# JITMM2014
# & FBPZ8

## Joint International Tropical Medicine Meeting 2014

# “3D Perspectives on Tropical Medicine: Drivers, Diversity, and Determination”

## The 8th Seminar on Food- and Water-borne Parasitic Zoonoses

2-4 December 2014

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


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Joint International Tropical Medicine Meeting 2014
"3D Perspectives on Tropical Medicine:
Drivers, Diversity, and Determination"
The 8th Seminar on Food- and Water-Borne Parasitic Zoonoses
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## ABSTRACTS
## ORAL Presentations

Centara Grand & Bangkok Convention Centre at CentralWorld,
Bangkok Thailand

# Tuesday 2nd December 2014

**Time:**

**9.30 – 10.30** **Convention**

## S1 The 20th Chamlong-Tranakchit Harinasuta Lecture

**Keynote Speaker:**

Dr. BT Slingsby

Keynote: Investing in health, creating an impact

*BT Slingsby*

Global Health Innovative Technology Fund

# INVESTING IN HEALTH, CREATING AN IMPACT

BT Slingsby, M.D., Ph.D., M.P.H.

*Global Health Innovative Technology Fund*

The GHIT Fund's catalytic first year changed the game for global health R&D. Since it first call for proposals, the Fund has allocated over US$33 million in more than 30 groundbreaking development partnerships between Japanese and international research institutions, as well as reinvigorated drug discovery for neglected and parasitic diseases by opening the door for the global health community to Japan's innovations and pharmaceutical technologies. In 2014, the Fund forges ahead with even more urgency. The accelerated re-emergence of the Ebola virus infection in West African coupled with an outbreak of Dengue Virus infection in Tokyo – the first in 70 years – makes global health R&D investment, innovation, and partnership more urgent than ever.

Dr. Slingsby's KEYNOTE SPEECH IN AN OPENING CEREMONY AT JITMM 2014 will provide an outline of GHIT's short but high-impact history and current work in the context of the "3D Perspectives" conference theme, illustrating the driving forces behind GHIT's establishment and funding of global health R&D, the challenges GHIT has overcome, and the determination that made its success to date a reality. Key "drivers" will include Japan's legacy of innovation and ODA, its globalizing pharmaceutical sector, the Japanese population's personal connection to addressing NTDs, and a global strengthening and prioritization of global health R&D efforts. This presentation will also emphasize the vibrant history of collaboration between Thailand and Japan around global health and innovation creation.

Keywords: Dr. Slingsby's KEYNOTE SPEECH IN AN OPENING CEREMONY AT JITMM 2014

# Tuesday 2nd December 2014

**Time:**

**11.00 – 12.30** **Convention**

## S2 "Amebiasis"

**Chairpersons:**

1. Porntip Petmitr

2. Tomoyoshi Nozaki

Invited Speakers:

1 Mass Spectrometric Analysis Of L-Cysteine Metabolism: Physiological Role And Fate Of L-Cysteine In The Enteric Protozoan Parasite *Entamoeba* histolytica

*Tomoyoshi Nozaki*

Department of Parasitology, National Institute of Infectious Diseases, Japan

2 Immunochromatographic dipstick test using recombinant pyruvate phosphate dikinase for rapid diagnosis of amoebic liver abscess

*Rahmah Noordin*

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

3 Production of specific monoclonal antibodies for differentiation of pathogenic *Entamoeba histolytica* and *E. moshkovskii* from non-pathogenic *E. dispar*

*Saengduen Moonsom*

Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Thailand

4 Genotyping Entamoeba strains and their differential tolerance to antiamebic drugs

*Jaishree Paul*

School of Life Sciences, Jawaharlal Nehru University, India

# MASS SPECTROMETRIC ANALYSIS OF L-CYSTEINE METABOLISM: PHYSIOLOGICAL ROLE AND FATE OF L-CYSTEINE IN THE ENTERIC PROTOZOAN PARASITE *ENTAMOEBA HISTOLYTICA*

Ghulam Jeelani[a], Dan Sato[b]*, Tomoyoshi Soga[b], Haruo Watanabe[c],
Tomoyoshi Nozaki[a,d]#

[a] *Department of Parasitology, National Institute of Infectious Diseases, Shinjuku, Tokyo, Japan;*
[b] *Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, Japan;*
[c] *National Institute of Infectious Diseases, Tokyo, Japan;*
[d] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan*

L-cysteine is essential for virtually all living organisms from bacteria to higher eukaryotes. Besides its role in the synthesis of virtually all proteins, taurine, cysteamine, glutathione and other redox regulating proteins, L-cysteine has other important functions under anaerobic/microaerophilic conditions. In anaerobic or microaerophilic protozoan parasites such as *Entamoeba histolytica,* L-cysteine was implicated in the growth, attachment, survival, and protection from oxidative stress. However, a specific role of this amino acid or related metabolic intermediates is not well understood. In this study, using stable isotopic labeled L-cysteine and capillary electrophoresis time-of-flight mass spectrometry, we investigated the metabolism of L-cysteine in *E. histolytica*. [U-$^{13}$C3, $^{15}$N] L-cysteine was rapidly metabolized into three unknown metabolites, besides L-cystine and L-alanine. These metabolites were identified as thiazolidine-4-carboxylic acid (T4C), 2-methyl thiazolidine-4-carboxylic acid (MT4C), and 2-ethyl-thiazolidine-4-carboxylic acid (ET4C), the condensation products of L-cysteine with aldehydes. We demonstrated that these 2-(R)-thiazolidine-4-carboxylic acids serve as storage of L-cysteine. Liberation of L-cysteine occurs when T4C were incubated with amebic lysates, suggesting enzymatically degradation of these L-cysteine derivatives. Furthermore, T4C and MT4C significantly enhanced trophozoites growth, and reduced intracellular ROS levels, when added into the culture, suggesting that 2-(R)-thiazolidine-4-carboxylic acids are involved in the defense against the oxidative stress.

# IMMUNOCHROMATOGRAPHIC DIPSTICK TEST USING RECOMBINANT PYRUVATE PHOSPHATE DIKINASE FOR RAPID DIAGNOSIS OF AMOEBIC LIVER ABSCESS

Rahmah Noordin[1], Syazwan Saidin[1], **Muhammad Hafiznur Yunus**[1],
Nurulhasanah Othaman[1], and Lim Boon Huat[2]

[1] *Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800 Penang, Malaysia;*
[2] *School of Health Sciences, Universiti Sains Malaysia, Kelantan, Malaysia*

Most cases of extraintestinal *Entamoeba histolytica* infection result in amoebic liver abscess (ALA). *E. histolytica* pyruvate phosphate dikinase (PPDK) has been shown to be a potential diagnostic marker for ALA. In this study, recombinant PPDK protein (rPPDK) was expressed, purified and initially evaluated by western blots. In parallel recombinant galactose-and-N-acetyl-D-galactosamine inhibitable (rGal/GalNAc) lectin was produced as a control antigen. Subsequently, an immunochromatographic dipstick test using rPPDK, named as "XEh Rapid", was developed and evaluated. Further purification of the protein was then performed to increase its diagnostic sensitivity. The results of the Western blots of rPPDK using sera from patients with ALA, healthy individuals and other diseases probed with anti-human $IgG_4$-HRP showed diagnostic sensitivity of 93.3% (14/15) and specificity of 100% (n=40). The specificity of rPPDK was found to be higher when compared to that of rGal/GalNAc lectin (95%) control antigen. The initial optimized XEh Rapid used 1.25 mg/ml of rPPDK and showed diagnostic sensitivity and specificity of 87% (26/30) and 100% (n=40) respectively. Enhancement of the purity of rPPDK allowed lining of membrane cards with rPPDK at 2.0 mg/ml, this resulted in an increase in diagnostic sensitivity of the rapid test to 97.8% (44/45) while maintaining its high diagnostic specificity (100%, n=60). Thus XEh Rapid using rPPDK showed good potential for rapid diagnosis of ALA, and may be also useful for diagnosis of other forms of extraintestinal amoebiasis. Next, the test will be converted into the more robust cassette format of the immunochromatographic test for multicenter evaluation.

Keywords: amoebic liver abscess, *Entamoeba histolytica*, immunochromatographic dipstick test, diagnosis

# PRODUCTION OF SPECIFIC MONOCLONAL ANTIBODIES FOR DIFFERENTIATION OF PATHOGENIC *ENTAMOEBA HISTOLYTICA* AND *E. MOSHKOVSKII* FROM NON-PATHOGENIC *E. DISPAR*

Saengduen Moonsom[1], Somphob Leetachewa[2], Narumol Khomkhum[1], Tomoyoshi Nozaki[3], Waroon Boonyaudomsart[4]and PorntipChavalitshewinkoon Petmitr[1]

*[1] Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,*
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*[3] Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan,*
*[4] Department of Biology, Faculty of Sciences, Mahidol University, Bangkok.*

About 25,000 people worldwide die annually due to amoebiasis caused by *Entamoebahistolytica*. *E. moshkovskii*is considered as a diarrhea causing protozoanin some endemic countries. *E. histolytica*and *E. moshkovskii* are morphology identicalto the non-pathogenic *E. dispar*. Such limitation leads to missed diagnosis of a true agent by gold standard microscopy and consequently unnecessary treatment. To overcome this problem, *Entamoeba* species specific monoclonal antibodies (mAbs) that may be potential for differential diagnostic development of *E. histolytica/E. moshkovskii and E. dispar*were produced. BalB/c mice were immunized with *Entamoeba*trophozoites and *Entamoeba* specific polyclonal antibodies were monitored by indirect-Enzyme Linked Immunosorbent Assay; ELISA.mAbs were then produced using standard hybridoma technique. The mAbswere tested for specificity against proteins extracted from each of *Entamoeba*species.Binding specificity of the mAbswasfurther tested with the whole cell and cellular fractions of the trophozoites by immunofluorescence, flow cytometry and ELISA, respectively. Fifty-five mAbswere successfully produced. Among them, there are eightof*E. histolytica* specific, eight of *E. moshkovskii*specific, 18 of *E. histolytica* and *E. dispar* reacting mAbs, one of the *E. histolytica*-*E. moshkovskii*mAb, and 17 of mAbs reacting to all of *E. histolytica*, *E. moshkovskii*and *E. dispar*. ELISA revealed two *E. histolytica*mAbs and at least three of *E. moshkovskii*mAbs that react to cell membrane. Thus, thesemAbs may be potential for development of a rapid and differential diagnosis for amoebiasis causative agents in the near future.

Keywords: *Entamoebahistolytica*, *Entamoebamoshkovskii*, amoebiasis, differential diagnosis, monoclonal antibody

# GENOTYPING ENTAMOEBA STRAINS AND THEIR DIFFERENTIAL TOLERANCE TO ANTIAMEBIC DRUGS

Jaishree Paul

*School of Life Sciences, Jawaharlal Nehru University, India*

The protistan parasite *Entamoeba histolytica* is the etiological agent of amoebiasis , causing high rate of morbidity and mortality in endemic countries including Indian subcontinent. Varied organ tropism affecting extra intestinal organs like liver, lung and brain causing abscesses is common in this parasite. Accumulating evidences indicate that degree of virulence varies among the strains of *Entamoeba histolytica* as shown by their differential cytopathic effects suggesting that virulence may be linked with genotype. We have explored the mobile genetic elements of *E. histolytica* genome, the retrotransposons in order to differentiate the strains of natural isolates from New Delhi and other places. Assessing the genomic distribution of SINE elements has proved to be a valid method for strain identification.

Metronidazole is the widely used drug for amebiasis. In order to understand the impact of the drug on the parasite harboring in our population, some of these natural isolates maintained in xenic culture are subjected to antiamebic drugs- Metronidazole and Auranofin (a FDA approved drug) to show their tolerance level. MIC values for HM-1 (lab strain) and two clinical isolates (MS 96 and 654) were 20, 25 and 50µM respectively when the cells were subjected to metronidazole whereas to auranofin, the isolates were much more sensitive at lower doses but their tolerance varied between 1-5µM among isolates. Metronidazole is known to create an oxidative stress in the parasite. We demonstrated the expression and the pattern of localization of an important antioxidant enzyme, peroxiredoxin in the clinical isolates by RT PCR and immunolocalization studies.

# Tuesday 2nd December 2014

**Time:**

**11.00 – 12.30** **Room A**

## S3 "International Center of Excellence for Malaria Research Studies in Asia"

**Chairpersons:**

1. Jetsumon Prachumsri

2. Ivo Mueller

Invited Speakers:

1 US NIH International Center of Excellence for Malaria Research: South Asia

*Pradipsinh Rathod*

University of Washington, Seattle, USA

2 Outcome of international center of excellence for malaria research studies in Thai-Myanmar-China borders

*Liwang Cui*

College of Agricultural Sciences, Penn State University, USA

3 Back from the brink and where to now? Outcome of the ICEMR studies in Papua New Guinea and Solomon Island

*Ivo Mueller*

Walter & Eliza Hall Institute in Melbourne, Australia and Institute for Global Health (ISGlobal), Barcelona, Spain

# US NIH INTERNATIONAL CENTER OF EXCELLENCE FOR MALARIA RESEARCH: SOUTH ASIA

Pradipsinh K. Rathod, PhD.

*Director, US NIH ICEMR (South Asia)*
*Professor of Chemistry and Global Health*
*University of Washington, Seattle, WA 98103*

The US National Institutes of Health Program Project "Malaria Evolution in South Asia" aims to understand how natural as well as man-made forces direct the evolution of malaria parasites on the subcontinent. Our work begins with an observational study of disease presentation at a tertiary care center in Southwest India and is gradually expanding to three additional sites reaching towards the India-Myanmar border. At each site, our aim is to develop a more in-depth description of local transmission, drug resistance, acquired and innate immunity, and pathogenesis. We rely heavily on phenotypic assays to characterize parasites isolated from well-characterized patient cases. Such work integrates our understanding of the powers of the parasite to mutate and reveals the challenges of implementing ambitious malaria control and elimination policies directed from higher governing bodies.

# OUTCOME OF INTERNATIONAL CENTER OF EXCELLENCE FOR MALARIA RESEARCH STUDIES IN THAI-MYANMAR-CHINA BORDERS

Liwang Cui

*College of Agricultural Sciences, Penn State University, USA*

The Southeast Asian International Center of Excellence in Malaria Research (ICEMR) focuses on malaria research in the Greater Mekong Subregion with emphasis on malaria in Thailand, China and Myanmar, which have drastic differences in epidemiology. At selected sentinel sites located along international borders, we closely follow the changes of malaria epidemiology using active case detection at endemic villages and passive case detection at hospitals, malaria clinics and posts. The demographic and epidemiological systems allow us to track the distribution and dynamics of malaria and its relation to both biotic and abiotic factors such as climatic changes, host movements, vector abundance. Investigations into the vector biology provide updated information about relative abundance, seasonal fluctuations, biting behaviors, as well as insecticide resistance. Furthermore, we closely monitor the treatment efficacy of antimalarial drugs and use advanced genomic tools to identify the molecular mechanisms of drug resistance. Through this interactive malaria research projects, we aim to develop an integrative strategy of malaria control and aid the local agencies in their strives towards malaria elimination in this region.

# BACK FROM THE BRINK AND WHERE TO NOW? OUTCOME OF THE ICEMR STUDIES IN PAPUA NEW GUINEA AND SOLOMON ISLAND

Ivo Mueller

*Walter + Eliza Hall Institute, Melbourne, Australia*
*Institute for Global Health (ISGlobal), Barcelona, Spain*

At the high of the last malaria elimination effort in the mid 1970s the burden of malaria in Papua New Guinea and Solomon Island had been reduced to relatively low levels (PR <10%, API = 50/1000). After independence both countries however abandoned their vector control programs and concentrated exclusively on case management, resulting in a massive resurgence of malaria in the 1980s and 1990s. With support from the Global Funds both countries have recently re-established malaria control and have subsequently seen dramatic reduction in the burden of malaria. The incidence of malaria was reduced by >95% in the Solomon Island and by >60% in Papua New Guinea. With a coordinated series of epidemiological studies, the SW Pacific International Centre of Excellence in Malaria Research is investigating the impact of these dramatic changes on burden of infections and disease, transmission, parasite genetic diversity and the natural acquisition of immunity. These studies show that while there was initial substantial progress against both *P. falciparum* and *P. vivax* in Papua New Guinea, the *P. vivax* remains entrenched but *P. falciparum* has almost completely disappeared from the Solomon Island. This highlights the greater challenge for the elimination of *P. vivax*.

# Tuesday 2nd December 2014

**Time:**

**11.00 – 12.30** **Room B**

## S4 HIV Preventive Strategies- Now and Next

**Chairpersons:**

1. Punnee Pitisuttithum

2. Supachai Rerks-Ngarm

Invited Speakers:

1 HIV epidemic in Asia: implications for HIV vaccine and other prevention trials

*Jean-Louis Excler*

US Military HIV Research Program, USA

2 Biomedical HIV prevention: What do we know and Where are we going

*Timothy Holtz*

HIV/STD Research Program, Thailand MOPH – US CDC Collaboration (TUC), Nonthaburi, Thailand and Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA

3 State of the art of HIV prevention special focus on PrEP

*Suwat Chariyalertsa*

Research Institute for Health Sciences (RIHES), Chiang Mai University, Thailand

4 Global overview of HIV pipelines and central role of Thailand for future HIV VACCINE development

*Robert O Connell*

Department of Retrovirology, U.S. Component of the Armed Forces Research Institute for Medical Sciences, Thailand

# HIV EPIDEMIC IN ASIA: IMPLICATIONS FOR HIV VACCINE AND OTHER PREVENTION TRIALS

Jean-Louis Excler,

*US. Military HIV Research Program*

According to UNAIDS, at the end of 2013,in Asia and the Pacific there were 4.8 million people living with HIV, China, India, Indonesia, Myanmar, Thailand, and Viet Nam—accounting for more than 90% of the people living with HIV in the region. An overall decrease of HIV prevalence is now observed in several key Asian countries due to effective prevention programs.Low national prevalence also masks higher HIV prevalence and incidence rates in certain key populations. New HIV infections are concentrated among key populations at higher risk, more difficult to reach due to stigma and legal barriers, which include PWID, FSW and their clients, MSM and TG, mostly concentrated in major cities. The estimated annual fraction of new HIV infections (FNI) among FSW remained low, with little variability by region and epidemic type.In contrast, the FNI among MSM was higher than in FSW, varied with countries and increased with MSM population size. The FNI among PWID was largest in early-phase epidemics with low overall HIV prevalence.HIV molecular epidemiology shows an increased diversification of HIV-1 subtypes and circulating recombinant forms, in particular at country borders.

The implementation of prevention trials may then face then important challenges. The opportunity to identify heterosexual populations at high risk such as FSW may rapidly wane. It is more likely that efficacy trials will occur in the MSM and TG populations because their higher HIV incidence would allow smaller and less costly trials. The constantly evolving patterns of HIV-1 diversity in the region suggest to closely monitoring the molecular HIV epidemic in potential target populations for HIV vaccine efficacy trials. While vaccines inducing non-neutralizing antibodies offer promising perspectives for protection against HIV acquisition, other vaccines designed to induce broadly neutralizing antibodiesand cell-mediated immune responses of greater breadth and depth in the mucosal compartments should be aggressively pursed and considered for testing in MSM and TG with a much higher rate of transmission than heterosexual populations.The rationale and design of efficacy trials of combination prevention modalities such as HIV vaccine and PrEPremains for the moment hypothetical and would require high adherence to PrEP, be more costly and challenging for the regulatory approval process to licensure.The prioritization of prevention interventions should be driven by the HIV epidemic and decided by the country-specific health and regulatory authorities. Modeling the impact and cost-benefits interventions may help this decision process.

# BIOMEDICAL HIV PREVENTION: WHAT DO WE KNOW AND WHERE ARE WE GOING

Timothy H. Holtz

*Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA*
*HIV/STD Research Program, Thailand MOPH – US CDC Collaboration (TUC), Nonthaburi, Thailand*

Background and purpose: The proof-of-concept of voluntary medical male circumcision (VMMC), antiretroviral (ARV) treatment as prevention (TasP), and pre-exposure prophylaxis (PrEP) have recently been demonstrated. These innovative new approaches have the potential to dramatically improve HIV prevention in high-risk groups. The purpose of this presentation is to review recent clinical trial results, and discuss remaining questions and possible population-based implementation strategies.

Methods: Literature review using PubMed and conference proceedings for clinical trials published between 2007–2014.

**Results and findings:** Over the past 7 years, a plethoraof clinical trialshave been published on innovative biomedical HIV prevention technologies, such as VMMC, oral or vaginal tenofovir-based PrEP, and ARVs for prevention. These trialshave demonstrated varying protection against HIV acquisition among heterosexual men, women, men who have sex with men (MSM), serodiscordant couples, heterosexualwomen, and people who inject drugs. The level of efficacy in these trials was significantly related to the level of adherence, both by self-report as well as pharmacokinetic measures. Some clinical trials among heterosexual women showed no effect. The CDC and WHO have released guidance for HIV prevention among key populations.

Conclusion: New biomedical HIV prevention methods are efficacious for the prevention of sexual and parenteral HIV acquisition. Critical issues include adherence and real world demonstration in implementation science projects, and developing next-generation agents that use innovative technology.

# STATE OF THE ART OF HIV PREVENTION SPECIAL FOCUS ON PrEP

Suwat Chariyalertsa

*Research Institute for Health Sciences (RIHES), Chiang Mai University, Thailand*

# GLOBAL OVERVIEW OF HIV PIPELINES AND CENTRAL ROLE OF THAILAND FOR FUTURE HIV VACCINE DEVELOPMENT

ROBERT J. O’CONNELl, MD

*Chief, Department of Retrovirology*
*U.S. Component of The Armed Forces Research Institute for Medical Sciences*
*Bangkok, Thailand*

Globally, although Human Immunodeficiency Virus (HIV) infection rates have fallen in recent years, the pandemic continues, with 1.6 million deaths attributed to the infection in 2012 in spite of massive expenditures for antiretroviral therapy programs. And while emerging biomedical interventions such as medical male circumcision, pre-exposure prophylaxis, microbicides, and treatment as prevention have proven value, the dual challenges of public health implementation and the requirement for ongoing adherence by the at-risk individual still strongly argue that an effective vaccine is needed. Six efficacy trials testing four preventive HIV vaccine concepts have been completed, and yet there remain no licensed HIV vaccines. Thailand hosted two such trials, including the first efficacy trial in a low or middle income country (Vax003), and the only trial to demonstrate efficacy (RV144). The current global portfolio of HIV vaccine candidates has never been more diverse, with dozens of concepts at various stages of maturity. This talk will provide an overview of the HIV vaccine field including a summary of the HIV vaccine candidates being investigated, with a focus on the most advanced concepts, including plans for the next efficacy trial in Thailand.

