

JITMM 2013

Joint International Tropical Medicine Meeting 2013
(JITMM 2013)

11-13 December 2013

Centara Grand & Bangkok Convention Centre at CentralWorld

Bangkok, Thailand

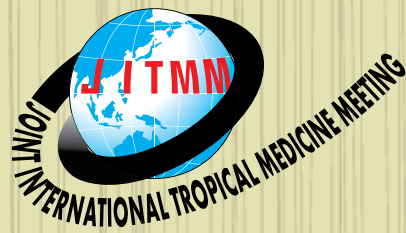


Towards Global Health:
an Asian Paradigm
of Tropical Medicine

ABSTRACT BOOK







ABSTRACTS

ORAL PRESENTATIONS

Joint International Tropical Medicine Meeting 2013

JITMM 2013

11-13 December 2013

Centara Grand & Bangkok Convention Centre at CentralWorld, Bangkok, Thailand

Join the largest regional conference on Tropical Medicine,
hosted by the Faculty of Tropical Medicine, Mahidol University

**“Towards Global Health: an Asian
Paradigm of Tropical Medicine”**



Wednesday 11th December 2013

09:00 – 09:25

Convention Center

BEFORE the Chamlong-Tranachit Harinasuta Lecture:

OPENING CEREMONY



Dean Yaowalark Sukthana
Report and Welcome Address



**Professor Nicholas Day and
Mr Henry Braun**
Welcome Address



President Prof. Rajata Rajatanavin
Opening Address

TROPMED Alumni Award
Presentation, presented by
Aruneek Sabchareon
President, TROPMED
Alumni Association

Award Recipients:

1. Dr. Tawee Chotpitayasunondh
2. Emeritus Prof. Major General Vanich Vanapruks
3. Leonard Ortega



Wednesday 11th December 2013

Time: 09:25 – 10:40

Convention Center

Keynote Pleanar I: The 19th Chamlong-Tranachit Harinasuta Lecture

Towards Global Health: An Asian Paradigm of Tropical Medicine



Keynote Speaker 1

Health in Asia Beyond 2015

Suwit Wibulpolprasert

Ministry of Public Health, Thailand

Profile

Dr. Suwit Wibulpolprasert is a general practitioner, a public health specialist, an administrator and a policy advocator. He began his career as a director and a practitioner in four rural district hospitals in Thailand from 1977 to 1985. Later he was the Director of the Northeastern Public Health College, Director of Technical Division of the FDA, Director of Bureau of Health Policy and Plan, Assistant Permanent Secretary, Deputy Permanent Secretary, and Senior Advisor at the Thai Ministry of Public Health.



Keynote Speaker 2

Health in Asia Beyond 2015

John E.L. Wong

National University of Singapore

Profile

Professor John Eu-Li Wong, Isabel Chan Professor in Medical Sciences, is Vice Provost (Academic Medicine), National University of Singapore (NUS) and Chairman, Board of Directors, Health Sciences Authority of Singapore. He is also the Deputy Chief Executive, National University Health System and Director, National University Cancer Institute, Singapore. A medical oncologist-haematologist, Professor Wong graduated from NUS and did his residency and fellowship at the New York Hospital-Cornell Medical Center, where he was the Chief Resident in Medicine, and Memorial Sloan-Kettering Cancer Center. He is actively involved in the development of Biomedical Sciences and Academic Medicine in Singapore. Professor Wong represents Singapore in the M8 Alliance of Academic Health Centers and the Association of Academic Health Centers-International. He is a member of the World Economic Forum Global Agenda Council on Personalized and Precision Medicine, the Nature Index Panel of Senior Medical Advisors, the International Editorial Board of the American Journal of Medicine, and the Editorial Board of the Journal of the American Medical Association.



Wednesday 11th December 2013

Time: 11:00 – 12:30

Room A

S1 Commemorative Symposium for the 100 years of ICTMM "The Paradigm Shift towards Parasitic Disease Elimination" (IFTM)

Chairperson 1



Santiago Mas-Coma

Chairperson 2



Srisin Khusmith

Symposium description:

The symposium is organized to celebrate Commemorative Event of the Centennial of the ICTMM in Asia. This symposium is about the IFTM and the 100 years of the ICTMM, followed by the talks on the issue of The Paradigm Shift towards "Parasitic Disease Elimination" As the present role in international and national committees, the frontline expertise of the speakers and their international recognition could furnish excellent symposium from different point of views.

The interruption of parasite diseases is one of the greatest challenges for international health and development communities. The current expert view suggests that, by aggressively scaling up control with currently available tools and strategies, much greater gains could be achieved against parasitic diseases, including elimination from a number of countries and regions. Therefore, identification of key knowledge gaps and defining the strategies and tools will result in controlling the diseases, with the ultimate aim of eradication of the parasite from the human population. Sustained commitment from policy makers, scientific community and local communities will be critical factors in the success of this agenda.

1. Introducing the Centenary of International Meetings of Tropical Medicine
Santiago Mas-Coma
2. Malaria Elimination from Asia/Pacific Areas
Shigeyuki Kano
3. Towards Control of Cysticercosis in Asia
Akira Ito
4. Tropical Medicine and Global Health: Lessons from a Parasitic Disease Distributed Worldwide
Santiago Mas-Coma

INTRODUCING THE CENTENARY OF INTERNATIONAL MEETINGS OF TROPICAL MEDICINE

Santiago Mas-Coma

*Professor and Chairman of the Parasitology Department and Director of the International Master's Course on Tropical Parasitic Diseases (University of Valencia), Spain
Vice-President / President Elect of IFTM*



MALARIA ELIMINATION FROM ASIA/PACIFIC AREAS

Shigeyuki Kano

*Director, Department of Tropical Medicine and Malaria, Research Institute, National Center for Global Health and Medicine (NCGM), Japan
Expanded Board of the International Federation for Tropical Medicine (IFTM)*



WHO reported that estimated number of malaria cases in South-East Asian Region (SEAR) and Western Pacific Region (WPR) in 2010 was 30 million, and that of deaths was 43 thousand. Thus it looks as if malaria is not so much of a serious health problem in SEAR and WPR as it is so in African Region (AR). However, if you look at the numbers of people estimated to be at low and high risk of malaria in SEAR and WPR, they are about 1,300 and 800 million respectively, which are much larger than that of AFR at around 700 million. Thus, in Asia and West Pacific, burden of malaria is much higher than you may have expected. Moreover, the emergence and spreading of drug resistant malaria in greater Mekong sub-region are making the problem more serious. Malaria elimination from Asia and West Pacific is now becoming a very distant goal.

However, dedicated efforts are being directed for the elimination of malaria in the regions, with great support of Global Fund, WHO, and many other stakeholders. Strategy of malaria control is also changing from specific approach which may be very straightforward, to comprehensive approach, inclusive of health system strengthening (HSS), to complement the vertical approach. Concept of universal health coverage (UHC) in the malaria control is also ensuring the provision of health services for all people who are really in need of them.

In this presentation, some successful examples of the malaria elimination project such as school-health-based malaria control in Thailand, community-based malaria control in the Philippines in terms of HSS, and UHC for malaria control in the Solomon island (all of them are directly or indirectly under JICA project) are introduced to be discussed.

TOWARDS CONTROL OF CYSTICERCOSIS IN ASIA

Akira Ito

*Emeritus and Visiting Professor, Asahikawa Medical University, Asahikawa, Japan
the Working group in Asia
Department of Parasitology and NTD Research Laboratory, Asahikawa Medical University, Asahikawa, Japan*



One of the most serious but neglected neurological diseases is neurocysticercosis (NCC). It had been expected to be common almost all Asian countries except Muslim societies, but almost completely neglected in 20th century. There are some reasons why it has been neglected. However, through globalization, NCC is becoming more common and serious everywhere including Muslim societies but still neglected. In Asia, we have many other helminthic diseases easily detectable by microscopy. In all areas where we are facing outbreaks of such helminthic diseases, either schistosomiasis, food- or fish-borne trematodiasis or meat- or fish-borne cestodiasis or soil-transmitted helminthiasis, cysticercosis is also common but just ignored or neglected due to the difficulty in identification of the species, *Taenia solium* from two other

species, *T. saginata* and *T. asiatica* and one more step for detection of asymptomatic NCC cases. Throughout our international joint project towards control of cysticercosis in Asia from 1994, we have developed the real-time detection or identification of the species and serology to detect human NCC cases. Through our collaboration mainly in Thailand, Indonesia, China, Lao PDR, Japan with several international consultants, and in Nepal, India, Cambodia Vietnam, Philippines, Malaysia and Papua New Guinea, we have confirmed NCC from all countries and summarize the ongoing project on “Towards control of cysticercosis in Asia” and stress the next step for detection of pigs and the importance of sustainable health education through real time detection of the infection itself, and the present chaotic situation of cysticercosis in Asia and Pacific.

TROPICAL MEDICINE AND GLOBAL HEALTH: LESSONS FROM A PARASITIC DISEASE DISTRIBUTED WORLDWIDE

Santiago Mas-Coma

*Professor and Chairman of the Parasitology Department and Director of the International Master's Course on Tropical Parasitic Diseases (University of Valencia), Spain
Vice-President / President Elect of IFTM*



In recent years, a new approach called “Global Health” has been slowly but progressively introduced in the different international forums of Tropical Medicine. Today, it is usual to hear specialists talking about “Global Health” and this term repeatedly appearing in programs of congresses and meetings, as well as in fact sheets and advertisements in many institutions and even universities.

The question is what are we intending to say with “Global Health”. Which is the message we want to pass with that term? How to combine this term with Tropical Medicine, which is in the present year of 2013 commemorating the Centenary of the first international meeting of the discipline? Are “Global Health” and Tropical Medicine two disciplines which should follow parallel ways? Are they antagonistic or complementary? There are opinions supporting all positions.

The “Global Health” perspective is undoubtedly appropriate to refer to the geographically spreading phenomenon many infectious diseases traditionally included within Tropical Medicine are showing due to the impact of climate change and the so called “global changes” which comprise the effects of man-made modifications of the environment on the diseases, such as irrigation systems, deforestation, transport of humans and animals by ships and flights from one continent to another, etc., just to mention a few. Thus, a trend of globalization may be referred to when considering given diseases. The recent introduction of Chagas disease in Europe is an example. But is it there sufficient scientific reasons to use such an example, when the specific triatomine vectors are absent from Europe?

Fascioliasis is a good model to analyze the aforementioned questions but from the opposite direction, as indeed this parasitic disease is distributed worldwide but has recently proved to be a serious public health problem in many tropical and subtropical developing countries. The pronounced differences this trematode disease shows when comparing developed and developing countries are related not only to poorness, but also to biogeography, ecology, climatic and other abiotic and biotic factors, among which many related to social aspects and local human traditions. This is why this disease has been included within the neglected tropical diseases to which priority has been given by the World Health Organization.

Wednesday 11th December 2013

Time: 11:00 – 12:30
Room B

S2 The Great Malaria Debate: Elimination of Artemisinin-Resistant Malaria from Southeast Asia

Chairperson 1



Srivicha Krudsood

Chairperson 2



Jeffery Smith

Symposium description:

The Great Malaria Debate: Elimination of Artemisinin-Resistant Malaria from Southeast Asia – Is It Possible?

The past decade has demonstrated the greatest advances in global malaria control and elimination since the WHO-initiated Global Malaria Eradication Program of the 1950s and 1960s. Since 2000, deaths resulting from malaria have fallen by more than 25%. However, we may not be able to sustain these achievements due to the presence of artemisinin-resistant malaria parasites in Southeast Asia. With artemisinin compounds being the foundation of the current therapeutic arsenal to control malaria, the presence of drug resistance in the Mekong region threatens the advances made in malaria control and elimination. Current efforts in Southeast Asia focus on controlling malaria in areas of transmission, containing artemisinin-resistant parasites within the region and implementing strategies for eliminating malaria where feasible. The goal of the culmination of these efforts is malaria elimination in Southeast Asia. This debate will provide a platform for experts to present the scientific, social, economic, and political challenges faced in trying to eliminate artemisinin-resistant *P. falciparum* malaria and provoke innovative ideas for overcoming these challenges.

Invited Speakers:



1. Investigator, Howard Hughes Medical Institute
Professor and Leader, Malaria Group Center for Vaccine Development,
University of Maryland School of Medicine
Christopher V. Plowe
-



2. Laboratory Head, Walter & Eliza Hall Institute, Melbourne
Ivo Mueller
-



3. Mahidol Oxford Tropical Medicine Research Unit
Nicholas J. White
-



4. Faculty of Tropical Medicine, Mahidol University
Jetsumon Prachumsri
-

(abstracts are not available for this debate session)

Wednesday 11th December 2013

Time: 11:00 – 12:30

Room C

S3 Food Security and Food Safety of Chicken Meat from Thailand
Organized by: World Poultry Science Association Thailand

Chairperson 1



Rungsun Tungtrongchitr

Chairperson 2



Dumrongkiet Arthan

1. The development of poultry genetic that's made food security to serve the world population
Borisuth Wunnasuth
2. New technology of poultry production and biosecurity system to assure food security and safety to consumer
Payungsak Somyanontanagul
3. Update nutrition technology that's made poultry growth without antibiotic growth promoter or without additional hormone
Yuwares Ruengpanich

THE DEVELOPMENT OF POULTRY GENETIC THAT'S MADE FOOD SECURITY TO SERVE THE WORLD POPULATION

Borisuth Wunnasuth
Cobb Asia



Abstract not available

NEW TECHNOLOGY IN POULTRY PRODUCTION AND BIOSECURITY SYSTEM TO ASSURE FOOD SECURITY AND SAFETY TO CONSUMER

Payungsak Somyanontanagul
CPF (Thailand PCL)

Abstract not available

UPDATE NUTRITION TECHNOLOGY THAT'S MADE POULTRY GROWTH WITHOUT ANTIBIOTIC GROWTH PROMOTER OR WITHOUT ADDITIONAL HORMONE



Yuwares Ruangpanit

Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University

The genetic selection of modern broiler emphasizes on improving feed conversion efficiency and maximal growth performance which could be supported by several nutritional and feeding approaches. To maximize performance, broiler diets are formulated to contain the correct balance of the essential nutrients required to meet the nutritional needs of modern-day genotypes. With a very precise nutrient digestibility study techniques, broiler dies are currently formulated base on metabolizable energy, ileal amino acid digestibility and ideal protein concept. Commercially available synthetic amino acids help to ensure an adequate and physiologically balanced of amino acid requirement, allowing broilers to reach their maximum muscle deposition. Feed manufacturing also plays an important role in improving nutrient utilization of broilers. Most feed mills possess effective feed quality control and feed quality assurance programs which guarantee a better feed quality being produced. Furthermore, feed processing improves availability of protein and energy, destructs anti-nutritional factors and toxins, improving nutrient utilization for broilers while reducing feed wastage. Since nutritionists do realize that different breeds of broiler have different nutrient requirements and growth patterns, a good design of nutritional and feeding programs are successfully established. Feeding strategies are used not only to maximize growth performances but also to improve gut health and optimize immune response. In addition, a wide variety of effective feed additives are used as alternative to antibiotic growth promoter to enhance production efficiency. These nutrition technologies make it possible for broiler to achieve their maximum genetic potential without the use of hormone and antibiotic growth promoter.

Wednesday 11th December 2013

Time: 11:00 – 12:30

Room D

S4 Reducing the Risk of Emerging Infectious Diseases Transmission:
Results from Research on Human-Animal Interactions in Southeast Asia
Organized by: Family Health International (FHI360)

Chairperson 1



Pratap Singhasivanon

Chairperson 2



Sara Woldehanna

Symposium description:

Many newly emerging diseases that pose a serious threat to human health in an increasingly globalized world are zoonotic in origin. Increased risks of emergence have been attributed to human development activities that are changing the natural environment and the interface of humans and animals. To understand and address the risk of disease transmission from animals to humans, it is therefore important to examine the human-animal interface from the perspectives of both human behavior and disease. The PREVENT Project is funded by USAID and implemented by FHI 360 to examine these connections.

Our symposium will first present a newly developed Human-Animal-Interface framework that characterizes relationships between different disease transmission routes and human activities, and discuss how initial human-animal exposure research was designed based on this foundation. In addition, initial results from three studies conducted by PREVENT will be presented: 1) human-animal interface survey in Bolikhamxay, Laos to measure human exposure to different animals, assess and identify populations or subgroups with particularly high rates of exposure, and the practices/locations associated with exposure (and therefore, risk); 2) a similar survey in KhonKaen Province, Thailand; and 3) a participatory rapid appraisal in KhonKaen Province to describe human exposure to peridomestic rodents and identify strategies people use (or might be able to use) to mitigate the risk of exposure. We anticipate that results of all of these studies will help inform the design and implementation of future interventions to reduce the risk of transmission of emerging infectious diseases.

- 1 Developing a Human-Animal Interface Framework to Help Reduce Transmission Risk of Emerging Infectious Diseases
Sara Woldehanna
- 2 Human-Animal Interactions in Khon Kaen Province, Thailand
Kanokwan Suwannarong
- 3 Characterizing Human-Animal Exposure in Hmong and Lao Ethnic Groups in Lao PDR
Cecile Lantican
- 4 Human Exposure to Peridomestic Rodents in Khon Kaen, Thailand: Results from a Participatory Rapid Appraisal
Laura Seckel

DEVELOPING A FRAMEWORK TO INFORM STUDIES ON THE HUMAN-ANIMAL INTERFACE

Sara Woldehanna
FHI 360



Sara Woldehanna¹, Susan Zimicki¹

¹FHI 360, Washington DC; USA

In this session a conceptual framework for understanding the human-animal interface – that is, direct and indirect interactions between humans and wild and domestic animals that could lead to zoonotic disease transmission – is presented. The framework accounts for social as well as epidemiologic factors that are important for determining risk of zoonotic disease transmission. Social factors such as gender, age, ethnicity, and socio-economic status affect social norms and the activities people engage in. These, in turn, determine opportunities for transmission (i.e., if, how, where, and when people may come in contact with different animals). Probability of transmission also depends on the rate, intensity, and duration of human contact with potentially infectious animals.

This conceptual framework informed the development of a human-animal exposure study, which ensures that information about the different ways that people come in contact with animals is firmly grounded in the specific social context in which data is collected. The study employs a mixed-methods approach that culminates in a representative, random sample survey to quantify exposure to animals in a variety of settings. The study is designed to obtain information that will allow comparison of exposures between men and women, adults and children as well as different cultural groups (e.g. ethnicities). The rationale behind the framework as well as key aspects of the human-animal exposure study that are informed by the framework are described.

HUMAN-ANIMAL INTERACTIONS IN KHON KAEN PROVINCE, THAILAND

Kanokwan Suwannarong
FHI 360



Kanokwan Suwannarong,^{1,3} Zo Rambeloson,² Sara Woldehanna,² R.S. Chapman R.S.,³ and Susan Zimicki²

¹FHI 360, Asian Pacific Regional Office (APRO), Bangkok, Thailand

²FHI 360, Washington, DC, USA

³College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand

Increasing human-animal interaction, along with environmental factors and expanding worldwide transportation, can promote emergence and spread of emerging infectious diseases (EIDs). Therefore, during November to December 2011, a cross-sectional study was conducted with the aim of ascertaining human-animal exposure characteristics in urban, agriculture and forest settings in four districts in Khon Kaen Province, Thailand. Trained field researchers used a questionnaire in face-to-face interviews to gather information on characteristics of human-animal contact, including several types of human-rodent interactions, as well as characteristics potentially associated with such interactions (e.g., gender, age group, site location, occupation, size of family, educational attainment level, economic status, household characteristics, perception of animals that could transmit diseases, source of water, waste disposal system, and belief and/or attitude toward EIDs). The 201 men and women who participated in this study reported contact with many types of animals, but rodents were the main animal species they reported encountering. Analyses showed that rice/field farmers who had to work outside the household are a subgroup at risk of encountering rodents; within this group, gender was not significantly associated with risk of encountering rodents. Three factors (occupation as farmer, perceiving that disease can be acquired from rodents, and always using rain water for drinking) were statistically significantly associated with rodent encounters, while one factor (had food waste collected from household by collectors) was associated with less contact with rodents. Such contact could increase the risk of rodent-borne infections, including rodent-borne EIDs. The analysis results indicate possible ways of reducing risk.

CHARACTERIZING HUMAN-ANIMAL EXPOSURE IN HMONG AND LAO ETHNIC GROUPS IN LAO PDR

Cecile Lantican
FHI360



Zo Rambeloson,¹ Sara Woldehanna,¹ Cecile Lantican,² Susan Zimicki¹

¹FHI 360, Washington, DC, USA

²FHI 360, Lao PDR Office, Vientiane, Lao PDR

To help inform the design and implementation of interventions to reduce the transmission of emerging infectious diseases, we conducted a quantitative survey in Lao PDR to provide estimated rates of human exposure to different animals and to identify populations or subgroups with particularly high rates of exposure, as well as the locations/activities that are associated with exposure. The population-based household survey among Hmong and Lao adult men (n=292), women (n=292), boys (n=203), and girls (n=189) was conducted in Khamkeut District, Bolikhamxay Province, Lao PDR in early 2013. Independent samples were obtained for men and women aged 18-50 in each ethnic group; up to one girl and one boy aged 10-14 years of age were selected from every adult household that had children who met the age criteria. Animals of interest were wild animals particularly likely to carry zoonotic viruses – bats, rodents, and primates – although the survey also noted other wild animals to which people were exposed. Selected domestic animals (pigs and chickens) were used as controls. Results revealed that there is nearly universal exposure to domestic animals and rodents by both Lao and Hmong groups, males and females, and adults and children. Overall, men/boys reported higher contact with rodents, primates, and wild boars than women/girls. This occurs because hunting is primarily a responsibility of men/boys; however, women/girls are just as exposed during slaughter, butchering, and preparation of wild and/or domestic animals. Other results will be shared during this session.

HUMAN EXPOSURE TO PERIDOMESTIC RODENTS IN KHON KAEN, THAILAND: RESULTS FROM A PARTICIPATORY RAPID APPRAISAL

Laura Seckel
FHI 360



Laura Seckel,¹ Kanokwan Suwannarong,² and Susan Zimicki¹

¹FHI 360, Washington, DC, USA

²FHI 360, Asian Pacific Regional Office (APRO), Bangkok, Thailand

Rodents are carriers and reservoirs of numerous pathogens that pose significant risk to humans, including many emerging diseases. In both urban and rural areas, peridomestic rodents (Family *Muridae*), such as rats and mice, pose a public health threat because they regularly come into contact with humans and domestic animals, providing opportunities for disease transmission. To prevent the spread of disease from these rodents to humans, feasible and acceptable strategies must be developed to minimize human exposure to rodents and their excrement. However, mitigation strategies will only be successful if they are framed within the context of the perspective, knowledge, skills, and resources of a given community. To inform the development of mitigation strategies, we used rapid appraisal and participatory research techniques to characterize peridomestic rodent exposure among men, women, and children in four sites in Khon Kaen province, Thailand, where peridomestic rodents are viewed as both a destructive nuisance and a source of food and income. Exposure to peridomestic rodents inside and outside of the home in two of these communities will be described, and variation due to gender, location (urban vs. rural), age, and occupation will be considered. Strategies people use to capture rodents for consumption or to protect themselves or their property from rodents will be presented. The way these strategies either contribute to or mitigate the risk of exposure and the relevance of the findings for future public health interventions to reduce disease transmission from peridomestic rodents to humans will be discussed.

Wednesday 11th December 2013

Time: 11:00 – 12:30
Room E

S5 Health Management System

Chairperson 1



Vicente Jr. Belizario

Chairperson 2



Jaranit Kaewkungwal

1. Effectiveness of teacher's training package for providing teacher's training for school based children's health check-up in Lao PDR
Sachi Tomokawa
2. Safety and Feasibility of Combined Mass Drug Administration for Soil-Transmitted Helminthiases and Schistosomiasis among School-age Children
Vicente Jr. Belizario
3. Prevalence and Pattern of Injuries Among Adolescent Students: Implication for Public Health Concern
Jephtha C NMOR
4. Multidisciplinary Collaboration Of Thailand One Health University Network, Responding To Emerging And Re-Emerging Diseases: Lesson Learned And Achievements
Saengduen Moonsom
5. Quality Improvement Project For Mobile Health Clinics
Andrew Silver

EFFECTIVENESS OF TEACHER'S TRAINING PACKAGE FOR PROVIDING TEACHER'S TRAINING FOR SCHOOL BASED CHILDREN'S HEALTH CHECK-UP IN LAO PDR

Sachi Tomokawa

Shinshu University, Nagano, Japan



Background and Aims: Lao Government officially mentioned that necessity of introduction of school health check-ups for children in its educational policy. However, applicable systems in Lao context and how to train the teacher for school based health checkup are not well known. Therefore, this study aimed to develop teacher's training package for school-based health check-ups and to evaluate its effectiveness.

Methodology: This study was conducted in September, 2013, Luang Prabang, Lao PDR. Teachers in Teacher Training Colleges (TTC) in Luang Prabang and its nearby primary and secondary schools teachers participated in the training. The training program consisted by lectures (importance of health check-up and its data utilization, methodology) and practical training in the school. In the health checkup, height, weight, eye-sight and hearing ability were tested. Moreover, effectiveness of the training was evaluated by pre-post test using a self-writing questionnaire to teachers.

Results: The data with unknown sex and with insufficient data was excluded. Teachers were 38.9 (10.5) years on average: 29(42.0%) were males and 40(58.0%) were females. 39 teachers from TTC and 30 teachers from nearby schools. After the training confidence of measurement skills in teacher's self, conducting health check up in the school, if trained teachers can teach to colleagues were significantly increased. Almost teachers evaluated that well understood the lecture and practical training.

Conclusion: It was cleared that the developed training package was applicable in the Lao context. However, it needs to follow up about teachers' practice in the school after the training.

SAFETY AND FEASIBILITY OF COMBINED MASS DRUG ADMINISTRATION FOR SOIL-TRANSMITTED HELMINTHIASES AND SCHISTOSOMIASIS AMONG SCHOOL-AGE CHILDREN

Vicente Jr. Belizario

University of the Philippines Manila - National Institutes of Health



Vicente Y. Belizario, Jr.^{1,2}, Jose Rafael Marfori, MD¹,
J. Mark Erfe, MPH^{1,2}, Paul Lester Chua, RN¹, and June Rose Naig, RMT¹

¹ National Institutes of Health, University of the Philippines Manila, 623 Pedro Gil St., Ermita, Manila 1000 Philippines

² College of Public Health, University of the Philippines Manila, 625 Pedro Gil St., Ermita, Manila 1000 Philippines

Soil-transmitted helminthiases (STH) and schistosomiasis remain public health problems in the Philippines. The WHO has recommended combined mass drug administration (MDA) for the two helminth infections in co-endemic areas, utilizing existing infrastructure in schools. This study aimed to demonstrate the safety and feasibility of school-based combined MDA using albendazole and praziquantel in two co-endemic areas in the Philippines. This study consisted of two phases: Phase I assessed the safety of combined MDA in two pilot schools in the Municipality of Calatrava, Province of Negros Occidental; Phase II assessed the safety and feasibility of teacher-assisted combined MDA in ten schools in the Municipalities of Carmen and Santo Tomas, Province of Davao del Norte. As part of this study, praziquantel and albendazole was co-administered to school-age children enrolled in the selected schools. School-based combined MDA covered 327 students in Calatrava and 2,410 in Davao del Norte with coverage rates of 80.1% and 75.5%, respectively. Of students treated, 5.2% in Phase I and 5.4% in Phase II were confirmed to experience adverse events (AEs), which were mostly mild and transient; all AEs resolved in the schools. AEs observed in this study were generally of lower incidence than those reported in other studies. The safety profile and feasibility demonstrated in this study may provide basis for a larger scale implementation of school-based, teacher-assisted combined MDA for schistosomiasis and STH in co-endemic areas in the country, provided standard operating procedures are established and followed.

Keywords: soil-transmitted helminthiases, schistosomiasis, combined mass drug administration, neglected tropical diseases, praziquantel, albendazole

PREVALENCE AND PATTERN OF INJURIES AMONG ADOLESCENT STUDENTS: IMPLICATION FOR PUBLIC HEALTH CONCERN

Jephtha C Nmor

National Mental Support Center for School Crisis, Osaka Kyoiku University, Osaka, Japan



Jephtha C Nmor^{1,2*}, Kehi H Nwaka^{2,3}, Kensuke Goto⁴, Junko Toyosawa¹ and Daisuke Fujita^{1*}

¹National Mental Support Center for School Crisis, Osaka Kyoiku University, Osaka, Japan.

²Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria.

³St. Anthony Model School Ubuluku, Delta State, Nigeria.

⁴Department of Eco-epidemiology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan.

This study describes the prevalence and pattern of injuries in a cross section population of students in Southern Nigeria, in order to provide data for use in developing priorities for injury prevention efforts. A school-based survey of students attending schools in Nigeria was conducted in 2013. The study sample involved 585 students (60.9% male, 39.2% female and overall mean age of 15 years). We inquired about participants self-reported injuries in the past one year. When reported, injuries were assessed according to cause, intent, nature, type, place, and number of days absent from school due to injury. Overall, there were 549 self-reported injuries in past one year (93.8%) among the respondents (95.5% for males and 90.8% for females, $p = 0.6696$). Sex wise, prevalence of injury differ by age, school setting and parents' occupation ($p < 0.05$). Falls 293 (53.4%) was the leading cause of injury. Falls and animal bites were significantly higher in males compared to females [206 (60.4%) vs 87 (41.8%) $p = 0.002$, and 10 (2.9%) vs 0 (0.0) $p = 0.016$] respectively. Traffic injuries and falls decreases progressively with age. Homes and schools were settings injuries occurred mostly. Over 68 % of the reported injuries were unintentional. On the average, 2 days of normal school activity were lost per injured persons because of an injury. Given the high prevalence of injury reported in this study, injury should be considered a significant public health problem in Nigeria. Also, there is need for safety education aiming to educate this young population on how to prevent injury.

Keywords: School-based, Self-reported, Injury, Prevalence, Students, Nigeria

MULTIDISCIPLINARY COLLABORATION OF THAILAND ONE HEALTH UNIVERSITY NETWORK, RESPONDING TO EMERGING AND RE-EMERGING DISEASES: LESSON LEARNED AND ACHIEVEMENTS

Saengduen Moonsom

Faculty of Tropical Medicine, Mahidol University



Saengduen Moonsom^{a,e}, Jeein Chung^b, Irwin Fernandez Chavez^c, Ronald Enrique Morales Vargas^d, Pratap Singhasivanon^{c,e}

^a Department of Protozoology, ^cDepartment of Tropical Hygiene, ^dDepartment of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Thailand

^b College of Veterinary Medicine, University of Minnesota, MN, USA

^e THOHUN Coordinating Office, Tranakchit Building Fl.9, Faculty of Tropical Medicine, Mahidol University, Thailand

New infectious diseases have emerged while seemingly “older” and sometimes “neglected” diseases continue to re-emerge, primarily from the interaction of multiple species in a constantly changing environment. The sheer complexity of these diseases' etiologies demands that these health problems are addressed more holistically and through the collaboration of multiple disciplines. The One Health (OH) framework seeks to address such complexity by fostering a trans-disciplinary approach that engages and employs, in addition to physicians, other allied health professionals including veterinarians, nurses, public health practitioners, environmental specialists, and other relevant disciplines.

The Southeast Asia One Health University Network (SEAOHUN) was established in December 2011 to implement its One Health vision of “fostering sustainable trans-disciplinary capacity building to respond to emerging and re-emerging infectious and zoonotic diseases.” The SEAOHUN is a network of medical, veterinary, and public health academic institutions and faculties in Indonesia, Malaysia, Thailand, and Vietnam.

In Thailand, multidisciplinary collaboration have taken place through the Thailand One Health University Network (THOHUN). Four objectives have been identified through the THOHUN mission:

1. Build, Develop, and expand One Health University Network of Thailand.
2. Develop and exchange skilled One Health professionals between universities and involved agencies.
3. Support trans-disciplinary collaboration within and between universities and involved agencies.
4. Promote research work using One Health Approach to promote the health of humans, animals and environment.
5. Coordinate with One Health Network members in Southeast Asia; the formation of One Health professionals.

This abstract will review and examine the extent of THOHUN’s activities and achievements since its establishment in 2012.

Keywords: One Health, multidisciplinary collaboration, THOHUN, capacity building, university network
Financial support provided by RESPOND-USAID through THOHUN
and underline is a presenter

QUALITY IMPROVEMENT PROJECT FOR MOBILE HEALTH CLINICS

Andrew Silver

Karen Department of Health and Welfare



Andrew G. Silver [Poo Maw Taw]

Karen Department of Health and Welfare, Mae Sod, Tak 63110, Thailand

The Karen Department of Health and Welfare operates, with partner community-based organizations, 45 mobile health clinics serving a target population of 167,000 internally displaced persons in southeastern Burma. Logbook review for randomly selected cases of malaria, diarrhea, and ARI was conducted for 2011 and 2012. Commonly found lapses were failure to record signs of dehydration or treatment for diarrhea, and unclear recording of RDT results for malaria. A pilot quality improvement project for correcting such lapses was initiated June, 2012. Major obstacles are multiple languages, remoteness of clinics, and lack of direct communication except during visits. Flip charts to facilitate remembering targeted procedures, like checklists, were given to two clinics. Volunteer QI coordinators at the clinics were instructed to fill out logbook review forms and return them when possible, about every 3 months. Also when possible, the project team would return data summaries and bar graphs to track improvement month to month. The pilot was meant to be limited to two clinics while methods were devised and tested, but it met with unanticipated enthusiasm. Although QI volunteers receive little or no compensation, 6 additional clinics requested materials and named 2 healthworkers each as QI coordinators. During the first year, data were received from 5 clinics for 1576 cases. Summaries and charts were returned. The immediate goal of engaging healthworkers to understand and work for quality improvement was achieved. Longer term goals of producing measurable quality improvement and institutionalizing the project lie ahead.

Keywords: logbook review, quality improvement, mobile health clinics, Karen, pilot, malaria, diarrhea, ARI, checklists, remoteness

Wednesday 11th December 2013

Time: 13:30 – 15:00

Room A

S6 Dengue Update

Chairperson



Chukiatt Sirivichayakul

1. DENVax - Tetravalent Dengue Vaccine, development status
Wolfgang Bender
2. A Spatial Model for Dengue Virus Infection in Urban Areas
Stephan Karl
3. Differentiation between dengue and non-dengue among acute febrile patients
Chukiatt Sirivichayakul

DENVAX - TETRAVALENT DENGUE VACCINE, DEVELOPMENT STATUS

Wolfgang Bender

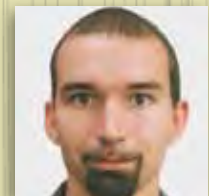


Abstract Not Available

A SPATIAL MODEL FOR DENGUE VIRUS INFECTION IN URBAN AREAS

Stephan Karl

School of Computer Science and Software Engineering, The University of Western Australia, Perth, Australia



Stephan Karl¹, Nilimesh Halder¹, Joel Kelso¹, Scott Ritchie², George Milne¹

¹ *School of Computer Science and Software Engineering, The University of Western Australia, Perth, Western Australia*

² *School of Public Health, Tropical Medicine & Rehabilitation Sciences, James Cook University, Cairns, Queensland, Australia*

There are recent indications that the health burden caused by dengue virus infection has been vastly underestimated. While World Health Organization (WHO) estimates for the global number of infections usually range between 50-100 million cases per year, recent studies estimated approximately

3 times higher case numbers. There are also indications that the geographic range of dengue virus transmission is extending with the disease now more frequently occurring in areas such as southern Europe. The far north of Australia is endemic for *Aedes aegypti*, one of the most prominent dengue vectors and the city of Cairns in northern Australia frequently experiences dengue epidemics.

We have developed a spatially explicit individual based mathematical model to predict Dengue transmission in the city of Cairns. The model incorporates detailed census and mosquito trapping data and accounts for the spread of infections through mosquito dispersion and human movement. The model is capable of realistically reproducing past dengue epidemics in Cairns and can be used for planning interventions and scaling the effectiveness and cost effectiveness of potential vaccination programmes. Our current model setup incorporates conventional interventions such as in house residual spraying and removal of mosquito breeding sites.

To our knowledge this is the most comprehensive, realistic and versatile model for urban dengue transmission which is currently available. The model can be transferred to other urban scenarios where census and mosquito trapping data are available. Our current work includes data collection to transfer of the model to the city of Madang, Papua New Guinea, which has a size similar to Cairns but more intense, perennial dengue transmission.

DIFFERENTIATION BETWEEN DENGUE AND NON-DENGUE AMONG ACUTE FEBRILE PATIENTS

Chukiat Sirivichayakul
Department of Tropical Pediatrics
Faculty of Tropical medicine
Mahidol University



Disease caused by dengue infection is an important public health problem in tropical countries. Although many confirmatory laboratory diagnostic tests have been developed, they are still expensive and not available in many dengue endemic areas. The clinical diagnosis is therefore still important. In 2009, WHO developed new guidelines for dengue diagnosis, treatment, prevention and control. However, only few studies validate these dengue diagnosis guidelines.

Data from a school-based cohort in children aged 3-10 years in Ratchaburi Province, Thailand conducted during 2005-2008 were analyzed. 297 dengue (including 112 undifferentiated fever, 140 dengue fever and 45 dengue hemorrhagic fever) and 1154 non-dengue acute febrile episodes were included in the analysis. It was found that higher proportion of dengue cases had anorexia, vomiting, myalgia, diarrhea, abdominal pain, rash, facial flush, leucopenia (WBC <4,000/mm³) and thrombocytopenia (platelet <150,000/mm³) compared to non-dengue cases. 278 (93.6%) of dengue cases and 847 (73.4%) of non-dengue cases met the probable criteria for dengue; and 230 (74.4%) of dengue cases and 613 (53.1%) of non-dengue cases had warning sign according to WHO (2009)'s guidelines. More accurate clinical criteria for dengue diagnosis will be discussed in the presentation.

Wednesday 11th December 2013

Time: 13:30 – 15:00

Room B

S7 Next-generation antimalarial drugs

Chairperson 1



Thanat Chookajorn

Chairperson 2



Pascal Ringwald

1. Molecular markers of delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia: First replication GWAS
Christopher V. Plowe
2. Knowledge for drug development against pyrimidine biosynthesis
Pradip Rathod
3. The challenges of developing next generation synthetic peroxide antimalarials
Stephen Ward
4. Next-generation antifolate drugs and drug targets
Yongyuth Yuthavong

MOLECULAR MARKERS OF DELAYED CLEARANCE OF *PLASMODIUM FALCIPARUM* FOLLOWING ARTEMISININ TREATMENT IN SOUTHEAST ASIA: FIRST REPLICATION GWAS

Christopher V. Plowe

Investigator, Howard Hughes Medical Institute

Professor and Leader, Malaria Group Center for Vaccine Development, University of Maryland School of Medicine



Shannon Takala Harrison¹, Christopher Pepin¹, Michael P. Cummings², Christopher Jacob¹, Cesar Arze³, Arjen Dondorp⁴, Mark Fukuda⁵, Tran Tinh Hien⁶, Myat P. Kyow⁷, Myat H. Nyunt⁷, Myaing M. Nyunt⁸, Mayfong Mayxay⁹, Paul N. Newton⁹, Francois Nosten¹⁰, Harald Noedl¹¹, Mallika Imwong¹², Delia Bethel⁵, Youry Se¹³, Chanthap Lon¹³, Stuart Tyner⁵, David Saunders⁵, Duong Socheat¹⁴, Aung Pyae Phyoh¹⁰, Peter Starzengruber¹¹, Paul Swoboda¹¹, Hans-Peter Fuehrer¹¹, Taane G. Clark¹⁵, Joana C. Silva³, Matthew Adams³, John C. Tan¹⁶, Michael T. Ferdig¹⁶, Olivo Miotto¹⁷, Magnus Manske¹⁷, Bronwyn Maclnnis¹⁷, Dominic Kwiatkowski¹⁷, Nicholas J. White⁴, Pascal Ringwald¹⁸, Christopher V. Plowe¹

¹ Howard Hughes Medical Institute/University of Maryland School of Medicine, Baltimore, MD, United States, ²University of Maryland, College Park, MD, United States, ³University of Maryland School of Medicine, Baltimore, MD, United States, ⁴Mahidol Oxford Research Unit, Bangkok, Thailand, ⁵Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, ⁶Centre for Tropical Medicine, Oxford University Clinical Research Unit, Ho Chi Minh, Viet Nam, ⁷Department of Medical Research (Lower Myanmar), Yangon, Myanmar, ⁸Johns Hopkins University, Baltimore, MD, United States, ⁹Wellcome Trust/Mahosot Hospital, Vientiane, Lao People's Democratic Republic, ¹⁰Shoklo Malaria Research Unit, Mae Sod, Thailand, ¹¹Medical University of Vienna, Vienna, Austria, ¹²Mahidol University, Bangkok, Thailand, ¹³Armed Forces Research Institute of Medical Sciences, Pnom Penh, Cambodia, ¹⁴Center for Parasitology, Entomology and Malaria Control, Pnom Penh, Cambodia, ¹⁵London School of Tropical Medicine, London, United Kingdom, ¹⁶University of Notre Dame, Notre Dame, IN, United States, ¹⁷Wellcome Trust Sanger Institute, Hinxton, United Kingdom, ¹⁸Global Malaria Programme, World Health Organization, Geneva, Switzerland

Containment of artemisinin-resistant *Falciparum malaria* required tools to measure the extent and direction of its spread. A molecular assay to detect markers of artemisinin resistance would be a highly valuable surveillance tool. An initial genome-wide association study (GWAS) with a relatively sparse set of markers identified single nucleotide polymorphisms (SNPs) on *Plasmodium falciparum* chromosomes 10, 13 and 14 that were associated with delayed parasite clearance, as well as several regions of the parasite genome under recent positive selection. We undertook a replication GWAS using a DNA microarray with ~33K SNPs to more finely map the location of genes involved in artemisinin resistance in an independent set of samples collected during artesunate efficacy studies conducted in Cambodia, Laos, Vietnam, and Myanmar. Linear mixed models and Random Forests were used to estimate associations between individual SNPs and parasite clearance half-life, while adjusting for important covariates and taking into account multiple comparisons. Genomic signatures of selection were also identified. Candidate markers of artemisinin resistance located in genomic regions will be presented, and implications for the dissemination and containment of artemisinin resistance will be discussed.

KNOWLEDGE FOR DRUG DEVELOPMENT AGAINST PYRIMIDINE BIOSYNTHESIS

Pradip Rathod

NIH International Center of Excellence for Malaria Research for South Asia (South Asia ICEMR), University of Washington



Finding good inhibitors against proliferating malaria parasites is increasingly easy. Pradip Rathod will review work on the use of high-throughput chemical libraries to find potent inhibitors of *P. falciparum*, some of which are now headed to clinical trials. Equally importantly, some of these compounds are powerful tools for target validation and for understanding population-based strategies of malaria parasites to acquire resistance in their genome at the right locus, without extensive damage elsewhere in their genome.

THE CHALLENGES OF DEVELOPING NEXT GENERATION SYNTHETIC PEROXIDE ANTIMALARIALS

Stephen Ward

Walter Myers Professor of Parasitology, Deputy Director Liverpool School of Tropical Medicine



Steve Ward¹, Richard Amewu² and Paul O'Neill³

¹ Liverpool School of Tropical Medicine

² Department of Chemistry University of Accra Ghana

³ Department of Chemistry University of Liverpool

At the turn of the last century the importance of artemisinin based antimalarials was beginning to emerge. Despite the fantastic performance of these molecules in killing parasites in a test tube, a mouse or a man there were potential areas of concern around drug supply, as semisynthetic molecules requiring a source of artemisinin starting material, physicochemical properties and dispositional characteristics. To try to address these challenges the team in Liverpool set out to develop a fully synthetic peroxide based antimalarial. Starting with one very enthusiastic Ghanaian Post Doc (RA above) a range of peroxide nuclei were evaluated. The tetraoxane core was selected as the most promising pharmacophore and from their evolved a rational medicinal chemistry programme that has generated over three hundred molecules most of which have single digit nanomolar IC₅₀'s *in vitro*. Potency was easy to achieve but delivery of a truly drug like molecule with good stability, excellent pharmacokinetics and simple cost-effective synthesis proved much more challenging. Over the following years we have systematically performed SAR analysis of lead – like drug molecules and optimized the key antimalarial and drug like features in order to generate a credible candidate drug for onward development. Along the way we have been able to generate some insight into how peroxides kill parasites, how parasites might become resistant to peroxides and importantly that not all peroxides are equal with subtle but mechanistically important differences in their mechanism of action. In the presentation we will look at the development of Tetraoxane E209.

NEXT-GENERATION ANTIFOLATE DRUGS AND DRUG TARGETS

Yongyuth Yuthavong

BIOTEC, National Science and Technology Development Agency



Yuthavong Y.¹, Vilaivan T², Tarnchompoo B¹, Thongpanchang C¹, Chitnumsub P¹, Vanichtanankul J¹, Yuvaniyama J³, Uthaipibull C¹, Shaw, P.¹, Leartsakulpanich U¹, Chookajorn, T.³ Yongkietrakul¹, S., Kongkasuriyachai¹, D. and Kamchonwongpaisan, S.¹

¹ National Centre for Genetic Engineering and Biotechnology/ National Science and Technology Development Agency, Thailand Science Park, Thailand

² Department of Chemistry, Chulalongkorn University, Thailand

³ Department of Biochemistry, Mahidol University, Thailand

We have focused on molecular biology and chemical approaches to obtain new effective antimalarials from structure-based design, and to identify new enzyme targets in the malarial folate pathway. Dihydrofolate reductase (DHFR) is a validated target which has been compromised by resistance to such inhibitors as pyrimethamine and cycloguanil through enzyme mutations. From the crystal structures of *P. falciparum* DHFR of both wild type and resistant mutants, we could understand the basis of resistance, and design new compounds to overcome the problem. These compounds bind tightly to the targets with a subsequent slow onset mechanism. They are orally highly active, with no *in vitro* or *in vivo* toxicity. One of them, P218, is in advanced pre-clinical phase of development. A new *in silico* screening method has allowed us to select designed DHFR inhibitors. Furthermore, we have designed drugs with dual binding modes, which are both effective and can prevent further resistance mutations. We have also explored other molecular targets, including Plasmodium dihydropteroate synthase (DHPS), serine hydroxymethyltransferase (SHMT) and GTP cyclohydrolase (GTPC). New methods to identify and validate new targets include inducible ribozyme-mediated gene knockdown. Crystal structures of DHPS and SHMT have allowed us to design new inhibitors to be tested. Since these enzymes are all in the *de novo* folate biosynthesis pathway, their inhibitors can be drugs with potential to be synergistic with the DHFR inhibitors, as already known for DHPS.

Supported by Medicines for Malaria Venture (MMV), Howard Hughes Medical Institute (HHMI), UNP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), Cluster Program Management Office (CPMO) of NSTDA and National Synchrotron Research Center.

Wednesday 11th December 2013

Time: 13:30 – 15:00

Room C

S8 Emerging/re-emerging Viral Infectious Diseases and Modern Therapeutics

Chairperson 1



Yuvadee Mahakunkijcharoen

Chairperson 2



Pornsawan Leangwutiwong

1. Hand, foot and mouth disease: enteroviral etiologies and seroepidemiology
Pilalpan Puthavathana
2. Predicting of dengue severity by immunodiagnostic assay, molecular detection and clinical data
Pornsawan Leangwutiwong
3. Personalized Medicine Based on Host-Viral Interaction
Wasun Chantratita

HAND, FOOT AND MOUTH DISEASE: ENTEROVIRAL ETIOLOGIES AND SEROEPIDEMIOLOGY

Pilalpan Puthavathana

Siriraj Influenza Cooperative Research Center, Department of Microbiology,
Faculty of Medicine Siriraj Hospital, Mahidol University



Hand, foot and mouth disease (HFMD) is a common disease in childhood under 5 years of age, particularly, in the Asia-Pacific region. The disease is characterized by acute febrile with papulovesicular rash in oral cavity and mouth and on palm of hands and feet. HFMD is caused by several virus members in the genus *Enterovirus*; however, enterovirus 71 (EV71) and coxsackie A16 (CA16) are the two most common etiologic agents. However, EV71 is associated with more severe illness and occasionally associated with brain stem encephalitis, meningitis and neurogenic pulmonary edema which may lead to death. After 2001, enteroviruses ever found to cause HFMD in our study include EV71, CA 16, CA6, coxsackie B virus and ECHO virus. Based on VP1 sequences, EV71 is further subdivided into 3 genotypes: A, B and C. There is only one lineage in genotype A; whereas genotype B is further subdivided into 5 subgenotypes: B1, B2, B3, B4 and B5 and genotype C is also subdivided into 5 subgenotypes: C1, C2, C3, C4 and C5. Range of nucleotide divergence among EV71 genotypes vary from 17-22% (mean: 18.99%); and it is 10-14% (mean: 12.02%) for inter-subgenotypes and 1-10% (mean: 3.92%) for intra-subgenotypes. On the other hand, CA16 is subdivided in 3 genogroups: A, B and C, or 2 genogroups as A and B in which B is further subdivided into 2 lineages, 1 and 2 based on designation from different groups of investigators. Phylogenetic analysis showed that genogroup C and lineage 2 of genogroup B belong to the same cluster.

Genotypic classification of EV71 and CA16 is important on epidemiological linkage for tracing the virus origin in an outbreak investigation. For example, EV71 subgenotype C4 that first appeared in Thailand in 2006 was mostly likely imported from Shenzhen, China. Genetic evolution of EV71 in China was further driven by selective pressure and resulted in the emergence of EV71 sub-subgenotypes C4a and C4b in 2008. The genotype C4a has been the major etiologic agent causing HFMD in several large outbreaks in many provinces in China; and the virus might have spread to Vietnam and Cambodia and remains there until present. On the other hand, EV71 subgenotypes B5 has been the major etiologic agent causing HFMD in Thailand for few years, except that occurred in 2012 in which CA6 played a major role. Prevalence of HFMD in Thailand is increasing in the past 5 years. One explanation may be the reporting system that looks carefully for the disease; but the more

importance could be the emergence of virus with antigenic change among children without immunity.

In term of immunity to EV71, it was originally demonstrated that neutralizing antibody can protect across subgenotypes of EV71. The finding leads to the development of inactivated EV71 monovalent vaccine of various subgenotypes in different countries; and those going on clinical trials include subgenotype C4 from few companies in China, subgenotype B3 from Singapore and subgenotype B4 from Taiwan. Nevertheless, genetic variability of EV71 is very remarkable during recent years; and it is uncertain whether this fact is still hold true. Our group conducts seroepidemiological study in Thai people of various age-groups using various EV71 subgenotypes. The result showed that most of our subjects contained neutralizing antibody to EV71 subgenotype B5/2011. Whether the antibody can protect against the other EV71 subgenotypes is to be further investigated. Seroepidemiological data will be useful for strategic plan on vaccine administration in Thai children, i.e., the target group for vaccination and the subgenotype of virus in vaccine that can confer the protection against the Thai virus isolates.

PREDICTING OF DENGUE SEVERITY BY IMMUNODIAGNOSTIC ASSAY, MOLECULAR DETECTION AND CLINICAL DATA

Pornsawan Leungwutiwong

Faculty of Tropical Medicine, Mahidol University



Tidtaya Sriburin^{1,2}, Krisana Pengsaa², Kriengsak Limkittikul², Nathamon Kosoltanapiwat¹, Narin Thippornchai¹, Pannamas Maneekarn³, Pornsawan Leungwutiwong¹

¹ Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Thailand

² Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Thailand

³ Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Thailand

Background: Dengue is an endemic disease throughout tropical and sub-tropical region as the principle vector, *Aedes aegypti*, expands its habitat across Asia, Africa, Central American, South America, and the Pacific. Infection with any of the four serotypes (DENV-1 to 4) may result in an undifferentiated fever or a mild illness known as dengue fever (DF). In some patients, infection evolves to the severe syndrome known as dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), because of a secondary heterologous dengue virus (DENV) infection. Other factors have been postulated as important in the pathogenesis of DHF such as specific virulent virus genotype that replicate to high levels resulting in an increased immune response and increase disease.

Objective: To find out the association between clinical and laboratory factors that affecting the severity of dengue infection

Material and Method: One hundred and twenty seven serum samples of dengue infected patient were collected from Hospital for Tropical Diseases. Acute and convalescent serum samples were classified as primary or secondary dengue virus infection by ELISA assay. Analysis of the viral load and dengue specific serotypes were done with real time-PCR and conventional RT-PCR, respectively. The genetic diversity of dengue virus, which may associate with severe dengue symptoms, was investigated by DNA sequencing.

Expected results: The study will provide the general demographic data of dengue infection in Hospital for Tropical Disease. The results in this study could be expanded the knowledge of antibody response pattern, dengue virus serotype, and viral load associated with severity of dengue infection.

