC



# Antifungal Effect of *Eleutherine americana* Merr. Extract and Its Combination with Clotrimazole against Clinical Isolates of *Malassezia pachydermatis*

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## Abstract

**Objective** To determine antifungal effect of *Eleutherine americana* Merr. extract and its combination with clotrimazole against clinical isolates of *Malassezia pachydermatis*.

**Materials and Methods** *Eleutherine Americana* Merr. was extracted from freshly collected plant using 70% ethanol. The organisms used in this study were 26 samples of *Malassezia pachydermatis* isolated from dogs in Khon Kaen province. The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of *E. Americana* and clotrimazole toward the tested organisms were evaluated by broth microdilution method. The synergistic effect between *E. americana* and clotrimazole were evaluated using checkerboard method.

**Results** The study revealed that the MICs/MFCs ranges of *E. americana* and clotrimazole were 50-100/50-100 mg/ml and 0.031-0.5/0.031-0.5 mg/ml, respectively. Synergistic effects of *E. Americana* and clotrimazole against 6 samples found to be indifferent interaction. The MIC values of the *E. americana* were reduced up to 1/2-1/32 MIC and the MIC of clotrimazole up to 1/2 MIC values.

**Conclusion** These results indicated that combination between *E. americana* extract and anti-fungal drugs could be useful in develop the preparation for canine dermatitis.

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**Keywords:** Herbal extract, Combination, Anti-fungal

# Synergistic Effect of Antioxidant Activity of Clove Essential Oil in Combination with Plai Essential Oil and Vitamin E

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## Abstract

**Objective** To study the synergistic effect of antioxidant activity of clove essential oil in combination with plai essential oil and vitamin E.

**Materials and Methods** The essential oils of clove and plai obtained from steam distillation, which purchased from Thai-China fragrant industry, Thailand. In vitro antioxidant activity for each substance and its combination was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. Antioxidant potential of plant combination was also comparable to that of standard vitamin E ( $\alpha$ -tocopherol). Statistical comparisons of the results were performed with one -way ANOVA using SPSS. A significant difference was set at p-value<0.05.

**Results** The concentration values ranging 0.125-0.5  $\mu$ l/ml were tested the combination antioxidant activity of plai essential oil and vitamin E with clove essential oil. The results showed that the combination of plai essential oil and vitamin E with clove essential oil can synergistically enhance antioxidant activity all fix ratios (1:1, 1:2, 2:1, 1:4, and 4:1) tested, which showed no significant differences at p<0.05. The lowest concentrations of both comparison gave enhance antioxidant activity which were 0.125:0.125  $\mu$ l/ml. The antioxidant activity of theoretical between plai and clove essential oil was 46.02%, while the experimental was 64.0%. In the same way, the theoretical and experimental antioxidant activity of vitamin E and clove essential oil were found to be 48.33% and 60.95%, respectively.

**Conclusion** Plai and vitamin E combination with clove essential oil were found to produce better antioxidant activity than individual agents.

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**Keywords:** Essential oil, Synergistic, Antioxidant

# The Efficacy of Essential Oils Ointment on *Malassezia* Causing Canine Dermatitis

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## Abstract

**Objective** To determine the efficiency of essential oils ointment on *Malassezia* causing canine dermatitis.

**Materials and Methods** The ointment containing 5% betel vine and 5% clove essential oils was evaluated for antifungal activity against 26 samples of *Malassezia pachydermatis* isolated from canine dermatitis by agar well diffusion method. The mixture of 5% betel vine and 5% clove essential oils in the solvent of 5% tween 80 and 5% absolute alcohol used as a negative control. Time kill assay was used to determine antifungal activity of essential oils ointment against *M. pachydermatis*.

**Results** The mean of inhibition zone (IZ) of ointment essential oils preparation was  $18.32 \pm 1.40$  millimeter and the IZ of essential oils in the solvent was  $18.35 \pm 1.20$  millimeter ( $p < 0.05$ ). Fungicidal effect can be seen by a 3 log reduction (99%) at 60 minutes.

**Conclusion** This study indicated that the ointment essential oils preparation had a potential against *Malassezia* causing canine dermatitis. Further study is required to evaluate in clinical trials.

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**Keywords:** Essential oil, Ointment, *Malassezia*

# Antifungal Activity of *Kaempferia parviflora* against *Microsporium gypseum*

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## Abstract

**Objective** To study antifungal activity of *Kaempferia parviflora* against *Microsporium gypseum* by agar dilution technique.

**Materials and Methods** *Kaempferia parviflora* was supplemented in Sabouraud dextrose agar (SDA) in volume by volume at 1%, 2%, 4% and 8% concentrations then inoculated *Microsporium gypseum* on the central of SDA plates. The colony diameters were measured and compared with control groups every 2 days for 8 days.

**Results** Colony diameters of *Microsporium gypseum* on SDA with *Kaempferia parviflora* at concentrations 4% (v/v) and 8% (v/v) were significant smaller than those of the control group all duration ( $P < 0.05$ ).

**Conclusion** With many bioactive compounds, *Kaempferia parviflora* can inhibit *Microsporium gypseum*.

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**Keywords:** *Kaempferia parviflora*, Antifungal activity, *Microsporium gypseum*

# Effect of Tryptic Soy Broth (TSB) and Luria-Bertani (LB) Broth on Production of PirA<sup>VP</sup> Toxin from *Vibrio parahaemolyticus* (*Vp*<sub>AHPND</sub>)

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## Abstract

**Objective** To study the effect of TSB and LB Medium on production of PirA<sup>VP</sup> toxin from *Vibrio parahaemolyticus* (*Vp*<sub>AHPND</sub>)

**Materials and Methods** Seven isolates of *V. parahaemolyticus* (*Vp*<sub>AHPND</sub>) and an regular *V. parahaemolyticus* isolate were isolated from sick culture Pacific white shrimp on TCBS agars. *V. parahaemolyticus* and *V. parahaemolyticus* strains causing acute hepatopancreatic necrosis disease (AHPND) were identified using PCR methods targeting the *ldh* and the *PirAB*<sup>VP</sup> genes, respectively. All isolates were cultured in Tryptic Soy Broth (TSB) and Luria-Bertani Broth (LB). The suspension was used to measure total *Vibrio* count and the supernatant was used to detect PirA<sup>VP</sup> toxin by western blotting technique.

**Results** The amount of PirA<sup>VP</sup> toxin produced from *Vp*<sub>AHPND</sub> grown in TSB was greater than that grown in LB culture medium. Therefore, it might indicate that TSB medium might support the production of PirA<sup>VP</sup> toxin of *Vp*<sub>AHPND</sub> better than LB broth.

**Conclusion** The elements of culture medium influence the production of PirA<sup>VP</sup> protein of *Vp*<sub>AHPND</sub>.

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**Keywords:** Shrimp, Early mortality syndrome, Acute hepatopancreatic necrosis disease, *Vibrio parahaemolyticus*, PirA<sup>VP</sup> toxin, Western blotting

## Introduction

Acute hepatopancreatic necrosis disease (AHPND) is an emerging disease in penaeid shrimp, causing a serious loss of shrimp production, approximately 600,000 metric tons in 2011 to less than 200,000 in 2014 in Thailand [1]. This disease was first reported in China in 2009 and then spread to many countries including Vietnam (2010), Malaysia (2011), Thailand (2012), Mexico (2013) and Philippines (2014) [2]. The affected shrimps were stop to eat and then die on the pond bottom within 35–45 days after stocking post-larva shrimp in grow out ponds. The gross lesions included empty stomach and midgut; moreover, a hepatopancrease was pale, atrophy and showing a typical sloughing characteristic of tubule epithelial cells in histopathological examination. This is an emphasis characteristic of AHPND; therefore, this disease is called AHPND [3, 4]. The causative agent of AHPND is *Vibrio parahaemolyticus* which containing two virulence-associated genes that harbored in pVA1 plasmid. These genes encodes Binary toxins called AHPND Photorhabdus insect-related toxins A (PirA<sup>VP</sup>) or ToxA (12.7 kDa or approximately 16 kDa on SDS-PAGE) and PirB<sup>VP</sup> or ToxB (50.1kDa) [4-7]. These proteins were produced by *Vp*<sub>AHPND</sub> that colonized on shrimp stomach and entered to hepatopancrease (HP) resulting in cell sloughing and necrosis. However the AHPND characteristic were presented by reverse gavage tests, shrimp were treated with the cell free culture broth of *Vp*<sub>AHPND</sub> that refer to PirAB<sup>VP</sup> were the soluble proteins which released from *Vp*<sub>AHPND</sub> [3]. However the toxicity assays showed that PirA<sup>VP</sup> (ToxA) or PirB<sup>VP</sup> (ToxB) proteins alone could not induce typical AHPND pathology [4]. According to the information, *Vp*<sub>AHPND</sub> colonization on the shrimp stomach, we hypothesized

that the feed ingredient might be influencing PirAB<sup>vp</sup> protein produced of *Vp*<sub>AHPND</sub>. So we design *in vitro* experiment by using western blotting technique to compare the presenting of PirA<sup>vp</sup> from *Vp*<sub>AHPND</sub> which culture in difference culture medium including Tryptic soy broth (TSB) and Luria-Bertani (LB) broth.

## Materials and Methods

### Bacterial identification:

Seven isolates of *Vp*<sub>AHPND</sub> (VP<sub>AHPND</sub>1-7) and a normal *V. parahaemolyticus* (non-AHPND) which isolate from clinical AHPND shrimps were cultured on Thiosulfate-citrate-bile salts-sucrose agar plates (TCBS agar) and incubated at 30 °C for 24 hrs. The lecithin dependent hemolysin (*ldh*) genes specific primers (*ldh*-F: 5'-AAAGCGGATTATGCAGAAGCACTG-3' and *ldh*-R: 5'-GCTACTTTCTAGCAT TTTCTCTGC-3') describe by Taniguchi et al (1985) was used for identified the species of *V. parahaemolyticus*. [8] Also, nested PCR for detection *pirAB*<sup>vp</sup> genes (AP4-F1: 5'- ATGAGTAACAATATAAAACATGAAAC -3', AP4-R1: 5'-ACGATTTTCGACGTTCCCCAA- 3', AP4-F2: 5'- TTGAGAATACGGGACGTGGG -3', AP4-R2: 5'-GTTAGTCATGTGAGCACCTTC-3') were applied for detect the present of PirAB<sup>vp</sup> gene in the isolated bacteria [9]. All PCR products were analyzed by 2% agarose gel electrophoresis and exam with an ultraviolet (UV) transilluminator.

### Production of Rabbit anti PirA<sup>vp</sup> polyclonal antibody:

Purified PirA<sup>vp</sup> (12.7 kDa) was provided from the Center for Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Biotec-NSTDA and Mahidol University. A Newzeland white rabbit was immunized with PirA<sup>vp</sup> protein at 0.1 ml (2 ug/ml) via intramuscular injected and subsequently boosted for 2 times at two-week intervals. Rabbit was bleeding for collect the anti PirA<sup>vp</sup> polyclonal antibody after 2 wks of last immunization and kept in -20 °C until using.

### Bacterial culture:

All bacterial isolates were culture on TCBS agar and incubated at 30 °C for 24 hrs. A colony of bacteria was picked up and culture in both of 10 ml of tryptic soy broth (TSB) and LB broth supplemented with 1.5% NaCl and incubated overnight at 30°C in shaking incubator at 160 rpm. This bacterial suspension use for total *vibrio* count and preparation of PirAB<sup>vp</sup> protein .

### Total *Vibrio* count (TVC):

Ten fold serial dilutions of bacterial suspension were prepared and culture on TCBS agar at 30°C for 24hrs. The numbers of colonies were count after the end of incubation period. The results are reported as colony forming units per ml. (CFU/ml).

### Preparation PirAB<sup>vp</sup> protien from culture broth:

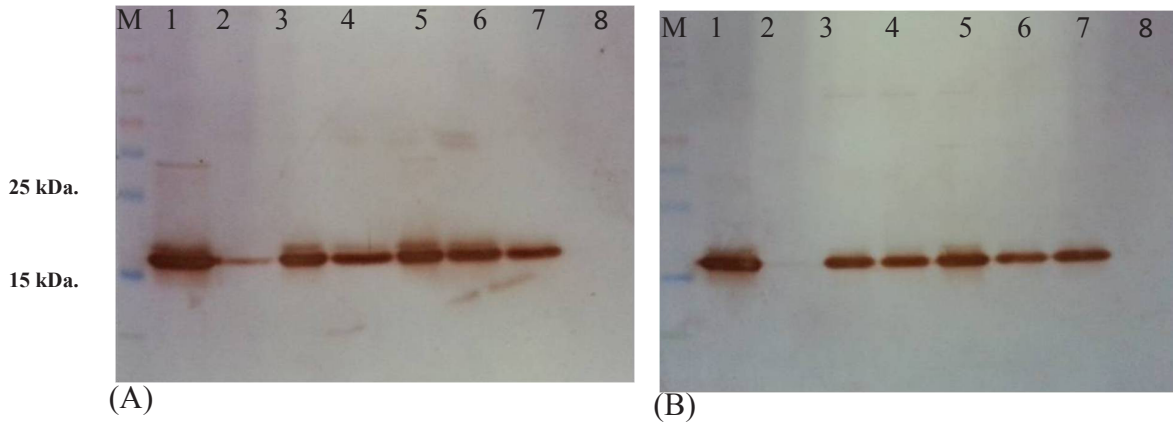
The cell free bacterial supernatant which containing PirAB<sup>vp</sup> protein was collected from bacterial suspension after centrifugation at 12,000 rpm for 3 mins at 4 °C and kept in -20 °C until using.

### SDS-PAGE and Western blotting:

Bacterial supernatants from VP<sub>AHPND</sub>1-7 and a normal *V. parahaemolyticus* (non-AHPND) were separated on 15% SDS-PAGE. Samples were electrophoresed for 120 mins at 90 V. The SDS-PAGE was transferred onto nitrocellulose membranes using a Trans-Blot® SD Semi-Dry Transfer Cell (BioRad). Nitrocellulose membranes were blocking in 5% skim milk solution for overnight at 4°C. The membranes was incubated with 1:1000 Rabbit anti PirA<sup>vp</sup> antibody for 1 hr then wash and incubated with Goat Anti-Rabbit IgG Antibody probe with HRP-conjugate for 1 hr. Then membranes was wash with PBS buffer before incubated for 10 mins in diaminobenzidine (DAB) and hydrogen peroxide in PBS.

## Results

Bacterial identification and Total *Vibrio* count were shown in Table 1 and 2, respectively. Western blotting revealed PirA<sup>vp</sup> protein bands. A prominent protein bands with 16-17 kDa of molecular weight (according to the gel marker) were observed from TSB and LB cell-free culture media when analyzed by western blotting (Figure 1A and 1B, respectively). Protein bands of TSB cell-free culture media (Figure 1A) shown more greater than it found in LB cell-free culture media (Figure 1B). Moreover *V. parahaemolyticus*



**Figure 1.** Western Blotting of VP<sub>AHPND</sub> 1-7 and VP<sub>non-AHPND</sub> (A) from TSB cell-free culture medium, and (B) from cell-free LB culture medium.

For both figures lane 1-8 were cell free culture media of VP<sub>AHPND</sub> 1-7 and VP<sub>non-AHPND</sub> respectively. There are a major bands of PirA<sup>vp</sup> protein, approximately size around 16-17 kDa., present in lanes of VP<sub>AHPND</sub> 1-7 but absent in VP<sub>non-AHPND</sub> lanes. Bands in figure 1A seem to be more greater than figure 1B

**Table 1.** Bacterial characteristic on TCBS agar and PCR results of lecithin-dependent hemolysin (*ldh*) and *pirAB<sup>vp</sup>* genes (AP4 primer) in VP<sub>AHPND</sub> 1-7 and VP<sub>non-AHPND</sub> isolates.

Sample	Bacterial characteristic on TCBS agar			PCR detection	
	Size	shape	Colony color	<i>ldh</i>	<i>pirAB<sup>vp</sup></i> (AP4 primer)
VP <sub>AHPND</sub> 1	large	round	Green	+	+
VP <sub>AHPND</sub> 2	large	round	Green	+	+
VP <sub>AHPND</sub> 3	large	round	Green	+	+
VP <sub>AHPND</sub> 4	large	round	Green	+	+
VP <sub>AHPND</sub> 5	large	round	Green	+	+
VP <sub>AHPND</sub> 6	large	round	Green	+	+
VP <sub>AHPND</sub> 7	large	round	Green	+	+
VP <sub>non-AHPND</sub>	large	round	Green	+	-

(+) = positive, (-) = negative

**Table 2.** Total Vibrio count.

Sample	Total Vibrio count (CFU/ml)
TSB suspension of VP <sub>AHPND</sub> 1	1.58 x 10 <sup>6</sup>
TSB suspension of VP <sub>AHPND</sub> 5	1.45 x 10 <sup>7</sup>
TSB suspension of VP <sub>AHPND</sub> 6	1.95 x 10 <sup>5</sup>
TSB suspension of VP <sub>non-AHPND</sub>	2.81 x 10 <sup>8</sup>
LB suspension of VP <sub>AHPND</sub> 1	1.36 x 10 <sup>8</sup>
LB suspension of VP <sub>AHPND</sub> 5	1.09 x 10 <sup>7</sup>
LB suspension of VP <sub>AHPND</sub> 6	3.28 x 10 <sup>6</sup>
LB suspension of VP <sub>non-AHPND</sub>	1.48 x 10 <sup>8</sup>

strain VP<sub>AHPND</sub> 2 produce a little PirA<sup>vp</sup> toxin in LB culture media if compare to TSB culture media (lane 2 of both figure).

## Discussion

Bacterial characteristic on TCBS agar shown large round greenish colonies which *V. parahaemolyticus* characteristic on TCBS [10]. All isolates positive to PCR detection of the lecithin-dependent hemolysin (*ldh*) gene which is known as a species-specific marker for *V. parahaemolyticus* [8, 11, 12] revealed that all isolates were *V. parahaemolyticus*.

The western blotting results revealed that all isolates which containing PirA<sup>vp</sup> gene could produce PirA<sup>vp</sup> protein but in different quantitation. The number of bacteria were consentaneousness to determine that it absolutely involved with the quantitation of PirA<sup>vp</sup> protein. Therefor the results indicating the PirA<sup>vp</sup> production ability of VP<sub>AHPND</sub> was individual. These may related to the immersion challenges test by using many isolates of VP<sub>AHPND</sub> resulting in the different mortality rate and histopathology of the HP characteristics. [12].

In addition, PirA<sup>vp</sup> protein bands which from TSB medium were shown more greater bands than LB medium. On the other hand number of Vibrio cell were larger in bacteria grew in LB broth more than grew in TSB. It indicated the production of PirA<sup>vp</sup> toxin was not related with the growth performance of bacteria in each culture media. According to the results, TSB medium might be provide more appropriated conditions to induce PirA<sup>vp</sup> production by the VP<sub>AHPND</sub> than LB medium. When the culture medium might promote the PirA<sup>vp</sup> protein productions thus we suggest that the type of culture medium is an influencing factor which relevant to the virulence of VP<sub>AHPND</sub> because PirA<sup>vp</sup> and PirB<sup>vp</sup> proteins were both required in a dose dependent manner to cause AHPND lesions[4].

Each culture medium formula was unique, such as TSB; compose of tryptone, soytone, glucose, sodium chloride and dipotassium phosphate) and LB broth; compose of peptone, yeast extract and sodium chloride). Therefore the elements of culture medium might affected to some bacterial activities which stimulate the growth performance as in a recent research which shown that the enrichment culture media effected to *V. parahaemolyticus* culture performance[13] or activated some bacterial mechanism to induce to production of the protein. Therefor in the future work is planned to study the effect of different ingredients in culture media on PirA<sup>vp</sup> protein of VP<sub>AHPND</sub> to discover and describe which components is the most influencing factor to produce PirA<sup>vp</sup> of VP<sub>AHPND</sub>.

## Acknowledgements

We would like to thank Centex Shrimp, Faculty of Science, Mahidol University, RamaVI Rd., Bangkok, Thailand Department, for provide us PirA<sup>vp</sup> purification protein and also thank to all staffs of Serology, Biotechnology (WWVLAB), Bacteriology, Virology laboratory of Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom for all support.

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# Infection of Nile Tilapia (*Oreochromis niloticus*) with *Streptococcus agalactiae* by Nares Inoculation

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## Abstract

**Objective** This study was to determine the mortality and morbidity of *Streptococcus agalactiae* infection in Nile tilapia by nares inoculation.

**Materials and Methods** Nile tilapia (n=15) were divided into 2 groups. Ten fish were inoculated into the both nares of each fish with 0.1 ml of bacterial concentration of  $3.5 \times 10^7$  CFU/ml for 1 min. Five control fish were inoculated with sterile 0.9% normal saline solution in a similar manner. Clinical signs, lesions and mortality were monitored daily for 14 days post-inoculation. Samples from kidney spleen eye and brain of all dead and surviving fish were confirmed of infection by bacteriological examination and PCR.

**Results** There was only 1 dead fish and 9 surviving fish. All fish were positively examined with *S. agalactiae* infection. Percentage of mortality and morbidity were 10 (1/10) and 100 (9/9), respectively. Dead fish was observed at day 5 and moribund fish showed darkening skin, surface swimming, ascites and/or splenomegaly by day 2 post-inoculation. No mortality, clinical signs, lesions and bacterial infection were observed in control group.

**Conclusion** Successfully experimental *S. agalactiae* infection by nares inoculation demonstrated that nares may be a potential route of *S. agalactiae* infection in Nile tilapia. It would be useful for studying the naturally-occurring disease in this species.

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**Keywords:** Infection, Nares inoculation, *Streptococcus agalactiae*, Nile tilapia

# Survey of Minute Intestinal Fluke Metacercariae in Cyprinoid Fish from Border Markets in Sa Kaeo, Thailand

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## Abstract

**Objective** The objective of this survey was to investigate the distribution of Minute intestinal fluke (MIF) metacercariae in cyprinoid fishes collected from the border and local markets in Sa Kaeo.

**Materials and Methods** Cyprinoid fish were collected from the border and local markets in three districts, Sa Kaeo province, eastern Thailand. The fish were classified and digested by pepsin HCl. The sedimented sample were identified the MIF metacercariae under microscope.

**Results** Total of 845 MIF metacercariae were found in five species of cyprinoid fish including *Barbonymus schwanenfeldii*, *Puntioplites proctozystron*, *Systemus rubripinnis*, *Cyclocheilichthys repasson* and *Barbonymus gonionotus* which collected from 2 important border markets located in Aranyaprathet and Khlong Hat districts and 1 local market located in Watthana Nakhon district. *B. schwanenfeldii* had the highest number (575 metacercariae) and intensity (108.49 cysts/100 g fish and 71.88 cysts/fish) of MIF metacercariae following *P. proctozystron*, *S. rubripinnis*, *B. gonionotus* and *C. repasson*, respectively. Moreover, the border market in Aranyaprathet district showed the various species of infected cyprinoid fish and the high intensity of MIF metacercaria infection.

**Conclusion** The result of this study reveals the update status of this fish-borne trematode in border and local markets in Sa Kaeo province. The consumption of raw or undercooked freshwater fish or fish products from this area may lead to human infection and public health problem issue.

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**Keywords:** Minute intestinal fluke, Heterophyidae, Metacercariae, Cyprinoid fish, Border markets, Sa Kaeo province

## Introduction

The minute intestinal fluke (MIF) is general name of trematodes in the genus Heterophyidae; because of their body size are minute and found in the small intestines of definitive host [1]. Several species of Heterophyidae trematodes have been reported in human; in many counties of Southeast Asia [2-3]. In Thailand, the heterophyid fluke, such as *Haplorchistaichui*, *H. pumilio*, *H. yokogawai*, *Centrocestus caninus* and *Stellantchasmus falcatus* have been found to cause human disease [4-6]. Human is infected by trematodes from eating raw or undercooked freshwater fish or fish products containing the heterophyid metacercariae. After excyst and development, the adult flukes can penetrate and irritate the intestinal mucosa that cause colicky pain and mucous diarrhea [7-8].