# Tuesday 2nd December 2014

**Time:**
**11.00 – 12.30** **Room C**

## S5 Dengue Vaccine Update I

**Chairpersons:**

1. Pornthep Chanthavanich

2. Chukiat Sirivichayakul

Invited Speaker

1 Update on Takeda's Live Attenuated Tetravalent Dengue Vaccine (TDV) Candidate
*Jeremy Brett*
Takeda Vaccines Pte Ltd, Singapore

2 Update on NIAID/ NIH Dengue Vaccine Candidates
*Louis Macareo*
Armed Forces Research Institute of Medical Sciences (AFRIMS)

3 An Update on Recombinant Dengue Vaccine Development in Thailand
*Nopporn Sittisombut*
Department of Microbiology, Faculty of Medicine, Chiang Mai University, Thailand

# UPDATE ON TAKEDA'S LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (TDV) CANDIDATE

Jeremy Brett

*Takeda Vaccines Pte Ltd. Singapore*

We have developed a live attenuated tetravalent dengue vaccine candidate based on an attenuated dengue virus-2 (TDV-2) and three chimeric viruses containing the pre-membrane and envelope genes of DENV-1, -3 and -4 expressed in the context of the attenuated TDV-2 genome (TDV-1, -3, & -4, respectively). This vaccine candidate is currently in phase II clinical trials in humans. Preclinical and clinical characterization of the immune response to this vaccine provides evidence that it stimulates innate immune responses, and elicits a humoral response and T-cell mediated immunity to dengue structural and non-structural proteins. Furthermore, clinical trials in dengue endemic and non-endemic geographic areas demonstrate that the vaccine is generally well-tolerated. A single dose elicits an immune response lasting to Day 120 to all four serotypes, with little improvement when a second dose is given at 90 days. We are currently planning a pivotal phase III study to investigate the efficacy of this vaccine candidate.

# UPDATE ON NIAID/NIH DENGUE VACCINE CANDIDATES

Louis Macareo[1], Stephen Whitehead[2]

[1] *Armed Forces Research Institute of Medical Sciences*
[2] *National Institutes of Health, Bethesda, Maryland, United States*

The NIAID has developed live attenuated dengue vaccine candidates shown to be both safe and immunogenic in monovalent and tetravalent studies in humans. These studies have enabled the down-selection of vaccine candidates to an optimal mixture of rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30. In admixture TV003, each component is delivered at a potency of 1000 PFU and neutralizing antibody data collected 90 days post-vaccination in flavivirus-naïve adults showed seroconversion to DENV1, DENV2, DENV3, and DENV4 in 92%, 76%, 97%, and 100% of vaccinees, respectively, after a single subcutaneous dose. 74% of vaccinees achieved a tetravalent antibody response. When the potency of the DENV2 component of the vaccine was increased 10-fold in admixture TV005 and administered in the same manner as TV003, frequencies of seroconversion in vaccinees to the individual serotypes 1 - 4 reached 92%, 97%, 97%, and 97%, respectively, after a single dose, with 90% of vaccinees achieving a tetravalent antibody response. In both studies, low level vaccine viremia was detected in 70 - 75% of vaccinees and mild asymptomatic vaccine-associated rash was observed in 55 - 68% of vaccinees. Following a second dose of vaccine given 180 days after the first dose, vaccine viremia, rash, or boosts in neutralizing antibody titers were not observed in any vaccinee, indicating that sterilizing immunity was elicited following the first dose. Importantly, the data suggest that admixtures such as TV005 can be administered as a single dose. This has positive implications for vaccine safety, compliance, cost, and dose sparing.

# AN UPDATE ON RECOMBINANT DENGUE VACCINE DEVELOPMENT IN THAILAND

Nopporn Sittisombut

*Department of Microbiology, Faculty of Medicine, Chiang Mai University, Thailand*

# Tuesday 2nd December 2014

**Time:**
**11.00 – 12.30** **Room D**

## S6 Information Systems for 3Ds

**Chairpersons:**

1. Jaranit Kaewkungwal

2. Wirichada Pan-Ngum

Invited Speakers:

1 Development and utilization of data in consolidated malaria reporting system in Thailand
*Amnat Khamsiriwatchara*
BIOPHICS, Faculty of Tropical Medicine, Mahidol University, Thailand

2 Improved Healthcare Supply Chain and Logistics Management
*Duangpun Kritchanchai*
Healthcare Supply Chain Excellence Centre (LogHealth), Mahidol University, Thailand

3 Informatics for health policy and systems research: Lessons learned from a study of healthcare financing cross-subsidization in Thai public hospitals
*Borwornsom Leerapan*
Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand

# DEVELOPMENT AND UTILIZATION OF DATA IN CONSOLIDATED MALARIA REPORTING SYSTEM IN THAILAND

Amnat Khamsiriwatchara

*BIOPHICS, Faculty of Tropical Medicine, Mahidol University, Thailand*

In Thailand, there are two malaria national reporting systems: 1) by Bureau of Epidemiology (BOE), and 2) by Bureau of Vector Borne Diseases (BVBD). The two systems have difference in methodology in data collections and difference in line of authorities of working units. Both systems have analyzed and reported their own data separately. The number of malaria report in each system may not reflect the real incidence of malaria in Thailand. In addition, the number of malaria report in the past may not reliable according to the figures from both system were aggregated numbers, highly fluctuated, and had difficulties to trace for accuracy.

Most of previous data analysis and reports of malaria in Thailand were using surveillance data either from BOE or BVBD; because there was no a single national database that reconciles data from the two national sources. Results from those studies may not truly reflect the real malaria situation in Thailand. Therefore, both BOE and BVBD have agreed to combine the data from the two different Health Information Systems into a single national database. The BIOPHICS under collaboration with the MOPH, has developed the data consolidation process. The best fit algorithm was developed to effectively detect and delete data redundancy between both systems. This data consolidation algorithm and process were approved by the MOPH.

After merging two systems, the redundancy cases can be cut off by 20%. The new figurewill be officially published and broadcasted publicly as the national malaria incidence in Fiscal year 2015.

# IMPROVED HEALTHCARE SUPPLY CHAIN AND LOGISTICS MANAGEMENT

Assoc. Prof. Dr. Duangpun Kritchanchai

*Healthcare Supply Chain and Logistics Excellence Centre , Mahidol University*

In the area of healthcare industry which is vital to life, there is a lack of supply chain information sharing capability among healthcare supply chain players. It does not only affect the product traceability, but also prevented the development and use of a standard national data exchange system which direct impacts on patient safety and supply chain efficiency.

A standardized healthcare product identification system can monitor the use of medical products and movement throughout the supply chain. It also shares and exchanges information as it allows hospitals and distributors create a single database of medical products.

The standard code with supporting of standard database and electronic data interchange (EDI) can provide the important information about healthcare products, there are not only the general information, but also the clinical information, logistics information and dynamic product information. In addition, traceability system and Vendor Managed Inventory (VMI) is being developed. The traceability system allows healthcare products can be tracked to the patients, traced back to the manufacturers and also supports the data for the product recall. VMI provides suppliers to take responsibility for maintaining drug replenishment to make sure that the hospital has the required level of drug inventory.

These will be benefit to both healthcare and supply chain management. The medication errors and harm to the patients is reduced, and the whole healthcare supply chain is brought together with higher system interoperability resulted in higher performance of health information system, product traceability and logistics efficiency.

# INFORMATICS FOR HEALTH POLICY AND SYSTEMS RESEARCH: LESSONS LEARNED FROM A STUDY OF HEALTHCARE FINANCING CROSS-SUBSIDIZATION IN THAI PUBLIC HOSPITALS

**Borwornsom Leerapan**

*Faculty of Medicine Ramathibodi Hospital, Mahidol University*

**Objective:** Policymakers, healthcare administrators and researchers require appropriate information to conduct health policy and systems research (HPSR) in the contexts of developing countries such as Thailand. This paper demonstrates opportunities and challenges in utilizing the existing information to conduct a study of healthcare financing cross-subsidization in Thai public hospitals. Lessons learned of informatics for HPSR are drawn from the case study.

**Methods:** This study employed a mixed-methods research design. Qualitative data were collected from focus-group interviews of 30 hospital administrators in three selected public hospitals. Content analysis was used to synthesize a mental model of how administrators making decisions related to cross-subsidization. Quantitative analysis was used to compliment on the issues of unit-cost difference, difference between charge and cost, and difference between reimbursement and cost across health schemes.

**Findings:** Despite no evidence suggests that hospitals cost-shift by increasing prices charged to out-of-pocket payment patients to compensate for the loss, three patterns of decision-making of hospital administrators related to cross-subsidization were found. Hence, financing policies of health schemes also impact other patients groups within the hospitals.

**Discussions:** The nature of exploratory research and its cross-sectional dataset limits this empirical study. Future research should utilize time-series data to investigate causational relationships between payment policies of health schemes and cross-subsidization at the hospital level. To analyze the data across hospitals and patient groups, standardization of cost allocation and unit-cost calculation methods is needed. Policymakers should invest on developing a systematic collection of longitudinal datasets from hospitals nationwide.

**Keyword :** health policy and systems research, health informatics, healthcare information systems, cross-subsidization, healthcare financing

# Tuesday 2nd December 2014

**Time:**

**13.30 – 15.00** **Convention**

## S7 Protozoan Infections

**Chairpersons:**

1. Saengduen Moonsom

2. Dar-Der Ji

Speakers:

1 Subtype Distribution of *Blastocystis* in Tak Province, Thailand
*Supaluk Popruk*
Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Thailand

2 Transcriptome changes and genomic analyses of *Naegleria fowleri* thermal isolate from a PAM patient in Taiwan
*Dar-Der Ji*
Department of Tropical Medicine, National Yang-Ming University, Taiwan

3 Loop-mediated isothermal amplification (lamp) assay for rapid molecular detection of *Enterocytozoon bieneusi* in fecal specimens
*Emelia Osman*
Department of Parasitology & Medical Entomology, Universiti kebangsaan, Malaysia

4 Metronidazole resistance in *Blastocystis* sp. – subtype-dependent?
*Kalyani Raman*
Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia

5 Molecular evidence of *Babesia caballi* in horses in Thailand
*Sasiwimon Yoo-eam*
National Institute of Animal Health, Thailand

# SUBTYPE DISTRIBUTION OF *BLASTOCYSTIS* IN TAK PROVINCE, THAILAND

Supaluk Popruk

*Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok, 10400, Thailand*

*Blastocystis* spp. is a common zoonotic intestinal protozoa which has been classified into 17 subtypes.To determine the prevalenceand subtype distribution of *Blastocystis* in villagers living on Thai- Myanmar border (Tak Province), where the risk of parasitic infection is high, a cross-sectional study was conducted.The prevalence of *Blastocystis* infection was 37.2 %. ST3 was the predominant subtype, followed by ST1, ST2 and ST4.Phylogenetic tree was reconstructed using the maximum likelihood (ML) method.Some *Blastocystis*positive samples were closely related to animals indicating the zoonotic risks. Further longitudinal studies will be required to investigate *Blastocystis* STs in animals living in this villages.

Keywords: *Blastocystis*, zoonotic risk, Thai- Myanmar border

# TRANSCRIPTOME CHANGES AND GENOMIC ANALYSES OF *NAEGLERIA FOWLERI* THERMAL ISOLATE FROM A PAM PATIENT IN TAIWAN

Roy Chen Chih Wu[1], Chen Chung Liao[2], Tze Tze Liu [3], **Yen-Hua Huang**[4], **Jian-Ying Chiu**[5], Men-Fang Shaio[6] and Dar-Der Ji[6]

[1] *Institute of Clinical Medicine, National Yang-Ming University (NYMU);*
[2] *Proteomic Research Center, NYMU;*
[3] *VYM Genome Research Center, NYMU;*
[4] *Institute of Biomedical Informatics, Center for Systems and Synthetic Biology, NYMU;*
[5] *Center for Systems and Synthetic Biology, NYMU;*
[6] *Department of Tropical Medicine, NYMU, Taipei, Taiwan*

The first primary amebic meningoencephalitis (PAM) patient caused by hot spring bath had been reported recently. A thermal strain of *Naegleria fowleri* had been isolated from patient's cerebrospinal fluid. Although the parasites can grow from 25° to 45°, their morphology and mobility varied in size and moving speed at different temperatures. *N. fowleri* ameba forms transformed to flagellate forms also could be seen in the monoxenic culture. Therefore, the transcriptome changes and genomic analyses of *N. fowleri* ameba were investigated to reveal the morphology and mobility changes under temperature control (25/37/45°). The raw RNA sequencing was performed by illumina, and the sequence results were filtered out the bases with quality lowered than Q30 and assembled with Trinity suite. The assembled transcriptome contains about 11,013 genes and about 80 differential displayed genes were identified in different temperature. Otherwise, the whole genome draft was estimated about 28MB bps and about 12,471 genes were predicted. The detail information of transcriptome and genome would be constructed and showed in an Ensembl based website. The cytosolic, exosome and cell membrane proteins from different temperature treatments were identified by Mass spectrometry. Hopefully, this information can improve our understanding to the biology of pathogenic *Naegleria* strains in thermal environments.

Keyword: Naegleria fowleri, PAM, hot spring, transcriptome, genome, thermal environment

# LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR RAPID MOLECULAR DETECTION OF *ENTEROCYTOZOON BIENEUSI* IN FECAL SPECIMENS

N Siti Nur Su'aidah[a], B Mekadina[a], **H Alfizah**[b], O Emelia[a]

[a] *Department of Parasitology & Medical Entomology,*
[b] *Dept of Medical Microbiology & Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaakob Latiff, Bandar Tun Razak, 56000 Kuala Lumpur, Malaysia*

Microsporidia infection due to *Enterocytozoon bieneusi* causes chronic diarrhoea in immunocompromised patients. Routine diagnosis of microsporidia relies on light microscopy but the interpretation of the slides can be very difficult since the spores are minute. Alternatively, molecular detection by polymerase chain reaction (PCR) is time consuming and require thermocycler, which precludes its use in low resource setting areas. In this study, a loop-mediated isothermal amplification (LAMP) assay was developed, targeting SSUrRNA gene using four primers to detect *E. bieneusi* DNA in human fecal samples. LAMP amplification for detection of *E. bieneusi* was performed at a constant temperature of 60°C for 1 hour. The amplified products were visualised using fluorescent intercalating dyes and confirmed by gel electrophoresis. Sensitivity, specificity and agreement between the LAMP assay was evaluated and compared with PCR. Out of 100 fecal samples, 39 (39%) were confirmed positive for *E.bieneusi* by LAMP as compared to 34% (34/100) by PCR. LAMP yielded a sensitivity and specificity of 72% and 90% respectively. No significant difference was found between the two methods ($X^2 = 0.35$, $p = 0.55$). A substantial agreement was demonstrated between LAMP and PCR (kappa = 0.60). In conclusion, microsporidia LAMP assay exhibited similar diagnostic accuracy to PCR with shorter reaction time and thus offers a good alternative for molecular diagnosis of *E. bieneusi* in fecal specimens.

Keywords: microsporidia, *E. bieneusi*, LAMP, PCR

# METRONIDAZOLE RESISTANCE IN *BLASTOCYSTIS* SP. – SUBTYPE-DEPENDENT?

Kalyani, R[1]., Tan, T.C[1]., Suresh,K[1] .

[1]*Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur*

*Blastocystis* sp. is one of the most common intestinal parasites found in human. Several reports have implicated the role of the organism in causing gastrointestinal diseases. Metronidazole has been the drug of choice for treating *Blastocystis* infection. The underlying mechanism involving metronidazole resistance in *Blastocystis* infection has yet to be studied. Since the presence of nitroimidazole (*nim*) gene has been associated with metronidazole resistance in some other organisms, the present study attempts to screen the presence of *nim* gene in various subtypes of *Blastocystis* sp. A total number of 7 symptomatic isolates were screened. It was found that *nim* gene was present only in *Blastocystis* sp. subtype 3 obtained from symptomatic patients. Analysis of metronidazole resistance in *Blastocystis* sp. for duration of 10 days in different concentrations of metronidazole was done. The results favour the postulation that metronidazole resistance could be subtype-dependent. Further analysis was done to observe the phenotypic changes in ST3 of *Blastocystis* isolates. Transmission electron microscopy of the isolate shows prominent difference in terms of the presence of mitochondrion-like-organelles, fuzzy coat, and size. Resistance within these symptomatic patients in this group when treated with metronidazole implies that the presence of *nim* gene in this particular group of isolates indicate the potential role of *nim* gene in conferring metronidazole resistance. Nevertheless the mechanism of *nim* gene warrants further studies.

Keyword: Blastocystis, metronidazole resistance

# MOLECULAR EVIDENCE OF *BABESIA CABALLI* IN HORSES IN THAILAND

**SasiwimonYoo-eam***, MunchareeTattiyapong, Supawan Ngamjiteue, Montakan Vongpakorn

Equine piroplasmosis is an important tick-borne disease of horses that debilitates their general health status and possibly causes economic impact. This study aims to determine *Babesia caballi* and *Theileria equi*, the causative agents of the disease. Collection of 84 horse blood samples, submitted to Parasitology section, National Institute of Animal Health, in the period of January through March 2014. The blood samples were examined by stained thin smear under light microscope and molecular technique. From blood smear, the results revealed that protozoal-like organisms were found in 39 samples which led to further investigation with nested Polymerase Chain Reaction (nested PCR) both specific for *B. caballi* and *T. equi* detection. As a result, target DNA from nested PCR of *B. caballi* positive for 6 samples were successfully amplified. The discrepancy between the results are discussed, phylogenetic analysis shows that the isolates from this study are classified into the same group with those reported from Mongolia, Puerto Rico and the in vitro isolate from USDA and Japanese laboratories. Analysis of the genetic diversity of the agent originated from different locations worldwide will be beneficial for the outbreak monitoring and preventive schemes.

Keywords: Equine piroplasmosis, *Babesia caballi*, *Theileria equi*, nested PCR

# Tuesday 2nd December 2014

**Time:**
**13.30 – 15.00** **Room A**

## S8 Emerging Resistance Mechanisms to Antimalarial Drugs

**Chairpersons:**

1. John Adams

2. Sasithon Pukrittayakamee

Invited Speakers:

1 *In vitro* drug susceptibility of *Plasmodium falciparum* in Thailand
*Kesinee Chotivanich*
Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand

2 Not all resistance is created equal: evolutionary paths and shortcuts toward malaria drug resistance
*Thanat Chookajorn*
Faculty of Tropical Medicine, Mahidol University, Thailand

3 Artemisinin-induced Dormancy and Malaria Treatment Failure
*Dennis Kyle*
Global Health University of South Florida, USA

# *IN VITRO* DRUG SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* IN THAILAND

Kesinee Chotivanich

Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand

Multi drug resistant falciparum malaria is an increasing problem in South East Asia, threatening global plans to control and eliminate malaria. Concern is now focused on the first line antimalarial drugs used in this region i.e. artesunate and piperaquine. To monitor malaria containment and elimination, *in vitro* monitoring of antimalarial drug susceptibility that correlates well with in vivo efficacy is required. We deployed the new TMI (Trophozoite Maturation Inhibition) test, to measure artesunate susceptibility. We used the conventional 48 hours test to assess piperaquine susceptibility of *Plasmodium falciparum* parasites isolated from 60 patients from the western and eastern borders of Thailand, The majority of the isolates (90%) had high 50% TMI inhibition concentration (IC50) for artesunate ( Mean (95%CI)= 9.17 (6.4-11.9) ng/mL, but all of them had normal 48 hours IC50s for piperaquine (mean (SD)= 3.51 (2.43-4.58) ng/mL. These results indicate that *Plasmodium falciparum* in Thailand is generally resistant to artesunate, but it is still sensitive to piperaquine. In vitro monitoring of the artemisinin resistance of Thai isolates by the TMI test is correlated significantly with in vivo efficacy assessed by the parasite clearance half-life (r=0.67, P=0.001).

# NOT ALL RESISTANCE IS CREATED EQUAL: EVOLUTIONARY PATHS AND SHORTCUTS TOWARD MALARIA DRUG RESISTANCE

Thanat Chookajorn

*Faculty of Tropical Medicine, Mahidol University, Thailand*

# ARTEMISININ-INDUCED DORMANCY AND MALARIA TREATMENT FAILURE

Dennis Kyle

*Global Health University of South Florida, USA*

# Tuesday 2nd December 2014

**Time:**
**13.30 – 15.00** **Room B**

## S9 Studies on Immuneresponse to Mosquito Borne Viruses

**Chairpersons:**

1. Eiji Konishi

2. Pongrama Ramasoota

Invited Speakers:

1 Pathogenesis of Epidemic Chikungunya Virus in the Common Marmoset
*Chang-Kweng Lim*
Department of Virology, National Institute of Infectious Diseases, Japan

2 Neutralizing antibody titers as a surrogate for protection against dengue: a revisit of neutralizing antibody titers of dengue patients using Fcgamma receptor expressing cells
*Meng Ling Moi*
Department of Virology, National Institute of Infectious Diseases, Japan

3 Dengue virus infection-neutralizing and enhancing antibody responses in central Thai populations against Indonesian and Thai strains
*Atsushi Yamanaka*
BIKEN Endowed Department of Dengue Vaccine Development, Faculty of Tropical Medicine, Mahidol University, Thailand

# PATHOGENESIS OF EPIDEMIC CHIKUNGUNYA VIRUS IN THE COMMON MARMOSET

Chang-Kweng Lim[1], **Yasushi Ami**[2], **Yoshiki Fujii**[1,3], Meng Ling Moi[1], Kazutaka Kitaura[1,3], Mutsuyo Takayama-Ito[1], Akira Kotaki[1], Kenji Shirai[1,3], Eri Nakayama[1], **Yukie Yamaguchi**[1], **Yuriko Suzaki**[2], Shigeru Morikawa[1], Masayuki Saijo[1], Ryuji Suzuki[3], Ichiro Kurane[1], Tomohiko Takasaki[1]

[1] *Department of Virology 1, National Institute of Infectious Diseases, Japan,*
[2] *Division of Experimental Animal Research, National Institute of Infectious Diseases,*
[3] *Department of Rheumatology and Clinical Immunology, Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, National Hospital Organization*

Chikungunya virus (CHIKV) virus has re-emerged as an important mosquito-borne pathogen causing epidemics in several parts of the world. The CHIKV belongs to the Alphavirus genus in the family *Togaviridae*. A large-scale epidemic of CHIK fever started in Kenya in 2004 and spread to Indian Ocean islands, India, Sri Lanka, Thailand, Malaysia, Indonesia, and the Philippines. During Jan. 2007-Aug. 2013, 43 imported CHIK cases were detected in Japan from South and Southeast Asia. As primates possess physiological characteristics more reflective of humans, we assessed a nonhuman primate model of CHIKV infection in adult, immunocompetent common marmosets. In this study common marmosets were inoculated subcutaneously with recent 2006 epidemic CHIKV strain. All marmosets developed viremias that persisted for 4-5 days. Neutralizing antibodies were detected after day 7 with the fading viraemia. At 4 dpi, focal necrosis and mononuclear cells infiltration were observed in the liver. The viral antigens were immunohistochemically detected in the hepatocytes. At 7 dpi, the proliferation of lymphocytes around the central artery and macrophage accumulation in red pulp were observed. In the cytoplasm of macrophages, viral antigens were detected. No viral antigens were detected on control animal. These results demonstrate that CHIKV effectively infects common marmosets. This is the first common marmoset model of CHIKV infection to allow for analyses of the molecular mechanisms of the CHIKV lifecycle directly in a non-human primate.