Keywords: Dengue fever, Dengue haemorrhagic fever, RT-PCR, Real time-PCR, ELISA

PERSONALIZED MEDICINE BASED ON HOST-VIRAL INTERACTION

Wasun Chantratita

Faculty of Medicine Ramathibodi Hospital, Mahidol University



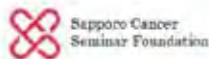
Abstract Not Available

Wednesday 11th December 2013

Time: 13:30 – 15:00

Room D

S9



Imperial College
London



Asian School Health Symposium: Beyond deworming I: 32nd
International Symposium on Cancer supported
by Sapporo Cancer Seminar Foundation

Organisers: Sapporo Cancer Seminar Foundation (SCSF), Japan Consortium for Global School Health Research (JC-GSHR), Partnership for Child Development (PCD) and Faculty of Tropical Medicine (FTM), Mahidol University.

Opening Remarks:

- Dean Yaowalark Sukthana, FTM, Mahidol University
- OBEC, Ministry of Education, Thailand
- DOH, Ministry of Health, Thailand
- Secretary General/Coordinator SEAMEO TROPMED NETWORK
- Executive Director, PCD
- Sapporo Cancer Seminar Foundation & JC-GSHR

SESSION 1: POLICY MANAGEMENT

Chairperson 1



Watanaporn Ra-Ngubtook
Office of Basic Education
Commission (OBEC),
Ministry of Education

Chairperson 2



Nuttaporn Wongsuttipakorn
Department of Health
(DOH) Ministry of
Public Health Thailand

Chairperson 3



Jitra Waikagul
FTM, Mahidol
University

1. Regional situation of school health policy management
Jun Kobayashi
2. FRESH: Achievements and next steps
Lesley Drake

REGIONAL SITUATION OF SCHOOL HEALTH POLICY MANAGEMENT

Jun Kobayashi

Department of Global Health, School of Health Sciences, University of the Ryukyus



Jun Kobayashi^{1,2}

¹ *Chair, Japan Consortium for Global School Health Research*

² *Professor, Department of Global Health, School of Health Sciences, University of the Ryukyus, Okinawa, Japan*

All countries in Southeast Asia have formulated the comprehensive school health policy with the strong ownership of each government. Global partners in this region have supported this movement since 2000 with promoting deworming program in primary schools. In 2008, World Health Organization reported that deworming is progressing well in the Mekong countries. On the other hand, peer education program has also promoted widely in higher education though the implementing HIV-AIDS control program. However, other activities in comprehensive school health; such as setting health policies in school, improvement of school environment including in safe water supply, skills based health education except the prevention education for HIV-AIDS, and any other health services except deworming, might be not always operated well in school level.

Current situation of the policy implementation for comprehensive school health will be shown in this symposium with the evidence from case study researches in Thailand and Lao PDR. First, we provided evidence that can support efforts to institutionalize the comprehensive school health program for sustainable implementation by suggesting three areas of focus: extensive planning, training, and a monitoring cycle. Especially national school health program must be considered the harmonization with the existing management cycle for general education. Second, our study highlighted the importance of understanding the key concept among stakeholders at all levels and integrating national program into teacher training colleges.

We also summarize the information related to the human capacity of central government for implementing national school health program on the basis of comparison among several countries. The Ministry of Education, Cambodia established the specific department to implement the national school health program, and have accumulated the knowledge and the experiences in the institution. The strengthening of institutional capacity building is recommended in order to sustain the capacity for the school health policy management.

FRESH: ACHIEVEMENTS AND NEXT STEPS

Lesley Drake

*Partnership for Child Development
Imperial College London*



FRESH: Achievements & next steps

Dr. Lesley Drake,

Executive Director, Partnership for Child Development, Imperial College London

Poor health and malnutrition are important underlying factors for low school enrolment, absenteeism, poor classroom performance, and early school dropout. Programmes to achieve good health, hygiene, and nutrition at school-age are therefore essential to the promotion of basic education for all children. School health and nutrition (SHN) programmes that improve children's health and ability to learn are now widely recognized as significantly contributing to the achievement of the Millennium Development Goals (MDGs) and Education for All (EFA). Using schools as a platform to deliver safe and simple interventions, these programmes can enable the poorest and most disadvantaged children, many of whom are girls, to participate in education, boosting enrolment, reducing absenteeism and dropout.

In 2000 the World Education Forum met in Dakar, Senegal, and on this occasion school health was

for the first time not just a speculative idea, but launched by UNESCO, UNICEF, WHO and World Bank and PCD as a formal effort to work together in a FRESH way; to Focus Resources on Effective School Health. In 2001, the World Health Assembly boosted this work further by formally adopting school based deworming in Resolution 54.19; this acts as a solid platform for school-based health activities. More recently in 2010 the High Level Group on Education for All made the Addis Ababa Declaration on the crucial role of health and nutrition in achieving quality education and high enrolment. These international policy movements have translated into strong programmes that benefit children: In Sub-Saharan Africa, for example, the percentage of countries implementing comprehensive school health programs rose from 4 percent in 2000 to nearly 50 percent by 2008, while in South and East Asia, coverage nearly doubled over the same period.

Looking forward, the growth in effective school health programs seems set to continue, but there will also be important new trends in school health in low income countries. Some trends will be a natural extension of what has gone before, such as extending school health services to adolescents, especially adolescent girls and future mothers. Others will be brand new, such as working to make schools inclusive of children with disabilities and other vulnerable groups who are likely to be excluded from education; promoting healthy eating to prevent NCDs and improving oral health. While others again will arise from rethinking of old certainties, as is happening with the recognition of a role for school feeding as a social safety net to help vulnerable societies deal with shocks.

Whether leading or following this trend, analytical work and a strong evidence-base must be at the heart of this endeavor as we move forward together.

Wednesday 11th December 2013

Time: 13:30 – 15:00

Convention Center

S10 Taking the Resistance Out of Elimination Organized by: Malaria Consortium

Chairperson

Ernest Smith

1. Malaria Elimination in Asia
John MacArthur
 2. Malaria Consortium's efforts in containment of drug resistance and the pathway to elimination for Asia
Sylvia Meek
-

MALARIA ELIMINATION IN ASIA

John MacArthur
US CDC, PMI Lead

Abstract Not Available

MALARIA CONSORTIUM'S EFFORTS IN CONTAINMENT OF DRUG RESISTANCE AND THE PATHWAY TO ELIMINATION FOR ASIA

Sylvia Meek
Malaria Consortium



Abstract Not Available

(Session to be continued in S15)

Wednesday 11th December 2013

Time: 15:30 – 17:00
Room A

S11 Helminth Intestinal Parasitic Genomics

Chairperson 1



Poom Adisakwattana

Chairperson 2



Qijun Chen

1. Harnessing high throughput technologies to tackle neglected parasitic diseases of major socioeconomic importance
Aaron R. Jex and Neil D. Young
2. Comparative and functional genomics of reference helminth species
Matthew Berriman
3. It's not a fluke: the planarian as a model for understanding schistosomes
Phillip Newmark

HARNESSING HIGH THROUGHPUT TECHNOLOGIES TO TACKLE NEGLECTED PARASITIC DISEASES OF MAJOR SOCIOECONOMIC IMPORTANCE

Aaron R. Jex, Neil D. Young
University of Melbourne



Abstract Not Available

COMPARATIVE AND FUNCTIONAL GENOMICS OF REFERENCE HELMINTH SPECIES

Matthew Berriman

the Parasite Genomics Group, Wellcome Trust Sanger Institute



Matthew Berriman

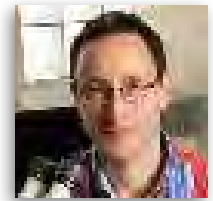
the Parasite Genomics Group, Wellcome Trust Sanger Institute, Cambridge, CB10 1SA, United Kingdom.

For more than a decade the availability of parasite genome sequences has been changing the face of parasitology. Due to the economics of producing larger-sized genomes, and the difficulty in obtaining material, some key areas of parasitology have lagged behind, particularly parasitic helminths. The parasite genomics group at the Sanger Institute are interested in the genomic basis for differences in the biology of helminths. This talk will give an overview of progress on our Helminth Genomes Initiative, which includes more than 50 draft genomes as well as 5 reference genomes. We have started with the establishment of high quality genomes for a few select species including some key human pathogens (or their model equivalents) such as whipworms, threadworms, Schistosomes and tapeworms. To make sense of these reference genomes we adopt two approaches: (i) comparative genomics over a range of evolutionary scales to map genes that are common or unique to individual species or clades and (ii) high throughput sequencing-based functional genomics. I will illustrate how comparative genomics is allowing us to study the evolutionary relationship between helminth genomes and how we are using detailed functional genomics to identify putative components of important host-parasite interactions.

IT'S NOT A FLUKE: THE PLANARIAN AS A MODEL FOR UNDERSTANDING SCHISTOSOMES

Phillip Newmark

HHMI/ University of Illinois at Urbana-Champaign



Planarians are free-living flatworms with amazing regenerative abilities. Their regenerative prowess is based upon a population of adult stem cells, called neoblasts, that serve as the source of new tissue during regeneration and tissue homeostasis. Using the functional genomics tools available for studying planarians, we have been investigating how these stem cells give rise to the various differentiated cell types and how the regenerative process is controlled systemically. I will discuss how our work on planarians has led us to study the biology of schistosomes, parasitic flatworms with great significance for global health. We have shown that, like planarians, schistosomes have neoblast-like stem cells in the adult stage of the life cycle, providing one potential explanation for their longevity. Extending this work to the intramolluscan stage of the schistosome life cycle, we find that the so-called germinal cells in the sporocysts, resemble neoblasts morphologically and express similar genes that are required for germinal cell proliferation and maintenance. Thus, applying the lessons learned from studying planarians will help inform the biology of their parasitic cousins.

Wednesday 11th December 2013

Time: 15:30 – 17:00
Room B

S12 Viral Infections

Chairperson 1



Nathamon Kosoltanapiwat

Chairperson 2



Tamaki Okabayashi

-
1. Genome sequence of chikungunya viruses derived from Ratchaburi Province, Thailand
Mikiko Sasayama
 2. Screening novel HIV-1 inhibitors targeting cyclophilin A by structure-based and ligand-based in silico screening
Norihito Kawashita
 3. The comparative analysis of the Dengue virus serotype 2 in Thailand and CYD tetravalent dengue vaccine strain
Tamaki Okabayashi
 4. A holistic approach for modeling the dynamic of chikungunya and developing control actions
Roger Frutos
-

GENOME SEQUENCE OF CHIKUNGUNYA VIRUSES DERIVED FROM RATCHABURI PROVINCE, THAILAND

Mikiko Sasayama

Faculty of Tropical Medicine, Mahidol University



Mikiko Sasayama¹, Natthanej Luplertlop², Surachet Benjathummarak³, Norihito Kawashita^{4,5}, Prasert Rukmanee², Suntaree Sangmukdanun², Promsin Masrinoul¹, Orapim Puiprom¹, Panjaporn Chaichana¹, Pitak Wuthisen², Pannamas Maneekan², Tamaki Okabayashi¹, and Pongrama Ramasoota³

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Chikungunya fever (CHIKF), an arboviral disease that is caused by chikungunya virus (CHIKV) and is transmitted by mosquitoes, was first recognized in epidemic form in East Africa in 1952-1953. Recent outbreaks of CHIKF occurred in coastal Kenya in 2004 and spread to several Indian Ocean islands including Reunion. In Thailand, CHIKF outbreaks occurred during 2008-2009 and CHIKV has subsequently been producing the highest number of infection cases among neighboring nations.

In this study, we isolated seven CHIKV from the sera of 50 CHIKF-suspected patients in Ratchaburi province of Central Thailand in 2010. These seven isolates were sequenced and analyzed for phylogeny of its structural region and growth kinetics.

We found that all sequences were clustered in the Eastern, Central, Southern, and African (ECSA) group, and kept the mutations E1-A226V and E2-I211T which provide a suitable background to allow CHIKV adaptation to *Aedes albopictus*. Furthermore, 4 novel substitutions have been observed in some of their clones. In growth kinetic analysis using Vero cell and C6/36 cell, viral growth speed was faster than that of the control viruses Ross strain and clinical isolates in 2008-2009. Moreover, cytopathic effect (CPE) caused by the seven isolates on C6/36 cell was milder compared to the control viruses.

It is suggested that CHIKV have been still circulating in Thailand in 2010, and significant observations in this study might be one of the main causes for continuous outbreaks. It would be an important event for CHIKV to have mutated and adapted to the local mosquitoes in Thailand.

SCREENING NOVEL HIV-1 INHIBITORS TARGETING CYCLOPHILIN A BY STRUCTURE-BASED AND LIGAND-BASED IN SILICO SCREENING

Norihito Kawashita

Graduate School of Pharmaceutical Sciences, Osaka University



Norihito Kawashita^{1,2}, Yu-Shi Tian¹, Chris Verathamjamras³, Kousuke Okamoto¹, Teruo Yasunaga², Kazuyoshi Ikuta², Masanori Kameoka^{3,4}, Tatsuya Takagi^{1,2}

¹⁾ Graduate School of Pharmaceutical Sciences, Osaka University

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³⁾ Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI)

⁴⁾ Department of Virology, Research Center for Infectious Disease Control, Research Institute for Microbial Diseases, Osaka University

Cyclophilin A has peptidyl-prolyl isomerase activity and binds to the capsid protein of Human Immunodeficiency Virus (HIV). Therefore, it is an attractive target of anti-HIV drugs. However, no drug against HIV-1 infection targeting cyclophilin A has been approved. We have found a number of active compounds by in silico structure-based screening applied to the database with 1,300 compounds recently. As a result, two compounds were identified as the most active. Specifically, both exhibited anti-HIV activity against viral replication at a low concentration and relatively low cytotoxicity at the effective

concentration inhibiting viral growth by 50 % while they show moderate toxicity. Hence, we try to identify novel compounds with more anti-HIV activity and less toxicity by in silico ligand-based screening using the structure of active compounds. In this screening, we used larger database which contains about 200,000 compound.

Keywords: HIV, cyclophilin A, docking simulation, computer-based drug design, in silico screening

THE COMPARATIVE ANALYSIS OF THE DENGUE VIRUS SEROTYPE 2 IN THAILAND AND CYD TETRAVALENT DENGUE VACCINE STRAIN

Tamaki Okabayashi

Mahidol-Osaka Center for Infectious Diseases (MOCID)



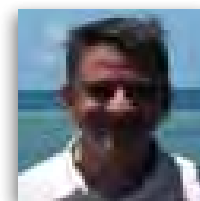
Miwa Kuwahara, PhD,¹ Akifumi Yamashita, PhD,^{1,2} Norihito Kawashita, Kriengsak Limkittikul, MD,¹ Mikiko Sasayama, Panjaporn Chaichana, Orapim Puiptom, Chukiat Sirivichayakul, MD,¹ Arunee Sabchareon, MD,¹ Kazuyoshi Ikuta, Pongrama Ramasoota, Tamaki Okabayashi, DVM, PhD¹

We recently reported the results of a phase 2b clinical trial of the dengue virus (DENV) vaccine at Ratchaburi Province, Thailand. Although the efficacies against the DENV-1, -3, and -4 serotypes were quite high, the efficacy against DENV-2 was poor. Here, we compared the sequences of the envelope genes (E) from the vaccine strains (CYD) and recent clinical isolates (CI) at this province. A clear difference in amino acid residues between CYD and CI was identified only in DENV-2; TQG in CYD versus KQE in CI at residues 226–228. Of note, this amino acid shift influenced the surface structure and charge of this site. The phylogenetic tree of DENV-2 revealed that the virus population with TQG had been circulating until around 2001. Since then, the KQE type has become predominant in Thailand. This KQE type emerged in Thailand since around 2001 and after that distributed southeast-asia, Austrarila, and China.

A HOLISTIC APPROACH FOR MODELING THE DYNAMIC OF CHIKUNGUNYA AND DEVELOPING CONTROL ACTIONS

Roger Frutos

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Objectives: We developed integrative approaches for the study of emerging diseases. Chikungunya virus (CHIKV), a mosquito-borne pathogen from the alphavirus genus transmitted by *Aedes* mosquitoes (*Aedes aegypti* and *Aedes albopictus*) causes acute infection in humans characterized by rash, high fever and, its hallmark feature is a severe arthritis that can persist for years. These disease was taken as a model system. Modeling the dynamic of the disease in Asia, where the CHIKV is actively propagating, is essential to develop prevention strategies for regions with high risk of propagation, including Europe where the vector

A. albopictus is now well established. Multidisciplinary and multi-scale approaches are considered in our solutions.

Methods: We have constructed an object model (UML class diagram) to describe the structure of the system. This object model has been extended to dengue. The modeling of the dynamic of the transmission of CHIKV was performed with a multi-agent system implemented on the Netlogoplatform. A case study has been selected in the region of Yogyakarta-Sukoharjo, Indonesia to validate both static and dynamic models. Information relative to geolocalisation, demography, environment (rural, urban, industrial), clinical information, vector population, has been collected on the field using standardized procedures.

Results: The class diagram defines the compartments of the model. We carefully describe compartments concerning environment description (description referring to geographic standards, ecology, anthropic influence), sample description (vector, human, virus triptych), clinical data (including media influence) and diagnostic tools. Data have been collected in a periurban zone of Yogyakarta (RT42) and a rural section of Sukoharjo district (RT02 and RT03). A questionnaire to collect clinical information has been set up following OBOE standard and presented to the local health centers. The index case was successfully established in the rural region. SEIR (Sensitive/Exposed/Infected/Resistant) compartments were considered for the host while SEI (Sensitive/Exposed/Infected) were selected for the vector to establish the dynamic model. We have been able to test the influence of medical care policies, vector control options and external income of virus. We also develop analyses on the fate and persistance of mosquitocidal agents post treatment.

Conclusion: We have tested the influence of medical practices in the development of the illness. Isolation of patients during the acute initial phase of the infection would avoid transmission and should drastically reduce the transmission of the virus. According to our study, the presence of large factories in the region of Sukoharjo is suspected to constitute the major source of CHIKV for the region. Whether factories provide suitable habitat for local populations of vector and whether industrial activity contributes to the introduction of populations originating elsewhere will be tested by further serological and molecular analyses.

Wednesday 11th December 2013

Time: 15:30 – 17:00
Room C

S13 Young Scientist

Chairperson 1



Waranya Wongwit

Chairperson 2



Urai Chaisri

1. Molecular detection of Spotted Fever Group Rickettsiae in *Amblyomma variegatum* ticks collected from cattle in Benin Republic (West Africa)
Paul Franck Adjou Moumouni
2. Prevalence and molecular characterization of *Giardia duodenalis* infection in humans and dogs from Cambodia
Tawin Inpankaew
3. Soil-transmitted helminthiases in Guimaras Island, the Philippines: Is elimination feasible?
Harvy Joy Liwanag
4. Modulatory role of *Plasmodium falciparum*-infected erythrocytes on the development of naïve lymphocytes derived from hematopoietic stem cells
Setthakit Chitsanoor

MOLECULAR DETECTION OF SPOTTED FEVER GROUP RICKETTSIAE IN *AMBLIOMMA VARIEGATUM* TICKS COLLECTED FROM CATTLE IN BENIN REPUBLIC (WEST AFRICA)

Paul Franck Adjou Moumouni

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Paul Franck Adjou Moumouni^{1,2}, M Alaa Terkawi¹, Tatsunori Masatani¹, Shinuo Cao¹, Ketsarin Kamyinkird¹, Ahmed A. Moussa¹, Yoshifumi Nishikawa¹, Hiroshi Suzuki^{1,2}, Xuenan Xuan¹

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Spotted Fever Group (SFG) *Rickettsias* are obligate intracellular Gram-negative bacteria causing acute flu-like symptoms, generalised maculopapular cutaneous rash and complications that can lead to human casualties. Recently, *Rickettsia africae*, a member of SFG *Rickettsia* has been identified as causative agent of African tick bite fever and ranked second most frequently identified cause of systemic febrile illness among travellers. The disease is transmitted by ticks of the genus *Amblyomma*. Despite the presence of *Amblyomma variegatum* in Benin Republic (West Africa), there is neither report of cases of African tick bite fever nor proof of existence of *Rickettsia africae* in the country. The current study aims to identify SFG *Rickettsia* in ticks infesting cattle in Benin Republic. Ticks were collected in 13 villages and from a total of 66 traditional farms during May-June 2011. In each farm 1 to 6 ticks were collected on cattle for a total of 326 ticks. Tick species identification was done using standard taxonomic keys and total DNA was extracted using ISOHAIR and Dr GenTLE precipitation carrier™ kits. Successful DNA extraction was assessed by PCR targeting ticks' 18S rRNA gene. Detection of *Rickettsia* spp. was carried out by using primers specific to 16S rRNA gene of SFG *Rickettsia*. All the ticks were identified as *Amblyomma variegatum* among which 60 were female and 266 male. Screening of tick DNA revealed that 81.60% (266/326) were infected with *Rickettsia* spp. Sequencing of PCR products revealed 98-99% and 96-97% similarity to *R. africae* strains and other members of SFG *Rickettsia*, respectively.

Keywords: African tick bite fever; *Rickettsia* spp., *Amblyomma variegatum*; Benin Republic.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF *GIARDIA DUODENALIS* INFECTION IN HUMANS AND DOGS FROM CAMBODIA

Tawin Inpankaew

Department of Veterinary Disease Biology, Faculty of Health and Medical Science, University of Copenhagen, Denmark



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Giardia duodenalis is a protozoan flagellate that infects the intestinal tract of vertebrate hosts including humans and a considerable variety of other mammals through ingestion of infective cysts. The aim of this study was to determine the prevalence and genotypes of *Giardia duodenalis* infection in humans and dogs living in the same villages in rural Cambodia. We compared isolates of *G. duodenalis* from humans and dogs on a genetic level to ascertain zoonotic transmission risk at the village level. In May 2012, a total of 218 human and 94 dog faecal samples were collected from 67 households in Dong village, Preah Vihear

province, Cambodia. Faecal samples were examined microscopically for the presence of *Giardia* cysts by the zinc sulphate floatation method and PCR based on amplification of the SSU rRNA gene. Prevalence of *G. duodenalis* infection in humans was 9.2% (20/218) and 18.3% (40/218) by microscopy and PCR, respectively. In dogs 2.1% (2/94) and 10.6% (10/94) were positive by microscopy and PCR, respectively. *G. duodenalis* molecular analysis characterized 27.5% (11/40) and 72.5% (29/40) of human samples as assemblage A and B, respectively. In dog 20.0% (2/10) and 80.0% (8/10) were characterized as assemblage B and C, respectively. Transmission of *G. duodenalis* occurs mainly among humans and among dogs. Latter pose minimal zoonotic transmission risk for *G. duodenalis*. Larger investigation might bring to light the origins of *G. duodenalis* infection in human and animal population in communities in Southeast Asia.

Keywords: *Giardia duodenalis*, dogs, humans, Assemblage, Cambodia

SOIL-TRANSMITTED HELMINTHIASES IN GUIMARAS ISLAND, THE PHILIPPINES: IS ELIMINATION FEASIBLE?

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Control of soil-transmitted helminthiasis (STH) in the Philippines includes school-based mass drug administration (MDA) of single-dose anthelmintics twice a year, among other strategies. In 2011, Guimaras Island was proposed as a pilot site for demonstrating the feasibility of eliminating STH as a public health problem by reducing prevalence of moderate-heavy intensity infections in school-age children to <1%. This study aimed to determine the feasibility of elimination by evaluating parasitological status and MDA coverage over a two-year period and conducting a situational analysis that included an assessment of WASH (access to safe water, sanitation, and hygiene) parameters.

In 2010 and 2012, 623 and 1,053 stool samples, respectively, from sentinel schools were examined using the Kato-Katz technique. Data on MDA coverage was collected using reports from the Department of Education. A map was also created to compare WASH and parasitological parameters.

Between 2010 and 2012, MDA coverage increased from 69.4% to 97.4%, exceeding the Department of Health's target of 85%. Reductions in cumulative prevalence (from 43.8% to 40.7%) and prevalence of moderate-heavy intensity infections (from 14.1% to 10.8%) were not significant despite high MDA coverage. No clear relationship between WASH and parasitological parameters was observed, suggesting the need for a more accurate assessment of sanitation conditions. However, a few sentinel sites had low prevalence, indicating that parasitological status varied across schools. The feasibility of elimination may be demonstrated by future studies that focus on low-prevalence schools and assess the impact of intensified WASH interventions on top of school-based MDA in these sites.

Keywords: soil-transmitted helminthiasis, school-age children, mass drug administration, WASH, Philippines

MODULATORY ROLE OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES ON THE DEVELOPMENT OF NAÏVE LYMPHOCYTES DERIVED FROM HEMATOPOIETIC STEM CELLS

Setthakit Chitsanoor

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³ Center for Emerging and Neglected Infectious Diseases, Mahidol University, Bangkok, Thailand.

Plasmodium falciparum is a dominant species causing human malaria, leading to cerebral complication in which the symptom during acute infection can range from asymptomatic to fatal. Immunity to malaria is an important factor determining these phenomena especially effector function of T lymphocytes. We recently produced naïve T lymphocytes derived from hematopoietic stem cells (HSCs) in cord blood. These naïve T lymphocytes were exposed to either total protein extracts of *P. falciparum*-infected erythrocytes (iRBCs) or to the freshly isolated intact iRBCs by an *in vitro* co-cultivation for 2 and 4 days. By using flow cytometric analysis, the results showed that the percentages of CD4 and CD8 lymphocytes in both conditions were lower than those of controls, there was no significant difference between the two types of antigens. However, the expression of CD95 (Fas receptor) on the surface of T lymphocytes exposed to iRBC was 2-fold higher than that exposed to the iRBC protein extracted. The newly developed-naïve lymphocytes used in this study has ruled out the complication of immune cross reactivity that might incur among the lymphocyte populations had they been taken from peripheral blood. These observations suggested that *Plasmodium falciparum* quickly depleted T lymphocytes via apoptotic process obviously through the function of Fas receptor in all lymphoid tissues, i.e. bone marrow, spleen and lymph nodes where lymphocyte progenitor development take place. Taken together, these findings verify for the first time that an immunosuppression by malaria has started far beyond than what we have known.

Key words: Malaria, hematopoietic stem cells, T lymphocyte, immunosuppression

Wednesday 11th December 2013

Time: 15:30 – 17:00

Room D

S14



Sapporo Cancer
Seminar Foundation



Imperial College
London

JC-GSHR

PCD
The Partnership for
Child Development

ACIPAC
Asian Child Injury Prevention
Association

Asian School Health Symposium: Beyond deworming II: 32nd
International Symposium on Cancer supported
by Sapporo Cancer Seminar Foundation

Organisers: Sapporo Cancer Seminar Foundation (SCSF), Japan Consortium
for Global School Health Research (JC-GSHR), Partnership for Child Development (PCD)
and Faculty of Tropical Medicine (FTM), Mahidol University.

Session 2: School Health & Nutrition Topics Beyond the MDGs in Asia

Chairperson 1



Pratap Singhasivanon
Secretary General/Coordinator
SEAMEO TROPMED NETWORK

Chairperson 2



Mithong Souvanvixay
Director General, Department of
Pre-School and Primary Education
and Sports, Lao PDR

1. School Health & Nutrition Promoting Inclusion
Natasha Graham
2. Need and approach for NCD prevention
Tetsuya Mizoue
3. Oral health
Hiroshi Ogawa
4. Nutrition transition
Napaphan Viriyautsahakul
5. Eco-health education
Takashi Asakura

Session 3: Drafting of Bangkok Declaration on School Health & Nutrition

Chairperson 1



Masamine Jimba
Professor, Tokyo University,
JC-GSHR

Chairperson 2



Lesley Drake
Executive Director,
PCD, Imperial College

Chairperson 3



Jitra Waikagul
FTM, Mahidol
University

Comments by Chairing panel

Session 2: School Health & Nutrition Topics Beyond the MDGs in Asia

SCHOOL HEALTH & NUTRITION PROMOTING INCLUSION

Natasha Graham

Disability Advisor, Partnership for Child Development (PCD), Imperial College



School Health Programs: Each year children in low-income countries miss up to 500 million school days due to common health problems. The school system provides a cost-effective platform for delivering simple health interventions to school children, thereby optimizing the benefits of education, increasing access for the most marginalized, and preventing millions of children from missing school and dropping out.

Children with Disabilities: Children with disabilities are more likely to drop out of school than any other vulnerable groups even in countries with high enrolment rates. Poverty, malnutrition, poor health and lack of access to proper sanitary conditions or clear water aggravate the consequences of existing disabilities. Most children with disabilities in low-income countries have never been seen by a doctor or have had any contact with an existing health care system. In addition, many children develop disabilities due to relatively simple but left untreated health conditions which then prevent them from going to school and learning.

Comprehensive Inclusive School Health Programs with Disability and Health Screening

More and more countries recognize that adding a comprehensive school-based health programs in the Education Sector Plan leads to preventing many children from dropping out of school, and that the benefits of school-based health programs can be multiplied when implemented as part of a more comprehensive and disability inclusive school-based health program.

NEED AND APPROACH FOR NCD PREVENTION

Tetsuya Mizoue

Department of epidemiology and prevention, National Center for Global Health and Medicine, Japan



Tetsuya Mizoue¹⁾, Daisuke Nonaka²⁾, Diyanath Samarasinghe³⁾

¹⁾ Department of Epidemiology and Prevention, Clinical Research Center, National Center for Global Health and Medicine, ²⁾ Department of Parasitology and International Health, Graduate School of Medicine, University of the Ryukyus, ³⁾ Department of Psychological Medicine, Faculty of Medicine, University of Colombo

Non-communicable disease (NCD) has become a serious burden in the world including developing countries. Given the difficulty of lifestyle change in adults, intervention at earlier ages is preferable and school would be an ideal setting to tackle this issue. Meta-analyses from randomized trial (mostly conducted in developed countries) have indicated that school-based behavioral intervention is effective in improving determinants of NCD including diet, physical activity, obesity, and tobacco smoking.

In developing countries, where health resources are limited, comprehensive and cost-effective approach targeting on both physical and mental health components would be desirable. In Sri Lanka, a controlled trial has been performed to examine the effect of school-based intervention on student knowledge, attitude, and behavior related to NCD prevention, observing significant differences between intervention and control schools. There was also a suggestion of the influence of the program on their family and community.

Increasing impact of NCD in developing countries and scientific evidence on school-based intervention could provide a rationale for the promotion of school health against NCD prevention. Further research and development are required to facilitate these activities and enhance their effect in developing countries, including collaboration between education and health sectors, development of curriculum and educational materials, training for teachers, and involvement of community.

ORAL HEALTH

Hiroshi Ogawa

Department of Oral health Science, Niigata University, Japan



Hiroshi OGAWA

*WHO Collaborating Centre for Translation of Oral Health Sciences
Niigata University, Japan*

Dental Caries is still major public health problem in majority of nations globally. Many epidemiological studies have shown that prevention of dental caries by fluoride is a basic and indispensable method to maintain and improve oral health. A school based fluoride mouthrinse (FMR) programme has become wide-spread since the 1970s in Japan. FMR has so far been the only community oriented health measure that has contributed to caries prevention of the permanent teeth, neither community water fluoridation nor dietary fluoride supplements in the form of drops or tablets are currently available. The guideline of FMR was released by the Ministry of Health Labor and Welfare, Japanese society for Oral Health recommends the use of a weekly fluoride mouthrinse (0.2% NaF) for primary and secondary school children, and a daily regimen (0.05% NaF) for preschool children. To avoid swallowing all of the rinse solutions, the children start practicing FMR after having trained by rinsing with tap water. Long-term FMR programmes in groups have been confirmed to markedly reduce caries prevalence. The effects of these programmes have been shown to continue after their completion. It is therefore reasonable to consider that FMR is to promote school dental health.

NUTRITION TRANSITION

Napaphan Viriyautsakul

Director of Bureau of Nutrition, Department of Health, Thailand



Prior to the 1980s, protein-energy malnutrition and micronutrient deficiencies predominantly affected mothers and children in Thailand. Nationwide community-based nutrition programs through primary health care were implemented with greatly improved results especially during the 1980s to mid-1990s. As the country moved from a low-income to a middle-income country, with rapid economic and social development, changes in lifestyles from agricultural to industrial careers resulted in a shift from rural farm tasks to sedentary factory or office work. Households tended to purchase ready-cooked or processed food rather than produced by their own even in rural areas. The rise of obesity is as a result while stunting, underweight and micronutrient deficiencies are still prevalence. Harmonized policy and strategic programs with multi-sectoral collaboration are needed to address this double burden of malnutrition.

ECO-HEALTH EDUCATION

Takashi Asakura

Department of School Health and Health Care, Tokyo Gakugei University, Japan



Eco-health education

Takashi Asakura, Professor, Tokyo Gakugei University, Faculty of Education

The Ottawa Charter for Health Promotion as a socio-ecological approach to health was declared in 1986. However, to date ecological approach to health or ecohealth has been put a peripheral position in the health promotion agenda, despite that our societies have been facing on new global risk patterns of health such as climate changes, deforestation, global imbalance in food supply, lack access to safe drinking water for more than four decades. Recently ecological approach to health has revived in public health because development of a new framework for understanding us and the world, values, principles, and ethics are inevitable for addressing with these health risks. Epistemologically, the central notion of ecological thinking is interdependent, interactional, or reciprocal relationships between social system, development, human health, human livelihood, and ecosystem.

In our presentation, first we briefly review literally a few points at issue regarding a concept of ecological approach to health. Second, we propose ecohealth education as an integrated framework, delineating key characteristics of ecohealth education based on the theory of ecohealth. To help the participants to understand more clearly ecohealth education and its challenges, we present our ecohealth education project in Lao PDR as an example which we are engaging for five years in collaboration with Shinshu University (Dr. Sachi Tomokawa), the National University of Laos and teacher training collages. Thereby we discuss challenges we may face on when we promote ecohealth education in schools in lower income countries or developing countries in an era of post-Millennium Development Goals.

Session 3: Drafting of Bangkok Declaration on School Health& Nutrition

COMMENTS BY CHAIRING PANEL

Abstract Not Available

Wednesday 11th December 2013

Time: 15:30 – 17:00
Convention Center

S15 Taking the Resistance out of Elimination (continuation from S10) Organized by: Malaria Consortium

Chairperson

Ernest Smith

1. Surveillance efforts in Cambodia
Arantxa Roca
 2. Positive Deviance: An innovative approach to improve malaria prevention and treatment practices among high risk communities (rubber tappers and fishermen) in Myanmar
Muhammad Shafique
 3. The Status and Preparation of Thailand Towards Containment of Drug Resistance and Elimination
Apinya Niramitsantipong
-

SURVEILLANCE EFFORTS IN CAMBODIA

Arantxa Roca
Malaria Consortium



In the Greater Mekong Region (GMR) and since the discovery of artemisinin resistant malaria parasites along the Thai-Cambodia border in 2007, containment activities started in 2008. In Cambodia, these resulted in the strengthening of systems for malaria surveillance through the development of a Malaria Information System (MIS) as a platform for village level case reporting-based on village malaria workers and incidence-based stratification. As no single tool was able to provide the mixture of routine and real time data necessary to respond to malaria outbreaks as well as responding to individual cases, a series of innovative tools such as real time (day 0 and day 3 SMS reporting tools) were piloted and eventually scaled up from 2009. The Cambodian malaria programme now has a number of tools available that has improved routine surveillance and are decentralized to the operational district level allowing district officials to have improved access to relevant information for their operational activities. The MIS platform is capable of producing a comprehensive automated report (“Malaria Bulletin”) which summarises malaria trends in Cambodia on quarterly basis. A full description of the several tools currently being used as well as recommendations and next steps will be discussed. There is an unprecedented momentum of malaria control and elimination activities in the GMR and preparations towards containment of drug resistance and elimination are ongoing in Cambodia. In this talk, an emphasis will also be given on the existing surveillance needs to meet the targets set by the Cambodia Malaria Elimination Strategy Plan.

POSITIVE DEVIANCE: AN INNOVATIVE APPROACH TO IMPROVE MALARIA PREVENTION AND TREATMENT PRACTICES AMONG HIGH RISK COMMUNITIES (RUBBER TAPPERS AND FISHERMEN) IN MYANMAR

Muhammad Shafique
Malaria Consortium



Authors: Muhammad Shafique, Celine Zegers de Beyl, Ernest Smith, Sylvia Meek, Dr. Myo Min

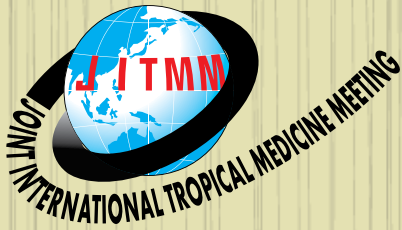
Positive Deviance: An innovative approach to improve malaria prevention and treatment practices among high risk communities (rubber tappers and fishermen) in Myanmar

Reaching high risk populations is one of the key strategies in the Myanmar Artemisinin Resistance Containment (MARC) Project, Myanmar. Positive Deviance (PD) is an asset-based behaviour change approach with the underlying notion that every community has certain individuals (positive deviants or champions) whose malaria prevention and treatment practices result in better health outcomes than their neighbours with whom they share similar resources. Malaria Consortium (MC) collaborated with Myanmar Medical Association (MMA), a local organization to pilot PD among high risk rubber tappers and fishermen in six villages in Kyun Su Township, Thaninthryi region, Myanmar. The PD pilot aims to identify already existing model preventive and health seeking behaviours and promote these beneficial practices during the PD informed intervention in six target communities. A survey was conducted in March 2013 (n=504) to establish baseline of the project. The data entry of the survey is at final stages. The survey was also complemented with a qualitative component i.e. PD process to understand the normative behaviours around malaria prevention and control. The PD process included 6 steps: pre-orientation meeting, community orientation, situation analysis, PD inquiry, participatory analysis, and community feedback. During the one week PD process, 13 focus group discussions and 18 in-depth interviews were conducted with target community members both male and female to identify the positive deviant individuals or champions. For example, we identified a female rubber tapper who has been working in rubber farm for more than 15 years but never gets malaria by always wearing long sleeved shirts, long trousers, rubber boots and always covering her head with a cloth during the rubber tapping to avoid mosquito bites. She always sleeps under the bed net when she is home and not working at the rubber farm. All identified PD practices are being shared with other community members by the 22 trained PD volunteers. After the one-year PD-informed intervention which includes training of volunteers, interactive health education sessions, role plays, art competitions and an advocacy seminar, a follow up survey will be conducted in March 2014 to better evaluate this intervention. The preliminary data suggests that PD can serve as 1) a malaria intervention to better reach out to the high risk communities; 2) an alternative or supplementary method to interpersonal communication to deliver existing BCC/IEC messages; and 3) an innovative model to ensure community participation and ownership and foster sustainability in the malaria interventions

THE STATUS AND PREPARATION OF THAILAND TOWARDS CONTAINMENT OF DRUG RESISTANCE AND ELIMINATION

Apinya Niramitsantipong
Bureau of Vector-Borne Diseases, Department of Disease Control, Ministry of Health, Thailand

Abstract Not Available



Thursday 12th December 2013

Time: 09:00 – 10:30
Convention Center

Plenary II HIV, Enteric diseases and HPV Vaccines

Chairperson 1



Punnee Pitisuttithum

Chairperson 2



Carl Mason

-
1. An update on enterics diseases vaccines
Carl Mason
 2. Beyond RV144 efficacy results: Update and Future Plan
Punnee Pitisuttithum
 3. HPV Vaccine – Update: Cost Effectiveness
Anuj Walia
-

AN UPDATE ON ENTERIC DISEASES VACCINES

Carl Mason

Dept of Enteric Diseases, Armed Forces Research Institute of Medical Sciences



Abstract Not Available

BEYOND RV144 EFFICACY RESULTS: UPDATE AND FUTURE PLAN

Punnee Pitisuttithum

Department of Clinical Tropical Medicine, Mahidol University



Pitisuttithum P¹

¹ *Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University*

Thailand has been involved and fully committed to HIV vaccine R&D for more than 16 years. There were two efficacy trials performed. The first used the first generation vaccine- r gp120 B/ E HIV vaccine involving injecting drug users showed no efficacy. The second efficacy trial was the community trial using ALVAC HIV vaccine priming and rgp120 (AIDSVAX B/E) involving 16,402 participants. This regimen had modest protection against HIV acquisition (31.2% at 42 months follow up) for the first time. However, the vaccine efficacy appeared to be higher up to 60% at 12 months, 6 months after the last boosting. This suggested early and nondurable immune response. Subsequent investigations demonstrated the potential immune correlates of risk antibodies directed against the V2 loop. The recent sieve study also uncovered genetic footprints in the V2 region associated with reduced risk of HIV. All these paved the way to new vaccine designs. Extension of the RV144 study-RV305 started in 2012 and vaccination phase is almost complete. Two more studies are underway adding the booster doses at one year. Future efficacy trials using a similar vaccine concept tested in high-risk groups are being planned.

HPV VACCINE – UPDATE: COST EFFECTIVENESS

Anuj Walia

AFFILIATION



Abstract Not Available

Thursday 12th December 2013

Time: 11:00 – 12:30
Room A

S16 Health in Asia Beyond 2015: Strategic Initiatives in Tropical Medicine

Chairperson



Jetsumon Prachumsri

-
1. Grant priority, direction to Asian Countries
Peter A. Singer
 2. Health priorities in the Mekong: Progress with Regional Cooperation
Aye Aye Thwin
 3. Topic to be announced
Thomas Kanyok
-

GRANT PRIORITY, DIRECTION TO ASIAN COUNTRIES

Peter A. Singer
Grand Challenges Canada



Grand Challenges Canada is dedicated to supporting Bold Ideas with Big Impact in global health. Funded by the Government of Canada, we support innovators in low- and middle-income countries and Canada. The bold ideas integrate science and technology, social and business innovation (we call this Integrated Innovation). We work to catalyze scale, sustainability and impact. We have a determined focus on results, and on saving and improving lives. Grand Challenges Canada's Stars in Global Health program supports Bold Ideas with Big Impact in global health from innovators and entrepreneurs in low- and middle-income countries and Canada, and our targeted challenges focus on Saving Lives at Birth, Saving Brains and Global Mental Health. Dr. Singer will discuss the Grand Challenges concept and funding opportunities to tackle grand challenges in global health.

HEALTH PRIORITIES IN THE MEKONG: PROGRESS WITH REGIONAL COOPERATION

Aye Aye Thwin
U.S. Agency for International Development



Abstract Not Available

TOPIC TO BE ANNOUNCED

Thomas Kanyok
Bill & Melinda Gates Foundation



Abstract Not Available

Thursday 12th December 2013

Time: 11:00 – 12:30
Room B

S17 Translation Research Of Insect Allergy
Organized by: Parasitology and Tropical Medicine Association of Thailand

Chairperson 1



Mathirut Mungthin

Chairperson 2



Padet Siriyasatien

1. Current status of insect allergy in Thailand
Nualanong Visitsunthorn
2. House dust mite & cockroach allergy: from lab to market
Anchalee Tungtrongchitr
3. Proteome and Allergome of predominant Thai wasp, *Vespa affinis*
Nitat Sookrung

CURRENT STATUS OF INSECT ALLERGY IN THAILAND

Nualanong Visitsunthorn

Division of Allergy, Department of Pediatrics, Faculty of Medicine, Siriraj Hospital, Mahidol University



The incidence of atopic diseases has been shown to increase in Thailand and all over the world. The prevalence of asthma in Thailand is 9-14% and of allergic rhinitis is 26-38%. The most common allergens causing allergic sensitization in children are house dust mites (57.8%) followed by cockroaches (21.7%). The prevalence of allergen skin test reactivity among Thai asthmatic children showed that at least one skin prick test was positive in 74% and two positive tests in 66% of cases. The prevalence of sensitization to various allergens in asthmatic children was 67% *Dermatophagoides pteronyssinus* (Dp), 62% *Dermatophagoides farinae* (Df) and 44% American cockroach. In allergic rhinitis adults, the positive skin tests to indoor allergens were 72% Dp, 79% Df, 60% American cockroach and 41% German cockroach. Eighty-five per cent of patients were sensitive to both Dp and Df, indicating substantial cross-reactivity. The study on the allergenicity and suitable allergen extracts of dust mite and cockroach for diagnosis and immunotherapy is going on.

The common stinging insects that cause allergy are the insect in Order *Hymenoptera* (bee, wasp, hornet and fried ant). Insect sting was the cause of anaphylaxis in 7-11% of anaphylactic cases in Thailand. The allergen extracts of these insects are expensive and periodically not available in Thailand. Further studies about the stinging insects and their allergenicity are important and will improve the diagnosis and the treatment in patients with insect sting allergy.

HOUSE DUST MITE & COCKROACH ALLERGY: FROM LAB TO MARKET

Anchalee Tungtrongchitr

Department of Parasitology, Siriraj Hospital, Mahidol University



Anchalee Tungtrongchitr¹, Nitat Sookrung², Nitaya Indrawattana³, Nawannaporn Sae-Lim¹, Yuwaporn Sakolvaree¹, Nualanong Visitsunthorn⁴, Chaweewan Bunnag⁵ and Wanpen Chaicumpa¹.

¹ Department of Parasitology; ²Department of Research and Development; ⁴Department of Pediatrics, ⁵Department of Oto-rhino-laryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, ³Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand.

Allergic diseases caused by household insects have become accentuated during the past few decades owing to the life style of urban people who spend most of their time in daily living in indoor. House dust mite (HDM) and cockroach (CR) allergens are recognized as the 2 most important indoor allergens and are mainly contained within relatively large particles (>10-40 µm in diameter). *Dermatophagoides* mites; especially *D. pteronyssinus* and *D. farinae*, are the most clinically important and predominant species found in house dust worldwide. Among several domiciliary CR, the most important species causing CR allergy are the German CR (*Blattella germanica*) and the American CR (*Periplaneta americana*).

Biological characterization of these relevant insect allergens and their epitopes have been studied using 2DE-based-proteomics, 2DE-immunoblotting and bioinformatics. Purified native and recombinant allergens of the respective allergens including their specific monoclonal antibodies were successfully produced. Various products were invented using those research outcomes. In regards to the poor quality and variability of allergenic activity of crude extracts, using refined native or recombinant allergens has been applied. This approach could assist a better standardized alternative for diagnosis and immunotherapy. ELISA test kits for detection and quantification of HDM and CR allergens in homes of the patients were developed for intervention of the allergic morbidity. Rapid tests for detection of these allergens are under developed.

Keywords: House dust mite, cockroach, allergen, allergy, IgE immunoblotting, proteomics.

PROTEOME AND ALLERGENOME OF PREDOMINANT THAI WASP, *VESPA AFFINIS*

Nitat Sookrung

Faculty of Medicine Siriraj Hospital, Mahidol University



Nitat Sookrung¹, Siriporn Wong-din-dam², Anchalee Tungtrongchitr³, Onrapak Reamtong⁵, Nitaya Indrawattana⁶, Yuwaporn Sakolvaree³, Nualanong Visitsunthorn⁴, Wiparat Manuyakorn⁷ and Wanpen Chaicumpa³

¹ Department of Research and Development; ²Graduate Program of Immunology, Department of Immunology; ³Department of Parasitology; ⁴Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand, ⁵Department of Molecular Tropical Medicine and Genetics; ⁶Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand, ⁷Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Vespa affinis (Asian wasp, Thai banded tiger wasp or local name: Tor Hua Seua) causes the most frequent incidence of medically important *Hymenoptera* sting in South and Southeast Asia. However, data on the venom components attributable to the sting derived-clinical manifestations (local reactions, IgE mediated-anaphylaxis and/or systemic envenomation) are lacking. This study provides first set information on *V. affinis* venom proteome, allergenome and IgE reactivity of individual venom components. From 2DE-gel based-proteomics, the venom revealed 92 protein spots of which proteins in 53 spots could be identified and classified into three groups: typical venom components and structural and housekeeping proteins. Venom proteins in 32 spots reacted with serum IgE of wasp allergic patients. Major allergenic proteins which reacted to IgE of >50% of the wasp allergic patients included PLA₁ (100%), arginine kinase (73%), GB19860 transcription protein (73%), antigen-5 (66.7%), enolase (66.7%), PLA₁ magnifin (60%), ENSAPMG transcripts (53.3%) and hyaluronidase (53.3%) and venom minor allergens were uncharacterized protein (26.7%), GB17291 transcript (20%), glyceraldehyde-3-phosphate dehydrogenase (20%), aconitate hydratase (6.7%) and phosphoglucosmutase (6.7%). The information has diagnostic and clinical implications for future improvement of case diagnostic sensitivity and specificity, component-resolve diagnosis (CRD) and design of specific *Hymenoptera* venom immunotherapy.

Keywords: allergen, allergenome, IgE immunoblotting, proteomics, *Vespa affinis*

Thursday 12th December 2013

Time: 11:00 – 12:30

Room C

S18 Therapeutic and Diagnostic Antibodies Against Viral Diseases

Chairperson



Tamaki Okabayashi

1. Development of rapid immunochromatography test kit to detect the chikungunya virus antigen
Tamaki Okabayashi
2. Human monoclonal antibodies showing broad cross-reactive neutralization against group 1 influenza A viruses including H5N1
Tadahiro Sasaki
3. Preliminary evaluation of a novel DNA vaccine candidate expressing dengue neutralizing antibody
Atsushi Yamanaka
4. Development of A New Murine Model of Dengue Virus Infection
Takeshi Kurosu

DEVELOPMENT OF RAPID IMMUNOCHROMATOGRAPHY TEST KIT TO DETECT THE CHIKUNGUNYA VIRUS ANTIGEN

Tamaki Okabayashi

Mahidol-Osaka Center for Infectious Diseases (MOCID)



Tamaki Okabayashi¹, Tadahiro Sasaki³, Promsin Masalinori¹, Orapim Puiptom¹, Mikiko Sasayama¹, Miwa Kuwahara¹, Panjaporn Chaichana¹, Mieko Kosaka⁴, Pongrama Ramasoota², Kazuyoshi Ikuta³

¹ Mahidol-Osaka Center for Infectious Diseases (MOCID), ² Center of Excellence for Antibody Research, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand

³ Department of Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, ⁴ Alfresa Pharma Corporation, Ibaraki, Osaka 567-0806, Japan

Chikungunya fever (CHIKF) is a mosquito-borne disease and an important public health concern in tropical and subtropical countries together with dengue fever (DENV). CHIKF usually starts 2–4 days after chikungunya virus (CHIKV) infection with the clinical symptoms of high fever, rash, headache, vomiting, myalgia, and joint pain. Although severe joint pain is the most prominent feature of CHIKF, the early diagnosis of CHIKF remains difficult because of the similar clinical symptoms of DENV. An IgM-detection kit is used to diagnose patients with CHIKF, but the sensitivity and specificity of the IgM detection kit are limited. An immunochromatography diagnosis kit for the detection of CHIKV antigen in serum samples is needed.

We produced a panel of mouse monoclonal antibodies (mAbs) against CHIKV isolated from a Thai patient (ECSA genotype). Two of the mAbs against CHIKV E protein were selected and characterized by an indirect immunofluorescence assay. We developed an immunochromatography-based kit for the detection of CHIKV antigen using these mAbs, and we evaluated it using clinical isolates.

Our kit was specific to CHIKV (ECSA and Asia) and does not react to dengue virus, Japanese encephalitis virus or Sindbis virus. The sensitivity of the kit was 1.0×10^5 PFU of CHIKV. Further studies using patient samples are ongoing to evaluate the performance of the kit.

HUMAN MONOCLONAL ANTIBODIES SHOWING BROAD CROSS-REACTIVE NEUTRALIZATION AGAINST GROUP 1 INFLUENZA A VIRUSES INCLUDING H5N1

Tadahiro Sasaki

Department of Virology, Research Institute for Microbial Diseases, Osaka University



Abstract Introduction: Influenza A hemagglutinin (HA) is a glycoprotein with high antigenicity located on the surface of the virus and is classified into 17 types (H1–H17). Neutralizing antibodies (Ab) against HA play important roles in protection against and recovery from influenza virus infection. However, it is thought that influenza virus induce epidemic annually or pandemic due to the antigenic variation of HA by mutation or by reassortment. Therefore, many researchers seek for anti-HA broad neutralizing Ab or induction method of the Ab in vivo. Recently, some groups reported broad neutralizing human monoclonal antibodies (HuMAbs) that recognize stem region of HA. We also have succeeded to generate broadly neutralizing HuMAbs using peripheral blood mononuclear cells (PBMCs) from H1N1 pdm (2009) virus infected patients and SPYMEG cell (product of MBL, Nagoya, Japan). In this presentation, I would like to show the detailed characteristics of the HuMAbs.

Materials and Methods: The cross-reactivity of HuMAbs, 1H11 and 5G2, was examined by immunofluorescence assay (IFA) and neutralization assay. To analyze the epitope, we performed Mass Spectrometry analysis using antibody-antigen complexes that were digested extra-epitope region of antigen by protease. HA sequences were downloaded from the NCBI influenza virus resource and were aligned by BioEdit program.

Result and Discussion: These HuMAbs react with H1N1, H5N1, and H9N2, but not react with H2N2 strains by IFA. Interestingly, virus neutralization activities against H1N1 2009 pdm strains isolated in 2011 were much weaker than these against H1N1 2009 pdm strains isolated in 2009. Epitope mapping by Mass Spectrometry analysis revealed the region at stem -helix (residues 40 to 58). Comparison of this epitope region gene sequencing between these strains showed that the Glu47 in 2009 isolates changes to the Lys47 in 2011 isolates. Based on the database information, H1N1 2009 pdm circulated in 2009 showed that most strains had Glu47, while most of recently circulating strains had Lys47. At least H1N1 2009 pdm virus may have already started evolution to escape from immune pressure against this epitope region.