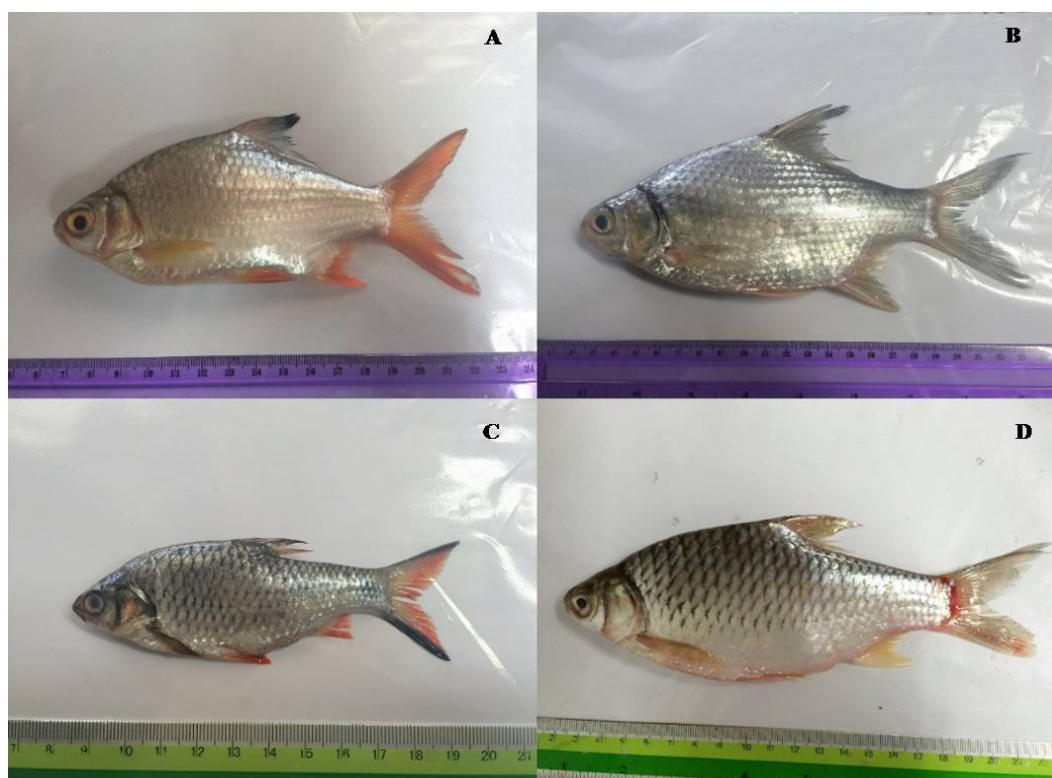
Cyprinoid fish are the second intermediated host of MIF, many species of Cyprinoid fish in North and Northeast regions of Thailand have been reported to harbor the heterophyid metacercariae [9-12]. People in these area likes to eat the raw and undercooked fish as a traditional food, therefore, the fish-borne trematode is a common public health problem [13]. However, there was a few report about MIF metacercariae survey in cyprinoid fish in eastern Thailand. Sa Kaeo province is in the eastern Thailand and bordering Cambodia. This province has an important border market to distribution the agricultural products including freshwater fish between Thailand and Cambodia. Therefore, objective of this survey was to investigate the distribution of MIF metacercariae in cyprinoid fish collected from the border and local markets in Sa Kaeo. The result of this study reveals the update status of this fish-borne trematode.

## Materials and Methods

The cyprinoid fish were obtained from the border and local markets in Aranyaprathet, Khlong Hat and Watthana Nakhon districts, Sa Kaeo province, eastern Thailand. This survey was studied in October to December 2016. The fish were placed on ice and transported to the laboratory. The species of cyprinoid fish were classified, then weighted and counted the number of fish in each species. Fish were mashed and digested by pepsin HCl at 37 °C for 1 hour, the digested material were filtered and sedimented with 0.85% sodium chloride solution. The MIF metacercariae was identified under the microscope by the morphological examination based on Scholz et al [14]. The number of metacercariae were recorded.

## Results

The MIF metacercariae were found in the collected cyprinoid fish from the border and local markets in Sa Kaeo province. These MIF metacercariae infected fish were collected from 2 important border markets located in Aranyaprathet and Khlong Hat districts, and 1 local market located in WatthanaNakhon district. A total of 845 MIF metacercariae were found in cyprinoid fish 5 species including *Barbonymus schwanenfeldii*, *Puntioplites proctozystron*, *Systomus rubripinnis*, *Cyclocheilichthys repasson* and *Barbonymus gonionotus* (Figure 1 and Table 1). The highest number of MIF metacercariae was found in *B. schwanenfeldii* following *P. proctozystron*, *S. rubripinnis*, *B. gonionotus* and *C. repasson*, respectively. The intensity of MIF metacercariae in the examined cyprinoid fish were 71.88, 12.69, 5.13 and 0.05 metacercariae per fish in *B. schwanenfeldii*, *P. proctozystron*, *S. rubripinnis* and *C. repasson*, respectively (Table 1). In addition, *B. gonionotus* collected from border and local markets had 0.45 and 2.00 metacercariae per fish, respectively. The results from this survey revealed that *B. schwanenfeldii* harbored the highest number and intensity of MIF metacercariae. The total number of metacercariae was 575 cysts and the intensity were 108.49 cysts/100 g fish and 71.88 cysts/fish (Table 1). Furthermore, the border market in Aranyaprathet showed the various species of infected cyprinoid fish and the high intensity of MIF metacercaria.



**Figure 1.** The species of cyprinoid fish harbored the MIF metacercariae found in Sa Kaeo province, Thailand. A; *B. schwanenfeldii*, B; *P. proctozystron*, C; *S. rubripinnis* and D; *B. gonionotus*.

**Table 1.** Number and intensity of MIF metacercariae in cyprinoid fish collected from border and local markets in Sa Kaeo province, Thailand.

Source	District	Fish species	Weight of fish (g)	Number of fish	MIF metacercariae		
					Total cysts	Cysts/100 g fish	Cysts/fish
1. Rattanaatham market	Aranyaprathet	<i>Barbonymus schwanenfeldti</i> **	530	8	575	108.49	71.88
		<i>Puntioplites proctozystron</i> **	1640	13	165	10.06	12.69
		<i>Systomus rubripinnis</i>	310	15	77	24.84	5.13
		<i>Cyclocheilichthys repasson</i>	550	223	12	2.18	0.05
2. Ban Khao Din border market	Khlong Hat	<i>Barbonymus gonionotus</i>	2065	22	10	0.48	0.45
3. Fresh market	Wattana Nakhon	<i>Barbonymus gonionotus</i>	1200	3	6	0.50	2.00

\*\*Cambodia imported fish; MIF=minute intestinal fluke

## Discussion

The infection of MIF metacercariae in cyprinoid fish is commonly found in northern and northeastern regions of Thailand. The distribution of MIF in eastern region was no update. This survey reveals the contamination of MIF metacercariae in cyprinoid fish from border and local markets in Sa Kaeo province, eastern Thailand. MIF metacercariae was found in five species of cyprinoid fish i.e. *B. schwanefeldii*, *P. proctozystron*, *S. orphoides*, *C. repasson* and *B. gonionotus* which similar to previous reports in many species of cyprinoid fish from northern and northeastern Thailand [11-12]. The heterophyid metacercariae showed broad ranges in host specific. In infective stage, these trematodes can live in several species of cyprinoid fish [9-12] that can cause the high risk of MIF infection in human who like to eat raw or undercooked freshwater fish.

In this study, we found that 2 species (*B. schwanefeldii* and *P. proctozystron*) of cyprinoid fish imported from Cambodia. Interestingly, these imported fish harbored very much higher number and intensity of MIF metacercariae especially *B. schwanefeldii* than the studied fish from Thailand. In the previous study of zoonotic trematode metacercaria contamination in freshwater fish in Cambodia, the distribution of MIF metacercariae was found in many kinds of fish including *P. proctozystron* [15]. The freshwater fish from Cambodia are currently famous because the low price and spreading to Thailand. Thus, the border markets are important reservoirs of MIF metacercariae and the infected freshwater fish can be distributed to eastern and other regions of Thailand. Therefore, the consumption of raw or undercooked freshwater fish as the traditional dishes may lead to a risk of human infection and public health problem issue.

## Acknowledgements

This work was financially supported by the Research Grant of Burapha University through National Research Council of Thailand (Grant no. 2/2559). The author would like to thank the Faculty of Agricultural Technology, Burapha University Sakaeo Campus for providing the laboratory facilitation and thank all staffs for supporting, especially Dr. Niramai Fangkrathok.

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# A Survey of Ectoparasites and Intracellular Blood Parasites and Hematological Changes in Snail-eating Turtle (*Malayemys* spp.) in Wat That Noi, Chawang District, Nakhon Si Thammarat Province

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## Abstract

**Objective** The objective was to study effect of ectoparasites and intracellular blood parasites on hematological changes of snail-eating turtle (*Malayemes* spp.) in Wat That Noi, Chawang District, Nakhon Si Thammarat Province.

**Materials and Methods** A total of forty blood samples were collected from subcarapacial vein or sinus about 0.4 - 0.8% of turtle body weight into lithium heparin tubes. Prior to analysis, fresh blood samples were kept in 4°C. Hematological analysis included packed cell volume (PCV), white blood cell differential count, red blood cell count and thin blood smear for hemoparasite detection. Association between PCV & ectoparasites and between hematology parameter & intracellular blood parasites were determine.

**Results** The results showed all of the turtles had lower PCV and RBCs; whereas, WBCs and eosinophil were higher than the normal range. There were intracellular blood parasites, *Haemogregarine* spp.. In a group that infected with this parasite had lower PCV and higher monocyte than the non-infected group and negative correlation between an average number of ectoparasites (leeches) and PCV with statistically significant difference at  $P < 0.005$ .

**Conclusion** Because leeches are a vector of *Haemogregarine* spp., many leeches and *Haemogregarine* spp. can induce anemia in snail-eating turtles in Wat That Noi, Chawang District, Nakhon Si Thammarat Province.

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**Keywords:** Ectoparasites, Intracellular blood parasites, Hematological changes, Snail-eating turtles (*Malayemes* spp.)



# Bioaccumulation of Lead Acetate in Rice Field Frogs (*Fejervarya limnocharis*) *in vivo*

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## Abstract

**Objective** The objective of the study was to determine the concentration of Pb in water and *F. limnocharis* after *in vivo* injection with different concentrations of lead acetate and exposure time of 24, 48 and 72 hours.

**Materials and Methods** Rice field frogs (*F. limnocharis*) were injected with four different concentrations (0, 5, 10 and 20 mg/L) of lead acetate for 24, 48 and 72 hours. Pb concentrations in water and *F. limnocharis* were measured by using inductively coupled plasma optical emission spectrometry (ICP-OES).

**Results** Pb concentrations were not detected in water and *F. limnocharis* of control groups. Pb concentration was detected at high levels in experimental groups. The highest Pb concentrations in water and *F. limnocharis* samples were found in the 20 mg/L for 72 hours (0.05±0.01 mg/L in water and 0.78±0.04 mg/kg in *F. limnocharis*, respectively). The water samples exposure to 5 and 10 mg/L Pb for 24 hours and 5 mg/L for 48 hours were significantly different from the control (p<0.05). Pb concentrations in *F. limnocharis* differed significantly between the control and experimental groups (p<0.05).

**Conclusion** The results from this study *F. limnocharis* have metabolized lead acetate in the body and then excreted in the urine and feces, so the concentration in water was low. *F. limnocharis* has accumulation and elimination Pb in their body.

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**Keywords:** Concentration, Lead acetate, *Fejervarya limnocharis*, Frog

## Introduction

Lead (Pb) is an element naturally found in the environment, which non-essential element for organisms [1]. It can be found in all parts of the environment, such as air, water and soil. Much of Pb exposure comes from human activities including industrial facilities, used car batteries, paints, mining sites, wastewater discharge and leachate from contaminated sites [2,3]. Pb tends to accumulate in living organisms and may become biomagnified up food chain trophic levels to toxic concentrations [4,5]. Low levels exposure of Pb can cause detrimental effects to health. High levels, Pb toxicity affects multiple organ systems including the haemopoietic, cardiovascular, hepatic, renal, respiratory, musculoskeletal and reproductive systems. Acute and chronic toxicity of Pb in organisms depends on the concentration and duration of exposure [6-12].

Amphibians have been shown to be good bioindicators and a sensitive model for environmental and ecotoxicological studies [13-15]. Amphibians including rice field frog are sensitive to environmental pollutants because of their extremely permeable skin, unshelled eggs and exposure to terrestrial and aquatic environments at different life stages [16-20]. There are exposed to several kinds of chemical contaminants such as those discharged by effluents into agricultural areas. Therefore, the organisms presented in the aquatic body can be exposed to both single and multiple contaminants. In previous studies, concentrations Pb have

been studied extensively in fish but information relating to frog is limited [21]. Frog (*F. limnocharis*) model was chosen for this study as well as frog species is abundant in aquatic ecosystem. This study focused on Pb concentration in water and frog after in vivo exposure to different concentrations of Pb including different times.

## Materials and Methods

### Experimental design and *F. limnocharis* specimens

Healthy specimens of *F. limnocharis* were collected from the paddy field in Khon Kaen province, Thailand. *F. limnocharis* samples were left to acclimatize for 5 days before laboratory conditions. *F. limnocharis* with similar weight and size (10-20 g and 4-8 cm) were divided into 12 experimental groups including 3 groups as control and 9 groups as treatment. The frog, *F. limnocharis* were kept in plastic bucket 15 L in capacity and containing 1 L of dechlorinated tap water.

### Lead acetate exposure concentration

Frogs were then divided into 36 buckets containing 5 frogs of each. 100  $\mu$ L of deionized water (control groups 1-3) or Pb (treatment groups 4-12) of varying concentrations (5, 10 and 20 mg/L). Each Pb concentration, frogs were left to be exposed to the injected solution for 24, 48 and 72 hours before being analyzed. The water in the buckets was not changed during the experimental period.

### Pb concentration in the water

The 25 mL of water samples were collected to a beaker and 1.25 mL of HNO<sub>3</sub> was added. The sample was covered with a watch glass and heated in a water bath at 90 $\pm$ 5 $^{\circ}$ C for 30 minutes and then left to cool. The water samples were then adjusted to a final volume to 25 mL with deionized water and then filtered. The samples were then analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) [22].

### Pb concentration in *F. Limnocharis*

The frogs were knocked by ice. The muscle samples were cut into small pieces. 1 gram of muscle tissue samples were placed into a 25 mL beaker. 7 mL of concentrated HNO<sub>3</sub> acid and 1 mL of concentrated H<sub>2</sub>O<sub>2</sub> were added into the beaker. The samples were heated in a water bath for 2 hours at 90 $\pm$ 5 $^{\circ}$ C and then left to cool. The digested muscle samples were adjusted to a final volume of 25 mL with deionized water and the mixture was then filtered. (The detection limit of Pb analyzed was 0.005 mg/L. The ICP-OES wavelengths were setup for analysis of Pb were 220.353 nm. The precision of the Pb concentration was evaluated with certified reference material (CRM) via the 3111C method [23].

### Statistical analysis

The Pb concentration in the water and the frog samples group were compared statistically significant differences between control and experimental groups. These data were checked using ANOVA, followed by Turkey's post hoc test. The results were expressed as the mean $\pm$ SD. All statistical tests were conducted at a 95% confidence level.

## Results

### Pb concentrations in the water and *F. limnocharis* samples

Pb concentrations in the water after injection with four different concentrations (0, 5, 10, 20 mg/L) for 24, 48 and 72 hours are shown in Table 1. Most Pb concentrations in water did not exceeded the standard of the Pollution Control Department of Thailand [24] of water quality standards (0.05 mg/L) except for the highest Pb concentration detected, which was found in the 20 mg/L for 72 hours of exposure experimental group. Pb concentration was not detected in water of the control group, whereas average Pb concentrations of the experimental groups were 0.01 $\pm$ 0.00, 0.02 $\pm$ 0.00 and 0.03 $\pm$ 0.01 mg/L, respectively for 24 hours; 0.01 $\pm$ 0.00, 0.03 $\pm$ 0.00 and 0.03 $\pm$ 0.01 mg/L, respectively for 48 hours; and 0.03 $\pm$ 0.00, 0.03 $\pm$ 0.01 and 0.05 $\pm$ 0.01 mg/L, respectively for 72 hours. The highest Pb concentration in the water was found in the 20 mg/L experimental groups for 72 hours of exposure (0.05 $\pm$ 0.01 mg/L). However, the statistical analysis indicated that differences in the Pb concentrations in the water samples between the experimental and control groups ( $p$ <0.05) of all Pb concentrations in different durations exposure except for

the 5 and 10 mg/L for 24 hours and 5 mg/L for 48 hours.

The Pb concentrations in *F. limnocharis* after exposure to three different Pb concentrations (5, 10 and 20 mg/L) for 24, 48 and 72 hours are shown in Table 2. Pb concentrations was not detected in *F. limnocharis* of the control group; however average Pb concentrations in *F. limnocharis* of the experimental groups were  $0.28 \pm 0.03$ ,  $0.27 \pm 0.09$  and  $0.35 \pm 0.03$  mg/kg, respectively for 24 hours;  $0.44 \pm 0.02$ ,  $0.34 \pm 0.03$  and  $0.63 \pm 0.04$  mg/kg, respectively for 48 hours; and  $0.32 \pm 0.06$ ,  $0.30 \pm 0.02$  and  $0.78 \pm 0.04$  mg/kg, respectively for 72 hours, which exceeds standard of the Pollution Control Department of Thailand (0.05 mg/kg) [25]. The highest Pb concentrations were found in the 20 mg/L experimental groups for 72 hours group  $0.78 \pm 0.04$  mg/kg. The statistical analysis indicated that differences in the Pb concentrations in *F. limnocharis* samples between the

**Table 1.** The Pb concentrations in the water samples

Lead acetate concentrations (mg/L)	Pb concentrations in the water (mg/L)			Thailand standard (mg/L)
	24 hours	48 hours	72 hours	
0	ND	ND	ND	
5	$0.01 \pm 0.00^a$	$0.01 \pm 0.00^a$	$0.03 \pm 0.00^b$	
10	$0.02 \pm 0.00^a$	$0.03 \pm 0.00^b$	$0.03 \pm 0.01^b$	
20	$0.03 \pm 0.01^b$	$0.03 \pm 0.01^b$	$0.05 \pm 0.01^b$	$\leq 0.05$

Remark: Data were analysed by one way ANOVA followed by Turkey's post hoc test. Number of *F. limnocharis* in each group n=5, All groups are compared with control group. ND=Not detected (Less than the detection limit of Pb); Different letters (a, b) indicate there is a significant difference at  $p \leq 0.05$

**Table 2.** The Pb concentrations in the *F. limnocharis* samples

Lead acetate concentrations (mg/L)	Pb concentrations in the <i>F. limnocharis</i> (mg/kg)			Thailand standard (mg/kg)
	24 hours	48 hours	72 hours	
0	ND	ND	ND	
5	$0.28 \pm 0.03^a$	$0.44 \pm 0.02^a$	$0.32 \pm 0.06^b$	
10	$0.27 \pm 0.09^a$	$0.34 \pm 0.03^b$	$0.30 \pm 0.02^b$	$\leq 0.3$
20	$0.35 \pm 0.03^b$	$0.63 \pm 0.04^b$	$0.78 \pm 0.04^b$	

Remark: Data were analysed by one way ANOVA followed by Turkey's post hoc test. Number of *F. limnocharis* in each group n=5, All groups are compared with control group. ND=Not detected (Less than the detection limit of Pb); Different letters (a, b) indicate there is a significant difference at  $p \leq 0.05$

experimental and control groups ( $p < 0.05$ ) for all Pb concentrations in different durations of exposure.

## Discussion

### Pb concentrations in the water and *F. limnocharis* samples

The results suggest that the Pb concentrations found in the water samples originated from *F. limnocharis*. The Pb concentrations in the water samples exceed the standards for the Pollution Control Department of Thailand. High concentration of injected Pb and longer exposure times resulted in increased Pb concentrations in the water and *F. limnocharis*. Pb accumulates mainly in the liver and kidney [26-28] and is excreted in urine and feces [29]. Consequently, *F. limnocharis* may not eliminate all of the Pb from their bodies and some of the Pb remains accumulated in the *F. limnocharis* muscles and other organs.

The muscles of *F. limnocharis* were used to detect the Pb concentration. Pb concentrations are higher and will then be transported to the various target organs such as liver and kidney than in the muscles

[30-32]. Pb movements across cells via active transport with the utilisation of ATP are facilitated by P-type ATPase ionic pump (mainly type IB ATPase subfamily) as both metals are divalent soft Lewis acids [33-35]. Pb has higher propensities to bioaccumulate in the frog organs that involve in the detoxification process.

In conclusion, Frog, *F. limnocharis* was exposed to three different concentrations (5, 10 and 20 mg/L) of Pb for 24, 48 and 72 hours comparison to the control group. Pb was not detected in water and *F. limnocharis* of control groups. Pb concentration was detected high levels in experimental groups. In *F. limnocharis* samples, Pb concentrations in all experimental groups differed from control group ( $p < 0.05$ ). *F. limnocharis* have metabolizing lead acetate in the body and then excreted in the urine and feces, so the concentration in water was low. *F. limnocharis* has accumulation and elimination. This study suggests that laboratory-based Pb concentration tests are essential to confirm the effects and bioaccumulation caused by this metal.

## Acknowledgements

This research was supported by the Research Group on Toxic Substances in Livestock and Aquatic Animals, Khon Kaen University, and the Graduate School of Khon Kaen University, Thailand.

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# Presence of Tyrosine Phosphorylated Proteins in Testis, Epididymis, and Seminal Vesicle of Adult Rats

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## Abstract

**Objective** The objective was to immuno-localize and identify tyrosine phosphorylated proteins in male reproductive tissues including testis, epididymis, and seminal vesicle of Sprague-Dawley rats.

**Materials and Methods** The monoclonal anti-phosphotyrosine (clone 4G10) antibody was used for immunohistochemistry to probe tyrosine phosphorylated proteins and also to examine the expression of tyrosine phosphorylated proteins using immuno-Western blotting in testis, epididymis, and seminal vesicle of adult Sprague-Dawley rats.

**Results** The positive reactivity of tyrosine phosphorylated proteins was clearly observed in Leydig cells, Sertoli cells, spermatogonia, spermatocytes, and spermatids (round and elongated), respectively. The expressions of testicular tyrosine phosphorylated proteins were 200, 131, 93, 70, 60, and 48 kDas, respectively. In addition, the positive reactivity of phosphorylated proteins was clearly observed in cytoplasmic principle cells, nuclei of apical & basal cells of epididymis and seminal vesicle. The profiles of such proteins were 182, 127, 80, 70, 57, 45, 34, and 31 kDas, respectively.

**Conclusion** The tyrosine phosphorylated proteins were specifically localized in male reproductive tissues such as germinal epithelium, interstitial endocrine cells, epididymis, and seminal vesicle of adult Sprague-Dawley rats. It is assumed that these proteins play important roles in productions of mature sperm, androgen hormones, and fertilizing factors.

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**Keywords:** Tyrosine phosphorylated proteins, Testis, Epididymis, Seminal vesicle, Rats

# Expression of Granulin, p53, BCL2 and PCNA in *Opisthorchis viverrini* Repeated Infections in a Hamster Model

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## Abstract

**Objective** This study aimed to investigate the relationship between expression of granulin, p53, Bcl-2 genes and proliferation index (PCNA) in *O. viverrini* infected hamsters and *O. viverrini* associated CCA in hamsters.

**Materials and Methods** Two groups of 5 male Syrian gold hamsters age 15 weeks were studied. Each hamster in the treatment group was orally fed with 50 *O. viverrini* metacercariae and then treated with praziquantel. This 5-week experiment was repeated for three rounds. The non-treatment group was carried out similarly but no treatment. The animals were euthanized at weeks 15. The liver tissue were stained with hematoxylin and eosin and immunohistochemistry of granulin, p53, Bcl-2 and PCNA for detection.

**Results** The hamsters in non-reinfection group, showed more severe histopathological changes comparing to the treatment group. Most of them were proliferative biliary lesions such as bile duct hyperplasia, bile duct dysplasia and cholangiofibrosis. Cystic formation could not be found in this group. In reinfection with praziquantel group, the hamsters showed lesions similar to the hamsters in non-reinfection group but lesion much less severe, especially very few cholangiofibrosis. CCA could not be detected in both groups. Average PCNA index and abnormal proliferation of biliary epithelial cells of animals in non-reinfection group (PCNA index = 72.50) were higher than those in reinfection group (PCNA index = 20.92). Significant difference of PCNA expression was found between non-reinfection and reinfection groups at p-value = 0.014. Average area of Bcl-2 stained biliary cell in non-reinfection (average area = 68.75) was higher than in reinfection group (average area = 24). There was no granulin and p53 staining in both groups.

**Conclusion** These suggested that three round repeated infections following by multiple treatments could not induce CCA development in a hamster model. Late response of CCA involving genes such as granulin and p53 have not been altered during the CCA induction in this study.