Keyword: chikungunya virus, animal model, alphavirus, common marmoset

# NEUTRALIZING ANTIBODY TITERS AS A SURROGATE FOR PROTECTION AGAINST DENGUE: A REVISIT OF NEUTRALIZING ANTIBODY TITERS OF DENGUE PATIENTS USING FC$\gamma$R-EXPRESSING CELLS

Meng Ling Moi[1], Jittraporn Rattanamahaphoom[2], Chang-Kweng Lim[1],
Chukiat Sirivichayakul[2], Masayuki Saijo[1], Arunee Sabchareon[2],
Tomohiko Takasaki[1], Ichiro Kurane[3]

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*[2] Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*
*[3] National Institute of Infectious Diseases, Japan*

Protective immunity to dengue is best reflected by the presence of neutralizing antibodies. The general understanding on the biological properties of antibody in protection against dengue virus (DENV) infection is based on neutralizing antibody levels determined by FcγR-negative cells using conventional plaque reduction neutralizing assay test (PRNT). In the absence of FcγR, conventional PRNT do not consider infection-enhancement activity, and the potential of these infection-enhancement activity that may hamper neutralizing activity. We have developed a novel PRNT test using FcγR-expressing BHK cells, to determine the sum of antibody infection-enhancement and neutralizing activity. Using FcγR-expressing BHK cells and FcγR-negative BHK cells, we examined the sum infection-enhancement activity and neutralizing activity in serum samples from dengue patients with primary and secondary DENV infection. A total of 112 serum samples from 28 dengue patients that were serially collected before and after acute DENV infection in Thailand were also used. Serum samples with low neutralizing activity demonstrated infection-enhancing activity, while those with high neutralizing activity demonstrated low or no infection-enhancement activity in FcγR-expressing cells. Neutralizing activity to the infecting DENV serotype was absent in FcγR-expressing cells during the early phase of infection. As FcγR-expressing cells measures the sum of infection-enhancement activity as neutralizing antibody titer, our findings suggest that in comparison to FcγR-negative cells, FcγR-expressing cells may better reflect the biological properties of antibodies in vivo. The newly developed assay provides a platform to define protective capacity of antibodies in vivo, as the major target cells of DENV infection are the FcγR-positive cells.

Keyword: dengue, protection, neutralizing antibody

# DENGUE VIRUS INFECTION-NEUTRALIZING AND ENHANCING ANTIBODY RESPONSES IN CENTRAL THAI POPULATIONS AGAINST INDONESIAN AND THAI STRAINS

**Atsushi Yamanaka**, Duangjai Oddgun, Nantarat Chantawat, Tamaki Okabayashi, Pongrama Ramasoota, Siti Churrotin, Tomohiro Kotaki, Masanori Kameoka, Soegeng Soegijanto, Eiji Konishi

Dengue fever and dengue hemorrhagic fever are the most important mosquito-borne viral diseases worldwide. Dengue virus, the causative agent, has four serotypes (DENV-1 to DENV-4), each with 4-6 genotypes. Predominantly circulating serotype/genotype differs according to countries/regions. The two top countries reporting a large number of dengue patients in ASEAN countries are Indonesia and Thailand. Thus, it is conceivable that currently circulating strains in Indonesia might be introduced into Thailand and vice versa. A shift of predominant serotype/genotype has been reported to have a potential to increase the number of patients and/or disease severity. Since infection-enhancing antibody is a hypothesized mechanism behind disease severity, we carried out in the present study a small survey in central Thailand to compare enhancing antibody activities against Indonesian and Thai strains. One obstacle to pursue this type of survey is the difficulty in preparing assay antigens derived from foreign virus: transportation of live virus materials beyond national borders is basically restricted. To solve this problem, we developed a recombinant system to simply generate functional antibody assay antigens based on viral sequence information. Briefly, single-round infectious dengue viral particles were obtained by co-transfection of 293T cells with two plasmids: one is to produce a Japanese encephalitis virus replicon and another is to express DENV nucleocapsid-free subviral particles. Among 23 healthy volunteers in Uthai Thani Province we have tested to date, eight were found to have experienced a single infection with DENV-1 or DENV-2, based on antibody titer differences of ≥4 times in a conventional neutralization test. Most serum samples showed similar enhancing activities against Indonesian and Thai strains, although the activities varied in some samples and/or against different serotypes/genotypes. Our system may be useful for risk assessment upon introduction of foreign DENV strains.

Keyword: Dengue; Neutralizing antibody; Antibody-dependent enhancement

# Tuesday 2nd December 2014

**Time:**
**13.30 – 15.00** **Room C**

## S10 School Health”

**Chairpersons:**

**1. Vicente Y. Belizario**

**2. Jitra Waikagul**

Invited Speakers:

1 Development of a School-Based Health Education Model to Prevent Opisthorchiasis and Cholangiocarcinoma in Primary School Children in Northeast Thailand

*Luxana Laithavewat*
Biomedical Science Program, Faculty of Associated Medical Science, Khon Kaen University, and Office of Disease Prevention and Control 6, Ministry of Public Health, Khon Kaen, Thailand

2 Effective School Health Program: Addressing an Animated Education for Oral Health Outcomes in Schoolchildren

*Rachanon Nguanjairak*
Faculty of Public Health, Nakhon Ratchasima Rajabhat University, Thailand

3 Parasitological and nutritional status of school-age and preschool-age children in four villages in southern leyte, Philippines: Lessons for monitoring and evaluation of community-led total sanitation outcomes

*Vicente Y. Belizario Jr*
Department of Parasitology, College of Public Health, and Neglected Tropical Disease Study Group, National Institutes of Health, University of the Philippines Manila, Philippines

# DEVELOPMENT OF A SCHOOL-BASED HEALTH EDUCATION MODEL TO PREVENT OPISTHORCHIASIS AND CHOLANGIOCARCINOMA IN PRIMARY SCHOOL CHILDREN IN NORTHEAST THAILAND

Luxana Laithavewat[1,2], Tinnakorn Srinang[3], Kannika Sutumma[3], Kesorn Thaewnongiew[2], Saowalux Kutchamart[2], Seri Singthong[2], Sasithon Tangsawad[2], Puangrat yongvanit[4,6], Paiboon Sithtihaworn[5,6]

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[2] *Office of Disease Prevention and Control 6, Ministry of Public Health, Khon Kaen, Thailand*
[3] *Khon Kaen Primary Educational Service Area Office 2, Ministry of Education, Khon Kaen, Thailand*
[4] *Departments of Biochemistry , Faculty of Medicine, Khon Kaen University*
[5] *Departments of Parasitology, Faculty of Medicine, Khon Kaen University*
[6] *Liver Fluke and Cholangiocarcinoma Research Centre, Faculty of Medicine, Khon Kaen University*

Cholangiocarcinoma (CCA) is a significant health problem in Southeast Asia particularly northeast Thailand. The major risk factor is liver fluke infection by *Opisthorchis viverrini* from raw fish consumption. Until now, campaigns against raw fish consumption in general population have had little success. The alternative and long lasting approach is to target children. School children have been increasingly recognized as a target group for future control of the liver fluke and CCA. Unfortunately, no formal model is available. The objective of this study was to develop a school-based liver fluke and CCA intervention package focusing on primary school children in high risk areas of CCA in northeast Thailand. The package includes teacher training, provision of supplementary material and exhibits concerning liver fluke transmission and carcinogenesis. The program was implemented via the “Mini Food and Drug Administration Club” to primary school children. After enrolment, children had self learning activities and were asked to inspect and record the dinner dishes prepared and consumed at home and neighbor. Data were collected from school children by means of interviews and a simplified structured questionnaire regarding knowledge and perception of the liver fluke and CCA. The intervention packages were applied in 4 schools (n=118 pupils) and another 4 schools (113 pupils) with no activity served as controls. The experimental schools showed significantly higher knowledge, perception of severity, vulnerability, response efficacy, and self efficacy than control schools. Initial results suggest that school-based health education programs have potential to be a model for a prevention and intervention package against liver fluke and CCA. Application of the model in further schools with a more systematic evaluation i.e. pre and post intervention evaluation is required to assess its utility and future use.

Keyword: Opisthorchis viverrini, School-Based Health Education Model , Primary School Children.

# EFFECTIVE SCHOOL HEALTH PROGRAM: ADDRESSING AN ANIMATED EDUCATION FOR ORAL HEALTH OUTCOMES IN SCHOOLCHILDREN

Rachanon Nguanjairak

*Faculty of Public Health, Nakhon Ratchasima Rajabhat University*

Poor oral hygiene has been threatened by dental plaque and leading to the great problem in schoolchildren. Lack of effective evidence-based method should be promoted and focused on reducing the condition by multimedia application. A randomized controlled trial, single blinded, school-based study aimed to investigate effects of animated oral health intervention programs (AOHIPs) with and without social support (SS) on improving oral health outcomes in children. A questionnaire was used for collecting oral health knowledge and oral examination was done for plaque index (PI) and gingival index (GI). Descriptive statistics were used to summarize characteristic and demographic data and generalized estimating equation (GEE) was used for the comparison. A total of 415 schoolchildren from ten schools were assessed for eligibility. Out of 133 children were allocated to AOHIP with SS (group A), 160 children to AOHIP without SS (group B) and 122 children to a control group (group C). At post-intervention, 132 children (99.2%) attended in group A, 158 children (98.7%) in group B and 121 children (99.2%) in group C. At 3-month follow-up, 128 children (96.2%) remained in group A, 152 children (95.0%) in group B and 118 children (96.7%) in group C. The study result showed that the program of AOHIP with SS was more likely effective than AOHIP without SS and a control group ($p<0.001$). However, AOHIP without SS provides a feasible and almost equally effective alternative strategy to AOHIP with SS and it was more than a control group.

Keywords: Oral Hygiene, School-Based Oral Health Program, Schoolchildren, Multimedia Education

# PARASITOLOGICAL AND NUTRITIONAL STATUS OF SCHOOL-AGE AND PRESCHOOL-AGE CHILDREN IN FOUR VILLAGES IN SOUTHERN LEYTE, PHILIPPINES: LESSONS FOR MONITORING AND EVALUATION OF COMMUNITY-LED TOTAL SANITATION OUTCOMES

**Vicente Y. Belizario Jr.**[a,b], Harvy Joy C. Liwanag[b], June Rose A. Naig[b], Paul Lester C. Chua[a,b], Manuel I. Madamba[c], and Roy O. Dahildahil[a,b]

[a] *Department of Parasitology, College of Public Health, University of the Philippines Manila, 625 Pedro Gil St., Ermita, Manila 1000, Philippines*
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While preventive chemotherapy remains to be a major strategy for control of soil-transmitted helminthiases, improvements in water, sanitation, and hygiene (WASH) comprise the long-term strategy to achieve sustained control of STH. This study examined the parasitological and nutritional status of school-age and preschool-age children in four villages in the province of Southern Leyte, Philippines where two of the villages attained Open-Defecation-Free (ODF) status after introduction of Community-Led Total Sanitation (CLTS).

A total of 341 children submitted stool samples which were examined using Kato-Katz technique. Between the two villages where CLTS was introduced, Buenavista had a significantly higher cumulative prevalence of STH at 67.4% and prevalence of moderate-heavy intensity at 23.5%, while Caubang had a significantly lower cumulative prevalence at 4.9% and prevalence of moderate-heavy intensity at 1.8%. The non-CLTS villages had similar rates for cumulative prevalence at 17% and prevalence of moderate-heavy intensity at 3%. The findings may be explained by factors that include possible reversion to open defecation and poor mass drug administration (MDA) coverage. The data indicate no clear pattern among the parasitological and nutritional parameters, as well as the presence of CLTS in the village, suggesting the need to monitor the ODF status of villages on a regular basis even after the end of CLTS activities to ensure the sustainability of the CLTS approach. In order to sustain effective control of STH, deeper collaboration between the WASH and STH sectors are recommended where partners can work together in the area of monitoring and evaluation.

Keywords: Intestinal Helminthiasis, School-Age Population, Preschool Child, Sanitation, Defecation, Tropical Medicine

# Tuesday 2nd December 2014

**Time:**
**13.30 – 15.00** **Room D**

## S11 High-throughput technology for diagnosis of parasitic diseases

**Chairpersons:**

1. Aaron Jex

2. Poom Adisakwattana

Invited Speakers:

1 Mass Spectrometry-based Parasitic Proteomics
*Onrapak Reamtong*
Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Thailand

2 Advance diagnostic tools for tropical pathogens: eletrophoresis to nanopore sequencing
*Aaron Jex*
Faculty of Veterinary Science, The University of Melbourne, Australia

3 Transcriptional studies on *P. vivax* transmission stage
*Ivo Mueller*
Walter & Eliza Hall Institute in Melbourne, Australia

# MASS SPECTROMETRY-BASED PARASITIC PROTEOMICS

Onrapak Reamtong, Ph.D.

Mass spectrometry (MS) is recently the most sensitive and high-throughput technique for peptide and protein analyses. Peptide mass fingerprinting (PMF) and MS shotgun proteomics are common approaches to accomplish protein identification. PMF is a technique used to identify relatively pure proteins by matching their peptide masses generated by trypsin digestion to the theoretical peptide masses generated from a protein or DNA database. Whereas MS shotgun proteomics is a method for protein identification from complex samples using a combination of liquid chromatography (LC) separation of tryptic digested peptides and their subsequent analysis by tandem mass spectrometry (MS/MS). Several studies have revealed the identification of parasite proteomes using MS based approaches for example *Plasmodium falciparum*, *Schistosoma mekongi* and *Brugia malayi*. In addition, MS technology is able to detect protein abundance changes in biological samples. Two main approaches are currently being used for MS-based quantitative proteomics: differential isotopic labelling and label-free quantification.

A biomarker refers to a component, which can indicate the severity or disease state. A number of studies have applied MS based approaches to identify protein biomarkers of parasitic infections. *Gnathostoma spinigerum* was investigated biomarkers for diagnostic purposes using two-dimensional gel electrophoresis, immunoblot analysis and proteomics. Recent research in mefloquine resistant biomarkers of *Plasmodium falciparum* was also explored by MS label-free quantification. Furthermore, mass spectrometry also enables the identification of post-translational modifications (PTMs), establishment of protein–protein interactions and determination of target proteins of active compounds. The efficiency of MS analytical concepts are important for bridging the genotype–phenotype gap, generating a better understanding to biological researches.

# ADVANCE DIAGNOSTIC TOOLS FOR TROPICAL PATHOGENS: ELETROPHORESIS TO NANOPORE SEQUENCING

Aaron Jex

*Faculty of Veterinary Science, The University of Melbourne, Parkville, Victoria,*

In recent years there have been major advances in the tools available to rapidly identify, diagnose and quantify parasites of major importance as neglected tropical diseases. As global poverty recedes and countries around the world begin to reach the millennium development goals, for many populations, parasite control is shifting from mass treatment to a focus on sustained control, and in some areas, even parasite eradication. Underpinning these efforts is a solid foundational understanding of pathogen epidemiology built on accurate and sensitive tools for pathogen detection and diagnosis. Even in regions and populations where neglected pathogens remain a major endemic problem, accurate diagnostic tools provide a backbone to support control efforts and evaluate the efficacy of, for example, mass drug administration programs and to track rates of reinfection or the emergent of treatment resistant pathogens. Classically, microscopy has been the central, and in many situations, unrivalled approach to pathogen diagnosis. However, skilled microscopists are in rapidly diminishing supply and technologies developed around molecular (e.g., DNA) based pathogen detection are emerging as genuine, viable and cost-effect alternatives. In this presentation, I will discuss several recent examples of advanced, cost-effective and user-friendly approaches to pathogen diagnosis we have been employing out our laboratory for pathogens of major agricultural, veterinary and medical important. I will also present some of our recent experiences with portable deep-sequencing technologies, specifically as a participant of the Oxford Nanopore Minion Access Program. Lastly, I will provide some perspectives on the utility and practicality of these tools for tropical research and the potential for their routine use in this field.

# TRANSCRIPTIONAL STUDIES ON *P. VIVAX* TRANSMISSION STAGE

*Ivo Mueller*

*Walter & Eliza Hall Institute in Melbourne, Australia*

# Tuesday 2nd December 2014

**Time:**
**15.30 – 17.00** **Convention**

## S12 Human Taeniases and cysticercosis

**Chairpersons:**

1. Akira Ito

2. Paron Dekumyoy

Invited Speakers:

1 The present situation of human taeniases and cysticercaosis in Asia
*Akira Ito*
Department of Parasitology, Asahikawa Medical University, Japan

2 Revisiting the Problem of *Taenia solium* cysticercosis in Malaysia: A possible Emergence Due to Rising International Tourism and Migration
*Priyadarshi Soumyaranjan Sahu*
Division of Pathology, School of Medicine, International Medical University, Malaysia

3 The species problem of *Taenia asiatica* and differentiation of three human taeniid species
*Munehiro Okamoto*
Center for Human Evolution Modeling Research, Primate Research Institute, Kyoto University, Japan

# THE PRESENT SITUATION OF HUMAN TAENIASES AND CYSTICERCOSIS IN ASIA

Ito, Akira[1*]; Wandra, Toni[1,2,3]; Swastika, I Kadek[1,4]; Dharmawan, I Nyoman[1,4]; Li, Tiaoying[1,5]; Dekumyoy, Paron[1,6]; Kusolsuk, Teera[1,6]; Nkouawa, Agathe[1]; **Yanagida,** Tetsuya[1,7]; **Sako, Yasuhito**[1]; Nakao, Minoru[1]; Okamoto, Munehiro[8]

[1] *Asahikawa Medical University, Asahikawa, Japan;*
[2] *Sulianti Saroso Infectious Diseases Hospital, Jakarta, Indonesia;*
[3] *Sari Mutiara Indonesia University, Medan, Indonesia;*
[4] *University of Udayana, Denpasar, Indonesia;*
[5] *Sichuan Centers for Disease Control and Prevention, Chengdu, China;*
[6] *Mahidol University, Bangkok, Thailand;*
[7] *Yamaguchi University, Yoshida, Japan*
[8] *Primate Research Institute, Kyoto University, Inuyama, Japan*

BACKGROUND: Neurocysticercosis due to accidental uptake of eggs of *Taenia solium* is common in developing countries where people eat pork. It is transmitted from humans (taeniasis carriers) toboth humans and pigs (cysticercosis), and emerging and reemerging worldwide. Therefore, this disease is based on consumption of pork full of cysticerciunder poverty and expected to be a local disease in rural and remote areas of developing countries where meat-inspection was not introduced, and was rare or not distributed in Muslim or Jewish societies in the 20 century.

PRESENT SITUATION: However, globalization with huge number of immigrants as labor and touristshas high risk to introduce cysticercosis everywhere even in Muslim or Jewish societies or developed countries including Japan. Nonetheless, we have almost no data on the real situation in any countries due to the lack of reliable tools to detect cysticercosis or identify the parasite. In Asia-Pacific, we have many other parasitic diseases including schistosomiasis, food- or fish-borne trematodiases, soil-transmitted helminthiases and fish- or meat-borne cestodiases. In any areas where we are facing these parasitic diseases, we are simultaneously facing unexpected outbreaks of cysticercosis. In this presentation, the present situation of cysticercosis of *T. solium*in Asia,most neglected among the neglected tropical diseases,are overviewed. The background information of the reason why it is neglected isstressed. Molecular identification of the species and serology for detection of cysticercosis in humans and pigs are discussed. Detection of circulating antigen(s) of *T. saginata*cysticerci might be useful for detection of human cysticercosis by cross responses among the three human *Taenia*species, but perhaps no use in scavenger pigs. Therefore, it is urgent to establish highly reliable tools for detection of pigs infected with *T. solium*.

# REVISITING THE PROBLEM OF *TAENIA SOLIUM* CYSTICERCOSIS IN MALAYSIA: A POSSIBLE EMERGENCE DUE TO RISING INTERNATIONAL TOURISM AND MIGRATION

Dr Priyadarshi Soumyaranjan Sahu

*Senior Lecturer Division of Pathology, School of Medicine, International Medical University, 57000 Kuala Lumpur, Malaysia*
*E-mail: Priyadarshi@imu.edu.my*

Larval *Taenia solium* infection or cysticercosis is a human to human transmitted disease and a major public health problem Worldwide. In Asia *T. solium* taeniasis/cysticercosis is known to be highly prevalent in most parts of Southeast Asia, China, and the Indian sub-continent. Although studies in the past have highlighted on *T. solium* taeniasis/cysticercosis occurrences in Malaysia, there have been persisting assumptions that this region is non-endemic.Becausecysticercosis results from the ingestion of tapeworm eggs hence either direct or indirect exposures to adult tapeworm carriers are the main risk factors for acquiring thisinfection. Vegetarians could also be at risk since*T. solium*eggs are microscopic and could be ingested without knowledge. Brain and eye cysts cause the most morbidity, with the brain being the most common location for cysts. Majority of neurocysticercosis cases do not show typical imaging features, hence often being underdiagnosed. However, considering the recent case reports of cysticercosis from Malaysia and serological evidence of exposure to the parasite, there is a strong suspicionof its emergence. Probably the consequences of rising international tourism and migration from and/or to areas known to be endemic for *T. solium*have caused an emergence of cases in Malaysia as in other developed countries where this infection was previously considered rare. With the continued increasing immigration of permanent and/or temporary foreign workers from *T. solium* endemic countries, will Malaysia still be safe from this neglected tropical disease?

## Invited Speaker Canceled

# THE SPECIES PROBLEM OF *TAENIA ASIATICA* AND DIFFERENTIATION OF THREE HUMAN TAENIID SPECIES

Munehiro Okamoto

*Section of Wildlife Diversity, Center for Human Evolution Modeling Research, Primate Research Institute, Kyoto University, Japan*

*Taenia solium, Taenia saginata* and *Taenia asiatica* are known as causative agents of taeniasis in humans. Eggs expelled from tapeworm carriers serve as a new source of infection for intermediate hosts, and humans acquire adult worms after eating uncooked or under cooked meet or viscera of intermediate hosts contaminated with cysticerci. Since Eggs of *T. solium* also cause cysticercosis in humans, this species is one of the most important cestodes for human health. In this paper, I will present molecular techniques used for identification of human taeniid cestodes.

There has long been a debate as to the specific status of*T. asiatica*, with some people regarding it as a distinct species and some preferring to recognize it as a strain of *T. saginata*. We examined the nucleotide sequences of one mitochondrial gene and of several nuclear genes from human taeniid cestodes, which were morphologically similar to *T. saginata* or *T. asiatica*. All phylogenetic trees showed that those worms were divided into clear two clades. These facts indicate that *T. saginata* and *T. asiatica* were separated into two independent groups once. Phylogenetic analyses also revealed that some worms showed nuclear-mitochondrial discordance, suggesting that they are derived from hybridization between *T. saginata* and *T. asiatica*. Surprisingly, most of *T. asiatica* endemic in Asian countries, which were identified by mitochondrial DNA, were not pure *T. asiatica* but originated from hybridization between pure *T. asiatica* and pure *T. saginata*. I will discuss genetic relationships between *T. saginata*, *T. asistica* and their hybrids.

# Tuesday 2nd December 2014

**Time:**
**15.30 – 17.00** **Room A**

## S13 Path towards drug discovery

**Chairpersons:**

1. Dennis Kyle

2. Kesinee Chotivanich

Invited Speakers:

1 The Discovery of Spiroindolone Antimalarials (KAE609)
*Bryan Yeung*
Novartis Institute for Tropical Diseases, Singapore

2 Natural products and the exploration of new chemical space for antimalarial chemotherapies
*Bill Baker*
University of South Florida, USA

3 The Success of Phase II Antimalarial Drug Clinical Trial: International Collaborative Experience at Faculty of Tropical Medicine
*Podjanee Jittmala*
Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Thailand

# THE DISCOVERY OF SPIROINDOLONE ANTIMALARIALS (KAE609)

**Bryan K. S. Yeung**, Ph.D.

*Associate Director*
*Novartis Institute for Tropical Diseases, Singapore*

Historicallythe antimalarial drug arsenal has benefitted from a phenotypicscreening approach toidentify lead molecules in the search for new drugs; with artemisinin being one of the most notable examples. As part of an effort to discover new antimalarial drugs we screened the Novartis natural product library to identify a spiroindolone hitcompound which was the focus of a lead optimization effort resulting in the discovery and development ofKAE609 (cipargamin, formerly NITD609). In a parallel effort, we applied afull genome analysis of spiroindolone-resistant parasites to discover that the spiroindolones work though a uniquemode of action, namely the inhibition of a P-type *Plasmodium falciparum* ATPase4 ($Na^+$ efflux pump). Disruption of $Na^+$ homeostasis by the spiroindoloneswas shown to rapidly lead to parasite death.KAE609 represents the first antimalarial chemotype working though a novel mechanism of action to enter Phase II clinical trials in over two decades.