PRELIMINARY EVALUATION OF A NOVEL DNA VACCINE CANDIDATE EXPRESSING DENGUE NEUTRALIZING ANTIBODY

Atsushi Yamanaka

BIKEN Endowed Department of Dengue Vaccine Development, Faculty of Tropical Medicine, Mahidol University and Research Institute for Microbial Diseases, Osaka University

Atsushi Yamanaka¹, ChayaneeSetthapramote¹, Pongrama Ramasoota², Pannamthip Pitaksajjakul², and Eiji Konishi¹

¹ BIKEN Endowed Department of Dengue Vaccine Development, Faculty of Tropical Medicine, Mahidol University and Research Institute for Microbial Diseases, Osaka University, ²Center of Excellence for Antibody Research, Faculty of Tropical Medicine, Mahidol University

Dengue vaccines currently under development can induce neutralizing antibodies, a major protective immune factor. However, most neutralizing antibody species can also show *in vitro* dengue infection enhancing activity at subneutralizing doses. To avoid potential vaccine-inducible increase in disease severity, we are now studying on a new concept of vaccine which expresses only neutralizing antibody. Its characteristic feature is to introduce a DNA plasmid expressing neutralizing antibody but not an antigen. The DNA plasmid can be arranged not to induce enhancing antibody activity by modification of the Fcγ receptor binding region. In this study, we have done preliminary evaluation of a DNA vaccine candidate expressing dengue neutralizing antibody by using a mouse model.

Mouse monoclonal antibody (MAb): 7F4 which shows high neutralizing activity against dengue type 1 virus (DENV1), but no enhancing activity, was used. **Antibody engineering:** The Fab gene of 7F4 was cloned into a series of pFUSE vector (InvivoGen, San Diego, CA). **In vitro expression test:** HEK293T cell monolayers were co-transfected with the pFUSE plasmids, whose culture fluids were harvested to analyze antibody levels and functional activities. **In vivo mouse experiment:** ICR mice (4 weeks, male) were injected with the DNA vaccine, whose blood samples were collected sequentially. **Antibody test:** a 50% plaque reduction neutralization test using Vero cells and ELISA were used.

One µg/ml of IgG was detected in the culture fluids of HEK293T cells co-transfected with the pFUSE plasmids. The recombinant IgG showed a neutralizing activity pattern similar to IgG produced by hybridoma. More than half of mice injected with the DNA vaccine showed increase in ELISA antibody level. Their neutralizing antibody levels were detectable, but not high.

Although active immunity may induce neutralizing antibodies accompanied by enhancing activity, our vaccine strategy involves an advantage of passive immunity (modification of Fcγ receptor binding region). In addition, interference among vaccine candidates will not appear under this strategy. However, improvements are also required to increase the amount of antibody generation *in vivo*.

DEVELOPMENT OF A NEW MURINE MODEL OF DENGUE VIRUS INFECTION

Takeshi Kurosu

Department of Virology, Research Institute for Microbial Diseases (RIMD), Osaka University, Japan

Takeshi Kurosu¹, Azusa Asai¹, Keiko Hanabara¹, Supranee Phanthanawiboon¹, Sabar Pambudi¹, Magot D. Omokoko¹, Tadahiro Sasaki¹, Surapee Anantapreecha⁴, Motoki Kuhara³, Pongraman Ramasoota², Kazuyoshi Ikuta¹

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⁴ National Institute of Health, Department of Medical Sciences, Muang, Nonthaburi

BACKGROUND: For the development of therapeutics and study of pathogenesis of dengue virus (DENV) infection, establishment of animal model is indispensable. Unfortunately, only humans and mosquitoes are susceptible to DENV. DENV and Japanese encephalitis virus (JEV) belong to genus *Flavivirus* and are mosquito-borne viruses. Although they share similar virological characteristics, they cause different symptoms in human. In addition, JEV is pathogenic to mice but DENV is not, because there is a critical difference in their infectivity in mice. Because of this, mouse model for DENV infection is not available, and which brings difficulty to develop a new drug and an efficient vaccine.

OBJECTIVES: We aim to develop a mouse model for DENV infection to evaluate effects of anti-dengue virus antibodies and understand the different infectivity between DENV and JEV.

METHODS: IFN- $\alpha/\beta/\gamma$ R double KO mice and other KO mice were infected with genetically engineered chimeric flaviviruses (DVChimeraVs) containing dengue virus genes by intraperitoneal, subcutaneous, and footpad injections.

RESULT: DVChimeraVs caused fetal infection in IFN- $\alpha/\beta/\gamma$ R double KO mice and were distributed in several organs, such as bone marrow, thymus, spleen, PEC, and so on. We have confirmed that the monocyte/macrophage and dendritic cells are the major targets for DVChimeraV. These observations are very similar as observed in human. When anti-DENV E antibody was introduced at an appropriate concentration one day prior to challenge, an ADE was clearly observed in this model system. Introduction of high concentration of anti-DENV E antibody protected mice from lethal infection.

CONCLUSION: This KO mouse model infected with chimera virus is highly useful to evaluate the therapeutic effect of neutralizing antibodies. For further study, this model is also useful to understand why antibody sometimes exaggerates condition.

Thursday 12th December 2013

Time: 11:00 – 12:30
Room D

S19 Vectors and Vector-Borne Diseases

Chairperson 1



Jiraporn Ruangsittichai

Chairperson 2



Suchada Sumruayphol

1. Mosquito Larvicidal and Pupicidal Properties of essential Oils From Some *Rutaceae* Plants Against *Aedes Aegypti* (Linn.) and *Culex Quinquefasciatus* (Say)
Ubol Phukerd
2. Malaria vector situation in ethnic minority villages of Ratanakiri and Mondulakiri provinces, northeastern Cambodia, 2008-2013
Wannapa Suwonkerd
3. Hemocytes changes in Bithynid snails infected with *Opisthorchis viverrini*
Kuwadee Suwannatrai
4. Biological control of vectors: An effective component for the control and elimination of mosquitoes and tropical diseases.
Roberto Sanchez Prieto

MOSQUITO LARVICIDAL AND PUPICIDAL PROPERTIES OF ESSENTIAL OILS FROM SOME *RUTACEAE* PLANTS AGAINST *AEDES AEGYPTI* (LINN.) AND *CULEX QUINQUEFASCIATUS* (SAY)

Ubol Phukerd

King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand



Ubol Phukerd^a and **Mayura Soonwera^a**

^aPlant Production Technology Section, Faculty of Agricultural Technology,
King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

This study was conducted to investigate the larvicidal and pupicidal properties of essential oils from some *Rutaceae* plants against fourth instar larvae and pupae of *Aedes aegypti* and *Culex quinquefasciatus*. The plants used in the study were *Citrus aurantifolia* Swing., *C. aurantium* Linn., *C. hystrix* DC., *C. medica*, *C. maxima* var. *racemosa*, *Citrofortunella microcarpa* (Bunge) Wijnands., and *Zanthoxylum limonella* Alston. The larval mortality was recorded at 1, 5, 10, 30, 60 minutes, and 24 hours while pupal mortality was recorded at

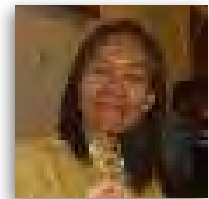
15, 30 minutes, 1, 12, 24 and 48 hours. The results revealed that *Z. limonella* essential oil exhibited the highest larvicidal activity against *Ae. aegypti* with LT_{50} values of 1 minute while *C. maxima* and *C. aurantifolia* essential oils showed the highest for *Cx. quinquefasciatus* with LT_{50} values of less than 1 minute. *C. medica*, *F. japonica* and *C. hystrix* essential oils showed high larvicidal activity with LT_{50} values of less than 5 minute against the two mosquitoes. *C. maxima* and *Z. limonella* essential oils also showed high larvicidal activity with LT_{50} values of less than 5 minute against *Aedes aegypti* and *Cx. quinquefasciatus* respectively. In addition. *C. medica*, *C. maxima*, *C. hystrix* and *Z. limonella* essential oils exhibited the highest pupicidal activity against *Ae. aegypti* with LT_{50} values of less than 1 hour while all essential oils except *C. aurantium* exhibited the highest pupicidal activity against *Cx. quinquefasciatus*. with LT_{50} values of less than 1 hour.

Keywords: larvicide, pupicide, Rutaceae plants, essential oil, *Aedes aegypti*, *Culex quinquefasciatus*

MALARIA VECTOR SITUATION IN ETHNIC MINORITY VILLAGES OF RATANAKIRI AND MONDULKIRI PROVINCES, NORTHEASTERN CAMBODIA, 2008-2013

Wannapa Suwonkerd

Department of Disease Control, Office of Disease Prevention and Control



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³ Provincial Health Department, Ratanakiri, Cambodia

⁴ National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

⁵ Department of Entomology, Kasetsart University, Bangkok, Thailand

This study aims to understand the situation of anopheline vectors over 5 years, and to strengthen the Village Malaria Workers (VMW) to perform a broader range of activities than blood collection and provision of treatment. VMWs were trained to perform mosquito collections. The collections were carried out from 2008, in Ratanakiri and Monduliri provinces. In each province, two houses were selected as collection sites. VMWs sat inside and outside the house to collect mosquitoes landing on their legs from 1800 to 0600 hr. For animal bait collections, one cow was tethered in a double-net trap. Mosquito collections were performed on three consecutive nights in each village. Collections were performed every three-four months.

In all, 19 species of *Anopheles* fauna were collected: *Anopheles minimus*, *dirus*, *maculatus*, *barbivirostris*, *aconitus*, *jamesii*, *jeyporensis*, *kawari*, *kochi*, *peditaeniatus*, *philippinensis*, *nivipes*, *sawadiwonporni*, *sinensis*, *splendidus*, *tesellatus*, *umbrosus*, *vagus*, *willmori*. Despite environmental and human-settlement changes over time, the first three species were commonly found over the five-year period in both provinces, but abundance was seasonal and focal-specific.

An. minimus showed higher abundance in dry season than in rainy season and started to bite at 19.00 hr to 22.00 hr and 04.00 hr in Ratanakiri province. But they showed early biting (19.00-20.00 hr) in Monduliri. *An. dirus* s.l. was mostly anthropophagic and only found in rainy season in both provinces. *An. maculatus* s.s. showed whole night biting, from 1900 to 0500 in Ratanakiri but half night biting (1900-2400 hr) in Monduliri.

HEMOCYTES CHANGES IN BITHYNID SNAILS INFECTED WITH *OPISTHORCHIS VIVERRINI*

Kulwadee Suwannatrai

Faculty of Medicine, Khon Kaen University



Kulwadee Suwannatrai^a, Smarn Tesana^a, Apiporn Suwannatrai^a, Kavin Thinkhamrop^b, Chalermmlap Donthaisong^a, Patpicha Arunsan^a, Pairat Tabsripair^c, Jariya Umka^d

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Bithynid snails serve as first intermediate hosts of *Opisthorchis viverrini*. The infectivity is strongly correlated with the destruction of helminths by hemocytes. *Bithynia funiculata*, *B. siamensis siamensis* and *B. siamensis goniomphalos* were individually exposed to ingest *O. viverrini* eggs. After infection, hemocyte was counted by using neutral red retention assay for evaluate differences in number hemocyte counts referred to total hemocyte counts and differential hemocyte counts at 11 designated periods of 0 (control), 2, 4, 6, 12, 24, 48 and 96 hours as well as 10, 30 and 60 days post infection. Three main types of hemocytes were identified; blast cell-like, hyalinocyte and granulocyte. After infection, temporary increasing of granulocytes whereas blast cell like and hyalinocytes were decreased. The total hemocyte counts were significant increased in 2 h after infection of *B. siamensis siamensis* (mean diff. = 68.55 cell x 10⁴ml⁻¹; 95%CI = 40.55 to 96.5; p-value = 0.001) and *B. funiculata* (mean diff. = 78.79 cell x 10⁴ml⁻¹; 95%CI = 45.71 to 111; p-value = 0.001). And number of hemocytes were reduced in 4 h after infection of *B. siamensis goniomphalos* (mean diff. = -62.32 cell x 10⁴ml⁻¹; 95%CI = -85.98 to -38.6; p-value = 0.001) and 12 h of *B. funiculata* (mean diff. = -119.93 cell x 10⁴ml⁻¹; 95%CI = -147.11 to 92.70; p-value = 0.001). Form 72 h to 60 day, number hemocytes was reestablished in the hemolymph, which indicates that defense cells in host species have different mechanisms for susceptibility or resistance.

Keywords: Bithynid snails, hemocyte, *Opisthorchis viverrini* infection.

BIOLOGICAL CONTROL OF VECTORS: AN EFFECTIVE COMPONENT FOR THE CONTROL AND ELIMINATION OF MOSQUITOES AND TROPICAL DISEASES

Roberto Sanchez Prieto

Labiofam

The vector-borne diseases occupy top place in areas where the endemic is high .Nowadays day is a challenge for professionals trying to reduce them. The vector control with the use of biological larvicides (*Bactiver*, *Bacillus thuringiensis* and *Griselesf*, *Bacillus spahericus*) and community participation is a weapon efficient within the integrated vector control. This presentation shows the results that prove its performance in vector control (*Aedes ssp.* and *Anopheles ssp.*) where have obtained a reduction over 98 % of the larvae stage in the breeding sites and the decrease of the transmission in areas with entomological and epidemic importance (Dengue Fever and Malaria) in Africa and Latin America countries.

Thursday 12th December 2013

Time: 13:30 – 15:00
Room A

S20 Hot issues in ASEAN Community
Organized by: Thailand Chapter of International Society
of Tropical Pediatrics

Chairperson 1



Krisana Pengsaa

Chairperson 2



Kriengsak Limkittikul

-
1. Emergence of Diphtheria
Tawee Chotpitayasunondh
 2. Adult Dengue Infection
Teerapong Tantawichien
-

EMERGENCE OF DIPHTHERIA

Tawee Chotpitayasunondh
Faculty of Medicine Siriraj Hospital, Mahidol University



Abstract Not Available

ADULT DENGUE INFECTION

Teerapong Tantawichien

Faculty of Medicine, Chulalongkorn University



Adult Dengue Infection

Teerapong Tantawichien, M.D.

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Dengue infection is re-emerging diseases that is endemic in the tropical world. The evidence of rising age of DF/DHF cases has been explained by association with demographic transition, modern housing, and commercial development. In Thailand, affected adults aged over 15 years old are reported to comprise 30–40% of dengue virus infected cases. Dengue is one disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome. Although shock and plasma leakage seem to be more prevalent as age decreases, the frequency of internal haemorrhage augments as age increases. Bleeding manifestations in adult patients including petechiae, menorrhagia were also frequently found, however, massive haematemesis may occurs in adult patients because of peptic ulcer disease and be not associated with profound shock as previous reports in children. Clinicians should be alert to the potential development of massive GI bleeding, particularly in patients with early altered consciousness, profound thrombocytopenia, prolonged PT. Increase in liver enzymes found in both children and adults indicated liver involvement during dengue infections. Pre-existing liver diseases in adults such as chronic hepatitis and cirrhosis may aggravate the liver impairment of adult dengue. Fatal patients were found to have significant frequencies of altered consciousness, hypothermia, massive GI bleeding, dengue shock syndrome (DSS), renal/hepatic failure, concurrent bacteremia. The early recognition of dengue infection, bleeding tendency, and signs of circulatory collapse would reduce mortality rate in adult patients with dengue infection.

Thursday 12th December 2013

Time: 13:30 – 15:00
Room B

S21 Best Student Presentation Award

Chairperson 1



Sasithon Pukrittayakamee

Chairperson 2



Viravarn Luvira

1. The potential use of recombinant protein in enzyme-linked immunosorbent assay and immunochromatographic test in the diagnosis of African trypanosomosis
Kennedy Mochabo
2. Phenotypic analysis of *Aspergillus fumigatus* strain lacking the NAD-dependent formate dehydrogenase encoding gene *fdh*
Teerada Ponpinit
3. Comparison of insect succession and decomposition patterns in laid and hanging carcasses in Nan province
Sutaporn Sukjit
4. Affinity Study of scFv-Fc Monoclonal Antibodies Specific to Hemagglutinin of H5N1 Influenza Virus Using SPR Biosensor
Khwanhit Boonha
5. Medical burden and economic burden of chronic Hepatitis B patients at Queen Savang Vadhana Memorial Hospital
Chuenrutai Yeekian

THE POTENTIAL USE OF RECOMBINANT PROTEIN IN ENZYME-LINKED IMMUNOSORBENT ASSAY AND IMMUNOCHROMATOGRAPHIC TEST IN THE DIAGNOSIS OF AFRICAN TRYPANOSOMOSIS

Kennedy Mochabo

National Research Center for Protozoan Diseases, Obihiro University



Kennedy Miyoro Mochabo^{1,2}, Thu-Thuy Nguyen¹, Keisuke Suganuma¹, Mo Zhou¹, Shin-ichiro Kawazu¹, Yasuhiko Suzuki³ and Noboru Inoue¹

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Trypanosomes are hemo-flagellate protozoan parasites that cause disease in humans and livestock called sleeping sickness and nagana, respectively. Recombinant protein Tc38630 (rTc38630) from *Trypanosoma congolense* was successfully expressed, characterized and found to be antigenic using ELISA in experimentally infected mouse model. According to amino acid domains structure, Tc38630 was assumed as a *T. congolense* orthologue of the *T. brucei* invariant surface glycoprotein (ISG) therefore, a potential diagnostic antigen. ELISA experiments though labor-intensive and time-consuming, require equipment and trained personnel to perform. However, they are suitable for epidemiological surveys. Among other serological tests, immunochromatographic test (ICT) has an advantage as a one-step rapid analysis, thus making it a convenient and sensitive diagnostic test. BALB/c mice were immunized with rTc38630 proteins. rTc38630 immunized serum was purified for IgG polyclonal antibodies using protein G affinity chromatography, dialyzed in PBS and used as control line in ICT. The ICT was optimized and assembled whereby the antigen-antibody reaction was detected by colloidal gold conjugated rTc38630 protein at test line. Indirect ELISA was performed according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals by using either PCF cell lysate or a recombinant antigen. The ICT result was found to be consistent with rTc38630 protein ELISA in the experimentally infected mice sera. The next plan is to apply rTc38630 protein in ICT and ELISA for field samples.

Key words: ELISA; Immunochromatographic test; Nagana; Serodiagnosis; Trypanosomosis

PHENOTYPIC ANALYSIS OF *ASPERGILLUS FUMIGATUS* STRAIN LACKING THE NAD-DEPENDENT FORMATE DEHYDROGENASE ENCODING GENE *fdh*

Teerada Ponpinit

Faculty of Medicine Siriraj Hospital, Mahidol University



TeeradaPonpinit, NadthananPinchai

Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

A*spERGILLUS fumigatus* is an important causative agent of invasive pulmonary aspergillosis, a common opportunistic fungal infection that causes morbidity and mortality in immuno-suppressed patients. Previous studies indicated that NAD-dependent formate dehydrogenase encoding gene *fdh* may play an important role in respiratory chain and oxidative phosphorylation which are crucial for alternative metabolic pathway and survival of the fungus under unfavorable conditions. However, the physiological function of *fdh* has not been fully investigated yet. The aim of this study is to clarify the physiological role of *fdh* in *A. fumigatus* by replacing the entire coding region of *fdh* by the hygromycin B resistance gene *hph* via homologous recombination and protoplast transformation. The deletion of *fdh* was confirmed by Southern

analysis. Radial hyphal growth, conidiation and germination were compared between Δfdh and wild-type strains. The results showed that the lack of *fdh* resulted in decreased hyphal growth and delayed germination but had no effect on conidiation, suggesting that *fdh* plays important role in certain phenotypic features of *A. fumigatus*. Further phenotypic analysis and gene expression studies will provide better understanding of fungal alternative metabolic pathways that facilitate survival in the host cells.

Keywords: *Aspergillus fumigatus*, fungal alternative metabolic pathway, invasive pulmonary aspergillosis, NAD-dependent formate dehydrogenase (FDH)

COMPARISON OF INSECT SUCCESSION AND DECOMPOSITION PATTERNS IN LAID AND HANGING CARCASSES IN NAN PROVINCE

Sutaporn Sukjit

Faculty of Science, Chulalongkorn University



The research aims to establish a base line database of forensic entomology in Thailand by identifying the key species, succession order and life history parameters that are associated with corpses. This preliminary study compared the insect succession and rate of decomposition of pig carcasses (*Sus scrofa domestica*) in two different spatial positions (hanging ca 1 m above and laid on the ground). The study was performed in May – August 2010, in a mixed deciduous forest, Nan province, Thailand. Over 40 taxa were collected and identified. *Chrysomya megacephala* and *Achoetandrus ruffifacies* were the dominant fly species found in carcasses in both positions, and were also the first species to colonize the hanging carcasses. In contrast, the first species to arrive on the laid carcasses were members of Formicidae (ants), with the green ant (*Oecophylla smaragdina*) being the first species found only on the laid carcass. The decomposition of the pig carcasses could be divided into the five classic discernible stages in both positions. However, the decomposition rate of the hanging carcasses was slower than the laid carcasses in stages 3 to 5.

Keyword: Forensic entomology, Decomposition, Succession, Insect

AFFINITY STUDY OF SCFV-FC MONOCLONAL ANTIBODIES SPECIFIC TO HEMAGGLUTININ OF H5N1 INFLUENZA VIRUS USING SPR BIOSENSOR

Khwanthit Boonha

Faculty of Tropical Medicine, Mahidol University



Khwanthit Boonha 5436341 TMTM/M

Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University

Advisor: Associate Professor Dr. Pongrama Ramasoota

Background: Rapid detection for deadly disease of H5N1 avian influenza virus is needed. Monoclonal antibody (MAb) is the main reagent of rapid test kit. Since, Fragment antigen binding (Fab) MAbs specific to Hemagglutinin (HA) of H5N1 was previously developed by our group. But Fab does not have Fc part to bind with strip test. So modified MAb with Fc is needed. Also the binding affinity of each candidate MAb is needed before apply as test kit. In this study, scFv-Fc MAbs were constructed from our developed Fab, and affinity binding of each scFv-Fc MAbs have been tested using Surface Plasmon Resonance (SPR) Biosensor.

Objective: To construct scFv-Fc from Fab MAbs and express in HEK293T cell. To study kinetic and affinity constant of the purified scFv-Fc with HA protein using Proteon XPR36 Biosensor.

Materials and Methods: Two Fab MAbs; one with influenza A specific and the other with H5N1 specific were selected for scFv-Fc construction and further expressed in HEK293T cell. Purified scFv-Fc was used as

ligand for interaction study with HA protein (as analyte) on biosensor chip. The affinity constant and kinetic values of the interaction will be analysed using Proteon manager software.

Results: Two scFv-Fc MAbs were successfully constructed and expressed in HEK 293T cell. The produced scFv-Fc is properly reacted with H5 hemagglutinin protein by western blot analysis. And the binding of purified scFv-Fc and HA on biosensor chip is under optimization.

Key words: H5N1, scFv-Fc, hemagglutinin, Influenza virus, affinity, Biosensor

MEDICAL BURDEN AND ECONOMIC BURDEN OF CHRONIC HEPATITIS B PATIENTS AT QUEEN SAVANG VADHANA MEMORIAL HOSPITAL

Chuenrutai Yeekian

Faculty of Tropical Medicine, Mahidol University



Chuenrutai Yeekian¹, Nopavut Geratikornsupak¹, Jongkol Lertiendumrong, Sirinart Tongsir², Jittima Dhitavat², Benjaluck Phonrat², Punnee Pitisuttithum²

¹ Queen Savang Vadhana Memorial Hospital, ² Faculty of Tropical Medicine; Mahidol University

This study was conducted prospectively among CHB patients at Queen Savang Vadhana Memorial Hospital in order to assess medical burden by using the EuroQOL-5D (EQ-5D), the Chronic liver Disease Questionnaire (CLDQ) and economic burden in a societal perspective. A total of 152, 140, and 129 CHB patients at D0, M6 and M12 were enrolled. Median (IQR) age was 39.0 (32.0-49.0) years. More than half (84 of 152 or 55.3%) treated ARV having median (IQR) month of treatment 21.0 (9.0-31.0).

Overall, mean (SD) average CLDQ score increased from 5.48 (0.89) scores at D0 to 5.79 (0.87) scores at M6 and 5.98 (0.88) scores at M12. The quality of life at D0, the most sequences of EQ-5D dimensions patients reporting problems were pain/comfort (54.6%), anxiety (51.3%), mobility (13.2%), activity (13.2%), 13.2% and self care (3.9%), respectively.

Direct medical cost, direct non-medical cost and indirect cost accounted for 70.25, 9.93, 19.82 percents of total cost, respectively. Mean (SD) of these costs were 45,719.12 (64,647.43), 32,115.04 (54,259.40), 4,539.17 (6,353.99), and 9,063.97 (19,068.75) Baht/patient/year. Mean (SD) total cost of CLDQ < 5 scores and CLDQ ≥ 5 scores groups were 57,494.83 (45,405.94) and 41,671.22 (69,798.64) Baht/patient/year. There was no difference of total cost and direct medical cost between CLDQ < 5 scores and CLDQ ≥ 5 scores. However, the CLDQ < 5 scores group had mean (SD) direct non-medical cost and indirect cost from work productivity loss more than the CLDQ ≥ 5 scores group significant differences.

Key Words: Medical Burden/Economic Burden/Chronic Hepatitis B

Thursday 12th December 2013

Time: 13:30 – 15:00

Room C

S22 Population Pharmacokinetic-Pharmacodynamic Modeling of Antimalarial Drugs
Organized by: Mahidol-Oxford Tropical Medicine Research Unit

Chairperson 1



Joel Tarning

Chairperson 2



Sompob Saralamba

Symposium description

Malaria is one of the most important infectious diseases in the world and kills approximately 2,000 people each day. Recent research has shown that many of the current antimalarial drugs were introduced at the wrong doses, particularly in young children and pregnant women. The only way to determine accurately the correct dose regimens for antimalarial treatment is to establish a dose-response relationship through pharmacokinetic-pharmacodynamic (PK-PD) modelling. It is necessary to identify the different demographic, physiological, disease related, and pharmaceutical variables that influence drug concentrations and thereby outcomes. Better precision can be obtained with a modelling approach, compared to traditional dose-group comparisons, since the individual concentration-time profiles are used to modulate the treatment outcomes. This powerful approach allows dose-optimisation in different patient groups and it facilitates considerably the interpretation of clinical trials and pharmacological studies. Population modelling also offers a higher power for statistical inference compared to traditional end-point analysis and is especially useful when dealing with small sample sizes, which is often the reality in antimalarial PK-PD studies.

The proposed symposium will present state-of-the-art modelling of antimalarial drugs and the translational advantages of such models. The symposium will also demonstrate the importance of population modelling for global health.

1. Introduction to population pharmacokinetics and pharmacodynamics
Joel Tarning
2. Pharmacokinetic properties of artesunate in pregnant women with falciparum malaria
Frank Kloprogge
3. Pharmacokinetic-pharmacodynamic properties of piperaquine in patients with vivax malaria
Praiya Thana
4. Drug-drug interactions and pharmacokinetics of primaquine in healthy volunteers
Kalayanee Chairat
5. Pharmacokinetic interactions of antiretroviral and antimalarial drugs
Richard Hoglund

INTRODUCTION TO POPULATION PHARMACOKINETICS AND PHARMACODYNAMICS

Joel Tarning

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand



Joel Tarning^{1,2}

¹ Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand;

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Malaria still kills approximately 2,000 people each day. Effective antimalarial drug treatment saves lives. Many of the antimalarial drugs developed in the past fifty years were introduced at the wrong dose, particularly in young children and pregnant women. This undoubtedly contributed to therapeutic failures and the development of drug-resistant parasites. The only way to determine accurately the correct dose regimens for antimalarial treatment is to establish a dose-response relationship through pharmacokinetic-pharmacodynamic modelling. Pharmacokinetic-pharmacodynamic modelling provide a mechanistic understanding of how a drug behave in the body and link that to the therapeutic outcome. It also have the advantage of quantifying the variability between and within patients as well as the influence of demographic and disease variables. Established models can be used for translational simulations in order to optimize treatments for populations at particular risk, such as pregnant women and children with malaria.

PHARMACOKINETIC PROPERTIES OF ARTESUNATE IN PREGNANT WOMEN WITH *FALCIPARUM* MALARIA

Frank Kloprogge

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand



Frank Kloprogge^{1,2}, Rose McGready^{1,2,3}, Aung Pyae Phyo³, Marcus J. Rijken³, Warunee Hanpithakpon², Hla Hla Than³ Nathar Hlaing³ Naw Thida Zin³, Nicholas J. White^{1,2}, François Nosten^{1,2,3} & Joel Tarning^{1,2}

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Background Pregnancy alters the pharmacokinetic properties of many antimalarial compounds and this may result in lower drug exposure. This can cause lower cure-rates and accelerate the development of resistance. In the current study, oral and intravenous artesunate and dihydroartemisinin pharmacokinetics were studied using a population pharmacokinetic approach in pregnant women on the Thai-Myanmar border with uncomplicated *Plasmodium falciparum* malaria and again post-partum (no malaria).

Methods A simultaneous drug-metabolite model was developed in NONMEM 7.2. Disease and pregnancy related covariates were evaluated on first-pass metabolism and elimination clearance. The final model was used for Monte-Carlo simulations to assess the effect of pregnancy and acute uncomplicated *Plasmodium falciparum* malaria.

Results Twenty pregnant patients in the 2nd and 3rd trimester were recruited and 15 returned 3 months later as post-partum volunteers. A simultaneous drug-metabolite model with first-pass artesunate metabolism described the data well. Both the drug and metabolite showed two-compartment disposition pharmacokinetics. Dihydroartemisinin elimination clearance was 24.3% higher during pregnancy and absolute oral bioavailability was 95.8% higher during the acute malaria phase.

Conclusions Lower drug exposure during pregnancy might have implications on cure-rates and the development of resistance.

PHARMACOKINETIC-PHARMACODYNAMIC PROPERTIES OF PIPERAQUINE IN PATIENTS WITH VIVAX MALARIA

Praiya Thana

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand



Joel Tarning^{1,2}, Praiya Thana¹, Aung Pyae Phyo³, Khin Maung Lwin³, Warunee Hanpithakpong¹, Elizabeth Ashley^{1,2,3}, Nicholas Day^{1,2}, François Nosten^{1,2,3}, Nicholas J White^{1,2}

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Dihydroartemisinin-piperaquine is a promising alternative antimalarial for the treatment of *Plasmodium vivax* malaria since the long half-life of piperaquine might be able to suppress emerging relapses of *P. vivax* malaria. The aim of this study was to characterize the pharmacokinetics and pharmacodynamics of piperaquine in patients with *P. vivax* malaria using a population modeling approach. Two hundred fifty Karen and Burmese patients received a standard 3-day regimen of dihydroartemisinin-piperaquine. Venous and capillary blood samples were collected in 116 patients at 6 random time points over 69 days and at the time of recurrent malaria. Drug quantification was performed using liquid chromatography followed by tandem mass spectrometry. Piperaquine pharmacokinetics was well described by a 3-compartment distribution model with a transit-compartment (n=3) absorption model. The pharmacokinetic parameter estimates were generally similar to published literature values with an elimination clearance of 54.1 L/h, a large volume of distribution of 39,270 L, and a long terminal elimination half-life of 28.8 days. The time to recurrent *P. vivax* malaria was well explained by a time-to-event model with a constant baseline hazard (8.94 infections per year) with an increased risk of relapsing *P. vivax* malaria every third week. Piperaquine had a significant protective effect against relapsing infections and completely suppressed the first relapse of *P. vivax* malaria.

Keywords: pharmacokinetics, pharmacodynamics, population modeling, recurrent malaria, *Plasmodium vivax*

DRUG-DRUG INTERACTIONS AND PHARMACOKINETICS OF PRIMAQUINE IN HEALTHY VOLUNTEERS

Kalayanee Chairat

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand



Kalayanee Chairat¹, Podjane Jittamala², Borimas Hanboonkunupakarn², Salwaluk Panapipat¹, Sasithon Pukrittayakamee^{1,2}, Nicholas P.J. Day^{1,3}, Nicholas J. White^{1,3}, Joel Tarning^{1,3}

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³ Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Primaquine is an effective gametocytocidal antimalarial drug for all species of human malaria and the only available drug for radical cure of *Plasmodium vivax* and *Plasmodium ovale*. Primaquine is now recommended to be given in combination with other antimalarials to reduce transmission, especially in areas of emerging artemisinin resistance. It is therefore essential to study the potential drug-drug interactions of these drugs in order to provide clinical guidance for future treatment regimens. This study will present enantiospecific population pharmacokinetics of primaquine and its major metabolite, carboxyprimaquine in healthy Thai subjects when given alone and in combination with 1) chloroquine, 2) fixed dose combination of pyronaridine and artesunate and 3) fixed dose combination of dihydroartemisinin and piperaquine.

Population pharmacokinetic modeling was performed using non-linear mixed effects modeling where the impact of the potential drug-drug interactions could be identified and quantified.

Keywords: malaria, primaquine, carboxyprimaquine, drug-drug interactions, population pharmacokinetics

PHARMACOKINETIC INTERACTIONS OF ANTIRETROVIRAL AND ANTIMALARIAL DRUGS

Richard Hoglund

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Richard M. Hoglund¹, Pauline Byakika², Michael Ashton¹, Warunee Hanpithakpong³, Nicholas P.J. Day^{3,4}, Nicholas J. White^{3,4}, Angela Äbelö¹, Joel Tarning^{3,4}

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There is a high risk of co-infection and concomitant treatment of malaria and HIV considering the overlapping geographical distribution of the two diseases. Substantial drug-drug interactions between the treatments of the two diseases could have consequences for efficacy and toxicity of the treatments. The aim of this study was to investigate the potential drug-drug interactions between the anti-malarial drugs; lumefantrine, artemether and their respective metabolites desbutyl-lumefantrine and dihydroartemisinin, and the HIV-drugs efavirenz, nevirapine and lopinavir/ritonavir. The pharmacokinetic analysis was based on data from two clinical studies which were pooled and analysed with a non-linear mixed effects modelling approach. Lumefantrine exposure was decreased by concomitant treatment with efavirenz or nevirapine and increased by lopinavir/ritonavir. Dihydroartemisinin exposure was decreased by all the anti-retroviral drugs. The final models were used for dose simulations which suggest an increased artemether-lumefantrine dose of 250% and 50% to achieve equivalent exposure to lumefantrine when co-administered with efavirenz and nevirapine, respectively. A standard artemether-lumefantrine dose could be given when combined with lopinavir/ritonavir.

Keywords: malaria, population pharmacokinetics, artemether, dihydroartemisinin, lumefantrine, desbutyl-lumefantrine, efavirenz, nevirapine, lopinavir/ritonavir, drug-drug interaction

Thursday 12th December 2013

Time: 13:30 – 15:00

Room D

S23 Prevention is Better Than Cure: Tips & Tricks

Chairperson



Jittima Dhitavat

1. You are what you eat
Daruneewan Warodomwichit
2. Where there's a will there's a way
Arth Nana
3. Beauty is only skin deep
Vesarat Wessagowit

YOU ARE WHAT YOU EAT

Daruneewan Warodomwichit

Division of Nutrition and Biochemical Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University



Abstract Not Available

WHERE THERE'S A WILL THERE'S A WAY

Arth Nana

College of Sports Science and Technology, Mahidol University



Prof. Dr. Arth Nana - Topic 2: Where there's a will, there's a way.

Session Title: "Prevention is Better than Cure: Tips & Tricks"

Date: Thursday 12 December 2013

Time: 13.30-15.00

WHO has identified 'physical inactivity' as a fourth-leading risk factor for global mortality and accounted for about 6 to 10 percent of deaths globally. In 2008, lack of exercise was responsible for the deaths of 5.3 million of the 57 million lives globally. People failing to exercise or physically inactive may be responsible for as many global deaths as 'smoking'. Evidence for the benefits of physical activity for health has been available since the 1950s but the promotion to improve the health of the populations by increasing daily physical activities or by exercise has lagged until only recently. According to the 1996 U.S. Surgeon General's Report on Physical Activity and Health, people of all ages who are generally inactive can improve their health and well-being by becoming even moderately active on a regular basis. Regular physical activity that is performed on most days of the week reduces the risk for developing or dying from some of the leading causes of illness. Being physically active helps combat problems that can result from a sedentary lifestyle such as obesity, diabetes and heart disease. Regular moderate intensity physical activity such as walking, cycling or participating in sports has significant benefits for health. It can reduce the risk of hypertension, stroke, colon and breast cancer, and depression. Adequate levels of physical activity will help control weight and reduce the risk of falls. Adults aged 18-64 should accumulate at least 150 minutes of moderate intensity aerobic physical activity throughout the week and also engaged in muscle training activities involving major muscle groups on 2 or more days a week. Inactive people should start with small amounts of physical activity and gradually increase duration, frequency and intensity over time. Doing some physical activity is better than doing none. The benefits of the above recommendations outweigh the harms and are major implications for primary prevention of non-communicable diseases (NCD) and the general health of the general population.

BEAUTY IS ONLY SKIN DEEP

Vesarat Wessagowit

Institute of Dermatology, Department of Medical Service, Ministry of Public Health, Thailand



The global increase in life expectancy brings about the expectation of better quality of life. We are unfortunately living in the age of digital media and, being bombarded with images of surreal beauty, we equate quality of life with youthful looks. Ageing is a natural phenomenon thus unpreventable. However, the extrinsic causes of ageing are something we control. Sun damage is the worst environmental factor which hasten ageing. The protection of the skin from the sun can prevent dermatoheliosis but, at the same time, reduce vitamin D synthesis. The direct benefits of vitamin D, apart from bone health, are uncertain but the reduction of vitamin D from the skin can be counteracted by oral intake.

Thursday 12th December 2013

Time: 15:30 – 17:00
Room A

S24 One Health

Chairperson 1



Serge Morand

Chairperson 2



Chalit Komalamisra

1. The social sciences issues of the One Health
Aurélie Binot
2. Does One Health fit the Biodiversity research agenda
Serge Morand
3. Implementation of the One Health concept in the new approaches in environmental law and policies
Claire Lajaunie
4. Veterinary health and the One Health
Sathaporn Jittapalapong

THE SOCIAL SCIENCES ISSUES OF THE ONE HEALTH

Aurélie Binot

Cirad/Kasetsart University, Faculty of Veterinary Medicine



By Aurélie Binot¹ Cirad/Kasetsart University

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Several initiatives (MEA, One Health etc.) have emphasized the importance of integrated approaches for managing zoonoses emergence risk within a One Health approach. But the implementation of a concrete One Health approach to public health at the animal/human/environment interface raises many research questions and communication gaps among disciplines and sectors. How are the global changes and their impacts perceived by different types of stakeholders, from international decision makers to natural resources users? How do these various actors understand the patterns of zoonotic diseases emergence and the interaction between environment and health?

Participatory approaches, like participatory modeling for example, have an important potential in terms of involving different interest groups in order to build shared and co-designed representations

of a dynamic socio-ecosystem. Participatory risk mapping could also lead to innovative GIS including information layers defined by local communities. They could be used in order to collect users' knowledge about environmental and health risks at local level, or to highlight the behaviors rules when people are facing risks, taking into account socioeconomic or sociopolitical stakes at play. Regarding risk governance and management involving various stakeholders, we really need to better understand, through social sciences inputs, what are the social and political spaces and networks where effective decision are taken for health and environmental resources' management.

In order to illustrate our point, we will refer to a case study at the level of a village of Northern Thailand.

DOES ONE HEALTH FIT THE BIODIVERSITY RESEARCH AGENDA

Serge Morand

CNRS - Université de Montpellier, France /Mahidol University, Faculty of Tropical Medicine, Bangkok, Thailand



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Biodiversity changes (species extinction, invasive species, land use changes, deforestation, etc) are hypothesized to modify the epidemiological environment, hence favouring the spread of infectious diseases and/or their emergence. Biodiversity is often presented as a source of pathogens, such as bat-borne diseases, and as an insurance against their spread to humans or their domestic animals. For example, the “dilution effect” is presented as a mechanism by which a rich biodiversity may reduce the spread of zoonotic diseases to humans. However, several recent papers have challenged the view that conservation is beneficial for human health.

The sciences of biodiversity (ecology and evolution) provide rich frameworks that greatly help at providing hypotheses that can be tested using various kinds of data and statistics. Particularly, these scientific domains are those regularly endorsed in the environmental part of the “One Health” (along with the human health and the animal health).

Here, I will first use several recent studies to show the complex relationships between biodiversity and health, emphasizing on infectious diseases. This will help at defining the conditions where biodiversity of various organizations (landscapes, species, genes) may affect the diversity and spread of infectious diseases.

Second, I will illustrate concretely how the links between biodiversity and health can be investigated in the fields using ongoing studies in several villages of Northern Thailand and Laos (ANR BiodivHealthSEA project).

IMPLEMENTATION OF THE ONE HEALTH CONCEPT IN THE NEW APPROACHES IN ENVIRONMENTAL LAW AND POLICIES

Claire Lajaunie

INSERM (National Institute for Health and Medical Research-France)



The link between health and the environment has been acknowledged in various ways in international declarations and agreements since the Stockholm Conference of 1972.

In the discourse promoting sustainable development it appeared obvious that the environment had a direct effect on health.

Principle 1 of the Rio Declaration of 1992 proclaims that:

“Human beings are at the centre of concerns for sustainable development. They are entitled to a healthy and productive life in harmony with nature.”

The WHO progressively recognized that a healthy environment is a prerequisite to a healthy life. It seemed increasingly certain (as a result of the Millenium Ecosystem Assessment) that the rapid changes in our ecosystems and the broader pressures on them had harmful consequences such as the emergence and spread of new infectious diseases.

The concept of One Health is not new but received a stronger support since the 2000s: it has “particularly great relevance for microbiology as a unifying discipline that connects health among humans, animals, and the environment” (Atlas et al. 2010).

After the adoption of the Millenium Development Goals, the new International Health Regulations of 2005 enhanced the role of the WHO and provided a new framework for the coordination in case of a public health emergency of international concern. The spread of Avian Influenza throughout Asia since 2003 led the FAO, the WHO and the OIE to develop the Global Early Warning and Response System for Major Animal Diseases, including Zoonoses (GLEWS) and later on with the Unicef and the UN System Influenza Coordination the Strategic Framework for Reducing Risks of Infectious Diseases at the Animal–Human–Ecosystems Interface, the title mentioning explicitly “Contributing to One World-One Health™”.

The involvement of international agencies within the UN framework, of the World Bank or of IUCN into the spread of the concept of One Health probably induced the introduction of this concept within legal documents regarding international environmental law such as the Convention on Biological Diversity, the Convention on Migratory species of Wild Animals or the UN Convention to Combat desertification.

We will examine this new approach in environmental law and policies and how the concept is integrated into the legal texts and to what extent it contributes to changes of perceptions.

We will consider how the concept is or could be implemented at the local level through the use of legal instruments such as the Environmental Impact Assessment or the Health Impact Assessment and how it could encourage new integrated policies at the local level.

VETERINARY HEALTH AND THE ONE HEALTH

Sathaporn Jittapalapong
Kasetsart University



Sathaporn Jittapalapong^a, Marc Desquesnes^{a,b}, Stephane Herder^{a,c}, Aurelie Binot^{a,b}, and Serge Morand^{a,c}

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Introduction. Environmental modification due to deforestation, flooding, global warming, and climatic changes create alteration of ecological conditions for rodents and pathogens. Rodent-borne diseases such as leptospirosis, toxoplasmosis, trypanosomosis, bartonellosis, cryptosporidiosis, giardiasis, and hanta virus infections were recognized as emerging diseases menacing other animals and humans. A wide variety of rodents correlated with various habitats was sensitive to conservational reformation. Changing of rodent behaviors might respond to adjust transmission of zoonotic pathogens linked to animal and human diseases.

Approach: The epidemiological surveys were carried out in the 7 different geographic locations for wild rodent trapping and sampling, insect trapping, livestock sampling and data collection survey. Seven sites are chosen: Ayuthaya, Kalasin, Nan, Nakhon sawan, Phrae, Songkla, and Tak; Kalasin, Nan, and Phrae were already investigated by the CERoPath project since 2007 and 2010. Ayuthaya, Nan, and Nakhon sawan were studied for seasonal effects in 2012-13. Sites and locations are assigned by the history of zoonoses and the recent transformation of environment. For examples, the floods have provoked a long stagnation of organic rich water, a huge increase of wet surfaces, and the migrations of animals potentially reservoirs (increasing the animal/human contact). These important modifications of the environment have probably favored the explosion of epidemics, with a likely increase of the lethality rate.

Results: For non vector-borne pathogens, *Cryptosporidium* spp, *Giardia* spp., *Leptospira* spp., and *Toxoplasma gondii* were identified in rodents as contaminants in the environment. The presence of these pathogens is strictly connected to the hydrometric conditions. Soils and water are contaminated by feces, urine, and carcass of reservoir animals. The floods are considered as favoring the appearance of epidemics. Consequences of floods influence the circulation and the incidence of some parasitic zoonoses, in ways which are hardly predictable and need data record and analyses. For vector-borne pathogens, *Babesia* spp., *Trypanosoma* spp., *Bartonella* spp., and *Rickettsia* were recognized and related to their vectors. Availability of vectors was abundant during the optimal temperature and humidity, as well as suitable habitats. The conditions of vectors are consisted of their population dynamic, densities, and interrelations with the environment and have an impact on transmission of diseases.

Conclusion: The knowledge on the consequences of environmental changes on the epidemiology of the diseases, their incidence and prevalence, the population dynamics of rodent, and vectors were assimilated to determine the predictive evolution of the rodent population dynamic and epidemiology of zoonoses in order to create advices, recommendations, and predictions for prevention of the infections by zoonoses in these areas.

Keywords: climatic changes, zoonoses, rodents, Thailand

Thursday 12th December 2013

Time: 15:30 – 17:00

Room B

S25 Severe Malaria Treatment and Its Complications

Chairperson 1



Arjen M Dondorp

Chairperson 2



Kesinee Chotivanich

1. Fluid treatment in severe malaria
Josh Hanson
2. Late haemolysis after artesunate for severe malaria
Arjen Dondorp and Kesinee Chotivanich
3. Plasma haemoglobin and heme in adult severe malaria
Hugh Kingston

FLUID TREATMENT IN SEVERE MALARIA

Josh Hanson
Menzies School of Health Research



Fluid resuscitation has long been considered a key and potentially life-saving intervention in the initial treatment of adults with severe *Falciparum malaria*. Hypovolaemia is common in this population, but recent data suggest that compared to microvascular sequestration, it has a limited role in the reduction of microcirculatory tissue flow and fluid resuscitation has little effect on microvascular obstruction. Conversely, liberal fluid management increases the risk of complications, particularly pulmonary oedema which is commonly fatal when managed in the resource-poor setting. Although clinicians managing patients with severe malaria must rely on clinical judgment to judge volume status, neither physical signs of hypovolaemia, nor central venous pressure, can reliably guide fluid resuscitation. Instead, given the proven potential harm of fluid loading, clinical findings should be assessed collectively and cumulatively in the context of available laboratory and radiological data to inform a generally conservative approach to fluid therapy. Adults with significant anaemia (haemoglobin <7g/dL) require blood transfusion, but in patients without anaemia colloid solutions should be avoided given their increased cost and potential for harm in other critical care populations. The optimal crystalloid for maintenance is unclear, but the use of balanced solutions requires exploration. Timely use of inotropic support and renal replacement therapy may reduce unnecessary and potentially harmful fluid loading. There are fewer data to guide the fluid management of severe vivax and knowlesi malaria, although a similar restrictive strategy would appear prudent.

LATE HAEMOLYSIS AFTER ARTESUNATE FOR SEVERE MALARIA

Arjen Dondorp and Kesinee Chotivanich

Mahidol-Oxford Tropical Research Unit, Faculty of Tropical Medicine, Mahidol University



In 2 separate large trials in Asian adults and African children, parenteral artesunate has proven to be far superior to quinine in saving lives in patients with severe falciparum malaria, which is not at the expense of an increase in neurological sequelae. In addition, hypoglycaemia after start of therapy is less common with artesunate. Parenteral artesunate is now the WHO recommended treatment for severe malaria in all age groups, independent of the endemic setting. Recent reports on returning travellers treated with intravenous artesunate for severe malaria describe a thus far not recognized adverse effect of the drug: late haemolysis with a significant drop in haemoglobin levels occurring more than a week after treatment. The drop in haemoglobin is in particular prominent in patients presenting with high peripheral blood parasitaemia. This is puzzling, since artesunate and its active metabolite dihydroartemisinin have very short half-lives of around an hour, so that the drugs have long gone when haemolysis occurs. The likely mechanism of this intriguing observation is probably the flip side of the initial life-saving properties of artesunate. This will be discussed during the presentation.

PLASMA HAEMOGLOBIN AND HEME IN ADULT SEVERE MALARIA

Hugh Kingston

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Abstract Not Available

Thursday 12th December 2013

Time: 15:30 – 17:00

Room C

S26 Migration and Health

Chairperson 1



Suparat Phuanukoonnon

Chairperson 2



Kamolnetr Okanurak

1. Migration and health of young adults 15-29 years old: evidence from Kanchanaburi Demographic Surveillance System (DSS), Thailand
Sureeporn Punpuing
2. Cross border migration and health
Malee Sanpuwan
3. Health impact monitoring and evaluation from resource development: experience from the ExxonMobil PNG LNG project in Papua New Guinea
Suparat Phuanukoonnon
4. Migrant and Thailand border health strategy
Supakit Sirilak

MIGRATION AND HEALTH OF YOUNG ADULTS 15-29 YEARS OLD: EVIDENCE FROM KANCHANABURI DEMOGRAPHIC SURVEILLANCE SYSTEM (DSS), THAILAND

Sureeporn Punpuing

Institute for Population and Social Research Mahidol University



Movements from other countries and/or rural villages to large cities are often undertaken with the hopes of improved opportunities for economic advancement. However, changes in health status, both physical and emotional, constitute an important set of potential consequences of movement in both non-monetary costs and benefits. For young adults in the prime ages of migration, one key dimension of health status that can be profoundly affected by migration is their physical and mental health. The paper aims to explain variability in health status that have consequences for health outcomes later in life, within population working age adults (15-29) living in the study areas of the Kanchanaburi Demographic Surveillance System (DSS) site. Health status was measured by a set of standardized and sensitive instruments (SF36) that have been used extensively for other populations. Key predictor variables of interest for this study include type of migration, individual characteristics, socio-economic status and social connectedness.

It is found that migration, particularly lifetime cross-border migration can explained variation in health status that is measured by social functioning, emotional well-being, role limitations due to emotional health and bodily pain more than the lifetime internal migration. However, this study only partially supports that migrants have worse health status than non-migrants. This is due to the unfamiliar with environment at the destination among the migrants compare with non-migrants. There is an opposite direction in relationship with health status between the lifetime cross-border and internal migration. The adaptation strategies seem

to play an important role in different group of migrants if the assimilation theory would explain that after an initial adjustment period, migrants become more and more like the native-born because of their experience in the new home. It is recommended for further investigation.

**Institute for Population and Social Research, Mahidol University*

CROSS BORDER MIGRATION AND HEALTH

Malee Sunpuwan

Institute for Population and Social Research, Mahidol University



It is known that migration is considered as social determinant of health for migrants and migrant health is not only influenced by health sector but also other factors. Therefore, this paper aims to explore working conditions of migrant children which may lead to their health problems. The data was collected from 177 migrant children working in rubber plantation in Southern Thailand, aged 9- 17 years old. It is found that almost half of child migrant workers worked longer than 8 hours a day and engaged in night work. Slightly more than one-fifth engaged in excessive hours worked per week. And almost one-fifth engaged in night work. Tasks performed of migrant children that may lead to health problems were tapping rubber, making rubber sheet, applying panel treatment, using grass herbicide and spraying insecticide. Reported health problems that might be related to working conditions were: cut wounds from tapping rubber; snake/scorpion/insect bites; lower back pain; accidents with acids/chemicals/sharp equipment; allergic reactions due to spilled latex ; and breathing problems and headaches due to the smell of chemicals or rubber . A majority of them could access to health service but they could access to private clinic rather than that of public health facilities. The findings suggested that implement mechanisms to monitor farms and family- based work environments that can be reduced hazardous work is necessary. In addition, health care providers should get training in order to detect and document injuries from works and illness of migrant children that related to work.

Keyword: Migration, Health, Child migrant workers

HEALTH IMPACT MONITORING AND EVALUATION FROM RESOURCE DEVELOPMENT: EXPERIENCE FROM THE EXXONMOBIL PNG LNG PROJECT IN PAPUA NEW GUINEA

Suparat Phuanukoonnon

Papua New Guinea Institute of Medical Research



Background: The PNG Liquid Natural Gas (PNG LNG) project is mostly located in two sites in Papua New Guinea (PNG); Hides in the Southern Highlands Province and the Hiri West district of Central Province. Anticipating major changes in these communities, Papua New Guinea Institute of Medical Research (PNGIMR) conduct a health and demography surveillance to better understand the impact these developments will have on the health and well-being of the populations proximal to the PNG-LNG sites.