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**Keywords:** Repeated infection, *Opisthorchis viverrini*, Granulin, p53, Bcl-2, PCNA, Hamster



# Molecular Characterization of *Plasmodium Juxtannucleare* in Thai Native Chickens (*Gallus gallus domesticus*) in Khon Kaen Province, Thailand

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## Abstract

**Objective** The aim of this study was to identify and characterize the *Plasmodium juxtannucleare* infecting Thai native chickens using molecular technique.

**Materials and Methods** Blood samples of Thai native chickens were collected and extracted for detection of the cox I gene of *Plasmodium* spp. The PCR was amplified using forward primers CoxI-F1 and CoxI-R1 followed previous study. The PCR products were purified and subjected to direct sequencing. The assembled cox I gene sequences were identified and analyzed by comparing with sequences of the matching *Plasmodium* spp. along with other *Plasmodium* spp. obtained from the GenBank database. Percent identity and multiple sequence alignment analyses of the nucleotide and deduced amino acid sequences were generated. The phylogenetic tree of nucleotide sequences was constructed using MEGA6 software version 6.06 by Neighbor-joining with the maximum likelihood method with 1,000 replications of bootstrap values.

**Results** The PCR products of positive samples displayed 588 base pairs. Sequence analysis of *P. juxtannucleare* partial cox I gene showed that all parasite isolates in this study possessed the same nucleotide sequences and 100% identity *P. juxtannucleare* including nucleotides and deduced amino acids. The phylogenetic analysis revealed that all *P. juxtannucleare* sequences were clustered in the same group and separated from other *Plasmodium* spp. In addition, the phylogenetic tree showed that the sequence was the most closely related to *P. juxtannucleare* from Japan (AB250415).

**Conclusion** This study presents the first molecular confirmation and characterization of *P. juxtannucleare* found in Thai native chicken in Khon Kaen province, Thailand.

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**Keywords:** Cytochrome c oxidase subunit I (cox1) gene, *Plasmodium juxtannucleare*, Thai native chicken

# Comparisons of Carcass Yields in Four Breeds (Cobb 500, Ross 308, Arbor Acres and Hubbard) of Broiler Chickens Raised under Standard Commercial Housing System

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## Abstract

**Objective** To compare carcass yields at market age of four broiler breeds (Cobb, Ross 308, Arber Acres and Hubbard) raised under standard commercial housing system.

**Materials and Methods** A total of 48 broiler chickens (n = 12 in each breed (six for male and six for female)), aged 39 days, were used in this study. Each breed was raised under identical conditions of the commercial housing and feeding system and in the same rearing period. For study eligibility, chickens must have their body weight within  $\pm 2.5\%$  of their standard body weight for each breed at 39 days of age. Measurements were made on live body weight, bleeding weight, feather weight, eviscerated carcass weight, and weight of the visceral organs (crop, proven-gizzard, intestine, liver, heart, spleen and cloacal bursa) and the abdominal fat. The whole chicken carcass was undergone standard carcass dressing resulting in seven parts (head and neck, feet, breast muscle, fillet muscle, thigh, and drumstick). Each part was weighted. Carcass yields, weight of the visceral organs, and other variables were calculated and expressed as a percentage of the body weight (without feed in crop and gizzard). One way analysis of variance (ANOVA) with Tukey's HSD test was used to compare carcass yields among chicken breeds for each sex.

**Results** In male broiler chickens, the percentage of eviscerated carcass was similar among four breeds ( $77.3 \pm 0.9$  (mean  $\pm$  SD) for Cobb,  $77.3 \pm 0.6$  for Ross 308,  $77.1 \pm 0.9$  for Arber Acres, and  $77.8 \pm 1.3$  for Hubbard). This was similar for female broiler chickens. However, the percentage of breast muscle weight was found the greatest in Cobb ( $25.1 \pm 2.5$ ) and the smallest in Arber Acres ( $20.5 \pm 2.0$ ) for male chickens and the greatest in Hubbard ( $24.4 \pm 1.7$ ) and the smallest in Arber Acres ( $20.4 \pm 1.4$ ) for female chickens. The percentage of abdominal fat weight was the greatest in Hubbard ( $1.6 \pm 0.4$ ) and the smallest in Arber Acres ( $1.1 \pm 0.2$ ) for male chickens and the greatest in Hubbard ( $2.2 \pm 0.4$ ) and the smallest in Arber Acres ( $1.5 \pm 0.2$ ). The percentage of each visceral organ weight was similar among breeds for each sex.

**Conclusion** Results from this study indicated that although the percentage of the eviscerated carcass was similar among four breeds, carcass yields of some parts were significantly different for some breeds.

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**Keywords:** Carcass yield, Broiler chicken, Cobb, Ross 308, Arber Acres, Hubbard

# Comparisons of Bone Lengths in Four Breeds (Cobb 500, Ross 308, Arbor Acres and Hubbard) of Male Broiler Chickens Raised under Commercial Housing System

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## Abstract

**Objective** To compare the length of particular bones starting from day old chick to market age of four broiler breeds (Cobb, Ross 308, Arber Acres and Hubbard) raised under commercial housing system.

**Materials and Methods** A total number of 1,200 male broiler chickens (n = 300 in each breed) were used in this study. Each breed was raised under identical conditions of the commercial housing and feeding system and in the same rearing period. For study eligibility, chickens must have their body weight within  $\pm 2.5\%$  of their standard body weight for each breed. Bone lengths including back bone length, keel length, middle toe length, and shank length were measured at day old chick (DOC), 7, 14, 21, 28, and 35 days of age. Live body weight was also measured. Bone lengths were calculated and expressed as a ratio (percentage) of the body weight. One way analysis of variance (ANOVA) with Tukey's HSD test was used to compare bone length among chicken breeds.

**Results** Unaware of breeds, the ratio of all bone lengths was significantly reduced with age. At DOC, the ratio of back bone length in Hubbard was significantly greater than that of the other breeds. However, the values of this variable were not significantly different at 21, 28, and 35 days of age.

**Conclusion** The ratio of all bone lengths was significantly reduced with age. When the age of broiler chickens increased, variation in the ratio of bone lengths was significantly decreased.

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**Keywords:** Broiler chicken, Bone length, Cobb, Ross 308, Arber Acres, Hubbard

# A Model Development for Native Fowl Health and Environmental Managements under the Sufficiency Economy Concept for Parasitic Prevention and Control in a Rural Village, Khon Kaen Province, Thailand

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## Abstract

**Objective** The objective was to develop a model of an educational program under the sufficiency economy concept to improve animal health and environmental health managements for prevention and control internal and external parasites in fowl for native fowl small-holders.

**Materials and Methods** A total of 10 native fowl small-holders in Bankudwa, Dondu district, Amphou Nongsonghong, Khon Khean province, Thailand were selected for this study. All the farmers had a fowl number at least 30 and were educated with the educational program. Some information was surveyed from each farmer such as fowl number, fowl housing, vaccination program, internal and external parasites control, feeding, marketing. In addition, 120 and 48 samples of feces and feather were taken for parasitic examination, respectively. The participant farmers were asked to take pre-test and post-test including meeting our staffs every week.

**Results** A fowl number of the participant farmers were 797 before receiving an educational program and sharply increasing to 1,129 (or 41.66%) after the program. Fecal and feather samples taken before the program were 101 (84.17%) positive for internal parasites and 3 (6.25%) positive for external parasites. Pre-test scores had been collected. At present, our research is on going to get more data of post-test scores including samples collection after the program.

**Conclusion** All poor farmers had not enough fund for having closing house, fence protecting animal from predators, vaccines and drugs. These problems were risk factors that mainly made the farmers losing animals. Their fowl kept and feed in open areas, particularly feeding on the ground. This increased a risk for parasitic infection. In addition, all fowl had no vaccine program including parasitic elimination.

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**Keywords:** Native fowl, Parasite, Sufficiency economy

# Prevalence and Antimicrobial Resistance of *Salmonella* spp. Isolated from Broilers in Khon Kaen Province

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## Abstract

**Objective** This objective in this study was determine to prevalence and antimicrobial resistance of *Salmonella* spp. isolated from broilers in Khon Kean Province.

**Materials and Methods** During December 2016 – October 2017, 400 samples of broilers were collected by cloacal swab from three poultry slaughterhouses in Khon Kaen province. The identification of *Salmonella* used ISO-6579: 2002 method. Antimicrobial susceptibility testis determine by agar disc diffusion tests following by CLSI with 9 antimicrobials: Ampicillin, Amoxicillin/Clavulanate, Ceftazidime, Chloramphenicol, Ciprofloxacin, Nalidixic, Norfloxacin, Tetracycline and Trimethoprim/Sulfamethoxazole.

**Results** The prevalence of *Salmonella* spp. in broiler in Khon Kaen province is 9% (n=36). 75% of isolates were resistant to Ciprofloxacin and Nalidixic acid (69.4%) and highly sensitive to Chloramphenicol (97.2%) and Tetracycline (97.2%) followed by Amoxicillin/Clavulanate (94.4%), Ceftazidime (94.4%), Norfloxacin (94.4%), Trimethoprim/Sulfamethoxazole (94.4%) and Ampicillin (86.1%).

**Conclusion** To prevent and control of Salmonellosis in broiler, well organized farm management is essential to keep the broiler healthy so the product is safe to consume. Slaughter house must be appropriate with good hygiene standards and also the well manage slaughter system to deliver the harmless product for the customers.

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**Keywords:** Prevalence, Antimicrobial resistance, *Salmonella* spp., Broiler

# Prevalence and Risk Factors of Foot and Mouth Disease on Pig Farms in Amphoe Mueang, Nakhon Pathom

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## Abstract

**Objective** To study the prevalence and identify potential risk factors of Foot and Mouth Disease on swine farms in Amphoe Mueang, Nakhon Pathom during June 2016 to May 2017

**Material and Methods** In this study, data were collected by questionnaires, interview provided by the swine farmers in the area of Amphoe Mueang, Nakhon Pathom about 107 farms took part in the study between January 2017 to May 2017, data collection is concerning general information about farms, farms management, history of FMD outbreak from June 2016 to May 2017 and risk factors information. Then risk factors were analyzed by multivariate logistic regression

**Results** From the data collected, there were 86 out of 107 farms that had FMD outbreaks. 80.37% was affected from June 2016 to May 2017. The major risk factors found were disinfecting the market place and (OR=3.281; 95%CI=0.99-10.87; p=0.05) and no preventive measures to get rid of rats (OR=3.141; 95%CI=1.079-9.139; p=0.036)

**Conclusion** The Good management and biosecurity improvement may prevent future Foot and mouth disease outbreak in pig farms.

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**Keywords:** Foot and Mouth Disease, Risk Factors, Pig farms

## Introduction

Foot and Mouth Disease (FMD) is a contagious disease that severely affects cloven hoofed animals such as buffaloes, beef cattles, dairy cattles, goats, sheeps, pigs and some wildlife ruminant [1] It can cause economic damage both because of the cost control the disease and due to the animal trade barrier. The stoppage of international distribution of animal products impacts the income of our country. FMD is caused by Foot and Mouth Disease virus (FMDV), a member of the genus *Aphthovirus* in the family *Picornaviridae*, single-stranded RNA, non-enveloped, about 26 diameter [4]. There are 7 serotypes of FMDV such as, O, A, C, South African Territory (SAT)1, SAT2, SAT3 and Asia1 where there is no cross immunity. Moreover, we had reports of the spread of serotype O, A and Asia1 in Thailand [5], especially these serotypes found spreading in South East Asia [6]. The symptoms are; a high fever of about 39-40 degree Celsius, depression, hypersalivation, the occurrence of vesicles on the nose, tongue, lips, oral cavity, between the toes, above the hooves, teats and pressure points on the skin. Pregnant sows may abort or deliver stillborn, infected young, a drop in milk production in dairy cattle and can cause sudden death by failure of blood circulation or myocarditis in young animal [7, 8]. Infected animals disseminate virus in their excretions and secretions (breath, saliva, milk, urine, feces and semen). Moreover it can be spread by directly contact to susceptible animals with contaminated mechanical vectors (human, footwear, clothing, vehicles, etc.), food products containing infectious pork and aerosol. [9]. Nakhon Pathom has many swine farms, 50% is located in Amphoe Mueang, Nakhon Pathom which consist of 210,000 pigs or 2.16% from domestic pigs [10]. Therefore, it's a high risk area to the spreading the disease rapidly. Making it essential to study the risk factors for how the disease is spreading and in order to find preventive measures for controlling the disease. The objective of this study

is to find the prevalence and identify potential risk factors of Foot and Mouth Disease on swine farms in Amphoe Mueang, Nakhon Pathom during June 2016 to May 2017 and to bring the results of controlling and spread of FMD. The study will also be beneficial for conducting effective controlling and preventative methods against the spread of disease.

## Materials and Methods

This study was conducted by questionnaire to gather sample informations from the swine farmers in the area of Amphoe Mueang, Nakhon Pathom during June 2016 to May 2017.

### Data collection:

Informations from questionnaire including general information about farm, number and type of swine, history of FMD outbreak in a period of time and asking about other risk factors in farm that can be increased or decreased opportunities of FMD . The information was collected in Microsoft Excel®.

### Data analysis:

Analyzed the prevalence of infected farms from total, definition infected farmis at least 1 pig in farm showing suspicious of FMD symptom. Analysis the risk factors of FMD by multivariate logistic regression in SPSS version 20.

## Results

From the study, the pig farm can be classified into three types naming a farrowing to finishing farm, farrowing to nursery farm, and finishing farm, respectively. The number of farrowing to finishing farm and finishing farm is about the same, 46 farrowing to finishing farm (43%) and 44 finishing farm (41%). There are 17 Farrowing to nursery (16%). From the questionnaire, there were 86 out of 107 farms in the area of this study that had the history of FMD infection from June 2016 to May 2017 (Table 1). All data were analyzed by multivariate logistic regression. It was found out that the relating two main factors were no appropriate disinfection at the market place and no effective measures to get rid of rats (Table 2)

Table 1 Number of FMD outbreak farms in Amphoe Mueang, Nakhon Pathom province

Farm Type	No. of surveyed farms	No. of FMD outbreak farms
Farrowing to finishing farm	46	36
Farrowing to nursery farm	17	9
Finishing farm	44	41

Table 2 Risk factors associated with FMD outbreak in Amphoe Mueang, Nakhon Pathom province

Factor	Odds Ratio	95% Confidence Limits	P-value
no appropriate disinfection at the market place	3.281	0.99-10.87	0.05
no effective measures to get rid of rats	3.141	1.079-9.139	0.036

## Discussion

From the study of FMD in swine farming in Amphoe Mueang, Nakhon Pathom area was found that there are 2 main kinds of swine farming. Most farms are farrowing to finishing farm run by independent farmers and solely finishing farm run by 29 contract farmers and 11 independent farmers. From the questionnaire, it was found that contract farming transport food all together with other farms nearby which may be a channel to spread diseases [11]. In June 2016 to May 2017, FMD was spread through 86 nearby farms. The FMD is an airborne disease and has spread throughout the farming area. The risk factor is farmers do not use disinfectant in swine marketplace and there is no preventive measure from rats that can be related to a circulation of disease [12]. The marketplace is high risk area because it is in contact with contaminated swine transport vehicles and traders who may bring disease to new farms [12]. This study has found that biosecurity is a significant factor not only to prevent FMD but also to help in development of preventive measure to controlling all disease in swine farming.

## Acknowledgements

First and foremost, thanks to farmers for cooperating and giving information and to Mr. Thanathorn Waraeksiri who provided me with the means to getting the data. Furthermore, I really appreciate Subdistrict Administration Organization of Amphoe Mueang, Nakhon Pathom for giving me a list of farms.

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# Energy Providing by Lecithin and L-carnitine Supplemented to Lactating Sow

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## Abstract

**Introduction** A study was conducted to investigate effect of Lecithin, L-carnitine and natural extract containing in Synerlac to stimulate milk production on improving litter growth performance and preventing losses in suckling piglets.

**Materials and Methods** Twenty lactating sows were presented by separating 10 sows with control group and 10 sows with treatment group. Feeding control group was normal feed and Treatment group was lecithin and l-carnitine mixed with normal feed. Lecithin and l-carnitine (Synerlac) were administered by mixed with feed in ration 1.5 kg/ton of feed from farrowing day until weaning.

**Results** The result demonstrated that ADLWG (Average daily litter weight gain: ADLWG) 1,767 g/piglet/day of Synerlac group had significantly difference from 1,430 g/piglet/day of control group (P<0.05). Synerlac group had no difference of %pre-weaning mortality (%PWM) from control group 8% and 6% (P>0.05). However, there is significantly difference in lactating period between 2 groups (P<0.05).

**Conclusion** This study demonstrated that lecithin and l-carnitine supplementation (SynerLac) in lactating sows can improve lactation performance of sows as a resulting in increasing litter performance or ADLWG.

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**Keywords:** Synerlac, Lactating period, Growth Performance, ADLWG

# Effects of Acidity and Somatic Cell Count on Alcohol Stability Test in Raw Milk

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## Abstract

**Objective** To determine a relationship between the alcohol stability test and other parameters that are acidity and somatic cell counts (SCC).

**Materials and Methods** A total of 75 composite milk samples were collected from individual milking cows of the smallholder dairy farms at milk in Khon Kaen province. All milk samples were measured by using the alcohol stability test and California Mastitis Test (CMT). Somatic cells in all milk samples were counted by using DeLaval™ cell counter (DeLaval, Sweden). In addition, the acidity of all milk samples was further determined using the digital pH meter (Beckman Coulter pH410 Meter, USA). The difference in milk pH, milk SCC between the milk samples negative and positive for the alcohol stability test was analyzed. Also, the correlation between milk pH and log-transformed milk SCC was determined. The significant difference was considered if the probability was below 0.05.

**Results** Forty-four (58.6%) of 75 composite milk samples were positive for the alcohol stability test. The mean pH of the negative and positive results was 6.67±0.01 and 6.58±0.02, respectively. The difference in milk pH between the samples that were positive and negative of the alcohol stability test was significantly statistical detected ( $P<0.01$ ). The higher variations of milk pH of the positive samples were higher than that the negative samples. The mean SCC of the negative and positive results were 540±882 (x 1,000 cells/mL) and 383±615(x 1,000 cells/mL), respectively. However, there was no difference in milk SCC between the groups of the alcohol stability test. Furthermore, the correlation between milk pH and log SCC was not statistically detectable.

**Conclusion** The alcohol stability test of milk was potentially influenced by its acidity but was not affected by the milk SCC.

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**Keywords:** SCC, Alcohol stability test, Raw milk, Acidity

# Relationship between Somatic Cell Counts and Concentrations of Aflatoxin M1 in Raw Milk

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## Abstract

**Objective** To determine a relationship between somatic cell counts (SCC) and AFM1.

**Materials and Methods** Quarter milk samples were collected from all quarters in 18 cows from a small holder dairy farm in Chiang Mai province. Milk samples were measured their SCC using CombiFoss7<sup>TM</sup>7 (Foss, Denmark) and their AFM1 concentration using Charm MRL Aflatoxin M1 Quantitative Test (Charm Sciences Inc., USA). A repeated measure analysis using Proc Mixed (SAS, student edition) was used to determine the relationship between SCC and AFM1. The significant difference was define at P<0.05.

**Results** A total of 69 samples were collected and 64 samples were contaminated with AFM1 ranging from 17 to 67ppt including 10.9% (7/64) samples with AFM1 above 50 ppt, the EU regulation limit of AFM1. SCC ranged from 6 to 7,991x10<sup>3</sup> cell/ml. Due to non-normal distributed data, both SCC and AFM1 were firstly logarithm transformed before the final analysis. A result from the mixed model analysis shows that SCC was significantly related to AFM1. Each unit of log-transformed SCC was decreased log-transformed AFM1 at 0.11 unit ( $\beta = -0.1137$ ).

**Conclusion** An increased SCC is related to decrease AFM1.

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**Keywords:** Somatic cell count, Aflatoxin M1, Raw milk

## Introduction

Aflatoxin M1 (AFM1), a hydroxylated metabolite of AFB1, has been found in the milk from lactating animals receiving feedstuffs contaminated with AFB1 [1] and is now classified as a group 1 human carcinogen [2]. The bioconversions of AFB1 is initiated by various oxidative reactions catalyzed by microsomal hepatic cytochrome P450 enzyme, and then metabolizes to AFM1 by hydroxylation in liver and consequently excrete to milk [3]. The excretion of AFM1 into milk vary greatly between individual cows and AFB1 intake ranging from 0.018 – 6.2% [1,4]. After metabolizing in liver, AFM1 is circulated in blood vessels and transported to milk through passive diffusion across mammary gland epithelium [5]. Previous reports indicated that the excretion of AFM1 is affected with mammary alveolar cell membrane health [1, 6-7]. Milk somatic cells directly represent the inflammatory status of the cow udder. While an inflammation occurs, the tight junction of alveolar cell membranes might be disrupted and increased blood-udder permeability resulting in an influx of somatic cell (SCC) [8] and diffuse of AFM1 from blood to milk [1]. Previous study indicated that increasing the number of somatic cells by increasing alveolar membrane permeability may affect AFM1 carry-over rate. [1,9]. However, more recent study suggests that the excretion of AFM1 was not affected by the SCC [6]. Up to date, there is limited information about the relation of somatic cell count which is the indicator of udder health to AFM1 in milk. Therefore, the objective of this study was to evaluate the relationship between somatic cell counts on concentration of AFM1 in raw milk.

## Materials and Methods

### Sample collection:

A total of 69 quarter milk samples from 18 cows were collected from a small holder dairy farm in Chiang Mai province. Thirty milliliters of milk were collected and then stored in styrofoam box with ice pack (4°C) and transferred to laboratory.

### SCC analysis:

All milk samples were measured their SCC using CombiFoss7<sup>TM</sup>7 (Foss, Denmark). The analysis procedure was described by a guide for Installation, Operation and Maintenance. The milk samples were heated to 40°C in a water bath and held at this temperature for 15 min. The samples were then double processed in a machine. The reagents were prepared following the manufacturer's instructions.

### AFM1 analysis:

AFM1 concentration was determined by using Charm MRL Aflatoxin M1 Quantitative Test (Charm Sciences Inc., USA). Samples were kept on ice and immediately transported to the milk quality laboratory, Faculty of Veterinary Medicine, Chiang Mai University. The samples were mixed well before testing. Three hundred microliters of each milk sample were added to the MRLAFMQ test strip and incubated at 40 °C on the ROSA incubator for 15 min. Subsequently, the test strips were removed and inserted into the Charm EZ READING instrument for 1 min to determine AFM1 concentrations.

### Statistical analysis:

A repeated measure analysis using Proc Mixed (SAS, student edition) was used to determine the relationship between SCC and AFM1. Cow identification was defined as a random factor. The significant difference was determined at  $P < 0.05$ .

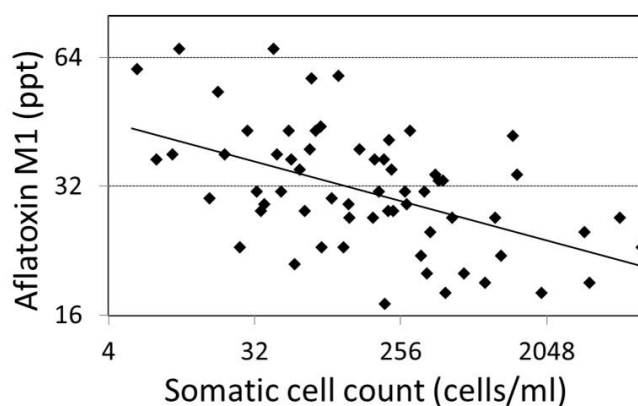
## Results

Results from AFM1 analysis showed that 64 (92.8%) of 69 samples were contaminated with AFM1, the concentrations ranged from 17 to 67 ppt (Table 1). Approximately eleven percent samples (7/64 samples) possess the AFM1 concentration more than 50 ppt which above level of EU regulation limit [9]. SCC ranged from 6 to  $7,991 \times 10^3$  cell/ml. Due to non-normal distributed data, both SCC and AFM1 were firstly logarithm transformed before the final analysis. A result from the mixed model analysis shows that SCC was significantly related to AFM1. An increased SCC is related to decrease AFM1 (Figure 1). Each unit of log-transformed SCC was decreased log-transformed AFM1 at 0.11 unit ( $\beta = -0.113$ ).