# NATURAL PRODUCTS AND THE EXPLORATION OF NEW CHEMICAL SPACE FOR ANTIMALARIAL CHEMOTHERAPIES

Bill Baker

*University of South Florida, USA*

# THE SUCCESS OF PHASE II ANTIMALARIAL DRUG CLINICAL TRIAL: INTERNATIONAL COLLABORATIVE EXPERIENCE AT FACULTY OF TROPICAL MEDICINE

PodjaneeJittamala, Borimas Hanboonkunuprakarn, Joel Leong, JorgMohrle, Joel Tarning, Kasinee Chotivanich, Salwaluk Panapipat, Nick Day, Francois Nosten, Nick White, Sasithon Pukrittayakamee .

New antimalarial drugs are urgently needed because of the increasing problem of artesunate resistant *P. falciparum* in South East Asia. Drug finding processes show a shift of efforts from mainly pharmaceutical companies to the collaboration among product development partnerships including the academic, biotech, funding agency and pharmaceutical company. The Faculty of Tropical Medicine (FTM) has commitment to support border aspects of research for tropical diseases from basic science through clinical trials. For antimalarial drug discovery innovation, FTM has the collaboration with Mahidol Oxford Research Unit (MORU), Medicine for Malaria Venture (MMV) and Novartis Institute for Tropical Diseases (NITD). Together we currently work on three new anti-malarial drug candidates reached phase II clinical trial development, namely KAE609, OZ439 and KAF156. The studies were conducted for the first time in patients with uncomplicated malaria. The proof-of-concept studies of KAE 609(spiroindolone) and KAF 156(Immidazolepiperazine, a new class of antimalarial drug exhibiting in vitro potency against blood, liver and gametocyte stages) from Novartis, exhibited a rapid parasite clearance in both *P. falciparum* and *P.vivax*malaria. The long terminal half-life in malaria patient would make KAE 609 suitable for once a day dosing regimen. The OZ 439, a fully synthetic peroxides designed to provide a single oral dose from MMV, showed rapid reduction in malaria parasitemia in all studied patients with a good dose-response relationship. The OZ439 has extended structure from artemisinin and would remain effective against emerging artemisinin resistant malaria. One of the malaria treatment problem is the under dosing, so in order to identify the suitable treatment dosage, a careful *in vivo* study design is required. FTM currently innovated an *in vivo* dose finding by using real time qPCR. The minimal inhibitory concentration trials of KAE 609 and OZ439 in patients with *P. falciparum* malaria are in progress.

These outstanding drug candidate clinical trials reassure the strong international collaborations and commitment leading to the development of valuable antimalarial therapy.

# Tuesday 2nd December 2014

**Time:**
**15.30 – 17.00** **Room B**

## S14 Best Student Presentation Award

**Chairpersons:**

1. Sasithon Pukrittayakamee 2. Kasinee Buchachart

Speakers:

1 2-aminoethyl diphenylborinate potentiates the antimalarial effect of chloroquine against chloroquine-resistant malaria parasites: a novel therapeutic strategy

*Ehab Mossaad*
National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Japan

2 Clinical presentations of influenza in adult hospitalized patients at bangkok hospital for tropical diseases

*Hisham Imad*
Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand

3 Detection of parasite antigen in urine and its diagnosis potential for human opisthorchiasis

*Chanika Worasith*
Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand

4 Effect of seasonality and host size-preference on transmission of *Opisthorchis viverrini* and Lecithodendriidae in the snail intermediate host, *Bithynia siamensis goniomphalos*, in Northeast Thailand

*Jutamas Namsanor*
Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand

5 Population pharmacokinetic-pharmacodynamic modeling of piperaquine for seasonal intermittent preventive treatment in pediatric patients

*Palang Chotsiri*
Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand

6 Impact of health education on knowledge, attitude, and acceptability of influenza vaccine among elderly thais, representative of developing country

*Rawipun Worasathit*
Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand

# 2-AMINOETHYL DIPHENYLBORINATE POTENTIATES THE ANTIMALARIAL EFFECT OF CHLOROQUINE AGAINST CHLOROQUINE-RESISTANT MALARIA PARASITES: A NOVEL THERAPEUTIC STRATEGY.

Ehab Mossaad[1,2,3], Wakako Furuyama[1], Masahiro Enomoto[4], Satoru Kawai[5], Katsuhiko Mikoshiba[6] and Shin-ichiro Kawazu[1]

[1] *National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan*
[2] *The United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan*
[3] *College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum, Sudan*
[4] *Princess Margaret Cancer Centre, Toronto, Canada*
[5] *Laboratory of Tropical Medicine and Parasitology, Dokkyo Medical University, Tochigi, Japan*
[6] *Laboratory for Developmental Neurobiology, RIKEN Brain Science Institute, Saitama, Japan*

Malaria is still a major public health problem in the world. After the emergence and spreading of drug resistance for malaria parasites, there is an urgent need to develop new drugs and novel agents to reverse the resistance to existing antimalarials. As such, calcium ($Ca^{2+}$) homeostasis and signaling pathways in the malaria parasite *Plasmodium* could be promising targets for these objectives. In our current study, we are presenting a novel therapeutic strategy against the chloroquine (CQ)-resistant *Plasmodium falciparum* and *Plasmodium chabaudi* in terms of $Ca^{2+}$ homeostasis by potentiating the effect of CQ. This was done by using the minimum concentration that affects the parasite growth of 2-aminoethyl diphenylborinate (2-APB), the inositol 1,4,5-trisphosphate ($IP_3$) receptor inhibitor, which inhibits the $IP_3$ pathway for $Ca^{2+}$ release. This resulted in a complete reversal of CQ-resistance in *P. falciparum* K-1 strain with an $IC_{50}$ value significant decrease from 955 ± 61 nM to 9 ± 1 nM, whereas its CQ-resistance reversing activity in *P. chabaudi* AS (30 CQ) strain has the same efficacy as verapamil. The strategy we are presenting here based on the partial blockage of $IP_3$ pathway and the involvement of CQ in $Ca^{2+}$ homeostasis disturbance may help resolve the longstanding problem of CQ-resistance.

Keywords: *Plasmodium*, calcium signaling, chloroquine, 2-APB, malaria

# CLINICAL PRESENTATIONS OF INFLUENZA IN ADULT HOSPITALIZED PATIENTS AT BANGKOK HOSPITAL FOR TROPICAL DISEASES

Hisham Ahmed Imad[1], Weerapong Phumratanaprapin[1], Benjaluck Phonrat[1],
Watcharapong Piyaphanee[1], Viravarn Luvira[1], Maleerat Sutherat[1]

[1] *Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Bangkok School of Tropical Diseases, Mahidol University*

This retrospective study was conducted at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The study aimed to analyze the clinical and laboratory presentations of influenza A, pandemic H1N12009 influenza A and influenza B, to compare the clinical presentations of influenza A and B, to determine the risk factors for severity of pandemic H1N1 2009 influenza A, and to describe seasonal variations in influenza. All patients were admitted to the Hospital for Tropical Diseases during the period January 2009-October 2013. The total number of influenza cases was highest in 2009. The clinical presentations among the patients in the study comprised fever (95.4%), cough (87%), rhinorrhea (67.9%), sore throat (58%), myalgia/arthralgia (56.4%), nausea/vomiting (30.5%), headache (29.7%), fatigue (25.2%) and chills (19.8%). 34.3% of patients had underlying co-morbidities. Most (80.3%) of the patients appeared normal on physical examination. Pneumonia and acute kidney injury were reported as influenza-associated complications. Common radiographic findings were alveolar infiltrates (61.5%). Some parameters were statistically significantly different between the influenza A and influenza B patient groups. 90.9% of the patients had a fever clearance of less than or equal to 3 days, and 61.1% of the patients were hospitalized for not more than 3 days. Our study suggests that early antiviral treatment leads to a favorable outcome.

Keywords: Influenza /Clinical Presentations/Hospitalized Adults

# DETECTION OF PARASITE ANTIGEN IN URINE AND ITS DIAGNOSIS POTENTIAL FOR HUMAN OPISTHORCHIASIS

Chanika Worasith[a,d], Kunyarat Duenngai[b], Jiraporn Sithithaworn[c], Paiboon Sithithaworn[a,d]

[a] *Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand*
[b] *Department of Public Health, Faculty of Science and Technology, PhetchabunRajabhat University, Phetchabun, Thailand*
[c] *Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand*
[d] *Liver Fluke and Cholangiocarcinoma Reserch Center, Khon Kaen University, Khon Kaen, 40002, Thailand*

Improved diagnostic methods for human opisthorchiasis are required to uncover people with light infections for effective treatment and control and this has important bearing for the E-Sarn agenda for “liver fluke elimination to reduce cholangiocarcinoma”. Within the available diagnostic methods, parasite antigen detection approach is the most attractive candidate to develop into a simple diagnostic kit for mass screening. We aimed to establish a capture ELISA protocol for quantitative detection of excretory-secretory antigen of *Opisthorchis viverrini* in urine specimens for diagnosis of opisthorchiasis. For the urine sample preparation, 4 methods of sample treatment were tested prior to antigen detection. Monoclonal antibody-based capture ELISA was optimized using known *O. viverrini* negative and positive samples. The final protocol was used to obtain a cutoff point by ROC analysis. The established protocol was used to construct the standard curve and determine a limit detection of antigen in urine. The antigen detection system was applied on field collect sample to evaluate its performance compared with a conventional diagnostic method. Antigen concentration in *O. viverrini* infected group was significant higher than parasite-negative group ($p< 0.001$) and levels of antigen significantly correlated with intensity of infection ($p< 0.001$). By logistic regression analysis using level of urinary antigen as independent variable, significant odd ratios (adjusted for age and sex) indicated risk for opisthorchiasis and the odds increase with intensity of infection. With reference to FECT method the sensitivity and specificity of the urinary antigen detection was 73.6% and 84.3%, respectively. The presence of *O. viverrini* antigen in urine indicated that parasite antigen entering hepatobiliary tissue and may diffuse into blood circulation before being excreted in urine in infected patients. The ease of collection and non-invasiveness in sample collection has revolutionized diagnosis of *O. viverrini* and providing a better and effective tool for diagnosis, evaluation of treatment and also elimination of the liver fluke.

Keywords: Human opisthorchiasis, Cholangiocarcinoma, Monoclonal antibody-based capture ELISA

# EFFECT OF SEASONALITY AND HOST SIZE-PREFERENCE ON TRANSMISSION OF *OPISTHORCHIS VIVERRINI* AND LECITHODENDRIIDAE IN THE SNAIL INTERMEDIATE HOST, *BITHYNIA SIAMENSIS GONIOMPHALOS,* IN NORTHEAST THAILAND

Jutamas Namsanor[1,2], Kulthida Kopolrat[1,2], Nadda Kiatsopit[1,2] ,Opal Pitaksakulrat[1,2] , Smarn Tesana[1], Paiboon Sithithaworn[1,2]

[1] *Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

[2] *Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

Environmental changes play an important role in the transmission success of fish-borne zoonotic trematodes (FZT), particularly in the snail intermediate host which is an amplification point in the life cycle. In this study we examined the seasonal transmission patterns and the interaction of *O. viverrini* sensu lato (s.l.) and a virgulate cercaria (family Lecithodendriidae) in the snail intermediate host, *Bithynia siamensis goniomphalos*. Samples of *B. s. goniomphalos* were collected during different seasons during 2012-2013 in an endemic area in northeast Thailand. The prevalence of *O. viverrini* s.l. in *B.s.goniomphalos* varied significantly with season, being 0.31, 1.05 and 0.37% in rainy, cool and hot seasons, respectively ($P < 0.05$). Similarly, the prevalence of virgulate cercariae was 3.11, 6.80 and 1.64% in the rainy, cool and hot seasons, respectively ($P < 0.05$). The intensity of infection, i.e. cercariae, sporocyst and rediae, also varied between seasons and peaked in the hot season ($P < 0.05$) in both trematodes. The snails infected with *O. viverrini* s.l. were significantly smaller ($P < 0.05$) and those infected with virgulate cercariae significantly were larger ($P < 0.05$) in size than uninfected snails. Variation in transmission success in different seasons and the different size selection of *B. s. goniomphalos* parasitized by *O. viverrini* s.l. and virgulate cercaria indicated complex host-parasite interactions with important implications for the epidemiology of *O. viverrini* s.l.

Keywords: *Bithynia siamensis goniomphalos*, *Opisthorchis viverrini*, Lecithodendriidae, Season, Shell size

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-GMS) and a grant of Graduate school KKU.

# POPULATION PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF PIPERAQUINE FOR SEASONAL INTERMITTENT PREVENTIVE TREATMENT IN PEDIATRIC PATIENTS

Palang Chotsiri (1), Issaka Zongo (2), Paul Milligan (3), Daniel Chandramohan (2), Brian Greenwood (2), Philip Rosenthal (4), Warunee Hanpithakpong (1), Nicholas J. White (1,5), Francois Nosten (1,5,6), Jean-Bosco Ouédraogo (2), Joel Tarning (1,5)

*(1) Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*
*(2) Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso*
*(3) Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, United Kingdom*
*(4) Department of Medicine, University of California, San Francisco, San Francisco, California, USA*
*(5) Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, United Kingdom*
*(6) Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand*

**Objectives:** This study aimed to investigate the pharmacokinetic and pharmacodynamic properties of piperaquine in young children and provide a practical dosing regimen.

**Methods:** Sparse piperaquine plasma sampling in 183 children (age between 2.33-58.1 months) were obtained after monthly dihydroartemisinin-piperaquine prophylaxis treatment. Piperaquine concentrations were measured using solid phase extraction followed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). The pharmacokinetic and pharmacodynamic properties of piperaquine were evaluated with non-linear mixed-effects modelling. Parasite density and time-to-malaria detection during the six months of follow-up was described by an interval-censoring time-to-event model. The final pharmacokinetic-pharmacodynamic model was used to simulate and evaluate optimal dosing strategies.

**Results:** Piperaquine pharmacokinetics was characterized by using three-distribution compartments with five-transit absorption compartments, stabilized by using prior information. Parasite density at the time of malaria detection was used for interpolation of the likely time interval of malaria acquisition. An interval-censoring time-to-event model with the sigmoid Emax inhibitory effect of piperaquine successfully described the recurrent malaria in this vulnerable population. Dose-optimization using the final pharmacokinetic-pharmacodynamic model suggested that small children had lower exposures of piperaquine after the standard body weight-normalised dose. A prospective dose increase predicted a decreased 2-months malaria incidence of 34% in small children weighting 5 to 10 kg.

**Conclusions:** The pharmacokinetic and pharmacodynamic properties of piperaquine were successfully described by the final model. Modelling conducted here suggests that small children are at risk of under-dosing and that they need a higher body weight-normalised dose compared to adults. A prospective dose increase in acute treatment and during intermittent preventive treatment of malaria might have a significant impact in this vulnerable population.

**Keywords:** Pharmacokinetic-pharmacodynamic modelling, dihydroartemisinin-piperaquine, intermittent preventive treatment, paediatric, malaria

# IMPACT OF HEALTH EDUCATION ON KNOWLEDGE, ATTITUDE, AND ACCEPTABILITY OF INFLUENZA VACCINE AMONG ELDERLY THAIS, REPRESENTATIVE OF DEVELOPING COUNTRY

Rawipun Worasathit[1], Wantanee Wattana[2], Kamolnetr Okanurak[1], Archin Songthap[3], Jittima Dhitavat[1], Punnee Pitisuttithum[1]

[1] *Faculty of Tropical Medicine*
[2] *Bangkok Metropolitan Administration (BMA)*
[3] *Sirindhorn College of Public Health, Trang*

**Objectives.** The study was to compare knowledge, attitude, and acceptability of an influenza vaccine before and after receiving educational video among the elderly in Thailand. Methods. The quasi-experimental study was conducted among 2693 community elderly who lived in Bangkok. They were divided into Education group (N=1402) or Control group (N=1291). Knowledge, attitude, and acceptability were assessed twice by using the same validated interview forms. The interview forms were given before and after education in the Education group. The similar method and forms were done in the Control group without giving education.

**Results.** Among the Education group, the proportion of participants who had knowledge in high level rose from 29.2% to 49.2% (P-value <0.001); mean score of knowledge rose substantially from 8.38 to 9.26 (P-value <0.001); the proportion of participants who had positive attitude increased from 52.4% to 70.7% (P-value <0.001); mean score of attitude was higher significantly from 3.72 to 3.86 (P-value <0.001) after education. The education impact on acceptability was obviously seen among elders who never had history of influenza vaccination. The percentage of those who expressed willingness to receive vaccination increased from 80.2% to 91.2% (p<0.001), and the percentage of the elderly who express willingness to pay for influenza vaccine ascended dramatically from 58.7% to 72.9% (p<0.001).

**Conclusions.** Educational video has demonstrated significant impacts on knowledge, attitude, acceptability of influenza and vaccine among the elderly Thais who are classified as high risk for severe influenza and death, especially those who had no history of prior influenza vaccination.

**Keyword:** education, influenza vaccine, elder, knowledge,attitude, acceptability

# Tuesday 2nd December 2014

**Time:**
**15.30 – 17.00** **Room C**

## S15 Toxoplasmosis

**Chairpersons:**

**1. Yaowalark Sukthana**

**2. Florence Robert-Gangneux**

Invited Speakers:

1 Toxoplasmosis: new diagnostic tools and implication on care management according to the clinical setting

*Florence Robert-Gangneux*
Faculty of Medicine, University of Rennes 1, France

2 *Toxoplasma gondii*–Cat, Man and Education

*Veeranoot Nissapatorn*
Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia

3 *Toxoplasma gondii* in Thailand: Seroprevalence by indirect fluorescent antibody and modified agglutination tests in free-range chickens

*Aongart Mahittikorn*
Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Thailand

# TOXOPLASMOSIS: NEW DIAGNOSTIC TOOLS AND IMPLICATION ON CARE MANAGEMENT ACCORDING TO THE CLINICAL SETTING

Florence Robert-Gangneux

*Laboratory of Parasitology and Mycology*
*University Hospital of Rennes and INSERM U1085 IRSET, University Rennes 1, Rennes, France*

The awareness of toxoplasmosis burden has led some European countries with high prevalence rates to implement measures to reduce the burden of disease in neonates and in immunocompromised patients. Serological screening of pregnant women has been implemented in France in 1992, in the aim to start rapidly a specific treatment in case of *Toxoplasma* seroconversion, and to propose prenatal diagnosis and/or postnatal follow-up of newborns. Similarly, serological screening of recipients and organ donors can be implemented to guide chemoprophylaxis prescription in the aim to prevent organ-transmitted infection or reactivation of past infection in the recipient.

Meanwhile, sophisticated diagnostic tools (IgG avidity, IgA detection, western-blotting, molecular diagnosis) have been developed, but their use differs according to the clinical setting: congenital toxoplasmosis, immunocompromised patient or ocular toxoplasmosis.

Detection of *Toxoplasma gondii* DNA by PCR is crucial for diagnosing congenital toxoplasmosis, as well as toxoplasmosis in immunocompromised patients. Various methods have been described over the past twenty years, with variable performances. Most ancient pitfalls of PCR have been circumvented by recent quantitative PCR methods, but there remains a huge diversity of methods and performances, which will be discussed.

Another important field of PCR use is the diagnosis of toxoplasmosis in transplant patients. Rapid and efficient diagnosis is particularly crucial in allogeneic hematopoietic stem cell transplant (allo-HSCT) patients or solid organ transplant patients, since early treatment is needed. Regular follow-up of allo-HSCT patients by blood PCR has been proposed to guide pre-emptive therapy. Such prevention policy needs evaluation, but could improve survival.

Keyword: toxoplasmosis, diagnosis, prevention, congenital toxoplasmosis, transplantation, PCR

# *TOXOPLASMA GONDII*–CAT, MAN AND EDUCATION

Veeranoot Nissapatorn

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*Toxoplasma gondii* (*T. gondii*) is an obligateintracellular protozoan parasite that causes the disease “toxoplasmosis”. Dated back in 1908, *T. gondii* was discovered and later in 1920s, it wasrecognized as a human pathogen. *T. gondii* is a cause of disease in animals of economically importanceand produces a variety of clinical manifestations from congenital, ocular to cerebral toxoplasmosis in humans. The complexity of its life cycle and geneticdiversity contribute to toxoplasmosis as a show case among parasitic diseases in humans. In spite of itssublime nature, the precise mechanisms of *T. gondii* in producing systemic dissemination and life-longpersistence in the human host remain poorly understood. Since 1948, it was marked as a greatachievement on developing the first-ever reliable Sabin-Feldman dye test for serological diagnosis. Sero-markerrepresents the first and the most widely used approach to determine the stage of *Toxoplasma*infection. However, the results may be affected by difficulties in interpretations, as the same antibodypattern may have different valence, studied subjects and clinical settings. Of this, the measurement ofIgG avidity is capable of discriminating recent from chronic infections and has significantly increased theefficiency of *Toxoplasma* serological tests. The recombinant antigens, SAG, MIC, ROP, GRA or MAG1,are among other potential antigens for the sero-diagnosis of *Toxoplasma* infection. Apart from the host immunestatus, molecular diagnosis includes PCR base-conventional, nested, multiplex, real-time or LAMPtechniques has effectively been used to directly detect *T. gondii* DNA from clinical samples. Thesemolecular techniques are rapid, sensitive for acute phase and less time consuming; however, they areexpensive that not applicable in resource limited settings, need to be performed in well-equippedlaboratories, insensitive for chronic infection, and required experience technician. Recently, the utilizationof molecular techniques has enabled correlation between clinical manifestations and outcome of humantoxoplasmosis with the virulence of specific genotypes I, II and III of *T. gondii*. In spite of the advancement in laboratory diagnosis, *T. gondii* remains one of the top priorities among parasitic diseasesthat still needsmuch more attention particularly in Southeast Asia. Health education, a basic principle of prevention and control strategies, is mandatory as a primary source of implementation especially among women in child-bearing age and during their pregnanciesto attain “the right to health”.