Methods: Health and demographic surveillance system (HDSS) is developed to monitor populations across four sites in PNG; two 'impact sites' and two other 'comparison sites' in Asaro, Eastern Highlands Province and Karkar Island, Madang Province. Populations of between 10,000 and 20,000 in each site are monitored by an annual census and biannually demographic update surveys.

Results: Populations are undergoing rapid growth and in some cases exhibit a high male to female ratio. Educational attainment and employment varies widely between sites. These results will be discussed in light of the unique contexts and cultures in each site. Population dynamics like population growth rates, birth and death rates will be presented for each of the sites, and the implications of findings explored to elaborate on the potential impacts of the LNG project in the subject populations.

Conclusions: HDSS not only provides the denominator for all health related research within the HDSS but is itself a sensitive monitoring system capable of capturing and identifying changes, trends and anomalies in the population which require closer examination

Keywords: Health impact, demography, resource development

MIGRANT AND THAILAND BORDER HEALTH STRATEGY

Supakit Sirilak
Ministry of Public Health, Thailand



Dr. Supakit Sirilak MD., MPH.
Senior advisor on preventive medicine, MoPH

Thailand is surrounded by four neighboring countries; Myanmar, Laos PDR, Cambodia and Malaysia; with over three thousand kilometers long of border. The mixed population residing in the border provinces needs specific intervention to cope their health problems. Although universal health care has been implemented successfully more than ten years for Thai citizen, later extended to the ethnic minority, and the compulsory migrant health insurance scheme (CMHIS) was applied for registered migrant workers but still left the irregular migrants behind. The poor accessibility to care not only causes the late treatment for migrant themselves but also increasing risk to spread the communicable diseases to the community. Some border areas are very remote and hilly especially the Northern part of the country shared with Myanmar and Laos PDR. The outreach health services are needed for both Thais and the migrants to improve their health status. During the two decades there were the re-emerging diseases found such as Meningococcal meningitis, Filariasis and the more resistant XDR Tuberculosis threatening not only the border people but also those living inner Thailand.

Several actors are playing their role in the areas sometimes without any collaboration and integration resulting in overlapping and inefficiency of achieving goal and resources used. The collaboration with the neighboring countries which is more important in the local level is not yet well established especially in some specific borders. The exchange of health information, the effective referral system, the cross border outbreak investigation cannot be done without the permission of the central. Several provinces has established the special unit in the provincial health office as the focal point and responsible unit for all border health matters. This has been proved to be one of an essential re-structure for the administration at the border.

The 2nd Border Health Development Master Plan (BHDMP) 2012-2016 is developed with the aim to be a collaborative framework among relevant offices within MOPH, other line ministries, local administrative organizations, non-governmental organization and international organizations to develop the public health work in border areas. Additionally it is hoped that the plan will serve as a practical tool for budget and resource requests from government and non-governmental sources for efficient resource mobilization, so that the quality of life of border populations in Thailand will be improved. The plan was developed with coordinated efforts and involvement from various partners and networks. It corresponds with the 11th National Economic and Social Development Plan, the National Security Policy, the 11th National Health Development Plan (2012-2016), and policy of the Ministry of Public Health. It also factors in the current and future situations that may have an implication on border health. It seeks to address current limitations of border health work, specifically in the areas of developing a quality health service system, increasing access to primary care services, and promoting participation from all relevant stakeholders within and cross border country.

The Ministry of Public Health hopes that relevant organizations and sectors will participate in endorsing and utilizing this strategic Master Plan in developing concrete actions that will minimize the public health gaps at the border areas.

Thursday 12th December 2013

Time: 15:30 – 17:00
Room D

S27 Rickettsia and Bacteria

Chairperson 1



Pornpan Pumirat

Chairperson 2



Yupaporn Wattanagoon

1. Loop-mediated isothermal amplification for murine typhus (*Rickettsia typhi*) – problems with diagnosis at the limit of detection
Sabine Dittrich
2. Trehalase Regulates Growth and Virulence in *Burkholderia pseudomallei*
Muthita Vanaporn
3. Population Pharmacokinetics of Isoniazid in Children With Tuberculous Meningitis
Thanaporn Wattanakul
4. PCR detection of *gyrA* markers of reduced susceptibility to fluoroquinolones in extracts of DNA from *Salmonella typhi* antigen rapid diagnostic tests.
Chaoimhe Nic Fhogartaig
5. Development of a Non-Human Primate Model of Scrub Typhus Using Intradermal Inoculation
Piyanate Sunyakumthor

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR MURINE TYPHUS (*RICKETTSIA TYPHI*) – PROBLEMS WITH DIAGNOSIS AT THE LIMIT OF DETECTION

Sabine Dittrich

Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit



Sabine Dittrich^{1,2}, Josée Castonguay-Vanier^{1,2}, Catrin E Moore^{1,2}, Narongchai Tongyoo^{1,3}, Paul N Newton^{1,2}, and Daniel H. Paris^{1,2,3}

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Murine typhus is a flea-borne disease of worldwide distribution caused by *Rickettsia typhi*. Although treatment with tetracycline antibiotics is effective, treatment is often misguided due to diagnostic difficulties. As the gold standard immunofluorescence assay is imperfect, the aim of this study was to develop and evaluate a loop-mediated isothermal amplification assay (LAMP). LAMP assays have the potential to fulfil the WHO 'ASSURED' criteria for diagnostic methodologies, as they can detect pathogen-derived nucleic acid with minimal technical expenditure.

The LAMP assay was developed using samples from bacterial isolates (n=41), buffy coat from *R. typhi* PCR-positive Lao patients (n=42), and diverse negative controls (n=47). A following prospective evaluation recruited consecutive patients with suspected scrub typhus or murine typhus (n=266). The limit of detection was ~40 DNA copies/ LAMP reaction, with an analytical sensitivity below 10 DNA copies/reaction based on isolate dilutions. Despite these low cut-offs, the clinical sensitivity was disappointing with 48% (95%CI 32.5 - 62.7) (specificity 100 % (95%CI 100 - 100)) in the developmental phase and 33% (CI 95%: 9.2 - 56.8) (specificity: 98.5% (CI95%: 97.0% - 100%)) in the prospective study. This low diagnostic accuracy was attributed to low *R. typhi* bacterial loads (median: 210 DNA copies/ mL blood, IQR: 130 – 500) in patients. LAMP-negative samples demonstrated significantly lower bacterial loads compared to LAMP-positive samples, in both independent datasets.

Our findings highlight the diagnostic challenges for diseases with low pathogen burdens and emphasize the need to integrate pathogen biology and improved template production into assay development strategies.

Keywords: *Rickettsia typhi*, murine typhus, loop-mediated isothermal amplification assay, diagnostic, bacterial load

TREHALASE REGULATES GROWTH AND VIRULENCE IN *BURKHOLDERIA PSEUDOMALLEI*

Muthita Vanaporn

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B*urkholderia pseudomallei* is an intracellular pathogen causing a fatal disease, melioidosis. It can survive in minimal media including water. The bacteria are able to invade mammalian cells and multiply inside both epithelial and macrophage. There is an enzyme called trehalase (Tre) which hydrolyzes trehalose to glucose. Previous studies in *E. coli* showed that trehalase is related to the stress responses such as osmotic stress and cold shock. *B. pseudomallei* has only one trehalase which similar to TreA in *E. coli*. Only studies in yeast were shown the important of trehalase to virulence. The objective of this study is to determine the essential of *B. pseudomallei* trehalase in growth and virulence. The *B. pseudomallei* strain K96243 with deletion of tre gene was constructed and confirmed by PCR. The bacterial growth in LB and M9 minimal media were determined as well as the response to oxidative and osmotic stresses. The bacterial virulence was determined by survival assay in J774A.1 macrophage cell line and in BALB/c mouse model. We found that tre mutant was unable to grow in minimal media and more sensitive to oxidative stress, but not osmotic stress. The tre mutant can not survive in J774A.1 cell line after 24hr post infection and give a hundred percent attenuation in mouse model. In summary, tre gene encoded for enzyme trehalose is essential to *B. pseudomallei* in order to survive in minimal media and response to oxidative stress. This is the first report to highlight the important of trehalase in bacterial virulence.

Keyword: trehalase, virulence factor, oxidative stress, minimal media

POPULATION PHARMACOKINETICS OF ISONIAZID IN CHILDREN WITH TUBERCULOUS MENINGITIS

Thanaporn Wattanakul

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Thanaporn Wattanakul¹, Thomas Pouplin^{1,3}, Toi Pham Van², Nicholas Day^{1,3}, Nicholas White^{1,3}, Jeremy Day^{2,3}, Jeremy Farrar^{2,3}, Joel Tarning^{1,3}

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Tuberculosis is a high burden disease in developing countries. Children are especially vulnerable to develop symptomatic disease after infected with *Mycobacterium Tuberculosis*. The World Health Organization reported that the incidence of tuberculosis among children was estimated at 490,000 cases resulting in 64,000 deaths in 2011. Tuberculosis Meningitis (TBM) is the most common form of tuberculosis in the central nervous system. Children aged <1 year has 10-20% risk of developing TBM following the primary infection of tuberculosis. Children with TBM also have high rates of morbidity and mortality. Early diagnosis and treatment are crucial to reduce the mortality in these patients. Isoniazid is one of the essential first-line anti-tuberculosis agents that have a rapid early bactericidal activity. Isoniazid is a lipophilic small molecule and considered to be the ideal agent for TBM treatment since it easily cross the blood-brain-barrier to the site of action. Nevertheless, Isoniazid in tuberculosis treatment shows somewhat complex pharmacokinetic properties such as polymorphic elimination and substantial first-pass metabolism. The population pharmacokinetics of isoniazid in adult pulmonary tuberculosis patients has been reported. However, information in children with TBM is limited. The aim of this study was to investigate the population pharmacokinetic properties of isoniazid in 99 Vietnamese children with TBM by using nonlinear mixed-effects modeling. A time-dependent increase in isoniazid clearance was found. Age as a maturation effect on clearance and body-weight as an allometric function on all clearance and volume parameters were identified as significant covariates. The final population pharmacokinetic model described the plasma and cerebrospinal fluid data well in these children. The developed model will be an invaluable tool in further dose optimization of this important drug.

Keywords: Isoniazid, tuberculous meningitis, population pharmacokinetics

PCR DETECTION OF GYRA MARKERS OF REDUCED SUSCEPTIBILITY TO FLUOROQUINOLONES IN EXTRACTS OF DNA FROM *SALMONELLA TYPHI* ANTIGEN RAPID DIAGNOSTIC TESTS

Chaoimhe Nic Fhogartaig

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Background: A *S. Typhi* antigen RDT accurately and rapidly detects *S. Typhi* from positive blood cultures. Combining RDT diagnosis in the field with resistance gene detection in a reference laboratory could help inform treatment during typhoid outbreaks in regions without such facilities, and improve surveillance of resistance.

Aim: To optimise DNA extraction methods from RDTs to enable PCR and RFLP detection of *gyrA* mutations associated with reduced susceptibility to fluoroquinolones (RSF) in a timely manner.

Method: RDTs were inoculated with blood culture fluid spiked with sensitive and resistant *S. Typhi* controls. DNA was extracted, from 5 different RDT sections, using a Qiagen kit or boiling method. Extracts underwent PCR targeting *gyrA*. DNA quantification was assessed by intensity of bands on gel electrophoresis for serial dilutions, and *gyrA* mutations were determined using RFLP. The optimised protocol was evaluated prospectively with positive RDTs from blood cultures alongside standard susceptibility tests.

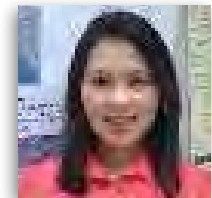
Results: For all RDT sections the boiling method yielded more DNA. Sample and conjugate pads yielded most DNA. However, they also had more inhibitors present, which could be overcome by adding bovine serum albumin. Prospective evaluation of the optimised protocol is in progress, and so far has correctly predicted fluoroquinolone susceptibility results for 8/8 *S. Typhi* cases (including one RSF).

Conclusion: A simple, relatively inexpensive, same-day method can detect RSF from positive *S. Typhi* RDTs, which could aid patient management and surveillance efforts in areas with inadequate laboratory facilities, provided samples can be transported to the reference laboratory.

DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL OF SCRUB TYPHUS USING INTRADERMAL INOCULATION

Piyanate Sunyakumthor

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Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi* for which no effective broadly protective vaccine is available. To develop and evaluate effective vaccine candidates, we need to understand the immune response against *O. tsutsugamushi* and a well-characterized animal model. In this study, we demonstrate initial evaluation of a NHP scrub typhus disease model based on the intradermal inoculation of *O. tsutsugamushi* in rhesus macaques (n=7). A series of time course investigations involving hematological, biochemical, bacterial quantitation and immunological bioassays were performed, until day 28, when tissues were collected for pathology and immunohistochemistry. In all NHPs with *O. tsutsugamushi* inoculation (n=4), the development of a classic eschar with central necrosis, regional lymphadenopathy, elevation of body temperatures was observed on day 7-21 pi, bacteremia was detected by qPCR on days 6-18 pi, and biochemical and hematological assays demonstrated alteration of liver enzyme function and increase of white blood cells on day 14 pi. Immune assays demonstrated raised serum levels of soluble cell adhesion molecules, anti-*O. tsutsugamushi*-specific antibody responses (IgM and IgG) and pathogen-specific cell-mediated immune responses in inoculated monkeys. At day 30 pi, qPCR detected *O. tsutsugamushi* in eschar, spleen, draining and non-draining lymph nodes, and immuno-double staining demonstrated intracellular *Orientia* in antigen presenting cells. These data show the potential of using rhesus macaques as a scrub typhus model, however further investigations into inoculum routes, dosages and immuno-pathophysiological responses are required to ultimately validate this animal model for future scrub typhus vaccine studies.

Keywords: scrub typhus, *Orientia tsutsugamushi*, non-human primate model, intradermal inoculation

Thursday 12th December 2013

Time: 15:30 – 17:00
Room E

S28 Helminthiasis

Chairperson 1



Paron Dekumyoy

Chairperson 2



Dorn Watthanakulpanich

1. Diagnosis of hydatid disease using *Echinococcus granulosus* recombinant paramyosin protein
Rahmah Noordin
2. Prevalence of ov infection and behavior for prevention and control of ov and cholangiocarcinoma among people, upper northeast Thailand, 2013
Narong Wongba
3. Dirofilariasis — a zoonosis in developed countries
Yukifumi Nawa
4. Molecular analysis of ropporin 1-like protein from human liver fluke, *Opisthorchis viverrini*
Sittichon Rattanachan
5. Ecological niche modelling of strongyloidiasis in endemic areas, khon kaen provinces, northeast Thailand
Apiporn Suwannatrai
6. A follow-up study of *Opisthorchis viverrini* infection after the 10-year implementation of control program in Baan Nayao, Chachoensao, central Thailand
Lalitpat Palakool

DIAGNOSIS OF HYDATID DISEASE USING *ECHINOCOCCUS GRANULOSUS* RECOMBINANT PARAMYOSIN PROTEIN

Rahmah Noordin

Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia



Rahmah Noordin¹, Zohreh Kazemi Moghadam¹, Akbar Khalilpour¹, Muhammad Hafiznur Yunus¹, Sabariah Osman¹ and Fatemeh Ghafarifar²

¹ Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 Penang, Malaysia;

² Department of Parasitology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran

Hydatic disease caused by *Echinococcus granulosus* is a public health problem in many parts of the world. We have shown that *E. granulosus* paramyosin has potential for use in diagnosis of cystic hydatid disease, and subsequently produced the recombinant form of the protein. Protein expression was induced by adding 1 mM IPTG for 5 hours at 37 °C. The cell pellet was resuspended in lysis buffer containing protease inhibitors and lysozyme and incubated on ice for 30 min. The cell suspension was lysed, and the supernatant was filtered and mixed with Ni-NTA resin, then loaded into a chromatography column. Gradient washings of the column were performed consecutively with 10 ml washing buffers containing four imidazole concentrations (10mM, 20mM, 30mM and 40mM imidazole) and 500 mM NaCl. The protein fractions were eluted using buffer containing 250 mM imidazole. The recombinant paramyosin showed 86% diagnostic sensitivity (42/49) with serum samples from hydatid cyst patients and 98% diagnostic specificity (58/59) with control sera from 24 healthy people and 35 patients with other parasitic infections. In an attempt to develop a rapid lateral flow dipstick test, the recombinant protein preparation was further purified by pH-based separation using an OFFGEL fractionator (Agilent) and IPG strip of pH range 3-10. Liquid fractions containing the purified recombinant protein were collected, concentrated, buffer exchanged and used in the development of a rapid lateral flow test. The results of the rapid test evaluation will also be presented.

Keywords: *Echinococcus granulosus*, recombinant paramyosin, Western blot, rapid test, diagnostic sensitivity and specificity

PREVALENCE OF OV INFECTION AND BEHAVIOR FOR PREVENTION AND CONTROL OF OV AND CHOLANGIOCARCINOMA AMONG PEOPLE, UPPER NORTHEAST THAILAND, 2013

Narong Wongba

The office of Disease Prevention and Control



Narong Wongba¹, Kesorn Thaewngiew¹, Laithavewat Luxana¹, Seri Singthong¹, Saowalux Kutcharat¹, Sasithorn Tangsawad², Sawai Phothimol³

¹ The office of Disease Prevention and Control 6 Khon Kaen

² The office of Disease Prevention and Control 2 Saraburi

³ Vector born Disease Control 6.2 Udonthani

Aliver fluke, *Opisthorchis viverrini* (OV), is a serious health problem of Northeast, Thailand. *O. viverrini* infection is the major etiology of cholangiocarcinoma. This study is a cross-sectional study, aimed to determine actual levels of *O. viverrini* infection and behavior for prevention and control of *O. viverrini* infection and cholangiocarcinoma. The subjects were chosen by using 30 Clusters Sampling technique from 7 provinces which represent the whole population in upper northeast Thailand. The data collected by questionnaire, 4 focus group discussion, 18 in-depth interview and stool examination by using method of Modified Kato Katz. A total of 3,916 subjects were obtained from 1,782 males and 2,134 females, mean age 43 years. Stool examination showed that 25.8% were infected with *O. viverrini*. High endemic area (prevalence

> 20 %) about 4 of 7 provinces due to the low priority in national plan. The subjects knew that eating raw freshwater fish was a cause of opisthorchiasis (91%), not knowing how to take praziquantal correctly and not knowing side effect of medicine 58.8%, 52.8% respectively. The people still enjoy eating raw fish 67.1. They perceived that it is way of life since their ancestors and more delicious. This study indicates that *O. viverrini* is still a problem in some areas of upper Northeast. The people not awareness for severity of *O. viverrini* infection and cholangiocarcinoma. Therefore, encouraging people to avoid health risk behavior should be undertaken more rigorously and supporting local communities to find ways to change eating behaviors.

Keywords: Prevalence of *Opisthorchis viverrini*, Behavior for Prevention and Control

DIROFILARIASIS — A ZONOSIS IN DEVELOPED COUNTRIES

Yukifumi Nawa

Research Affairs, Faculty of Medicine, Khon Kaen University



Yukifumi Nawa³, Nobuaki Akao², Masahide Yoshikawa³,

¹ Research Affairs, Khon Kaen University, Khon Kaen, Thailand

² Section of Environmental Parasitology, Graduate School of Tokyo Medical and Dental University, Tokyo, Japan

³ Department of Parasitology, Nara Medical University, Kashivara, Japan

Dirofilariasis is a vector-borne zoonotic disease transmitted from dogs to humans by mosquitoes. Of the two zoonotic *Dirofilaria* species, *D. immitis* and *D. repens*, the former is mainly distributed in East Asia and North America, while the latter is distributed in Europe and South and Southeast Asia. The most common clinical manifestation of *D. immitis* infection in humans is a solitary nodular lesion in the lungs, and the patients have mainly been reported from Japan and the United States. In contrast, nearly all cases of *D. repens* infection in humans are recognized as a nodular lesion in the skin, with the majority of patients being reported from Europe, while some also reported from India and Sri Lanka. In Southeast Asia, both *D. immitis* and *D. repens* are endemic among dogs with relatively high prevalence, whereas human cases have rarely been presented. For example, over 40% of stray dogs in Thailand were reported to be microfilaria positive in blood smear tests, but only 2 cases of *D. immitis* infection and 1 case of *D. repens* infection in humans were reported. The basis of the discrepancy for prevalence of dirofilariasis between developing and developed countries should be explored in future.

MOLECULAR ANALYSIS OF ROPPORIN 1-LIKE PROTEIN FROM HUMAN LIVER FLUKE, OPISTHORCHIS VIVERRINI

Sithichon Rattanachan

Department of Biology, Faculty of Science, Mahidol University



Sithichon Rattanachan¹, Hans Rudi Grams², Smarn Tesana³, Suksiri Vichasri Grams¹

¹ Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

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³ Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Human opisthorchiasis remains a major disease in Southeast Asia. The pathogenesis is associated with hepatobiliary system. Clinical effects and epidemiology have been intensively investigated. However, the reproductive biology of this parasite is less researched. In this study, ropporin 1-like (OvROPN1L) protein coding cDNA was isolated from adult stage cDNA library of *O. viverrini*. DNA sequence analysis confirmed that this cDNA contained the complete open reading frame encoding 222 amino acid residues.

OvROPN1L amino acid sequence was highly similar to cAMP dependent RII subunit of PKA from *Schistosoma mansoni* and *Clonorchis sinensis* with more than 70% identity. RT-PCR result demonstrated active expression of OvROPN1L already in 2-week-old juveniles and transcripts were then continuously detected in up to 8-week-old adult worms. The antigenicity of recombinant OvROPN1L protein was assessed by immunoblot analysis with sera from *O. viverrini* infected hamster sampled at 2, 4, 6, and 8 weeks post infection. The approximately 25.4 kDa native OvROPN1L protein was detected in the crude worm extract using mouse polyclonal anti-OvROPN1L antibody. No cross reactivity of this antibody was found among relative parasites and sperm proteins from mammalian hosts. For the immunolocalization result, Native OvROPN1L was detected in the testes, the sperm mass in the seminal vesicle, and sperms in the seminal receptacle of 6-week-old juveniles and 8-week-old adult worms. In 2-week-old juveniles, native OvROPN1L was only found in the testes. Overall studies implied that OvROPN1L contributes to male reproductive system of *O. viverrini*, however, crucial role of OvROPN1L in sperm of *O. viverrini* is unknown.

Keywords: Trematoda, *Opisthorchis viverrini*, ropporin 1-like protein

Acknowledgements:

This experiment was supported a grant from The Royal Golden Jubilee Ph.D Program, Thailand Research Fund. A financial support for Mr. Sitthichon Rattanachan during the study was contributed by Mahanakorn University of Technology.

ECOLOGICAL NICHE MODELLING OF STRONGYLOIDIASIS IN ENDEMIC AREAS, KHON KAEN PROVINCES, NORTHEAST THAILAND

Apiporn Suwannatrai

Food-Borne Parasite Research Group, Department of Parasitology, Faculty of Medicine, Khon Kaen University



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³ Tropical Disease Research Laboratory (TDR), Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand,

Strongyloidiasis remains one of the most public health problems particularly in Northeast Thailand. This disease caused by *Strongyloides stercoralis*. The correctly defining disease distribution and disease risk map are important information for the prevention and control strategy. We used geographical information systems (GIS) and remote sensing (RS) technologies within the MaxEnt ecological niche modeling program to determine the relationships and transmission patterns of strongyloidiasis in endemic areas based on climate and other environmental factors (climate, bioclim, altitude and soil surface salt) and 142 locations of disease occurrence are from 2008 to 2013. Altitude was the most important variable in the prediction model (81.4 % contribution). Annual precipitation (Bio12), temperature seasonality (Bio4), maximum temperature of warmest month (Bio5) and mean diurnal range (Bio2) were also important model variables (5%, 4.9%, 4.4% and 4.3% contribution, respectively). These results indicate that, altitude and bioclimatic regime have important influences on current strongyloidiasis in endemic areas and suggest environmental risk surfaces generated by Maxent ecological niche models can be used to guide better targeted control programs in Northeast Thailand.

Keywords: Strongyloidiasis, GIS, Ecological niche modelling

A FOLLOW-UP STUDY OF *OPISTHORCHIS VIVERRINI* INFECTION AFTER THE 10-YEAR IMPLEMENTATION OF CONTROL PROGRAM IN BAAN NAYAO, CHACHOENSAO, CENTRAL THAILAND

Lalitpat Palakool
Phramongkutklao College of Medicine



Sahapat Charoenratana¹, Lalitpat Palakool¹, Sarunkorn Promboon¹, Saris Chalermpongchai¹, Anchittha Intharapin¹, Nagoon Sangphoo¹, Natthaphat Chanmano¹, Thanaporn Srisuppachaiya¹, Piyatida Haruehansavasin¹, Wit Jeamwjitkul¹, Veerawat Lertlitdech¹, Summa Lertheerakul¹, Sirinporn Sawasvirojwong¹, Onteera Somjid¹, Awassya Choe-ngern¹, Saovane Leelayoova², Paanijit Taamasri², Tawee Naaglor², Ram Rangsin³, Picha Suwannahitatorn², Mathirut Mungthin²

¹ 4th year Medical student, ²Department of Parasitology, ³Department of Military and Community Medicine, Phramongkutklao College of Medicine, 315 Ratchawithi Rd., Ratchathewi, Bangkok 10400, Thailand

Background : *Opisthorchis viverrini* infection is still one of the public health problems in Thailand. Two cohort studies were conducted in Baan Nayao, a rural community in central Thailand. In 2002-2004, the first cohort study showed the prevalence of *O. viverrini* infection of 21.3% and the incidence of 21.6/100 person-years. Since the first survey, conventional control program including case diagnosis and treatment, health education and promotion for hygienic defecation and cooked fish consumption has been implemented. In 2007-2009, the second cohort study, the prevalence and the incidence of *O. viverrini* infection remained high which was 18.6% and 21.4/100 person-years, respectively. In 2011, our survey indicated that the prevalence of this infection was decreased (6.2%). Thus we were interested to determine the trend of the incidence of this infection.

Objective : To determine the incidence and risk factors of *O. viverrini* infection in Baan Nayao, Chachoensao, central Thailand.

Study design : We conducted both quantitative study using a retrospective cohort design and qualitative study to assess the situation of *O. viverrini* infection in 2011-2013.

Materials and Methods : A prospective cohort study was conducted to evaluate the incidence and risk factors of *O. viverrini* infection. Stool examination methods including wet preparation, Kato and formalin-ethyl acetate concentration technique were performed for the detection of *O. viverrini* eggs. A standardized questionnaire was used to assess risk behavior. In addition, qualitative information was collected from both *O. viverrini* negative and positive villagers using focus group discussions.

Results: The incidence of *O. viverrini* infection was 7.05/100 person-year. Consumption of chopped raw fish salad (Koi-pla) was independently associated with *O. viverrini* infection which was similar to the previous studies. From qualitative study, Koi pla consumption in this population was decreased especially in younger age groups. However, consumption of other raw fish dishes remained in the older adults. Inadequate knowledge and misbeliefs were the important factor leading to their risk behavior. Besides, unhygienic defecation and insufficient diagnosis and treatment may facilitate *O. viverrini* infection.

Conclusion : After 10-year implication of control program, the incidence of *O. viverrini* infection was decreased in this population. Precise and regular health education and promotion targeting the main risk factor, Koi pla consumption has been continuously used. From the qualitative study, avoiding consumption of other raw fish dishes should be put in the control program. Improving diagnosis and treatment, and promoting hygienic defecation should be used in the prevention and control program

Keywords: *Opisthorchis viverrini*, Incidence, Risk factors, Koi pla, Qualitative study

Thursday 12th December 2013

Time: 17:00 – 18:30

Meet The Professor (light meal provided)

Room	A	B	C	D
				
Professor	Pradip Rathod	Stephen A. Ward	Phillip Newmark	Matthew Berriman
Affiliation	University of Washington	Liverpool School of Tropical Medicine	HHMI/ University of Illinois at Urbana-Champaign	the Parasite Genomics Group, Wellcome Trust Sanger Institute
Expertise/Areas of interest	Pradip Rathod is a Professor of Chemistry and Adjunct Professor of Global Health at the University of Washington, and Director at NIH International Center of Excellence for Malaria Research for South Asia. His research interest is drug development against Plasmodium Malaria. The Rathod group at the University of Washington is currently working on four main areas: 1) targeting Pyrimidine Biosynthesis, 2) mutator phenotypes, 3) System-Wide differences in gene regulation, and 4) New tools for malaria research.	Drug discovery, drug development and tropical pharmacology. Understanding the chemical, molecular and cellular basis of drug action and drug resistance in tropical pathogens with an emphasis on human malaria, TB and more recently the Neglected Tropical Diseases (NTDs). This fundamental information is then employed in the rational design and evaluation of novel chemotherapeutic agents and in improving the effectiveness of existing chemotherapeutic agents. Linked to this interest there is a focus on understanding fundamental pathogen-specific biological processes with a view to identifying drug development unique targets for chemotherapy.	Phillip Newmark is a Professor in the Department of Cell and Developmental Biology at the University of Illinois at Urbana-Champaign and an Investigator of the Howard Hughes Medical Institute. He initiated postdoctoral work on planarians with Jaume Bagu at the University of Barcelona, and continued his postdoctoral research with Alejandro Sanchez Alvarado at the Carnegie Institution's Department of Embryology. They applied cellular and molecular tools to studies of planarians, helping to revitalize work on these organisms and permitting analyses of the mechanisms underlying regeneration. Current research in his laboratory focuses on regeneration and germ cell development in planarians, as well as the developmental and reproductive biology of parasitic flatworms.	Professor Berriman leads a programme in the genomics of Neglected Tropical Disease parasites, including helminths such as Schistosomes, tapeworms, roundworms, hookworms, threadworms and whipworms. Matt graduated in 1994 from the University of Manchester with a degree in biochemistry. Since 2003, Prof. Berriman has been leading more than 20 eukaryotic pathogen sequencing projects at the Wellcome Trust Sanger Institute. His research is primarily focused on the medically important Apicomplexan and Kinetoplastid protozoa, which include malaria parasites and trypanosomes. He is leading a programme in the genomics of Neglected Tropical Disease parasites, which include parasitic helminths such as Schistosomes, tapeworms, roundworms, hookworms, threadworms and whipworms.



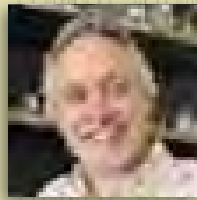
Friday 13th December 2013

Time: 09:00 – 10:30

Room A

S29 Enteric Protozoa: Trends and Challenges

Chairperson 1



Andrew Thompson

Chairperson 2



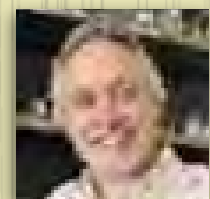
Supaluk Popruk

1. *Giardia* and *Cryptosporidium* – paradigm shifts in understanding their ecology
Andrew Thompson
2. Are animals considered as zoonotic risks of intestinal protozoa? Part 1:
Wildlife and livestock
Aongart Mahittikorn
3. Are animals considered as zoonotic risks of intestinal protozoa? Part 2:
Companion animals and monkeys living in town
Hirotake Mori
4. Subtype distribution of *Blastocystis* in humans
Supaluk Popruk

GIARDIA AND CRYPTOSPORIDIUM – PARADIGM SHIFTS IN UNDERSTANDING THEIR ECOLOGY

Andrew Thompson

Professor of Parasitology in the School of Veterinary and Life Sciences, Murdoch University, Western Australia



Giardia and *Cryptosporidium* are ubiquitous enteric protozoan pathogens of vertebrates. Although recognised as the aetiological agents of disease in humans and domestic animals for many years, fundamental questions concerning their ecology have been unresolved. Molecular tools have helped to better understand their genetic diversity and in so doing have helped to resolve questions about their transmission patterns and associated impacts on public health. However, taxonomic issues have, until recently, made it difficult to determine the epidemiology of infections with both *Giardia* and *Cryptosporidium*. Similarly, improved understanding of their respective phylogenetic relationships has helped to resolve questions about zoonotic potential and to improve opportunities for the development of new anti-cryptosporidial drugs. In the case of *Cryptosporidium*, imaging technologies have complemented phylogenetic studies and have further supported its extracellular developmental capability and potential role as an environmental pathogen. These new developments will be discussed.

ARE ANIMALS CONSIDERED AS ZONOTIC RISKS OF INTESTINAL PROTOZOA? PART 1. WILDLIFE AND LIVESTOCK

Aongart Mahittikorn

Department of Protozoology, Faculty of Tropical Medicine, Mahidol University



Host specificity of the pathogens and habitat of the animals are two major key components for the transmission of zoonotic pathogens. We conducted a series of surveillances in central and western Thailand focusing on intestinal protozoa in humans and animals. Fecal samples of humans and a variety of animals (Companion, domestic animals and wildlife) were collected. Pathogenic intestinal protozoa, including *Cryptosporidium*, *Giardia*, *Enterocytozoon*, and *Blastocystis* were investigated. The prevalence and host specificity of the organisms were determined by PCR, direct sequencing, and phylogenetic analysis. Investigated animals were classified by their characteristics and habitats; the results were compared with previous molecular epidemiological surveys around the world. We found non-human primates as high risk animals for transmitting various intestinal protozoa in both wildlife and community settings. Our surveillances will be reported by dividing into 2 parts: Part 1. Wildlife and livestock, and Part 2. Companion animals and monkey living in town.

Keywords: Wildlife, livestock, *Cryptosporidium*, *Giardia*, *Enterocytozoon*, *Blastocystis*

ARE ANIMALS CONSIDERED AS ZONOTIC RISKS OF INTESTINAL PROTOZOA? PART 2: COMPANION ANIMALS AND MONKEYS LIVING IN TOWN

Hirotake Mori

Medical Board of Internal Medicine (Japan)



Host specificity of the pathogens and habitat of the animals are two major key components for the transmission of zoonotic pathogens. We conducted a series of surveillances in central and western Thailand focusing on intestinal protozoa in humans and animals. Fecal samples of humans and a variety of animals (Companion, domestic animals and wildlife) were collected. Pathogenic intestinal protozoa, including *Cryptosporidium*, *Giardia*, *Enterocytozoon*, and *Blastocystis* were investigated. The prevalence and host specificity of the organisms were determined by PCR, direct sequencing, and phylogenetic analysis. Investigated animals were classified by their characteristics and habitats; the results were compared with previous molecular epidemiological surveys around the world. We found non-human primates as high risk animals for transmitting various intestinal protozoa in both wildlife and community settings. Our surveillances will be reported by dividing into 2 parts: Part 1. Wildlife and livestock, and Part 2. Companion animals and monkey living in town.

Keywords: Wildlife, livestock, *Cryptosporidium*, *Giardia*, *Enterocytozoon*, *Blastocystis*

SUBTYPE DISTRIBUTION OF *BLASTOCYSTIS* IN HUMANS

Supaluk Popruk

Department of Protozoology, Faculty of Tropical Medicine, Mahidol University



Blastocystis is an intestinal protozoan parasite commonly found in humans and animal hosts. The majority of *Blastocystis* infections are transmitted by the fecal-oral route and causes blastocystosis. Several techniques are used to examine *Blastocystis* infections, such as microscopy, culture and molecular methods. Based on the characterization of the small-subunit ribosomal RNA (ssrRNA) gene by molecular studies, at least 17 subtypes (STs) of *Blastocystis* have been identified. ST1-ST9 have been found in humans, and ST1-ST4 are the most common subtypes. All STs (ST1-ST17) have also been found in various animals hosts such as birds, pigs, monkeys, and cattle, and may be linked to zoonotic transmission. Several molecular epidemiological studies of *Blastocystis* infections reveal that differences in subtype distribution of *Blastocystis* vary depending on reservoir hosts and geographical areas. Non-specificity and the variety of symptoms of *Blastocystis* infection have led to a lack of understanding of its potential pathogenicity. Therefore, pathogenicity of this parasite remains unclear and needs further studies for better understanding.

Keywords: *Blastocystis*, humans, subtypes

Friday 13th December 2013

Time: 09:00- 10:30

Room B

S30 Panel Discussion on *P. vivax* Challenge Model: Risks VS Benefits for Vaccines and Drugs Development

Chairperson 1



John Adams

Chairperson 2



Pratap Singhasivanon

Session Description:

The panel will discuss different model available for evaluation of vaccines or drugs for Plasmodium vivax. Because of the difficulty to culture *P. vivax in vitro*, the subsequent studies that required live parasites will have to depend on parasite isolates from patients, thus clinical evaluation of vaccine candidate or new compound efficacy is a challenge.

1. The challenge of *P. vivax* sporozoite challenge model
Jetsumon Prachumsri
2. *P. vivax* Blood stage human challenge model: new tools for vaccine and drug development
James McCarthy
3. Animal VS human model: Benefit to vaccine/drug development: will it worth to take risk?
Arturo Reyes-Sandoval
4. Consideration of Benefit and Risk of *P. vivax* challenge model
Yupaporn Wattanagoon

THE CHALLENGE OF *P. VIVAX* SPOROZOITE CHALLENGE MODEL

Jetsumon Prachumsri

Faculty of Tropical Medicine, Mahidol University



P. VIVAX BLOOD STAGE HUMAN CHALLENGE MODEL: NEW TOOLS FOR VACCINE AND DRUG DEVELOPMENT

James McCarthy

Queensland Institute of Medical Research, University of Queensland, Australia



ANIMAL VS HUMAN MODEL: BENEFIT TO VACCINE/DRUG DEVELOPMENT: WILL IT WORTH TO TAKE RISK?

Arturo Reyes-Sandoval

Uniservsity of Oxford



CONSIDERATION OF BENEFIT AND RISK OF *P. VIVAX* CHALLENGE MODEL

Yupaporn Wattanagoon

Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University



(Abstracts are not available for this panel discussion)

Friday 13th December 2013

Time: 09:00 – 10:30

Room C

S31 Aging

Chairperson



Yaowalark Sukthana

1. Why population aging matters
Worawet Suwanrada
2. Elderly and their families in Thailand: past, present and future
Pramote Prasartkul
3. Quality of aging and death
Chammongsri (Rutnin) Hanchanlash

WHY POPULATION AGING MATTERS

Worawet Suwanrada

Dean, College of Population Studies and Associate Professor, Faculty of Economics, Chulalongkorn University



Thai society has been apparently facing demographic change during last 4 decades. Thai women have fewer children in average. At the same time, Thai people live longer. After the start of the voluntary family planning based population policy in the former half of 1970s until now, the level of fertility has kept on decreasing. According to the Survey of Population Change in the past conducted by Thailand's National Statistical Office, the total fertility rate (TFR) decreased from 4.9 in 1974-76, 2.7 in 1985-86, 2.0 in 1995-1996 to 1.7 in 2005-06. The office of the National Economic and Social Development Board (NESDB) has estimated that, TFR will continue dropping to 1.5 in 2021. Life expectancy of both Thai male and female has been increasing. In case of male, life expectancy at birth has increased from approximately 63.8 in 1974-76 to 68.9 in 1985-86, 77.6 in 2005-06. On the other hand, female's life expectancy at birth has also increased from 58.0 in 1974-76 to 63.8 in 1985-86, 69.9 in 2005-06. The NESDB has estimated that, life expectancy at birth of female will increase to 77.7 and 79.4, and that of male will increase to 70.6 and 72.8 in 2011 and 2021 respectively.

As a result of fertility decline and increase of life expectancy, Thai society has stepped into the era of aging society. The number and proportion of older population (generally defined as the population age 60 and over) continue increasing apparently after 1980. According to the National Population and Housing Census conducted by the National Statistical Office, from 1970, the proportion of older population has

increased from 4.9% in 1970, 5.5% in 1980, 7.4% in 1990, 9.5% in 2000 to 13.2% in 2010. From the NESDB population projection, the proportion of the older population will keep on increasing to 18.4% in 2020, 25.9% in 2030 and 32.1 in 2040. Such population aging is matter to Thai society because it has caused both country-wide social and economic impacts. My presentation will review existing data and literatures to explore how Thai society and economy have been and will be affected by population aging.

ELDERLY AND THEIR FAMILIES IN THAILAND: PAST, PRESENT AND FUTURE

Pramote Prasartkul

Institute for Population and Social Research, Mahidol University



Pramote Prasartkul, Ph.D.

The population of Thailand has been aging dramatically since the last decade due to the rapid decline of fertility and longer life expectancy. It is projected that Thailand will become complete aged society in about 10 years and be super-aged society with population 65 years and over reached 20% in 2021. The changes in age structure have affected to the types of living arrangement in families. The size of Thai families is getting smaller and transforming from extended to nuclear family. Some related factors such as the change in marital pattern, declining fertility and value and attitude toward independent life cause the increasing trend of elderly living alone and/or by themselves. The long-term care among elderly living alone will be one of the most concerned in the Thai aging society in the future. The roles of family care may be decreasing while those of community and institutional cares will increase.

QUALITY OF AGING AND DEATH

Khunying Chamnongsri

Rutnin Eye Hospital



Aging and Dying are an integral part of life. The quality of aging and dying, includes aspects of the physical, social, mental and spiritual aspects. This presentation will focus on spiritual aspects based on the Buddhist views of the universal condition of all things is the foundation for quality of aging and quality of dying.

Friday 13th December 2013

Time: 09:00 – 10:30

Room D

S32 Bacterial Infection

Chairperson 1



Wirongrong Chierakul

Chairperson 2



Daniel Paris

1. Overview of *Leptospira* spp. surveillance in environment
Janjira Thaipadungpanit
2. Unresolved issues related to improving the diagnosis of tropical rickettsial illnesses
Daniel Paris
3. Cell Biology of *Orientia tsutsugamushi*
Jeanne Salje
4. Human immune response to bacteria and implications for vaccine design
Susanna Dunachie
5. Vaccine for melioidosis, would it be applicable for Thai people?
Direk Limmathurotsakul

OVERVIEW OF *LEPTOSPIRA* SPP. SURVEILLANCE IN ENVIRONMENT

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Leptospirosis is an acute febrile illness caused by pathogenic bacteria in genus *Leptospira*. It is a zoonotic disease with a worldwide distribution but most common in tropical and subtropical regions. The bacteria, which commonly colonize the kidney of infected animals or carrier animals, spread into the environment via excreted urine. Human leptospirosis mainly results from indirect contact with contaminated environmental water. Outdoor activities such as rice farming and daily activities in bad sanitary conditions such as living in flooded areas or in urban slums are related to the risk of getting human leptospirosis. The global

burden of leptospirosis is unknown as lacking the true incidence in many countries.

Surveillance data of pathogenic *Leptospira* species in reservoir animals or infected animals is essential for tracking sources of human infections, and for designing of appropriate interventions targeted towards decreasing the burden of leptospirosis. However, abilities of surviving in environment among the pathogenic *Leptospira* species in the genus are different. Therefore, *Leptospira* species or strains dominating a rodent population might not represent a major cause of human infections as they might not survive for long in the environment. Comparisons of surveillance data from *Leptospira* spp. from the environment should include surveillance of reservoir animals as well. Little is known about presence and molecular epidemiology of *Leptospira* in environment mainly due to the difficulty of isolating and genotyping *Leptospira*. Surveillance of *Leptospira* species in environment from several tropical countries will be summarized with a focus on the methodology of bacterial isolation and molecular genotyping methodologies.

Key words: *Leptospira*, Leptospirosis, environment

UNRESOLVED ISSUES RELATED TO IMPROVING THE DIAGNOSIS OF TROPICAL RICKETTSIAL ILLNESSES

Daniel H. Paris, Piyanate Sunyakumthorn, Eric Lombardini, Susanna J. Dunachie, Jeanne Salje, Stuart D. Blacksell and Nicholas P.J. Day

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Scrub typhus and murine typhus are rickettsial illnesses that represent a major cause of acute undifferentiated febrile illness in Southeast Asia. The current understanding of the immunopathophysiology and host-pathogen interactions of these diseases is very limited. Investigations into these aspects are important, as data from both pathogen and host are required to support the advancement of diagnostic development, which represents a central pillar of research for any treatable or preventable infectious disease.

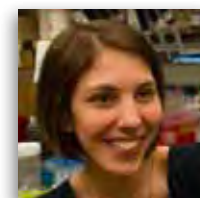
The current knowledge gaps regarding rickettsial illnesses in the tropics revolve around the development of improved diagnostics and preventive measures, such as vaccine design. The current “gold standard” diagnostic remains serology, specifically the indirect immune-fluorescent assay, which is very suboptimal. Characterizing pathogen antigens for their capacity as diagnostic targets, defining the antigen-specific immune responses they elicit, and their roles in bacterial immune-modulation at the cellular level will provide valuable data towards improving pathogen detection and diagnosis in patients with febrile illness.

However, the quest for defining targetable antigens during human disease and the roles of antigens in the natural human immune response is no minor task and requires better understanding of the bacterial dissemination dynamics as well as the cellular tropism of *Orientia tsutsugamushi* and *Rickettsia typhi*. In order to improve diagnostic and vaccine development for these easily treatable diseases, a concerted research effort into the immune-pathology, cell biology and clinical pathophysiology is required. We summarize current standings and highlight unresolved issues.

CELL BIOLOGY OF ORIENTIA TSUTSUGAMUSHI

Jeanne Salje

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Abstract Not Available

HUMAN IMMUNE RESPONSE TO BACTERIA AND IMPLICATIONS FOR VACCINE DESIGN

Susanna Dunachie

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Vaccines for intracellular pathogens are difficult to make and are likely to require induction of a cellular immune response. Effective vaccine design needs an understanding of how people's immune systems react to the pathogen in natural infection. *Burkholderia pseudomallei*, the bacterial of melioidosis, is a Tier 1 select agent and a vaccine is highly desirable. Ubon Ratchathani in North-east Thailand has the highest reported rate of melioidosis in the world, and local researchers have over 30 years' experience in studying the disease.

This presentation will present the latest findings of an observational study of humeral and T-cell responses to melioidosis. To date a cohort of over 80 patients acutely unwell with culture-confirmed melioidosis have been enrolled and are being followed up 12 and 52 weeks later. Their responses were compared to those from healthy subjects in the region with and without diabetes, the major risk factor for melioidosis.

Fresh *ex vivo* interferon-gamma ELISPOT responses to heat inactivated whole *B. pseudomallei* were found for patients in the Melioid Cohort, with a significant difference between the Melioid Cohorts and the Diabetes or the Healthy Cohorts (by 2-tailed Mann-Whitney). For the Melioid Cohort, responses were lower in diabetics compared to non-diabetics, and in the 15 patients who died from disease compared to survivors.

The results demonstrate T-cell activity in the natural response to *B. pseudomallei* infection, and provide a foundation for epitope mapping and immunogenicity monitoring in future clinical trials of candidate vaccines.

Keywords: melioidosis, immunogenicity, T-cell, ELISPOT, vaccine, epitope

VACCINE FOR MELIOIDOSIS, WOULD IT BE APPLICABLE FOR THAI PEOPLE?

Direk Limmathurotsakul

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Direk Limmathurotsakul,^{1,2*} Sharon J Peacock,^{2,3} Yoel Lubell,² Gavin C. K. W. Koh,^{2,3} Lisa White,^{2,4} Nicholas PJ Day^{2,4} and Richard W. Titball⁵

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Background: *Burkholderia pseudomallei* is a Category B select agent and the cause of melioidosis. Research funding for vaccine development has largely considered protection within the biothreat context, but the resulting vaccines could be applicable to populations who are at risk of naturally acquired melioidosis. Here, we discuss target populations for vaccination, consider the cost-benefit of different vaccination strategies and review potential vaccine candidates.

Methods and Findings: Melioidosis is highly endemic in Thailand and northern Australia, where a biodefense vaccine might be adopted for public health purposes. A cost-effectiveness analysis model was developed, which showed that a vaccine could be a cost-effective intervention in Thailand, particularly if used in high-risk populations such as diabetics. Cost-effectiveness was observed in a model in which only partial immunity was assumed. The review systematically summarized all melioidosis vaccine candidates and studies in animal models that had evaluated their protectiveness. Possible candidates included live attenuated, whole cell killed, sub-unit, plasmid DNA and dendritic cell vaccines. Live attenuated vaccines were not considered favorably because of possible reversion to virulence and hypothetical risk of latent infection, while the other candidates need further development and evaluation. Melioidosis is acquired by skin inoculation, inhalation and ingestion, but routes of animal inoculation in most published studies to date do not reflect all of this. We found a lack of studies using diabetic models, which will be central to any evaluation of a melioidosis vaccine for natural infection since diabetes is the most important risk factor.

Conclusion: Vaccines could represent one strand of a public health initiative to reduce the global incidence of melioidosis.

Friday 13th December 2013

Time: 09:00 – 10:30
Room E

S33 Malaria: Country Experiences

Chairperson



Srivicha Krudsood

1. Malaria Consortium's experiences on mHealth in Cambodia
Pengby Ngor
2. Lessons Learned from Village Malaria Workers in Western Cambodia: from malaria control to containment of artemisinin resistance
Sara Elena Canavati de la Torre
3. Malaria Prevalence and Treatment-Seeking Behaviors in an Area of Low Transmission in Nigeria
Kehi H Nwaka
4. High Risk of *Plasmodium Falciparum* Infection Among Jhum Cultivators in The Chittagong Hill Districts of Bangladesh
Wasif Khan

MALARIA CONSORTIUM'S EXPERIENCES ON MHEALTH IN CAMBODIA

Pengby Ngor
Malaria Consortium Cambodia



Pengby Ngor; Mellor, Steve; Sovannaroth Siv; Suy, Channe; Poly, and Roca-Feltrer, Arantxa

Malaria Consortium Cambodia, #113 (6th Floor of Parkway Square), Mao Tse Toung Blvd., Chamcar Morn, Phnom Penh, Cambodia

As the malaria programme moves towards elimination phase there is a need to react promptly to every *Plasmodium falciparum* (Pf) case (and ultimately every *Plasmodium vivax* case) and not wait for information to flow through the routine system. Building upon the success of the SMS model of reporting cases in the day 3 positive system (a real time reporting system to rapidly identify potential artemisinin resistant parasites) Day 0 alert system was designed to support the move towards malaria elimination in Pailin Province.

The system was designed by Malaria Consortium and CNM staff in collaboration with InSTEDD and required a web-based system to cope with large volume of SMS per day. A partnership was established with Mobitel (Cambodia's largest telecommunications company) which provided free SIM cards and free SMS making the system extremely cost effective and easy to maintain..

The system is being implementing in Pailin province since July 2012 covering 116 VMWs and 6 health centres to report all cases of Pf. Simple SMS are sent by Village Malaria Workers (4 digits) and Health Center staff (12 digits) for Day 0 cases and the software automatically alerts appropriate district officials (based on the location of the village) to take action. This presentation will focus on describing the lessons learned from during the design and implementation phases and will provide recommendations on how it can be further improved for a potential full scale up in the country.

Keywords: Malaria, Day 3 positive System, Day 0 Alert System.

LESSONS LEARNED FROM VILLAGE MALARIA WORKERS IN WESTERN CAMBODIA: FROM MALARIA CONTROL TO CONTAINMENT OF ARTEMISININ RESISTANCE

Sara Elena Canavati de la Torre
Faculty of Tropical Medicine, Mahidol University



Sara E. Canavati de la Torre^{ab}, Chea Nguon^b, Po Ly^b, Arantxa Roca-Feltrer^c, Saranath Lawpoolsri^a, David Sintasath^d, Sasithon Pukrittayakamee^{oe}, Pratap Singhasivanon^e, Maxine Whittaker^f

^a Faculty of Tropical Medicine, Mahidol University, Thailand; ^b The National Centre for Parasitology, Entomology and Malaria Control, CNM, Cambodia; ^c Malaria Consortium Cambodia, Cambodia; ^d Malaria Consortium Asia, Thailand; ^e Mahidol-Oxford Research Unit, Thailand; ^f University of Queensland, Australia

Introduction: Village/ Mobile Malaria Workers (VMWs/MMWs) are a critical component in Cambodia's national strategy for malaria elimination by 2025. The VMW project has recently expanded considerably to provide a crucial surveillance function for Cambodia's artemisinin resistance containment effort. As a consequence it now covers 1,523 villages with a population of approximately one million.

This study aimed to understand if VMW/MMWs job performance and satisfaction had changed as low malaria prevalence levels have been achieved. It also sought their and the communities perspectives on their work and communities needs when malaria levels are low.

Methods: A mixed methods study was conducted in six provinces of Western Cambodia in the artemisinin resistance containment zones 1&2. A total of 498 semi structured interviews, 60 FGDs (416 participants) and 65 IDIs were conducted through October 2011 till January 2012, including VMWs/MMWs, public health facility staff, village chiefs, villagers, migrants and malaria patients.

Results: This study found that the community and workers felt that performance of services by the VMWs was high. However both the VMWs and the communities desire a broader range of services being provided by the VMWs as the malaria burden has declined significantly but there are still a lot of health needs for these communities who have poor access to routine services. VMWs perceive their role in their communities quite limited and their motivation for work diminishing. Re-engaging them in surveillance activities could service as a motivational factor. Other challenges to performance and motivation discussed were systems issues such as poor supply chain, need for further training and supervision. The implications of expanding their roles should be considered, especially in terms of resource allocation, training and monitoring and evaluation.