**Table 1.** Somatic cell counts and AFM1 concentration in milk from different quarters of udder

Udder quarters	SCC ( $\times 10^3$ cell/ml)			AFM1 (ppt)		
	Mean	S.E	Range	Mean	S.E	Range
All quarters	687.1	179.2	6-7,991	33.4	1.7	17-67
RF	778.7	411.1	8-6,327	31.9	3.3	27-43
LF	756.6	411.3	6-5,851	31.9	3.3	17-60
RH	657.8	361.4	21-1,279	32.1	3.0	19-57
LH	636.3	361.4	10-7,991	32.6	3.2	18-67

RF: Right front quarter, LF: Left front quarter, RH: Right hind quarter, LH: Left hind quarter



**Figure 1.** The relation of somatic cell counts and concentrations of aflatoxin M1 in raw milk

## Discussion

The detection levels of all quarter milk samples were below US FDA regulatory limit at 0.5 ppb [10]; however, few samples were higher than that of EU regulatory limit at 0.05 ppb [10]. AFM1 from blood are rapidly adsorbed through alveolar epithelial membrane to milk by passive mechanism because their low molecular weight characteristics [5]. Hence, there are assumptions that during udder inflammation, the functional integrity of the blood-udder barrier may be temporarily damaged, leading to an accelerated influx of SCC which is directly representation of inflammatory status of mammary gland and may leading to more diffuse of AFM into milk [8]. Therefore, a previous report suggested a positive correlation between SCC and milk AFM1 [7]. However, our results show a negative relation between SCC and AFM1 indicating an increased SCC is related to decreased AFM1. The negative relations between SCC and AFM1 might be explained by the effect of aflatoxin to overall immune system of cattle. Aflatoxin B1 is able to affect the inflammatory response by inhibits phagocytosis and general mechanism responsible for the broad immunosuppressive effect. Therefore, increasing of AFB1 intake resulting in an increasing of AFM1 in milk, whereas, decreasing of SCC from immunosuppressive effect [11]. Furthermore, the increased mammary gland permeability as a consequence of inflammatory processes alone does not seem to explain the effect of the AFM1 concentration [1, 6]. Several studies indicated that the excretion of AFM1 is affected by numerous factors including the state of lactation, health status, capacity of transformation of aflatoxin in liver and milk yield. Veldman et al [1] and Pettersson et al. [12] also found that the main factor contributing to the total AFM1 concentration is milk yield but not SCC. In addition, the amount of AFM1 in milk is major positively related to the amount of AFB1 ingestion from feeds [13].

## Acknowledgements

We would like to thank dairy farmers in Mae-Wang dairy cooperatives, Chiang Mai for their grateful participant. We are also grateful to Charm Sciences Inc, USA for providing the analytical instruments to fulfill this research.

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# Prevalence of Mastitis Pathogens from Clinical and Subclinical Mastitis in Dairy Cows

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## Abstract

**Objective** The aim of current study was to assess clinical and subclinical mastitis and determined the bacterial causes of subclinical mastitis in dairy cows.

**Materials and Methods** A cross sectional study was conducted on a total of thirty three dairy cows from dairy farm in Chumphon province, Thailand. The milk samples were tested by California Mastitis Test (CMT) for detection of subclinical case. Mastitis pathogens were identified based on colony characteristic, Gram's staining and biochemical tests.

**Results** Prevalence of clinical mastitis and subclinical mastitis on CMT basis was 5.71% (n=33) and 94.29% (n=80) in milk sample and quarter basis, respectively. Identification bacterial culture showed eighty isolates of seven genera i.e. Staphylococci, Streptococci, Corynebacterium, Klebsiella, Micrococcus, Bacillus and Escherichia. The prevalence of mastitis pathogen was Corynebacterium (15.00%), *Staphylococcus aureus* (10.00%), *Streptococcus uberis* (22.50%), *Staphylococcus epidermidis* (6.25%), *Staphylococcus haemolyticus* (5.00%), *Staphylococcus chromogenes* (7.50%), Micrococcus (12.50%), *Klebsiella pneumoniae* (5.00%), Bacillus (6.25%), *Escherichia coli* (7.50%) and Staphylococci (2.50%).

**Conclusion** The CMT test is simple and easy for dairy farmer. We recommended the CMT to dairy farmer. It should be for screening test before milking process to good milk quality.

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**Keywords:** Clinical mastitis, Subclinical mastitis, Prevalence, Dairy cow

## Introduction

Mastitis is a costly disease in dairy cows. The disease is damaged milk producing tissues of the mammary gland which caused by bacteria pathogens and induce inflammation of the mammary gland leading to decreased milk yield and impaired milk composition (Harmon, 1996). Bacteria penetrate to the teat canal in several ways between milkings, bacteria pass through the teat canal by multiplying inside the canal. During machine milking, bacteria may be propelled into the teat canal, teat cistern and udder. The potential invasion is greatly increased by bacteria that reside in or colonize the teat duct. Such colonizations occur in both lactating and dry cows, and the colonizing bacteria may survive for months, serving as sources of bacteria for infecting the gland (Auld and Hubble, 1998). The inherent virulence of the bacterial species is often associated with an ability to adhere to mammary epithelium. *St. agalactiae* and *S. aureus* adhere well but *E. coli* does not; however, multiplies rapidly (Auld and Hubble, 1998). As part of the cow's defense mechanism, the new intramammary infection is quickly followed by an influx of leukocytes into udder and an increase of the somatic cell counts (Bruckmaier and Blum, 2004). Mastitis also results in a reduction in synthesis of the main components of milk, such as lactose, milk fat, solids not fat and casein (NMC, 1996) and caused to increase in milk somatic cell count (Forsback et al., 2009). Furthermore, the bacteria produce virulence factors, toxins and irritants causing swelling and death of alveoli (Bruckmaier and Blum, 2004). The cellular damage can produce holes within the mammary epithelium that can lead to changes in milk composition and short circuit the blood-milk electrical potential in the same manner as opening of tight junctions. For example, lactose which is synthesized by mammary epithelial cells, partially leaks into blood circulation through the damaged blood-milk barrier (Bruckmaier and Blum, 2004). Simultaneously, there is an increase of the concentrations of blood borne components in the milk of affected quarters, such as serum albumin, sodium and chloride ions.

Subclinical mastitis is an important more than clinical mastitis because it shows no visible changes in the milk or the udder but decreased in milk yield and bacteria are presented in mastitis milk (Erskine, 2001). California Mastitis Test (CMT) is a screening method for assess subclinical mastitis in dairy farms (Khanal and Pandit, 2013). So, identification bacterial culture is calculated prevalence of mastitis pathogen which interest in treatment and farm management. In the present study, incidence of clinical mastitis and subclinical mastitis was assess in milk samples in Chumphon and determined the bacterial causes of subclinical mastitis in dairy cows.

## Materials and Methods

Milk samples were collected in thirty three dairy cows from thirteen dairy farms in Chumphon province. Chumphon is a small province in southern part of Thailand. The people are a gardener and dairy farmer in Pathiu and Thasae districts. Therefore, dairy farms, dairy cows and milk samples are a few. At the farm, the California Mastitis Test (CMT) was used to screening the quarters of the udder and the results were classified by CMT score which Somatic Cell Count (SCC) estimation (Table 1.). Questionnaires were used for interview owner's farm about mastitis in dairy cows and environment in dairy farms. The most informations were used for assessed clinical mastitis and subclinical mastitis. Eighty milk samples were collected from quarters by aseptic technique after CMT screening test. These samples were numbered and marked as right front, right hind, left front and left hind. After that, the milk samples were stored in ice cooler box and transferred to laboratory. Bacterial identification was performed according to Osbaldiston (1973). Pipette one microliters of milk sample was streaked on blood agar plates and MacConkey 's agar plates. Plates were incubated at 37 °C for 24 to 48 h. Colonies were identified on the basis of colony characteristic, gram stain, morphology, hemolysis patterns and the numbers of each colony type were recorded. Selected colonies were subcultured on blood agar plates and MacConkey 's agar plates, and incubated at 37 °C for 24 to 48 h. to obtain pure cultures. Catalase production was tested for gram positive cocci. Gram negative isolates were identified by colony morphology, gram staining, oxidase and biochemical reaction test on MacConkey's agar plates

**Table 1.** CMT score and estimation of Somatic Cell Count

CMT Score	Somatic cells level (cells/ml)
0 (Negative)	< 200,000
Trace	150,000 – 500,000
+1	400,000 – 1,500,000
+2	800,000 – 5,000,000
+3	> 5,000,000

## Results

Prevalence of clinical mastitis and subclinical mastitis on CMT basis was 5.71% (n=33) and 94.29% (n=80) in milk sample and quarter basis, respectively. Identification bacterial culture showed eighty isolates of seven genera i.e. *Staphylococci*, *Streptococci*, *Corynebacterium*, *Klebsiella*, *Micrococcus*, *Bacillus* and *Escherichia*. The prevalence of mastitis pathogen was *Corynebacterium* (15.00%), *Staphylococcus aureus* (10.00%), *Streptococcus uberis* (22.50%), *Staphylococcus epidermidis* (6.25%), *Staphylococcus haemolyticus* (5.00%), *Staphylococcus chromogenes* (7.50%), *Micrococcus* (12.50%), *Klebsiella pneumoniae* (5.00%), *Bacillus* (6.25%), *Escherichia coli* (7.50%) and *Staphylococci* (2.50%) (Table 2). The bacterial isolated in milk sample is similar to other studies in other countries. The potential factors have greater risk of mastitis such as number of parturition, age, hoof health, teat dip. However, the most subclinical mastitis cases are chronic mastitis and dangerous to consumer more than clinical mastitis



**Table 2.** Isolated mastitis pathogens from dairy milk quarter

Isolated bacteria	Number of isolated	Percentage (%)
<i>Corynebacterium</i>	12	15.00
<i>Staphylococcus aureus</i>	8	10.00
<i>Streptococcus uberis</i>	18	22.5
<i>Staphylococcus epidermidis</i>	5	6.25
<i>Staphylococcus haemolyticus</i>	4	5.00
<i>Staphylococcus chromogenes</i>	6	7.50
<i>Micrococcus</i>	10	12.50
<i>Klebsiella pneumoniae</i>	4	5.00
<i>Bacillus</i>	5	6.25
<i>Escherichia coli</i>	6	7.50
<i>Staphylococci</i>	2	2.50
Total	80	100

## Discussion

The milk samples were tested by California Mastitis Test (CMT) for detection of subclinical case. The CMT test is simple and easy for dairy farmer. We recommended the CMT to dairy farmer. It should be for screening test before milking process to good milk quality. The mastitis pathogens were identified based on colony characteristic, Gram's staining and biochemical tests. Subclinical mastitis was found higher in late lactation stage compare to early lactation. The farm management is a method for good preventing the disease. For example, milk hygiene, udder health management and nutritional management. However, the potential factors are the most important associated severity in subclinical mastitis in dairy cows and dairy farm. If, the farmer found the causative agent of mastitis. The farmer should be control mastitis in dairy cows.

## Acknowledgements

The authors sincerely wish to thank the staff of the Department of Veterinary Public health and Diagnostic Service and The Animal Disease Diagnostic Unit, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus for providing the facilities and all instruments. The authors also grate thanks to the King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon campus for the financial support in this study.

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# Prevalence and Antimicrobial Resistance Pattern of *Streptococcus agalactiae* Isolated from Mastitic Cows in Lamphun, Thailand

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## Abstract

**Objective** The objectives of this study were to determine prevalence of Streptococcal intramammary infection (IMI) and antimicrobial resistance pattern of *S. agalactiae* in mastitic cows kept on smallholding, Lamphun, Thailand.

**Materials and Methods** A cross-sectional study was conducted between January 2015 to December 2017 in Lamphun, Thailand. A total of 162 milk samples from mastitic cows were examined microbiologically according to the National Mastitis Council. Drug susceptibility testing was performed by disk diffusion method. The epidemiological data and the patterns of antimicrobial resistance of *S. agalactiae* were evaluated by descriptive statistical analysis.

**Results** The quarter-level prevalence of streptococcal mastitis was 67.9%. Overall prevalence of contagious *S. agalactiae* (36.4%) clearly predominated followed by environmental streptococci including *S. uberis* (30.9%), *S. bovis* (5.5%) and *S. dysagalactiae* (3.6%). The *S. agalactiae* exhibited high percentages of resistance to 100.0% gentamicin (CN), 100.0% streptomycin (S), 92.5% kanamycin (K), 90.0% sulfamethoxazole-trimethoprim (SXT), 52.5% tetracycline (TE) and 22.3% chloramphenicol. Moreover, 100.0% of the strains showed resistance to more than one antimicrobial agent. Eleven antimicrobial resistance patterns were demonstrated. The highest frequency antimicrobial resistant pattern was “CN, S, K, SXT TE” (42.5%) followed by “CN, S, K, SXT” (30.0%) and “CN, S, K, SXT, C” (7.5%).

**Conclusion** The present study concluded that prevalence of mastitis particularly the IMI of *S. agalactiae* was major problem of smallholder dairy farms. Alarmingly, antimicrobial therapy of streptococcal mastitis should be used with caution. Therefore, efforts should be directed to the decreased contagious mastitis by improving post-milking teat dipping, proper milking practice and dry cow therapy.

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**Keywords:** Antimicrobial resistance, *Streptococcus agalactiae*, Mastitis, Bovine

# Investigation of Factors Affecting Milk Composition in Holstien-Friesian Cows, Nakhon Ratchasima, Thailand

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## Abstract

**Objective** The purpose of this study was to investigate whether seasonal-lactation and protein levels in feeds may affect Holstein-Friesian cow's milk composition.

**Material and Methods** The study was conducted on 225 healthy Holstein-Friesian cows between the age of 3-7 years old fed with modified feeds containing 16% (%CP16) or 18% (%CP18) protein. The observed seasons were *Cold or Winter Season* (November to January), *Summer Season* (February to April) and *Rainy Season* (May to October). Milk samples (30mL) were aseptically collected and analyzed using an Automatic Milk Analyzer (Speedy Lab, Italy). The observed data were the percentage of fat, SNF, lactose, total solids and total protein.

**Results** %Fat, %total solid and %protein significantly increased during the summer time ( $P<0.05$ ), while the variation of %SNF and %lactose during the observing period has not been elucidated. These parameters also elevated during the early- and late-lactation periods, but they reduced during the mid-lactation time. Moreover, supplemental feeds with either %CP18 or %CP16 did not affect milk protein, fat, lactose and other solid materials. The significant elevation of SNF content was observed in milk from cows fed with %CP16 ( $P<0.05$ ).

**Conclusion** Both factors (seasons and lactation-period) affected milk composition, especially the %fat, %total protein, and %total solid. The highest values of these parameters were detected during the summer and the late-lactation period. Supplemental feeds with either %CP18 or %CP16 do not affect the milk composition, with the only exception being SNF.

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**Keywords:** Milk composition, Cow, Season, Lactation period, Feed protein

## Introduction

The appeal of milk composition analysis has significantly developed throughout time. The interests include nutritional improvement, added-manufacturing procedure, and modifying raw milk into various finished milk products [1]. These investments are the response to the increasing demand of modern consumers. Additionally, milk composition (Milk Component Yield or MCY) can also determine the market price [2].

Many studies have tried to determine the factors that affect milk components, which decided that milk composition, calories, protein, or carbohydrates may be the significant controller of milk-production process. Other aspects such as breed, age, pregnancy order, disease, and environment factors could also affect milk composition [3-5].

The purpose of this study is to investigate whether seasonal-lactation and protein level in feeds may affect milk composition in 225 Holstein-Friesian Cows at various ages and pregnancy periods within a 12 month time period (May 2016 – April 2017). The nurturing method of subjects was not modified as it was performed regularly. Once complete, the outcomes of this study may become a productive tool to enhance the quality of milk in the future.

## Materials and Methods

### Animals and Samples:

This study tested 225 Holstein-Friesian Cows between the age of 3-7 years old nurtured by Pak Chong Dairy Cooperative Ltd., Nakhon Ratchasima, Thailand. The observed seasons included *Cold or Winter Season* (November to January), *Summer season* (February to April) and *Rainy Season* (May to October). Milk samples were collected from cows that have been separated from their calves for at least 10 days after calving. These cows were fed with modified feeds containing 16% or 18% protein for at least 7 days prior to the sample collection period. The milk collection procedure was performed with aseptic technique at 07.00 and 18.00 daily. Sample analysis was conducted within 24 hours after collection.

### Milk composition analysis:

Milk samples were delivered to the Veterinary Technology Laboratory, NRRU at less than 4°C. Before each analysis, milk samples were adjusted to room temperature or 25 °C in a water bath. The analysis was performed using an Automatic Milk Analyzer (Speedy Lab, Italy) with ultrasonic technique calibrated with the standard method. Briefly, the %fat (Gerber method), %lactose (Lactometer), and solid-not-fat contents of milk samples were carried out as described previously [6]. Milk compositions analyzed were the percentage of fat, Solid-not-fat (SNF), lactose, total solids and total protein.

### Statistical Analysis:

All parameters were expressed as mean  $\pm$  SD. The collected data include the percentage of fat, SNF, lactose, total solids and total protein. Statistical analysis of difference was carried out by analysis of variance (ANOVA) followed by Scheffe's *post hoc* test using SPSS version 11 (SPSS Institute, Inc., Chicago, IL, USA). A probability level less than 5% ( $P < 0.05$ ) was considered statistically significant.

## Results

A diversity of milk compositions was observed depending on the season. Compositions of milk in each season are illustrated on Table 1. %Fat, %total solid and %protein raised during the summer ( $P < 0.05$ ). The variation of %SNF and %Lactose during the observing period has not been elucidated. The highest % protein and %total solid were also observed in the summer time ( $P < 0.05$ ). The lowest values of %fat and %lactose exhibited during the raining season ( $3.22 \pm 0.72$  and  $3.86 \pm 0.54$ , respectively).

**Table 1.** Compositions of milk during Raining, Cold (Winter) and Summer (Nakhon Ratchasima, Thailand).

Seasons	%Fat	%SNF	%Lactose	%Total Solids	% Protein
Raining	$3.22 \pm 0.72^a$	$9.84 \pm 0.77^a$	$3.86 \pm 0.54^a$	$12.49 \pm 0.08^a$	$3.73 \pm 0.64^a$
Cold (Winter)	$3.51 \pm 0.96^a$	$9.50 \pm 0.38^a$	$3.90 \pm 0.58^a$	$12.47 \pm 0.09^a$	$3.56 \pm 0.38^a$
Summer	$4.19 \pm 0.81^b$	$9.75 \pm 0.71^a$	$3.94 \pm 0.50^a$	$12.48 \pm 0.07^a$	$4.55 \pm 0.34^b$

Data express as the mean  $\pm$  SD. Different letters in the same column indicates statistical differences ( $P < 0.05$ ).

Many factors can vary milk composition; lactation period is an interest aspect which affected these parameters. Compositions of milk in each lactation period are presented on Table 2. The average milk yield in each cow during early-lactation, mid-lactation and late-lactation periods are  $9.45 \pm 2.23$ ,  $12.78 \pm 2.13$  and  $10.03 \pm 3.21$  L/day, respectively.

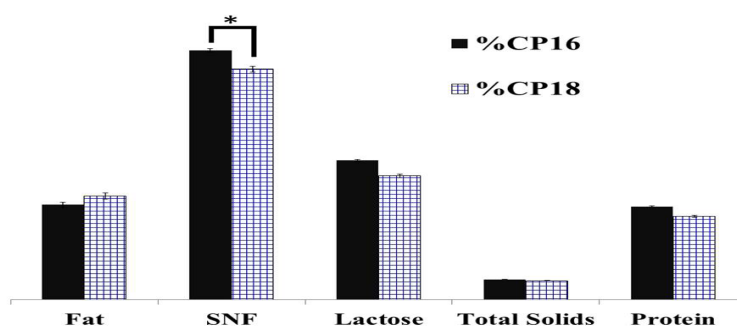
As demonstrated on table 2, % Fat, SNF, total solid and protein increased during early- and late-lactation period, but these data dropped during mid-lactation time. Only %lactose has the tendency to increase throughout the lactation time until at the end of the experiment ( $P > 0.05$ ).

**Table 2.** Effects of lactation period on milk composition

Lactation Period	%Fat	%SNF	%Lactose	%Total Solids	%Protein
Early-Lactation	3.65 ± 0.86 <sup>a</sup>	9.80± 0.51 <sup>a</sup>	3.89± 0.51 <sup>a</sup>	12.63 ± 0.38 <sup>a</sup>	4.17± 0.64 <sup>b</sup>
Mid-Lactation	3.47± 0.54 <sup>a</sup>	9.56± 0.55 <sup>a</sup>	3.94± 0.55 <sup>a</sup>	12.48± 0.07 <sup>a</sup>	3.86± 0.65 <sup>a</sup>
Late-Lactation	3.73±0.94 <sup>a</sup>	10.35 ± 0.67 <sup>a</sup>	4.08± 0.67 <sup>b</sup>	13.01 ± 0.73 <sup>b</sup>	4.79± 0.62 <sup>b</sup>

The data express as the mean ± SD., different letters in the same column indicates statistical differences (P<0.05).

The study of protein concentration on milk composition throughout lactation time was also observed (Figure 1). The result showed that supplemental feeds with either %CP18 or %CP16 do not affect the milk protein or other components such as fat, lactose and other solid materials. A significant elevation of SNF content was observed in milk from cows that were fed with %CP16 feed. In addition, %CP16 supplementation could and tend to increase lactose total solid and protein (P>0.05).



**Figure 1.** Effects of protein-concentrated feeds on milk composition.\* illustrated as the statistical differences between the observed data (P<0.05).

## Discussion

Prior studies reported various factors that influence milk composition. However, providing nutritional-balanced feeds during lactation period is still a significant concern for farmers. This study shows the importance of the early lactation period, as milk composition significantly fluctuate. The post partum-negative energy imbalance of the mother cow could be the cause for the lack of energy during the post-delivery period.

In addition, the ability to produce milk during the early period of post-delivery is naturally low, which could affect milk composition more than the mid-lactation period. This study shows the fluctuation of %fat, total solid, and protein significantly depends on the season. During the intensive lactation period, especially during the summer, the level of %fat and protein significantly increased. The cause of such increase may be due to the rise of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) [7] and  $\alpha$ -Casein [8], respectively. As result, heat stress in ruminants could also lead to a change in milk fat[9] and protein [10] compositions. In this study, %lactose did not significantly change throughout the lactation. The cause of such occurrence may be due to lactose synthesis and the amount of water drawn into milk that makes lactose a stable milk component [11].

Additionally, protein overload did not influence milk composition, with the only exception of SNF. Interestingly, milk of %CP16-fed cow, %fat, SNF, lactose, total solid and protein tend to increase compared to milk from cows fed with %CP18 feeds (P>0.05). Feed restriction during early- and mid-lactation period may lead to reduced energy intake, but there is no significant change in energy balance in both low-protein and high-protein milk. The result collaborates with previous studies regarding protein supplementation in feeds could affect on milk production but not its composition [8,12]. This result could derive from the influence of insulin and glucagon responding to the change of available amino acid pool in circulation [13-14].

## Conclusion

This study demonstrated that the season and lactation period can affect milk composition, especially %fat, %total protein and %total solid. The highest values of these parameters were detected during the summer and the late-lactation period. Therefore, the general compositions of cow milk is influenced by environmental conditions and lactation period more than protein concentration in feeds. Farmers should concentrate on achieving nutritional balance and maintain energy level in order to improve the quality of milk in addition to the improvement of the cows' reproductive system, which has the natural leading role in producing milk.

## Acknowledgements

The authors would like to acknowledge the generous financial support from the Research and Development of the Faculty of sciences and Technology, Nakhon Ratchasima Rajabath University. Finally, the authors also thank the local farms by members of Muak Lek Dairy Cooperative Ltd. for the access to the farms, support on samplings and sample transportation as well as local veterinarians in the region of Nakhon Ratchasima Province, Thailand.

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# Effect of Probiotic *Bacillus subtilis* Supplementation on Lactating Performance and General Health during Post-partum in Dairy Cows

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## Abstract

**Objective** In this experiment, we intend to study the effects of probiotic *Bacillus subtilis* supplementation on milk productive performances and post-partum health in dairy cows under field condition.

**Material and Methods** Thirty-four of cross-bred Holstein-Friesian cows in 6 small holder dairy farms in Mae On dairy cooperative were randomly separated into two groups as control group (n=16) and probiotic *Bacillus subtilis* supplemented group (n=18). Both groups within the same farm were fed by the same basal diet. Supplemented cows were fed probiotic *Bacillus subtilis* at  $1.2 \times 10^7$  cfu/cow/day for 8 weeks interval, from 2<sup>nd</sup> weeks before expected calving date through 6<sup>th</sup> weeks post-partum in morning meal. Milk yield and milk samples were collected every week. Blood samples were collected at -2, 1, 3 and 7 weeks post-partum. Body weight and body condition score were measured at -2, 1, 3, 5 and 7 weeks post-partum.