# *TOXOPLASMA GONDII* IN THAILAND: SEROPREVALENCEBY INDIRECT FLUORESCENT ANTIBODY AND MODIFIED AGGLUTINATION TESTS IN FREE-RANGE CHICKENS

Aongart Mahittikorn[1], Ruenruetai Udonsom[1], Patcharee Chaichan[2],
**Yaowalark Sukthana**[1]

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[2] *Department of Parasitology, University of Limoges / Inserm UMR 1094 Tropical Neuroepidemiology, France*

*Toxoplasma gondii* infection in free-range chickens isa good indicator of environmental contamination of *T. gondii* oocysts because they feed from the ground. Moreover, chicken meat is a good source of infection for humans and other animalsif undercooked infected meat is ingested. Serological tests are commonly usedin epidemiological surveys and considered to be sensitive methods for the diagnosis of *T. gondii* infection in chickens.However, there is littleinformation on their acceptability for detecting antibodies in chickens. In the preliminary study, we validated the two serological diagnostic tools, indirect fluorescent antibody test (IFAT) and modified agglutination test (MAT), for *T. gondii* infection with the positive chicken control sera, and then the methods were evaluated with 150 free-range chicken sera samples. Our results showed a high agreement for *T. gondii* IgG antibody detection by the two methods after testing with positive chicken control sera. The seroprevalence of *T. gondii* by IFAT was 27.3% (41/150) with titer ranging from 1:16 to 1:256. MAT showed 7.3% (11/150), with a titer from 1:10 to 1:40. A further study is needed to compare the seropositive results with the detection of *T. gondii* in the organs of the chickens by using PCR.

# Tuesday 2nd December 2014

**Time:**

**15.30 – 17.00** **Room D**

## S16 Malaria Elimination

**Chairpersons:**

1. Jetsumon Prachumsri

2. Wang Nguitragool

Speakers:

1 Antimalarial drugs in the modern malaria elimination era: a perspective from the south pacific
*Harin Karunajeewa*
Walter and Eliza Hall Institute, Australia

2 Monitoring and evaluation of the therapeutic efficacy and safety of pyronaridine-artesunate for the treatment of uncomplicated falciparum malaria in western Cambodia, an area of artemisinin-resistant *Falciparum malaria*
*Sara Canavati*
Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand

3 The Solution Lies Within: Evaluating Positive Deviance As A Means of Promoting Community-driven Malaria Preventive Behaviours Among Hard-to-reach Populations in Myanmar
***Shafique Muhammad***
Malaria Consortium Asia, Faculty of Tropical Medicine, Mahidol University, Thailand

4 Ivermectin mass drug administration for malaria elimination in the greater mekong subregion
*Kevin Kobylinski*
Armed Forces Research Institute of Medical Sciences, Thailand

5 Intensified intervention packages for populations at higher risk of malaria elimination in Cambodia
*Harriet Lawford*
The National Centre for Parasitology, Entomology and Malaria Control, Thailand

6 Artemether-lumefantrine in vulnerable patient populations: getting the dose right
*Frank Kloprogge* *(Please see Abstract on P. 289)*
World Wide Antimalarial Resistance Network, Bangkok, Thailand

# ANTIMALARIAL DRUGS IN THE MODERN MALARIA ELIMINATION ERA: A PERSPECTIVE FROM THE SOUTH PACIFIC

Harin Karunajeewa[1], Lyndes Wini[2], George Taleo[3], Inoni Betuela[4] and Ivo Mueller[1]

[1] *Walter and Eliza Hall Institute, Melbourne, Australia*
[2] *Vector Borne Disease Control Unit, Solomon Islands Ministry of Health, Honiara, Solomon Islands*
[3] *Vector Borne Disease Control Unit, Vanuatu Ministry of Health, Port Vila, Vanuatu*
[4] *Papua New Guinea Institute of Medical Research*

The malaria-endemic countries of the South Pacific, including Papua New Guinea (PNG), the Solomon Islands (SI) and Vanuatu, present unique challenges and opportunities for malaria control and elimination. Following a long period of effective control during the pre-independence era, these countries experienced disastrous increases in malaria prevalence and morbidity, fuelled at least in part by the emergence and spread of drug resistance. In some areas transmission of both *Plasmodium vivax* (Chesson strain) and *Plasmodium falciparum*, approached some of the highest in the world. Fortunately, much progress has been made to reverse this situation, with particularly impressive reductions in malaria morbidity (>95%) over the last 2 decades in SI and Vanuatu. However *P.vivax* is particularly challenging to treat in this region. In particular, there are high levels of chloroquine resistance, very high rates of early hypnozoite relapse and uncertainties regarding the efficacy, safety and optimal dosing of primaquine therapy. This may represent a crucial final “roadblock” to future malaria elimination. Notwithstanding these challenges, the unique geography of these island archipelago states may be advantageous for malaria elimination strategies. Both SI and Vanuatu have now committed to malaria elimination as the explicit objective of their national malaria programs and are actively considering strategies incorporating mass drug administration (MDA). An important precedent for this exists following a successful malaria elimination experiment that utilized MDA on the Vanuatu island of Aneityum. Here we present recent data on anti-malarial drug efficacy that will help inform the approach to possible future MDA interventions.

Keyword: Malaria, Vivax, Falciparum, Antimalarial drugs, malaria elimination

# MONITORING AND EVALUATION OF THE THERAPEUTIC EFFICACY AND SAFETY OF PYRONARIDINE-ARTESUNATE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN WESTERN CAMBODIA, AN AREA OF ARTEMISININ-RESISTANT *FALCIPARUM MALARIA*

Sara E Canavati, Rithea Leang, Harriet Lawford, Tol Bunkea, Denis Mey Bouth, Huy Rekol, Arjen Dondorp, Pascal Ringwald

*Mahidol-Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand*
*The National Centre for Parasitology, Entomology and Malaria Control, Cambodia*
*The World Health Organisation, Geneva*

**Background:** Emerging resistance to artemisinins and to its partner drugs severely threaten the treatment of falciparum malaria in western Cambodia. Pyronaridine-artesunate was recently approved by the European Medicine Agency in countries with documented artemisinin resistance including Cambodia, where the drug was tested in 2005 and 2008. Here we present the findings of an open-labelled clinical trial being conducted to assess the efficacy and safety of pyronaridine-artesunate for the treatment of uncomplicated falciparum malaria in western Cambodia.

**Methods:** The primary analysis is conducted on an 'intention to treat' basis, including all patients enrolled into the study. Primary outcome are the Kaplan Meier analysis up to day 42 of follow-up. As per the protocol, analysis is based on all enrolled patients complying with all inclusion criteria who received a full course of the study drugs. Secondary analyses include the 'adequate parasitological and clinical response' (APCR) at day 28 and day 42. Safety and tolerability measurements are summarized for the ITT patient population.

**Results:** Preliminary results show a trend of increasing levels of eosinophils after treatment. Patients with high baseline differentials have had some sort of co-existing infection. Preliminary data show low rise of either post baseline ALT/AST or both. There have been no recurrent infections so far and no major side effects have been documented.

The main primary and secondary endpoints used in this on-going study will be also presented in full detail including the factors influencing these endpoints.

**Discussion:** The trend of increase in eosinophils after treatment has previously been observed. Pyronaridine-artesunate was first used as a fixed combination in 2005 in the context of the Phase II development. In a multi-centre trial there was a 10% recrudescence rate on D42 in the 211 patients recruited at the western Cambodian site treated with pyronaridine-artesunate, which contrasted with the overall D42 genotyping corrected efficacy rate of the ACT in the trial of 99.2%. The parasite clearance time was also slower for pyronaridine-artesunate in Pailin.

It is of high relevance to verify whether this truly reflects a genuinely lower treatment efficacy of combination therapy in areas where artemisinin resistance is prevalent. The results of this study will be used to inform the Cambodia National Malaria Treatment Guidelines.

**Keywords:** artemisinin resistance, western Cambodia, treatment policy

# THE SOLUTION LIES WITHIN: EVALUATING POSITIVE DEVIANCE AS A MEANS OF PROMOTING COMMUNITY-DRIVEN MALARIA PREVENTIVE BEHAVIOURS AMONG HARD-TO-REACH POPULATIONS IN MYANMAR

**Shafique Muhammad**

*Affiliation: Malaria Consortium Asia*

Reaching mobile populations, such as fishermen and forest-goers, is central to Myanmar's artemisinin resistance containment strategy. However, traditional inter-personal communication often fails because these populations are located in rural areas; work nocturnally; and often work in even more inaccessible locations. Innovative behavior change communication (BCC) approaches are therefore needed to successfully target them. For 1 year, starting March 2013, Positive Deviance (PD) was piloted in hard-to-reach populations of six villages in southern Myanmar. PD is a BCC approach that identifies "Positive Deviants" (i.e. role models who never had malaria due to easily replicable good health practices) in each village, and promotes them with the help of Village Health Volunteers (VHVs). In each village, one Positive Deviant was identified who never got malaria by effectively implementing malaria prevention measures. Over 12 months the Positive Deviants' practices were shared with the community through interactive community sessions and household visits. Baseline and endline data were collected to measure changes in key outcome indicators. The evaluation showed statistically significant increases in wearing of long-sleeved clothing. At baseline, 21.7% of rubber-tappers and fishermen wore long-sleeved clothing to avoid malaria. At endline, in villages where VHVs communicated this message without referring to a Deviant, long clothing use increased to 38.8%. In villages where VHVs also referred to a Deviant, long clothing use increased to 56.4%. Mosquito net usage also increased from 69.9% to 78.5% among female household members. Additionally, 30.6% of respondents indicated they would go to a VHV for malaria advice/treatment, compared to 11.4% at baseline. These findings were also confirmed by qualitative results, indicating that PD may be a cost-effective alternative to traditional BCC methods for hard to reach populations; a means to improve attitudes towards VHVs as sources of malaria advice/treatment; and a model to promote community ownership and sustainability of health interventions.

Keywords: Positive Deviance, Malaria Prevention, Behavior Change, Myanmar

# IVERMECTIN MASS DRUG ADMINISTRATION FOR MALARIA ELIMINATION IN THE GREATER MEKONG SUBREGION

Kevin Kobylinski[1], Alongkot Ponlawat[1], Ratawan Ubalee[1], MAJ Wes McCardle[1], LTC Anthony Schuster[1], Joel Tarning[2], Daniel Szumlas[3], and Jason Richardson[4]

[1] *Armed Forces Research Institute of Medical Sciences: Department of Entomology*
[2] *Mahidol-Oxford Tropical Medicine Research Unit: Department of Clinical Pharmacology*
[3] *Walter Reed Army Institute of Research: Entomology Branch*
[4] *Armed Forces Pest Management Board*

Novel vector control tools are urgently needed for the malaria elimination and artemisinin resistance containment efforts in the Greater Mekong Subregion (GMS). These vector control tools must target outdoor-feeding malaria vectors and integrate with current malaria control efforts. Ivermectin mass drug administration (MDA) has been shown to reduce both the survivorship and sporozoite rate of wild *Anopheles gambiae* in Senegal, Liberia, and Burkina Faso. Current *in vitro* data demonstrates that important GMS vectors *An. dirus* (LC$_{50}$ = 55.6 ng/ml), *An. minimus* (LC$_{50}$ = 16.3 ng/ml), *An. campestris* (LC$_{50}$ = 26.4 ng/ml), and *An. sawadwongporni* (LC$_{50}$ = 27.1 ng/ml) are ivermectin susceptible. Sublethal impact of ivermectin are currently being investigated such as delay of vector re-feeding, sporontocidal effect, and impact of compounded ivermectin blood meals on survivorship. Ivermectin MDA would make treated humans lethal to GMS vectors regardless of mosquito feeding location or time, thus directly targeting residual malaria transmission. Ivermectin MDA could easily integrate with antimalarial drug MDAs already occurring in the GMS which would target both human and vector reservoirs, thus synergistically reducing transmission burden. Furthermore, ivermectin MDA will affect numerous neglected tropical diseases such as lymphatic filariasis, scabies, lice, and soil-transmitted helminths (STHs). Combining albendazole with ivermectin will maximize impact of MDAs against lymphatic filariasis and STHs, thus delivering a greater overall health benefit than malaria control alone which should help maintain high compliance in treated populations.

Keyword: Ivermectin, Anopheles, malaria

# INTENSIFIED INTERVENTION PACKAGES FOR POPULATIONS AT HIGHER RISK OF MALARIA ELIMINATION IN CAMBODIA

Harriet L.S. Lawford, Sara E. Canavati, Lek Dysoley, Philippe Guyant, Shunmay Yeung, Maxine Whittaker

Background: In response to the threat of artemisinin resistance in the Greater Mekong Subregion (GMS), the Emergency Response to Artemisinin Resistance in the GMS (ERAR) Regional Framework for Action 2013-2015 was developed. At risk populations are commonly defined as: seasonal workers, construction/mine workers, forest workers, security personnel, and visitors; nevertheless, these are not mutually exclusive groups. These groups should be redefined around their activities in as opposed to their individuality.

Methods: Two stakeholder workshops were conducted in Cambodia; the first gathered representatives from the National Malaria Control Program, WHO, NGOs and research institutions at national level and participants presented current research and/or implementation projects focusing on MMPs. The second workshop gathered, in addition to representatives from the first workshop, representatives from Provincial Health Departments to collect their feedback and expert opinion on the outcomes of the first workshop and on the way forward.

Results: We will present the results of the workshops and the development of an operational definition of different sub-groups of Mobile and Migrant Population. We will also show the package of strategic interventions that were tailored for each group per strategic area (prevention, early diagnosis and treatment, behaviour change communication, research and surveillance).

Discussion: Defining the package of strategic interventions for populations at higher risk of malaria meets the objectives of the ERAR framework to develop country-specific artemisinin resistance elimination plans; the objectives of the plans overlap and include ‘improving access to services for mobile and migrant populations’ and ‘promoting elimination of resistant parasites’.

# Wednesday 3rd December 2014

**Time:**

**9.30 – 11.00** **Convention**

## S17 One Health: Food and Feed Safety

**Chairperson:**

1. Parntep Ratanakorn

Invited Speaker:

1 One Health: Food and Feed Safety

*Phatanee Leksrisompong*

Executive Vice President of Feed Technology Office at Charoen Pokphand Group

2 One Health: Food and Feed Safety

*Jon Ratcliff*

Food & Agriculture Consultancy Services (F.A.C.S Ltd)

# ONE HEALTH: FOOD AND FEED SAFETY

Phatanee Leksrisompong

The fields of agriculture and food animal production have been overshadowed by more attractive fields and the reality of food animal production has been shielded from the consumers by the advent of modern supermarkets. Misconceptions constantly propagated in the social media of today have turned the basic food animal production into something out of a horror movie.

The starting point of food animal production is the raw materials that go into making feeds for the animals. First misconception is that any materials unfit for human consumption will go into animal feeds. Food animals are a starting point in the food chain and undesirable substance that may be present in the raw materials can be concentrated further down the food chain raising the toxicity to the end consumers. Animal feed producers are well aware of the risk involved and standards and guidelines are set up in each organization. Work on common standard is underway by the Codex Alimentarius Commission and will apply to animal feed producers around the globe. Measures to prevent undesirable substances from getting into the food chain have been adopted at great expense to the feed producers. Costly instruments such as liquid chromatography and mass spectrometry are routinely used to analyze such molecules. With the added cost comes innovation to improve the prevention schemes. Automated system in collecting samples and routine analyses of these samples are employed to reduce the risk of contaminations to the animal feeds. Visitors to the laboratories in modern feed mills will be surprised to see modern, expensive analytical instruments that have improved the accuracy of the analyses.

Second misconception we still hear from time to time is the use of growth enhancing hormones in today's meat production. Work conducted within the animal genetic companies over the past many decades negates the necessities of such use. Modern-day broiler chickens have the ability to grow to market weight in less time than in the past with the reduction in days to a fixed body weight still maintained at half a day per year over the past forty plus years. The body conformation of modern-day chickens is also very much different from those of years past with higher percentage of edible meat. With modern genetic comes better nutrition required to support such growth and current understanding of what and how to feed these animals for the best growth with their welfare always an important factor. Prudent use of antibiotics has always been a major consideration in food animal production. Natural products with claims of enhancing immunity of the animals are being introduced and are under test to determine their viability.

Modern food animal production needs to guarantee safety of the food to be sold to the consumers. Evolution of meat animal production to what we see today was not an easy path and every detail of what is practiced today has some basis in the need to ensure that food is free of pathogenic agents. Rapid urbanization and lack of integrating agriculture into basic education curriculum result in further disconnect of the general population from the science of food production. The result that can be seen today is the general criticism of 'industrial' animal farming. Where food safety is a main concern, 'factory-farmed' meat is still the better choice. Current supple chains of meat products are routinely audited with more stringent standards being tagged on as needed. Some of these standards set by customers are more rigid than government regulations.

People who do not think twice about shelling out a lot of money for the latest technological gadgets will not pay premium price for what they consider to be commodities especially agricultural products. This scenario makes it difficult for agriculture in general to be an attractive choice for the young generation to pursue as a career. Plus the image of farm environment versus clean office makes the decision easier but it effectively shields the general population from the real science behind food animal production. Future educational system should include the science of food animal production so that future generation can conduct a healthy scientific debate on such topic whenever necessary.

Safe food for the consumers remains the main objective of major food animal producers. As the societies move forward, health is becoming more of an issue and food producers are the first to respond. As quality of life has become more important than just simply longer life, all players in the food production chain must understand their roles in improving the quality of life and must take their responsibilities seriously.

# ONE HEALTH: FOOD AND FEED SAFETY

Jon Ratcliff: F.A.C.S. Ltd

Prior to the mid Eighties there was very little attention directed at the animal feed industry in terms of implications for food safety. The emphasis at that time was very much focused on food processing and further processing based upon Hazard Analysis and Critical Control Point (HACCP) and Good Manufacturing Practice (GMP). However, events were to quickly change in Europe with the onset of Bovine Spongiform Encephalopathy (BSE) closely followed by salmonella contamination in eggs. The feed industry suddenly found itself under close scrutiny with regard the use of animal by- products and a perceived lack of accountability.

Once in the spotlight other millpractices were called into question, particularly the use of antibiotic medications and growth promoters. The concern in Europe about the link between antibiotics in animal feed and thepotential for antibiotic resistance in animals and therefore the general public, led to the EU banon the use of all in feed antibiotic growth promoters (AGPs) in 2006. During the preceding period the animal feed industry in Europe faced increasing legislation as well as independent audits by retailers concerned at the apparent lack of government control. This in turn led to the feed industry in a number of member statescollectively deciding to move towards an Assurance Scheme system of approval, independently audited to recognized standards in an effort to stem the tide of criticism and also to reduce the increasing frequency of second party audits. In the UK the Universal Feed Assurance Scheme (UFAS) was launched in 1998 andEurope also saw the implementation of the Dutch GMP+ system andthe German QS system. These were followed by Assurance schemes for Transport, Raw Material suppliers and traders and Feed Additive producers. By 2006 certainEuropean countries had established a comprehensive Feed Assurance scheme that covered much of the feed supply chain.

In Asia the impact of European Feed regulations was felt directly by companies exporting chicken and seafood products to Europe because of retailer expectations that the same standard of assurance apply to producers outside of Europe. Thailand is a good example of how proven food and feed safety systems can be voluntarily assimilated into existing legislation to provide equivalence in terms of standards and outcome. The Thai Poultry Exporters Association, supported by the Department of Livestock Development has worked diligentlyacross the complete food chain from breeding stock through to further processing to establish food and feed safety procedures that mirror those required in Europe. External auditing procedures are applied at all levels of production to cover not only feed and food safety but welfare and health management at farm, slaughter and further processing. The challenges in terms of feed safety are considerable given the infrastructure, the climate and the extensive supply chain. Approved Supplier systems underpin the feed operation because of the lack of equivalent assurance certifications for much of the raw material supply chainand its suppliers. Many of the undesirable hazards associated with feed will originate in the raw material or additives and extensive screening and testing procedures are required to ensure they do not become a problem in the finished feed. Within Thailand many feed companies choose not toproduce feed with antibiotics and similarly the government has banned AGPs in line with European legislation. Those that do produce medicated feeds or feeds with zootechnical additives such as anticoccidials must implement strict segregation and line cleaning procedures to meet theEU carryover limits. In terms of biological contamination, testing for a wide range of pathogens is implemented throughout the entire production chain. Feed materials are subjected to routine testing for salmonella and other known microbial hazards and technologies utilised in the feed production process to eliminate the threat in the finished feeds.

In conclusion, the Thai Feed Industry has demonstrated how the concept of One Health: Food and Feed is not just an aspiration in Asia but a reality that enhances the quality and safety of food both domestically and internationally.

# Wednesday 3rd December 2014

**Time:**
**11.00 – 12.30** **Convention**

## S18 Community based study on fishborne trematode infections

**Chairpersons:**

1. Dorn Watthanakulpanich

2. Poom Adisakwattana

Invited Speaker:

1 Infection pattern of liver fluke in Savannakhet, Lao PDR
*Tiengkham Pongvongsa*
Section of Malariology, Parasitology and Entomology, Savannakhet, Lao PDR"

2 Situation and mass drug administration as preventive measure for fish-borne zoonotic infections in Vietnam
*Dung Trung Do*
Department for Parasitology, National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi, Vietnam

3 Cross-sectional study onliver fluke infection in rural communities, Northeast Thailand
*Sirintip Boonjaraspinyo*
Department of Community Medicine and Department of Parasitology, Faculty of Medicine, KhonKaen, Thailand

4 A follow-up study of Opisthorchis viverrini infection in a rural community of central Thailand
*Suppawat Thongbor*
Phramongkutklao College of Medicine

# INFECTION PATTERN OF LIVER FLUKE IN SAVANNAKHET, LAO PDR

Tiengkham Pongvongsa[1], Chitsawang Chanthavisouk[2], Megumi Sato[3], Surapol Sanguankiat[4] ,Tipparayat Yoonuan[4] , Jitra Waikagul[4]

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*[2] World Health Organization to Lao PDR*
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*[4] Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand*

Foodborne Trematode, a recognized major public health problem in Lao PDR, is due to *Opisthorchiasis* caused by infection with *Opisthorchis viverrini*. Wide spread cultural practice of eating raw fish is the main risk factor of *O.viverrini* infection in the country. In 2007, surveys conducted in the entire country, 6 provinces have infection rates over 20% of the national threshold, and almost 90% of the total population is currently at risk of infection. Savanakhet province is ranked as the third highest of *O.viverrini* infection in Lao PDR with overall prevalence rate of 40%. Recent research findings showed the positivity rates for expelled worms among 207 participants after Praziquentel treatment, 65 people (31.4%) were infected with *O. viverrini. The infection rate* was highest in the age group 21-30 years. Of a total of 1,773 people were examined by using portable sonographer machines, 26 cases (1.48%) were suspected to have cholangio carcinoma resulting from heavy *O.viverrini* infection. A clear national policy and strategies for control of *O.viverrini* exist in Lao PDR. Passive case detection and treatment has been in place in a few provinces while MDA has not been conducted regularly due to funding constraints.

Keyword: Opistorchis Viverrini, Savannakhet, Lao PDR.

# SITUATION AND MASS DRUG ADMINISTRATION AS PREVENTIVE MEASURE FOR FISH-BORNE ZOONOTIC INFECTIONS IN VIETNAM

Dung Trung Do[1], Thi Hop Nguyen[1], Nguyen Thu Hien[1], Tore Lier[2]

[1] *Department for Parasitology, National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi, Vietnam.*

[2] *Department for Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway.*

The cross-sectional surveys for determining the helminth infection were conducted from 4,731 human in 9 provinces in Vietnam using the Kato-Katz thick smears for identifying the eggs of helminths. The result showed that small trematode prevalence was 24.6%. Forty-five infected persons were treated to expel liver and intestinal trematodes for specific identification. Totally, 46,651 adult flukes were collected. Mixed and diverse trematodes collected indicated that the small liver opisthorchiid fluke and the intestinal trematodes of Heterophyidae and Echinostomatidae are endemic in the localities examined. The adult of small intestinal trematodes were identified by morphology and confirmed by molecular techniques.