Discussion: Surveillance at the community level is now being piloted in Pailin province through the D0 system and as active case detection and treatment activities. At the official border point VMWs are being deployed to screen asymptomatic crossers. In unofficial border points, VMWs are also screening asymptomatic crossers. The VMW role in these pilot projects needs to be evaluated in order to re-direct the role of VMWs from malaria control to malaria elimination. Operational research on the expanding the role of VMWs to Integrated Vector Surveillance (IVS)—that is malaria and other infectious diseases (especially dengue) could result in a cost-effective intervention in a malaria elimination setting. Further research is needed to understand the role of VMWs as surveillance agents in their communities especially in moving from intensified control to elimination (from passive surveillance to active surveillance).

Keywords: malaria elimination, active surveillance, village malaria workers, Western Cambodia, artemisinin resistance

MALARIA PREVALENCE AND TREATMENT-SEEKING BEHAVIORS IN AN AREA OF LOW TRANSMISSION IN NIGERIA

Kehi H Nwaka

Department of Animal and Environmental Biology, Delta State University



Kehi H Nwaka¹, Judith Nmor², and Jephtha C Nmor^{1*}

¹ Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria

² Department of Nutrition Sciences, Siebold University, Nagasaki, Japan

A study of the prevalence of malaria was carried out in Ibusa, rural community in Oshimili North Local Government Area of Delta State, Nigeria. Thick blood films of 600 individuals attending the Fenlab Diagnostic and Research Laboratory Ibusa were used to determine the prevalence. Structured questionnaire were also administered to 600 individuals in order to obtain information on malaria management practices of the people. The study was carried out between the months of June and November, 2006 which corresponds to the wet season in Nigeria. The prevalence rate was found to be 73.3% and all were infections of *Plasmodium falciparum*. The age prevalence though showed a gradual increase with age followed by a slight decrease in age group 30 and above, the prevalence was highest among subjects of age group 20 – 29 years (31.81%) and least in age group 40-49 years (8.64%). Chi-square analysis showed no significant difference ($P>0.05$). The prevalence by sex showed higher prevalence in females (60.22%) than male (39.78%), this difference was not statistically significant at ($P>0.05$). The malaria management practices showed that (20.83%) buy antimalarials from shop (17.33%) visits hospitals, while (16.33%) employ traditional medicine from local healers. However, some of the subjects employ a combination of approach. (13.83%) get antimalaria from shops, hospital and local healers, (10.50%) get antimalarials from shops and hospitals, (9.67%) visits local healers and hospitals, while (0.33%) don't employ any management practices. Proper education of the people on the need to employ proper malaria management practice is essential.

Keywords: Malaria, Prevalence, Treatment, Seeking-behaviors, Nigeria, Africa.

HIGH RISK OF *PLASMODIUM FALCIPARUM* INFECTION AMONG JHUM CULTIVATORS IN THE CHITTAGONG HILL DISTRICTS OF BANGLADESH

Wasif Khan

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh



Wasif A. Khan¹, Sean R. Galagan², Chai S. Prue¹, Jacob Khyang¹, Sabeena Ahmed¹, Malathi Ram², Mohammad S. Alam¹, Mohammed Z Haq¹, Jasmin Akter¹, Peter K. Streatfield¹, Gregory Glass², Douglas E. Norris², Myaing M. Nyunt², Timothy Shields², David J. Sullivan², David A. Sack²

¹ International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

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Malaria is endemic in the Chittagong Hill Districts of Southeastern Bangladesh. Previous epidemiological analyses identified the agricultural practice of “jhum cultivation” as a potential risk factor for malaria infection. We conducted qualitative interviews with jhum cultivators and surveillance workers to describe jhum cultivation and used demographic and malaria surveillance in two study unions from May 2010 to August 2012 to determine the relationship between jhum cultivation and malaria infection. Qualitative interviews revealed jhum cultivation is conducted on remote, steep hillsides by ethnic tribal groups. Quantitative analyses found that adult jhum cultivators had significantly higher incidence rates of symptomatic *P. falciparum* infection than non-cultivators (1.27 compared to 0.55 infections per 1,000 per month). These results suggest jhum cultivation is a risk factor for malaria infection and underscore the need for malaria testing and treatment services to reach remote populations in the Chittagong Hill Districts.

Keywords: Jhum cultivation; malaria; *Plasmodium falciparum*; occupational health; Bangladesh; agriculture; epidemiology; Bandarban

Friday 13th December 2013

Time: 11:00 – 12:30

Room A

S34 Health Informatics: Spatial-Temporal Modeling for Tropical Diseases in the Asian Region

Chairperson 1



Jaranit Kaewkungwal

Chairperson 2



Wirichada Pan-ngam

1. Spatio-temporal pattern of leptospirosis in Thailand: is flooding a risk factor?
Sarin Suwanpakdee
2. Application of Remote Sensing Technology for the Classification of Malaria Risk Areas in a Thai-Myanmar border Province
Chotipa Kulrat
3. Spatially explicit transmission dynamic models for malaria elimination in the Greater Mekong Sub-Region
Lisa White
4. Strengthening control of neglected tropical diseases in the Asia-pacific: implications for health information system priorities and strategies
Maxine Whittaker

SPATIO-TEMPORAL PATTERN OF LEPTOSPIROSIS IN THAILAND: IS FLOODING A RISK FACTOR?

Sarin Suwanpakdee

Faculty of Tropical Medicine, Mahidol University



Sarin Suwanpakdee¹, Jaranit Kaewkungwal², Lisa White³, Norberto Asensio⁴, Parntep Ratanakorn¹, Pratap Singhasivanon², Wirichada Pan-ngum^{2,3}

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⁴ Faculty of Environment and Resource Studies, Mahidol University, Thailand.

Background: Flooding is believed to have significant influence over incidence of leptospirosis, mainly by increasing human and reservoir animal contacts. Since Thailand has experienced many severe flooding events in the past few years, concerns with post-flooding leptospirosis outbreaks have been raised. Consequently leptospirosis has been closely monitored in the disease surveillance system, Bureau of Epidemiology, Ministry of Public Health, Thailand. We study the temporal and spatial pattern of leptospirosis in Thailand and further determine the association between the disease incidence, flooding and some related environmental factors.

Materials and methods: Flooding data during 2010 and 2012 were extracted from satellite images available from Geo-Informatics and Space Technology Development Agency (GISTDA). Incidence Rate Ratio (IRR) estimated from the negative binomial regression model was used to determine the influence of flooding on the number of human leptospirosis cases reported to the Bureau of Epidemiology spatially and temporally. In addition, size of flooding coverage, duration of water logging, time lags between flooding, and some environmental factors including number of possible animal reservoirs were also considered in the sub-analysis.

Results: Occurrence of flooding varied in magnitude and coverage during the study period. The influence of flooding on the incidence of leptospirosis varied significantly by year and region. Leptospirosis tends to occur repeatedly in the same areas irrespective of flooding, particularly in the northeastern part of Thailand. Neither the size of flooding coverage area, duration of water logging nor the lag times of flooding and disease occurrence were significantly associated with the incidence. The number of buffaloes may be associated with the rising of incidence.

Discussion: Our analysis suggests that flooding plays less of a role in leptospirosis transmission than previously thought. In fact, direction and magnitude of the association between flooding and incidence varied in space and time. Occurrence of cases may be explained by various factors such as rice farm activities which take place mostly during the rainy season. In addition, we found that the number of buffalo in an area was associated with the incidence of leptospirosis reported in that area throughout the study period. This highlights buffalo may be a potentially significant animal reservoir in Thailand. Some genetic studies suggest that serovars of *Leptospira* found in Thai buffalo have a strong link with the severe form of human leptospirosis.

Keywords: Leptospirosis, Flooding, environment, Thailand

APPLICATION OF REMOTE SENSING TECHNOLOGY FOR THE CLASSIFICATION OF MALARIA RISK AREAS IN A THAI-MYANMAR BORDER PROVINCE

Chotipa Kulrat

Faculty of Tropical Medicine, Mahidol University



Chotipa Kulrat, Surapon Yimsamran, Patiwat Sa-Angchai and Natefa Rukmanee

Malaria remains an important health threads in the border areas of Thailand to neighboring countries. This study aimed to model malaria transmission in the endemic area of SuanPhung district, Ratchaburi province which borders to Myanmar. Malaria data, as the outcome variable, were selected from The Vector-Borne Disease Control Center 4.4, Ratchaburi Province, during January and May of every year from 2006 to 2010. The predictor variables used in the model were environmental and meteorological factors. Environmental data i.e., vegetation index and land surface temperature, were extracted from the Terra MODIS satellite images that pertaining to the study areas in the corresponding time periods. Meteorological data mainly used in the model were relative humidity and precipitation. The relative humidity data were also extracted from satellite images while precipitation data were collected from seven meteorological stations in the study area. We modeled the data from 2006-2009 to get the coefficients of these predictors, while the 2010 data were used to validate the accuracy of models. This study was initiated to test how useful of remotely-sensed data for modeling of malaria transmission in order to use in the further sophisticated models.

Keywords: Remote sensing, LST, MODIS, NDVI

SPATIALLY EXPLICIT TRANSMISSION DYNAMIC MODELS FOR MALARIA ELIMINATION IN THE GREATER MEKONG SUB-REGION

Lisa White

Mahidol-Oxford Tropical Research Unit, Faculty of Tropical Medicine, Mahidol University



After a decade of gains in malaria control, elimination is a feasible aim in many endemic areas. This is not only a laudable aim, but also a pressing necessity as failure to eliminate will result in the continual and inevitable loss of our current control resources to drug and insecticide resistance in a future where malaria continues to inflict human and economic losses. There is no “one size fits all” intervention for *P. falciparum* elimination due to the spectrum of available sub-optimal interventions acting at different stages of the parasite life-cycle and the heterogeneous transmission landscape. The answer to the question “which intervention is best?” will always be different for every country and from every perspective (e.g. front-line medicine, public-health, health economics, drug-resistance, etc.). Every district of every country has its own unique challenges, conditions and solutions as do cross-country border areas and high risk populations such as migrants and other marginalised communities. A paradigm shift in the way that interventions are considered is required to move away from the search for the “Holy Grail” single intervention and towards a practical marshalling and harmonisation of all the resources available for the efficient and timely solution to the problem. Mathematical modeling is the best available tool for combining the many interacting factors that must be considered in the design of optimal elimination strategies. Put simply, a model is a simplified description of a system or process used to aid understanding. A mathematical model uses equations to achieve this goal. The more familiar statistical models are used to describe observed data and trends. Mathematical models differ from statistical models in that they include knowledge about the underlying biology of the system and can therefore not only describe the observed data and trends but also predict the most likely outcome of intervening and changing aspects of the system which is precisely what happens during elimination programs. The role of mathematical models in the design of cost-effective malaria elimination strategy in the Greater Mekong Sub-Region will be discussed.

STRENGTHENING CONTROL OF NEGLECTED TROPICAL DISEASES IN THE ASIA-PACIFIC: IMPLICATIONS FOR HEALTH INFORMATION SYSTEM PRIORITIES AND STRATEGIES

Maxine Whittaker

Australian Centre for International and Tropical Health, School of Population Health, The University of Queensland



Maxine Whittaker,¹ Robert Bergquist² and Michael Buttsworth¹

Over two billion people in the world are affected by infections collectively known as the neglected tropical diseases (NTDs): tropical because of their location; and neglected because of the insufficient interest and funding allocated to them. A common denominator for being at risk for NTDs is poverty.

Health information systems (HIS) play a pivotal role in disease control by providing evidence for decisions and interventions. Without HIS to inform decision-makers of where the health problems are, and if the health of the population is improving or getting worse, sound judgements cannot be made. To gain control over NTDs, tools of recognition and discovery, such as diagnostics, geospatial sciences and health metrics, must be used and adapted to work with stronger surveillance and early warning systems. Progress also needs to be linked to cross-cutting issues, such as socio-economic status and climate change. Health information for NTD control will require links to such datasets.

Based upon an expert workshop and extensive literature reviews, this presentation will discuss several, often coinciding, reasons why NTD indicator data are poorly collected, leading to significant underestimations of burden. Emerging tools to support health information processes, including spatial epidemiology, decision-support systems, and methods and tools to quantify hazard exposure and health impacts due to global changes will also be discussed. Finally, the implications for HIS development will be detailed using the Health Metrics Network framework.

Friday 13th December 2013

Time: 11:00 – 12:30
Room B

S35 Host-Pathogen Interactions: specific interactions that regulate malaria parasite infections

Chairperson 1



John Adams

Chairperson 2



Wang Nguitragool

1. The heparin-binding proteome of *Plasmodium falciparum*
Qijun Chen
2. A panel of *Plasmodium vivax* merozoite ligands
Julian Rayner
3. Invasion inhibition assay of *Plasmodium vivax*; Tool for vaccine screening
Wanlapa Roobsoong

THE HEPARIN-BINDING PROTEOME OF *PLASMODIUM FALCIPARUM*

Qijun Chen

Chinese Academy of Medical Sciences and Key Laboratory of Zoonosis, Jilin University, Changchun, China



P. falciparum merozoites invade and multiply within host erythrocytes during the blood-stage of their life cycle. The prerequisite for host invasion of the parasites is the attachment of the parasite ligands to the host cell receptors. Heparin-like molecules, such as heparin sulfate, are distributed on RBC surface, which have been regarded as important receptors for invasion. Heparin and its derivatives can block the invasion of the *P. falciparum* merozoites into host cells, albeit the parasite ligands in the host-parasite interplay as well as the molecular mechanisms remain poorly understood.

Here, we presented a new approach by combination of receptor-based purification with high-throughput proteomic identification for a systematic and comprehensive research of *P. falciparum* merozoite-derived heparin-binding proteome. In the functional categorization of the heparin-binding proteome, 47 *P. falciparum*-derived proteins with highest affinity to heparin were found to be associated with the cell invasion organelle, the rhoptry, which is a unique structure, contains several critical cell adhesins being functionalized in forming the junction between the invading parasite and host cell. Our study proposed that heparin can interact with multiple *P. falciparum*-derived ligands and consequently has a profound inhibitory effect on cell invasion compared to other GAGs. The data not only revealed the mechanism of heparin inhibition on *P. falciparum* invasion, but also further supports the exploration of heparin derivatives as anti-parasite drugs.

Keywords: malaria, *Plasmodium falciparum*, invasion, heparin

A PANEL OF *PLASMODIUM VIVAX* MEROZOITE LIGANDS

Julian Rayner

Wellcome Trust Sanger Institute

Jessica Hostetler^{1,2}, Gavin Wright², Rick Fairhurst¹, Julian Rayner²¹ National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States² Wellcome Trust Sanger Institute, Hinxton, United Kingdom

A vaccine targeting the illness-inducing blood stage of parasite development is an essential component of any worldwide malaria eradication campaign, but major gaps in our understanding of *P. vivax* biology, including critical events during reticulocyte invasion, hinder the search for a universal vaccine. Currently, only a single receptor-ligand interaction is known, that between *P. vivax* Duffy Binding Protein (PvDBP) and its erythrocyte receptor, DARC, and strain-specific immune responses to PvDBP make it a challenging vaccine target. At the Wellcome Trust Sanger Institute we have expressed a library of recombinant cell surface and secreted *P. falciparum* merozoite proteins in a mammalian cell expression system (Crosnier *et al.*, Mol Cell Proteomics 2013). More than 50 full-length *P. falciparum* ectodomains of up to 200kDa have been successfully expressed by optimizing codon usage, removing predicted N-linked glycosylation sites and exchanging the *Plasmodium* signal sequence for a mouse Ig light chain signal sequence. These *P. falciparum* proteins have been useful for studying known merozoite-erythrocyte interactions (Wanaguru *et al.*, J Biol Chem. 2013) as well as identifying new ones (Crosnier *et al.*, Nature 2011; Bartholdson *et al.*, PLoS Pathogens 2012), and have clear applicability to vaccine and immunoepidemiology studies. We are now applying the same approaches to carry out a comprehensive study of *P. vivax* ligands that may mediate *P. vivax* reticulocyte binding and invasion, in order to identify additional non-PvDBP vaccine candidates. As a first step, we generated a library of full-length *P. vivax* proteins to test for erythrocyte binding and immunoreactivity. We mined existing *P. vivax* microarray data and homology comparisons with *P. falciparum* to produce a list of 38 candidate merozoite proteins, the majority of which are predicted to localize at the merozoite surface, micronemes or rhoptries. More than 30 proteins were expressed at usable levels ($\sim >0.1 \mu\text{g}/\text{mL}$). Where a function was known or predicted, such as a protein-protein interaction between merozoite surface proteins Pv12 and Pv41, it was confirmed. Pilot sera screens using 21 pairs of acute and convalescent Cambodian patient sera showed that all six antigens tested are recognized by immune sera, but with a range of response levels. Initial *P. vivax* protein expression data, erythrocyte and protein interaction data, and immunoreactivity data will be presented.

INVASION INHIBITION ASSAY OF *PLASMODIUM VIVAX*; TOOL FOR VACCINE SCREENING

Wanlapa Roobsoong

Mahidol Vivax Research Unit, Faculty of Tropical Medicine

Wanlapa Roobsoong, Nattawan Rachaphaew and Jetsumon Sattabongkot

Mahidol Vivax Research Unit, Faculty of Tropical Medicine

Due to the unavailable *in vitro* continuous culture of *Plasmodium vivax*, an *in vitro* vaccine/drug screening test for this parasite has to rely on fresh/cryopreserved primary isolates. Two major factors contribute to the success of the experiments. (i) Parasitemia at the time of admission is always very low ($<0.05\%$) and becomes even lower after reinvasion. This makes it difficult to count the parasite from Giemsa-stained thin smears and to get statistical significant result. (ii) Parasites are normally obtained in mixed stages which are not ideal for determination of the effects of vaccines/antimalarial drugs. Synchronous ring and schizont stage parasites are more suitable for drug and vaccine testing, respectively. Although vaccines and

antimalarial drugs against *P. vivax* have been widely studied, the reproducible and reliable standard protocol for such those experiments has been established. The optimisation of invasion inhibition assay of *P. vivax* is aimed. After optimization of standard invasion assay, the invasion inhibition assay was performed by co-cultured Percoll-purified *P. vivax* schizont-infected erythrocytes (60,000 schizonts/well) with varied percentage of reticulocytes (1%, 4% and 8%) in 96-well plate in the presence of antibody against Duffy Binding Protein (a well known antibody that could inhibit invasion). The culture well with out antibody was used as a non inhibitory control. After 18 h of incubation, ring parasitemia was determine by flow cytometry (SYBR green staining) and counting of ring stage from Giemsa stained thin smears. Percent reduction of ring parasitemia from the culture wells in the presence of antibody compare to the control was determined. This optimized standard invasion inhibition assay will benefit the research on vaccine development of *P. vivax*.

Friday 13th December 2013

Time: 11:00 – 12:30
Room C

S36 Entomological Challenges and Strengths Towards ASEAN Global Health Paradigm

Chairperson 1



Ronald Enrique Morales Vargas

Chairperson 2



Sungsit Sungvornyothin

1. Mosquito Insecticide Resistance: ASEAN status with emphasis in Thailand
Narumon Komalamisra
2. Plant extracts strengths of the department of Medical Entomology: an alimentary supply and bio-insecticide novel alternative
Kaewmala Palakul
3. Unresolved entomological issues of tropical rickettsioses in Asia
Daniel Paris
4. Analyzes of Tick Microbial Populations by Next Generation Sequence Technology
Chihiro Sugimoto

MOSQUITO INSECTICIDE RESISTANCE: ASEAN STATUS WITH EMPHASIS IN THAILAND

Narumon Komalamisra

Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University



Narumon Komalamisra, Raweewan Srisawat, Ronald E Morales Vargas and Theerawat Phanphoo Wong
Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

Asean Economic Community (AEC) will be implemented by 2015. This will enhance infrastructure and communications connectivity, allow travel and trade between these countries to become quicker and easier. Exotic insect vectors can also distribute faster and more distance. Vector-borne diseases are still important health problems in this region. The vector control programs mainly rely on insecticides. Insecticide resistance has been continuing reported that compromised the effectiveness of insecticides used in the control program. Information on insecticide susceptibility status in the Asean countries should be addressed.

Primary malaria vectors in Asean countries are *Anopheles dirus* and *An. minimus*. Bioassays using standard WHO susceptibility test with diagnostic concentrations showed that malaria vector in Cambodia, Myanmar, Laos, Vietnam and Thailand are still susceptible to deltamethrin and permethrin. Except *An. dirus* in central Vietnam showed possible resistance to deltamethrin. *An. epiroticus* is highly resistant to all pyrethroids in the Mekong delta, while resistance to deltamethrin and permethrin of *An. sondaicus* was reported in some areas in Myanmar.

In Thailand *Aedes aegypti* and *Ae. albopictus*, the dengue vectors, are susceptible to malathion. *Ae. aegypti* is highly resistant to permethrin. Decreasing susceptibility to deltamethrin occurred in many sites as well as the sporadic areas of temephos resistance in mosquito larvae.

There is not specific control program for *Culex quinquefasciatus*, the lymphatic filarial vector. However, other vector control programs had impact on the susceptibility of the *Culex* mosquito. Insecticide susceptibility test of 14 localities in 3 provinces showed resistance to all classes of insecticides except malathion.

PLANT EXTRACTS STRENGTHS OF THE DEPARTMENT OF MEDICAL ENTOMOLOGY: AN ALIMENTARY SUPPLY AND BIO-INSECTICIDE NOVEL ALTERNATIVE

Keawmala Palakul

Faculty of Tropical Medicine, Mahidol University



Plants derived insecticides have been studied in the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University since 1987. *Euphobia antiquarum* Linn., *Agave maginata* Linn., *A. americana*, *A. sisalana* and *A. midiopicta* had larvicidal effect on *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles dirus* and *Mansonia bonnea*. *An. dirus* was susceptible to all tested Agave species. *A. americana* was the most promising with LC₅₀ of 0.008% against *An. dirus* and also found that 4% of crude extract of *A. americana* kill larvae stage 100% in 48 hours and persistence effect last for 9 days in small field trial. *Mansonia bonnea* was susceptible to *Eu. antiquarum* with LC₅₀ of 0.04% (ml/l).

Beside larvicides, we found that volatile oil from *Litsea cubeba*, *Citrus hystrix* DC., and *Artemisia annua* Linn. had repellency effect. Formulation as cream and balm gave the repellent product that can repel *Ae. aegypti* 3 hours and 2 and a half hours respectively.

Keywords: Bio-insecticide

UNRESOLVED ENTOMOLOGICAL ISSUES OF TROPICAL RICKETTSIOSES IN ASIA

Daniel Paris

Mahidol-Oxford Tropical Research Unit, Faculty of Tropical Medicine, Mahidol University



Authors: Daniel H. Paris, Nicholas P.J. Day

The tropical rickettsioses scrub typhus (*Orientia tsutsugamushi*) and murine typhus (*Rickettsia typhi*) are important, under recognized and preventable causes of acute febrile illness in Asia.

Unresolved issues include the incomplete understanding of vector species involved in transmission. Leptotrombidium vectors dominate scrub typhus transmission, but the significance of other reported species remains unclear. Rats and fleas (*Xenopsyllacheopis*) are important reservoirs and vectors in murine typhus, however new flea species and mites serving as vectors of *R. typhiraise* questions especially in Asia where scrub and murine typhus are sympatric diseases. The role of rodents as true reservoirs is questionable, as vector co-feeding on rodents seems more relevant for effective host-to-vector transmission, than independent feeding on rickettsaemic hosts.

The role of selective pressure on pathogens from the immune response of intermediate hosts and/or during vertical transmission within vectors in the evolution of antigenic diversity is unclear. This diversity

is a major source of difficulties encountered in the development of diagnostics, strain characterization and identification of vaccine targets. To what extent do non-pathogenic strains maintained and transmitted by vectors contribute to these difficulties? What are the roles of vectorsalivary proteins? Do they exert anti-inflammatory, immunosuppressive, analgesic and anticoagulant effects similar to tick saliva? Investigations of early processes at the bite site pose multiple challenges, as eschar formation, bacterial dissemination and early innate immune recognition can be affected by multiple factors, including bacterial strain and inoculum load, inoculation depth and natural host immune response dynamics.

ANALYZES OF TICK MICROBIAL POPULATIONS BY NEXT GENERATION SEQUENCE TECHNOLOGY

Chihiro Sugimoto

Research Center for Zoonosis Control, Hokkaido University



Tick can transmit a variety of viral, bacterial and protozoan pathogens, which are often zoonotic. The fact that there are up to 9 rickettsial diseases caused by spotted fever Rickettsia newly reported between 1984 and 2001 indicates there are many potential pathogens which have not been unidentified or found in ticks. We are aiming at building an all-encompassing database of tick-borne pathogens to improve preparedness to emerging tick-borne diseases. In this presentation, I illustrate our recent studies on analyzes of tick-borne pathogens from two different approaches by using high-throughput DNA sequencing technologies. Our first approach was to analyze genomic DNA fragments purified from bacteria-enriched fractions prepared from whole tick homogenates. Purified genomic DNA samples from 6 tick species were subjected to sequencing by the next generation sequencer (Roche FLX454). Sequence reads were analyzed by using Batch Learning Self-organizing Map (Abe et al.) and BLAST. Our second approach was to analyze 16s rRNA gene products amplified from whole tick DNA by using bacterial 16s rRNA gene-specific primers. Amplified products were subjected to the next generation sequencer and BLAST searched against bacterial ribosomal DNA database. These results help us to construct a database of tick microbes which may contain zoonotic pathogens. Our efforts to develop the tick pathogen database may lead to the empowerment to preempt climate-change-associated emergence of tick-borne diseases.

Friday 13th December 2013

Time: 11:00 – 12:30

Room D

S37 Infectious diseases

Chairperson



Polrat Wilairatana

1. Effect of tyrosine kinase inhibitors on the STAT6 signaling of host ARPE-19 cells infected with *Toxoplasma gondii*
Howoo Nam
2. Flooding Effects on *T. Gondii* Infection of Cattle in Thailand
Sathaporn Jittapalapong
3. Molecular Characterization of *Entamoeba* Species in The Patients of Suspected Amoebiasis by Microscopic Examination
Dar-Der Ji
4. Methamphetamine Use Prio to Sex and the Risk Factors Associated With Hiv Prevalence Among Men Who Have Sex With Men (Msm) In Thailand, 2006-2010
Phunlerd Piyaraj
5. Water Footprint, Water Globally: Pathways to Sustainability
Voranuch Wangsuphachart

EFFECT OF TYROSINE KINASE INHIBITORS ON THE STAT6 SIGNALING OF HOST ARPE-19 CELLS INFECTED WITH *TOXOPLASMA GONDII*

Howoo Nam

Dept. of Parasitology, College of Medicine, Catholic University of Korea



Ho-Woo Nam¹, Kyoung-Ju Song¹, Zhaoshou Yang¹, Hye-Jin Ahn¹, Chom-Kyu Chong², Tong-Soo Kim³ and Sung-Jong Hong⁴

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The spontaneous STAT6 phosphorylation and the following specific gene expressions of host cells are major strategy for the survival of the protozoan intracellular parasite *Toxoplasma gondii* against the parasitocidal events through the STAT1 phosphorylation by the interferon-gamma provoked in the infection. Several tyrosine kinase inhibitors (TKI) are tested the effect on the growth inhibition of intracellular growth of *T. gondii* and the relationship with STAT1 and 6 phosphorylation in the retinal cell line, ARPE-19. Among the tested TKIs, Afatinib (pan ErbB/EGFR inhibitor, 5mM) inhibits the growth of *T. gondii* of 98.0%, which is comparable to pyrimethamine (5mM) of 96.9%, and followed by Erlotinib (ErbB1/EGFR inhibitor, 20 mM) of 33.8% and Sunitinib (PDGFR or c-Kit inhibitor, 10 mM) of 21.3% in the counting the number of *T. gondii* per parasitophorous vacuolar membrane within host cells at 12-hour interval for 72 hr. In the early stage of infection (2, 4, and 8 hr after *T. gondii* challenge), Afatinib inhibits the spontaneous phosphorylation of STAT6 in western blot and immunofluorescence assay, but still the STAT1 phosphorylation is not affected by interferon-gamma stimulation. On the while, Jak1/Jak3, the upper hierarchical kinase of the cytokine signaling, are strongly phosphorylated at 2 hr and then disappear entirely after 4 hr. These suggest the tyrosine kinase inhibitors, belonging to a kind of anti-cancer drug, might play an important role in inhibition of intracellular replication of *T. gondii* through the inhibition of direct phosphorylation of STAT6 by *T. gondii*.

Key words: *Toxoplasma gondii*, tyrosine kinase inhibitor, STAT1, STAT6, Jak1, Jak3

FLOODING EFFECTS ON *T. GONDII* INFECTION OF CATTLE IN THAILAND

Sathaporn Jittapalpong

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Sathaporn Jittapalpong^a, Soichi Maruyama^b, Sinsamuth Saengo^a, Nongnuch Pinyopanuwat^a, Wissanuwat Chimnoi^a, Chanya Kengradomkij^a, Aurelie Binot^{a,d}, Stephane Herder^{a,c}, Serge Morand^d, Sayamchai Suksai^a, Noppawan Thapraphom^a, Sarawut Yangtara^a, and Marc Desquesnes^{a,d}

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Introduction. Floodings generate modifications of ecological conditions for pathogen, hosts, reservoirs, and vectors, which will condition their population dynamic, densities, and interrelations with the environment. Consequences of floods influence the circulation and the incidence of some parasitic zoonoses, in ways which are hardly predictable and need data record and analyses. *Toxoplasma gondii* infections are widely prevalent in animals and humans worldwide particularly in Thailand.

Objective. The objective of this study is to compare the seroprevalence of *T. gondii* infections in cattle in flooding areas between dry (D) and rainy (R) season in Thailand.

Method. Cattle sera of Nan (70&116), Nakhon Sawan (81&110), and Ayutthaya (90&103) were collected respectively during rainy/ dry season and tested by using latex agglutination test (cutoff \geq 1:64, Eiken, Japan).

Results. The overall seroprevalence of *T. gondii* infections in cattle from Nan, Nakhon Sawan, and Ayutthaya was 18.3 % (34/186), 10.9% (21/191), and 27.5% (53/193), respectively. The effect of season was compared and shown as the prevalence in Nan (R: D =17.7: 18.9), Nakhon Sawan (R: D=4.9:15.5), and Ayutthaya (R: D=25.6:29.1). The animal with 0-5 year had the higher prevalence (19.66%, 101/514) than > 5years group (12.28%, 7/56). All animal age groups (0-5 years and >5 years) had the higher *T. gondii* infections in dry season compared to the rainy season. The result indicated that possible factors associated with transmission might be disappeared or inactive during rainy season. One potential factor, rodents as reservoir hosts were forced to leave their habitats during the flooding period. The dry season might influence rodents to be more active for food and other activities leading to increasing prevalence. Finally, a delay between implementation of the epidemiological cycle and seroconversion might also partly explain the increasing prevalence recorded with samples collected during the dry season.

Keywords: flooding, Nan, Nakhon Sawan, Ayutthaya, *Toxoplasma gondii*, cattle

MOLECULAR CHARACTERIZATION OF *ENTAMOEBIA* SPECIES IN THE PATIENTS OF SUSPECTED AMOEBIASIS BY MICROSCOPIC EXAMINATION

Dar-Der Ji

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Entamoeba histolytica can cause human amoebiasis that is still a major public health issue especially in the developing world and the men who have sex with men. The purpose of this study was to evaluate the microscopic diagnosis of *E. histolytica* by real-time PCR and to characterize the *Entamoeba* species in suspected amoebiasis patients in Taiwan. A total of 167 suspected patients examined positive to *E. histolytica*/*E. dispar* by Merthiolate-Iodine-Formalin microscopy were then evaluated by the real-time PCR targeting to *E. histolytica*/*E. dispar*. Among them, 48.5% (81 of 167) patients were positive to *E. histolytica* and 31.7% (53 of 167) to *E. dispar*. About 19.8% (33 of 167) patients were undetectable and further analyzed by a common PCR of *Entamoeba* species targeting to SSU rRNA genes and sequencing. Interestingly, two phylogenetically distinct clusters of *E. hartmanni* were identified and correlated to their genetic variation. These two clusters were also morphological distinct groups especially in size. Furthermore, three distinct groups of *E. polecki* were also identified according to the transmission routes: human-human, animal-human and animal only. However, there were still 13.8% (23/167) suspected patients were all PCR negative that might correlate to low parasite concentration. These new findings may have a significant impact in the microscopic and molecular diagnosis of amoebiasis, and the importance of genetic diversity of *E. hartmanni* correlated to morphology need to be further investigated.

Keywords: *Entamoeba histolytica*, *E. hartmanni*, *E. polecki*, microscopy, PCR

METHAMPHETAMINE USE PRIOR TO SEX AND THE RISK FACTORS ASSOCIATED WITH HIV PREVALENCE AMONG MEN WHO HAVE SEX WITH MEN (MSM) IN THAILAND, 2006-2010

Phunlerd Piyaraj

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Objectives: To examine the prevalence of methamphetamine use prior to sex and to investigate the risk factors associated with HIV prevalence including methamphetamine use to enhance sex pleasure among MSM participating in BMCS in Bangkok, Thailand.

Methods: A cross-sectional study of 1,744 MSM participating in a cohort of MSM in Bangkok, Thailand between April 2006 to November 2010 was evaluated for HIV prevalence and associated risk factors. Men were tested for STI/HIV infection at baseline. Demographic and behavioral data were collected by audio-computer-

assisted self-interview. Logistic regression analysis was used to investigate the risk factors associated with HIV prevalence.

Results: Baseline prevalence of Methamphetamine use was 11.2% (195/1,744), respectively. The overall HIV prevalence was 21.3% (372/1,744). Reported methamphetamine use was not associated with HIV prevalence in multiple logistic regressions. After adjusting for potential confounders, risk factors for HIV prevalence were self-identified as homosexual/gay, practice dual role taking of anal intercourse, ever had forced sex, younger age when having first sex, ever diagnosed STI, practice anal douched, recruited sex partners from park, used poppers, clinically appearance of anal warts, and *Treponema Pallidum* (TP), hepatitis B surface antigen, anti-HSV-1 and anti-HSV-1 and 2 positivity. The inversely factors associated with lower HIV prevalence were being student, a history of HIV testing, and ever paid for sex.

Conclusion: Behavior of methamphetamine use to enhance sex was common and high prevalence of HIV infection among these men. It is underscores the need for innovative and increased efforts to prevent HIV infection in this population.

Keywords: HIV, Prevalence, Risk factors, MSM, Methamphetamine, Bangkok, Thailand

Acknowledgement

This study was conducted under a larger project entitled “Bangkok MSM Cohort Study” which is funded by the US CDC. Additional support was provided in kind of partial financial support for Dr. Phunlerd Piyaraj’s doctoral dissertation study as of a student investigator. The support of the Ministry of Science and Technology, Thailand, and the Johns Hopkins University Fogarty AIDS International Training & Research Program (Hopkins AITRP) was provided a doctoral scholarship to Dr. Phunlerd Piyaraj (Grant Number: D43 TW000010-25).

WATER FOOTPRINT, WATER GLOBALLY: PATHWAYS TO SUSTAINABILITY

Voranuch Wangsuphachart

Faculty of Tropical Medicine, Mahidol University



Globally, freshwater resources are becoming scarcer due to an increase in population, economic development, subsequent increase in water misuse and deterioration of water quality. Problems of water scarcity, water excess and deterioration of water quality would be solved if the resource ‘water’ were properly treated as an economic good. The logic is clear: clean fresh water is a scarce good.

Do you know how much water was used to make a cup of hot Cappuccino? Simply, Water footprint of a cup of black hot coffee was 140 litres. One 150 gram burger uses 2400 litres of embedded water, of one cow which produced 200 kg beef.

Concept of the Water footprint of a product is the volume of freshwater used to produce the product, summed over the various steps of the product chains. The water footprint of a nation has been defined as the total volume of freshwater that is used to produce the goods and services consumed by the inhabitants of the nation. Most of the time, price of water consumption and pollution is hardly or not at all included in the price of merchandises produced. The impact of consumption of people on the global water resources can be mapped with the concept of the ‘water footprint’, a concept introduced by Hoekstra-Hung (2002) and subsequently by Chapagain-Hoekstra (2004). The global average water footprint is found to be 1385 m³/yr per capita in the period 1996-2005, translating into the green, blue and grey water footprint of production and consumption. There are large differences between countries. In the USA the average water footprint is 2842 m³/yr per capita. In China the average water footprint is 1071 m³/yr per capita. Sustainable use of water should be considered to avoid global water crisis.

Keywords: Water Footprint, water supply, economic good, sustainable development

Friday 13th December 2013

Time: 11:00 – 12:30
Room E

S38 Malaria: Clinical Laboratory Studies

Chairperson 1



Porntip Petmitr

Chairperson 2



Kesinee Chotivanich

1. Factors influencing the misdiagnosis of microscopists in malaria clinics along the Thai-Myanmar and Thai-Cambodia border provinces
Supawadee Pounsombat
2. An Open-Label Crossover Study to Evaluate Potential Pharmacokinetic Interactions of Orally Administered Primaquine and Dihydroartemisinin-Piperaquine in Healthy Adult Thai Subjects
Borimas Hanboonkunupakarn
3. Monitoring a Key Folate Enzyme in *Plasmodium falciparum* During Changes in Metabolic States
Narisa Dawar
4. Investigation of Halotag Function in *Plasmodium Spp.*
Golam Ahmed
5. Comparison of the Efficacy and Safety of Two Acts Plus Primaquine for Uncomplicated *Plasmodium vivax* Malaria in North Sumatera, Indonesia: 1 Year Follow-Up
Ayodhia Pitaloka Pasaribu
6. Histidine-rich proteins as tools for the diagnosis and management of *Falciparum malaria*
Charles Woodrow

FACTORS INFLUENCING THE MISDIAGNOSIS OF MICROSCOPISTS IN MALARIA CLINICS ALONG THE THAI-MYANMAR AND THAI-CAMBODIA BORDER PROVINCES

Supawadee Pongsombat

Bureau of Vector Borne Disease, Department of Disease Control, Ministry of Public Health, Thailand



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The purpose of this study was to determine the factors influencing the misdiagnosis of microscopists in malaria clinics along the ten provinces of Thai-Myanmar and Thai-Cambodia border provinces. There were 164 microscopists were selected by using random sampling technique. The questionnaires, pre-test and the unknown blood slide examination test for determining malaria knowledge and slide readings skill of microscopists were the instruments for data collection and analysis according to the process of research methodology.

The results have shown that there was no significantly difference in the accurate malaria diagnosis between the microscopists who have been working at the Thai-Myanmar and Thai-Cambodia Border provinces. This result has shown that there was the homogenously basic knowledge of malaria with the same standard and protocol of the training for malaria microscopists. The results revealed that female microscopists, old age, having family member with color blindness and have been trained for the training course on malaria microscopists more than 5 years gave the high percentage of the error in diagnosis. The malaria misdiagnosis also determined that the percentages of the error rate in diagnosis and false positive were 4.82 and 2.44 respectively whereas the percentage of false negative was zero. The result of this study may gave considerably helpful for the refreshment course on malaria microscopy which was found to be necessary and urgently need to be conducted in the border provinces where the occurrence of malaria transmission was still high.

AN OPEN-LABEL CROSSOVER STUDY TO EVALUATE POTENTIAL PHARMACOKINETIC INTERACTIONS OF ORALLY ADMINISTERED PRIMAQUINE AND DIHYDROARTEMISININ-PIPERAQUINE IN HEALTHY ADULT THAI SUBJECTS

Borimas Hanboonkunupakarn

Faculty of Tropical Medicine, Mahidol University



Borimas Hanboonkunupakarn¹, Elizabeth Ashley², Podjane Jittamala^{2,3}, Joel Tarning², Salwaluk Panapipat², Sasithon Pukrittayakamee¹, Nicholas Day², Nicholas J. White²

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Dihydroartemisinin-piperaquine (DHA-PQP) is one of the artemisinin-based combination treatments (ACTs) recommended by WHO for treatment of uncomplicated *P. falciparum* malaria. Recently, WHO has recommended to add primaquine (PQ) to all ACTs with the aim of reducing transmission of artemisinin-resistant strains. The co-administration of these 2 drugs is inevitable and could result in a significant drug-drug interaction. To characterise their potential interactions, we studied the pharmacokinetics of PQ and DHA-PQP in 16 healthy Thai adult volunteers in a crossover study. All volunteers were randomised to two groups of three sequential hospital admissions to receive 30 mg PQ, three tablets of DHA-PQP (960-120 mg) and the combined drugs. The pharmacokinetic properties of all drugs were evaluated using a

non-compartmental approach. An analysis of variance (ANOVA) was carried out on the log-transformed pharmacokinetic parameters for exposure to assess the drug-drug interactions. No drug-drug interaction was assumed if the 90% confidence intervals of the geometric mean ratio (combination/alone) of C_{MAX} , AUC_{0-LAST} and $AUC_{0-\infty}$ were contained within 80% to 125%. The treatment was well-tolerated. There were no statistically significant differences in DHA and PQP pharmacokinetics when administered with or without PQ. In contrast, significant drug-drug interactions were observed on PQ pharmacokinetics with DHA-PQP co-administration as demonstrated by geometric mean ratios (90% CI) for C_{MAX} , AUC_{0-LAST} and $AUC_{0-\infty}$ of 148% (117-187%), 129% (103-163%) and 128% (102-161%), respectively. In conclusion, co-administration of DHA-PQP and PQ did not result in any significant pharmacokinetic alterations on DHA and PQP, but changes in PQ pharmacokinetics were observed.

Keywords: Antimalarial drugs, *Plasmodium falciparum*

MONITORING A KEY FOLATE ENZYME IN PLASMODIUM FALCIPARUM DURING CHANGES IN METABOLIC STATES

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Plasmodium falciparum, a major human malaria parasite, switches between two hosts in order to complete their life cycle. The parasite requires large amount of nucleic acid to generate thousands of new progenies. The folate pathway of *P. falciparum* produces one-carbon transfer unit needed in the conversion of dUMP to dTMP. Folate derivatives are also used for amino acid metabolism, protein translation and iron-sulfur cluster synthesis. GTP cyclohydrolase I (GCH1) is the first enzyme in the folate pathway. It converts GTP into pterin which is subsequently incorporated into folate derivatives. Recent findings revealed that *gch1* gene amplification causes antifolate resistance and drives the course of drug resistance evolution. The importance of GCH1 suggests that this enzyme could be one of the master metabolic hubs in malaria parasites. Here we monitored the presence of GCH1 in the parasites under various states. Antibody-based detection methods were employed to determine the amount and the subcellular localization of GCH1 in malaria parasites. The studies were performed in various stages and under metabolic pressures from anti-malarial drugs.

Keywords: malaria, GTP cyclohydrolase I, folate, *Plasmodium falciparum*, drug resistance

INVESTIGATION OF HALOTAG FUNCTION IN *PLASMODIUM SPP.*

Golam Ahmed

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HaloTag is a modified bacterial haloalkane dehalogenase enzyme, which has recently being developed as a tool for studying protein function, purification, intracellular protein localization, time-dependent labeling and control of intracellular protein levels. Ligand mediated control of

protein level is an attractive tool for determining protein function, including the validation of drug targets. We propose to test the function of HaloTag in the blood stages of *Plasmodium berghei* malaria parasite using Green Fluorescent Protein (GFP) as a target protein. To date, in bacteria, a GFP-HaloTag fusion protein was successfully expressed and detected by Western analysis. It was also labeled with halogenated TMR ligand specific to HaloTag. However, In *P. berghei*, we are unable to detect any GFP signal from the same gene. Moreover, no protein was detected for other HaloTag fusion genes in other transgenic experiments in *P. berghei* and *P. falciparum*. Therefore, we hypothesized that HaloTag cannot be expressed, or it is not functional in the context of *Plasmodium*.

Keywords: haloalkane dehalogenase, HaloTag, TMR ligand.

COMPARISON OF THE EFFICACY AND SAFETY OF TWO ACTS PLUS PRIMAQUINE FOR UNCOMPLICATED PLASMODIUM VIVAX MALARIA IN NORTH SUMATERA, INDONESIA: 1 YEAR FOLLOW-UP

Ayodhia Pitaloka Pasaribu

Faculty of Tropical Medicine, Mahidol University



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Because of high prevalence of chloroquine resistant *P. vivax* in Sumatera, first-line treatment has shifted to artemisinin-based combination therapies (ACTs), combined with primaquine for radical cure. However, which combination is most effective and safe, against a background of significant prevalence of G6PD-deficiency needs to be established. We conducted a prospective open-label randomized comparison of artesunate/amodiaquine with primaquine (AAQ+PQ) versus dihydroartemisinin-piperavaquine with primaquine (DHP+PQ) for the treatment of uncomplicated *P. vivax* in Sumatera, Indonesia. Febrile patients with microscopically confirmed *P. vivax* mono infection were randomised to either standard 3 days AAQ (n = 167) or 3 days DHP (n=164), both combined with 14 days primaquine (0.25 mg/kg) without prior testing for G6PD-status. Follow-up for safety and efficacy was 1 year. Between December 2010 and April 2012, 331 patients were included. Recurrent infection occurred in 0/167 within 42 days and in 15/130 (11.5%, C.I. 6.6% to 18.3%) within a year in patients treated with AAQ+PQ and in 1/164 (0.6%, C.I. 0.01% to 3.4%) within day 42 and 13/143 (9.1%, C.I. 4.9% to 15.0%) within a year with DHP+PQ treatment. Intravascular hemolysis occurred in 5 patients, of which 3 males were hemizygous for G6PD-Mahidol. Minor adverse events were more frequent with AAQ+PQ. In North Sumatera, Indonesia, both AAQ and DHP, both combined with 0.25 mg/kg primaquine for 14 days, are safe and efficacious treatments for uncomplicated *P. vivax* without prior testing for G6PD deficiency, but DHP+PQ was better tolerated.

Clinical Trials Registration. NCT01288820.

Keywords: Artemisinin-based combination therapy; Primaquine; Radical cure; *Plasmodium vivax*; Indonesia

HISTIDINE-RICH PROTEINS AS TOOLS FOR THE DIAGNOSIS AND MANAGEMENT OF *FALCIPARUM MALARIA*

Charles Woodrow

Faculty of Tropical Medicine, Mahidol University



The histidine-rich proteins (HRP2 and HRP3) are a pair of soluble, highly antigenic proteins produced specifically by the hominid malaria parasites (*P. falciparum* and *P. reichenowi*), being absent from other human malaria parasites such as *P. vivax*.

HRPs are expressed in very high quantities particularly in the asexual (human) phase of malaria infection where HRP2 is one of the most highly transcribed *P. falciparum* genes. The biological functions of HRPs are thought to include a role in parasite haemoglobin digestion.

The amino acid sequences of the repeat motifs of HRPs make them highly antigenic, and detection of HRPs via antibody forms the basis of an ever-expanding range of applications, including lateral flow-based rapid diagnostic tests, measurement in plasma and cerebrospinal fluid to assess severity, and quantification of parasite growth in ex vivo drug susceptibility assays.

This review aims to summarise more than a quarter of a century of experimental work on HRPs and relate this basic biological foundation to the modern application of HRPs in the management and control of *Falciparum malaria* at or close to the point of care.

Friday 13th December 2013

13:30 – 14:40
Convention Center

Keynote Plenary III Closing Session - "Sornchai Looreesuwan Medal"

Chairperson



Yaowalark Sukthana

Moderator



Parnpen Viriyavejakul

Award Recipient



Louis H. Miller
*Malaria Cell Biology Section
National Institute of Allergy and
Infectious Diseases, NIH*

Profile:

Dr. Louis H. Miller, of the National Institutes of Health, USA, for his distinguished achievements in malaria research, specifically in identifying the basic genetic mechanisms employed by malaria parasites to infect and survive in their hosts, and discovering molecular targets that support malaria-vaccine development.

ACCEPTANCE OF THE 2013 SORNCHAI LOOAREESUWAN MEDAL

I spent the earliest period of my scientific career and the beginning of my malaria research in Thailand from 1965 to 1967. I was a young investigator like many of you and went to Siriraj Hospital to read the literature. This was before the internet that we all take for granted. For the first year, I worked closely with Professor Visith Sitprija, a renal physiologist at Chulalongkorn Hospital. I also became familiar with the work of Sompone Punyagupta on *Gnathostoma spinigerum* and became his close friend. During the second year, Bob Desowitz, my mentor, introduced me to Professor Chamlong Harinsuta, the director of the Thai School of Tropical Medicine. This led to my work with Tan Chongsuphajaisiddhi on severe malaria in monkeys and my lunches with three generations of Thai scientists, S. Daengswang who at that time was the expert on Fasciola

buski, Chamlong and Tan. Tan had trained in malaria pathophysiology with Brian Maegraith who had a strong link to the Thai School of Tropical Medicine. He was famous for questioning facile conclusions by saying 'post hoc ergo propter hoc (this therefore that)' meaning that many temporally related events may not be causally related. Maegraith believed that parasitized erythrocytes in the brain do not relate to the etiology of cerebral malaria. Boy was he wrong. Ian McGregor, a dear friend, told me that when he was young he met the greater leader in malaria immunology, William Taliaferro, who believed in cellular immunity. Ian told him about his interest in antibody in protection from malaria. Taliferro said nonsense and turned away, not taking this opportunity to talk with Ian. Boy was he wrong. Ian's great contribution, working with Sidney Cohen, showed that passive transfer of immune immunoglobulin controlled malaria. It was like Paul Ehrlich trying to talk with Elie Metchnikoff. I guess the lesson for young scientists is never be intimidated by authorities who have their own prejudices. I have enjoyed young scientists who question my dogmas and especially young scientists from endemic countries where the problems of malaria and other parasitic diseases affect their own people. Wouldn't it be wonderful if a scientist from SE Asia solved the problem of continuous culture of *P. vivax*, identified its reticulocyte receptor for red cell invasion or developed a detailed understanding of hypnozoites, the latent *P. vivax* infections, and a treatment for eliminating hypnozoites. As old scientists are prone to say, if I were young, I would love to work on these problems. Most importantly, you young scientists can solve problems of malaria that will enlighten all of us. I treasure the intelligence and energy of young scientists the world over.

Although I started my career in Thailand in 1965, I worked most of my life at the National Institutes of Health in the US. I had many wonderful moments such as the discovery of the red blood cell receptor for *P. vivax*, but I will never forget those two formative years that cemented my interest in malaria. I thank all my Thai colleagues here who had such a profound effect on my life in science.

Award Recipient



Stephen A. Ward

Liverpool School of Tropical Medicine

Profile:

Professor Dr. Stephen A. Ward, Liverpool School of Tropical Medicine, UK, for his key achievements in anti-malarial drug discovery, and in the application of pharmacokinetic principles to the rational design and investigation of anti-parasite dosage regimens, a cornerstone of many current global research programs.

FROM MICE TO MEN AND THE EVOLUTION OF QUANTITATIVE MALARIA INFECTION MODELS TO ACCELERATE ANTIMALARIAL DRUG DISCOVERY

Abstract:

Steve Ward

Liverpool School of Tropical Medicine, UK

Since the definitive discovery of malaria parasites scientists have been trying to find ways to kill them with small organic molecules. At the turn of the last century scientists were using avian malaria models to probe the mechanism of action of quinine. Even in these early days it was clear that different parasite stages had different drug susceptibilities. Screening models progressed in the 1940's towards more relevant rodent and primate models and with the important exception of the establishment of continuous in vitro culture in 1976 by Trager and Jensen, there have been few significant advances in our screening strategy until quite recently. This period saw the introduction of only a handful of new antimalarials, principally acting

against asexual parasites and with only two or possibly three mechanisms of action, despite considerable efforts albeit originating from a small number of labs to discover more. On reflection it could be suggested that the type of drug selected was strongly influenced by the screening models employed.

The last decade has seen an unprecedented acceleration in antimalarial drug discovery, catalysed in large part by the Medicines for Malaria Venture. The dearth of good chemical starting points and the failure of targeted drug screens triggered investment in establishing high throughput screening platforms for asexual *P. falciparum* parasites. This in turn has resulted in over 5 million compounds being screened and some 20,000 nanomolar hits being identified. Failure to replicate *in vitro* *P. falciparum* activity with some compounds *in vivo* has resulted in the generation of a humanized *falciparum* mouse model and the need to get to clinic quickly has promoted the re-establishment of human challenge models. Even our use and interpretation of the simple *in vitro* and *in vivo* dose response relationship has seen radical change. The move from treatment of *P. falciparum* to eradication of malaria has promoted investment into screens for stage specific drugs and cross species -active compounds and drug resistance selection coupled with “omics” technologies have rapidly generated knowledge on new mechanisms of action and resistance. Again it is interesting to see how the screening strategy has had a strong influence on the types of molecule that are being selected. This concerted effort is helping shape the largest and most diverse antimalarial portfolio in history, is providing unique insight into parasite biology and importantly has opened the door for many in the scientific community to get involved in translation science in a way never possible before. In this presentation we will review the successes, discuss the remaining challenges and consider the potential impact of these new approaches and the resulting new therapies on malaria at the patient and population level.