**Results** No significant difference ( $P > 0.05$ ) was observed in milk yield and compositions.

However, the average milk yield in probiotic *Bacillus subtilis* supplemented group was higher than control group at approximate 1 kilogram. Even though not statistically significant difference but may economically acceptable. Post-partum period was played a major effect ( $0.05 > P < 0.10$ ) on milk yield, milk compositions and serum metabolic profiles. Moreover, probiotic *Bacillus subtilis* supplementation was not found negative effects on serum metabolic profiles.

**Conclusion** Probiotic *Bacillus subtilis* supplementation had no statistical significant difference in milk yield and compositions. However, the supplementation may be used as an alternative way in terms of economical satisfied value of milk yield and safety on serum metabolic profiles.

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**Keywords:** Probiotic, Lactating performance, Post-partum health, Dairy cow



## Live Yeast *Saccharomyces cerevisiae* Supplements for Increased Milk Production

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### Abstract

**Objective** To determine the beneficial effect of live yeast supplement on milk quality in dairy cows.

**Materials and Methods** Twenty milking cows in three small holder farms in Khon Kaen dairy co-operative were used in a randomized block by parity and DIM. Cows assigned into treatment group were supplemented with 10 mg of live yeast (*Saccharomyces cerevisiae*, Yea-Sacc®, Alltech, USA) mixed with the concentrate while cows in the control groups received the placebo for 30 days. The milk samples were collected every weeks for five times and measured for somatic cell count and milk composition by Fossomatic® 5000 (Foss Electric, Hillerød, Denmark).

**Results** The average milk production was significantly increased in treatment group. However, the milk composition was not different between treatment and control groups.

**Conclusion** This study showed that live yeast supplement increased the milk production but had no positive effect on the milk composition.

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**Keywords:** Yeast supplementation, Milk quality, Dairy cows

# Ovulation Induction in Non-pregnant Dry Cow and Repeat Breeder Cows

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## Abstract

**Objective** To investigate an ovulation induction protocol for improving fertility in the non-pregnant dry cows and repeat breeder cows.

**Materials and Methods** Eleven repeat breeder cows and nine non-pregnant dry cows at the Khumjareon dairy co-operative were subjected to an ovulation induction to initiate pregnancy. The CIDR®-B device (Eazi-breed™ CIDR® B containing 1.38 g progesterone, Zoetis Animal Health, USA) was inserted intravaginally for 9 days and 500 IU of eCG (Folligon®, Intervet, MSD) and 25 mg of prostaglandin F2α (Lutalyse®, Pharmacia & Upjohn Company, Kalamazoo, MI, USA) were intramuscularly injected on the day of CIDR device removal. All cows were inseminated with TAI (timed-AI) with an injection of 1,500 IU of hCG (Chorulon®, Intervet, MSD) at 72 hours after CIDR removal. Cows return to estrus after TAI were re-inseminated by conventional AI. Pregnancy was determined by rectal palpation at 60 days after AI.

**Results** Two CIDR®-B devices were lost before the end of the protocol. The overall pregnancy rate was 33% (6/18). Four out of 18 cows (22 %) were pregnant after TAI. Two additional cows (2/14) were pregnant after the following conventional AI.

**Conclusion** Ovulation induction and timed-AI was shown to be an alternative mean for resolving infertile dairy cows. This ovulation induction protocol could be used for dairy cows with reproductive problems.

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**Keywords:** Repeat breeder, Ovulation induction, Dairy cows

# Supplementation of Hormones in Repeat Breeder Dairy Cows

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## Abstract

**Objective** To evaluate the efficiency of human chorionic gonadotropin (hCG) and gonadotropin releasing hormone (GnRH) administered at artificial insemination time on pregnancy rate in repeat breeder cows.

**Materials and Methods** Thirty repeat breeder cows from small holder farms in Khon Kaen dairy co-operative were assigned into two groups. All cows were observed for natural estrus and bred with conventional artificial insemination with an injection of hCG or GnRH. Fifteen cows were injected with 1500 IU of hCG (Chorulon<sup>®</sup>, Intervet, MSD) at the time of artificial insemination. The other 15 cows were injected with 10 µg of GnRH (Receptal<sup>®</sup>, Veterinaria AG, Zurich, Switzerland) at artificial insemination. Pregnancy was determined by rectal palpation at 60 days after AI.

**Results** The pregnancy rates were 46.7% (7/15) and 33.3% (5/15) for hCG and GnRH treated cows, respectively.

**Conclusion** Administration of hormones to promote ovulation was helpful for repeat breeder cows. Human chorionic gonadotropin was found to more efficient in improving pregnancy rates in repeat breeder cows compared to gonadotropin releasing hormone.

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**Keywords:** Repeat breeder, Hormone, Dairy cows

# A Complete Investigation of Dairy Herd Health and Production Management Program Reflected Farm Problems

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## Abstract

**Objective** To demonstrate the using of a complete Dairy Herd Health and Production Management program (DHH&PM) as a tool to investigate dairy farm problems.

**Materials and Methods** Nine dairy farms in Khon Kaen province were investigated for productivity using a complete DHH&PM program in 2017. The two visits in one week had been performed in each farm. The information of farm practices were collected and analyzed for weak and strong points. General information of number of total cattle in farm, cows, replacement and breeding records (ID card or cow card) were analyzed. Days in milk (DIM), proportion of dairy cows (pregnant, lactating, non pregnant, non lactating), semen (Sire) using in farms were recorded. Pregnancy diagnosis was done in cows which were bred (AI) more than 60 days. Reproductive examination was performed in cows that showed no signs of oestrus within 60 days after calving, cows that were rebred more than three times and heifers that never been bred when older than 15-18 months. Information of milk quality from dairy collecting center and milk production of farm were recorded. Milk from all lactating cows were checked by California Mastitis Test (CMT) and bulk milk of farm was collected and analyzed. Milking system and cleaning steps of milking instruments were observed. Body condition score, hoof score and faeces score of individual cows and heifers were recorded. Approximate analyzed of feed composition for lactating cows, dry cows, heifers, replacement and calves were done for crude protein, energy and proportion of roughage to concentrate. Cost of milk production, drug use and good agricultural practice (GAP) were also investigated.

**Results** Overall results in this study showed reproductive problems and longer average days of calving to conception. Most farms (seven out of nine farms) had good milk composition (total solid > 12.25) and only two farms had somatic cells higher than 500,000 cells/ml. Laminitis/acidosis was found in every farms. Body condition score of most cows in every farm showed poor feeding management on pre and post calving period. All farms need to have a regular visit (D HH&PM) to improve productivity.

**Conclusion** A complete Dairy Herd Health and Production Management programme (DHH&PM) could clarify dairy performance and productivity. Problems could be clearly detected and take a better care by regular farm visit.

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**Keywords:** Dairy herd health and production management programme, Dairy cattle, Body condition score

# The Impact of Foot-and-Mouth Disease on Milk Quality and Fertility on Dairy Cattle

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## Abstract

**Objective** The objective was to investigate the impact of Foot-and-Mouth Disease (FMD) outbreak in 2016 on milk quality and fertility of dairy cows in small scale dairy farms.

**Materials and Methods** Dairy farms in Khon Kaen were randomly selected into the study after FMD outbreak in 2016. Milk quality (for eight months) after the FMD outbreak from 20 FMD infected farms and 20 non infected farms in same area were evaluated. Data of reproductive performance of each cows during 2016-2017 retrieved from individual cow card from ten FMD infected farms and from other ten farms which had no FMD infection were analyzed. Data of milk quality were analyzed by repeated measurement linear mixed model. The model included the fixed effect of FMD infected, time of milk quality collection and their interactions, the random effect was considered at farm level and time of data collection was assigned as covariance matrix for repeated effects. Comparing of reproductive performance between farms with FMD infection and without FMD infection was performed by two level random effect linear mixed model to deal with fixed effect as FMD infected and random effect of cows nested in the farm.

**Results** Milk quality was negatively affected by the FMD outbreak. Both somatic cells count and methylene blue reduction test (MB) were higher in FMD infected farms than the non infected farms (440,000 versus 314,000 cells/ml, and 1.54 versus 1.13, respectively,  $p < 0.05$ ) but the percentages of solids not fat and fat were not different. Reproductive performance of dairy cows in FMD infected farms was significantly poorer than those from non infected farms. Days open and calving interval were longer in FMD infected farms than the non infected farms (206.58 versus 163.33 days, and 443.41 versus 413.71 days, respectively,  $p < 0.05$ ), whereas calving to first service, calving to conception and service per conception were similar.

**Conclusion** FMD had an negative impact on fertility and milk quality on dairy farms.

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**Keywords:** FMD, Dairy cow, Fertility, Milk quality

# An Outbreak of Bovine Coronavirus and Rotavirus in Dairy Cows in Khon Kaen Province, Thailand: Case Report

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## Abstract

**Case Description** A total of ten clinical dairy cows with acute diarrhea from four small holder farms in Khon Kaen province during July 2017-February 2018 were reported in this study. Six adult cows and two calves showed bloody diarrhea and were positive for bovine coronavirus. One calf was positive for bovine rotavirus. Co-infection both bovine coronavirus and rotavirus was found in one adult cow.

**Clinical Finding** All cows were presented clinical sign with a diarrhea. The faecal samples were collected from diarrheic cows by directly swab in the rectum. All specimens were submitted in veterinary diagnostic laboratory from Veterinary Research and Development Center (Upper Northeastern Region) and were processed within 24 h and were stored in a freezer at -80 °C. Faecal samples were examined by microscopy for *Cryptosporidium parvum* and coccidia, bacterial culturing for *Salmonella* spp., *E.coli* and *Clostridium perfringens*. The reverse-transcription polymerase chain reaction (RT-PCR) was used to detect bovine viral diarrhea virus (BVDv), bovine coronavirus (BCoV) and rotavirus (ROTAV).

**Treatment and Outcome** Clinical cows were treated with proper antibiotic and supportive fluid. The specimens from clinical cows were quickly confirmed to diagnosis and supportive treatment. The calves with diarrhea recovered about three to four weeks. Adult dairy cow recovered in a few days after treatment for enteritis. Other enteritis pathogens were negative.

**Clinical Relevance** BCoV and ROTAV infections were well known to play an important role with the diarrhea. All cows showed clinical sign with diarrhea syndrome and clinical cows were quickly confirmed to diagnosis and supportive treatment enteritis from the diarrhea. So these were important criteria for clinician to appropriate services including cows and calves good hygienic management should be considerable to prevent and control the disease.

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**Keywords:** Dairy cows, Bloody diarrhea, Coronavirus, Rotavirus, RT-PCR

# Year-Round Survey of Gastrointestinal Parasitic Infestation Prevalence of Chital Deer in an Organic Deer Farm of Phetchaburi, Thailand

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## Abstract

**Objective** To examine the year-round prevalence of gastrointestinal parasite in Chital deer (*Axis axis*) in an organic deer farm of Phetchaburi, Thailand.

**Materials and Methods** The gastrointestinal parasitic investigation was carried out from 40 chital deer of Nongkheurn Wildlife Sanctuary, Phetchaburi Province, Thailand for 6 intervals. Total 240 samples were examined microscopically by Simple floatation, Simple sedimentation, and Modified Mc Master techniques. Data were run by using Multivariate test and the results were expressed in percentage with P-value <0.05 was considered statistically significant.

**Results** Our study found that 22.9% of deer were positive for the eggs of *Paramphistomum* sp., 1.3% was infected with *Fasciola* sp. and 4.6% were infected with *Eimeria* sp. The high prevalences of trematodes infestation were seen in the farmed deer during the early and middle raining season and *Eimeria* sp. infestation showed the highest prevalence in summer. There is no prevalence of nematode infestation occur since ivermectin (50 mg/kg) has been administered to all deer tri-annually.

**Conclusion** A high prevalence of flukes was seen in the deer farm studied, and after a proper management program for control trematodes infection was set, the prevalence of the eggs of flukes and *Eimeria* sp. was significantly decreased. These suggested that good husbandry and health management can enhance deer productivity and enhance disease and/or parasitic resistance which would significantly improve deer immunity for against an infestation of parasites.

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**Keywords:** Chital deer, *Fasciola* sp., *Paramphistomum* sp., *Eimeria* sp.

## Introduction

The deer farming can be more profitable than traditional livestock farming. Deer consume fewer amounts of feedstuff than cattle, fewer pastures damaging, mature more quickly, and can reproduce for up to 20 years in captivity. Farmers can produce a good quality herd on a small area of land because the animals are well adapted to multiple terrains. In Thailand, the deer farming industry has been dramatically increased as a mean of produce several parts of deer for alternative medicinal ingredients as well as for human consumption. An organic deer farm, Nongkheurn Wildlife Sanctuary, is one of the modern farms established 6 years ago aiming for conserve endangered species, center, produce deer products such as antlers and placenta processing, and educational and entertainment center.

Deer are hosts to a wide range of ecto- and endoparasites [1]-[3]. Outbreaks of parasitic diseases among farmed deer in a limited area and intensive management practices lead to more heavily infestation of parasites than wild deer [4]. Parasite in animals, especially in young animals leading to poor condition, lowered body weight gains and reproductive disorders, besides parasitic infestation affect the quality of animal products [5]. Good husbandry and health management not only enhance deer productivity but also enhance disease and/or parasitic resistance which would significantly reduce economic losses due to gastrointestinal

infestation, dividends for deer farming industry [6].

Thus, this study aimed to determine the prevalence of gastrointestinal parasites and to assess the effectiveness of a proper husbandry and health management for controlling different species of deer gastrointestinal parasites in Nongkheurn Wildlife Sanctuary in the period of 1 year.

## Materials and Methods

### Sampling site:

The study was carried out at an organic deer farm, Nongkheurn Wildlife Sanctuary, Cha-am district, Phetchaburi province, Thailand (12°47'59"N 99°58'1"E). The weather in this area is classified as hot and humid, with a long monsoon season and has 3 official seasons: hot (from March through to June), cool (from November to February), and wet (from July to October). The monsoon season usually is accompanied by heavy rain [7].

### Animals and husbandry:

The farm raises 4 types of 320 deer: Chital deer (*Axis axis*), Rusa deer (*Rusa timorensis*), Sambar deer (*Cervus unicolor equinus*) and Hog deer (*Axis porcinus*). The farm consists of 20 paddocks. Each paddock is divided by a 3 m height wire fence. All types of deer are raised in a paddock all year round. The herd was kept in an extensive system, veterinarians came only when veterinary service was required.

### Background of management and the parasite control in the farm:

The handling facilities of animals including ear tag identification, vaccination, parasite control and general farm management. Deer are fed fresh grasses such as Sweet grass jumbo, Napier grass, and Lucy grass each morning and supplementary feeding is common practice during the dry season.

The farm-based deworming program before we started our study was performed triannually with 1% ivermectin injection subcutaneously at the folds of skin behind the shoulder in all animals.

### Collection of fecal samples:

The study was conducted in the period from June 2016 to October 2017, 40 deer dropping were collected randomly from the ground immediately after defecation of deer in paddock at the morning of the day in the period of 1) late of June 2016 2) early of October 2016 3) early of January 2017 4) late of April 2017 5) late of July 2017, and 6) early of October 2017, totally 6 times. Due to the fact that animals of different ages and sexes were kept together, it was impossible to analyze the result with respect to the group of age or sex. After collection of the fecal sample, about 20-25 grams then was separately placed in a dark polyethylene bag and numbered properly. The properly labeled of sealed bags containing fecal sample then kept in a cooling box until brought to the laboratory and refrigerated at 4°C and examined within 2 days.

### Examination of fecal samples:

All samples were examined in the laboratory of the Department of Parasitology, Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya, Thungyai, Nakorn Si Thammarat. The samples were processed for microscopic examination [8]. The ova, cyst, oocyst, and larvae of different parasitic species were identified according to the morphology by Simple floatation and Simple sedimentation techniques for a qualitative screening and counting number of parasites by Modified Mc. Master technique for a quantitative screening.

### Interpretation of data:

Data were run by using SPSS program. Multivariate test was performed and the result was expressed in percentage with P-value <0.05 was considered statistically significant [9].

### Ethical consideration:

This study was conducted after approval of the RMUTSV Institutional Animal Ethics Committee as a non-invasive observation.



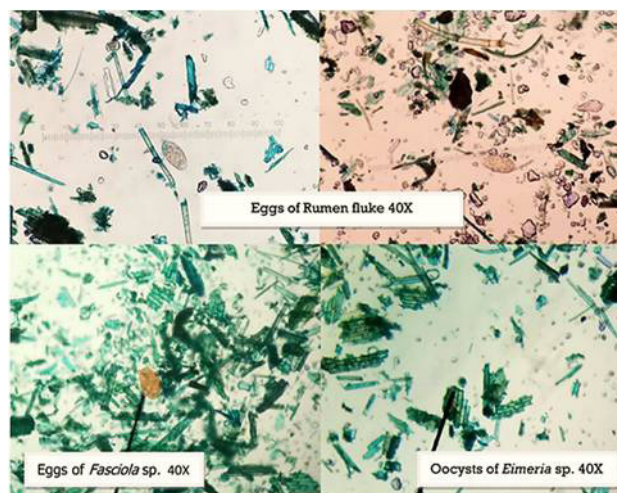
## Results

Total of 240 deer fecal samples collected from June 2016 to October 2017 were examined. For the qualitative fecal examination by using Simple floatation technique showed the negative result. On the other hand, the result from Simple sedimentation technique revealed total 6 intervals of sample collection, 55 deer (22.9%) were positive for the egg of rumen fluke, *Paramphistomum* sp., 3 deer (1.3%) was positive for the egg of liver fluke, *Fasciola* sp. in the first period of the study and 11 deer (4.6%) were positive for the oocyst of *Eimeria* sp. in the 4<sup>th</sup>-6<sup>th</sup> of this study. (Table 1, and Figure 2-3)

For the quantitative fecal examination by using Modified Mc Master technique was negative for both the egg and oocyst.



**Figure 1.** Represent deer paddock condition of the 6 periods in this study: 1) late of June 2016 2) early of October 2016 3) early of January 2017 4) late of April 2017 5) late of July 2017, and 6) early of October 2017

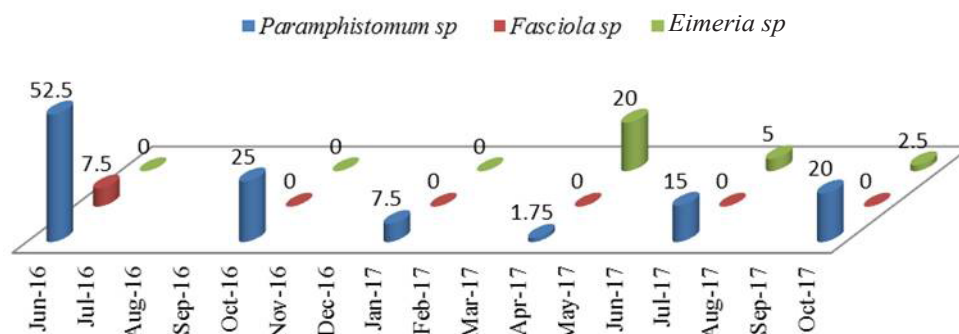


**Figure 2.** The eggs of trematodes and the oocyst of *Eimeria* sp. by using the Simple sedimentation technique

**Table 1.** Number and percentage of deer infected trematodes and protozoa and mean scores for the 6 intervals of the Chital deer fecal sample collection at Nongkheurn Wildlife Sanctuary.

Type of parasites	Period of time	No. of deer	No. of infection	Percent of positive (%)	Mean±Std. Deviation
<i>Paramphistomum</i> sp.	1	40	21	52.5	0.53±0.51*
	2	40	10	25	0.25±0.44
	3	40	3	7.5	0.08±0.27
	4	40	7	1.75	0.18±0.38
	5	40	6	15	0.15±0.36
	6	40	8	20	0.20±0.41
	Total	240	55	22.9	0.23±0.42
<i>Fasciola</i> sp.	1	40	3	7.5	0.08±0.27*
	2	40	0	0	0.00±0.00
	3	40	0	0	0.00±0.00
	4	40	0	0	0.00±0.00
	5	40	0	0	0.00±0.00
	6	40	0	0	0.00±0.00
	Total	240	3	1.3	0.01±0.11
<i>Eimeria</i> sp.	1	40	0	0	0.00±0.00
	2	40	0	0	0.00±0.00
	3	40	0	0	0.00±0.00
	4	40	8	20	0.20±0.41*
	5	40	2	5	0.05±0.22
	6	40	1	2.5	0.03±0.16
	Total	240	11	4.6	0.05±0.21

**Prevalence of gastrointestinal parasitic infestation in Chital deer**



**Figure 3.** Summarised results of percentage of deer infected trematodes and protozoa

The first two highest prevalence of *Paramphistomum* sp. (52.59% and 25%) were in early and middle of rainy season, respectively. The negative result of the egg of *Fasciola* sp. might be correlated with dry weather and a better grass-field management after the first time *Fasciola* sp. positive alert in June 2016. The high prevalence of *Eimeria* sp. in April might be correlated with warm and wet litter which appeared to be the highest suitable in the middle of summer.

## Discussion

The highest prevalence of *Paramphistomum* sp. (52.59%) infestation deer showed in the rainy season

support the earlier reports [10]-[13] which might commonly found in farms which used only ivermectin triannually but not for annually [14]. The low incidence rate of *Fasciola* sp. in this study (1.3%) was also related to the previous report [3], [12], [15] -[8]. 1 month after the data of total deer fecal examination in June with a high prevalence rate of trematode infection, 71.9% (data not shown), farm's veterinarians gave all farmed deer excepted the fragile and high stress sensitive Chital deer with closantel injection (5 ml/kg BW) or ivermectin-F for trematodes controlling. That was the result why we study the year-round prevalence of gastrointestinal parasite only in Chital deer.

*Paramphistomum* sp. is belonged to the trematode phylum of helminth, parasites are most common parasites in the rumen and reticulum of ruminants including deer [19]. *Fasciola* sp. also belongs to the trematode phylum, lives in bile duct of deer. A life-cycle of *Paramphistomum* sp. and *Fasciola* sp were involved with water environment. Adult trematodes live on the ruminal lining of ruminants and release eggs into the lumen of the gastrointestinal tract, then the egg shedding exposes the external environment with the host's feces. Once the eggs hatch, the temporarily free-moving miracidia are released within the environment. Then miracidia locate and penetrate an intermediate host, typically a small snail living in a wet environment until develop into cercariae and shed before develop to be the infectious metacercarial cysts and ingested by the definitive host. The immature flukes then penetrate the duodenal mucosa and migrate to the rumen. The adult parasites attach to the ruminal surface with their acetabula, feeding ruminal contents. Low intensity of adult fluke do not cause clinical signs in infected animals, but the infected animal with high numbers of immature fluke have been shown some clinical signs, such as diarrhea, weight loss, stunting, and death [19].

*Eimeria* sp. is primarily parasites of terrestrial birds and mammals. A life cycle is started when the host ingests a sporulated oocyst then the oocyst release the 8 sporozoites within the intestine. Sporozoites penetrate epithelial cells in the gastrointestinal tract at a specific location of each species. The sporozoite becomes a schizont, in which nuclear division followed by cytokinesis produces masses of merozoites. The infected cell ruptures and the merozoite penetrates an advance epithelial cell. The process is called schizogony which repeats 1-4 times, depending upon the species involved. After the penetration of a late epithelial cell, the merozoite develops into a macrogametocyte or a microgametocyte. Microgametocytes provide microgametes and leave the infected cell to the macrogametocytes containing cells, and fertilize into the zygotes. Then zygotes further discarded its cyst wall and become to the oocysts. After oocysts rupture from the host cell and excreted in the host's expenditures. The unsporulated oocyte contains a non-infective zygote to a new host. A few days later, 8 sporozoites are formed within the oocyst become to a sporulated and infective. Infections with *Eimeria* sp. are self-limiting and the host develops some degree of immunity against reinfection [20].

The most common method of parasitic control is the utilization of chemotherapeutic agents. The study showed that deworming of deer in an organic deer farm tri-annual with 1% ivermectin showed the result in removal of gastrointestinal nematodes in deer. This result supported by work of [14] showed that the efficacy of deworming would be last longer for 45 days of a biannual nematode prevention program. The trematode control program in the rainy season did not completely protect animals against gastrointestinal trematodes because the deer were kept in a limited area of paddocks lead to the accumulation of parasite eggs which was a perfect condition for reinfection. Therefore, the information of the period of a high prevalence of gastrointestinal parasitic infestation from this present study and the data in previous works indicate that systematic parasitological monitoring in farmed deer should be intensive.

## Conclusion

The results of this study can be said that gastrointestinal trematodes and *Eimeria* sp. infestation increase in the rainy and summer season, respectively. This data might help farmers for decreasing the prevalence and intensity of gastrointestinal parasitic infestation prevention program in farmed deer at the accuracy period along with the restrict hygienic management at the period of high infection rate is arrived.