In other side, an intervention study with purpose was to investigate the effectiveness of preventive chemotherapy to control fish borne zoonotic trematodes in an endemic area. We followed a cohort of 396 people who fulfilled the criteria for receiving preventive chemotherapy. Stool samples were examined for the presence of trematode eggs before, and two, 16, 29 and 60 weeks after preventive chemotherapy. The prevalence of trematode eggs in stool was 40.2% before, 2.3% two weeks after and increased to a cumulative prevalence of 29.8% sixty weeks after preventive chemotherapy.

The effectiveness of preventive chemotherapy as a main component in control of fish borne zoonotic trematodes is not well documented in most endemic areas. We found a high reinfection rate within the first year after preventive chemotherapy. Since these trematodes are zoonoses, preventive chemotherapy may not have sufficient impact alone on the transmission to have a lasting effect on the prevalence.

Keywords: small trematode infection, vietnam

# CROSS-SECTIONAL STUDY ONLIVER FLUKE INFECTION IN RURAL COMMUNITIES, NORTHEAST THAILAND

Sirintip Boonjaraspinyo[1,2] , Thidarut Boonmars[2,3], Nuttapon Ekobol[4],
Porntip Laummaunwai[2,3], Ratchadawan Aukkanimart[2,3], Nadchanan Wonkchalee[2,3],
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Parasitic infections remain a major public health problem in Southeast Asia including Thailand. In rural communities, continuing infection is often reinforced by dietary habits that have a strong cultural basis and by poor personal hygiene. This study presents a survey of the prevalence of liver fluke infections among the people in rural Thailand. The community-based cross-sectional study was conducted in villages in KhonKaenProvince, the Northeast Thailand, from July to August 2013. A total of 253 stool samples from 102 males and 140 females, aged 2-80 years, were preparedusing formalin-ethyl acetate concentration methods and examined using light microscopy. Ninety-four individuals (37.2%) were infected with one or more parasite species. Presence of parasitic infection was significantly correlated with gender ($p$ = 0.001): nearly half of males in this survey (49%) were infected. Older people had a higher prevalence than younger members of the population. The most common parasite found was *Opisthorchisviverrini*(26.9%). The present results show that the prevalence of liver fluke infection in this region is still high even through government campaign has well done “do not eat raw fish”.Therefore, eating behavior is the most importantfactor and difficult to change.

Keywords: Parasitic infection,*Opisthorchisviverrini*, Prevalence, Rural communities

# A FOLLOW-UP STUDY OF *OPISTHORCHIS VIVERRINI* INFECTION IN A RURAL COMMUNITY OF CENTRAL THAILAND

Thanawin Tattamanas[1], Ploywarong Reangket[1], Natnicha Kanjanavaikoon[1], Sawanya Prammanakul[1], Kanvara Chaibenjapol[1], Jaturon Poovieng[1],Charinun Detjinda[1], Chanon Sutheparank[1], Nuttajak Onlaaied[1], Dithapol Wongcharoendham[1], Nipat Chantradilokrat[1], Badeesorn Techajanta[1], Pimrada Hongsawat[1], Suppawat Thongbor[1], Akarawin Tweekittikul[1], Saovanee Leelayoova[2], Paanjit Taamasri[2], Phunlerd Piyaraj[2], Picha Suwannahitatorn[2], Tawee Naaglor[2], Ram Rangsin[3] and Mathirut Mungthin[2]

*[1] 4th year Medical Cadet, Phramongkutklao College of Medicine*
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*[3] Department of Military and Community Medicine, Phramongkutklao College of Medicine*

**Background:** *Opisthorchis viverrini* (OV) is the public health problem in Thai rural area. The major risk factor is consumption of uncooked Cyprinoid fish. Recent study in 2012 showed that the OV prevalence is 9.3 %( 6.6-11.9, 95% CI).

**Methods:** A retrospective cohort study was conducted to evaluate incidence and risk factors. Stool examination methods including wet preparation, Kato thick and formalin-ethyl acetate concentration techniques were performed to diagnose OV egg. A standardized questionnaire was used to assess risk behaviors. Qualitative information was collected from 4 sessions of focus group discussion; local village health volunteers, control group, curative group and newly infected group. In-depth interview method was used in specific population to address interesting issues regarding OV infection included re-infection group and cured group.

**Results:** The incidence rate was 6.8 person-years (4.7-9.5, 95% CI). Consumption of chopped raw fish salad (*Koi pla*) was significantly associated risk factor of OV infection as same as previous study in this area. Qualitative study indicated that OV infection was a result of continuation of raw fish consumption due its taste, social and cultural influence.

**Conclusion:** Although conventional intervention and National Control Program has been applied to the study population, OV infection still emerged. Qualitative study also provided details of infection regarding bio-psycho-social aspect. The results could be potentially used to improve the health promotion program, providing more precise and effective intervention. Established risk such as consumption of *Koi pla* should be specifically incorperated in the prevention and control program.

**Keywords:** *Opisthorchis viverrini*, Incidence, Re-infection, Risk Factor, Qualitative study, Central Thailand

# Wednesday 3rd December 2014

**Time:**
**11.00 – 12.30** **Room A**

## S19 Enabling technologies to promote drug development for liver stage *P. vivax*

**Chairpersons:**

1. John Adams

2. Rapatbhorn Patrapuvich

Invited Speaker:

1 The Bill & Melinda Gates Foundation Malaria Culture Consortium
*Omar Vandal*
Bill & Melinda Gates Foundation, USA

2 The humanized mouse liver model as a as a drug discovery platform for *P. vivax* and *P. falciparum*
*Sebastian Mikolajczak*
Seattle Biomed Research Institute, USA

3 An in vitro human liver model as a drug discovery platform for *P. vivax* and *P. falciparum*
*Steven Maher*
University of South Florida, USA

# THE BILL & MELINDA GATES FOUNDATION MALARIA CULTURE CONSORTIUM

Omar Vandal

*The Bill and Melinda Gates Foundation*

I will give a brief overview of The Bill & Melinda Gates Foundations early-stage investments in platforms, models, and other approaches to enable the discovery of new malaria drugs for malaria eradication. I will describe the scientific challenges inherent in discovery of next generation medicines and the need for novel and creative partnerships between academia and industry to streamline these efforts. I will focus on Gates Foundation Malaria Culture Systems Consortium, an early-stage discovery program to develop new assays, platforms and technologies for malaria drug discovery.

# THE HUMANIZED MOUSE LIVER MODEL AS A DRUG DISCOVERY PLATFORM FOR *P.VIVAX* AND *P. FALCIPARUM*

Sebastian Mikolajczak

*Seattle Biomed Research Institute, USA*

The ability of *P. vivax* to form dormant liver stages (hypnozoites) that can be activated weeks or months after initial infection to cause relapse of malaria is of crucial importance for the unprecedented epidemiological success of the parasite. Yet, little progress has been made to understand the unique biology of hypnozoite formation and activation. Due to the parasite's strong preference for nonhuman primate and human tissue, the availability of models to study *P. vivax* liver stages is extremely limited. Here we report that the FRG KO mouse model transplanted with human primary hepatocytes (huHep) efficiently supports the development of *P. vivax* liver stages as well as the formation of hypnozoites for Thai isolates of *P. vivax*. Using a series of *P. vivax* specific polyclonal and monoclonal antibodies, we were able to evaluate the liver stage progression and maturation in the infected liver. The ability of the exo-erythrocytic merozoites to establish blood stage infection upon transfusion with human reticulocytes is being currently evaluated. Furthermore, *P. vivax* infections in the FRG huHep mice carried beyond the time of the liver stage maturation showed that persistence and activation of *P. vivax* hypnozoites can be further investigated in the model to determine the biological basis for liver stage dormancy. Successful evaluation of the antimalarial drugs with known activities on *P. vivax* liver stage infection confirmed that the FRG huHep/*P. vivax* infection model could be used as an efficient platform for testing new antimalarial drugs in vivo in quest to accelerate the development of interventions for the radical cure of *P. vivax* infection.

# AN IN VITRO HUMAN LIVER MODEL AS A DRUG DISCOVERY PLATFORM FOR *P. VIVAX* AND *P. FALCIPARUM*

Steven Maher

*University of South Florida, USA*

# Wednesday 3rd December 2014

**Time:**
**11.00 – 12.30** **Room B**

## S20 Dengue Vaccine Update II

**Chairpersons:**


1. Chukiat Sirivichayakul


2. Pornthep Chanthavanich

Invited Speaker:

1 Efficacy results of Sanofi Pasteur Chimeric Dengue Vaccine in Asian population

*Punnee Pitisuthitham*
Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand

2 Sanofi Pasteur's Dengue Vaccine Candidate Successfully Completes Final Landmark Phase III Clinical Efficacy Study in Latin America

*Danaya Chansinghakul*
Sanofi Pasteur, Clinical Research and Development

3 Predicting of dengue severity by immunodiagnostic assay, molecular detection and clinical data

*Pimolpatchr Sriburin*

4 By-stander effects of dengue specific T cell activation on endothelial cell morphology and functions

*Jittraporn Rattanamahaphoom*

5 Molecular diversity in envelope proteins of dengue virus isolates from patients in Ratchaburi, Thailand

*Supawat Chatchen*

# EFFICACY RESULTS OF SANOFI PASTEUR CHIMERIC DENGUE VACCINE IN ASIAN POPULATION

Punnee Pitisuttithum[1], Usa Thisyakorn[2], Tawee Chotpitayasunondh[3], , Krisana Pengsaa[4], Angkana Uppapong[5], Supachoke Trongkamolchai[6], Anongrat Tiawilai[7], In-Kyu Yoon[5], Swangjai Pungpak[1] Sriluck Simasathien[8], Kamchai Rangsimanpaiboon[9], Supat Chamnanchanunt[1], Darunee Buddhari[9], Danaya Chansinghakul[10], Anh Wartel[10], Alain Bouckenooghe[10] for the International Principal Investigators and the CYD14 Study Group

*[1] Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University, [2]Department of Pediatrics, Chulalongkorn University, [3]Queen Sirikit National Institute of Child Health, [4]Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, [5]Department of Virology, Armed Forces Research Institute of Medical Sciences, [6]Banpong Hospital Ministry of Public Health, [7]Photharam Hospital Ministry of Public Health [8]Phramongkutklao Hospital, [9]Kamphaeng Phet Provincial Hospital, [10]Sanofi Pasteur Clinical Development*

The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion are now at risk from dengue. Cases across the Americas, South-east Asia and Western Pacific have exceeded 1.2 million cases in 2008 and over 2.3 million in 2010. An estimated 500,000 people with severe dengue require hospitalization each year, a large proportion of whom are children. About 2.5% of those affected die. Among hospitalized patients, students lost 5.6 days of school, whereas those working lost 9.9 work days per average dengue episode. Overall mean costs were I$514 and I$1,394 for an ambulatory and hospitalized case, respectively. So dengue vaccine is urgently needed.

10,275 healthy children from five countries in the Asia-Pacific region between June 3, and Dec 1, 2011, aged 2–14 years were randomly assigned (2:1), to receive three injections of a recombinant, live, attenuated, tetravalent dengue vaccine (CYD-TDV), or placebo, at months 0, 6, and 12. Randomisation was stratified by age and site. Participants were followed up until month 25. The primary objective of the study was to assess protective efficacy against symptomatic, virologically confirmed dengue, irrespective of disease severity or serotype, that took place more than 28 days after the third injection. The primary endpoint was for the lower bound of the 95% CI of vaccine efficacy to be greater than 25%. Analysis was by intention to treat and per protocol.

There were 250 cases of virologically confirmed dengue reported after more than 28 days after the third injection (117 [47%] in the vaccine group and 133 [53%] in the control group) with 56·5% (95% CI 43·8–66·4) efficacy. Vaccine efficacy against dengue of any one of the four dengue virus (DENV) serotypes in this period (the per-protocol (PP) analysis) was estimated as 57% (95% CI 44, 66). However, the vaccine efficacy for serotype 2 was not statistically significant. In an exploratory analysis (ITT population) vaccine efficacy was higher in those vaccinated at older ages: 74% (95% CI 59, 84) in participants aged 12-14 years, 60% (95% CI 49, 68) in participants aged 6-11 years, and 34% (95% CI 12, 50) in participants aged 2-5 years. In a further exploratory analysis, an additional finding was that vaccinated children were found to be at reduced risk of severe disease (based on a definition by an independent data monitoring committee) with a PP vaccine efficacy of 81% (95% CI 43, 95). There were no singles of an increase in serious adverse events in the trial during the two years following the administration of vaccine.

CYD, live attenuated, tetravalent dengue vaccine appeared to be safe, moderately efficacious with 81% reduction in r risk of severe dengue infection. If the vaccine is licensed and introduced in countries, Phase 4 trials will be necessary for long-term follow-up of the duration of protection, vaccine safety, impact on dengue transmission and other questions that cannot be answered with pre-licensure clinical trials.

# SANOFI PASTEUR'S DENGUE VACCINE CANDIDATE SUCCESSFULLY COMPLETES FINAL LANDMARK PHASE III CLINICAL EFFICACY STUDY IN LATIN AMERICA

Danaya Chansinghakul[1], CYD15 Principal Investigators, and CYD15 Study Group

[1] *Sanofi Pasteur, Clinical Research and Development*

Dengue is a threat to over 2.5 billion people, nearly half the world's population, and is a pressing public health priority in over 100 countries in the Americas and in Asia.[1]Every year, an estimated 500,000 people, including children, are hospitalized due to severe dengue, which puts a huge strain on health care systems particularly during outbreaks.[1]Dengue has dramatically increased over the past 30 years with anacceleration over the last decade due to travel and urbanization.

Sanofi Pasteur, the vaccines division of Sanofi, on 3rdof November 2014 announced the publication of the detailed results of the final landmark phase III clinical efficacy study in Latin America in *The New England Journal of Medicine.*[2]A total of 20,869 children aged 9 to 16 years from dengue endemic areas of Brazil, Colombia, Mexico, Honduras and Puerto Rico participated in the study and were randomized to either receive three injections of the dengue vaccine or a placebo (2 to 1 ratio) at 0, 6, and 12 months.[2] Overall efficacy against any symptomatic dengue disease was 60.8% (95% confidence interval [CI], 52.0 to 68.0 in children and adolescents 9-16 years old who received three doses of the vaccine. Analyses show a 95.5% (95% CI, 68.8 to 99.9) protection against severe dengue and an 80.3% (95% CI, 64.7 to 89.5) reduction in the risk of hospitalization during the study. The results of this second phase III efficacy study confirm the high efficacy against severe dengue and the reduction in hospitalization observed during the 25-month active surveillance period of the first phase III efficacy study conducted in Asia[3], highlighting the consistency of the results across the world.

Safety analyses (solicited reactions, unsolicited events and Serious Adverse Events (SAEs)) during the study showed similar reporting rates between the vaccine and control groups and are consistent with the favorable safety profile observed during the 25-month active surveillance period of the previous efficacy study conducted in Asia.

Sanofi Pasteur's phase III efficacy clinical study program for its dengue vaccine candidate was conducted in over 31,000 participants across 10 endemic countries in Asia and Latin America. The phase III evaluations provide pivotal data on efficacy, safety, and immunogenicity of the vaccine candidate in a broad population and different epidemiological environments and assess the potential impact of the vaccine on the disease burden. This second phase III efficacy study confirms and share similar outcomes as in the recently concluded phase III study conducted in Asia.

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

Pimolpatchr Sriburin

# BY-STANDER EFFECTS OF DENGUE SPECIFIC T CELL ACTIVATION ON ENDOTHELIAL CELL MORPHOLOGY AND FUNCTIONS

Jittraporn Rattanamahaphoom

# MOLECULAR DIVERSITY IN ENVELOPE PROTEINS OF DENGUE VIRUS ISOLATES FROM PATIENTS IN RATCHABURI, THAILAND

Supawat Chatchen

# Wednesday 3rd December 2014

**Time:**
**11.00 – 12.30** **Room C**

## S21 Tuberculosis

**Chairpersons:**

1. Srisin Khusmith

2. Prasit Palittapongarnpim

Invited Speaker:

1 The wiring diagram of *Mycobacterium tuberculosis*
*David Sherman*
Seattle Biomdedical Research Institute, USA

2 Naive pooled-data approach for a pharmacokinetic evaluation of the first-line antituberculous drugs in children with Tuberculous meningitis
*Thomas Pouplin*
Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand

3 The contribution of target deconvolution in early TB drug discovery
*Pablo Bifani*
Novartis Institute for Tropical Diseases

4 Elucidating functions of hypothetical proteins of *Mycobacterium tuberculosis*
*Prasit Palittapongarnpim*
Department of Microbiology, Faculty of Science, Mahidol University, Thailand

# THE WIRING DIAGRAM OF *MYCOBACTERIUM TUBERCULOSIS*

David R. Sherman, PhD

*Professor, Seattle Biomedical Research Institute*

The bacterial pathogen *Mycobacterium tuberculosis* (MTB) infects 30% of all humans and kills someone every 20 – 30 seconds. In the push for new interventions, we have recently taken the first steps towards a completereconstruction of the*Mycobacterium tuberculosis* (MTB) gene regulatory network. We developed a high-throughput system based on ChIP-Seq for mapping transcription factor (TF) binding, and assayed genome-wide expression following induction of each TF. Using this method we report on the DNA binding and transcriptional regulatory profile of ~80% of all predicted MTB DNA binding proteins (>150 genes). We identify thousands ofstrong candidate interactions for direct transcriptional regulation associated with DNA binding; however, we also note thousands of DNA binding events that cannot be linked directly with transcriptional control. We propose a model in which TFs act on a spectrum from specific local control of gene expression to widespread binding with little or no direct impact on proximal genes, and suggest that many prokaryotic TFs bind DNA prolifically yet still home to and directly regulate a limited number of targets. This work updates current concepts of prokaryotic transcriptional control and also should form the basis for iterative rounds of modeling, prediction and refinement in pursuit of badly needed new TB therapies.

# NAIVE POOLED-DATA APPROACH FOR A PHARMACOKINETIC EVALUATION OF THE FIRST-LINE ANTITUBERCULOUS DRUGS IN CHILDREN WITH TUBERCULOUS MENINGITIS

Thomas Pouplin

*Centre for Tropical Medicine, Oxford University Clinical Research Unit Vietnam, Ho Chi Minh City, Vietnam*
*Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400 Thailand*
*Centre for Clinical Vaccinology & Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK*

**Objective:** To describe the exposure of first-line antituberculous drugs Pyrazinamide, Isoniazid and Rifampicin in the plasma and cerebrospinal fluid (CSF) of children with Tuberculous Meningitis (TBM).

**Method:** This study was a prospective descriptive study of 100 children less than 15 years of age presenting with suspected TBM in Ho Chi Minh City. The population was stratified into 2 age groups: 0 to 4 years old (young) and over 4 years old (old). Each patient enrolled in the TBM study was asked to provide plasma and CSF at several occasions: first day of treatment, steady-state and after 1 and 3 months. A naïve-pooled data (NPD) methodology was used for the 2 age groups in plasma at treatment initiation and steady state. Age group median concentration-time data were implemented in non-compartmental analysis to evaluate the pharmacokinetics of Pyrazinamide, Isoniazid and Rifampicin in plasma. The exposure in CSF was only descriptive based on the observed data.

**Results:** Despite the simplification using NPD, our findings fully confirmed that younger children needed higher dosage than older children. Based on the minimum inhibitory concentration (MIC) as the target to reach for drug concentrations, the exposure of PZA, INH and RIF were undoubtedly lowered in the young age group, obviously suggesting that increasing dosage is recommended to put all patients at the same exposure. In CSF, our approach confirmed that PZA and INH cross the blood-brain barrier effectively. The weak brain penetration of RIF was also established in children with all values well below the MIC.

# THE CONTRIBUTION OF TARGET DECONVOLUTION IN EARLY TB DRUG DISCOVERY

Pablo Bifani

*Novartis Institute of Tropical Diseases*

The mortality rate associated with *M. tuberculosis* has dropped by 45% since 1990, while the emergence of increasingly drug-resistant forms of tuberculosis continues to rise. The last two-decade have witness major developments in our understanding of the mechanisms of action of antimicrobials and mechanisms resistance of anti-tuberculosis drugs. These discoveries have led to the development of new cellular and molecular tools for diagnosis and treatment regiments with direct impact on patient care.

In contrast the contributions of mechanisms of resistance in early drug discovery have often been overlooked. Here we discuss some of the potential applications in unraveling the mechanisms of resistance in early drug discovery starting with target deconvolution, evaluation of cross-resistance, biological pathways, bacterial fitness, relative rate of resistance, mutation prevention concentrations and combination therapy.

# ELUCIDATING FUNCTIONS OF HYPOTHETICAL PROTEINS OF *MYCOBACTERIUM TUBERCULOSIS*

Prasit Palittapongarnpim, M.D.,

*Department of Microbiology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok, Thailand.*

Functions of a significant proportion of most bacterial genes are still unknown. Some of them may not be translated. Orthologs of some genes are identified in many genomes. The genes are, therefore, usually labeled as conserved hypothetical. Elucidating their functions is crucial for better understanding of the biology of the bacteria and is a major challenge in current bacteriology. Gene translation may be inferred from proteomics data while the evidence of transcription can be found in transcriptomics data. Primary elucidation of the gene functions is usually done by various bioinformatics methods, such as search for homologous proteins or proteins with similar 3-D structure. High-throughput experiments may provide information regarding the general functional categories of the proteins. Common methods include detection of preferential expressions in some conditions such as during intra-macrophage growth of *Mycobacterium tuberculosis*or detection of binding to specific targets. Nevertheless, these methods usually provide only a broad functional category but may not be good enough to correctly give the specific functions of the gene. Definite elucidation of the specific function generally requires gene-by-gene experimental confirmation and requires a concerted effort of communities of bacteriologists, such as TBCAP (Tuberculosis Community Annotation Project).

# Wednesday 3rd December 2014

**Time:**
**11.00 – 12.30** **Room D**

## S22 Frontiers of the Rat Lungworm *Angiostrongylus cantonensis*

**Chairpersons:**

1. Mathirut Mungthin
2. Padej Siriyasatien

I

Invited Speakers:

1 The genome of the rat lungworm *Angiostrongylus cantonensis*
*Hoi-Sen Yong*
Institute of Biological Sciences, University of Malaya, Malaysia

2 Clinical issues of the rat lungworm
*Kittisak Sawanyawisuth*
Department of Medicine, Faculty of Medicine, and Research Center in Back, Neck, Other Joint Pain and Human Performance (BNOJPH), Khon Kaen University, Thailand

3 Current state and future expectations for diagnostic methods of *Angiostrongylus cantonensis*
*Praphathip Eamsobhana*
Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

# THE GENOME OF THE RAT LUNGWORM *ANGIOSTRONGYLUS CANTONENSIS*

Hoi-Sen Yong

*Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia*

The rat lungworm*Angiostrongylus cantonensis* is a bursate, neurotropic nematode parasite.Presently, it has spread from its typical endemic regions of Asia and the Pacific to many other regions of the world, including the Americas, Australia, Caribbean islands and Africa.It is an important emerging pathogen causing human eosinophilic meningitis (or meningoencephalitis) with thousands of cases in many parts of the world.Because of its importance in public health, *A. cantonensis* has received great attention in laboratory and clinical studies. In particular, immuno- and molecular diagnosis of human angiostrongyliasis has been explored extensively. Recently a number of studies have been reported on *A. cantonensis* high throughput sequencing, microRNAsand transcriptome profiling, but to date, no novel miRNAs have been found in adult worms. Finding novel miRNAs and other gene products is expected only when a reference genome becomes available. Here we report the draft whole genome (estimated to be 261 Mb) of this food-borne zoonotic parasite. This genome is the first to be reported for the Metastrongyloidea superfamily of bursate nematodes and adds to the list of *Wolbachia*-free nematode parasites. The genomic data from *A. cantonensis* will form a useful resource for comparative genomic and chemogenomic studies to aid the development of diagnostics and therapeutics.