14:40 - 15:10 Presentation of Awards, Best Poster Award and Closing remarks



ABSTRACTS

POSTER PRESENTATIONS

Joint International Tropical Medicine Meeting 2013

JITMM 2013

11-13 December 2013

Centara Grand & Bangkok Convention Centre at CentralWorld, Bangkok, Thailand

Join the largest regional conference on Tropical Medicine,
hosted by the Faculty of Tropical Medicine, Mahidol University

**“Towards Global Health: an Asian
Paradigm of Tropical Medicine”**

Poster No. 1

EFFECT OF NANOENCAPSULATED CURCUMIN ON PERIDUCTAL FIBROSIS RESOLUTION AND BILE CANALICULI ALTERATION IN *OPISTHORCHIS VIVERRINI*-INFECTED HAMSTERS AFTER PRAZIQUATEL TREATMENT

Lakhanawan Charoensuk^{1,5}, Porntip Pinlaor^{2,5}, Supason Wanichwecharunguang³, Rucksak Rucksaken^{1,5}, Kitti Intuyod^{1,5}, Sudarat Onsurathum^{1,5}, Puangrat Yongvanit^{4,5}, Somchai Pinlaor^{1,5}*

¹ Department of Parasitology, ²Department of Biochemistry, and ³Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand, ⁴Centre for Research and Development in Medical Diagnostic Laboratory, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand, ⁵Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. ⁶Biomedical Science Program, Graduate School, Khon Kaen University, Khon Kaen 40002, Thailand.
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Opisthorchis viverrini infection induces inflammation and liver injury leading to increase periductal fibrosis with time and increase a risk for cholangiocarcinoma (CCA) development. Although praziquantel (PZQ) treatment would be valuable for *O. viverrini* elimination; however, periductal fibrosis usually remains for a long time after treatment at chronic infection. To reduce periductal fibrosis induced by *O. viverrini* infection, encapsulated curcumin was used to enhance fibrolysis of fibrotic tissue after praziquantel treatment in *O. viverrini*-infected hamsters. Forty-eight hamsters were divided into 4 groups: (1) normal hamsters, (2) *O. viverrini*-infected hamsters (OV), (3) OV treated with praziquantel (OP), and (4) OV+PZQ and treated with encapsulated curcumin at 50 mg/kg BW (OPC). Each experimental group was divided into two sub-group, acute (1 month) and chronic (3 months) post-infection. Encapsulated curcumin was orally administered daily for one month after PZQ-treatment. Acute and chronic groups showed similar results. Picrosirius red staining revealed that encapsulated curcumin reduced periductal fibrosis. The changes were related to the reduction of the expression of fibrotic markers (Matrix metalloproteinase (MMP) 9, collagen I, tissue inhibitor of matrix metalloproteinase (TIMP) 1, TIMP3, alpha-smooth muscle actin (α-SMA) and the increase of MMP2. Moreover, bile canaliculi feature was recovered by encapsulated curcumin treatment as demonstrated by the increasing of Nephrilysin (CD10) and Multidrug resistance-associated protein 2 (Mrp2) expressions at the transcription and translation levels. **In conclusion**, encapsulated curcumin is a promising chemopreventive agent that can reduce periductal fibrosis and improve bile calicular structure after praziquantel treatment in opisthorchiasis which may be useful to reduce risk factor of CCA.

Keywords: *Opisthorchis viverrini*, periductal fibrosis, encapsulated curcumin, *bile canaliculi*

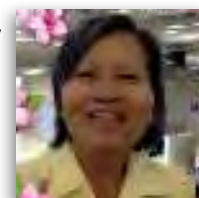
Acknowledgements: This work was supported by The Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Health Cluster (SHeP-GMS) and a grant of Graduate school KKU.

Poster No. 2

PREVALENCE AND RISK FACTOR OF *OPISTHORCHIS VIVERRINI* INFECTION IN UPPER NORTHEAST THAILAND, 2013

Kesorn Thaewongiew¹, Narong Wongba¹, Laithavewat Luxana¹, Seri Singthong¹, Saowalux kutchamart¹, Isarate Swangieang, Supan Sailugum², Sasiithorn Tangsawad³

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Opisthorchis viverrini (OV) infection is one of the major health problems in Thailand. Even though the government has tried to solve this problem, but there are still exist. This study is a cross-sectional analytic study, aim to study prevalence of *O. viverrini* infection and risk factors of *O. viverrini* infection during February - July 2013. A total of 3,916 subjects from 7 provinces in upper northeast Thailand were chosen by 30 Clusters Sampling technique. Data collected through questionnaires, group interview, in-depth interview and stool examination by Modified Kato Katz method. Descriptive and analytical statistics were introduced to explain the relationship of factors related to *O. viverrini* infection using odds ratio, and multiple

logistic regression analysis. The ratio of male and female among population who has 25% of prevalence was 1:1.25. *O.viverrini* infection was most frequent in the 50-59 year age group. The positive results were high endemic area (prevalence > 20 %) about 97 of 210 Tumbon in 7 provinces (46.19%), and the highest prevalence were 80% (range: 0-80%). Statistically significant factors found from multivariate analysis were: eating raw fish (OR 9.24, 95%CI 1.69, 50.49), eating uncooked fish salad (OR 4.10, 95%CI 1.68, 9.98), eating praziquantel (OR 3.62, 95%CI 1.57, 8.31) and drinking alcohol (OR 4.8, 95%CI 1.41, 2.32). Reducing of risk OV infection in community should be reducing of health risk behavior because of behaviors of people in village to become an important mechanism for sustainable prevention and controlling liver fluke in the community.

Keywords: Factors, *Opisthorchis viverrini*, upper northeast

Poster No. 3

IDENTIFICATION OF EXPRESSED PROTEINS ASSOCIATED WITH PERIDUCTAL-FIBROSIS IN CHRONIC EXPERIMENTAL OPISTHORCHIASIS BY PROTEOMIC ANALYSIS

Ornuma Haonon^{a,b}, Rucksak Rucksaken^{a,b}, Sudarat Onsurathum^{a,b}, Porntip Pinlaor^c, Thidarut Boonmars^{a,b}, Somchai Pinlaor^{a,b*}

^a Department of Parasitology, ^bLiver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Thailand,

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A small human liver fluke, *Opisthorchis viverrini* is endemic in northeastern Thailand, and causes periductal fibrosis during chronic infection contribution to the potential risk for cholangiocarcinoma (CCA). The histopathological feature of opisthorchiasis in animal is similar to the patient. Previously, many proteins have been successfully identified in hamster opisthorchiasis and could be applied in the patient for diagnostic marker. In this study, we aimed to identify proteins expression in relation to periductal fibrosis in chronic opisthorchiasis hamsters by proteomics analysis. Liver tissue extracted from normal and chronic opisthorchiasis hamsters were subjected to two-dimensional gel electrophoresis (2DE). The results showed that the protein expression pattern of infected hamsters were different when compared with normal control group. Identified protein spots were investigated by software analysis, and MS/MS analysis. Candidate proteins will be verified in the hamster liver and plasma, and then apply in opisthorchiasis patient for diagnostic purpose. In addition, histopathological features revealed that chronic opisthorchiasis hamsters showed the thickening of fibrosis surrounding the biliary system. In conclusion, *O. viverrini* infection induces the alteration of proteins expression in relation to periductal fibrosis. The altered proteins may be served as the novel fibrotic markers for opisthorchiasis to predict risk of CCA in the future.

Keywords: opisthorchiasis, periductal fibrosis, proteomic, biomarker

Acknowledgements: This work was supported by The Invitation Research from the Faculty of 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 Health Cluster (SHeP-GMS1165), Khon Kaen University, Thailand.

Poster No. 4

DISTRIBUTION PATTERNS OF *OPISTHORCHIS VIVERRINI* AND *HAPLORCHIS TAICHUI* INFECTIONS IN TWO VILLAGES OF SAVANNAKHET PROVINCE, LAO PDR

Megumi Sato¹, Tiengkham Pongvongsa², Surapol Sanguankiat³, Tipparayat Yoonuan³, Jun Kobayashi⁴, Boungnong Boupha⁵, Futoshi Nishimoto⁶, Kazuhiko Moji⁶, Marcello Otake Sato⁷, Jitra Waikagul³

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The liver fluke, *Opisthorchis viverrini*, and the minute intestinal fluke, *Haplorchis taichui*, are prevalent in many Asian countries. In this study, the pattern of infection with *Opisthorchis viverrini* and *Haplorchis taichui* in Lahanam and Thakhamrien Villages (Savannakhet Province, Lao PDR) was analysed in two cross sectional investigations. Intermediate hosts fishes acquired in the study area were also analysed. Post-anthelmintic treatment positivity rates for expelled worms were 170 (82.1%) for *H. taichui* and 65 (31.4%) for *O. viverrini* from a total of 207 participants; these 2 species co-existed in the study villages. Opisthorchiid egg findings initially increased with age, but decreased again from age 30 years. However, when each parasite was analysed separately, *H. taichui* infection reached a plateau among the age group > 20 years, and the *O. viverrini* infection rate was highest in the age group 21-30 years, with decreasing infection rates after age 30. Our results indicate that fish-borne trematode infections in particular are more prevalent among adults. Examination of fish intermediate hosts found *O. viverrini* metacercariae in only 6 species of the 35 examined, and these were mostly found during November. At this time, many farmers stay in the ricefields and obtain their food nearby, including intermediate-host fish, and may be at greater risk of *O. viverrini* infection. By contrast, *H. taichui* metacercariae were found in 3 species of fish, and only from the market, which means that anyone could consume them and become infected. If people working in ricefields limit the fish species they consume or avoid consuming raw fish around November, it may help reduce the risk of *O. viverrini* infection.

Poster No. 5

PARKIN-LIKE PROTEIN WITH E3 UBIQUITIN LIGASE ACTIVITY IN *CLONORCHIS SINENSIS*

Bai XL, Lee JY, Hong SJ

Department of Medical Environmental Biology, Chung-Ang University College of Medicine, Seoul 156-756, Korea



Parkin is associated with autosomal recessive juvenile Parkinsonism which was characterized by the loss of dopaminergic neurons in substantia nigra. Human Parkin consists of an N-terminal ubiquitin-like domain and two RING fingers separated by IBR structure. Function of Parkin is ubiquitin ligase catalyzing interaction of substrates with E2 conjugating proteins Ubc7 and Ubc8 then ubiquitin to the substrates. In present study, a cDNA clone of *Clonorchis sinensis* (CsParkin) showed high homology with Parkin of other animals and conserved RING-IBR-RING structure. Recombinant CsParkin protein was expressed and purified and was used for mouse immunization. Immunohistochemical staining with mouse immune sera showed CsParkin localized in oral sucker, sperm and testes of adult *C. sinensis*. Qrt-PCR revealed that CsParkin was expressed higher in metacercaria than in adult. Recombinant CsParkin revealed ubiquitin ligase function *in vitro* ubiquitination experiment. All these findings indicate that CsParkin may play an role in protein degradation in high metabolic organs of *C. sinensis* such as organogenesis and sperm production.

Keywords: *Clonorchis sinensis*, E3 ubiquitin ligase, Parkin

Poster No. 6

EFFECTS OF TEMPERATURE ON THE LIFE CYCLE OF *SCHISTOSOMA MANSONI* AND ITS SNAIL INTERMEDIATE HOST, *BIOMPHALARIA GLABRATA*, IN LABORATORY SETTING

Yanin Limpanont, Phiraphon Chusongsang, Yupa Chusongsang, Jareemate Limsomboon, Prasasana Chareonjai, Suwalee Worakhunpiset, Waranya Wongwit

Southeast Asian Center for Medical Malacology (SEACMM)
Department of Social and Environmental Medicine, Faculty of Tropical Medicine
Mahidol University



The increasing of water temperature due to global warming may impact the life cycle of schistosome as well as its snail intermediate host. This study aimed to investigate the effects of water temperature on reproductive biology and development of *Biomphalaria glabrata*, cercarial production and infectivity of *S. mansoni* cercaria in mice. *B. glabrata* were reared in the aquaria where the water temperatures were at 25, 28, 30 and 32°C. *B. glabrata* reared at 28 °C gave the highest egg outputs and hatching rate. Survival rate at 25 °C was highest while the growth of snail at 32 °C was significantly higher than the other groups. In terms of cercarial emergence, *B. glabrata* infected with *S. mansoni* miracidia reared at 28 °C shedded the highest number of cercariae followed by those reared at 30, 32 and 25 °C. The duration, onset and peak of cercaria shedding and survival of infected snails from each temperature were different. Cercariae shedded from *B. glabrata* of different temperatures were used to infect the mice by tail exposure. Weekly fecal examination revealed that the mice infected with cercariae shedded from 28 °C group released the *S. mansoni* eggs one week earlier than the other groups. Adult worm burden of the mice infected with cercariae was highest in 25 °C group followed by 28, 30 and 32 °C groups. Water temperature affects the reproductive biology, development and survival of *B. glabrata* and also the life cycle of *S. mansoni* which may alter the pattern of disease transmission in final host and disease control strategy.

Keywords: *Schistosoma mansoni*, *Biomphalaria glabrata*, Temperature, Climate change

Poster No. 7

OBSERVATIONS ON LARVAL DEVELOPMENT OF *SCHISTOSOMA JAPONICUM* IN *ONCOMELANIA HUPENSIS* SNAILS

Theerakamol Pengsakul*^[1, 2], Yassir A Suleiman^[2], Zhe Cheng^[2]

¹ Faculty of Medical Technology, Prince of Songkla University, Hatyai 90112, Songkhla Province, Thailand
² School of Life Sciences, Xiamen University, Xiamen 361005, Fujian Province, People's Republic of China



At present, blood fluke infection caused by *Schistosoma japonicum* remains a major public health concern in Asia and specifically in some regions of China. The disease is transmitted to human and some other vertebrates by the invasion of infective cercarial stage of the parasite through skin of the vertebrate hosts. The cercariae are released from infected fresh-water snail, *Oncomelania hupensis*, and an important intermediate host of *S. japonicum*. In this study, post-infection gradual development of *S. japonicum* larvae was investigated and illustrated through histological serial sections of *S. japonicum* in its host *O. hupensis*.

Keywords: *Schistosoma japonicum*, *Oncomelania hupensis*, intermediate host, development

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Poster No. 8

PARAGONIMUS WESTERMANI FOUND IN MANIPUR, THE NORTHEASTERN INDIAN STATE BORDERED WITH MYANMARDr. T. Shantikumar Singh¹, Dr. Sugiyama Hiromu², Dr. L. Deben Singh³¹Sikkim Manipal Institute of Medical Sciences, Sikkim, India²National Institute of Infectious Diseases, Tokyo, Japan³Jawaharlal Nehru Institute of Medical Sciences, Manipur, India

Paragonimus metacercariae were isolated from the fresh water crab species, *Alcomonsiliosum* collected at Lailok mountain stream in Moreh town, Manipur (India) bordered with Myanmar. The metacercariae being round to oval measuring 599 x 514µm on an average were inoculated intra-peritoneally into an experimental cat. Four mature adult flukes were obtained from the worm cysts in the lungs of the infected animal. The adult flukes, used for preparing borax carmine stained mounted specimens, had an ovary that is simply divided into five to six lobes. On the basis of the morphological features the species were identified as *Paragonimuswestermani* in spite of atypical morphological features of metacercariae. DNA sequencing and or molecular phylogenetic analyses of the metacercariae and or adult flukes will be required to determine their relationship with the similar species occurring in other parts of India as well as in Southeast and East Asian countries.

Keywords: *Alcomonsiliosum*, Morphological features, *Paragonimuswestermani*, India

Poster No. 9

ORIGIN AND SPREAD OF ASPERMIC FASCIOLA SP. IN ASIA

Tadashi Itagaki, Madoka Ichikawa-Seki, Takuya Shoriki, Pannigan Chaichanasak, Peng Mao and Keisuke Sakaguchi

Laboratory of Veterinary Parasitology, Faculty of Agriculture, Iwate University, Morioka, Iwate, JAPAN



Fascioliasis is an important parasitic disease of animal and human and caused by *Fasciola hepatica* and *F. gigantica*. However, aspermic *Fasciola* sp., which seems to reproduce parthenogenically and can't be accurately identified, is distributed in Asian countries. The purpose of this study is to discuss the origin and spread of aspermic *Fasciola* sp. on the basis of molecular phylogenetical data of *Fasciola* flukes obtained from Asian countries.

[Materials and Methods] Aspermic *Fasciola* flukes obtained from Japan, Korea, China, Vietnam, Thailand, Myanmar, Nepal, Bangladesh and the Philippines, and additionally spermic *F. hepatica* and *F. gigantica* flukes were used. Nuclear ITS1 types and mitochondrial *nadI* sequences of the flukes were determined by using PCR-RFLP and direct sequencing, respectively, and the sequences were analyzed phylogenetically.

[Results and Discussion] Three ITS1 types, Fh, Fg and Fh/Fg, were discriminated among aspermic flukes and identical to fragment patterns of *F. hepatica* and *F. gigantica*, and mixed pattern of both species, respectively. Occurrence of Fh/Fg type suggests that aspermic *Fasciola* sp. is hybrid descendents between *F. hepatica* and *F. gigantica*. Two main *nadI* haplotypes, Fg-C2 and Fh-C4, were detected in aspermic *Fasciola* sp. and belonged in *F. gigantica*- and *F. hepatica*-haplogroup, respectively and differed by 45/535 nucleotides. Further, both of the haplotypes were also detected in *F. gigantica* and *F. hepatica* from China but not from the other countries. These findings suggest that aspermic *Fasciola* sp. originated in China due to interspecific hybridization between *F. hepatica* and *F. gigantica*, and spread to neighboring countries together with host animals, possibly domestic cattle.

Keywords: Aspermic *Fasciola* sp., Asia, ITS1, *nadI*, phylogeny

Poster No. 10

EVIDENCE OF A SPECIES COMPLEX AND GENETIC STRUCTURE OF SOUTHEAST ASIAN *ECHINOSTOMA REVOLUTUM* (DIGENEA: ECHINOSTOMATIDAE) IN THAILAND AND LAO PDR

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^a Division of Cell Biology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Rangsit Campus, Pathumthani 12121, Thailand

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Objective - This study aims to explore the genetic structure and genetic variation of *Echinostoma revolutum* in Thailand and Lao PDR and also compare to the isolates from North America and Europe.

Methods - Totals of 41 adult *E. revolutum* from Thailand and Lao PDR were collected from domestic ducks. Partial sequence of cytochrome c oxidase subunit 1 (CO1) gene was sequenced. The 20 sequences of *E. revolutum*, 19 sequences of the isolates from North America and one sequence from Europe were retrieved from GenBank database. Phylogenetic tree and haplotype network were constructed.

Result - The variation within the 188 nucleotide positions of mitochondrial CO1 sequences after trimming was observed at 29 positions (15.43%). The 11 haplotypes of the isolates from Southeast Asia, 14 haplotypes of the isolates from North America and one haplotype of an isolate from Europe were generated. The phylogenetic tree and haplotype network revealed that *E. revolutum* was classified into three different lineages. The first lineage comprises of four haplotypes from Thailand, which was closely aligned with the second lineage of 14 haplotypes of the isolates from North American. The third lineage consists of seven haplotype from Thailand and Lao PDR and one haplotype from Europe.

Keywords: *Echinostoma revolutum*, species complex, genetic structure, CO1

Poster No. 11

FISHBORNE TREMATODE METACERCARIAE DETECTION IN FERMENTED FISH DISHES FROM 20 PROVINCES IN NORTHEASTERN THAILAND

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Fishborne trematode (FBT) including *Opisthorchis viverrini* and minute intestinal fluke (*Haplorchis* spp. and *Centrocestus formosanus*) are endemic in northeastern Thailand. FBT infection is caused by eating raw or inadequately cooked dishes contaminated with infective stage, metacercaria. In the present study, to increase public health awareness for prevention of FBT caused by eating raw cooked dishes, we investigated FBT metacercaria in fermented food (*pla-ra* and *pla-som*) obtained from 73 markets in 20 provinces of northeastern Thailand between April and November 2011. A cross-sectional survey was conducted from 93.9 kg of fermented food consisting of 3,607 fishes. Fermented food was weighed, identified fish species, counted, and digested using pepsin-HCl. Samples were examined for FBT metacercaria by a sedimentation method, and identified under a stereomicroscope. The result revealed that FBT metacercariae were detected in 5 provinces: Si Sa Ket, Sakon Nakhon, Mukdahan, Khon Kaen and Udon Thani. The prevalence of FBT metacercariae in fermented food was 9.6 % (7/73) of markets. Among these, 11.1 % (6/54) was found in *pla-ra* and 5.3% (1/19) was found in *pla-som*. FBT metacercariae were identified in four fish species—*Henicorhynchus siamensis*, *Puntius bimaculatus*, *Puntius orphoides* and *Hampala dispar*. Based on morphological identification, *O. viverrini* metacercariae were not identified in *pla-som* and *pla-ra* in this survey. In conclusion, FBT metacercariae could be detected in fermented food preparation from natural cyprinid fishes which may have a risk for infection with the infective stage FBT.

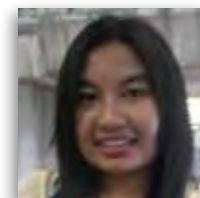
Keywords: Fishborne trematode metacercariae, freshwater cyprinid fish, *pla-ra*, *pla-som*, fermented food
Acknowledgements: The Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Health Cluster (SHeP-GMS681).

Poster No. 12**CERCARIAL INFECTIONS OF BRACKISH WATER SNAILS IN THE EAST COAST OF SOUTH THAILAND**

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Brackish water snails are suspected, as intermediate host of trematode diseases in vertebrates. The aim of this study investigated cercarial infections of brackish water snails in the east coast of South Thailand. Snails were collected from fifteen locations in three provinces (Phachuap Khiri Khan, Chumphon and Suratthani) in June 2013. Selected by two criteria studied areas were; they were mangrove forest and estuary of river. Five collectors picked the snails by hand for 10 minutes each station. The emerged cercariae by shedding and crushing methods were identified based on their morphological characteristics. A total of 2,382 brackish water snails were classified into six families, consisted of Neritidae, Littorinidae, Stenothyridae, Thiariidae, Potamididae and Assimineidae. Two families found trematode infections, they were family Potamididae (*Cerithidea cingulata*) and family Thiariidae (*Sermyla riqueti*). The infection rates were 3.33% (3/90) and 0.9% (1/111), respectively. The cercariae were categorized into two species, consisted of *Cercariae megulura* and *Euhaplorchis californiensis*.

Keywords: brackish water snail, infection, cercaria, trematode, *Cercariae megulura*, *Euhaplorchis californiensis*, Southern Thailand

Poster No. 13**TREMATODE INFECTIONS OF FRESHWATER SNAILS AT POND OF SILPAKORN UNIVERSITY SANAMCHANDRA PALACE, NAKHONPATHOM, THAILAND**

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Freshwater snails have been reported as the first and second intermediate hosts of trematodes such as minute intestinal flukes and liver flukes, which cause human and animal diseases. The aim of this study invested trematode infections of snails at Silpakorn University. Freshwater snails were collected between September 2012 and January 2013 from 12 locations of Srakaew pond at Silpakorn University, Nakhonpathom, Thailand. Counts per unit of time method was used for collecting snails, and the samples of snails were collected every 10 minutes per sampling by five collectors. The snails were classification by morphology. A total of 3,567 freshwater snails samples from 10 species were found: *Bithynia siamensis siamensis* (601), *Filopaludina javanica* (533), *Filopaludina sumatrensis polygramma* (264), *Tarebia granifera* (1463), *Clea* sp. (127), *Thaira* sp. (112), *Melanoides tuberculata* (453), *Promacea* sp. (8), *Thiara scaba* (6) and *Pila* sp. (55). The snails were examined cercariae infection by shedding and crushing methods. Six species of cercariae were categorised; they were *Acanthatrium hitaense*, *Loxogenoides bicolor*, *Haplorchis pumilio*, *Astiotrema monticellii*, *Paramphistomum* sp. and *Cadicola alseae*. The infection rate was 2.89% (103/3,567). *Bithynia siamensis siamensis* was the most species that found high infection rate. It was 12.59% (74/601).

Keywords: Trematode infection, snails, cercariae, infection rate

Poster No. 14

TREMATODE INFECTIONS IN BRACKISH WATER AND SOME MARINE SNAILS FROM THE COAST OF ANDAMAN SEA IN THAILAND

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The various brackish water and some marine snails inhabiting along the coast of Andaman sea in Thailand were examined larval stage of trematodes. This work collected snails from 12 areas, 4 provinces (Ranong province, Phangnga province, Krabi province and Trang province), in February 2013 and June 2013. The sampling areas were studied in mangrove forests, estuary of river and canal with salinity between 0.5 – 30.0 ppt. A total of 985 snails were classified into nineteen species. They were *Nerita (Nerita) articulata*, *Nerita (Theliostyla) planospira*, *Clithon (Pictoneritina) oualaniensis*, *Clithon (Clithon) sowerbyana*, *Nerita (Dostia) violacea*, *Littorinopsis melanostoma*, *Littorinopsis scabra*, *Assimineia (Sphaerassimineia) breviculata*, *Cerithidea (Cerithideopsilla) cingulata*, *Cerithidea (Cerithideopsilla) alata*, *Cerithidea (Cerithidea) weyersi*, *Cerithidea (Cerithidea) obtusa*, *Cerithidea (Cerithidea) quadrata*, *Telescopium telescopium*, *Faunus ater*, *Cassidula mustelina*, *Chicoreus capucinus*, *Planaxis sulcatus* and *Pagilina cochlidium*. *Cerithidea (Cerithideopsilla) cingulata* was the most common species. Larval stages of trematodes were examined by shedding and crushing methods, they were identified for species of trematodes. The infection rate was 0.20% (2/985). Two species of trematodes were categorized, they were *Parochis avitus* and *Echinoparyphium recurvatum*. Two species of collected snails were found trematode infections; *Cerithidea weyersi* and *C. alata*. The infection rates were 1.06% (1/94) and 0.58% (1/171), respectively.

Keywords: brackish water snails, intermediate host, trematode infection, Andaman sea

Poster No. 15

DEVELOPMENT OF MULTIPLEX PCR FOR DETECTION OF SOIL-TRANSMITTED HELMINTHS IN HUMAN FECAL SAMPLES

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Ascariasis, Trichuriasis, and hookworm infections are an important soil-transmitted helminth (STH) infection caused by three major species of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Necator americanus*, respectively. Microscopic examination of fecal samples has been a gold standard for diagnosis of Ascariasis, Trichuriasis, and hookworm infections for many years. However, is time-consuming and low sensitivity, which may result in misdiagnosis in case of light infection. Recently, multiplex real-time PCR has been introduced for detection of STH infection. However, in a routine diagnostic laboratory of these STH is costliness when compare with microscopic examination. Therefore, we aim to developed conventional multiplex PCR assay, which lower cost, simple, and rapid method by detected major STH infection including *A. lumbricoides*, *T. trichiura*, and *N. americanus* in fecal samples. In this study, the multiplex PCR assay was validated the sensitivity and specificity for detection of STH infections in fecal samples by comparing with microscopic examination. The result showed positive in 133 of 156 samples (85.26%) for microscopic examination and 120 of 156 samples (76.92%) for multiplex PCR assay. When compare with microscopic examination, multiplex PCR assay showed 87.22% and 82.61% for sensitivity and specificity, respectively. In conclusion, the multiplex PCR assay described here provides; a high sensitivity and specificity diagnostic tool for detection three major species of STH in fecal sample, which can be used as an alternative method for routine diagnostic laboratory. Moreover, the method can be applied for the epidemiological study of STH in endemic areas and monitoring of treatment programs.

Keywords: Soil-transmitted helminth, Multiplex PCR, Specificity, Sensitivity

Poster No. 16

NON-ENCAPSULATED *TRICHINELLA* SPP., *T. PAPUAE*, DIMINISHES SEVERITY OF DSS-INDUCED COLITIS IN MICEPoom Adisakwattana¹, Supaporn Nuamtanong¹, Teera Kusolsuk¹, Montri Chairoj², Pa-Thai Yenchitsomanas³ and Urai Chairiri⁴

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Background: Helminths use various mechanisms to avoid host immunity and protect themselves from being eliminated. Despite evading host immune responses, immunosuppression and regulation mechanisms elicit functions that diminish the adverse effects of unrelated inflammatory diseases.

Objective: We investigated whether helminthic infections can ameliorate inflammatory diseases.

Methods: Mice were infected with *Trichinella papuae* and then subjected to induced colitis through the oral administration of dextran sulfate sodium (DSS). Macroscopic and microscopic examinations measured weight loss, stool consistency, gross bleeding, colon length, and tissue inflammation. In addition, cytokine expression was observed in colon tissue by SYBR real-time RT-PCR to investigate the Th1, Th2, and regulatory cytokines.

Result: The results showed that *T. papuae* infection decreased the severity of DSS-induced colitis, including weight loss, bloody diarrhea, shortening of colon, and colon tissue damage in mice ($p < 0.05$). The expression level of IL-4 was high in the colons of DSS-treated mice without helminthic infection, while infected mice with DSS treatment had lower IL-4 levels ($p < 0.05$). Uninfected DSS-treated mice failed to produce IL-10 mRNA in colon tissue, which may cause more severe colitis. In contrast, prior to *T. papuae* infection DSS-treated mice had IL-10 levels in the colon significant lower than the normal and infected control groups.

Conclusion: Our data provide the evidence that prior *T. papuae* infection can ameliorate DSS-induced colitis in mice and may be considered for a novel therapeutic strategy against immunological diseases in the future.

Keywords: *Trichinella papuae*, colitis, inflammatory bowel diseases, Th1 and Th2 cytokines, regulatory cytokines

Poster No. 17

SUSCEPTIBILITY TO IVERMECTIN IN INFECTED MICE BY *TRICHINELLA SPIRALIS*.

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Trichinellosis is a zoonosis with world-wide distribution caused by *Trichinella spiralis*, which the important problem in global public health and not yet completely successful treatment by antihelminthic drugs, such as Albendazole, Mebendazole, etc. The previous research showed that these antihelminthic drugs could not be completely eliminating and preventing the complication such as inflammatory reactions in tissue and internal organs especially encyst larvae formation in muscles in which caused, be chronic myalgia and myositis. Therefore, any researchers try to study, how to treatment of Trichinellosis, effectively. This objective was concerned to assess susceptibility of Ivermectin treatment performed by ICR mice with 300 *Trichinella spiralis* larvae under treatment with the single dose of Ivermectin (200 µg/kg) via subcutaneous injection during study periods, on day post infection (DPI). Detection of counting number of parasites under stereomicroscopic examination and inflammatory reaction was evaluated under histological technique as hematoxylin and eosin

staining examination. The effects of Ivermectin could be reduced the number of parasites yield 6% to 66% on the 1th DPI to 32nd DPI respectively, on the 1th DPI was statistical significantly at p-value = 0.00. However, the inflammatory reaction in various tissue and internal organs was not reduced, not statistical significantly at P-value > 0.05. These results suggest that the effective of single dose Ivermectin (200 µg/kg) depending on the date of treatment after infection. Therefore, optimizing time for treatment should be within 6th DPI according to efficacy to reduce the number of parasites by Ivermectin treatment up to 54% to 66%.

Keywords: *Trichinella spiralis*, Ivermectin, inflammatory reaction, parasites number

Poster No. 18

EVALUATION OF DOT IMMUNOGOLD FILTRATION ASSAY (DIGFA) FOR RAPID DIAGNOSIS OF EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* (NEMATODA: METASTRONGYLOIDEA) USING SERUM SAMPLES FROM ENDEMIC AREAS IN THAILAND

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Objective: This study tested serum samples from eosinophilic meningitis patients and individuals at risk of infection with *Angiostrongylus cantonensis* but with no evidence of eosinophilic meningitis, in the parasite endemic areas of northeast Thailand, to evaluate a recently developed simple, rapid, sensitive and specific dot-immunogold filtration assay (DIGFA) versus dot-blot enzyme-linked immunosorbent assay (ELISA) for detection of specific antibodies against *A. cantonensis*.

Materials and Methods: Purified 31-kDa glycoprotein of *A. cantonensis* was used as specific antigen and protein A colloidal gold conjugate as color developing agent. DIGFA was employed to detect a corresponding 31-kDa anti-*A. cantonensis* antibody in patients sera from endemic areas in Thailand. The results were compared with those obtained by enzyme-coupled, dot-blot ELISA with a 31-kDa *A. cantonensis* antigen.

Results: The overall positivity rate of DIGFA and dot-blot ELISA for *A. cantonensis* infection in the sera from 98 clinically diagnosed cases with eosinophilic meningitis from three highly endemic districts in Khon Kaen province, *i.e.* Phu Wiang (n=39), Waeng Yai (n=26), and Phra Yuen (n=33), were 39.79% (39/98) and 37.75% (37/98), respectively. Among 86 sera of subjects at risk of infection with *A. cantonensis* - Phu Wiang (n=37), Waeng Yai (n=27), and Phra Yuen (n=22) - 24.41% (21/86) were positive by DIGFA and 23.25% (20/86) by dot-blot ELISA. There were good correlation between the visual grading of DIGFA and dot-blot ELISA in both groups of defined sera.

Conclusion: DIGFA is as sensitive and specific as dot-blot ELISA for detecting eosinophilic meningitis due to *A. cantonensis* infection, but has the advantages of simplicity, rapidity and without the use of specific/expensive equipment, and can be used in field settings.

Keywords: *Angiostrongylus cantonensis*, eosinophilic meningitis, serodiagnosis, rapid test, DIGFA, dot-blot ELISA

Poster No. 19

A REAL TIME PCR WITH HIGH RESOLUTION MELTING ANALYSIS FOR DIAGNOSIS OF LYMPHATIC FILARIAL INFECTION

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Lymphatic Filariasis (LF) or elephantiasis is caused by filarial nematodes i.e. *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. Over a billion people are at risk of contracting lymphatic filariasis (LF) and about 40 million people suffer from severe disfigurement and disability. Diagnosis is an essential element in the management of disease, both at the level of individual patient care and at the level of disease control in endemic populations. Because of the anticipated success of filariasis elimination programs, decreasing the diagnosis sensitivity of microfilariae detection. We applied a real time PCR with high resolution melting analysis (HRM) to detect and identify *B. malayi* and *W. bancrofti* in human blood samples. The assay can be performed using a single pair of primers targeting the mitochondrial partial 12S rRNA genes of these filarial parasites. Amplification and detection steps can be conducted in the same reaction well, and no post-PCR processing is required. The HRM assay showed a specific melting temperature for each species. The assay showed high sensitivity and specificity in comparison with microfilaria detection method after assessment with 30 blood samples. Thus, the assay could be helpful for diagnosis of lymphatic filariasis.

Keywords: *Brugia malayi*, *Wuchereria bancrofti*, real time PCR, High Resolution Melting, lymphatic filariasis

Poster No. 20

IDENTIFICATION OF ANISAKID NEMATODE SPECIES IN CLINICAL SPECIMENS BY USING NESTED POLYMERASE CHAIN REACTION

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Anisakid nematode larvae cause anisakiasis, a zoonotic helminthiasis, following the consumption of raw or undercooked fish. Anisakid nematodes isolated from infected patients are sexually immature and lack detailed morphological characteristics, and molecular methods have been established to identify their species. Generally, the ribosomal DNA internal transcribed spacer (ITS) region or the mitochondrial cytochrome *c* oxidase subunit 2 gene is used as a genetic marker for the molecular identification methods. However, the marker sequences are difficult to amplify using conventional polymerase chain reaction (PCR) with DNA templates prepared from formalin-fixed nematodes because formalin fixation causes DNA fragmentation. Therefore, we developed a nested PCR assay to amplify the ITS1 region. The primer pair employed for the second-round PCR was newly designed based on DNA sequences in the ITS1 region specific to several anisakid species such as *Anisakis simplex* sensu stricto, *A. pegreffii*, *A. physeteris*, and *Pseudoterranova decipiens*, which cause human illnesses. Using this nested PCR assay, amplicons (e.g., 208 bp for *A. simplex* sensu stricto) were generated from specimens isolated from patients and were then fixed in formalin. This method was applicable to paraffin-embedded anisakid nematode sections obtained from clinical specimens. To date, nucleotide sequencing of the PCR amplicons has revealed that the predominant causative species of anisakiasis is *A. simplex* sensu stricto.

Keywords: nested PCR, anisakiasis, foodborne helminth, molecular diagnosis

Poster No. 21

PREVALENCE AND IMPACT OF INTESTINAL PARASITIC INFECTIONS IN PREGNANT WOMEN IN 3 HEALTH CENTERS ALONG THE THAI-MYANMAR BORDER, SAUN PHUNG DISTRICT, RATCHABURI PROVINCE: FIELD BASE STUDY

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This prospective cohort study aimed to determine prevalence and the impact of intestinal parasite among pregnant women. The study was conducted in three health centers: Tanowsri, Ban Borwee, and Ban Huaypak, of Suanphung district, Ratchaburi province. All participated pregnant women who attended the antenatal care clinic (ANC) were test for stool examination and Hemoglobin level three times at gestational age of 1-6 months, 7-8 months, and 9 months. Risk factors for intestinal parasitic infection, including demographic characteristics, socioeconomic status, and behaviors were also collected. Data on baby birth weight was also collected. These factors were then used to compare between pregnant women who had intestinal parasite and those who had not intestinal parasitic infection. All infected cases were advice to consult physician for appropriate treatment.

Overall, 155 pregnant women were included in the study. The majority of the study population (73%) were Karen/Karan ethnicity (N=113); the rest of the population were Thai (N=42). The participants aged between 16 and 43 years old, with mean age of 25.37 (SD=6.47). About 60% of the pregnant women were labor, and about 35% were housewife. More than half of the pregnant women have no education. Most of pregnant women (87%) had latrine at their house. Stool examination revealed that half of the pregnant women (49.7%) had intestinal parasitic infection (N=77). The parasites found include hookworm (15%), Trichuris (9%), and fluke (N=4%). Factor significantly associated with intestinal parasitic infection was age ($p < 0.05$). Pregnant women who aged older than 25 years old were about 6-7 times higher risk of parasitic infection than those who aged 16-19 years. Other factors such as occupation, ethnicity, education, type of latrine, hand washing, using spoon, wearing shoes, eating vegetable, or eating meat did not show significant association with parasitic infection ($p > 0.05$). The mean hemoglobin level among the pregnant women was 11.07 (SD1.47) g/dl (range 6.4-14.3 g/dl). Pregnant women who had parasitic infection were more likely to have Hb less than 12 g/dl ($p < 0.05$). However, the association between parasitic infection and low birth weight was not statistically significant ($p > 0.05$). Findings of this study suggested that parasitic infection is still a major health problem, especially in remote area along the international border.

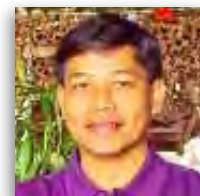
Keywords: intestinal parasitic, pregnant women

Poster No. 22

PREVALENCE OF HELMINTHIASIS IN 7 PROVINCES, IN THE UPPER NORTHEASTERN OF THAILAND, 2013

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Helminthiasis were situated in Thailand long times. Especially fishborne trematode (FT) named *Opisthorchis viverrini* (*O. viverrini*) infection was a serious public-health problem in the Northeastern of Thailand. It had evidence strongly associated with cholangiocarcinoma (CCA). This study aimed to determine of *O. viverrini* and others intestinal helminthes infection in the study area. This cross-sectional study was conducted in 2013. Participants, 15 years old or more from 7 provinces were enrolled by using 30 clusters sampling technique and stool were examined by modified Kato-Katz technique, using descriptive statistics. 4,439 stool samples were obtained from 2,028 males and 2,411 females, aged 15-84 years old, mean = 47.33 ± 15.15 years. Stool examination showed that 25.07% were infected with at least 1 parasite. Males were slightly more be infected than females (56.13% and 43.87%). Prevalence of *O. viverrini*, *Taenia spp.*, *Strongyloides stercoralis*, Echinostome, Hookworm and others were 22.08%, 1.55%, 2.75%, 0.65%, 0.50 and 0.22% respectively. *O. viverrini* infection was most frequent in the 51-60 age group (28.15%), the highest prevalence was in Nakorn Phanom (40.94%,). Highest of positive rate was 80.00%, however, there were 94 clusters (44.76%) that more than 20%. For mean egg per gram feces (MEPG) was 323.81 eggs, most of intensity was low level (94.08%). The study suggests that Opisthorchiasis is still a problem in the study area, and were loaded by the elder groups more than the younger. There were some areas had high positive rate (>20%) most of them loaded in low level. Furthermore studying for the way to change risk of infectious behaviors for decreasing morbidity of opisthorchiasis and CCA were necessities.

Keywords: Helminthiasis, Prevalence, Cholangiocarcinoma

Poster No. 23

PYROSEQUENCING FOR RAPID MOLECULAR IDENTIFICATION OF
TAENIA SAGINATA, *T. SOLIUM* AND *T. ASIATICA*

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PCR coupled with DNA pyrosequencing was developed for discrimination of human taeniid, *Taenia solium*, *T. saginata*, and *T. asiatica* samples collected from various countries: France, Brazil, Sudan, Ethiopia, Nepal, India, Vietnam, Japan, Laos and Thailand. New primers targeting partial sequences of the human Taeniid mitochondrial *cox1* gene were designed. This region reveals nucleotide variations at 26 positions were used for differentiation. The method is a rapid and high throughput. The technique is a valuable tool for the species-specific differentiation of taeniids eggs without the requirement of proglottid samples. It is valuable used for the parasite DNA diagnosis in suspicious cysts, suspect lesions or cyst residues, in domestic animals at the slaughterhouse or humans.

Keywords: pyrosequencing, *Taenia solium*, *T. saginata*, *T. asiatica*, identification

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Poster No. 24

COMPLEMENTATION ANALYSIS OF *PLASMODIUM FALCIPARUM* GTP
CYLOHYDROLASE I IN A BACTERIAL SURROGATE SYSTEM

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GTP cyclohydrolase I (GCH1) is the first enzyme in the folate pathway. It plays a key role in the development of antifolate resistance in *Plasmodium falciparum*. Surprisingly, little is known about the structure and function of *P. falciparum* GCH1. To characterize the function of *P. falciparum* GCH1, we developed a complementation method for the study of the gene function in a bacteria surrogate model. DNA plasmid containing wild-type and *gch1* mutants were constructed by DNA cloning in pBAD expression vector with a tightly regulated promoter. Growth analysis was observed in the bacteria without endogenous GCH1 under genetic complementation by *P. falciparum* GCH1. We studied the condition that can test the activity of *P. falciparum* GCH1 in bacteria. The complementation method can be further used in structure-activity relationship studies of *P. falciparum* GCH1.

Keywords: GTP cyclohydrolase I and complementation

Poster No. 25

INNOVATIVE AND INTEGRATIVE MALARIA EDUCATION PROGRAM IN TRIBAL REGIONS OF INDIA AFFECTED BY FALCIPARUM MALARIA

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Background: Rampachodavaram, a densely forested tribal area, has one of the highest incidence of malaria in India [1]. Our past surveys and fieldwork recognized the lack of malaria education as a major contributing factor [2]. We conducted Malaria education workshops for high school students and tribal healthcare workers.

Materials and Methods: The workshop consisted of a pretest and posttest, a PowerPoint presentation, a 3D graphic movie dubbed into the natives' language (Telugu) [3], a demonstration of homemade mosquito trap built with local materials, and an open discussion. Take home handouts consisted of a malaria vocabulary list, malaria lifecycle, and instructions for making mosquito trap.

Results: Statistical analysis of pre and posttests revealed 4% increase in correct answers among the high school students. However, no pattern of information retention can be found after test analysis.

Furthermore, 42.6% of the malaria health workers missed the question: "What is malaria?" This shows insufficient malaria literacy.

There was an overwhelming positive response from the participants that the program was unique and comprehensive in the delivery of malaria education.

Conclusions: Integration of an educational video with 3D graphics, lecture, and hands on activities provided a platform of multiple learning styles, engaging the participants providing great learning experience.

The workshops revealed the knowledge gaps within the community. Continued effort must be focused on education and active community engagement in the malaria control, prevention, and treatment.

Keywords: Malaria, educational work shop, 3D video graphics

Poster No. 26

ACTIVATION OF MAST CELLS IN THE SKIN OF *P. FALCIPARUM* PATIENTS

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Background: Mast cells (MCs) play an important role in immune and inflammatory processes. MCs can be stimulated to degranulate and release histamine upon binding to immunoglobulin E (IgE), and have been linked to IgE-antimalarial antibodies. This study investigated the response of MCs in the skin of *P. falciparum* malaria patients.

Methods: The skin tissues of *P. falciparum* malaria were classified into uncomplicated (10), complicated (20) malaria and normal skin tissues (29) which served as controls. Tissues from pre- and post-treatment were included. MCs were investigated by toluidine stain. Other histopathological features evaluated were extravasation of blood, perivascular edema and leukocyte infiltration. The percentage of MCs degranulation was compared among groups and correlated with clinical data.

Results: MCs degranulation was seen highest in complicated *P. falciparum* ($44.15\% \pm 3.20$) group as compared to uncomplicated ($33.25\% \pm 2.99$) and control groups ($19.84\% \pm 1.42$), ($p < 0.05$). Associated pathological features including extravasation of blood, perivascular edema, and leukocytic infiltration were significantly increased between malaria groups and when compared with control group (all $p < 0.001$).

Conclusions: MCs are activated during malaria infection and degree of degranulation is correlated with disease severity.

Keywords: Mast cells, *P. falciparum*, malaria, skin, toluidine blue stain, immunohistochemistry

Poster No. 27

CHARACTERIZATION OF *PLASMODIUM VIVAX* CALCIUM DEPENDENT PROTEIN KINASE (PvCDPK)

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Sexual stage in mosquito intestine is the most abundant physiological-biochemical phenomena in the complex malaria life cycle. In sexual stage, gametocytes secrete variety biochemical signals for induction of flagellum of microgametocyte and fertilization of nucleus from microgametocyte and macrogametocyte is occurred. Calcium dependent protein kinase (CDPK) is the key regulators that mediate flagella induction from microgametocyte to fertilization with macrogametocyte in sexual stage. In *P. falciparum*, CDPKs have three kind isoform with similar structure and express in sexual stage. We extracted genomic DNA of Korea *P. vivax* strain from patients' blood and cloned CDPK gene. PvCDPK gene is 1,762 bp of open reading frame (ORF) and deduced 587 amino acids. PvCDPK has an intron between two exons and constructs serine/threonine domain, junction domain and calmodulin like domain (EF-hand). In N-terminus, variety domains for serine/threonine and multi consensus phosphorylation sites coded protein kinase were identified. We expressed recombinant protein of about 62 kDa and measured kinase activity used Kinase-Glo Reagent (Promega). Protein kinase activity of PvCDPK proportionally increased the recombinant protein, ATP and Ca²⁺ concentration, and gradually decreased the EGTA, Ca²⁺ chelator. PvCDPK activity depended on ATP and Ca²⁺ ion and inhibited by EGTA. These data suggest that PvCDPK is functional serine/threonine kinase, regulated by calcium, and may have a role in Ca²⁺-mediated signaling in *P. vivax*.

Keywords: *Plasmodium vivax*, calcium dependent protein kinase, kinase activity

Poster No. 28

INCREASED DETECTION OF *PLASMODIUM KNOWLESII* IN SANDAKAN DIVISION, SABAH AS REVEALED BY PLASMONEX™

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Plasmodium knowlesi is a simian malaria parasite that is widespread in humans in Malaysian Borneo. However, little is known about the incidence and distribution of this parasite in the Sandakan division, Malaysian Borneo. Therefore, the aim of the present epidemiological study was to investigate the

incidence and distribution of *P. knowlesi* as well as other *Plasmodium* species in this division based on a most recent developed hexaplex PCR system (PlasmoNex™). A total of 189 blood samples were collected from BFMP-positive patients who attended Telupid Health Clinic, Sabah, Malaysia from 2008 to 2011. Identification of *Plasmodium* species was conducted using PlasmoNex™ targeting the 18S SSU rRNA gene. Results showed that a total of 178 samples were positive for *Plasmodium* species by using PlasmoNex™. *Plasmodium falciparum* was identified in 68 samples (38.2%) followed by 64 cases (36.0%) of *Plasmodium vivax*, 42 (23.6%) cases of *P. knowlesi*, two (1.1%) cases of *Plasmodium malariae* and two (1.1%) mixed-species infections (i.e, *P. vivax/ P. falciparum*). Thirty-five PlasmoNex™ positive *P. knowlesi* samples were misdiagnosed as *P. malariae* by microscopy. *Plasmodium knowlesi* was detected in all four districts of Sandakan division with the highest incidence in Kinabatangan district. The discovery of *P. knowlesi* in Sandakan division showed that prospective studies on the epidemiological risk factors and transmission dynamics of *P. knowlesi* in these areas are crucial in order to develop strategies for effective malaria control. The availability of advanced diagnostic tool PlasmoNex™ enhanced the accuracy and accelerated the speed in the diagnosis of malaria.

Keywords: *Plasmodium knowlesi*, Sandakan division, PlasmoNex™

Poster No. 29

GENETIC POLYMORPHISMS AND POPULATION STRUCTURE ANALYSIS OF GENE ENCODING CIRCUMSPOROZOITE PROTEIN (CSP) OF *PLASMODIUM KNOWLESI* ISOLATES FROM MALAYSIA

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There are limited studies conducted on genetic polymorphisms and population structure of CSP gene of *Plasmodium knowlesi* (*PkCSP*). Amplification of *PkCSP* gene was done by PCR on DNA extracted from clinical and primate *Plasmodium knowlesi* isolates from peninsular Malaysia (n=72). The PCR product was purified and cloned using pGEM®-T Vector System. Three positive clones were sent for sequencing. For further analysis, *PkCSP* sequences from other regions of Malaysia from Genbank databases were included (n=51). Sequence that encode the non-repeat N-terminal and C-terminal regions of the *PkCSP* gene were analysed and aligned with CLUSTAL-W using MEGA 5 programme. Phylogenetic tree was constructed using neighbour joining methods and bootstrap replicates of 1000. Measures of polymorphic sites (S), haplotypes diversity (Hd), nucleotide diversity (π), number of segregating sites (S), average number of pairwise nucleotide differences within the population (K), π diversity, fixation index (F_{ST} value), recombinant parameter, minimum number of recombinant event (Rm) and linkage disequilibrium (LD) between polymorphic sites were calculated using DnaSP version 5 programme. The numbers of synonymous (dS) and nonsynonymous (dN) substitutions were estimated and compared using Z-test ($P < 0.05$) in MEGA5 programme using the Nei and Gojobori's method with the Jukes and Cantor correction. Tajima's D test statistic was applied to measure the neutral theory of evolution. 73 haplotypes and 58 amino acid changes are identified among *PkCSP* isolates analyzed. Phylogenetic and population structure analysis showed that *PkCSP* of both peninsular Malaysia and Borneo population belong to the same population group.

Keywords: *Plasmodium knowlesi*, PkCSP, CSP, Malaysia, phylogenetic, polymorphic

Poster No. 30

ALTERATION OF ZONULA OCCLUDENS-1 (ZO-1) IN ACUTE KIDNEY INJURY IN SEVERE MALARIA

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Background: Zonula occludens-1 (ZO-1) proteins are scaffolding proteins providing the permeability barrier at the cytoplasmic surface of intercellular junctions. Maintenance of cell junction is important to balance the fluids and substances between compartments. In *Plasmodium falciparum* malaria, AKI is a major complication causing fatality and it will be valuable to investigate ZO-1 in kidney tissues and endothelial cells (ECs) in severe *P. falciparum* malaria.

Methods: Using immunohistochemistry, paraffin-embedded kidney tissues of fetal malaria were stained with rabbit polyclonal anti-ZO-1. For immunofluorescence, ZO-1 was demonstrated in ECs-malaria parasite co-culture. The expression of ZO-1 and degree of intensity were compared.

Results: Significant decreased in ZO-1 expression of AKI and non-AKI cases was observed in comparison to control kidney samples (21.8 ± 4.3 , 85.9 ± 10.8 vs 171.4 ± 17.5 ; $p < 0.001$, $p = 0.006$, respectively). AKI cases also showed significant decreased in ZO-1 expression as compared to non-AKI cases ($p < 0.001$). Immunofluorescence study showed significant alteration of ECs tight junction in malaria cases when compared with control.

Conclusions: The loss of protein tight junction (ZO-1) is associated with AKI. This is confirmed by alteration of ECs tight junction demonstrated in ECs-malaria parasite co-culture system.

Keywords: *Plasmodium falciparum*, kidney, acute kidney injury, Zonula occludens-1, ZO-1, immunohistochemistry

This research work was supported by the Office of National Research Council of Thailand (NRCT). BW received The Outbound Exchange Student Programme 2013 from the Faculty of Tropical Medicine, Mahidol University, Thailand to work at Liverpool School of Tropical Medicine, UK under the supervision of Prof. Steve Ward.