## Acknowledgments

The authors are grateful to Principal Chief of Nongkheurn Wildlife Sanctuary for kind permission for undertaking this work and Dean of Veterinary Science, RMUTSV for providing necessary facilities for our study. We would also like to acknowledge financial support from Research and Development Institute,

RMUTSV funds to carry out the present research work and support our work progress presentation submission fee for The World Parasitology Congress, WAAVP, 2017.

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# Effects of Egg Yolks from various Avian Species in Extender on Goat Semen Cryopreservation

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## Abstract

**Objective** This study was designed to determine the effects of egg yolks from various avian species in extender on the quality of goat spermatozoa after cryopreservation.

**Materials and Methods** The experimental design was Completely Randomized Design (CRD); treatments are egg yolks from 4 avian species: 1) chicken, 2) duck, 3) goose and 4) Japanese quail. Briefly, semen was collected from 1 fertile Saanen buck by electroejaculation once a week (8 replications). After collection, ejaculate was washed by centrifugation at 800 x g for 10 min. The pellet was re-suspended with Tris citric glucose solution (TCG) and divided into 4 aliquots. Then, each aliquot was added with TCG that composed of glycerol and egg yolk from various avian species, the final concentrations of spermatozoa, glycerol and egg yolk were 100 million/ml, 5 and 10%, respectively. All samples were chilled for 3h at 4 °C and then frozen to -196 °C. After thawing, the semen was assessed the motility, viability and membrane integrity.

**Results** Our study indicated that using egg yolks from Japanese quail, goose and duck in semen extender did not showed superior results on semen quality than semen that used egg yolk from chicken ( $p>0.05$ ).

**Conclusion** Due to the used of egg yolks from goose and Japanese quail in extender showed the interesting data on some semen parameters, the further studies in fertility trials should be performed.

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**Keywords:** Egg yolk, Avian, Frozen semen, Goat

# Anticancer Effects of Cepharanthine on Canine Lymphoma Cell Line

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## Abstract

**Objective** In this study, we investigated the anti-tumor effects of cepharanthine (CEP) and doxorubicin on cytotoxicity, apoptosis using canine lymphoma cells.

**Materials and Methods** The cells were treated with 10, 20 and 40  $\mu$ M of CEP and 2  $\mu$ M of doxorubicin. Cytotoxic and apoptosis studies were done after 6, 12 and 24h incubations, respectively. The percentage of apoptotic cells (annexin V<sup>+</sup>/PI<sup>+</sup>) were determined by annexin V-FITC/PI staining assay using fluorescent flow cytometer.

**Results** CEP had cytotoxicity on cells with an IC<sub>50</sub> value of 13.77 $\mu$ M. CEP at 10, 20 and 40  $\mu$ M could significantly induce canine lymphoma cells apoptosis in a concentration- and time-dependent manner.

**Conclusion** These findings demonstrated that CEP could potentially be used as a novel anticancer agent for canine lymphoma cells.

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**Keywords:** Cepharanthine (CEP), Apoptosis, lymphoma

## Introduction

Canine lymphoma is one of the most common cancers in dogs. The majority of lymphoma is adenocarcinomas, which originate from the lymphoid tissues of the hematologic lymphoid organs. Since clinical presentation, biological behavior, and response to chemotherapy of this canine neoplastic disease closely resembles those of human non-Hodgkin's lymphoma, it has been considered as a naturally occurring animal model for human non-Hodgkin's lymphoma [1]. This neoplastic disease is controllable by aggressive chemotherapy with a combination use of several antineoplastic, which results in a high rate of remission. However, relapses are frequently seen despite the aggressive initial treatment, and at that time, these neoplastic tissues often become refractory to the chemotherapy [2]. Success in achieving durable second remission in canine lymphoma is therefore much more difficult and many affected dogs have a poor prognosis. The most common treatment for canine lymphoma is chemotherapy, followed by adjuvant therapy with antineoplastic combination therapy which is composed of cyclophosphamide, doxorubicin, vincristine, and prednisone [3]. Although chemotherapy has been widely used, its use often limited due to drug resistance and serious side effects. Therefore, a novel compound that has potent anticancer activity and minimal side effects is urgently needed.

Cepharanthine (CEP), a natural compound isolated from *Stephaniacepharantha* Hayata possesses many pharmacological effects such as anti-inflammation, anti-retrovirus, anti-oxidant and anticancer [4]. CEP could inhibit production of pro-inflammatory cytokines (tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6) and nitric oxide via suppressing NF- $\kappa$ B [5]. Many studies also showed that CEP has anticancer activity against several types of cancer such as oropharynx cancer, leukemia, hepatocarcinoma and cholangiocarcinoma [6-8]. It has been reported that CEP inhibit tumor growth through multiple mechanisms, including increasing host immune response [9], inducing cancer cell to undergo apoptosis [10-11] and stimulating cell cycle arrest [12]. Although there were reports regarding to cytotoxicity of CEP against several tumors, the anticancer activity of this compound has not been evaluated in canine lymphoma cells. The aim

of this study was to determine anticancer effect of cepharanthine and doxorubicin against canine lymphoma cells and investigate the mechanism underlying the anticancer effect of cepharanthine.

## Materials and Methods

### Preparation of tested compounds:

Freshly prepared cepharanthine (Sigma-Aldrich, St. Louis, MO) was diluted in DMSO to various final concentrations with constant 0.2% DMSO. Doxorubicin was freshly prepared from doxorubicin injection solution (Pfizer, New York, NY) by diluting in sterile double distilled water to the required concentrations. DMSO of 0.2% was used as a negative control

### Culture of canine lymphoma cell line:

The cell line was used in this study as a parent tumor cell line to produce the sublines characterized by strong drug resistance. Cell line was derived from spontaneous canine B-cell lymphoma and has been maintained in a RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/ml), and streptomycin (0.1 mg/ml) (Gibco BRL, Gaithersburg, MD) at 37 °C in a humidified atmosphere of 5% carbon dioxide.

### Cytotoxicity assay:

Cell viability was evaluated by the ability of mitochondrial reductase enzyme in living cells to reduce resazurin into resorufin. Cells were seeded in a 96-well plate at a density  $3 \times 10^5$  cell/well and incubated overnight at 37°C, 5% CO<sub>2</sub>. Cells were then treated with at different concentrations (10, 20 and 40 µM) and doxorubicin (2 µM) or 0.2% DMSO (vehicle control) in culture medium for 24 hours. Then, 15 µL of resazurin solution (0.05 mg/ml) was added to each well and incubated at 37°C for another 5 hours. The colorimetric was quantified by measuring the absorbance at 570 and 600 nm using microplate reader (Thermo). The percent of cell viability was calculated using the following equation: (Abs. sample/ Abs. control) x 100. The values of half inhibitory concentration (IC<sub>50</sub>) were calculated using the fitted line by GraphPad prism software.

### Apoptosis assay:

Canine lymphoma cells were seeded in a 6-well plate at density of  $3 \times 10^5$  cell/well and incubated overnight. The cells were then treated with CEP at different concentrations (10, 20 and 40 µM) and doxorubicin (2 µM) in culture medium for 6, 12, 24 hours. At the end of treatment, the cells were washed twice with cold phosphate buffer saline solution (PBS). The cells were then stained with 1 µl Annexin V FIT-C (Invitrogen, USA) and 1 µl of 0.05 µg/ml PI (Santa Cruz Biotechnology, USA) for 15 min at room temperature in dark. The stained cells were analyzed using flow cytometry (BD LSR II, Biosciences). Four populations of cells can be distinguished, including viable cells (annexin V<sup>-</sup>, PI<sup>-</sup>), early apoptotic cells (annexin V<sup>+</sup>, PI<sup>-</sup>), late apoptotic cells (annexin V<sup>+</sup>, PI<sup>+</sup>) and necrotic cell (annexin V<sup>-</sup>, PI<sup>+</sup>), which are located in the lower left, lower right, upper right, and upper left quadrants of the cytograms, respectively.

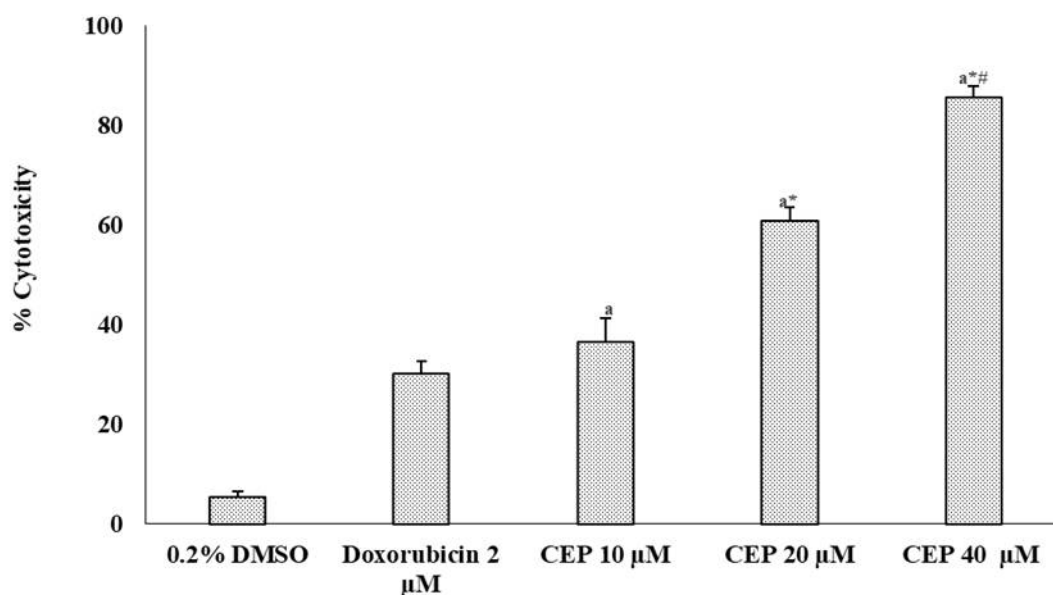
### Statistical analysis:

All data are presented as mean ± standard error of mean (SEM). Statistical analysis of data was performed by one-way analysis of variance (ANOVA) followed by LSD post hoc test using SPSS statistics software. Difference is considered significant if  $P \leq 0.01$ .

## Results

### Effect of cepharanthine and doxorubicin on cytotoxicity in canine lymphoma cells:

We then evaluated anticancer effects of CEP (alkaloids exhibiting both anti-inflammatory and anti-cancer activities) and doxorubicin (an anticancer drug) against canine lymphoma cells using resazurin reduction assay. At 24 h of treatment, doxorubicin at 2 µM significantly inhibited the growth of canine lymphoma cells, while the cytotoxic effects of CEP were detected at 10 - 40 µM in a concentration dependent manner ( $p < 0.05$ ) (Figure 1). The IC<sub>50</sub> value of CEP was 13.77 µM, suggesting that CEP exhibits the potent anticancer effect against canine lymphoma cells.



**Figure 1.** The effect of CEP on cytotoxic activity against canine lymphoma cells. The cells were treated with doxorubicin (2  $\mu$ M) and CEP at 10, 20, and 40  $\mu$ M for 24h. Cytotoxicity was determined by the resazurin reduction assay. The data is expressed as mean  $\pm$  S.E. of three independent experiments (n=3).<sup>a</sup> $p$ <0.01 denotes statistically significant difference from 0.2% DMSO. <sup>\*</sup> $p$ <0.01 denotes statistically significant difference between 20 and 40  $\mu$ M CEP compared with 10  $\mu$ M CEP. <sup>#</sup> $p$ <0.01 denotes statistically significant difference when compared between 40  $\mu$ M and 20  $\mu$ M of CEP.

#### Effect of cepharanthine on apoptosis induction of canine lymphoma cells:

Most of the currently chemotherapeutic drugs induce cancer cells to undergo apoptosis. We therefore examined the apoptosis induction effect of cepharanthine (CEP) in canine lymphoma cells using annexin V-FITC and PI double staining. At 6, 12 and 24 h after incubation, CEP at 10, 20 or 40  $\mu$ M could significantly induce apoptotic cell death in a concentration- and time-dependent manner (Table 1, Figure 2 and 3). Treatment of 20  $\mu$ M of CEP reduced cell viability about 3 times with respect to the vehicle control group. It however should be noted that the percentage of late apoptotic cells are greater than early apoptotic cells. Treatment of CEP at 10, 20 and 40  $\mu$ M could induce cells to undergo late apoptotic about 11.50 $\pm$ 0.85, 26.07 $\pm$ 1.44 and 41.10 $\pm$ 1.93% (at 6 h), 40.72 $\pm$ 4.30, 65.47 $\pm$ 2.91 and 83.30 $\pm$ 1.49% (at 12 h), 53.53 $\pm$ 2.62, 71.83 $\pm$ 3.16 and 61.97 $\pm$ 3.37 % (at 24 h), respectively. These results suggest that CEP effectively induced canine lymphoma cell death via apoptosis induction (Figure 2 and 3).



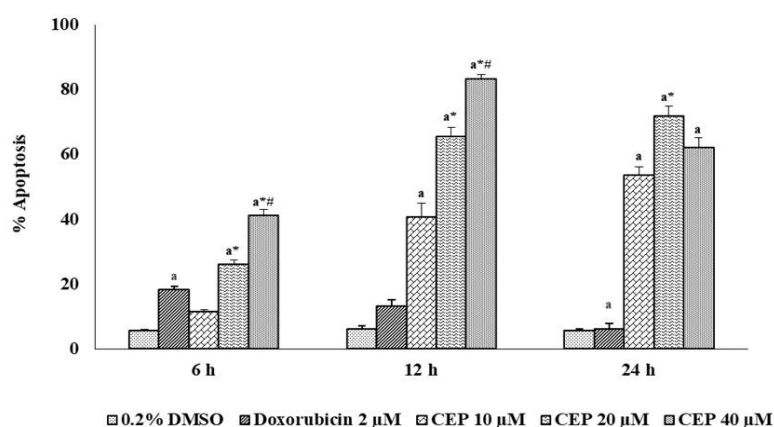
**Table 1:** The effect of CEP on canine lymphoma cells death. The cells were treated with 10, 20 and 40  $\mu$ M of CEP for 6, 12 and 24 h. The patterns of cell death were determined by annexin V-FITC/PI staining assay using fluorescent flow cytometer. The data is expressed as mean  $\pm$  S.E. of three independent experiments (n=3).

Treatment	% Viable cells	% Death cells			% Total death
		Apoptosis	PI positive	Double positive	
<b>6 h</b>					
0.2% DMSO	93.77 $\pm$ 0.42	5.73 $\pm$ 0.37	0.10 $\pm$ 0.00	0.33 $\pm$ 0.03	6.17 $\pm$ 0.38
Doxorubicin 2 $\mu$ M	80.83 $\pm$ 1.03 <sup>a</sup>	18.23 $\pm$ 1.25 <sup>a</sup>	0.23 $\pm$ 0.09	0.73 $\pm$ 0.12	19.20 $\pm$ 1.07 <sup>a</sup>
CEP 10 $\mu$ M	87.70 $\pm$ 0.82	11.50 $\pm$ 0.85	0.50 $\pm$ 0.12	0.33 $\pm$ 0.12	12.33 $\pm$ 0.80
CEP 20 $\mu$ M	72.57 $\pm$ 1.09 <sup>a*</sup>	26.07 $\pm$ 1.44 <sup>a*</sup>	0.63 $\pm$ 0.24	0.73 $\pm$ 0.13	27.43 $\pm$ 1.10 <sup>a*</sup>
CEP 40 $\mu$ M	56.97 $\pm$ 1.90 <sup>a*#</sup>	41.10 $\pm$ 1.93 <sup>a*#</sup>	1.10 $\pm$ 0.11 <sup>a</sup>	0.83 $\pm$ 0.12	43.03 $\pm$ 1.90 <sup>a*#</sup>
<b>12 h</b>					
0.2% DMSO	93.33 $\pm$ 1.13	6.22 $\pm$ 1.05	0.10 $\pm$ 0.05	0.32 $\pm$ 0.13	6.63 $\pm$ 1.15
Doxorubicin 2 $\mu$ M	84.77 $\pm$ 2.47	13.17 $\pm$ 2.18	0.62 $\pm$ 0.28	1.43 $\pm$ 0.68	15.22 $\pm$ 2.47
CEP 10 $\mu$ M	56.80 $\pm$ 4.68 <sup>a</sup>	40.72 $\pm$ 4.30 <sup>a</sup>	0.72 $\pm$ 0.22	1.77 $\pm$ 0.87	43.20 $\pm$ 4.69 <sup>a</sup>
CEP 20 $\mu$ M	29.17 $\pm$ 2.87 <sup>a*</sup>	65.47 $\pm$ 2.91 <sup>a*</sup>	3.85 $\pm$ 0.71 <sup>a</sup>	1.52 $\pm$ 0.56	70.83 $\pm$ 2.87 <sup>a*</sup>
CEP 40 $\mu$ M	9.40 $\pm$ 2.27 <sup>a*#</sup>	83.30 $\pm$ 1.49 <sup>a*#</sup>	4.72 $\pm$ 0.99 <sup>a*</sup>	2.55 $\pm$ 0.79	90.57 $\pm$ 2.28 <sup>a*#</sup>
<b>24 h</b>					
0.2% DMSO	94.13 $\pm$ 0.58	5.63 $\pm$ 0.60	0.03 $\pm$ 0.03	0.17 $\pm$ 0.03	5.83 $\pm$ 0.59
Doxorubicin 2 $\mu$ M	73.97 $\pm$ 1.50 <sup>a</sup>	6.10 $\pm$ 1.85	3.23 $\pm$ 1.77	16.70 $\pm$ 1.96 <sup>a</sup>	26.03 $\pm$ 1.50 <sup>a</sup>
CEP 10 $\mu$ M	31.63 $\pm$ 4.37 <sup>a</sup>	53.53 $\pm$ 2.62 <sup>a</sup>	12.07 $\pm$ 2.12	2.77 $\pm$ 0.46	68.37 $\pm$ 4.37 <sup>a</sup>
CEP 20 $\mu$ M	6.97 $\pm$ 1.49 <sup>a*</sup>	71.83 $\pm$ 3.16 <sup>a*</sup>	17.03 $\pm$ 3.56 <sup>a</sup>	4.20 $\pm$ 1.36	93.07 $\pm$ 1.47 <sup>a*</sup>
CEP 40 $\mu$ M	1.17 $\pm$ 0.03 <sup>a*</sup>	61.97 $\pm$ 3.37 <sup>a</sup>	17.57 $\pm$ 3.99 <sup>a</sup>	19.33 $\pm$ 2.40 <sup>a*#</sup>	98.87 $\pm$ 0.03 <sup>a*</sup>

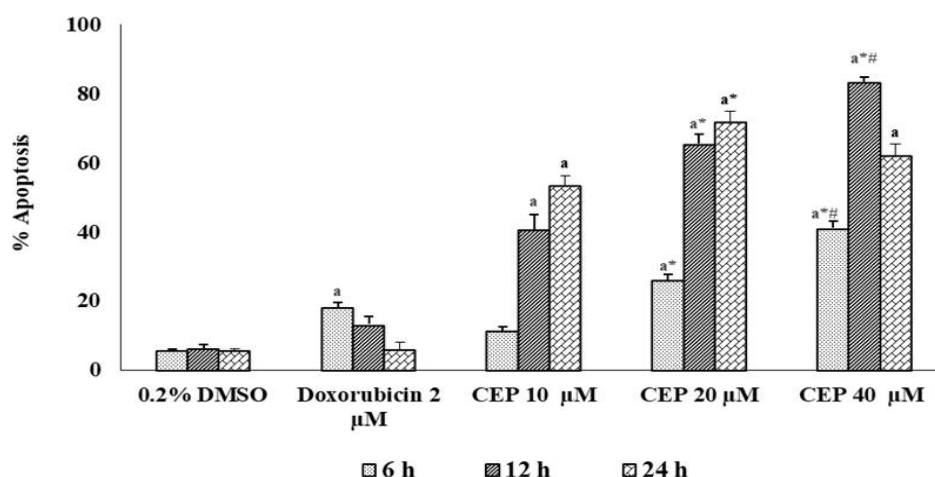
<sup>a</sup> $p$  < 0.01 denotes statistically significant difference from 0.2% DMSO.

<sup>\*</sup> $p$  < 0.01 denotes statistically significant difference between 20 and 40  $\mu$ M CEP compared with 10  $\mu$ M CEP.

<sup>#</sup> $p$  < 0.01 denotes statistically significant difference when compared between 40  $\mu$ M and 20  $\mu$ M of CEP.



**Figure 2.** The concentration-dependent effect of CEP on canine lymphoma cells apoptosis. The cells were treated with 10, 20 and 40  $\mu$ M CEP for 6, 12 and 24 h. The percentage of apoptotic cells (annexin V<sup>+</sup>/PI<sup>-</sup>) were determined by annexin V-FITC/PI staining assay using fluorescent flow cytometer. The data is expressed as mean  $\pm$  S.E. of three independent experiments (n=3).<sup>a</sup> $p$  < 0.01 denotes statistically significant difference from 0.2% DMSO. <sup>\*</sup> $p$  < 0.01 denotes statistically significant difference between 40 and 20  $\mu$ M CEP compared with 10  $\mu$ M CEP. <sup>#</sup> $p$  < 0.01 denotes statistically significant difference when compared between 40  $\mu$ M and 20  $\mu$ M of CEP.



**Figure 3.** The time-dependent effect of CEP on canine lymphoma cells apoptosis. The cells were treated with 10, 20 and 40  $\mu\text{M}$  CEP for 6, 12 and 24 h. The percentage of apoptotic cells (annexin V<sup>+</sup>/PI<sup>-</sup>) were determined by annexin V-FITC/PI staining assay using fluorescent flow cytometer. The data is expressed as mean  $\pm$  S.E. of three independent experiments (n=3). \*  $p < 0.01$  denotes statistically significant difference as compared to 6 h. # $p < 0.01$  denotes statistically significant difference when compared between 24 h. and 12 h.

## Discussion

Treatment for lymphoma patients has been improved dramatically. Benefit of combining one targeted cancer drug such as rituximab, a monoclonal antibody against CD20, with a gold standard regimen, CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) is an example of successful combination anticancer therapy. However, its use is restricted due to high costs of target-based antibodies. Recently, several natural-derived compounds have been explored and could serve as effective anticancer compounds in combination therapy when coupled with standardized cancer drug treatment. The natural compound would not only reduce the dose of concurrent chemotherapy to achieve effective anticancer activity but may also improve upon the adverse events of potent therapeutic agents such as doxorubicin. In the present study, we therefore investigated the effect of cepharanthine (CEP) on cytotoxicity and apoptosis induction against the canine lymphoma cells. The cytotoxicity of doxorubicin, a commonly chemotherapeutic drug used for lymphoma, was also tested. We found that CEP could effectively inhibit the growth of canine lymphoma cells in a concentration- and time-dependent manner. The  $\text{IC}_{50}$  value of CEP was approximately 13  $\mu\text{M}$  which was much more potent than doxorubicin, the anticancer agent commonly used for lymphoma. In the present study, we found that CEP and doxorubicin effectively induced apoptotic cell death in canine lymphoma cells. CEP has also shown to induce cancer cells to undergo apoptosis through several mechanisms including i) activating caspase-3 and 9 [8, 11-12], ii) stimulating pro-apoptotic signaling pathways such as JNK, ERK and p38 MAPK [13], and iii) inhibiting expression of anti-apoptotic gene Bcl-xl. The results in the present study clearly demonstrated that cepharanthine (CEP) has a potent anticancer activity against canine lymphoma cell line. Mechanistic studies indicated that CEP effectively induced canine lymphoma cells to undergo apoptosis. These findings suggest that CEP could potentially be used as a novel anticancer agent for lymphoma cancer cells which are commonly resistant to currently chemotherapeutic agent.

## Acknowledgements

We would like to thank grant from Faculty of Veterinary Science, Rajamangala University of Technology Srivijaya.

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# Glutathione Levels in Canine Lymphoma

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## Abstract

**Objective** The purpose of this study was to compare the mean glutathione levels between clinically healthy dogs and dogs suffering from spontaneous lymphoma.

**Materials and Methods** Blood samples were obtained from eighteen clinically healthy dogs and eighteen dogs suffering from spontaneous lymphoma at Veterinary Teaching Hospital, Khon Kaen University. The diagnosis of lymphoma was made from histopathological findings. Serum glutathione level was determined by spectrophotometric assay. The glutathione concentrations were calculated by comparing with standard glutathione. The data were presented as mean  $\pm$  standard deviation. Parametric data were determined using independent-sample T test. P value of less than 0.05 was considered significant.