# CLINICAL ISSUES OF THE RAT LUNGWORM

Kittisak Sawanyawisuth, MD, PhD

*Department of Medicine, Faculty of Medicine, and Research Center in Back, Neck, Other Joint Pain and Human Performance (BNOJPH), Khon Kaen University, Khon Kaen, Thailand*

There are three main types of human angiostrongyliasis caused by *A. cantonensis* including eosinophilic meningitis, eosinophilic meningoencephalitis, and ocular angiostrongyliasis. The main clinical issue is missed or delayed diagnosis of the rat lungworm infection in clinical practice. The main clinical feature of eosinophilic meningitis from *A. cantonensis* is a non-specific headache. A triad of meningitis is not typically presented; less than 10% in adult patients. History of larval exposure is crucial for the rat lungworm infection which may be missed by physicians. The identification of *A. cantonensis* larva is rare in human tissues or cerebrospinal fluid. The diagnosis can be made mostly by evidence of cerebrospinal fluid eosinophils and history of the rat lungworm exposure. Serological tests may be helpful to differentiate the rat lungworm infection from other causes of eosinophils in the cerebrospinal fluid.

Secondly, are anthelminthic agents effective for the rat lungworm treatment? For eosinophilic meningitis form, corticosteroid treatment is effective. The duration of headache after treatment with albendazole alone is longer than corticosteroid treatment. The combinations of corticosteroid and albendazole or mebendazole treatment are not superior to corticosteroid treatment alone.

Lastly, who will develop meningoencephalitis or ocular angiostrongyliasis if infected with the rat lungworm? Risk factors for meningoencephalitis in patients with the rat lungworm infection are older age, presence of fever, and prolonged headache. There is no clinical study on risk factors for ocular angiostrongyliasis. No effective treatment is warranted for both forms resulting in high mortality and morbidity.

Keyword: Rat lungworm; clinical; Angiostrongylus cantonensis; diagnosis; treatment; risk factors

# CURRENT STATE AND FUTURE EXPECTATIONS FOR DIAGNOSTIC METHODS OF *ANGIOSTRONGYLUS CANTONENSIS*

Praphathip Eamsobhana

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

Immunological testing has been very helpful in supporting the clinical diagnosis of eosinophilic meningitis or eosinophilic meningoencephalitis caused by the neurotropic parasite, *Angiostrongylus cantonensis* as direct observation of the parasite in infected patients is not common. Antibody detection methods focus primarily on antibodies to the 29 and 31 kDa proteins of the worm. Currently, proteomics approaches of producing the 31-kDa glycoprotein complex are under extensive study with the collaboration of the US Centers for Disease Control and Prevention. Antigen detection based on specific monoclonal antibodies to detect circulating parasite antigens may ultimately prove to be more reliable than antibody detection but no method has been adopted for clinical diagnostic use. A more conclusive diagnosis can be established by direct detection of the parasite DNA in clinical cerebrospinal fluid (CSF) or blood samples. Molecular diagnosis using PCR amplification of DNA sequences specific to *A. cantonensis*, e.g. a conventional nucleic acid amplification test based on the small ribosomal subunit (18S rRNA) and the 66-kDa muscle-associated protein gene, and real-time PCR or quantitative PCR (qPCR) assays targeting the internal transcribed spacer-1 (ITS-1) have been developed for detection of *A. cantonensis* DNA from snail-derived tissues, rat droppings or human CSF samples. The assays have not yet been fully validated for clinical use at this time. Immunological testing is thus still recommended to support nucleic acid amplification test results. The talk will discuss the need for an international collaborative network for multi-center blind testing to evaluate diagnostic methods and development of well standardized diagnostic systems for this global, emerging food-borne angiostrongyliasis.

# Wednesday 3rd December 2014

**Time:**
**13.30 – 15.00** **Convention**

## S23 Helminthic Diseases

**Chairpersons:**

1. Paron Dekumyoy

2. Yukifumi Nawa

Invited Speakers:

1 Morphological and molecular characterization of *Paragonimus* spp. in freshwater crabs of Costa Rica
*Roderico Hernández Chea*
National University of Costa Rica/ Universidad Nacional de Costa Rica (UNA), Costa Rica

2 Gnathostomiasis – an update –
*Yukifumi Nawa*
Research Affairs, Faculty of Medicine, Khon Kaen University, Thailand

3 *Strongyloides stercoralis* In Rural Cambodia
*Hanspeter Marti*
Swiss Tropical and Public-Health Institute, Switzerland

# MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *PARAGONIMUS* SPP. IN FRESHWATER CRABS OF COSTA RICA

R. Hernández-Chea

*Universidad Nacional de Costa Rica (UNA), PCVET, 304-3000 Heredia, Costa Rica*

Paragonimiasis is a food borne parasitic disease caused by flukes of the genus *Paragonimus*. According to the World Health Organization the most common species of *Paragonimus* transmitted to man in the American continent are *P. kellicotti* and *P. mexicanus*. In Costa Rica *P. mexicanus* was reported for the first time in the lungs of a gray fox in 1956, since then, *P. mexicanus* and *P. caliensis* were identified in the country only by morphological features. The first two human cases in Costa Rica were reported in 1968 in adult males, with pulmonary complications, one of which died. Summarizing the data published since then, a total of 27 clinical cases in humans have been recorded in Costa Rica, occurring mainly in rural areas, and usually affecting children (90%). In 1999, as a part of a Master thesis investigation, an intradermal test was applied to 100 Indigenous children attending the Alto Katsi school of Talamanca, and 23% reacted positive to the test. The last case of paragonimiasis was reported in 2013 in an Indigenous child, presenting a tumoral mass in the left temporal lobe, which was removed by neurosurgery, and sent for histopathological analysis, revealing the presence of eggs of *Paragonimus* spp. We believe that the few reports of clinical cases in Costa Rica can be attributed to the lack of specific diagnostic techniques, and due to ignorance of the disease, resulting in confusion with other diseases. The objective of the present study is to identify the *Paragonimus* species present in Costa Rica using parasitological and molecular techniques, and to determine the distribution of the parasite in the country, to implement prevention strategies.

Fresh water crabs (*Potamocarcinus magnus* and an unidentified decapod species) were collected from Alto Katsi, Talamanca, Costa Rica, a locality known to be an endemic area of *Paragonimus* spp. In two out of ten captured decapods infection with metacercariae of *Paragonimus* spp. was detected. Each decapod harboured two metacercariae. One metacercariae of each decapod was subjected to morphological identification, and body length, body width, oral sucker, ventral sucker, stylet and cecum were measured. The remaining metacercariae were subjected to DNA extraction, and will be analysed by PCR (ITS2, CO1 and NADH1) and sequenced for confirmation of the *Paragonimus* species. Results will be discussed.

Keyword: Paragonimus Costa Rica molecular Characterization Freshwater crabs

# GNATHOSTOMIASIS – AN UPDATE –

Yukifumi Nawa[1], Pewpan M. Intapan[2,3], Wanchai Maleewong[2,3], Sylvia Paz Diaz Camacho[4,5]

*[1]Research Affairs, [2]Research and Diagnostic Centre for Emerging Infectious Diseases and [3]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand, [4]Facultado de Ciencias Quimicobiologicas y [5]Unidad de Investigaciones en Salud Publica "Kaethe Willms", Universidad Autonoma de Sinaloa, Culiacan, Sinaloa, Mexico*

Gnathostomiasis is an important but neglected fish-borne parasitic zoonosis. Infection occurs where people preferentially consume uncooked/undercooked freshwater fish harboring advanced 3rd stage larvae of a nematode parasite species in the genus *Gnathostoma*. Until now, 7 species are recorded in the Americas and 6 species in Asia. Among those 13 species, only 5 species, *G. spinigerum*, *G. hispidum*, *G. doloresi*, *G. nipponicum* and *G. binucleatum*, have been proven as the pathogens for humans. While *G. spinigerum* is the major pathogen in Asia, *G. binucleatum* is responsible for the diseases in Latin American countries. The patients infected with other 3 species have been reported only from Japan in spite of wide distribution of these parasites in Asia. Thailand and Japan are the major endemic countries, but the numbers of patients in these countries has been decreasing. Instead, the disease is increasing in Mexico and Ecuador. Also, increasing number of cases has been reported in China. While neuro-gnathostomiasis have been recorded almost exclusively in Thailand, cases of ocular involvement have been reported in several endemic countries like Thailand, Japan and Mexico. In India, the number of ocular cases is comparable with other endemic countries, a cuntaneous gnathostomosis case was reported only very recently. For the pathogenesis and diagnosis, matrix metallo-proteinases and galectins are reported to be valuable molecules in both *G. spinigerum* and *G. binucleatum*. Albendazole followed by ivermectin has been used for treatment but their therapeutic efficacy remains uncertain.

Keyword: Gnathostoma, gnathostomiasis, food-borne parasitosis, neglected disiease

# *STRONGYLOIDES STERCORALIS* IN RURAL CAMBODIA

Virak Khieu [1,2], Fabian Schär [2], Hanspeter Marti [2], Sinuon Muth [1] and Peter Odermatt [2]

[1] *National Centre for Parasitology, Entomology and Malaria, Phnom Penh, Cambodia.*
[2] *Swiss Tropical and Public Health Institute, Basel, Switzerland.*

Strongyloidiasis, a neglected tropical disease, is endemic in tropical and temperate climates. Its diagnosis requiring specific methods, information on its geographic distribution is sketchy at best. We performed a cross-sectional study among 2,396 participants from 60 villages in Preah Vihear Province, northern Cambodia. A total of 44.7% of the participants were infected. The prevalence of infection increased with age and males were significantly higher infected than females. Itchy skin, muscle pain, diarrhea and urticaria were associated with infection, while having a latrine at home and possession of shoes were negatively associated. The cure rate of ivermectin was 98.3%. After treatment, all individuals stopped excreting larvae within 3 days. Larvae were not detected during the following 18 days.

We further determined S. stercoralis re-infection rates among 302 schoolchildren in Kandal province two years after ivermectin treatment. We observed infections in 24.2% and 22.5% of the children at baseline and at follow-up, respectively. At baseline, 73 children were treated for S. stercoralis infection. At follow-up, one-third of those treated were reinfected, while 19.6% of the 229 healthy children were newly infected. 68.5% of non-infected children remained free of infection for at least two years.

S. stercoralis is highly prevalent in rural Cambodia. Access to adequate diagnosis and treatment is urgently needed.

# Wednesday 3rd December 2014

**Time:**
**13.30 – 15.00** **Room A**

## S24 Systems Biology: Host-parasite interaction

**Chairpersons:**

1. Wang Nguitragool

2. Rachanee Udomsangpetch

Invited Speakers:

1 Human Regulator Recruitment as a Novel Complement Evasion Strategy for Blood Stage *Plasmodium falciparum* Infection

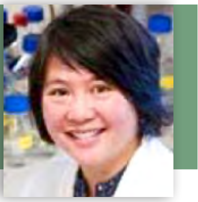
*Wai-Hong Tham*
Division of Infection and Immunity, Walter and Eliza Hall Institute of Medical Research, Australia

2 Development of a *Plasmodium vivax* malaria vaccine for clinical applications using transgenic parasites, virus-like particles and recombinant viruses

*Arturo Reyes-Sandoval*
The Jenner Institute, University of Oxford, United Kingdom

3 Development of a pan-species, multistage 'universal' vaccine for malaria

***Louis Schofield***
Walter and Eliza Hall Institute of Medical Research, Australia



# HUMAN REGULATOR RECRUITMENT AS A NOVEL COMPLEMENT EVASION STRATEGY FOR BLOOD STAGE *PLASMODIUM FALCIPARUM* INFECTION

Alexander T. Kennedy[1,3], Christoph Q. Schmidt[2], Paul N. Barlow[4], Alan F. Cowman[1,3], Wai-Hong Tham[1,3]

[1] *The Walter and Eliza Hall Institute of Medical Research, Australia,*
[2] *Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm University, Germany,*
[3] *Department of Medical Biology, The University of Melbourne, Australia,*
[4] *Schools of Biological Sciences and Chemistry, University of Edinburgh, Scotland*

The human complement system is a front line defence mechanism against invading pathogens. Of the 30-40 proteins involved in the complement cascade some are responsible for clearing pathogens either by direct membrane lysis or by opsonisation leading to phagocytosis. Pathogens that are exposed to serum subsequently have evolved numerous evasion mechanisms to prevent complement-mediated clearance, including the ability to recruit host regulators of complement for their own protection. One such host regulator is factor H, which acts as a negative regulator of complement to protect host tissues from aberrant complement activation. Until now it is not known if *Plasmodium falciparum* uses similar mechanisms to prevent complement-mediated lysiswhen merozoites, the invasive form of malaria parasite, are exposed to the host serum prior to erythrocyte invasion. We have identified that merozoitesactively recruit human complement regulator factor H. We have mapped the factor H binding site that recognizes merozoites and also identified a member of the 6-cys familyof surface proteins as its direct interaction partner. When bound to the merozoites factor H retains co-factor activity, a key function which allows it to down regulate the alternative pathway both in solution and on cell surfaces, thus affording *P. falciparum*merozoites protection from complement-mediated assault.

# DEVELOPMENT OF A *PLASMODIUM VIVAX* MALARIA VACCINE FOR CLINICAL APPLICATIONS USING TRANSGENIC PARASITES, VIRUS-LIKE PARTICLES AND RECOMBINANT VIRUSES

Arturo Reyes-Sandoval,

*The Jenner Institute, University of Oxford, The Henry Wellcome Building for Molecular Physiology, Roosevelt Drive, Oxford, OX3 7BN, UK;*

As plans for malaria eradication continue, a vaccine targeting *Plasmodium vivax* is of major importance due to the fact that it is the most widely distributed human malaria parasite in the world and the most difficult to eliminate due to its ability to hide in the liver forming hypnozoites. Malaria liver-stage vaccines are one of the leading strategies and the only approach that has demonstrated complete, sterile protection in clinical trials. We have developed vaccine candidates using platforms suitable for human use, consisting on virus-like particles (VLPs), chimpanzee adenovirus (ChAd63) and modified vaccinia Ankara (MVA) containing malaria pre-erythrocytic antigens, and several novel challenge models using transgenic mouse-malaria *P. berghei* parasites expressing *P. vivax* antigens that permit the assessment of vaccine efficacy in small animal models, thus providing a suitable platform for vaccine development in non-endemic countries and permitting the progress of vaccine that rely in assessment of efficacy rather than measurement of immunogenicity, in the absence of a conclusive correlate of protection.

We present results of two leading malaria vaccine candidates, a universal vivax circumsporozoite antigen (PvCSP) and the thrombospondin-related adhesion protein (PvTRAP) to create recombinant adenovirus, MVA and a platform consisting on VLPs to enhance efficacy of our malaria vaccines. All have been tested using transgenic parasites expressing the P. vivax CSP VK210, VK247 and TRAP, thus permitting the assessment of protective efficacy in various mouse strains and yielding important information to address cross protection against CSP using a challenge with VK210 or VK247 parasites in mice vaccinated with homologous, heterologous or chimeric CSP antigens.

Our studies indicate that our vaccines are able to induce outstanding T-cell responses and antibody titers that can protect against a stringent challenge with high doses of fully virulent transgenic parasites in mice. Our group is currently committing a substantial effort in developing further transgenic parasites to allow the discovery of new vaccine candidates able to afford protection.

# DEVELOPMENT OF A PAN-SPECIES, MULTISTAGE ‘UNIVERSAL’ VACCINE FOR MALARIA

Qiao Ye TAN[1], Julia C. Cutts[1], Vanessa Mollard[2], Anton Cozijnsen[2], Geoff I. McFadden[2], Stewart Campbell[3], JetsumonPrachumsri[5], Krystal J. Evans[1], **Louis Schofield**[1, 4]

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[4] *Australian Institute of Tropical Health and Medicine, James Cook University, Queensland 4811, Australia*
[5] *MahidolVivax Research Unit, Mahidol University, Bangkok, Thailand*

Malaria vaccine development faces formidable obstacles. The ten malaria life stages (sporozoites, hypnozoites, exoerythrocytic liver forms, merozoites, blood-stage trophozoites, schizonts, gametocytes, gametes, ookinete/zygotes and oocysts) are each highly differentiated, presenting completely different surface antigens, and occur variously in recondite locations. Immunity is necessarily stage-specific. Moreover, for the five different species of malaria infecting humans, immunity is species-specific, meaning a vaccine for one species will not work against another. The ideal vaccine target would therefore be allelically invariant, highly conserved across all five malaria species, be expressed on the surface of all differentiated life stages, provide clinical protection and also elicit transmission blocking immunity! Most vaccine candidates fail to meet even a portion of these stringent criteria.

However, we have defined a viable subunit candidate that does so. Our target has these properties because the life-stage differentiated, pathway redundant, allelic (and taxonomic) diversity strategy pursued by malaria applies to protein antigens; our target in contrast is a universally conserved surface oligosaccharide, the glycosylphosphatidylinositol moiety that anchors many surface proteins in different life stages and in all species of malaria. When rendered immunogenic by conjugation to carrier protein and formulation in adjuvant, vaccinating against this oligosaccharide target blocks sporozoites (prevents infections), inhibits blood-stage replication, prevents clinical disease and blocks onward transmission to the mosquito, in pre-clinical models. The vaccine is amenable to inexpensive synthesis and may be conjugated to generic carriers and formulated in generic adjuvants. These considerations indicate the possibility of developing an inexpensive saccharide-conjugate pan-species, multi-stage vaccine against malaria.

Keywords: malaria, host-pathogen

# Wednesday 3rd December 2014

**Time:**
**13.30 – 15.00** **Room B**

## S25 Therapeutic and Diagnostic Antibodies Against Viral Diseases

**Chairpersons:**

1. Tamaki Okabayashi

2. Pongrama Ramasoota

Invited Speakers:

1 Development and clinical evaluation of a new rapid immunochromatographic diagnostic test kit to detect Chikungunya virus antigen

*Tamaki Okabayashi*
Mahidol-Osaka Center for Infectious Diseases (MOCID), Faculty of Tropical Medicine, Mahidol University, Thailand

2 Antibody germline characterization of cross-neutralizing human IgGs against 4 serotypes of Dengue virus

*Pannamthip Pitaksajjakul*
Department of Social and Environmental Medicine and Center of Excellence for Antibody Research (CEAR), Faculty of Tropical Medicine, Mahidol University, Thailand

3 Epitope mapping of dengue specific human monoclonal antibodies

*Chonlatip Pipattanaboon*
Center of Excellence for Antibody Research, Faculty of Tropical Medicine, Mahidol University, Thailand

# DEVELOPMENT AND CLINICAL EVALUATION OF A NEW RAPID IMMUNOCHROMATOGRAPHIC DIAGNOSTIC TEST KIT TO DETECT CHIKUNGUNYA VIRUS ANTIGEN

Tamaki Okabayashi[1], Tadahiro Sasaki[2], Promsin Masrinoul[1], Nantarat Chantawat[1], Orapim Puiprom[1], Panjaporn Chaichana[1], Takeshi Kurosu[2], Pongrama Ramasoota[3], Kazuyoshi Ikuta[2]

*[1] Mahidol-Osaka Center for Infectious Diseases, Faculty of Tropical Medicine, Mahidol University, Thailand*
*[2] Research Institute for Microbial Diseases, Osaka University, Japan*
*[3] Center of Excellence for Antibody Research, Faculty of Tropical Medicine, Mahidol University, Thailand*

BACKGROUND: Chikungunya fever (CF) is a mosquito-borne disease of key public health importance in tropical and subtropical countries. Although severe joint pain is the most distinguishing feature of CF, diagnosis remains difficult because the symptoms of CF are similar to those of dengue fever.

OBJECTIVES: The present study aimed to develop a new immunochromatographic diagnosis kit (IC kit) for detection of chikungunya virus (CHIKV) antigen in serum.

METHODS: Mice were immunized with Thai CF-patient isolates, East Central South African genotype, to produce mouse monoclonal antibodies (MAbs) against CHIKV. Using these MAbs, an IC kit was developed and evaluated for the detection of CHIKV using clinical samples.

RESULTS: The MAbs and the newly developed kit reacted with ECSA and Asian genotypes of CHIKV. Testing of sera from CF-suspected patients in Thailand (n=50), Laos (n=54), and Indonesia (n=2) revealed sensitivity, specificity, and RT-PCR agreement values of 88.6%, 94.4%, and 93.2%, respectively. In our study using serial samples, IC kit showed high agreement with the RT-PCR within the first 5 days after onset.

CONCLUSIONS: A rapid IC kit was developed using mouse MAbs that react with CHIKV envelope proteins. The diagnostic accuracy of our IC kit is clinically acceptable for CF diagnosis in the acute phase.

# ANTIBODY GERMLINE CHARACTERIZATION OF CROSS-NEUTRALIZING HUMAN IgGs AGAINST 4 SEROTYPES OF DENGUE VIRUS

Pannamthip Pitaksajjakul [1, 2], Surachet Benjathummarak[1], Chonlatip Pipattanaboon[1], Waranya Wongwit[2], Tamaki Okabayashi [3, 5] , Motoki Kuhara[5], Ryo Misaki [4] , Kazuhito Fujiyama [4], Pongrama Ramasoota [1, 2]*

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*[2] Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*
*[3] Mahidol Osaka Center for Infectious Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*
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*Telephone: (6681) 5527589*

Dengue virus (DENV), a re-emerging virus, constitutes the largest vector-borne disease virus, with 50-100 million cases reported every year. Although DENV infection induces lifelong immunity against viruses of the same serotypes, the subsequent infection with the heterologous serotypes can cause more severe form of the disease, such as Dengue Haemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). However, there is neither approved vaccine nor specific drugs available to treat this disease. In this study, previously developed 19 human monoclonal antibodies (HuMAbs) showing strong to moderate cross neutralizing activity were selected. Most of them (13/19) were targeted to domain II of envelop glycoprotein. To understand and clarify the recognition properties, the maturation mechanisms comprising Variable/Diversity/Joining (VDJ) recombination, Variable Heavy (VH)/Variable Light (VL) chain pairing, variability at junctional site, and somatic hypermutation (SHM) of those antibodies were studied and compared with their predecessor germline sequences. IMGT/V-QUEST database was applied to analyze the isolated VH and VL sequences. To confirm the correction of isolated VH/VL, 3 HuMAbs (1A10H7, 1B3B9, 1G7C2) was transiently expressed in HEK293T cell. All three clones of the expressed recombinant IgG (rIgG) showed the same binding and neutralizing activity as same as those from hybridomas. The data obtained in this study will elucidate the properties of those HuMAbs for further genetic modification, and its binding epitopes.

Keywords: Recombinant IgG, Neutralization, Dengue virus, cross-neutralizing, germline

# EPITOPE MAPPING OF DENGUE SPECIFIC HUMAN MONOCLONAL ANTIBODIES

Chonlatip Pipattanaboon, Ph.D.