Poster No. 31

DEVELOPMENT OF SALIVARY IGA ANTIBODY DETECTION KIT FOR ACCURATE DIAGNOSIS OF AMEBIASIS

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An amebiasis detection kit was developed based on detection of anti-*Entamoeba histolytica* IgA antibodies present in the saliva of patients. The ELISA-based detection kit was tested along with microscopy and PCR detection on saliva and stool samples from 110 asymptomatic individuals from the Public Health Department of Manila City Hall. A receiver operating curve (ROC) was constructed to compare the kit results with PCR results as the "gold standard". *E. histolytica* infection was detected in 18 of the 110 individuals. The developed kit had a sensitivity of 83.3%, specificity of 94.6%, and accuracy of 92.7% if 1:2 dilution of crude saliva sample in phosphate buffer saline (PBS) were used for diagnosis. The developed method presents an easy and accurate method of detecting amebiasis in patients that makes use of saliva samples instead of stool or blood samples.

Keywords: Amebiasis, *Entamoeba histolytica*, salivary IgA, ELISA, diagnosis

Poster No. 32

SCREENING OF ANTI-AMOEBIC ACTIVITY ON *NAEGLERIA FOWLERI* IN EXTRACTS OF THAI MEDICINAL PLANTS

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Background: *Naegleria fowleri*, the causative agent of primary amoebic meningoencephalitis (PAM) which most of patients died within 5-7 days after infection. Owing to the treatment is not success therefore a better guideline for the chemotherapy of PAM should be improved. Aims of this study were to evaluate the efficacies of plants-based extract against *Naegleria fowleri* trophozoites.

Method: Present studies investigated the anti-amoebic activity of standardized extracts of *Andrographis paniculata*, *Curcuma longa*, *Momordica charantia* and *Zingiber officinale* on *Naegleria fowleri* trophozoites by staining method and MTT assay.

Results: In this study, the maximum non-toxic dose (MNTD) of the four medicinal plants was determined by testing the plants-based extracts against LLC-MK2 and SK-N-MC *in vitro*. The maximum non toxic dose across two cell lines tested was exhibited by *Andrographis paniculata*, *Curcuma longa*, *Momordica charantia* and *Zingiber officinale* extracts at 0.5, 0.5, 5 and 1 mg/ml, respectively. The activity of crudes extracts against *N.fowleri* trophozoites revealed that IC₅₀ of *Andrographis paniculata* and *Momordica charantia* were 0.12 and 3.11 mg/ml whereas *Curcuma longa* and *Zingiber officinale* were 0.69 and 1.72 mg/ml at 6 h post incubation.

Conclusions: The extracts of *Andrographis paniculata* and *Momordica charantia* showed anti-amoebic activity in *in vitro* assay. Both of these plants are worth to be further investigated and might be advantageous as an alternative for PAM.

Keywords: *Naegleria fowleri*, Plant extracts, anti-amoebic activity, toxicity

Poster No. 33

A FIRST CASE OF CHAGAS DISEASE DETECTED AT BLOOD DONATION EPIDEMIOLOGICAL SURVEY IN JAPAN

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Chagas disease, an infectious disease caused by *Trypanosoma cruzi* (*T. cruzi*), affects an estimated 7–8 million people worldwide, mainly in 21 countries in Central and South America. With improved transportation systems and increased movement of people across the globe, transmission to non-endemic areas via the immigration of carriers, or via transfusion or transplantation of infected materials, is a serious concern.

A male resident of Japan who immigrated from an endemic area around 25 years ago visited our hospital in August 2013 after anti-*T. Cruzi* antibodies were detected by an epidemiological survey conducted with patient consent at the time of blood donation in June 2013. He had no subjective symptoms, but electrocardiography and echocardiography at initial examination confirmed second-degree atrioventricular

block and concentric impairment of left ventricular wall motion, respectively. *T. Cruzi* DNA was detected from a serum sample by PCR, suggesting exacerbation of parasitemia in the chronic phase of infection.

Of approximately 300,000 people originally from Chagas disease-endemic areas now living in Japan, 3,000 are thought to be *T. Cruzi* carriers. Transmission of Chagas disease by blood-derived products reported in Canada, America, and Spain have clearly indicated the need for preventive measures against transfusion-transmitted infection in non-endemic areas, and countermeasures have been advanced. In Japan, since October 2012, blood donated by people from the disease-endemic areas, by their mothers, or by those who stayed in such areas for 4 or more weeks is used only for plasma fraction preparation, and the epidemiological survey for anti-*T. Cruzi* antibodies was introduced in April 2013. Greater numbers of immigrants from Chagas disease-endemic areas are expected in the future. Therefore, tests for anti-*T. Cruzi* antibodies should be actively encouraged, not limited only those who donate blood but introduced for all such new residents.

Keywords: Chagas disease, *Trypanosoma cruzi*, Transfusion, Blood donation, Neglect tropical disease

Poster No. 34

RAPID MOLECULAR DIFFERENTIAL DETECTION OF *BABESIA CANIS VOGELI*, *HEPATOZOON CANIS*, *EHRlichia CANIS* AND *ANAPLASMA PLATYS* IN CANINE BLOOD SAMPLES

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The common tropical canine tick-borne diseases that cause morbidity and mortality are infected by *Babesia canis vogeli*, *Hepatozoon canis*, *Ehrlichia canis* and *Anaplasma platys*. Microscopic examination of blood smear is the classical method for detection which was limited by the requirement of professional examiners. This study is trying to differentiate these canine tick-borne pathogens using high throughput pyrosequencing technique. The target regions for differentiation are 18S rRNA for protozoa (*B. canis vogeli* and *H. canis*) and 16S rRNA for rickettsia (*E. canis* and *A. platys*). These target regions were initially amplified by specific PCR primers and the pyrosequencing were subsequently performed using amplified-fragments as templates. Nine nucleotide positions can discriminate *B. canis vogeli* and *H. canis* whereas 15 nucleotide positions can discriminate *E. canis* and *A. platys*. Resulting from pyrogram and sequence analyses, this study presents an alternative method for rapid differential detection and diagnosis of four tick-borne haemoparasite species in infected dog.

Keywords: *Babesia canis vogeli*; *Hepatozoon canis*; *Ehrlichia canis*; *Anaplasma platys*; pyrosequencing; differential detection

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Poster No. 35

REAL-TIME FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) PCR ASSAY AND MELTING CURVE ANALYSIS DETECTION OF *BABESIA CANIS VOGELI* AND *HEPATOZOON CANIS* IN CANINE BLOOD SAMPLES

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A real-time fluorescence resonance energy transfer polymerase chain reaction (real-time FRET PCR) combined with melting curve analysis was developed for differential detection of *Babesia canis vogeli* and *Hepatozoon canis* infections in canine blood samples in single tube assay. The 18S ribosomal RNA gene, 267-bp of *B. canis vogeli* and 278-bp of *H. canis* infected dog blood samples, were amplified by single primer pair and the fluorescence melting curve analyses were generated by heating the hybrid of amplicons and single pair of fluorophore-labeled probes. Both *B. canis vogeli* and *H. canis* in infected dog blood samples were differentiated and detected with high (100%) sensitivity. Minimum detection limit was 15 copies/reaction of either *B. canis vogeli* or *H. canis* positive control plasmids. This assay could distinguish the DNA of both parasites from the DNA of controls other than *B. canis vogeli* and *H. canis* as well as with blood samples from non-infected dogs, which indicated that the high specificity. It can reduce labor time and misdiagnosis of microscopic examination and is not prone to carry over contamination of conventional PCR technique. Our real-time FRET PCR method would be beneficial to determine the accurate range of endemic areas and/or to discover the co-endemic areas of two parasites. In addition, it possible be used for differentiation/detection not only in infected patients but also in the hard tick, the important vector.

Keywords: *Babesia canis vogeli*, *Hepatozoon canis*, real-time FRET PCR assay, differential detection

Acknowledgement: This research was funded by grants from the National Science and Technology Development Agency (Discovery Based Development Grant); the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission through the Health Cluster (SHep-GMS) Thailand, the Faculty of Medicine, Khon Kaen University, and the TRF Senior Research Scholar Grant, Thailand Research Fund grant number RTA5580004.

Poster No. 36

HIGHER RATE OF APOPTOSIS IN *BLASTOCYSTIS* SPP. ISOLATES OBTAINED FROM SYMPTOMATIC PATIENTS

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Several previous studies have reported on the apoptosis-like features observed in *Blastocystis* spp., an intestinal protozoan parasite, when exposed to metronidazole, a cytotoxic drug. The distinct differences in the phenotypic characterization reported previously have prompted us to compare the rate of apoptosis in symptomatic and asymptomatic isolates. This study reports that the *Blastocystis* spp. isolates obtained from symptomatic patients exhibited the highest rate of apoptosis upon being exposed to the drug. The symptomatic isolates showed highest significant increase after 72 h of *in vitro* culture at 0.01 mg/ml (79%; p<0.01) and 0.0001 mg/ml (89%; p<0.001). This warrants further research on the apoptotic machinery of the parasitic protozoa. More research is required for

the identification of the effectors of apoptosis in *Blastocystis* spp. belonging to both symptomatic and asymptomatic isolates. These findings can then be exploited for the development of novel therapeutic drugs.

Keywords: *Blastocystis* spp., metronidazole, symptomatic, asymptomatic, apoptosis – like features

Poster No. 37

DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR DETECTION OF *TRICHOMONAS VAGINALIS*

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A loop-mediated isothermal amplification (LAMP) assay targeting the 2-kbp repeated DNA species-specific sequence was developed for detection of *Trichomonas vaginalis*, the causative agent of trichomoniasis. The analytical sensitivity and specificity of the LAMP assay were evaluated using pooled genital swab and urine specimens, respectively, spiked with *T. vaginalis* trophozoites. Genital secretion and urine did not inhibit the detection of the parasite. The sensitivity of the LAMP was 10 to 1,000 times higher than the PCR performed. The detection limit of LAMP was 1 trichomonad for both spiked genital swab and urine specimens. Also, LAMP did not exhibit cross-reactivity with closely-related trichomonads, *Trichomonas tenax* and *Pentatrichomonas hominis*, and other enteric and urogenital microorganisms, *Entamoeba histolytica*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. This is the first report of a LAMP assay for the detection of *T. vaginalis* and has prospective application for rapid diagnosis and control of trichomoniasis.

Keywords: *Trichomonas vaginalis*, LAMP, analytical sensitivity and specificity, diagnosis

Poster No. 38

HOUSEFLIES WITH HELMINTHIC OBJECTS, A GOOD INDICATOR FOR UNSANITARY ENVIRONMENT

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Houseflies are a mechanical vector of helminthic infections, and it is common to find all stages (egg-larva-adult) of helminths in flies. In communities where people harbor high prevalence of worm infection, and the use of outside latrines is common, houseflies may play an important role in the mode of transmission. People in the area with high contaminated flies are risk for many helminth infections due to *Ascaris*, *Trichuris*, *Taenia* and *Angiostrongylus*. For this study, helminth objects were compared from two study areas: Tak and Yasothon provinces in Thailand. In Tak province (western Thailand) where defecating in outside latrines is common, the prevalence of helminthiasis was 55.2%. The majority of infections were *Ascaris* (47.8%) and some villagers were even diagnosed with heavy ascaris infestation. Upon examination of 622 houseflies that were captured in this area, the number of eggs detected was as follows: 1,951 (*Ascaris*), 369 (*Taenia*), 147 (*Trichuris*), 52 (hookworm) and 1 (*Toxocara*). In Yasothon province (northeastern Thailand), indiscriminate defecation is rare, therefore the prevalence of helminthic infection was low with no observances of ascaris infection among the population. Examination of houseflies showed that *Dicrocoelium* eggs were common and other eggs such as *Strongyloides*, *Trichuris* and *Opisthorchis* were also noted. These eggs are commonly found in dogs and cats so it is possible that they belonged to animal parasites.

The examination of helminth objects on the external body of houseflies can be performed easily and the agents can be examined by using a light microscope. This simple method can be used as an indicator of an environment's unsanitary condition caused by human and animal waste.

Keywords: helminth objects, houseflies, environment

Poster No. 39

PUPICIDAL ACTIVITY OF HERBAL ESSENTIAL OILS AGAINST HOUSE FLY (*MUSCA DOMESTICA* L.)

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The insecticidal activity of essential oils of *Citrus sinensis* (sweet orange), *Cymbopogon citrates* (lemongrass), *Eucalyptus globulus* (eucalyptus), *Illicium verum* (chinese stars anise), *Lavandula angustifolia* (lavender), *Mentha piperita* (peppermint), *Myristica fragrans* (nutmeg), *Pelargonium graveolens* (geranium), *Rosmarinus officinalis* (rosemary) and *Zingiber cassumunar* (phai) against pupa stage of house fly (*Musca domestica* L.) using tropical application method. One hundreds pupae were exposed to essential oils at 1%, 5% and 10% v/v concentrations. The mortality rates were observed after 10 days. The criteria for mortality were defined as adult house fly not emergence included adult abnormal. The result revealed essential oils from *M. piperita*, *P. graveolens* and *L. angustifolia* were the most toxic to pupae, with LC₅₀ value of 7.80, 8.78 and 9.38%, respectively. At 10%, they gave the mortality rate which 65.00, 57.00 and 55.67, respectively. While, *R. officinalis* oil exhibited the lowest toxicity (LC₅₀ = 20.59%). Peppermint, geranium and lavender oils were effective in inhibiting adult emergence therefore should be recommended for house fly control activities.

Keywords: pupicidal, essential oil, house fly, *Musca domestica* L.

Poster No. 40

EXPRESSION AND CHARACTERIZATION OF THE ANTIMICROBIAL PEPTIDE GAMBICIN FROM *CULEX QUINQUEFASCIATUS*

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Antimicrobial peptides (AMPs), which can be found in many organisms, are generally low molecular weight peptides containing less than 100 amino acid residues. Due to ineffective conventional antibiotic treatments causing numerous mortalities of infected patients in the world, AMPs are an interesting approach to the development of novel therapeutics against pathogens, particularly antibiotic-resistant bacteria, because they are safe, non-toxic to mammals, no bacterial resistance and showing broad-spectrum antimicrobial activity. Interestingly, an antimicrobial peptide gambicin isolated from *An. gambiae* cell lines possess antimicrobial activity against gram-positive and -negative bacteria, filamentous fungi, and *Plasmodium* species. However, the isolation of *Culex* gambicin and expression of recombinant mosquito gambicins remain unreported. This study aimed to express recombinant *Culex* gambicin in *Pichia pastoris* and/or baculovirus expression system, and successfully cloned and expressed. SDS-PAGE of *Culex* gambicin in *Pichia pastoris* revealed an obvious protein band with a molecular weight of 7.0 kDa, indicating high production of recombinant *Culex* gambicin. Testing of antimicrobial activity against *E. coli* DH5a strain by agar-well diffusion assay exhibited no activity. A refolding approach to the recovery of antimicrobial peptides is required for further studies, while the expression of recombinant gambicin in the baculovirus expression system is on the process which will be also discussed.

Keywords: antimicrobial peptide, gambicin, *Culex quinquefasciatus*

Poster No. 41

PRESERVATION OF BLOOD FED *AEDES ALBOPICTUS* FROM FIELD TO LABORATORY AND ITS INCIDENCE ON HOST SPECIES IDENTIFICATION

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Aedes albopictus is the major vector of Chikungunya virus in Thailand and Southeast Asian countries. Blood meal characterizations are necessary to determine its natural feeding behaviour and on which host the females are feeding. This research determined the best laboratory preservation method for blood source detection of field mosquitoes. Female *Ae. albopictus* were fed with human blood and harvested at 0, 24, 48, 72 and 96 hours afterward. Then, females were either: (1) preserved at -20°C, (2) air-dried, (3) dried by lantern, (4) immersed in absolute ethanol, (5) placed in Carnoy's solution or (6) their abdomen were crushed on filter paper. After 24 hours, every sample was stocked at -20°C. The blood meal was detectable up to 24 hours post-feeding with ELISA tests. The optimal preservation method for ELISA test was ethanol. All the preservation methods were positive with PCR techniques up to 48 hours, except the Carnoy's solution method which was less efficient. We propose the use of the filter paper or ethanol as most convenient and efficient methods to preserve field mosquitoes until laboratory analysis either by ELISA or PCR.

Keywords: Blood meal, sample preservation, ELISA, PCR, *Aedes albopictus*

Poster No. 42

CHARACTERIZATION OF THE SALIVARY GLAND PROTEINS OF THE *AEDES AEGYPTI*, *AEDES ALBOPICTUS* AND *AEDES SCUTELLARIS*

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Objective: To characterize the protein component in salivary gland extract of *Aedes aegypti*, *Ae. albopictus* and *Ae. scutellaris*.

Methods: The pool of 100 glands was homogenized in phosphate saline buffer pH 7.0 was homogenized on ice. The supernatant was collected and subjected to filtration using Amicon Ultra®, 30K (molecular weight cut off 30kD). The fractions were analyzed by size-exclusion HPLC using Zorbex® G-250 column as a stationary phase and 100 mM sodium phosphate buffer pH 7.0 as a mobile phase and monitored at wavelength 200-400 nm using a photodiode array detector. As well as analyzed by gel electrophoresis on 12.5% SDS-PAGE and stained with Coomassie Brilliant Blue (CBB).

Results: Chromatograms demonstrated common five peaks at retention times 12, 13, 15, 16 and 17 minutes in the three species, the calculated molecular weight was approximately 60 - ≥ 90 kD, 20 - 35 kD, 10 kD, 2 kD and 0.5 - 1 kD, respectively. The most abundant was low molecular weight component (0.5 - 2 kD), accounting for 50-65% of total peak area. Although chromatograms did not reveal differences in the major peak components of the three species, analysis of UV spectrum indicated the variation in the composition of salivary gland extracted from the different species. SDS-PAGE stained with CBB demonstrated the unique band of 17 kD in *Ae. albopictus* and 150 kD in *Ae. aegypti*, and absence of some common proteins to the other two species. Furthermore, the protein band components between 20-35 kD were distinctive among the species.

Keywords: *Ae. aegypti*, *Ae. albopictus*, *Ae. scutellaris*. saliva gland, size-exclusion chromatography

Poster No. 43

LARVICIDAL ACTIVITY OF *PELTOPHOLUM PTEROCARPUM* EXTRACT AGAINST *AEDES AEGYPTI*, *CULEX QUINQUEFASCIATUS* AND *ANOPHELES DIRUS*

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An Ethanolic crude extract from the dried stem bark of *Peltophorum pterocarpum* was previously screened for the larvicidal activity against *Aedes aegypti*. The strong larvicidal activity of this extract showed high potential to be developed as a mosquito larvicide's product. To investigate the effectiveness of the crude extract, 3 species of mosquito larvae such as *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus* were tested. The bioassay was determined against the early fourth instar larvae by following the standard procedures from WHO, 1998. The crude extract was prepared and tested in various concentrations. The results showed a highly toxic effect against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles dirus* larvae with LC50 values of 15.39, 28.12 and 34.19 ppm, respectively and the larvae mortality could be detected within 24 h. Moreover, only in *Anopheles dirus* specie that the crude extract affect to the period of larval growth and development. Some abnormal characteristics were presented in pupae stage such as darker in color, size and shape deformation and abnormal pupae could not develop to be adult mosquito.

Keywords: *Peltophorum pterocarpum*, Larvicidal activity, *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles dirus*,

Poster No. 44

EFFECTS OF BODY SIZE AND BLOOD-FEEDING DURATION ON EGG PRODUCTION BY SEVEN *ANOPHELES* SP.

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Using an artificial membrane feeding system, we compared body size, blood-feeding duration, and blood meal size to egg production in seven laboratory maintained anopheline mosquitoes: *Anopheles campestris*, *An. cracens*, *An. dirus*, *An. kleini*, *An. minimus*, *An. sawadwongporni*, and *An. sinensis*. Overall, there were significant differences in body size as determined by wing length between the seven anopheline species. Adult females were allowed to feed uninterrupted on blood from 0 to 5 min, or were disturbed at 1, 3 and 5 minute intervals. The number of eggs produced by an individual female increased with the size of blood meal consumed. All seven species showed an increase in blood meal weight and fecundity with uninterrupted feeding duration. As body size increased the blood meal volume also increased, as did egg production. The average blood meals of *An. kleini* and *An. sinensis* were significantly higher than the other 5 species. This information is critical to understanding these species role in the transmission of malaria parasites in Thailand and Korea. We believe this is the first study of this type with these species.

Keywords: *Anopheles*, blood meal size, feeding duration, body size, egg

Poster No. 45

CURRENT STATUS OF TICK BORNE PATHOGENS IN DOMESTIC DOGS IN KHON KAEN, NORTHEAST THAILAND

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We determined the prevalence of tick-borne pathogens in domestic dogs using microscopy and PCR technology. A total of 303 EDTA-bloods were collected from domestic dogs in Khon Kaen Province, Thailand during May 2013. Microscopic observation using Giemsa's staining and molecular diagnosis using conventional polymerase chain reaction (PCR) were performed. Infected dogs were treated with Imidocarb and/or assigned drugs. Seventy two (23.76 %) out of 303 cases were positive with DNA of tick borne pathogens. A single infection with *Babesia canis* and *Ehrlichia canis* by 13.20 % and 1.32% were positive with microscopy respectively whereas 19.47% and 2.97% were positive with PCR technique. One cases of *Hepatozoon canis* infection were observed with microscopy and PCR technique. Co-infection with *B. canis* and *E. canis* was 0.66% and co-infection with *H. canis* and *E. canis* was 0.33%. Infected dogs were treated as assigned drugs and elimination of the pathogens was demonstrated by PCR. These results indicated that both microscopic and PCR diagnostic technique are useful for tick-borne pathogen detections. *Imizol* and *doxycycline* are effectiveness for treatment of babesiosis and ehrlichiosis respectively. The present study suggests that PCR technique was shown to be high sensitivity and a specific for *Babesia* and *Ehrlichia* diagnosis, detecting DNA of *B. canis*, *E. canis* and *H. canis* in EDTA-blood specimens. Moreover, detection tick borne pathogens DNAs from tick is more convenient than collection of blood from animal.

Keywords: Tick, blood parasite, *Babesia*, *Hepatozoon*, *Ehrlichia*, domestic dogs, KhonKaen, Thailand

Poster No. 46

CURRENT STATUS OF MISDIAGNOSIS OF TICK-BORNE PATHOGENS IN DOMESTIC DOGS IN KHON KAEN, NORTHEAST THAILAND

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We determined the incidence of tick-borne blood disease in domestic dogs using polymerase chain reaction (PCR) technology. A total of 280 blood samples from domestic dogs were collected from animal hospitals in Khon Kaen, Thailand, during May 2012–April 2013. All samples had a negative result from light microscopy using Giemsa staining. Most dogs had the following signs and symptoms: loss of appetite and slight fever. Other dogs appeared to be in normal condition, and some had no history of tick infection. Eighty (28.57%) out of 280 cases were positive for DNA of tick-borne pathogens. The most prevalent tick-borne disease was *Babesia canis* (48.75%), followed by *Ehrlichia canis* (33.75%) and a mixed infection of *B. canis* and *E. canis* (17.5%). No cases of *Hepatozoon canis* infection were observed. The present results indicated that detection of DNA of tick-borne pathogens using PCR had high sensitivity and specificity for diagnosis of *B. canis* and *E. canis*. Moreover, microscopic results remain problematic diagnosis of tick-borne pathogens, especially in cases of light infection or at an early stage of infection, which can result in misdiagnosis and lead to mistreatment. Therefore, to ensure accurate detection at a molecular level may need to be used in animal clinics or hospitals.

Keywords: Tick, blood parasite, *Babesia*, *Hepatozoon*, *Ehrlichia*, domestic dogs, KhonKaen, Thailand

Poster No. 47

TICK-BORNE PATHOGENS IN DOGS AT NAKHON PRANOM ANIMAL QUARANTINE STATION, NAKORN PRANOM PROVINCE, THAILAND

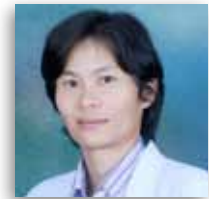
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To know the prevalence of tick-borne pathogens in dogs at Nakhon Pranom animal quarantine station, Nakorn Pranom province, Thailand during September, 2013. About 5% (43 dogs) of EDTA-blood from stay and domestic dogs in Nakhon Pranom animal quarantine station were collected and extracted DNA for detection of *Babesia* sp, *Ehrlichia* sp and *Hepatozoon* sp. using conventional polymerase chain reaction (PCR). The result found that 29 out of 43 were infected with tick borne pathogens. There were single infection with *B.canis* (10.35%), *E.canis* (13.80%) and *H.canis* (65.50%). Co-infection with *B.canis*, *E.canis* and *H.canis* was 3.45%, co-infection with *E.canis* and *B.canis* was 3.45% and co-infection with *E.canis* and *H.canis* was 3.45%. This study suggests that tick-borne pathogens remain the health problem in dog which may cause of sick and death at Nakhon Pranom animal quarantine station, Thailand. Therefore, treatment and prevention of tick-borne disease are needed.

Keywords: Tick, blood parasite, *Babesia*, *Hepatozoon*, *Ehrlichia*, Nakhon Pranom, Thailand

Acknowledgements: This work was supported by a grant from Thailand Research Fund (RTA5580004) and we also wish to thank the Nakhon Pranom animal quarantine station and Department of livestock development, Thailand for their assistance.

Poster No. 48

SIMULIUM (ASIOSIMULIUM) FURVUM, A NEW SPECIES OF BLACK FLY (DIPTERA: SIMULIIDAE) FROM THAILAND

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Simulium (Asiosimulium) furvum sp. nov. (Diptera: Simuliidae) is described from female, male, pupal, and larval specimens collected from Maewa National Park, Lampang Province, Thailand. This new species represents the fourth member of the subgenus *Asiosimulium* Takaoka & Choochote, one of two small black fly subgenera endemic in the Oriental Region. It is characterized by a pear-shaped spermatheca in the female; a ventral plate in the male with a laterally compressed median keel directed ventrally and with a deep notch posteromedially, and aedeagal membrane with stout spines; and by 22 gill filaments in the pupa. Taxonomic notes are provided to separate this new species from three known species, *Simulium (Asiosimulium) oblongum* Takaoka & Choochote and *Simulium (Asiosimulium) wanchaii* Takaoka & Choochote, both from Thailand, and *Simulium (Asiosimulium) suchitrae* Takaoka from Nepal.

Keywords: black fly, *Simulium*, *Asiosimulium*, new species, Thailand

Poster No. 49

ACTIVITY OF HERBAL ESSENTIAL OILS AS REPELLENT AGAINST GERMAN COCKROACH (*BLATTELLA GERMANICA* (L.): BLATTELLIDAE)Sirawut Sittichok^a and Mayura Soonwera^a^a Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

This study was conducted to investigate the repellent activity of herbal essential oils against *Blattella germanica* adult. The herbal essential oils derived from *Cymbopogon citratus* (lemon grass), *Eucalyptus globulus* (eucalyptus), *Mentha piperita* (peppermint), *Zingiber officinale* (ginger) and compared them with chemical insecticide (naphthalene). The repellency was observed at 24 hrs. The results showed that, all herbal essential oils in ethyl alcohol without 2% vanillin showed higher percentage of repellency (64-74%) against *B. germanica* than all of the essential oils in ethyl alcohol with 2% vanillin (30-68% repellency). The essential oil of *C. citratus* oil without 2% vanillin exhibited the highest repellency (74%) lower than naphthalene (80% repellency). This study indicates the potential of *C. citratus* oil to be used as botanical insecticide against *B. germanica*.

Keywords: repellent, herbal essential oil, *Blattella germanica*

Poster No. 50

USE OF SOLID CARBON DIOXIDE FOR CONTROLLING BED BUG *CIMEX HEMIPTERUS* (FABRICIUS)

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Use of solid carbon dioxide (dry ice) which provided carbon dioxide gas against bed bug *Cimex hemipterus* (Fabricius) (Hemiptera: Cimicidae) was conducted in laboratory at room temperature range from 25 to 30°C and 75%RH. In experiment 1, the tolerance of bed bug to dry ice treatment was studied among egg, nymph and adult stages exposed to 2.5 g dry ice pellets for 9 hr in 2,000 ml flask, which provided 40.87 % CO₂ in average. The complete mortality was found in nymphal stage, while the mortality in egg and adult stages were 22.50 and 90.00%, respectively. The result indicated that bed bug egg was the most tolerance to carbon dioxide gas. In experiment 2, the combination of dry ice treatment at 4 different amount (5, 10, 15 and 20 g) and for 3, 6, 9, 12 and 15 hrs (total 20 combinations) were treated on bed bug eggs in 2,000 ml flask condition which provided 86.02, 97.88, 98.51 and 99.07 % CO₂ in average. The hatchability was examined 10 days after treatment. In untreated control (no dry ice) bed bug survival rate was 86.30%. The insect mortality was started at 3 hrs after the CO₂ exposure (97.88% CO₂). Mortality of insects was increased with exposure time increasing. Insects were completely killed at least in combination treatment of 96.2% CO₂ (15 g of dry ice in 2000 ml chamber) and for 6 hrs.

Keywords: *Cimex hemipterus*, solid carbon dioxide, dry ice, bed bug, tolerance

Poster No. 51

DETECTION OF AUTOANTIBODIES AS BIOMARKERS OF CHOLANGIOCARCINOMA

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Cholangiocarcinoma (CCA) is a malignant cancer of cholangiocytes, and its incidence is highest in northeastern Thailand where the prevalence of *Opisthorchis viverrini* infection is widespread. Due to an ineffective diagnostic marker, patients usually come with unresectable tumor and have a short survival outcome. Chronic inflammation is an important mechanism leading to CCA. Since inflammation was found to be related with autoantibody production. Therefore, autoantibody may occur during CCA development. Autoantibodies have potential roles in disease initiation, diagnosis and prognosis. Thus, they are promising as new disease biomarkers. In order to investigate autoantibodies in CCA, immortal human cholangiocyte cell line (MMNK1) was extracted and separated by two-dimensional (2D) gel electrophoresis. Gels were transferred to PVDF membrane and probed with CCA patients plasma to screen for autoantibody reaction by 2D-Blot. In addition, other gels were run in parallel and stained with coomassie brilliant blue (CBB). Compared to CBB staining gel, corresponded spots were excised and proteins were identified using LC-MS/MS mass spectrometry. We have successfully detected eleven immunoreactive spots of autoantibodies. Functional analysis reveals most of targeted proteins mostly involved in transcription regulation process followed by mRNA processing, stress response, angiogenesis, calcium ion binding, lipid metabolism and unknown function. Candidate autoantibodies will be further validated using ELISA in human plasma samples. Our results suggested that autoantibodies will provide a potential in diagnosis of CCA.

Keywords: cholangiocarcinoma, autoantibody, biomarker

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Poster No. 52

ANTICANCER PROPERTIES OF *ASTRAEUS ODORATUS* C. PHOSRI, R. WATLING, M.P. MARTIN & A.J.S. WHALLEY AQUEOUS EXTRACT ON CHOLANGIOCARCINOMA CELL LINE (KKU-M213) AND BREAST CANCER CELL LINES (MCF-7)

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Objective – To investigate the anticancer properties of an aqueous extracts from *Astraeus odoratus* using cholangiocarcinoma cell line (KKU-M213), human hepatocarcinoma cell lines (HepG2), and breast cancer cell line (MCF-7).

Methods – The aqueous extract from fruiting bodies of *Astraeus odoratus* were prepared and lyophilized. The cytotoxic activities of the *Astraeus odoratus* aqueous extract against KKU-M213, HepG2, MCF-7 and Vero cell were assessed using the (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Result – *Astraeus odoratus* aqueous extract had cytotoxic effects with 50% cell growth inhibition (IC₅₀) against KKU-M213, HepG2, MCF-7, and Vero cell at 35.63±9.87, 241.93±8.99, 63.88±2.88, and 264.90±12.59 µg/mL, respectively, whereas the cytotoxic effects of doxorubicin against KKU-M213, MCF-7 and HepG2

showed an IC_{50} at 1.192 ± 0.12 , 0.99 ± 0.20 , 3.50 ± 0.60 , and 0.99 ± 0.20 $\mu\text{g/mL}$, respectively. The results showed that the *Astraeus odoratus* aqueous extract have strong cytotoxic activity on KKU-M213 and MCF-7 cells, but no cytotoxic activity on HepG2 and normal cells (Vero cell).

Conclusion- The results showed that an *Astraeus odoratus* aqueous extract reveals potent anti-cancer properties against cholangiocarcinoma cell line (KKU-M213) and breast cancer cell line (MCF-7). Our findings reveal that *Astraeus odoratus* aqueous extract might have therapeutic potential for cholangiocarcinoma and breast cancer and/or resource for pharmaceutical or food industry as a natural anticancer ingredient. However, further studies would be conducted in order to characterize the active components and the mechanisms underlying of the anti-cancer properties of *Astraeus odoratus*.

Keywords: *Astraeus odoratus*, aqueous extract, anticancer properties, breast cancer, cholangiocarcinoma

Poster No. 53

A CASE OF SCRUB TYPHUS COMPLICATED WITH GLOMERULONEPHRITIS

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Scrub typhus caused by *Orientia tsutsugamushi* transmitted through infected larval thrombiculid mite is one of the most important undifferentiated acute febrile illness. The disease is widespread, extending from Japan to Australia and from India to the Pacific. It is prevalent in many parts of India and has been reported in the sub-Himalayan belt extending from Jammu Kashmir to Northeast states. Recently several cases of scrub typhus were reported from Sikkim since 2003 through 2013. We report a case of a 2 year old male child who attended Paediatric OPD, Central Referral Hospital, Gangtok with complains of low grade fever, abdominal distension, lower limb swelling and low urine output. USG revealed mild hepatosplenomegaly with B/L pleural effusion. History of endemic area, clinical manifestations, laboratory test results and presence of Eschar helped us to establish a diagnosis of scrub typhus complicated with glomerulonephritis which was finally confirmed by IgM based ELISA. The child responded very well to Doxycycline therapy and recovered completely.

Keywords: *Orientia tsutsugamushi*, Glomerulonephritis, Doxycycline

Poster No. 54

CHOLERA IN NEPAL

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Nepal is a land-locked country in South Asia. The total population is approximately 29.8 million. A large proportion of people rely on unimproved sources for drinking water. Around half of the population (49 %) opts for open defecation, more so in the rural areas. Only 17 % of populations have adequate access to proper sewerage conditions in urban areas, whereas it is almost non-existent in rural areas.

We conducted a thorough literature search on cholera cases reported between 1990 and 2011. We primarily searched online databases like PubMed, WHO library and information for knowledge database (WHOLIS), MEDLINE, Med Explorer, Medscape, Med Hunt, Google Scholar. We used key words like “Cholera” and “Nepal”, “Acute Watery Diarrhea and Nepal”, “Acute Gastroenteritis and Nepal”.

We found most of the studies were conducted in Kathmandu valley. Yet, cholera outbreaks affect many regions of the country during August – October every year. Many studies report *Vibrio cholerae* O1 El

Tor Ogawa to be the sole causative agent. The proportion of stool samples positive for *Vibrio cholerae* 01 El Tor Ogawa range from 2.4 % to 42.8 %. According to World Health Organization (WHO), the highest number of cases and deaths due to cholera occurred during 1990 and 1991. However, there are major limitations in these reports due to incomplete data, and irregular and inconsistent data reporting, so that the burden of disease is seriously under-reported.

The Government of Nepal needs to strengthen surveillance and consider use of the Oral Cholera Vaccine as a supplement to other preventive measures (i.e. provision of clean water, sanitation and personal hygiene) and the provision of treatment, particularly in high risk areas.

Keywords: Cholera, Nepal, Surveillance, Vaccine

Poster No. 55

VANCOMYCIN SUSCEPTIBILITY TESTING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATES AT THAMMASAT UNIVERSITY HOSPITAL

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Risk associated with vancomycin treatment failure in Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is first indicated by increasing vancomycin MICs (Minimal inhibitory Concentrations: MICs creep). The “MICs creep” is considered in a susceptible range described in previous studies. The MRSA strains with vancomycin MIC creep have not been intensively investigated in Thammasat University Hospital. It is crucial for medical professionals to realize the importance of strain detection in order to design effective treatment of the MRSA with vancomycin MIC creep infection. The objective of this study was to examine MICs of vancomycin against clinical isolates of MRSA by broth microdilution method. Eighty-two clinical isolates of MRSA were collected at Thammasat University Hospital during August 2012 to July 2013. All isolates were tested by broth microdilution method. Vancomycin MICs were interpreted based on Clinical Laboratory Standards Institute (CLSI) susceptibility breakpoints. The MRSA strains were isolated from sputum (72.05% of patients), pus (14.70%), blood (10.29%) and urine (2.94%). We found that all isolates were susceptible to vancomycin. MICs of vancomycin of the tested strains were ranged from 0.25 to 2 µg/ml. Vancomycin MIC values of 0.25 µg/ml, 0.5 µg/ml, 1 µg/ml, and 2 µg/ml were 3.65%, 39.02%, 56.09% and 1.21%, respectively. The results indicated that vancomycin MIC range between 1 – 2 µg/ml need to be carefully examined for MIC creep and heteroresistant vancomycin intermediate *Staphylococcus aureus* (hVISA).

Keywords: Minimal inhibitory Concentration (MIC), Vancomycin, Methicillin-resistant *Staphylococcus aureus* (MRSA)

Poster No. 56

PREVALENCE OF BACTERIAL BLOOD STREAM INFECTION AND ANTIBIOGRAM OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AT THAMMASAT UNIVERSITY HOSPITAL

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Bacterial infection has been a serious problem worldwide. Methicillin-resistant *S. aureus* (MRSA) is one of the most important bacteria that cause high mortality. The notable failure of MRSA infection treatment has been reported in many studies. However, this has not been fully scrutinized at Thammasat University Hospital. This preliminary research provided useful and clarifying information for further study on prevention and control unknown impact on treatment of MRSA infection. This is a retrospective study of MRSA isolates susceptibility and prevalence of MRSA and other bacteria isolated from blood stream at Thammasat University Hospital between January 2008 and December 2012. A total of 1,469 bacterial strains were isolated from blood stream infection of 1,216 patients analyzed for preference study. The susceptibility of twelve antibiotics against MRSA by disk diffusion method was accessed from the database of Microbiology laboratory for antibiogram. The data analysis of blood stream infection revealed that *Escherichia coli* (28.15%) and MRSA (9.36%) were the major isolates. All tested strains of MRSA were susceptible to vancomycin, teicoplanin and linezolid. These isolates were resistant to penicillin and oxacillin. Eleven different antibiograms were found among sixty-five isolates of MRSA. The most common antibiogram being Te^RE^RFO^RD^SDA^RSXT^S designated pattern III (26 out of the total of 65 isolates). The antibiogram revealed that few effective antibiotics for MRSA treatment remain at medical practitioners' disposal. Therefore, overuse of antibiotics needs to be strictly under an efficient control.

Keywords: Bacterial blood stream infection, Antibiogram, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin, Teicoplanin, Linezolid

Poster No. 57

PREVALENCE OF ENTEROTOXIGENIC AND SHIGA TOXIN *ESCHERICHIA COLI* ISOLATED FROM DIARRHEA SWINE IN RATCHABUREE AND KANCHANABUREE, THAILAND

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Enterotoxigenic *Escherichia coli* (ETEC) and shiga toxin *E. coli* (STEC) are important causes of diarrhea disease in swine including human. To investigate the potential of ETEC and STEC may associated to human disease, 327 rectal swabs were collected from diarrhea swines from Ratchaburee and Kanchanaburee province, Thailand. By conventional bacteriology, 2,060 *E. coli* isolates were detected and submitted to determine the virulence genes of ETEC and STEC (LT, ST, stx1 and stx2 family) by PCR which specific primer pairs. The results showed that 192 isolates from 27 samples were positive for LT, 126 isolates from 26 samples were positive for LT and 29 isolates from 4 samples were positive for stx2 family gene. None isolate was positive for stx1 family gene. These data indicate that ETEC and STEC can also found in diarrhea swine which can cause the disease in human who consume improperly swine product. However, these study will further investigate the antibiotic susceptible profile and the other virulence gene to emphasized the diarrheagenic *E. coli* (DEC) surveillance.

Keywords: *Escherichia coli*, ETEC, STEC, LT, ST, stx1 family, stx2 family, swine

Poster No. 58

A REVIEW OF SYMBIOTIC AND POTENTIALLY PATHOGENIC BACTERIA OF MITES (SUBCLASS: ACARI): IMPLICATIONS FOR PATHOGEN TRANSMISSION

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A dataset of bacterial diversity found in mites was extracted from 131 publications (1946-2013). A hundred and fourteen mite species belonging to three taxa (Parasitiformes, Sarcoptiformes and Trombidiformes) were recorded, which were associated with 64 bacteria species (in 44 genera, 28 families and 19 orders). The intracellular symbiont *Cardinium* was the most prevalent mite-associated bacterium (30 species infected), followed by *Orientia* (the causative agent of scrub typhus) and *Wolbachia* (the most prevalent symbiont within the Arthropoda), with 27 and 23 species infected, respectively. Moreover, a number of bacteria of medical importance were also present in mites, such as *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Bartonella*, *Francisella*, *Coxiella*, *Borrelia* and *Staphylococcus*. The differences in bacterial infection pattern among mite taxa will be analysed and discussed.

These data are not only useful to increase awareness of the potential for mites to transmit disease, but deeper understanding of the impact of symbionts on their arthropod hosts may also facilitate the development of intervention tools for vector control. Due to their small size and the limited taxonomic descriptions available, the study of mite-associated bacteria is extremely challenging and few molecular protocols have been published. Therefore, we are beginning to conduct metagenomic studies focusing on the mite-associated bacteriome of “chiggers” (the parasitic larval stage of trombiculid mites), which are important vectors of scrub typhus in South-East Asia. This disease affects 1 million people per year, causing severe fever and potentially fatal complications. Manipulation of the bacteriome may lead to future opportunities to decrease vector competence in chiggers.

Keywords: Acari, Mite, Bacteria, Symbiont, Pathogen

Poster No. 59

RAPID LATEX AGGLUTINATION ASSAYS FOR SERODIAGNOSIS OF HUMAN MELIOIDOSIS

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Burkholderia pseudomallei is the etiologic agent of melioidosis, a significant community-acquired and severe disease in endemic regions. Diagnosis and treatment of human infections caused by this organism is challenging. In the absence of chemotherapeutic intervention, acute disease is frequently fatal. The objective of this project is to identify and characterize immunogens expressed by *B. pseudomallei* for developing rapid serodiagnostic tests. Recently, we have demonstrated that the O-polysaccharides (OPS) and capsular polysaccharides (CPS) are suited for this task. We evaluated the use of OPS- and CPS-based latex agglutination assays as a rapid approach for serodiagnosing human melioidosis. OPS and CPS antigens were isolated from *B. pseudomallei* Bp82, using a modified hot aqueous-phenol extraction procedure. Following purification by sequential rounds of ultracentrifugation and size-exclusion chromatography, the carbohydrate antigens were chemically activated with sodium periodate and covalently linked to aliphatic amine-derivatized latex beads via reductive amination to produce two distinct immunoassays. Utilizing rabbit antiserum samples raised against formalin fixed *B. pseudomallei*, *B. thailandensis* and *B. cenocepacia* isolates, the resulting OPS- and CPS-coated beads exhibited agglutination patterns consistent with those that would be predicted. Using 156 culture-confirmed human melioidosis patients and 143 healthy donor serum samples, the sensitivity and

specificity of OPS-coated beads were 82.1% and 62.9% respectively, and of CPS-coated beads were 65.4 and 69.2% respectively. The performance of these assays was equal to or greater than those associated with indirect hemagglutination assays (sensitivity 68.0%, specificity 67.1%). Our rapid latex agglutination assays may be useful as stable, affordable, point-of-care tests for serodiagnosing human melioidosis.

Keywords: *B. pseudomallei*, melioidosis, capsular polysaccharide, CPS, O-polysaccharide, OPS, latex agglutination assay, serum, serodiagnosis

Poster No. 60

AMINO ACID SUBSTITUTIONS IN THIOREDOXIN REDUCTASE FROM METRONIDAZOLE RESISTANT AND SENSITIVE STRAINS OF CLINICAL ISOLATED *HELICOBACTER PYLORI*

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An emergence of metronidazole resistance of gastric pathogen *Helicobacter pylori* was previously proposed as a result of mutations in oxygen-insensitive NAD(P)H nitroreductase (RdxA) and NAD(P)H flavin oxidoreductase (FrxA). However, a recent proteomic study of a resistant strain without the RdxA and FrxA mutations demonstrated not only up- or down-regulation of some proteins but changes in isoelectric points (pI) of a few redox enzymes including thioredoxin reductase, a probable enzyme in a reduction step of the metronidazole to an active form. Thus, an amino acid substitution that causes a change in the pI of the enzyme may result in a profound effect on the drug activation. In this study, sequencing of *thioredoxin reductase* gene from both metronidazole resistant (n=43) and sensitive (n=23) *H. pylori* revealed that, besides silent mutations, 5 amino acid replacements were observed in more than half of the resistant strains but less in the sensitive strains. It is notable that the substitutions occur at neither positions of functional residues nor homologous positions of the proposed thioredoxin reductase : thioredoxin interface. These substituted residues have been analysed based on the multiple sequence alignment of members of the bacterial thioredoxin reductase family. All of the replacements take place at the positions that are variable. Three of them are conservative substitutions implying that these replacements could produce less impact on the enzyme structure and function. In contrast, the others are non-conservative substitutions and might be questionable due to their amino acid substitution types.

Keywords: Amino acid substitutions, thioredoxin reductase, metronidazole resistance, *Helicobacter pylori*

Poster No. 61

IDENTIFICATION OF *ENTEROCOCCUS* BACTERIA ISOLATED FROM MOSQUITO

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Objective The insects including mosquito are known to possess microflora. Previous studies have shown that Lactic acid bacteria (LAB) constitute a part of mosquito microflora. Recently some novel LAB, including *Enterococcus* bacteria were isolated from insects and were identified. However, the identification of LAB from mosquito has never been reported. Therefore, we attempt to identify LAB isolated from mosquito.

Methods and Results In September 2012, 20 female *Aedes albopictus* were collected at Otori Park (Fukuoka, Japan) by human landing catch. Each mosquito midguts was collected and was homogenized. The homogenate were plated onto the GYP-CaCO₃ agar and were incubated at 30°C for 96 h. After incubation,

the colonies with halo were detected from two homogenates. One colony named m-13 was characterized by morphological, genetical, and physiological analysis. Morphological analysis shown that m-13 was Gram-positive and ovoid cocci, occurring in pairs, short chains or small groups. The phylogenetic analysis of 16S rRNA gene sequence of *Enterococcus* bacteria suggested that m-13 was placed in the same cluster of *E. faecalis* and the highest homology was observed between m-13 and *E. termitis* isolated from termite. However, the low homology of *pheS* gene sequence between these species was confirmed. In physiological analysis, m-13 grew slowly at 45°C and did not grow 6.5% NaCl. Moreover, m-13 had positive on acetoin and the acid production from D-mannitol in API test. These physiological characteristics of m-13 were different from that of *E. termitis*. Therefore, m-13 isolated from mosquito may be classified in a novel *Enterococcus* bacteria.

Keywords: mosquito, Lactic acid bacteria (LAB), *Enterococcus*, identification

Poster No. 62

CONTROVERSIAL FINDINGS OF MONOCLONAL ANTIBODIES SPECIFIC FOR *AEROMONAS* SPP. AGAINST HEMOLYSINS

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Objective - *Aeromonas* is a causative agent of gastroenteritis, wound infection and septicemia. The pathogenesis is mediated by their toxins, one of which is hemolysin. We have produced monoclonal antibodies (MAbs) from mouse spleen cells immunized with different species of *Aeromonas*. This study aimed to characterize their activities against the *Aeromonas* hemolysin.

Methods - First, the hemolytic activity of hemolysin of different *Aeromonas* spp. was tested against sheep red blood cells (SRBC). Second, two *Aeromonas*-specific MAbs, 88F2-3F4 (IgG2a) and 111E8-3G1 (IgG2a) produced in culture supernatants or protein G affinity chromatography-purified immunoglobulins were then tested for inhibition of *Aeromonas* hemolysins.

Results - Most isolates of *Aeromonas* species produced hemolysins to lyse SRBC. MAbs produced in culture supernatants was shown to inhibit SRBC lysis by *Aeromonas* hemolysins. However, MAbs purified using protein G affinity chromatography could inhibit a few hemolysins on SRBC lysis and seemed to promote most of them.

Conclusion - These findings are still controversial of inhibition and enhancement of *Aeromonas* hemolysin and studies on the mechanisms of their activities against hemolysin are underway.

Keywords: *Aeromonas* spp, monoclonal antibody, hemolysin

Poster No. 63

CONSTRUCTION OF RECOMBINANT *LACTOBACILLUS CASEI* EXPRESSING TETANUS TOXIN FRAGMENT C OF *CLOSTRIDIUM TETANI*

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Lactic acid bacteria (LAB) composed of a heterogeneous group of bacteria that can be found in a variety of environments and as normal flora in humans and animals. At present, the most interesting application of LAB in human and veterinary medicine is to use LAB as antigen delivery system. In this study, *Lactobacillus casei* TIRSTR1341, the probiotic bacteria isolated from chicken, was genetically modified to express the tetanus toxin fragment C (TTFC), the non-toxic fragment of tetanus toxin widely used as a

model antigen for expression in many different LAB. The amplified TTFC was cloned into pLC13.9:LDH-PRO1:GFPuv, the recombinant expression plasmid containing the green fluorescent protein (GFPuv), by replacing the GFPuv gene in the plasmid and resulting in pLC13.9:LDH-PRO1:TTFC. The TTFC containing plasmid under the control of the lactate dehydrogenase (*ldh*) promoter was further electrotransformed into *L. casei* RCEID02, the plasmid-free *L. casei* TIRSTR1341. Based on Western blotting analysis, the specific TTFC band with molecular weight of 46 KD was clearly detected in the selected transformants. In conclusion, we have successfully constructed the TTFC-containing plasmid and recombinant *L. casei* expressing TTFC. This recombinant plasmid and recombinant *L. casei* can further be used in the development of antigen delivery vehicle for various applications.

Keywords: Lactic acid bacteria, Recombinant *Lactobacillus casei*, TTFC

Poster No. 64

CONSTRUCTION OF THE RECOMBINANT *L. CASEI* EXPRESSING M2E:HBC FUSION PROTEIN UNDER THE CONTROL OF CONSTITUTIVE PROMOTER

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The extracellular domain matrix protein 2 (M2e) is highly conserved in influenza A virus and is one of the potential candidate antigens for the development of universal influenza vaccine. Due to its small size, some studies had successfully increased its immunogenicity by fusion the M2e with Hepatitis B core protein (HBc) (M2e:HBc). With respect to antigen delivery vehicles, *Lactobacillus casei* is the interesting bacteria to serve for this purpose as it can be used for the expression of various heterologous proteins. The aim of this study is to construct the recombinant *L. casei* expressing M2e:HBc fusion protein under the control of a constitutive promoter, the aggregation-promoting factor promoter (*Papf*). The *Papf* promoter was cloned into pLC13.9:M2eHBc:TT, the recombinant *E.coli/L. casei* shuttle vector containing M2eHBc, resulting in pLC13.9:APF:M2eHBc:TT. This recombinant plasmid was introduced into *L. casei* RCEID02 by electroporation. After verification for the correct construct by sequencing, the recombinant *L. casei* was determined for M2e expression by Western blot analysis. It was found that our recombinant *L. casei* was successfully expressed the M2e in the form of M2e:HBc fusion protein.

Keywords: *Lactobacillus casei*, constitutive promoter, extracellular domain matrix protein 2, influenza A virus

Poster No. 65

GREEN FLUORESCENT PROTEIN EXPRESSION IN *LACTOBACILLUS FERMENTUM* ISOLATED FROM HUMAN

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Lactic acid bacteria (LAB) are the member of gram positive bacteria present in many environments, and some species of LAB are also the endogenous microbiota of humans and animals. Most of LAB are generally recognized as safe and have probiotic properties. Currently, many LAB are being utilized as mucosal delivery vehicles for a variety of therapeutic and prophylactic molecules. In this study, LAB isolated from human feces were screened for *in vitro* probiotic properties and selected for using as host for expression of green fluorescent protein (GFPuv). The expression vector containing GFPuv, designated

as pLC13.9:LDH-PRO1:GFPuv, was constructed with the use of replicon derived from *L. casei* TISTR 1341 and lactate dehydrogenase (ldhL) promoter derived from *L. casei* ATCC393 and electrotransformed into the selected LAB isolate. It was found that the transformant LAB isolate, rLAB:GFP, could expressed the GFP as determined by visualization under the fluorescence microscope and by Western blot analysis using specific antibody against GFP. By identification using 16s RNA sequencing, this LAB isolate was identified as *L. fermentum*. In conclusion, LAB isolated from human feces and selected for *in vitro* probiotic properties can be genetically modified for GFP expression and identified as *L. fermentum*. This isolate can possibly be further applied as mucosal delivery vehicles for a variety of heterologous proteins used for therapeutic and prophylactic purposes.