**Results** The mean of glutathione levels were  $1.29 \pm 2.91$  mg/dL in canine lymphoma group and  $12.24 \pm 1.39$  mg/dL in clinically healthy group. Dogs with lymphoma had significantly lower glutathione levels compared with controls ( $P < 0.01$ ).

**Conclusion** The results may reflect an antioxidant depletion and a response to oxidative stress in canine lymphoma.

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**Keywords:** Glutathione, Dog, Lymphoma

# Occurrence of Antimicrobial Resistant Bacteria Isolated from Urinary Tract Infection Dogs and Cats at the Veterinary Teaching Hospital, Khon Kaen University, Thailand during 2011 to 2017

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## Abstract

**Objective** The objective was to investigate bacteria isolated from urinary tract infection (UTI) dogs and cats and to reveal the antimicrobial resistance.

**Materials and Methods** This retrospective study reviewed urine culture records (298 from UTI suspected dogs and 87 from cats) examined at the veterinary clinical diagnostic laboratory between 2011 and 2017. The bacteria identification was carried out using RapID™ systems and ERIC® software. Antimicrobial susceptibility testing (AST) using representative of five antimicrobial classes included sulfonamides/pyrimidines (sulfamethoxazole/trimethoprim, SXT), aminoglycosides (amikacin, AK), fluoroquinolones (enrofloxacin, ENR), tetracyclines (doxycycline, DO), and β-lactams (amoxicillin-clavulanate, AMC) was determined by Kirby Bauer disc diffusion method, together with using oxacillin (OX) to denote OX-resistant *Staphylococcus spp.*.

**Results** The urine culture of all urines were positive by 59.2% (228/385), whereby 64.8% (193/298) of dog urines while 40.2% (35/87) of cats were positive. A total of 295 strains of bacteria were isolated, where 122 strains (41.4%) were gram positive (GPBs) and 173 strains (58.6%) were gram negative (GNBs). The GPBs included *Staphylococcus spp.* (29.2%), *Streptococcus spp.* (7.8%), *Enterococcus spp.* (2.7%), and other GPBs (1.7%), on the one hand, GNBs consisted of *Escherichia coli* (19.3%), *Proteus mirabilis* (18.0%), *Klebsiella pneumonia* (4.8%), other *Enterobacteriaceae* (2.0%), *Pseudomonas spp.* (4.4%), *Alcaligenes spp.* (3.7%), *Enterobacter spp.* (3.4%), and other non-lactose fermenting GNBs (3.0%). The OX-resistant *Staphylococcus spp.* was encountered by 30.0% (24/80). The AST results showed that the overall bacteria resistance to SXT, ENR, DO, AMC, and AK were 58.8% (154/262), 57.0% (58/277), 50.2% (118/235), 31.3% (91/291), and 12.3% (33/268), respectively. Out of all GPBs, 53.0% (61/115) was resistant to ENR and 51.0% (49/96) exhibited resistant to SXT followed by AMC (20.8%, 25/120), DO (19.2%, 19/99), and AK (15.9%, 17/107). While, there was 72.8% (66/171) of GNBs resist to DO, 63.3% (105/166) resist to SXT, and 59.9% (97/162) resist to ENR followed by AMC (38.6%, 66/171), and AK (9.9%, 16/161). *Staphylococcus spp.*, was 50.6% (40/79) resistant to ENR and 45.1% (32/71) resistant to SXT followed by AMC (16.5%, 14/85), DO (8.5%, 7/71), and AK (3.8%, 3/80). There was 75.9% (41/54) of *E. coli* resist to ENR, 73.6% (39/53) resist to SXT, and 73.3% (33/45) resist to DO followed by AMC (36.8%, 21/57), and AK (7.8%). *Proteus spp.* resistant to DO revealed to be 100% (43/43) followed by SXT (59.3%, 32/54), ENR (45.1%, 23/51), and AMC (18.5%, 10/54), whereas none of the *Proteus spp.* (0/51) resist to AK.

**Conclusion** *Staphylococcus spp.*, *E. coli*, and *P. mirabilis* were the majorities isolated from UTI dogs and cats, and oxacillin-resistant *Staphylococcus spp.* emerged in high frequency. The UTI causing bacteria have been frequently resistant to broad-spectrum antimicrobials, which widely used in veterinary, *i.e.*, sulfamethoxazole/trimethoprim, enrofloxacin, and doxycycline, whereas amikacin seem to be effective and could be a drug of choice for canine and feline UTI treatment.

**Keywords:** Antimicrobial susceptibility, Urinary tract infection, Antibiotic resistance

# Health Status and Social Behavior of Community Dogs in Population Control Program of Chiang Mai University Extension Service

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## Abstract

**Objective** This study aimed to determine clinical parameters and social aggressive score of community dogs in extension service for population control program.

**Materials and Methods** A cross-sectional study was conducted between May 2016 to March 2018 in 150 community dogs at Chiang Mai University, Chiang Mai, Thailand. A total of 40 sampling dogs were of population control program at CMU evaluated for the social aggressive scoring and health status including complete blood count and blood chemistry profile.

**Results** Mean lymphocytes of community dogs was  $3,588.16 \pm 2,039.74$ , while mean thrombocytes in this study was  $165.20 \pm 75.14$ . The most common social aggression was non-aggressive dog (22/40; 55%). The second common was aggressive dog (12/40; 30 %). Remaining dogs were moderately aggressive.

**Conclusion** The friendly behavior of community dog enhances the population control program for extension service. However, their common problems are thrombocytopenia, lymphocytosis, and slightly increased creatinine. Therefore, anesthetic risk and perioperative complications should be particularly concerned.

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**Keywords:** Community dogs, Social behavior, Population control program

# Ocular Manifestation of Ehrlichiosis in a Dog: Case Report

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## Abstract

**Case Description** A six-year-old *Yorkshire Terrier* male dog weighing 5.1 kg was referred to Small Animal Teaching Hospital, Mahanakorn University of Technology. He bumped into objects for two months. The previous hospital treatments involved increased intraocular pressure, conjunctivitis and vision loss.

**Clinical Findings** Physical examination result was normal. According to complete blood count and serum biochemistry, there were mild anemia and increased alkaline phosphatemia. *Ehrlichia canis* antibody was strong positive by *SNAP 4Dx Plus Test*. Ocular examination menace, dazzle, pupillary light reflex and obstacle course were negative in both eyes. In the right eye, there were mucous ocular discharge, moderate conjunctivitis, mild corneal edema, aqueous flare (+1) and immature cataract. Schirmer tear test result was 12 mm/min. Intraocular pressure was 11 mmHg and fluorescein was negative. In the left eye, there were moderate conjunctivitis, mild corneal edema, nonclassified of cataract, hyphema (33%), aqueous flare (+2). Schirmer tear test result was 19 mm/min. Intraocular pressure was 49 mmHg and fluorescein was positive (indolent ulcer). Ocular ultrasonography result showed that retinal detachment (OU) and bleeding in vitreous chamber (OS). Final diagnoses were panuveitis (OU), retinal detachment (OU), immature cataract (OU), hyphema (OS), secondary glaucoma (OS) and blindness (OU).

**Treatment and Outcome** The dog was treated with oral doxycycline, a combination of oral prednisolone, corneal debridement (OS) and topical oxytetracycline HCL and polymyxin B sulfate for treating indolent ulcer (OS), topical antiglaucomaused Brinzolamide (OS) for treating control intraocular pressure. Enucleation was recommended if treatments did not control intraocular pressure and inflammation.

**Clinical Relevance** Ehrlichiosis is a tick - borne disease. The pathogenesis of ehrlichiosis begins with an incubation period of 8 to 20 days. The most common ocular manifestation is retinal detachment, vasculitis, unilateral or bilateral anterior or posterior uveitis. Other ocular signs involve retinal hemorrhage, hyphema, conjunctival petechial. Frequent hemorrhage may induce secondary retinal detachment.

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**Keywords:** Ehrlichiosis, Retinal detachment, Uveitis, Dog

# Splenic Soft Tissue Sarcoma (STS) with Hepatic Metastasis in a Dog: Case Report

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## Abstract

**Case Description** A twelve-year-old male, weighting 21.5, Thai dog was presented at Small Animal Teaching Hospital, Mahanakorn University of Technology with a one month history of a anorexia, weight loss and a lump at the cranial to middle part of the abdomen.

**Clinical Findings** Abdominal X-ray was shown a large abdominal mass and the abdominal ultrasound found the mass size approximately 10.2 cm in diameter which located adhesion to the spleen and multiple nodules were also observed in liver parenchyma. Thoracic X-ray was not shown metastasis into the lung parenchyma. The complete blood count was show mild normocytic normochromic anemia and serum biochemistry profiles were in a normal range.

**Treatment and Outcome** Laparotomy was performed for making diagnosis. Whole blood transfusion was given to the dog during operation. The large mass at the distal part of spleen and multiple nodules in all liver lobes were observed from laparotomy. Total splenectomy and liver nodule biopsy were performed and sent to histopathology. In the first day after surgery the electrocardiograph showed bradycardia (heart rate 60 bpm) combine with the second degree AV block. Atropine dose 0.2mg/kg (single dose) was prescribed for treatment of second degree AV block. Amoxicillin Clavulanate (25 mg/kg, iv, q 12 h) and Morphine (0.3 mg/kg, SC, q 4-6h) were prescribed for antimicrobial prophylaxis and pain control. The dog was discharge from the hospital after 1 week. The result from histopathology was soft tissue sarcoma in the spleen, which suggested of leiomyosarcoma. The nodule biopsy from liver was suspected metastasis tumor from the spleen.

**Clinical Relevance** Primary splenic tumor are relatively common in old dogs. Hemangiosarcoma and hemangiomas are the most common neoplasm of the canine spleen. Moreover, leiomyosarcoma, liposarcoma, and histiocytoma were also encountered. Dogs with splenic tumor commonly presented with distended abdomen or palpable abdominal mass. Abdominal radiographs may or may not identify the splenic mass due to loss of detail from abdominal effusion. Abdominal ultrasound is a superior even in the presence of effusion in evaluation for intra-abdominal mass. Thoracic radiographs should be obtained to evaluate for evidence of pulmonary metastasis. An exploratory laparotomy is mostly performed for definitive diagnosis and splenectomy is the first choice in treating splenic tumor. The goals of surgical excision is to removal the mass and biopsy of appropriate tissues (e.g., liver and lymph node[s]), which can be done by complete or partial splenectomy. In addition, platelet count, platelet function (BMBT), a coagulation profile and cross matching are recommend to evaluate prior to surgery. The most common complication in surgery of the spleen is cardiac arrhythmias, and internal bleeding which can occurred during splenectomy, or post-operative period, therefor electrocardiographic, pack cell volume (PCV) monitoring are recommended. Chemotherapy protocols containing doxorubicin (DOX) may adjuvant setting in group for metastasis, however chemotherapy alone is unlikely provide a complete treatment of most STS, but it can prolong disease-free intervals.

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**Keywords:** Abdominal mass, Splenic mass, Soft tissue sarcoma, STS



# Causal Relationship of the Antimicrobial Resistant *Salmonella* spp. Isolated from Pet Dogs and Their Owners

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## Abstract

**Objective** To investigate the causal relationship of antimicrobial resistance between pet dogs and humans via *Salmonella* spp.

**Materials and Methods** Fecal samples were collected from 141 families, complete pet dogs (n=140) and complete humans (n=91) from Thailand. All samples were isolated and identified for *Salmonella* spp., following the ISO standard 6597:2002. Then, the positive *Salmonella* spp. colonies were performed for antimicrobial susceptibility tests and statistical analysis.

**Results** There were 24.82% *Salmonella* spp. risk families (n=141), 20.62% *Salmonella* spp. infected humans (n=97) and 13.57% *Salmonella* spp. infected dogs (n=140). An identified antimicrobial resistance of total *Salmonella* spp. were ampicillin (42.10% in dog and 65.00% in human), ciprofloxacin (15.79% in dog and 30.00% in human), chloramphenicol (5.26% in dog and 20.00% in human), nalidixic acid (5.26% in dog and 20.00% in human), streptomycin (21.05% in dog and 30.00% in human), sulfamethoxazole-trimethoprim (15.79% in dog and 30.00% in human) and tetracycline (36.84% in dog and 65.00% in human). The relative risk of causal relationship is 11.43% from total *Salmonella* spp. risk families (n=35).

**Conclusion** The dynamic transmission of antimicrobial resistance genes between pet dogs and humans via *Salmonella* spp. is a serious potential risk. Further researches to prevent antimicrobial resistant *Salmonella* spp. transmission between household animals and humans are really needed, we suggest the pet owners to provide a good living environment for family health.

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**Keywords:** *Salmonella* spp., Antimicrobial resistance, Pet dogs, Humans.

# Risk Factors of Methicillin-Resistant *Staphylococcus aureus* in Swine-Production Personnel in Chiang Mai-Lamphun Province, Thailand

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## Abstract

**Objective** This study aimed to address the risk factors associated with Methicillin-Resistant *Staphylococcus aureus* (MRSA) in people whose work related with pigs, in Chiang Mai-Lamphun province, Thailand.

**Materials and Methods** Total of 202 nasal swab samples were collected from swine farm workers, swine farm owners, animal sciences and veterinary students, veterinarians and animal husbandman. Those samples were cultured for screening of MRSA and confirmed results by detection of *mecA* gene using multiplex PCR. The data including age, gender, occupation, education level, health status, hygiene practice, time to contact to swine and history of antibiotic usage were collected by using questionnaires to determine the risk factors.

**Results** The results showed that MRSA in swine-production personnel in Chiang Mai-Lamphun province, Thailand was 10.3% (21/202). Farm owner and veterinarians and animal husbandman had higher MRSA prevalence than other sample groups (13.3% and 12.5%, respectively). The high number of working hour and working with only pigs were risk factors ( $p=0.036$  and  $p=0.030$ , respectively). Meanwhile, high education level and good self-sanitation were protective factors ( $p=0.020$  and  $p=0.029$ , respectively).

**Conclusion** This study indicated that MRSA has circulated in people who are working related with pigs. Contact with pigs was risk factor, while education and sanitation can reduce the risk.

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**Keywords:** Methicillin-Resistant *Staphylococcus aureus* (MRSA), Pigs, Personnel, Risk factor

# The Critical Thinking, Metacognitive and Executive Functions of Working Memory Abilities in Veterinary Students

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## Abstract

**Objective** To examine the critical thinking, metacognitive and executive function of working memory ability in veterinary students after the intervention of constructivism, metacognitive, and neurocognitive based (CMEN) (an experimental group) or traditional teaching models (a control group).

**Materials and Methods** The total participants were 84 and equally distributed to 42 students in each group on a voluntary basis from Faculty of Veterinary Medicine, Khon Kaen University, Thailand. Researchers employed experimental research pre-test and post-test control group design.

**Results** There was no significant difference between groups on the dependent variables before the intervention. However, after the intervention, all the dependent variables namely medical terminology and anatomical knowledge achievement, metacognitive ability, executive function of working memory ability and critical thinking ability in the experimental group were significantly higher than those in control groups.

**Conclusion** The CMEN model has been successfully promoted students' learning outcomes (i.e. abilities in understanding medical terminology, anatomical knowledge, metacognitive ability, executive function of working memory ability and critical thinking).

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**Keywords:** Anatomical knowledge, Constructivism, Critical thinking, CMEN teaching model, Executive function of working memory ability, Medical terminology, Metacognitive ability, Traditional teaching model

## Introduction

Traditionally universities employ instructive forms of teaching such as lectures and laboratory part in the pre-clinical courses of veterinary medicine and related disciplines [1]. However, past researchers found that the traditional teaching model not only encourages a superficial approach to learning [2] but also many students are unable to effectively reason and their application of knowledge to real-life situations is less than optimal [1]. In addition, it seems that advances in medical knowledge and biotechnology are escalating the curricular content of veterinary schools to massive sections, to the point that it could be considered impracticable to ensure that all students have sufficient knowledge base [3]. The constructionism, metacognition and neurocognitive-based (CMEN) teaching model was developed by Uopasai [4] by utilizing the three emerging fields i.e. the constructivist philosophy of science teaching and learning, neurocognitive learning theory and metacognitive knowledge. Constructivist philosophy of science teaching and learning is about students' mental models and their misconceptions which have important implications for teachers who wish to model scientific reasoning in an effective fashion for their students [5] that includes aspects of Piagetian [6], Ausubelian [7], and Vygotskian [8] learning theories. According to Chauhan and Singh [9], metacognition is student's ability to use prior knowledge to plan a strategy for approaching a learning task, take necessary steps to solve their problem, reflect on and evaluate results, and modify his/her approach as needed. Metacognition approach assists students choose the appropriate tool for the task and plays a critical role in successful learning. Neurocognitive learning theory is a combination of three traditionally separate

strands of inquiry, namely neurophysiology with an emphasis on the biological bases of brain and neural activity; cognitive science with a focus on information processing and internal representations of experience, and learning theory that explains how students cumulatively interact with, and adapt to, our environments [10]. Critical thinking (CT) is reasonable and reflective thinking focused on deciding what to believe or do. According to Ennis, [11], CT comprised 8 factors include 1) judge the credibility of sources 2) update new knowledge 3) identify conclusions, reasons, and assumptions 4) judge the quality of an argument, including the acceptability of its reasons, assumptions and evidence 5) develop and defend a position on an issue 6) plan experiments and judge experimental designs and select approaches clarifying appropriate 7) define terms in a way appropriate for the context and 8) draw conclusions when warranted, but with caution.

Effective teaching in learning medical terms or vocabulary in health sciences would assist veterinary medicine students to understand the origin of words, rules of creating words from etymology, memorization, radical, and finally connected words to ease the difficulties and complication of their learning. According to Veach and Holtsberry [12], students have to understand the relationships between the terms with anatomy, physiology and clinical significance.

## **Materials and Methods**

### **Research Design and Study Samples**

A total of 84 second year undergraduate students who enrolled in Small Animal Anatomy course in the first semester of academic year 2014 from Faculty of Veterinary Medicine from a public university located at KhonKaen province were selected as participants. These 84 participants were equally distributed into experimental and control groups on the volunteering basis. A 2 (CMEN vs traditional teaching model) x 2 (time of measure: pretest vs posttest) design was utilized in this study. Participants' learning outcomes namely achievement on veterinary anatomy test, critical thinking ability, metacognitive ability, and executive function of working memory accuracy and reaction time were measured both before and after intervention. After finish intervention, critical thinking ability was be measured in order to compare the effectiveness of the two teaching models.

### **Pedagogical Manipulation**

This study aimed to study the learning outcomes of two teaching models, namely the constructivism, metacognition and neurocognitive-based model (CMEN) and the traditional model(TM). The learning outcomes were achievement on understanding the skeletal system of veterinary anatomy, metacognitive ability, and working memory ability. Both teaching models provided the equal opportunity for instructor and students to learn how their knowledge, cognition, and emotions interact with the environment and how both groups change occurred through the learning process (Joyce, Weil & Calhoun, 2015). The CMEN model used to teach the experimental group and the TM model was used for control group. Both groups were taught the same content of the canine anatomy skeletal system, consisted of four chapters including a skull, fore limb bone, hind limb bones, and vertebrae. Both groups attended their lessons for a total of 20 hours. The CMEN model is an innovative teaching model which integrated the three major components of constructivism, metacognition, and educational neuroscience [4]. Consequently, the CMEN model composed of six phases as follows: (i) perception and attention; (ii) objective of planning and monitoring; (iii) multisensory integration; (iv) rehearsal and practice, and (vi) summary and evaluation.

### **Research Instrument**

Research instruments were mainly used as tests to measure students' learning outcomes. A total of five types of instrument were utilized in this study namely medical terminology test, anatomical knowledge test, metacognitive ability, executive function of working memory battery test, and critical thinking ability test. The medical terminology test was used to measure the understanding of the medical terminology terms used to describe the dog skeletal system accurately. It was comprised of 30 items selected from the item bank of Department of Anatomy, Faculty of Veterinary Medicine, Khon Kaen University, Thailand. The reliability (KR20) was 0.91; discrimination index was 0.27 to 0.61, and difficulty index was 0.27 to 0.79. The anatomical knowledge test was used to measure the understanding the canine skeletal anatomy which consisted of 30 multiple choice items and selected from the Department of Anatomy, Faculty of Medicine, Khon Kaen University, Thailand. The reliability (KR20) was 0.86; discrimination index was 0.22 to 0.46, and difficulty index was 0.26 to 0.79.

The Metacognitive Awareness Inventory (MAI) is a rating scale used to measure the two components of 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 and Dennison [13] and translated from English to Thai language to ensure that the participants were clear about the statements. The reliability (KR20) was 0.95.

The executive function of working memory battery test was originally in Thai version and adopted from Bunterm et al. [14] which comprised of 5 tasks. The 5 tasks covered: i) switching task; ii) stroop; iii) 0-back; iv) 1-back, and v) 2-back. This working memory battery test allowed the researchers to measure working memory accuracy and reaction time. Students were given 10 trials for each task, giving a total of 100 trials. The reaction time below 200 milliseconds will be excluded and data was analyzed in the range of  $\bar{X} \pm 3S.D.$  The 'switching' task are considered as binary choice reaction time task (CRTs) which required the participants to respond as fast as possible without errors by pressing one of the two keys that required the participants to meet the stimulus of left-right and up-down tasks. While the 'stroop' task is an inhibition test that is also one of the most long standing, having been reported by John Ridley Stroop in the published version of his dissertation in 1935 [15] used to assess the ability to suppress responses which are inappropriate in a particular context. The stimulus of 'stroop' task consisted of a set of five arrows, with the target stimulus placed at the central position. The subjects must respond that the words represent the color and meaning of the words are consistent. The n-back task is a continuous performance task that comprised of 0, 1, and 2 back tasks. Pictures of the characters in the Ramayana such as Rama, Lakshmana, Sita, and Hanuman are used as stimulus to match the one from n steps earlier in the sequence. Again, all the 5 tasks of executive function of the working memory battery test were in the Thai language and the goodness of fit test for construct validity purpose had been evaluated by Bunterm et al. [14]. The test-retest reliability values of these tasks were ranged from 0.822 to 0.979.

The critical thinking ability test is a rating scale used to measure 8 factors include 1) judge the credibility of sources 2) update new knowledge 3) identify conclusions, reasons, and assumptions 4) judge the quality of an argument, including the acceptability of its reasons, assumptions and evidence 5) develop and defend a position on an issue 6) plan experiments and judge experimental designs and select approaches clarifying appropriate 7) define terms in a way appropriate for the context and 8) draw conclusions when warranted, but with caution. This instrument was adapted from Ennis [11] and translated from English to Thai language to ensure that the participants were clear about the statements. The reliability (KR20) was 0.91.

#### **Data Analysis**

Repeated measures multivariate analysis of variance (Repeated MANOVA) and MANOVA were used in analyzing the effect of time, teaching model, and interaction between time and teaching model on three dependent variables: medical terminology and anatomical knowledge achievement, metacognitive ability, and working memory ability and critical thinking ability. Wilks' lambda, a direct measure of the proportion of variance in the combination of dependent variables that is unaccounted for the group variable [16], is used to test whether there are differences between the means of identified groups of students on a combination of dependent variables.

## **Results**

The purpose of using MANOVA is to test whether the vectors of means for the two groups are sampled from the same sampling distribution [17]. However, researchers could not utilize randomization procedure to manage the groups for the intervention because this would obstruct the daily operation of the learning. Therefore researchers had taken into consideration the confounding factors such age, gender, and handedness to ensure the participants were the equally distributed over the two groups. The outcomes of the tests showed that there were no age ( $t_{(82)} = .267, p = p > .05$ ), gender ( $\chi^2 = 1.248, df = 1, p > .05$ ), and handedness ( $\chi^2 = .553, df = 1, p > .05$ ) differences between the experimental and control groups. Thus, the experimental and control groups were identified as the same sampling distribution and appropriate to follow up with the intervention.

#### **Medical Terminology and Anatomical Knowledge**

Before intervention, there is no mean difference of both dependent variables (ability in medical terminology and anatomical knowledge) between groups, Wilks'  $\lambda = 0.09$ ,  $F_{(2,81)} = 0.24$ ,  $p = 0.79$ , partial  $\eta^2 = 0.01$ . After intervention, finding indicated that there was a significant mean difference between groups, Wilks'  $\lambda = .71$ ,  $F_{(2,81)} = 15.94$ ,  $p < .01$ , partial  $\eta^2 = .28$ . The experimental group scored higher in medical terminology ( $F_{(2,81)} = 18.36$ ,  $p < .01$ , partial  $\eta^2 = .28$ ) as well as anatomical knowledge ( $F_{(2,81)} = 10.64$ ,  $p < .01$ , partial  $\eta^2 = 0.12$ ) than control group. The Box's M test for equality of variance-covariance matrices was not significance ( $p > .05$ ) implied that the assumption of homogeneity across the group was met. Repeated-measures MANOVA analysis confirmed that there was a significant multivariate effect across the interaction between the groups and reaction time: Wilks'  $\lambda = .89$ ,  $F_{(2,81)} = 18.53$ , ( $p < .05$ ), partial  $\eta^2 = .10$ . In addition, finding indicated that there was a significant multivariate effect for between learning outcomes encompassed the abilities in understanding medical terminology and anatomical knowledge across the groups regardless of their reaction time: Wilks'  $\lambda = .81$ ,  $F_{(2,81)} = 9.68$ ,  $p < .01$ , partial  $\eta^2 = .19$ . There was a significant multivariate effect across within-subjects time point (regardless of student group): Wilks'  $\lambda = .03$ ,  $F_{(2,81)} = 1426.05$ ,  $p < .01$ , partial  $\eta^2 = .97$ . The mean scores and standard deviation of the second year veterinary medicine students either medical terminology or anatomical knowledge test before and after intervention and univariate tests were shown in table 1.

**Table 1.** Pre-test vs post-test of skeletal system of veterinary anatomy achievement and univariate tests

Dependent variables	Experimental group (N=42)				Control group (N=42)				F
	Pre-test		Post-test		Pre-test		Post-test		
	M	SD	M	SD	M	SD	M	SD	
Medical terminology	7.05	3.09	22.51	1.97	6.64	2.81	20.84	1.69	7.18**
Anatomical knowledge	6.48	2.39	22.69	2.35	6.36	7.83	20.36	4.16	10.53**

\*\* $p < 0.01$

### Metacognitive Ability

Before intervention, result showed that there was no difference between the groups in all components. After intervention, the experimental group had possessed significantly better in all components of metacognitive ability than the control group.

**Table 2.** Pre-test vs post-test of metacognitive ability and univariate tests

Dependent variables	Experimental group (N=42)				Control group (N=42)				F
	Pre-test		Post-test		Pre-test		Post-test		
	M	SD	M	SD	M	SD	M	SD	
Declarative knowledge	26.67	3.19	28.79	2.61	27.33	3.30	28.17	3.43	11.35**
Procedural knowledge	13.17	2.23	14.29	1.73	13.10	2.09	13.55	1.95	25.92**
Condition knowledge	17.00	2.46	18.69	1.94	17.50	2.32	18.21	2.09	60.72**
Planning	22.62	3.12	61.76	5.23	22.64	2.93	59.93	6.24	21.81**
Monitoring	21.17	4.08	25.76	2.58	21.21	3.37	23.57	3.03	32.06**
Evaluating	18.33	3.87	24.64	2.65	18.98	2.52	22.98	3.16	30.53**
Information management	33.64	5.21	21.98	2.51	35.52	4.22	19.93	3.02	6.86*
Debugging	17.98	2.82	19.52	2.74	18.71	2.45	18.52	3.59	12.83**

\* $p < .05$ , \*\* $p < .01$

A 2x2 repeated MANOVA was utilized to examine the effect of the two teaching models on metacognitive ability of the veterinary medicine students. Result revealed that there was a significant multivariate effect across the interaction between student group and time point: Wilks'  $\lambda = .56$ ,  $F_{(8,75)} = 7.28$ ,  $p < .01$ , partial  $\eta^2 = .44$ . There was a significant multivariate effect for between-subjects (of the combined mean score of 8 components) across student group (regardless of time point): Wilks'  $\lambda = .86$ ,  $F_{(8,75)} = 1.49$ ,  $p < .05$ , partial  $\eta^2 = .14$ . There was also a significant multivariate effect across within-subjects time point (regardless of student group): Wilks'  $\lambda = .46$ ,  $F_{(8,75)} = 11.25$ ,  $p < .01$ , partial  $\eta^2 = .55$ .

When univariate tests were performed on the dependent variables, results indicated that all the components of metacognitive ability of the experimental group were significantly higher than control group

(regardless of time point) at .05 significant level, except procedural knowledge of experimental group was significantly higher than control group at .01 significant level.

**Executive Function of Working Memory Ability**

Working memory was measured based on accuracy and reaction time. The mean score and standard deviation of accuracy and reaction time measured by each working memory tasks between experimental group and control group was shown in Table 3 and 4.

*Accuracy*

A repeated measure of time (before and after intervention) as the independent variables and the accuracy percentage of performing the executive function of working memory tasks including switching, stroop, 0-back, 1-back, and 2-back task as dependent variables. A 2x2 multivariate analysis of variance (MANOVA) findings confirmed that there was a significant multivariate effect for between-subjects (of the combined accuracy of 5 tasks) across student group (regardless of time point): Wilks'  $\lambda = .96$ ,  $F_{(5, 79)} = 115.9$ ,  $p < .01$ , partial  $\eta^2 = .99$ . There was also a significant multivariate effect across within-subjects time point (regardless of student group): Wilks'  $\lambda = .696$ ,  $F_{(5, 79)} = 6.89$ ,  $p < .01$ , partial  $\eta^2 = .304$ . When univariate tests were performed on the dependent variables, results indicated that the accuracy percentage of performing the following most executive function of working memory tasks were significantly higher accuracy than control group (regardless of time point) at  $p < .05$ , see at table 3.

Table 3. Pre-test vs post-test of working memory ability (accuracy) and univariate tests

Dependent variables	Experimental group (N=42)				Control group (N=42)				F
	Pre-test		Post-test		Pre-test		Post-test		
	M	SD	M	SD	M	SD	M	SD	
Switching	86.76	5.84	93.43	5.32	86.14	9.44	86.57	8.03	4.854*
Stroop	89.83	4.25	93.14	3.88	91.9	4.56	92.76	4.1	14.64**
0 back	70.57	16.51	86.81	7.15	71.29	15.38	71.62	15.34	11.993**
1 back	66.29	17.96	81.38	11.65	66.1	15.64	65.81	16.37	3.783*
2 back	40	15.46	60.24	17.46	40.71	17.31	45	19.29	4.128*

\* $p < .05$ , \*\* $p < .01$

*Reaction Time*

The reaction time of performing the executive function of working memory tasks including switching, stroop, 0-back, 1-back, and 2-back task as dependent variables, are repeated measure of time (before and after intervention) as the independent variables. A 2x2 multivariate analysis of variance (MANOVA) findings confirmed there was a significant multivariate effect across the interaction between student group and time point: Wilks'  $\lambda = .951$ ,  $F_{(5, 79)} = 12.85$ ,  $p < .01$ , partial  $\eta^2 = .995$ . There was a significant multivariate effect for between-subjects (of the combined reaction time of 5 tasks) across student group (regardless of time point): Wilks'  $\lambda = .69$ ,  $F_{(5, 79)} = 3.15$ ,  $p < .01$ , partial  $\eta^2 = .30$ . There was also a significant multivariate effect across within-subjects time point (regardless of student group): Wilks'  $\lambda = .321$ ,  $F_{(5, 79)} = 15.47$ ,  $p < .01$ , partial  $\eta^2 = .68$ . When univariate tests were performed on the dependent variables, results indicated that the reaction time when perform switching, and stroop task were significant shorter than control group as shown in table 4.

Table 4. Pre-test vs post-test of the executive function of working memory ability (reaction time) and univariate tests

Dependent variables	Experimental group (N=42)				Control group (N=42)				F
	Pre-test		Post-test		Pre-test		Post-test		
	M	SD	M	SD	M	SD	M	SD	
Switching	863.24	111.72	751.96	91.85	868.1	104.9	831.69	123.46	44.02**
Stroop	616.19	64.6	554.34	49.47	607.41	57.97	610.63	62.25	12.52**
0 back	478.27	76.53	408.7	63.25	477.31	81.1	488.75	83.71	4.66*
1 back	510.14	91.96	421.04	74.44	503.77	103.11	496.08	90.99	2.42*
2 back	554.93	131.44	501.89	122.29	588.59	144.4	582.24	142.82	1.69*

\* $p < .05$ , \*\* $p < .01$

### Critical Thinking Ability

After intervention, finding indicated that there was a significant mean difference between groups, Wilks'  $\lambda = 0.715$ ,  $F_{(8,75)} = 23.56$ ,  $p < 0.01$ , partial  $\eta^2 = 0.98$ . The experimental group scored higher in all components of critical thinking, the mean scores and standard deviation of them and F test of MANOVA including partial  $\eta^2$  were shown in were shown in table 5.

**Table 5.** Pre-test vs post-test of the critical chinking ability and univariate tests

Factors	Experimental group		Control group		F
	M	SD	M	SD	
1) judge the credibility of sources	27.48	2.13	23.57	3.03	46.65**
2)update new knowledge	13.05	1.34	10.38	1.91	54.71**
3)identify conclusions, reasons, and assumptions	22.12	2.33	18.86	2.32	41.31**
4) judge the quality of an argument, including the acceptability of its reasons, assumptions and evidence	31.79	2.55	27.69	3.51	37.41**
5) develop and defend a position on an issue	12.19	1.67	10.60	1.59	20.05**
6)plan experiments and judge experimental designs and select approaches clarifying appropriate	29.62	2.24	27.95	2.43	10.68**
7)define terms in a way appropriate for the context	32.21	2.40	29.36	3.46	19.34**
8)draw conclusions when warranted, but with caution	27.86	2.04	26.19	1.92	14.87**

\*\* $p < .01$

## Discussion

Results of this study indicated that there was a significant different effect from the two teaching models. As a result, great emphasis has been laid on the instructors to use effective teaching models for improving students' learning outcomes. With the passage of time, the importance of university instructors' teaching style is being spread and they are taking initiative to improve their teaching strategies using appropriate teaching model for students' improved learning skills [18].

The results of this study are found to be consistent with several previous findings about constructivism [19]. Lin used constructivism on vocabulary teaching to undergraduate students in Dalian University to make their own meanings and found to be more effective teaching compared to traditional approach. Moreover, findings also reinforced the concept of metacognition as emphasized by Tanner [20]. Tanner found that metacognitive based teaching enables students to discover their own strengths and weaknesses thus made them know how to learn, being able to monitor their own understanding and found their strategies to resolve their confusions.

Finally, results of this study would provide further evidence in support of the need to develop university instructors' abilities to deliver and guide students using constructionism, metacognitive approach as well as how to apply neurocognitive with educational practice as a new concept to Thai lecturers. Effective training program that related to constructionism, metacognitive and neurocognitive based is suggested to Thailand Ministry of Higher Education.

## Conclusion

After experiment, the experiment group yield statically higher means score of dependent variables that included i.e. abilities in understanding medical terminology, anatomical knowledge, metacognitive ability, executive function of working memory ability and critical thinking than the control group. This result indicated that the treatment group had cognitive performance improvements than other one.



## Acknowledgements

This work was supported by Research Fund of Faculty of Veterinary Medicine, Khon Kaen University, Thailand and the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Cluster of Research to Enhance the Quality of Basic Education.

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# The P300 Wave and Brain Topography in Veterinary Students with Trained and Untrained Working Memory

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## Abstract

**Objective** To compare the ERP and topographical brain area between veterinary students who have trained and untrained working memory

**Materials and Methods** A total of 40 students equally distributed into experimental and control groups and attended to the two different teaching models, respectively. An experimental research pretest and post-test control group design was employed and analyzed using 2X2 multivariate analysis of variance.

**Results** The students in experimental group had significant higher voltage at fronto-parietal cortices than the control group. They had short latency and higher amplitude all electrode site than the control one.

**Conclusion** The effect of CMEN model used in the working memory training was greater than the TM model and proved to be an innovative teaching model to enhance working memory.

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**Keywords:** CMEN model, P300, Amplitude, Latency

## Introduction

The event-related potential (ERP) is a waveform that is commonly determined by averaging brain activity in the electroencephalogram (EEG) time-locked to a specific event including picture or auditory stimulus [1,2]. The ERP consists of a series of components that can be distinguished based on their latency (ms), polarity (positive/negative), amplitude ( $\mu$ V), and scalp distribution [1]. The P300 event related potential is a positive potential that occurs approximately 300 milliseconds (ms) after presentation of a stimulus, requiring detection, counting or cognitive processing by the participant [3,4]. It represents higher cognitive function of information processing like stimulus categorization, attention, working memory, and executive function [1,2]. One of the most prominent hypotheses linking the P300 amplitude to cognitive functioning is the context-updating hypothesis [1,2]. The P300 component of event-related potentials (ERPs) has been extensively investigated in clinical practice as it provides an easily obtained, noninvasive index of general cognitive functioning [5]. Longer P3 latencies, related to an increased delay in stimulus classification in a discrimination task, have been found in elderly subjects [6, 7], and in patients with dementia [8, 9], early Alzheimer's disease [10] or in cases of alcoholism [11], among others. A decrease in P3 amplitude has also been clinically observed in depressive [12], schizophrenic and alcoholic subjects [13] and has been identified as a risk marker for alcoholism or the development of a psychopathology [14, 15].

In addition, P300 wave is an ERP component stimulated in the human's decision making process and considered as an endogenous potential [2,7]. ERP's occurrence links not only shown as the physical attributes of a stimulus but also indicated as human's reaction on it. More precisely, the P300 wave is thought to reflect processes involved in stimulus evaluation using P300 amplitude and P300 latency. P300 amplitude refers as the voltage difference between a pre-stimulus baseline and the largest positive-going peak of the ERP waveform within a latency range such as 250-400 ms, although the range can differ depending on subject characteristics, stimulus modality, task conditions, etc [7]. Therefore, P300 amplitude is the thought to brain activity index that is required in the maintenance of working memory when the context is updated [16]. On the other hand, P300 latency is defined as the time from stimulus onset to the point of maximum positive amplitude within the latency window [7]. P300 latency is the thought to classification speed index, which is

proportional to the time required to detect and evaluate a target stimulus [2,7]. On this line of reasoning, the ERPs will provide an idea about the time course of information processing which encompasses expectancy, attention, cognition search, decision making and memorization. A distributed anterior posterior cerebral network is used specifically for updating, involving prefrontal cortex (e.g., BA 10 and BA 9/46) and parietal cortex as core regions in updating [17,18].

## **Materials and Methods**

### **Design and procedure**

In the beginning, all participants completed the focus picture task. For the next four weeks, the experimental group received CMEN teaching model that include 15 minutes daily working memory training sessions (excluding weekends) of content of dog bone in power point presentation, while the control group received TQF manual of Veterinary Medicine teaching model that without training. After approximately from 4 weeks from the first assessments, all participants attended a post-test like pre-test.

### **Subjects**

Experiments were performed in 40 healthy, right-handed 2<sup>nd</sup> year students of Faculty of Veterinary Medicine, Khon Kaen University (age range 19-20 years) with corrected-to-normal vision and no history of neurological or psychiatric conditions. They were randomly placed into two groups. While 20 participants (10 male, no left-handed) took part in the training program, 20 participants (10 male, no left-handed) were assigned to a control group that did not attend training; these group sizes exceeded the size in most of the trainings studies included in a recent review in the field of cognitive training (Morrison and Chein, 2011). Participants in both groups were equally rewarded with a monetary compensation of 1,000 baht and all had normal or corrected-to-normal vision. The study was approved by The Khon Kaen University Ethics Committee In human research (HE 551159).

### **Task and stimulation procedure**

A stimulus oddball paradigm was tested in EEG session which frequent non-target stimuli occur with 80% probability, novel irrelevant stimuli 10%, and novel task-relevant stimuli 10%. The subjects were presented a sequence of the pattern pictures which had different number of dots and the position in the grid 2x2. The dot on the left upper side of the grid 2x2 was defined as target. While the dot on the other side were defined as novel irrelevant stimuli, and two or more dots were defined as frequent non-target were distracter. The Target and distracter were presented in a 21" CRT monitor randomly. The participants were press the keyboard number "1" for target and "2" for distracter.

### **Data acquisition**

The EEG was recorded with DC-amplifiers (Neuroscan® EEG Nuamps device) from 32 positions referenced to linked mastoids using an Quik Cap system (Neuroscan® Inc) that placed according to the 10-20 International system. Impedance values were kept at 5 K $\Omega$  for all electrodes. We used three external flat electrodes to monitor eye movements (two above and below the left eye and one 3 cms next to the outer canthus of the right eye). Data were recorded continuously and stored for off-line analysis with SCAN 4.3 - Vol. I (Compumedics-Neuroscan®), software. We segmented continuous EEG into 500 ms. and the baseline used for the ERP analysis was 100 ms. previous to the appearance of the target stimuli. For accuracy analysis, we eliminated the undesired eye movements and eye blink artifacts waveform by using a semiautomatic and manual block rejection procedure. We included only corrected or match stimuli in our analysis and then quantified ERP components, at each recording site for each participant and probe type, by selecting the amplitude and latency of the largest deflection within a specified latency range around the peak. To investigate the influence of serial position on recognition, researchers examined the accuracy and reaction time of participants' responses to probe items followed by the amplitude, latency, and topographic scalp amplitude distribution of the P300 at Pz electrode site

### **Data Analyses**

EEG and EOG were epoched off-line into periods of 500 ms, starting 100 ms prior to stimulus onset. To investigate the influence of serial position on recognition, we examined the amplitude, latency, and topographic scalp amplitude distribution of the P300 at Pz electrode site.

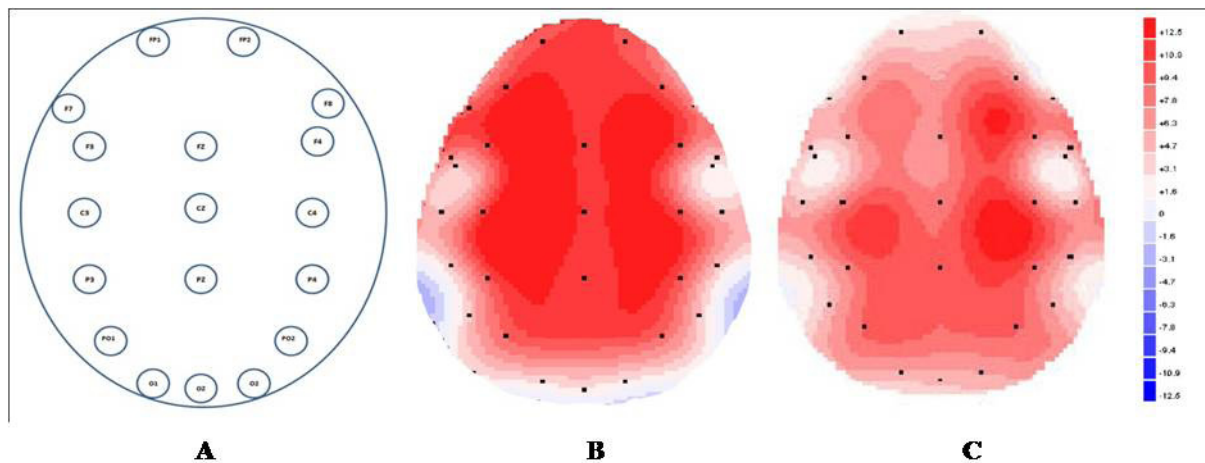
### **Statistical analyses**

Values of were expressed as mean  $\pm$  SD. Analysis for statistical significance of the amplitude of P300 waveform on pretest-posttest was performed using repeated MANOVA, while t-test and pair t-test the Pz latency was performed, also.

## Results

### Topographical data

According to Torres et al., [19], after working memory training, the students in experimental group had high voltage (+10.9 microvolts) at median electrode sites (i.e. frontal, central, and parietal cortices or Fz, Cz, and Pz ), while the control group had only moderate voltage at Pz site (+6.3 microvolts). The experimental group had highest voltage (+12.5 microvolts) in left side of prefrontal, frontal, and primary somatosensory in parietal lobe according to Fp1, F3,F7,and C3 electrode sites as well as the right prefrontal, frontal, and primary somatosensory in parietal lobe according to Fp2, F4, F8, and C4. Contrast with experimental group, the control group had lower voltage ( $\leq$  +6.3 microvolts) at paramedian cortices. In addition, they the left cortices had lower voltage than the right side one. However, the both group had the similar results at occipital cortex.



**Figure 1.** Two dimensional topographical of the brain. A: A schematic diagram depicting dorsal views of the human brain based on the international 10–20 EEG system. Electrodes on the right side of the brain are denoted with even numbered electrodes, whereas electrodes on the left size of the brain are denoted with odd numbered electrodes. The denotation “z” is for zero or midline electrode placement.; B and C: Two dimensional topographical distribution of the evoked P300 potentials at midline parietal Pz sites of experiment group (B) and control group (C) after working memory training.

### Amplitude data

Before intervention, there is no mean difference of all dependent variables (Fz, Cz and Cz amplitude) between groups, Wilks'  $\lambda = 0.16$ ,  $F_{(3,36)} = 2.28$ ,  $p = 0.09$ , partial  $\eta^2 = 0.16$ . After intervention, finding indicated that there was a significant mean difference between groups, Wilks'  $\lambda = 0.616$ ,  $F_{(3,36)} = 7.495$ ,  $p < 0.01$ , partial  $\eta^2 = .384$ . The experimental group manifest higher all amplitude sites than control group. The mean scores and standard deviation of the three amplitude electrode sites and univariate analysis of second year veterinary medicine students after intervention were shown in Table 1.

**Table 1.** The mean scores and standard deviation of the three amplitude electrode sites and univariate analysis

	Experimental group		Control group		F	Pvalue	partial $\eta^2$
	Mean	SD	Mean	SD			
Fz_amplitude	12.560	1.234	11.565	.886	8.573	0.006**	0.184
Cz_amplitude	12.851	0.938	12.213	0.901	4.806	0.035*	0.112
Pz_amplitude	14.908	1.653	13.023	1.237	16.671	0.0000**	0.305

\*p<0.0  
\*\*p<0.01

The repeated MANOVA was performed to compare the effect of training working memory on electrode amplitude site, the analysis indicated that there was a significant multivariate effect between-subjects factor and amplitude sites (Fz, Cz and Cz amplitude) across student group (regardless of time point): Wilks'  $\lambda = .668$ ,  $F(3, 36) = 5.968$ ,  $p < .01$ , partial  $\eta^2 = 0.332$ . There is also a significant multivariate effect across within-subjects time point (regardless of student group): Wilks'  $\lambda = 0.631$ ,  $F(2, 37) = 20.56$ ,  $p < 0.01$ , partial  $\eta^2 = 0.631$ . We also have a significant multivariate effect across the interaction between student group and time point: Wilks'  $\lambda = 0.515$ ,  $F(2, 37) = 11.292$ ,  $p < 0.01$ , partial  $\eta^2 = 0.485$ .

#### Latency data

Before intervention, there is no mean difference of PZ latency between groups (experimental group =  $339.25 \pm 15.47$  vs control group =  $348.8 \pm 16.27$ ),  $t = 0.06$ ,  $p > 0.05$ . After intervention, finding indicated that the latency shorter latency than control group (experimental group =  $331.7 \pm 18.83$  vs control group =  $349.7 \pm 21.94$ ),  $t = 2.783$ ,  $p < 0.01$ . When we have using paired t-test to compare the effect of training working memory on latency, the analysis indicated that the experimental group had a significant decrease the latency time than the other one ( $t = 2.7421$ ,  $p < 0.05$ ).

## Discussion

According to prior researchers [20, 2,7], the P300 amplitude appears to index the operation of an attentional gradient that modulates stimulus encoding for memory storage and sensitive to the amount of attentional resources engaged during dual task performance that varies cognitive demands is performed while the subject also is engaged in a secondary task of mentally counting target oddball stimuli, and might index neural power or cognitive resources, which increase with maturation [21]. The P300 amplitude of all electrode sites of the experimental group was greater than control group, so the students in experimental group had improve their intentional control to greater working memory capacity than the control group. The topographical brain of the students in experimental group showed that many area that involve working memory especially prefrontal and parietal cortices [21,22] had greater voltage than the other one. This implies that the information processing of the cortical networks in the brain of the experiment group have improved in correspond to encoding, retention, and retrieval of information held in working memory [23]. P300 peak amplitudes of both groups were largest at parietal, decreasing through central and frontal electrode sites, consistent with previous results such as Uopasai et al.[23], Li et al.,[24] and Rusiniak et al.[25].

## Conclusion

The results indicated that the information processing of the cortical networks in the brain of the experimental group had improved in encoding, retention, and retrieval of information held in their working memory. The practical implications suggest that the CMEN teaching model should be integrated into working memory training in order to upgrade the working memory capacity and transfer effect in academic achievement.

## Acknowledgements

This work was supported by Research Fund of Faculty of Veterinary Medicine, Khon Kaen University, Thailand and the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Cluster of Research to Enhance the Quality of Basic Education.

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