Keywords: lactic acid bacteria, expression vector, green fluorescent protein, mucosal delivery vehicle

Poster No. 66

EVALUATION OF IGM/IGG ANTIBODIES SPECIFIC LIPL32 DOMAIN(S) FOR DEVELOPMENT OF EARLY-PHASE SERODIAGNOSIS OF HUMAN LEPTOSPIROSIS

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Simple and accurate diagnosis for leptospirosis is still needed in order to differentiate the disease from other causes of acute febrile illness. LipL32-based enzyme-linked immunosorbent assay (ELISA) provides rapid and sensitive serologic diagnosis of human infection. It has been reported that the C-terminus LipL32 portion is an immunodominant and elicited both LipL32-specific IgM and IgG antibodies during earlier course of infection. In this study, LipL32-domain specific human IgM and IgG antibodies reactivity were evaluated from MAT-positive sera by using recombinant LipL32, full-length LipL32₁₋₂₇₂ and two deletion mutant, *i.e.* LipL32_{Δ1-90} and LipL32_{Δ1-170} proteins as antigens in ELISA assay. Diagnosis efficacy of individual antigens was evaluated.

Preliminary results reveal LipL32_{Δ1-90} is an immunodominant recognized by IgM whereas C-terminus LipL32_{Δ1-170} proteins was recognized by IgG antibodies in early phase of disease. However, more number of samples are needed in order to definite the use of LipL32 truncation proteins in development of LipL32-based ELISA.

Keywords: Leptospirosis, LipL32, diagnosis, immunodominant domain, ELISA

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Poster No. 67

MINING THE *BURKHOLDERIA* GENOME FOR NOVEL BIOACTIVE NATURAL PRODUCTS

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To date, most of the clinically used antibiotics have been discovered from Gram-positive bacteria, in particular *Streptomyces* and other related actinobacteria, as well as filamentous fungi. However, the success rate has diminished to almost zero in recent years with very few new antibiotics discovered in the last two decades. Meanwhile, the world wide antibiotic resistance urgently demands new sources of antibiotics to treat life threatening infections caused by MRSA/VRSA. Therefore, it is important to search for new antibiotics in other organisms beyond the traditional sources. *Burkholderia* are Gram-negative soil bacteria that many are opportunistic human pathogens. They are known to produce antifungal natural products such as 4-quinolones, cepalydin, cepaciamide, maculosin and banegasin. Little is known about the production of antibacterial natural products in *Burkholderia*. However, the wealth of *Burkholderia* genome sequences has revealed many high GC content genomic islands containing putative polyketide and nonribosomal peptide biosynthetic gene clusters. This observation has prompted us to investigate the production of antibacterial natural products by *Burkholderia* species. We have discovered that enacyloxin IIa as the product of an unusual cryptic polyketide biosynthetic gene cluster, identified via transposon mutagenesis and correlation to the genome of *Burkholderia ambifaria* AMMD. Enacyloxin IIa is active towards multidrug resistant Gram-negative bacteria such as *B. dolosa*, *B. multivorans*, *B. anthina* and *B. pyrrocinia* and *Acinetobacter baumannii*. The identification of enacyloxin IIa as the product of the cryptic gene cluster and insight into enacyloxin biosynthesis will be discussed. This genome mining approach has been successfully applied in the discovery of Enacyloxin IIa from *Burkholderia ambifaria*. Similar methodology could be adopted to investigate other *Burkholderia* strains or other bacterial species for the discovery of novel antibiotics.

Keywords : *Burkholderia*, genome mining, antibiotics, natural products

Poster No. 68

DEVELOPMENT OF *BURKHOLDERIA PSEUDOMALLEI* DETECTION BASED ON LAMP-TTS1 GENE IN CRUDE SOIL SAMPLE

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Melioidosis is the third most common cause of death from infectious diseases, after HIV/AIDS and tuberculosis in Northeastern Thailand. It caused by gram negative bacterium *Burkholderia pseudomallei* which their natural habitats are soil and surface water. Detection of *B. pseudomallei* in soil is important for defining geographical areas and indicating a risk of melioidosis in human and animals. Bacterial culture is conventional method for *B. pseudomallei* detection from soil sample. Although, it is simple but laborious and time consuming for large number of soil sample such as soil surveys or screening. Loop-mediated isothermal amplification (LAMP) targeting the TSS1 gene was developed to detect *B. pseudomallei* in soil sample collected from Northeastern Thailand. 10 g. of soil was suspended in 10 ml TBSS-C50 broth at 40 °C for 48 hr. 250 µl of soil suspension was boiled at 100 °C for 10 min and then used for direct detection of *B. pseudomallei* without prior extraction. In addition, we performed methods to eliminate inhibitors in soil by adding 10 mg/ml bovine serum albumin (BSA) into the reaction. The results demonstrated that the detection of LAMP product was clearly seen when adding BSA with boiling methods.

Keywords: *Burkholderia pseudomallei*, Soil survey, Loop-mediated isothermal amplification

Poster No. 69

ASSOCIATION OF GRANULYSIN, PERFORIN, IFN- γ AND LYMPHOCYTE SUBSETS INFLUENCES THE CLINICAL OUTCOME IN THAI PATIENTS WITH TB AND HIV/TB COINFECTION

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Host effector mechanism against *Mycobacterium tuberculosis* infection is dependent on innate immune response by macrophages and neutrophils and alterations in balanced adaptive immunity. Coordinated release of cytolytic effector molecules from NK cells and effector T cells and the subsequent granule-associated killing of infected cells have been documented; however, their potential role in clinical tuberculosis is still controversy. This study aims to investigate whether circulating granulysin and other effector molecules are associated with the number of NK cells, α NKT cells, $V\gamma 9^+V\delta 2^+$ T cells, $CD4^+$ T cells and $CD8^+$ T cells, and such association influences the clinical outcome of the disease in patients with pulmonary TB and HIV/TB coinfection who have been living in Chiang Rai, north of Thailand where TB is endemic. Granulysin and perforin levels in TB patients were lower than healthy controls, whereas much higher granulysin levels in HIV/TB coinfection than in any other groups, TB and HIV with or without receiving HAART were noted. Such higher levels corresponded to the number of $CD8^+$ T cells which kept high, but not with NK cells and other possible cellular sources of granulysin. In addition, the 17kDa, 15kDa and 9kDa isoforms of granulysin were recognized in plasma of HIV/TB coinfection. Increased granulysin and decreased IFN- γ levels in HIV/TB coinfection and TB after completion of anti-TB therapy were observed. The results suggested that the alteration of circulating granulysin has potential function in host immune response against TB and HIV/TB coinfection. This is the first demonstration so far of granulysin in HIV/TB coinfection.

Keywords: Granulysin, TB, HIV, HIV/TB Coinfection, Lymphocytes Subsets

Poster No. 70

ALCOHOL CONSUMPTION AND HIV RISK BEHAVIORS AMONG UNMARRIED YOUNG MEN (18 TO 24 YEARS), KHANH HOA PROVINCE, VIET NAM

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Alcohol use and sexual risk is almost exclusively cross-sectional in Aisa.

The aim is focused on the associated between alcohol use and HIV risk behaviors among young unmarried Vietnamese men between ages of 18 and 24. The longitudinal quantitative survey utilized a randomized sampling strategy and collected the data from four communes, Textile Company and Nha Trang University from February 2009 and May 2010 with three times at 6 month intervals.

The mean age of 1068 respondents was 20.9 years at baseline, 66.9% were in school and 35.5% were employed. A total of 93.7% reported "ever" using alcohol. Over 24% respondents reported ever having vaginal sex. There was a significant relationship between intoxication and vaginal sex, and 30.9% sexually active respondents reported consuming alcohol before most recent episode of sex. Between baseline and 12 month follow-up, 28.3% initiated sexual touching and 12.0% initiated engagement in vaginal, oral, and/or anal sex. More than 50% of respondents using condoms half time or less, however 62.8% reported condom

use at last sexual encounter. The reporting intoxication in the past 6 months had more lifetime sexual partners than those not and those sexually active respondents reporting intoxication in the last 6 months were significantly. This study showed a significant relationship between alcohol use, intoxication, and engagement in sexual risk behaviors. Nearly 50% of sexually active respondents use of condoms half time or less suggest a potential risk for both HIV/STIs, and unwanted pregnancy. These data are essential toward the development implementation intervention programs.

Keywords: 1. Alcohol consumption, 2. HIV risk behaviors, 3. Unmarried young men (18 to 24 years)

Poster No. 71

DETECTION OF HEPATITIS E VIRUS IN DEER AND MONKEY IN THAILAND

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Hepatitis E virus (HEV) infection in human leads to an acute viral hepatitis that is transmitted via fecal-oral route through consumptions of contaminated water or food. Four genotypes of HEV (HEV-1 to -4) have been described worldwide. HEV-1 and HEV-2 are found in human. HEV-3 and HEV-4 are found in both human and animals such as pig, wild boar and deer, indicating a zoonotic transmission. There were evidences showed that human got HEV zoonotic infections by contact with contaminated animal materials or eating contaminated undercooked animal products. In this study, a prevalence of HEV was surveyed in deer and monkey. Deer feces (n=47) were collected from a forest ground in Kanchanaburi Province where human and animal areas are superimposed. Monkey feces (n=55) were collected from an area in Lopburi Province where monkeys live in close contact with human. By semi-nested RT-PCR of 30% fecal suspension with primers specific to a region in 5' non-coding region (5'NCR) of HEV genome, 21.3% of deer and 25.5% of monkey fecal samples were positive for HEV RNA. An incidence of diseases in the animals was not inspected. However, all feces were normal in their gross appearances. The results suggest that deer and monkey could be potential reservoirs of HEV, although a possibility of the animals acquired the virus from human is not ruled out. The risk of HEV contamination from wild animals to human environment such as water and food also needs to be examined in human habitats.

Keywords: hepatitis E virus, deer, monkey, semi-nested RT-PCR, Thailand

Poster No. 72

CONSTRUCTION OF JAPANESE ENCEPHALITIS VIRUS MULTIEPITOPE GENE AND EXPRESSION IN PLANTS

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The E gene is responsible for producing E proteins which protects the viral nucleocapsid. In addition, the immune response to the E protein can protect against JEV infection. Previous researchers revealed that only humoral immune response was elicited from immunized mice with inactivated JEV vaccine strain SA14-14-2 whereas both humoral and cellular immune response were shown in immunized mice with recombinant multiepitope (MEP) peptide of JEV E protein expressed in *Escherichia coli*. Therefore, genetic engineering of JEV-MEP from E gene of JEV produced JEV-MEP protein in plants instead of culturing JEV in mice, animal tissue cultures and expressing of JEV-MEP proteins in *E.coli*. Hence, this research is the pioneer for plant derived JE vaccine.

The various steps were summarised as follows. First, six B cell epitopes and two T cell epitopes from the E protein of JEV vaccine strain SA14-14-2 were constructed and showed both antibody and CTL titres compared with inactivated vaccine in other research group. This JEV-MEP gene was constructed by private company and added the suitable promotor for cloning in *E. coli* and *Agrobacterium tumefaciens*. Then *A. tumefaciens* was inoculated and expressed in *Nicotiana benthamiana* leaves using the pEAQ-HT vectors. This vector provided an easy and quick tool for the production of milligram quantities of recombinant proteins. Second, the JEV-MEP construction was showed the bands which were detected by chemiluminescence immunoassay. Finally, this JEV-MEP proteins will be characterized and purified for the plant based vaccine and the immune response will be investigated further.

Keywords: *Japanese encephalitis virus*, plant based vaccine, immune response

Poster No. 73

CROSS SECTIONAL STUDY OF PATIENTS WITH COMMUNICABLE DISEASES IN GEMBE, KENYA –USING SYMPTOM-BASED QUESTIONNAIRE FROM INHABITANTS

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Health and demographic surveillance system (HDSS) was introduced in Suba district from 2009 by Institute of Tropical Medicine, Nagasaki University. This system was operated by fieldworkers' visit of all the compounds in the district and update information on events (e.g. pregnancies, births, deaths, etc.). We used this system to investigate the behavior research for patients with communicable disease. The study was conducted from April to November 2011. Study areas were Gembe, Suba district, Nyanza Province, Kenya. The fieldworkers visited all of the households in Gembe, and asked whether presence of acute infectious disease patients or not. If there were patients, fieldworkers asked following items; symptoms, onset date, utilization behavior in health care and the reason, the cost of treatment, and the outcome. Total 4398 inhabitants were answered. 1090 (24.7%) inhabitants were acute infectious diseases obtained at the time to answer. Most of them (1021/1090 patients) had symptom of high fever and approximately a half of them (575/1090) visited medical facilities. The major reason of not visited medical facilities was "not have enough money". The patients did not visit medical facilities were prolonged sickness day compare with those who visited medical facilities. These preliminary results show that accessibility of medical facility is important to keep inhabitants health. We will research other areas of Suba district and compare near future.

Keywords: Health and demographic surveillance system (HDSS), Communicable diseases, Suba district, Gembe

Poster No. 74

RISK FACTORS RELATED TO DENGUE INFECTIONS IN PRIMARY SCHOOL STUDENTS: EXPLORING STUDENTS' BASIC KNOWLEDGE OF DENGUE AND EXAMINING THE LARVAL INDICES IN SOUTHERN THAILAND

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Dengue is a major problem in southern Thailand.

Objectives: 1) To determine students' basic knowledge of dengue; and 2) to examine the larval indices in primary schools and in the students' households.

Methods: This study employed a cross-sectional quantitative and qualitative approach involving

meetings with students, discussions with groups of teachers, a questionnaire investigating students' basic knowledge of dengue, and a survey of the larval indices in primary schools and in the students' households. The study consisted of three stages: 1) community preparation, 2) data collection and analysis, and 3) feedback.

Results: A total of 306 students (from primary education levels 4, 5, and 6) from five primary schools in the community were included in the study. Of a total of 15 items on the basic dengue questionnaire, only five were answered correctly by more than 80% of the students. Most of the knowledge items showed statistically significantly different distributions of correct, incorrect, and unknown answers ($P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$). The larval indices surveyed in the five schools and in 302 student households showed a high risk of dengue, with high indices in the five schools (Breteau Index: BI=200; House Index: HI=60; and Container Index: CI=7.94) and in the students' households (BI=754; HI=77; and CI=35).

Conclusion: Risk factors for dengue were related to the students' basic knowledge of dengue and to the larval indices in both the schools and the students' households. Additionally, a coordinated effort will be required to eliminate *Aedes aegypti* mosquito breeding sites in the community.

Keywords: Risk factors, Dengue, Primary school, basic knowledge of dengue, Nakhon Si Thammarat province, Thailand

Funding agency: The Health Fund of Kamphaeng Sao sub-district, Nakhon Si Thammarat province, Thailand

Poster No. 75

MIMOTOPE IDENTIFICATION OF HUMAN NEUTRALIZING MONOCLONAL ANTIBODIES AGAINST DENGUE VIRUS USING RANDOM PEPTIDE PHAGE DISPLAY LIBRARY

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Background: Dengue virus is the fastest emerging arboviral infection spread by *Aedes* mosquitoes with major public health consequences in over 100 tropical and sub-tropical regions. The DENV complex consists of four distinct but related viruses, designated as serotypes. Currently, there is no specific antiviral or vaccine is available for dengue virus infection. However, many researchers have been trying to develop the therapeutic agents against DENV. Phage display technology is a powerful method recently developed to screen selective repertoires from large diversity of combinatorial peptide libraries displayed on the surface of bacteriophage.

Methods: Two Human monoclonal antibodies (huMAbs) that show cross-neutralizing activity to 4 serotypes of DENV were produced from previous study (Setthapramote et al., 2012) and used as target antibodies for epitope study. Random 7 and 12 position peptide libraries were used for selection by 3 rounds of panning, and checked the target-specific phage clones by ELISA, before sending the M13 phage plasmids for sequencing.

Results: The MAbs 19 and 54 recognized phages displaying peptides with the consensus motif LXXXG and LXGXG, respectively, that matched to conserved region of dengue E protein at position (107) LFGKG(111). However, for further specificity confirmation of the peptide, it need to be synthesized and checked the specificity by ELISA. The results described in this study will open avenues of development for dengue diagnostic assays and the technique can be applied for other antibodies specific to other tropical diseases.

Keywords: phage display, dengue virus, peptide, neutralizing, monoclonal antibodies, epitope

Poster No. 76

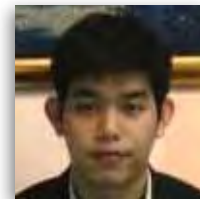
EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES MELLITUS ON OPISTHORCHIASIS HAMSTERS

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Diabetes mellitus (DM) is the disease which has hyperglycemic state leading to many complication such as increases risk of infection especially bacteria and fungus. However, information about interaction of parasitic infection and DM is limited. The prevalence of DM is high in northeastern Thailand where opisthorchiasis caused by liver fluke *Opisthorchis viverrini* infection is also endemic. The aim of this study was to investigate the effect of DM on opisthorchiasis at 1 month post-infection both on parasite and hamster. The diabetic condition was induced by streptozotocin injection. The present study was carried out on four groups of hamster: (1) normal group, (2) group infected with *O. viverrini*, (3) diabetic without infection group, and (4) group injected with streptozotocin and then 2 months post-treatment infected with *O. viverrini*. The results revealed that liver per body weight ratio in the diabetic with infection group was significantly higher than in other group. For the worm size of liver fluke in the diabetic with infection group was smaller than in non-diabetic group. These results suggest that diabetic condition has the effect on both host and *O. viverrini* fecundity by changing in physiological conditions.

Keywords: *Opisthorchis viverrini*, opisthorchiasis, diabetes mellitus, streptozotocin

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Poster No. 77

ANTI-INFLAMMATORY EFFECT OF ANTHOCYANINS COMPLEX ON OPISTHORCHIS VIVERRINI-INFECTED HAMSTERS

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Chronic *Opisthorchis viverrini* infection causes hepatobiliary diseases leading to cholangiocarcinoma (CCA) development via chronic inflammation. Although praziquantel is the most effective drug for worm elimination; however, re-infection occurs more frequently. Therefore, inhibition of inflammation by natural antioxidant/anti-inflammatory compound may be used as strategy for secondary prevention of opisthorchiasis-associated CCA. Several studies revealed that anthocyanins have anti-oxidant and anti-inflammatory activities. In this study, we developed anthocyanins complex from cob of purple waxy corn and investigated the anti-inflammatory effect of this complex in *O. viverrini*-infected hamster. Histopathological study, biochemical testing, real-time RT-PCR and Western blot analysis were performed. Histopathological study showed that anthocyanins complex reduced the accumulation of inflammatory cells in around of bile duct compared with untreated control. Furthermore, anthocyanins complex treatment significantly decreased nitrate stress marker (level of nitrate and nitrite) and reduced liver injury (alanine

aminotransferase activity) while serum level of ferric reducing antioxidant power significantly increased in dose-dependently. Moreover, anthocyanins complex treatment significantly reduced the expression of oxidant-related genes including NF- κ B and iNOS but induced the expression of antioxidant-related genes including CAT, SOD, and GPx. Relevantly, Western blot analysis revealed that translational level of NF- κ B and iNOS were decreased in anthocyanin-treated hamster. In conclusion, anthocyanins complex reduces inflammation, nitrate stress and prevent of liver injury via balancing of oxidant/antioxidant mechanism in *O. viverrini*-infected hamster.

Keywords: Opisthorchiasis, Cholangiocarcinoma, Anthocyanins Complex, Prevention and Treatment
Acknowledgements: This work was supported by The Royal Golden Jubilee Ph.D. Program (to KI and SP), Thai Agricultural Research Development Agency (Public Organization) and the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Health Cluster (SHeP-GMS1165).

Poster No. 78

ANTI-CHOLANGIOCARCINOMA PROPERTIES OF AQUEOUS EXTRACT OF *FICUS BOTRYOCARPA* MIQ

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Objective: To evaluate the anticancer activities of *Ficus botryocarpa* Miq on human cholangiocarcinoma cell line (KKU-M213), human hepatocarcinoma cell line (HepG2), human breast cancer cell line (MCF-7).

Material and methods: The (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was applied to compare the anti-tumor activity of an lyophilized aqueous extract of *Ficus botryocarpa* Miq on three human cell lines including KKU-M213, HepG2, MCF-7 and Vero cell lines (normal cell). Various concentration of aqueous extract of *Ficus botryocarpa* Miq were treated to 1×10^4 cells/well of each cell type.

Results: The aqueous extract of *Ficus botryocarpa* Miq exhibited cytotoxicity against on KKU-M213, HepG2, MCF-7 and normal cell lines (Vero cells) with IC₅₀ values of 59.54 ± 10.92 , 132.46 ± 25.38 , 183.23 ± 33.1 and 258.23 ± 37.74 μ g/mL, respectively. The results demonstrated that aqueous extract of *Ficus botryocarpa* Miq possessed a potent cytotoxic activity against human cholangiocarcinoma cell line KKU-M213 but less effects against human hepatocarcinoma cell line (HepG2), human breast cancer cell line (MCF-7) as well as no effect on normal cell (Vero cell).

Conclusion: The results obtained from the present study suggest that the aqueous extracts of *Ficus botryocarpa* Miq may have a potential natural source for anti-cholangiocarcinoma therapeutics. Further investigation need to be done to identify the active compounds.

Keywords: *Ficus botryocarpa* Miq, aqueous extract, anti-cholangiocarcinoma properties anticancer properties, cholangiocarcinoma

Poster No. 79

ETHNOBOTANICAL STUDY OF INSECT/MOSQUITO REPELLENT PLANTS AMONG THE MALAY ETHNIC IN KOTA TINGGI DISTRICT, JOHOR PROVINCE MALAYSIA

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An ethnobotanical survey was carried out to assess the knowledge and usage of repellent plant that traditionally used against mosquito or insects among Malay ethnic in Kota Tinggi district, Johor, Malaysia. This study was conducted between October to December 2012 using standardized and pre-test questionnaires. A random sampling was used for selection of 350 households from the total of 2291 households. From this study, 44% of the respondents had adequate knowledge and usage custom concerning traditional insect/mosquito repellent plants. Sixteen plant species were reported to have repellent effect in the study area. These species belong to 15 genera from 15 families. Graminae (13.4%), Alliaceae (8.9%) and Rutaceae (8.5%) were the most represented families with one species each. The most frequent recorded species were *Cymbopogon nardus* (13.7%), *Allium sativum* (8.9%), *Cinnamomum zeylicum* (7.4%), *Ilicium verum* (7.1%), *Pelargonium redula* (5.7%), *Pandanus amaryllifolius* (5.4%), *Citrus aurantifolia* (5.4%), *Alpinia galangal* (3.4%), *Citrus grandis* (3.1%), *Annona muricata* (2.6%), *Etilingera eliator* (1.1%), *Pogostemon cablin* (1.1%), *Sesbania grandiflora* (0.9%), *Murraya koenigii* (0.9%) and *Tagetes erecta* (0.9%). Growing such a plant around the house was one of the most common methods applied by the local community to repel mosquito and other nuisance insects since leaves were the most part known to have repellent effect. In spite of this, knowledge and usage of mosquito/insect repellent plants had no significant association with gender ($p=0.147$), age ($p=0.369$) and monthly income ($p=0.771$). However, there was a significant association with educational status ($p=0.003$). This study plays a part in documenting and conserving the knowledge and usage of repellent plants for future use. This kind of research also provides the basis for the research in developing new formulation of repellent product in the future.

Keywords: Ethnobotanical, knowledge, repellent, mosquito, plant

Poster No. 80

HEALTH STATUS OF IMMIGRANT CHILDREN AND ENVIRONMENTAL SURVEY OF THE CHILD DAY CARE CENTERS IN SAMUTSAKORN PROVINCE, THAILAND

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Samutsakorn Province is one of the popular provinces attracted by immigrant workers rushing to work in the factories and Thai food industries particularly Myanmar natives. The poor hygiene of personal behaviours, congested accommodation and limited sanitation lead to the health problems of the immigrant workers due to various infectious diseases undertaken and looked after by the Samutsakorn general hospital. The concern of intestinal parasitic infections on public health has been noted which can be spread from the infected immigrant areas to the virginal areas through the close contact and fecal-oral transmission of contaminated food and water. Our study was focused on total 372 immigrant children who resided in 8 child-daycare centers in terms of physical examination, stool examination, environmental examination surrounded the centers. The results showed high prevalence of intestinal parasitic infections at 70.2%. which was composed of both helminths and protozoa as follows: *Trichuris trichiura* (51.3%), *Enterobius vermicularis* (25.2%), *Ascaris lumbricoides* (14.2%), hookworms (11.6%), *Giardia lamblia* (10.2%), *Endolimax nana* (3.5%), *Entamoeba coli* (2.7%) and *Blastocystis hominis* (0.5%). The physical examination was un-remarkable except head lice and nail examination were positive in some cases. The environmental survey showed numerous house flies nearby the accommodation positive with some helminthic eggs such as *A. lumbricoides*, *E. vermicularis*, hookworms, *Taenia* spp. and minute intestinal flukes. The main factor of high prevalence of intestinal parasites has been hypothesized whether they were infected along with their parents during their daily lives before and after settle down in Thailand. The problem of intestinal parasites in immigrant children is quite epidemiologically impact as they can serve as the carriers to spread the diseases.

Keywords: immigrant children, intestinal parasitic infections, Thailand, carriers

Poster No. 81

DNA BARCODING FOR THE IDENTIFICATION OF EIGHT SPECIES MEMBERS OF THE THAI HYRCANUS GROUP AND INVESTIGATION OF THEIR STENOGRAMOUS BEHAVIOR

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Eight species members of the Thai Hyrcanus Group were identified based on the intact morphology and molecular analysis (COI barcoding, 658bp) of F₁-progenies. Five iso-female lines of each species were pooled in order to establish stock colonies. A stenogamous colony of each species was investigated by making 200 and 300 newly emerged adult females and males co-habit in a 30cm cubic cage for one week. After oviposition, the spermathecae of females were examined for sperms. The results revealed that *Anopheles argyropus*, *Anopheles crawfordi*, *Anopheles nitidus*, *Anopheles pursati*, *Anopheles sinensis*, *Anopheles nigerrimus*, *Anopheles paraliae* and *Anopheles peditaeniatus* yielded insemination rates of 0%, 0%, 0%, 31%, 33%, 42%, 50% and 77%, respectively. Continuous selection to establish stenogamous colonies indicated that *An. sinensis*, *An. pursati*, *An. nigerrimus*, *An. paraliae* and *An. peditaeniatus* provided insemination rates of 33–34%, 27–31%, 42–58%, 43–57% and 61–86% in 1, 2, 5, 6 and 20 generations of passages, respectively.

Keywords: *Anopheles*, Hyrcanus Group, DNA barcodes, Stenogamy, Eurygamy

Poster No. 82

DNA BARCODING OF THE PARASITIC FLIES, STOMOXYS FLIES (DIPTERA: MUSCIDAE), FROM NATURAL SOURCES IN THAILAND

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The *Stomoxys* flies are known as parasitic flies affecting livestock and wildlife. They have determined as high impact in economic lost especially dairy farm. Adult flies feed on blood causing blood loss and reducing milk product. They are major irritant pests of livestock and transmit many pathogens to cattle. The *Stomoxys* flies were collected from many parts of Thailand. Species were firstly identified based on morphological characteristics. There are some *Stomoxys* species, *Stomoxys indicus*, *Stomoxys pulla* and *Stomoxys uruma*, which are poorly identified by morphology. DNA barcoding has been developed to solve this problem. A 657 bp of COI barcodes from five species, *Stomoxys calcitrans*, *Stomoxys indicus*, *Stomoxys pulla*, *Stomoxys sitiens* and *Stomoxys uruma*, could be amplified. Their DNA sequences were made phylogenetic analysis and calculated sequence divergence by MEGA 5.1 program. The average of interspecific sequence divergence ranged 5.2–12.0%. The neighbor-joining (NJ) analysis among five species of the Stomoxyini flies was analysed using K2P parameter. All of *Stomoxys* flies were clearly separated into each species. The DNA barcoding can be used to confirm the poorly discriminated species and is applied for managing control programs of parasitic flies.

Keywords: DNA barcode, Parasitic flies, *Stomoxys*, Neighbor-joining, Interspecific sequence

Poster No. 83

THE STUDY OF MODEL DEVELOPMENT OF PREVENTION AND CONTROL OF LIVER FLUKE AND CHOLANGIOCARCINOMA IN THE SUB-DISTRICT LEVEL.

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This research aimed to study the model of resolution liver fluke and cholangiocarcinoma in the sub-district level in northeast Thailand. These were performed in seven provinces included of 37 sub-districts and carried out by following procedures; 1) determined the health behaviors of community, 2) feedback data to the community, 3) design and planning by participatory concept, 4) implemented for behaviors changing, and 5) evaluation. The duration is 2013-2014.

The first step: determined the health behaviors using simple random sampling method. Samples were aged 15 and over. 6,380 samples were interviewed by questionnaires. The results showed mean age 44 years. 25 % had experience of family died from cholangiocarcinoma. 78 % showed risk behaviors of liver fluke infection. 89 % consumed food containing nitrosamines. 27 % used to take praziquantel by themselves. Risk behaviors of liver fluke infection related to experience about family died from Cholangiocarcinoma, self-treatment of praziquantel, knowledge, and perception ($p < 0.05$). Feedback data to community, the sub-district conducted periodically 1-4 times. Most of activities got budget from sub-district Health Fund and designed related to health behaviors analysis.

At this moment, the study is step 4 and each sub-district is going to carry out their designed activities. The evaluation will be done at the end of this year. Then the researchers will analyze and synthesis the model development of prevention and control of liver fluke and cholangiocarcinoma and transfer appropriate solutions to other sub-districts around northeast.

Keywords: liver fluke, cholangiocarcinoma, model development

Poster No. 84

NOVEL POLYMER NANOSPHERES FOR SEROLOGICAL DIAGNOSIS OF MALARIA

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Objective – Malaria is a major public health problem especially in tropical and sub-tropical regions of the world. The methods most commonly used to diagnose malaria patients sera, such as IFAT and ELISA, are not easy methods to examine in the bedside and the clinical laboratory. Here, we wish to report novel diagnostic nanospheres for the detection of specific antibodies in malaria patients' sera.

Methods – The peptide antigens were designed from two Plasmodium specific sequences in lactate dehydrogenase (pLDH) and in enolase (AD22). Each test-material is prepared by chemical coupling of peptides to the surface of the diethyleneglycol dimethacrylate and methacryloyl-OSu copolymer nanosphere. Serum samples were 2-fold serially diluted (1/16...1/16384) in 96-well microplates. Then, the nanospheres suspension was added to the microplates, which were followed by agitation. Agglutination patterns can be observed almost at 4-6 h after the reaction.

Result – We have compared the reactivity against malaria patients' sera collected in endemic regions of the Philippines: (a) Mixed infected-, (b) falciparum-, (c) vivax-malaria patients, and (d) feverish patients (malaria-negative). Successful results were observed for the pLDH-material, which showed good specificity for three kinds of malaria patients compared with feverish patients. Interestingly, in the case of AD22-material,

malaria patients did not show distinct titer values from feverish patients. This is due to the difference of antibody persistence against two antigens in the endemic area where residents are sequentially infected by *Plasmodium* parasites. Therefore, these results indicate that pLDH and AD22 are suitable to detect present and recent-past infections, respectively.

Keywords: Synthetic Peptide, Polymer Nanosphere, Serological Diagnosis, Malaria.

Poster No. 85

OPTIMIZING DIHYDROARTEMISININ-PIPERAQUINE DOSE REGIMENS IN A PAEDIATRIC POPULATION: A PHARMACOKINETIC-PHARMACODYNAMIC APPROACH USING MONTE-CARLO SIMULATIONS

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Objective: To evaluate and optimize the dose regimen of dihydroartemisinin-piperaquine in a paediatric population using Monte Carlo simulations.

Methods: Different dosing regimens (i.e. the WHO recommended dose, manufacturers' recommended dose, and a proposed increased dose) were investigated in 1,000 hypothetical paediatric patients. The pharmacokinetic model of piperaquine was assumed to follow a three-compartment distribution model with five transit-absorption compartments. The pharmacodynamic model was implemented as a time-to-event model with a constant hazard of malaria infections and a sigmoid Emax function for the protective effect of piperaquine. The pharmacokinetic and pharmacodynamic parameters were fixed according to previous modelling results and implemented in Berkeley Madonna for simulations. Simulated day 7 piperaquine concentrations and the incidence of malaria infections during 2 months of follow-up were evaluated as pharmacokinetic and pharmacodynamic outcomes.

Results: Low day 7 piperaquine plasma concentrations were observed in the paediatric population and were strongly correlated with a higher number of malaria infections when using a standard body weight-based dose regimen (18 mg piperaquine/kg). The standard three day regimen in the small children (5-10 kg weight) resulted in a two-month incidence of malaria of 30.7% when using the manufacturers' dose recommendation as compared to 20.3% when using the proposed higher increased dose regimen. This is a 33.8% relative increase in piperaquine protective efficacy after the increased dose regimen. Additionally, a full 3-day regimen once a month for 3 consecutive months (i.e. seasonal intermittent preventive treatment) resulted in a 19.3% increased 5 months protective effect when using the proposed increased dose regimen as compared with the standard body weight-based dose in small children.

Conclusion: In conclusion, the suggested increased piperaquine dose regimen in small children resulted in an increased protective efficacy after treatment (33.8%) and after seasonal intermittent preventive treatment (19.3%). Current piperaquine dose recommendations in small children should be urgently reviewed.

Keywords: Malaria, Piperaquine, Pharmacokinetic, Pharmacodynamic, Paediatric population, Dose optimization

Poster No. 86

THE CORRELATION OF BEHAVIORAL CONSUMPTION ON HUMAN PARASITIC INFECTION IN EPIDEMIOLOGICAL EVIDENCE

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Parasitosis and related diseases are public health problem in Southeast Asia. Hence, community based cross-sectional study was conducted in the rural community in northeast of Thailand to evaluate the prevalence and behavioral consumption factors of parasitic infection. A total of 371 residents age from 2-80 years (age mean 53.5 ± 13 ; 42.8% male, 57.2% female) were questioned using questionnaires and fecal specimens were collected and examined by formalin-ethyl acetate concentration methods. The prevalence of parasitic infection was 127 cases (34.3%) with monoparasitized (28.6%) or polyparasitized (5.7%) cases. Helminthic infection in this area was 31.8%, the most of helminths was *Opisthorchis viverrini* (25.1%) followed by *Strongyloides stercoralis*, *Taenia* spp., Hookworm, Minute intestinal fluke and Echinostome. The prevalence of intestinal protozoa infection was 2.4%, there were *Blastocystis hominis*, *Entamoeba histolytica*, *E. coli*, *Balantidium coli*, *Iodamoeba butschlii*, and *Sarcocystis* sp. The uncooked meat consumption were correlation to parasitic infection in this area ($p = 0.049$). The highest consumption of uncooked food was fish (66.5%), followed by beef (28.5%) and pork (14.2%). The residents who ever drink alcohol showed more frequently uncooked meat consumption ($p < 0.001$) similar to residents with ever smoking ($p < 0.001$). Although the food consumption behavior was difficult to change but the appropriate and effective health education is need for controlling the parasitic infection in this area. The epidemiological survey in endemic areas of parasitic infection may improve a chance of success of control parasites.

Keywords: parasitic infection, food consumption behavior, alcohol consumption, smoking

Poster No. 87

EATING BEHAVIORS AMONG UNIVERSITY STUDENTS: EVALUATION OF BODY MASS INDEX DIFFERENCES

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Unhealthy eating behaviors have health implications, thus understanding the correlates of dietary habits are essential. This study seeks to investigate the eating behaviors of university students in relation to body mass index differences with the view to determine the relationship between these habits and BMI status. Anthropometric measurements, eating behaviors and personality traits were determined in a sample of university students from Southern Nigeria. The participants, 108 students (48.15% male and 51.85% female), aged 24.1 ± 4.1 filled out a self-reported questionnaire. The present study revealed that the mean factor structure scores in the underweight, normal weight and overweight students appear to be similar but no significant difference was found with respect to weight status. In the overweight category, anxiety showed significant positive associated with sleeping hour per night ($r = 0.36$) and also a negative correlation with sleeping condition ($r = -0.36$). Furthermore, emotional eating and personal interest in food was negatively correlated with snacking for all weight categories but the association was strongest for underweight students ($r = -0.81$), followed by the overweight students ($r = -0.35$). Another interesting finding was the strong negative relationship between anxiety and eating of breakfast observed only in the underweight students ($r = -0.65$). The results indicate that the same practices can have different effects

depending on the BMI of the subject, suggesting that is important to disentangle the subscales of dietary practices. Personality traits showed no significant relationship with BMI, additional research is needed to clearly understand the modulating factors.

Keywords: Weight status; Eating behaviors; University students; Body mass index differences; Nigeria.

Poster No. 88

THE *IN-VITRO* ANTIBACTERIAL EFFECT OF COLORED RICE CRUDE EXTRACTS AGAINST COMMON BACTERIA ASSOCIATED WITH SKIN AND SOFT-TISSUE INFECTIONS

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Skin and soft tissue infections (SSTIs) are caused mainly by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* bacteria. Due to the increasing emergence of bacterial resistance to existing antimicrobial agents, plants are being used as an alternative source to develop safe, effective, and inexpensive new agents for the treatment and prevention of bacterial infections. Recent studies show that *Oryza sativa* L., or rice, an important source of nutrients and consumed by most of the world's population, has exhibited suppressive effects on bacterial infections. There are many varieties of rice, e.g. white, brown, black, and red; however, the relationship between rice colors and antibacterial properties remains to be elucidated. This study aimed to investigate the antibacterial activity of colored-rice crude extracts against bacteria causing SSTIs. Ethanol extraction was performed to prepare crude extracts from four different types of colored rice: Hom Nil (HN), Neaw Dum (ND), Mun Poo (MP), and Sang Yod (SY). The paper disc diffusion method was used in this study. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of colored-rice extracts were determined, and bacterial survival percentage was also examined to assess antibacterial activity. Our results revealed that colored-rice crude extracts exhibited anti-Staphylococcal action, and more notably, crude extracts of differently colored rice restricted diverse antibacterial activities. This study provides a basic understanding of antibacterial properties found in colored rice, against skin and wound pathogens. An understanding of these properties would be invaluable in the development of alternative, natural and safe methods for controlling SSTIs.

Keywords: *Oryza sativa* L., colored rice, antibacterial effect, skin and soft tissue infections

Poster No. 89

IMPROPER PILL INTAKE COMPLICATES MALARIAPROPHYLAXIS

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U.S. and German military forces are deployed to regions in the world where chloroquine-resistant malaria is endemic like Afghanistan and Iraq. Doxycycline is more and more often used for malaria chemoprophylaxis in these areas. But the use of doxycycline can be complicated by oesophagitis, oesophageal ulceration and candida infection. We present a case of a 25-year-old U.S. soldier in Afghanistan, who developed a candida esophagitis after improper pill intake taking his medication immediately before going to bed, often without any fluid. A Medline search revealed 49 reports of oesophagitis linked to the intake of doxycycline and the WHO adverse drug reaction (ADR) database has 352 suspected reports of doxycycline-induced oesophagitis for the period 1969 to 2003. Drug-induced (candida)oesophagitis should be taken into consideration in all patients taking doxycycline presenting with chest pain and dysphagia. Physicians must warn the patients to take the pills and capsules with enough liquid and in the upright position.

Keywords: doxycycline, oesophagitis, candida, malariaprophylaxis

Poster No. 90

ORIGIN AND INTERFERENCE OF ROBUSTNESS IN MALARIA DRUG RESISTANCE

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Biological robustness allows mutations to accumulate while maintaining functional phenotypes. Despite its crucial role in evolutionary processes, the mechanistic details of how robustness originates remain elusive. Using an evolutionary trajectory analysis approach, we demonstrate how robustness evolved in malaria parasites under selective pressure from an anti-malarial drug inhibiting the folate synthesis pathway. A series of four nonsynonymous amino acid substitutions at the targeted enzyme, dihydrofolate reductase (DHFR) render the parasites highly resistant to the antifolate drug pyrimethamine. Nevertheless, the stepwise gain of these four *dhfr* mutations results in trade-offs between pyrimethamine resistance and parasite fitness. Here we report the epistatic interaction between *dhfr* mutations and amplification of the gene encoding the first upstream enzyme in the folate pathway, GTP Cyclohydrolase I (GCH1). *gch1* amplification confers low level pyrimethamine resistance and would thus be selected for by pyrimethamine treatment. Interestingly, the *gch1* amplification can then be co-opted by the parasites because it reduces the cost of acquiring drug-resistant *dhfr* mutations downstream in the same metabolic pathway. The compensation of compromised fitness by extra GCH1 is an example of how robustness can evolve in a system and thus expand the accessibility of evolutionary trajectories leading towards highly resistant alleles. The inhibitors against GCH1 were identified and tested for their potential as antimalarial drugs. The evolution of robustness during the gain of drug-resistant mutations has broad implications for both the development of new drugs and molecular surveillance for resistance to existing drugs.

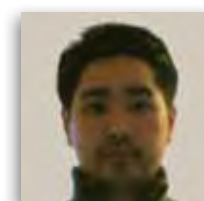
Keywords: Drug resistance, malaria, robustness

Poster No. 91

A NEW SHUTTLE VECTOR SYSTEM TO EVALUATE POTENTIAL MUTAGENIC RISKS OF ANTI-MALARIAL DRUGS

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It is generally believed that the rapid accumulation of several point mutations leads to drug resistance in the malaria parasite *Plasmodium falciparum*. While mutational processes might be explained by considering the selective pressure of anti-malarial drugs, the mutation rate of the parasites itself is actually low, at 10^{-9} . Therefore, we are reluctant to believe that spontaneous mutations alone contribute to drug resistance. Here, we hypothesize that anti-malarial drugs themselves could induce unexpected mutations at rates greater than spontaneous mutation rates.

To tackle this issue, we established a new shuttle vector system to measure the mutant frequency of cultured malaria parasites under various conditions, particularly exposure to anti-malarial drugs. The shuttle vector contains a monitor gene derived from *E. coli*, which should be maintained neutrally in parasites, but which makes *E. coli* clones sensitive to an antibiotic. Mutations that occur in the monitor gene of transfected parasites can be screened in a bacterial system.

Our new system detected mutant frequencies of 10^{-6} – 10^{-7} , while the error rate of next-generation sequencing is commonly in the order of 10^{-2} – 10^{-3} . Pilot experiments confirmed the dose-dependent increase in mutant frequency when parasites were exposed to a series of concentrations of the widely used mutagen *N*-ethyl-*N*-nitrosourea (ENU).

Using this system, we plan to evaluate the mutagenicity of anti-malarial drugs, which are potential risks as demonstrated in mutagenic tests using *Salmonella*. We also plan to apply the new system to wild isolates from Southeast Asia, which might show the accelerated resistance to multiple drugs (ARMD) phenotype reported by Rathod *et al.* (1997).

Keywords: *Plasmodium falciparum*, drug resistances, mutation frequency

Poster No. 92

CAMBODIAN ETHNIC MINORITIES AND INTERACTION ON MALARIA PREVENTION AND CONTROL IN CAMBODIA: A STUDY IN RATANAKIRI AND MONDOLKIRI

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Background: The Cambodia-Thailand Malaria Control project, was launched in 2007 in Ratanakiri and Mondolkiri provinces.

Objective: To describe the Cambodian ethnic minorities group and the interaction on malaria prevention and control.

Methodology: This qualitative study was conducted in February 2013. Observation, informal interview (malaria patients, community leaders and public health experts), in-depth interview (malaria patients, village malaria workers) and archival information were used for obtaining the data. This study observed the community environment and the villagers' lifestyle among the ethnic groups in Ratanakiri (Tum-poun community) and Mondolkiri (Pha-nong community).

Results: The findings revealed that the infrastructure, social and lifestyle of the people in both study communities were rapidly changing due to the economic growth. Health services accessibility are increasing. The prevalence of malaria infection among ethnic minority groups in both provinces are decreased. We found that some of well-trained village malaria volunteers quit the job to earn more income, some malaria health centers were closed.

The public health experts suggested that “community capacity building is the key success factor for prevention and control malaria in the ethnic minority groups. This included strengthening key capacities for community-based (community-led) disease control activities. The key principle is a respect for local cultures in encouraging healthy behavior for disease control, including community-based surveillance and routine vector monitoring. Socio-cultural skills are needed to understand the target population”.

Conclusion: Working with the ethnic minority groups for malaria prevention and control, building trust, respect and community capacity building need to be taken into account.

Keywords: malaria, prevention and control, ethnic group,

Poster No. 93

EFFECT OF SAMPLING PAPER ON DRIED BLOOD SPOTS SPECIMEN COLLECTION

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Dried blood spots (DBS) as a sampling technique has been in use for a long time. It is now increasingly common in preclinical and clinical sample collection especially the studies involving antimalarial drugs. In the beginning, its purpose was to screen newborn for phenylketonuria. For antimalarial drugs, it is a simple collection technique, particularly useful in rural areas where resources are limited for sampling, storage and shipping of venous samples. However, there are several factors to be considered when

using the DBS technique and this poster will describe some of those and what impact it may have on the final analysis of drug levels in those blood spots. Five different type of filter paper (31ET Chr, FTA DMPK-C, 903 Protein, Ahlstrom 226 and 3MM Chr) were evaluate on paper properties, blood absorption properties and blood spot distribution, hematocrit and price. The impact on measurement of drug analysis including influence on storage conditions and drying conditions on DBS sample was investigated.

Keywords: Dried blood spot, Filter paper, hematocrit, capillary blood sampling

Poster No. 94

DURATION OF FISH STORAGE IN LOW TEMPERATURE BEFORE RAW-FISH-PROCESSING DISH PREVENTS *OPISTHORCHIS VIVERRINI* METACERCARIAE INFECTION IN HAMSTERS

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Opisthorchis viverrini metacercariae (OVMC) is a high distribution and abundance in freshwater cyprinid fish in northeastern Thailand. People prefer to eat raw-fish dishes such as *pla-som* and *pla-ra* leading to a high prevalence of infection. Previously, OVMC obtained from fermented food was found only in *pla-som*. In the present study, we investigated the raw-fish-processing dish in *pla-som* to reduce risk of infection. Cyprinid freshwater fish was used to prepare raw-fish-processing dish, *pla-som*, for seven days in duplicate experiment. OVMC in fermented fishes were digested by artificial pepsin, examined by sedimentation method, and identified under stereomicroscope. The result revealed that morphology and viable of OVMC was observed for 1-3 day(s) of *pla-som* and they were destructed thereafter. Infection rate of OVMC obtained from *pla-som* in hamsters was 25.5%, 24% and 7% for day(s) 1, 2 and 3, respectively. In order to reduce OVMC contaminated in *pla-som* infection to animal, fishes were stored at refrigerator (4°C) for 1-5 day(s) and then a raw-fish-processing was fermented for 3 days, subsequently they were digested and examined. Fish storage for day(s) 1 and 2, OVMC morphology completely distinguished and was able to infect in hamsters. In contrast, fish storage for day(s) 3, 4 and 5, all OVMC were destructed and couldn't infect to hamster. In conclusion, OVMC from *pla-som* fermented during 1 to 3 day(s) is able to infect and fish storage at refrigerator more than 3 days before raw-fish-processing can prevent OVMC infection.

Keywords: *Opisthorchis viverrini* metacercariae, freshwater cyprinid fish, raw-fish dishes, fermented food

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Poster No. 95

EFFECTIVENESS OF AN ADDITIONAL BIRTH DOSE OF HEPATITIS B VACCINE TO THE CONVENTIONAL 3-DOSE OF PENTAVALENT (DTP+HEP-B+HIB) VACCINE AMONG THE INFANTS IN RURAL BANGLADESH

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Objective: To compare the effectiveness of hepatitis B vaccine with and without a birth dose among the Bangladeshi infants.

Materials and Methods: A multicenter open label randomized controlled trial where 486 newborn delivered by 480 mothers were randomly assigned to either receive conventional 3 doses of hepatitis B containing pentavalent (DTP+Hep-B+Hib) vaccine (control group) at 6,10 and 14 weeks or receive monovalent hepatitis B vaccine at birth in addition to the 3 doses of pentavalent vaccine (intervention group). Blood samples were drawn one month after the 3rd dose of pentavalent vaccine to assess seroprotection against hepatitis B and the seroprevalance of HBsAg among the vaccinated infants.

Results: Out of 486 infants, 463 completed the study, where 234 and 229 infants were randomly assigned to the intervention group and the control group respectively. Overall, 99.4 % of infants achieved protective (anti-HBs: ≥ 10 mIU/ml) level of antibody. The seroprotection rate was 99.2 % and 99.6 % among infants in the intervention group and the control group respectively. In general, the geometric mean titer (GMT) was 39.4 mIU/ml. This GMT was 38.7 mIU/ml in the intervention group and 40.9 mIU/ml in the control group. Gestational age and mother's education level were associated with antibody level among the infants ($p=0.03$). No infant was found to be positive with HBsAg.

Conclusions: Both schedules (with and without birth dose) of hepatitis B vaccine were well effective in terms of immune response.

Keywords: Effectiveness, hepatitis B vaccine, birth dose, infants, Bangladeshi

Poster No. 96

HYPOMETHYLATION OF ALU ELEMENTS IN POST-MENOPAUSAL WOMEN WITH OSTEOPOROSIS

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A decrease in genomic methylation commonly occurs in aging cells; however, whether this epigenetic modification leads to age-related phenotypes has not been evaluated. *Alu* elements are the major interspersed repetitive DNA elements in humans that lose DNA methylation in aging individuals. *Alu* demethylation in blood cells starts approximately 40 years of age, and the degree of *Alu* hypomethylation increases with age. Bone mass is lost with aging, particularly in menopausal women with lower body mass. Consequently, osteoporosis is commonly found in thin postmenopausal women. Here, we correlated the *Alu* methylation level of blood cells with bone density in 323 postmenopausal women. *Alu* hypomethylation was associated with advanced age and lower bone mass density, ($P<0.05$). The association between the *Alu* methylation level and bone mass was independent of age, body mass, and body fat, with an odds ratio [1]=0.4316 (0.2087-0.8927). Individuals of the same age with osteopenia, osteoporosis, and a high body mass index have lower *Alu* methylation levels ($P=0.0005$, 0.003, and ≤ 0.0001 , respectively). Finally, when comparing individuals with the same age and body mass, *Alu* hypomethylation was observed in individuals with lower bone mass ($P<0.0001$). In conclusion, there are positive correlations between *Alu* hypomethylation in blood cells and several age-related phenotypes in bone and body fat. Therefore, reduced global methylation may play a role in the systemic senescence process. Further evaluation of *Alu* hypomethylation may clarify the epigenetic regulation of osteoporosis in post-menopausal women.

Keywords: *Alu* elements, *Alu* methylation, methylation pattern, global hypomethylation, osteoporosis, menopause, aging, senescence, epigenomics

Poster No. 97

MASSIVE *TAENIA SOLIUM* TAPEWORM INFECTION IN A PATIENT IN THAILAND: A REPORT OF A CASE

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A 67-year-old Karen-male patient, who was confirmed to have harbored nineteen *T. solium* tapeworms. He was tall and had slim body, looked healthy, no history of underlying diseases and never took anti-parasitic drug before this treatment. He had a history of consuming uncooked meat, especially pork and beef, for over 50 years since 15 years of his age. He frequently consumed a traditional dish made from raw pork, called “Lahb Moo” and ate during an alcohol drinking with his neighbors. Two months before this examination and treatment, he recognized some proglottids of tapeworms were contaminated in his stool during defecation. He only complained about some clinical symptoms of abdominal discomfort, odd feeling like something moving in his abdomen. After deworming with Nicrosamide, proglottids with scolices of 19 tapeworms were recovered from his whole stool collection. All scolices of the worms were examined under a microscope for morphological identification of species, and all the worms were identified as *T. solium*. Nine-proglottids from each scolices were separated for species identification by PCR/sequencing at Department of Parasitology, Asahikawa Medical University, Japan and molecularly confirmed as *T. solium* (Asian type).

Keywords: massive Tapeworm infection, 19 scolices of *Taenia solium*, Thasongyang District, Thailand

Poster No. 98

METHOD VALIDATION OF MULTIDOT INDIRECT FLUORESCENT ANTIBODY FOR DETECTING FIVE ZONOTIC DISEASES

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Some zoonotic pathogens such as *Brucella melitensis* and *Burkholderia pseudomallei* are bioterrorism agents while other zoonotic genera act as emerging pathogens i.e., pathogenic *Leptospira* spp., *Orientia tsutsugamushi*, and *Rickettsia typhi*. Almost these pathogens play an important role of human infection and become one of public health problems in Thailand since closely relate of human and animals. In this study, we adapted a multidot indirect fluorescent antibody (multidot-IFA) by separately applying five antigens of *L. interrogans*, *B. melitensis*, *B. pseudomallei*, *O. tsutsugamushi*, and *R. typhi* onto one well of a slide. The slide test was validated using standard method for diagnosis of 125 cases; leptospirosis, brucellosis, murine typhus, scrub typhus, and melioidosis, including 70 sera from blood donors as positive and negative control samples, respectively. By resulting, it found that the sensitivity was ranging from 90-100% on both IgM and IgG. The specificity using blood donors was 91.4% (64/70). In conclusion, this technique referring to the principle method of IFA can diagnose five diseases in one experiment, less consuming time, and cost effectiveness.

Keywords: multidot-IFA, zoonotic pathogens, *Brucella melitensis*, *Burkholderia pseudomallei*, *Leptospira interrogans*, *Orientia tsutsugamushi*, *Rickettsia typhi*, brucellosis, melioidosis, leptospirosis, scrub typhus, murine typhus.

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