*Center of Excellence for Antibody Research, Faculty of Tropical Medicine, Mahidol University*

Epitope mapping is an important process in the selection and characterization of antibodies to identify binding sites or epitopes on target antigens. Exact epitope is a specific binding site information, which is to be included in regulatory dossiers for novel therapeutic antibodies following FDA and EMEA guidelines. Moreover, understanding the antibody-epitope interaction provides a basis for the rational design of preventive vaccines. After successfully generating 136 anti-dengue human monoclonal antibodies (HuMAbs) at Center of Excellence for Antibody Research (CEAR), target protein detection and truncated protein approach have been designed to screen binding regions of all HuMAbs. Most of these HuMAbs have the predominant binding region on first DII of dengue envelope protein. Now, site directed mutagenesis, phage display technique, and escape mutant strategy have been developed in our center to specify critical binding sites of neutralizing HuMAbs. This is our first step in developing epitope-based vaccines.

# Wednesday 3rd December 2014

**Time:**
**13.30 – 15.00** **Room C**

## S26 For Your Eyes Only
## (Interactive Case Discussion)

**Moderators:**


1. Yupaporn Wattanagoon


2. Jittima Dhitavat

# Wednesday 3rd December 2014

**Time:**

**13.30 – 15.00** **Room D**

## S27 HIV/STDs and associated infections

**Chairperson:**

Timothy H. Holtz

Invited Speakers:

1 Prevention of HPV-Associated Cancers and Diseases in Men Who Have Sex With Men (MSM)

*Eileen Dunne*

Thailand MOPH – U.S. CDC Collaboration, Thailand and Centers for Disease Control and Prevention, USA

2 Bad bed-bugs coming our way? *Neisseria gonorrhea* and drug resistance in Asia

*Marcel Curlin*

HIV/STD Research Program, Thailand MOPH – US CDC Collaboration (TUC), Nonthaburi, Thailand and Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA, USA

3 HIV and syphilis infection among MSM in Thailand

*Anupong Chitwarakorn*

Department of Disease Control, Ministry of Public Health

4 Ending AIDS in Thailand

*Sumeth Ongwandee*

Director of the Bureau of AIDS and STI (BATS), Department of Disease Control (DDC), Minister of Public Health, in Thailand

# PREVENTION OF HPV-ASSOCIATED CANCERS AND DISEASES IN MEN WHO HAVE SEX WITH MEN (MSM)

Eileen F. Dunne

[1] *Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA*
[2] *HIV/STD Research Program, Thailand MOPH – US CDC Collaboration (TUC), Nonthaburi, Thailand*

**Background and purpose:** HPV-associated cancers and diseases including anal cancer and anogenital warts are more common in men who have sex with men (MSM), especially MSM with HIV infection. The purpose of this presentation is to review data on HPV-associated cancers and diseases in MSM and prevention opportunities.

**Methods:** Systematic review of available data on burden of cancer and diseases in MSM, and primary efficacy data from clinical trials on HPV vaccine.

**Results and findings:** A variety of data, including estimates, modeling and cancer registry data, have demonstrated a substantial global burden of HPV-associated cancers and diseases in MSM; some cancers have been on the rise. This data on burden is very useful for cost-effectiveness evaluations assessing prevention approaches. Per-protocol efficacy of quadrivalent HPV vaccine for prevention of anal intraepithelial neoplasias 2/3 (anal precancer) in MSM was 74.9% (95% Confidence Interval 8.8, 95.4); Per-protocol efficacy for prevention of HPV 6-, 11-, 16-, 18-condyloma in men was 89.3% (95% Confidence Interval 65.3, 97.9).

**Conclusion:** There is a substantial burden of HPV-associated cancers and diseases in MSM and there are opportunities for prevention with HPV vaccine. Although some academic centers are screening MSM for anal cancers using an anal Pap test, more data is needed on the efficacy and cost-effectiveness of this approach.

# BAD BED-BUGS COMING OUR WAY? *NEISSERIA GONORRHEA* AND DRUG RESISTANCE IN ASIA

Marcel E. Curlin

[1] *Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA*
[2] *HIV/STD Research Program, Thailand MOPH – US CDC Collaboration (TUC), Nonthaburi, Thailand*

*Neisseria gonorrhea* has been the cause of sexually-transmitted infections in humans since antiquity. Several bacterial factors promote bacterial evasion from host defenses, adherence to the urogenital epithelium, and local tissue invasion. Untreated NG infection may result in serious health consequences including local genitourinary symptoms, infertility, sepsis, and disseminated infection with wide-ranging effects including articular damage and cardiac involvement. Modern attempts to treat and control the spread of NG infection have been challenged by successive waves of resistance to each newly recommended treatment regimen. While ceftriaxone has become the mainstay of treatment for multi-drug resistant NG, the recent appearance of extensively resistant strains that are resistant to third-generation cephalosporins has raised fears of "untreatable NG superstrains" that are unresponsive to all standard antibacterial agents. Efforts to address untreatable NG infection must include expansion of global NG surveillance programs, routine screening of high-risk populations, imple mentation of point-of-care resistance testing, promotion of best clinical practices to prevent induction of resistance to currently active agents, new drug innovation, and novel approaches to elicit protective immunity to *Neisseria gonorrhea.*

# HIV AND SYPHILIS INFECTION AMONG MSM IN THAILAND

Anupong Chitwarakorn M.D.

Although Thailand has been successfully controlled the HIV infection among heterosexual transmission since late 1990s, the epidemic of HIV still continued to spread among MSM and injecting drug users. In 2003, a survey found that HIV prevalence among MSM was 17.3% and had risen to 28.3% in 2005. Recent surveillance showed that HIV prevalence among MSM was around 30%. A clinic (Silom Community Clinic, SCC, now becomes SCC@Tropmed) for MSM has been established by Thailand Ministry of Public Health and US CDC Collaboration (TUC) since 2005 to provide VCT and STI screening and treatment. During 2005 to 2014, HIV prevalence among MSM visiting the clinic was 24-31% while prevalence of syphilis was increasing from 3-8% during 2005- 2009 to 11-16% (p value < 0.001) during 2009-2014. HIV and co-infection of Syphilis among MSM also has significantly increasing since 2010, from 1.6 to 5.3% during 2005- 2009 to 10.3 % in 2014 (p value < 0.001). The incidence of HIV among MSM during 2006-2014 was 2.7 -6.5%, p value 0.07, while the incidence of Syphilis was significantly increased from 0% in 2006 to 4.4 % in 2014 (p value < 0.001). Regarding to the outcome of treatment of syphilis, we analyzed data form 4,879 MSM visiting SCC during October 2010 – September 2014. We found that 776 had positive TP test and was diagnosed of having syphilis infection. Only 349 syphilis infection MSM was included in the analysis. The response to treatment was observed in 273/349 cases or 78.2%, 11/349 or 3.1% was possible reinfection. The failure to response to treatment was found in 65/349 cases or 18.6%. Among 65 cases of which showing failure to treatment, 41.5 had less than 4 folds decrease in RPR titer, 40% had > 4 folds increase in RPR titer, and 18.5% had stable high titer on follow up visit. The failure to treatment was not significantly observed with the stage of syphilis infection, treatment regimen (benzathine penicillin VS doxycycline, or HIV status.

The increasing HIV prevalence and re emerging of syphilis infection among MSM attending SCC has been observed and likely due to unprotected sex, multiple partners and unawareness. Effort to increase HIV and syphilis screening and early treatment, partner management, condom use promotion should be promoted through community involvement. Access to friendly services for HIV and STI for MSM should be implemented through existing STI services facility. More data on MSM is needed especially from other region to understand the epidemic of HIV/STI and sexual behavior among MSM in Thailand.

# ENDING AIDS IN THAILAND

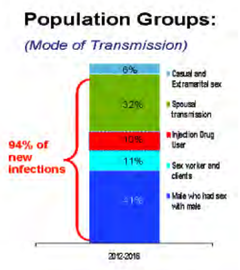
Sumet Ongwandee

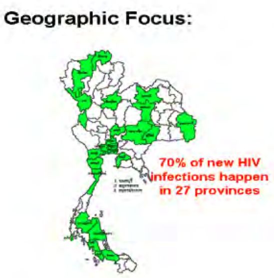
*Director of the Bureau of AIDS and STI (BATS), Department of Disease Control (DDC), Minister of Public Health, in Thailand*

Background: Thailand has committed to the targets of the 2011 High Level Meeting on AIDS and has a prioritized National Strategy which pledges a two-thirds reduction in incidence in sexual transmission, and through injecting drug use, by 2016.

The Thai epidemic is a very mature one; at its peak in the mid-1990's Thailand had around 160,000 new HIV infections per year. It's well known 100% condom programme addressing the client-sex worker relationship brought a dramatic change in incidence reduction. Thailand managed to reduce the incidence to less than 10,000 new HIV infections per year (2012) – this means more than 90% from its peak 20 years ago. In 2010, there was an estimated 500,000 people living with HIV in Thailand.

However, data consistently show high prevalence among injecting drug users (IDU over 20%), and MSM men who have sex with men (MSM - 8%-25%) during the last 5 years. Because of the large size of the MSM population, MSM will contribute around 40% of new infections in then next 5 years.





94% of new infections between 2010 and 2016 are estimated to come from MSM (41%), sex worker and clients (11%), injecting drug users (10%), and from members of key populations to their respective spouses (31%). Only 6% of new infections will occur through casual sex in the general population.

Moreover, Thailand's epidemic is also concentrated around "hot spots" in certain provinces and districts.

Thailand has virtually eliminated mother-to-child transmission of HIV, and currently has around 250,000 people on treatment.

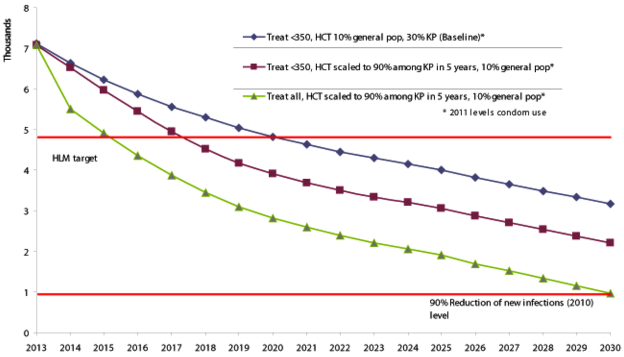
Current Response: The current response is characterized by peer-led behaviour change interventions for MSM, SW and IDU ("key populations"), and includes a range of prevention and care activities also for labour migrants, Young people, and the private sector. Despite this investment, behavioural variables (condom use and needle sharing behaviour) have remained stable over several years and uptake of HIV testing and counseling is limited. At the same time, treatment initiation with ARVs is late (median CD4 cell count at treatment initiation is reported to be 77 cubic mm). These factors together suggest that with the current response Thailand will not reach the High Level Meeting targets, and may fail to achieve its goal of reducing the number of new infections by two-thirds.

(*Continued*)

Strategic Options: Thailand has the potential to be one of the first countries in Asia to ‘End AIDS’ and reach the first milestone on the road to end AIDS by meeting the 2015 HLM targets.

The end of AIDS can become a reality if Thailand is able to effectively utilize the preventive effects of antiretroviral treatment. Thailand as has already shown this is possible through the virtual elimination of HIV infections from mother to child.

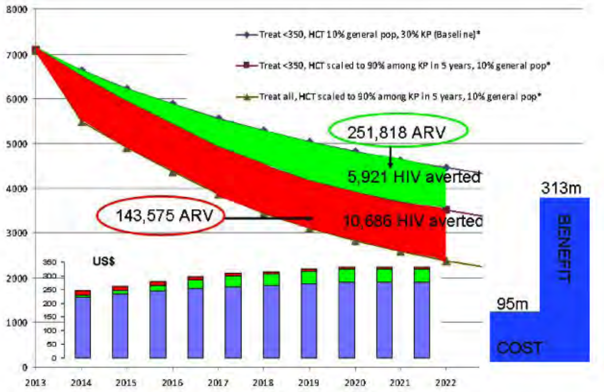
Sustaining current positive behaviour change among key populations, making use of alternative service delivery models for HIV testing, early treatment and adherence support, adequate monitoring, quality assurance, and positioning AIDS as a chronic and treatable medical condition, are critical components of Thailand’s investment to end AIDS.



This new model of combination prevention, which includes the strategic use of ARVs, needs to be focused on regions and ‘hot spots’ with the highest number of new infections. This will ensure better coverage and optimal use of resources.

### A re-focused response will avert 20,000 new HIV infections and 22,000 deaths by 2022.

Initial marginal cost-benefit analysis suggest that a modest investment of US$ 100 million over the next decade into HIV testing, early treatment (independent of CD4 cell count), and adherence support can translate **into potential benefits of more than US$ 300 million** in saved hospitalization and treatment costs, and productivity gains.



The yearly additional marginal costs of around 5% of present expenditures can be further reduced by focusing financial and human resources on globally accepted high impact interventions.

### Action now will pay off in the future

Every baht spent now can generate a return of 3 Baht. The critical action includes: scaling-up behaviour change interventions and HIV counseling and testing focusing on key populations, early treatment for people found to be HIV positive, community adherence support, and a new generation of data management and reporting.

# Wednesday 3rd December 2014

**Time:**

**15.30 – 17.00** **Convention**

## S28 Fish-Borne Trematodes

**Chairpersons:**

1. Aeron Jex

2. Paiboon Sithithaworn

Speakers:

1 Phototaxis of various wavelength of light on *Opisthorchis viverrini* cercariae

*Charinan Puifai*

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

2 Emerging parasitic infection of fish in irrigation management areas in Thailand

*Nuanpan Veeravechsukij*

Department of Biology, Faculty of Science, Silpakorn University, Thailand

3 Patterns of *Opisthorchis viverrini* infections in first intermediate host, *Bithynia siamensis goniomphalos* in endemic areas

*Monticha Chaiyasaeng*

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

4 The prevalence of liver fluke, *Opisthorchis viverrini* in the first intermediate host, Bithynia snails from Kampong Cham province, Cambodia

*Dusit Boonmekam*

Department of Biology, Faculty of Science, Silpakorn University, Thailand

5 Prevalence of *Haplorchis pumilio* cercaria in freashwater snails from Pasak Chonlasit reservior, Thailand

*Chatapat Chuanprasit*

Department of Biology, Faculty of Science, Silpakorn University, Thailand

6 Influence of salinity on trematode infections in *Bithynia siamensis goniomphalos* (Morelet, 1866), first intermediate host of the liver fluke, *Opisthorchis viverrini* in endemic areas in Northeast Thailand

*Apiporn Suwannatrai*

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# PHOTOTAXIS OF VARIOUS WAVELENGTH OF LIGHT ON *OPISTHORCHIS VIVERRINI* CERCARIAE

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The important stimuli (trigger cues) for cercariae to move toward direction to find out second intermediate hosts in freshwater habitats are light, gravity, temperature, turbulence, and host factors. Although responses to light and gravity are well known for cercariae of many digenetic species, but there are few experimental evidences on the effect of these environmental cues to *Opisthorchis viverrini* cercariae in the aspects of transmission to, and infection of, the second intermediate host, cyprinid fish. *Opisthorchis viverrini* cercariae present a pair of eye spots and active response to light. The eyespots consist of two cup-shaped pigment cells surrounding the parallel rhabdomeric microvilli of one or more retinular cells. The mitochondria of the retinular cells are packed in a mass near the rhabdomere. Because the rhabdomeres are the photoreceptors, the cup shape of the pigment cells allows the organism to distinguish light direction. To investigate the response of *O. viverrini* cercariae to various light wavelength in the visible spectrum, violet light (400 nm), indigo light (445 nm), blue light (475 nm), green light (510 nm), yellow light (570 nm), orange light (590 nm) and red light (650 nm), cercariae were tested those wave length stimuli. We used chamber that closed with black sheet paper and opened one side for light source. The chamber was trilled for 6 holes (hole number 0-5) on the paper for every 5 cm (0-30 cm.) and put cercariae into the chamber at the hole number 0. Then withdraw cercariae randomly by using auto-pipette 1 ml of water at every time point of 1 min then 5 min interval (5- 30 min). The most attractive wavelengths were orange and following by green. No light found the least attractive for *O. viverrini* cercariae.

Keywords: Phototaxis, *Opisthorchis viverrini* cercaria, wavelength

# EMERGING PARASITIC INFECTION OF FISH IN IRRIGATION MANAGEMENT AREAS IN THAILAND

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Irrigation has caused environmental changes and affected humans and their livelihood. There are increased parasitic infections in irrigated areas. Fish-borne trematode infections are important public health problems that have been reported in many Asian countries. The objective of the present study was to determine the status of trematode infections in various species of freshwater fish at Pasak Cholasid Reservoir in Thailand. The fish were collected from fish markets between June 2011 and May 2012. A total of 789 fish samples were classified into 25 species. Metacercariae, the larval stage of trematodes, were evaluated using the compression method. Ten of the collected fish species had trematode infections. These species were *Oxyeleotris marmoratus, Cyclocheilichthys enoplus, Henicorhynchus siamensis, Labiobarbus siamensis, Puntioplites proctozystron, Hypsibarbus wetmorei, Osteochilus hasselti, Barbodes schwanenfeldi, Barbodes altus,* and *Hampala dispar.* The infection rates were 20% (2/10), 49.53% (53/107), 23.36% (25/107), 77.78% (56/72), 53.97% (34/63), 85.29% (58/68), 16.28% (7/43), 88.71% (55/62), 100 % (14/14), and 66.67% (6/9), respectively. Polymerase chain reaction (PCR) of the internal transcribed spacer I and II was performed on the trematode species. The metacercariae were identified based on the size of the PCR products. From the morphology and molecular results, the trematodes were categorized into two species, *Haplorchis pumilio* and *Centrocestus formosanus*.

Keywords: Irrigation Management, Metacercariae, *Haplorchis pumilio, Centrocestus formosanus*.

Acknowledgments: This study was supported by the Office of the Higher Education Commission, Ministry of Education, and the Department of Biology, Faculty of Science, Silpakorn University, Thailand.

# PATTERNS OF *OPISTHORCHIS VIVERRINI* INFECTIONS IN FIRST INTERMEDIATE HOST, *BITHYNIA SIAMENSIS GONIOMPHALOS* IN ENDEMIC AREAS

Chaiyasaeng M.[1], Tesana S.[1], Suwannatrai A.[1]

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*Opisthorchis viverrini* is a human liver fluke causes a major public health problem in Greater Mekong subregion including Thailand, Lao PDR, Vietnam and Cambodia. *Bithynia siamensis goniomphalos* snail is the major first intermediate host in northeast Thailand and distribute throughout the region. The snails were collected in rice paddy fields in 2 localities in Khon Kaen Province during March 2013 to August 2014. The snails were identified to species based on the morphology of the shells. The infections of *O. viverrini* were examined by cercarial shedding method. Cercariae were identified morphologically and confirmed by using PCR specific primers. To investigate of snail infection, gender and size of snails were recorded. *O. viverrini* infection rates in *B. s. goniomphalos* were analyzed by Odds ratio between sex and size of snails. Total of 35,781 snails from 2 localities, only 0.54% (132/24,307) and 0.55% (63/11474) were found to be infected with *O. viverrini* cercariae in locality 1 and 2, respectively. Moreover, other trematode infection were found i.e. xiphidiocercaria, armatae, longifurcate, cystophorous, brevifurcate,amphistomes, monostome, mutabile, Unknown type 1 and Unknown type 2 cercariae. For snail populations were found that, female snails were higher infection rate than males (Odd ratio 1.52, 1.39; 95% CI 0.84-2.33). And adult snails over 8.0 mm in length the average of infection was higher than smaller snails (Odd ratio 4.73, 3.16; 95% CI 1.77-7.87). The result indicated that the infection rates of *O. viverrini* infected in *B. siamensis goniomphalos* were very low but it remained in round year.

Keyword: *Opisthorchis viverrini* cercaria, *Bithynia siamensis goniomphalos*, infection rate, Trematode, Odd ratios.

# THE PREVALENCE OF LIVER FLUKE, *OPISTHORCHIS VIVERRINI* IN THE FIRST INTERMEDIATE HOST, *BITHYNIA* SNAILS FROM KAMPONG CHAM PROVINCE, CAMBODIA

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Liver fluke, *Opisthorchis viverrini*, caused the major public health problem in Southeast Asia in particular around Mekong Basin including Cambodia. In rural area of Cambodian, most people did not have the toilet at home. So, *O. viverrini* had more chance to infect in *Bithynia* snails. Recently, fecal examinations of Cambodian people showed that the infection rates were very high especially in Kampong Cham province. The objective of this study was to investigate the prevalence of *O. viverrini* in *Bithynia* snail in Kampong Cham. During August 2012- December 2013, *Bithynia* snails were collected by count per unit of time from 33 localities around Kampong Cham province. First, the snail samples were identified by their shell morphologies. The shedding method was performed to obtain *O. viverrini* cercariae and stained with 0.5% neutral red. The cercarial morphology was also drawn under camera lucida. Ethanol preserved cercariae were extracted genomic DNA. Internal Transcribe Spacer 2 (ITS2) and NADH dehydrogenase I (NAD1) genes were amplified and sequenced. A total of 4,939 samples were identified into 2 species, *B. siamensis siamensis* and *B. siamensis goniomphalos*. The infection rates were 0.55% (17/3093) and 1.30% (24/1,846), respectively. The highest infection rate was 7.14%. Both morphology and molecular results revealed that all of cercariae samples were *O. viverrini*. The results of this study showed that the prevalence of *O. viverrni* in Bithynia snails was very high according to the previous report about stool examination.

Keyword: prevalence, *Opisthorchis viverrini*, *Bithynia* sp.

This work were supported by KAKENHI of Miyamoto, Dokkyo Medical University, Japan and Department of Biology, Faculty of Science, Silpakorn University, Thailand

# PREVALENCE OF *HAPLORCHIS PUMILIO* CERCARIA IN FREASHWATER SNAILS FROM PASAK CHONLASIT RESERVIOR, THAILAND.

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Pasak Cholasid reservoir was the largest dam in the central part of Thailand. It located in Lopburi and Saraburi Provinces. Since it was constructed, there was no any report about parasite infection in this dam. Our study focused on the diversity of freshwater snails and their infection rates of human fluke. During June 2011 to May 2012, the freshwater snails were collected by hand picking and scooping method based on counts per unit of time technique. Those snails were identified by only shell morphology. Shedding and crushing method were performed to study cercarial morphology. Later, internal transcribed spacer I and II genes were amplified by Polymerase Chain Reaction to diagnose types of trematode. Nine species of freshwater snails were found; *Pila ampullacea, Pomacea sp., Bithynia siamensis siamensis, Clea (Anentome) helena, Lymnea (Radix) rubiginosa, Melanoides tuberculata, Tarebia granifera, Filupaludina javanica, F. sumatrensis polygramma*. Both morphology and molecular method showed that only one type of human parasite was found in *M. tuberculata* and *B. siamensis siamensis*, *Haplorchis pumilio*. And their infection rate were 25.60% (62/250) and 0.14% (2/1,441) respectively.

Keywords: *Haplorchis pumilio*, Intermediate host, freashwater snails,PCR

This work was supported by the Office of the Higher Education Commission, Department of Biology, Faculty of Science, Silpakorn University, Thailand.

# INFLUENCE OF SALINITY ON TREMATODE INFECTIONS IN *BITHYNIA SIAMENSIS GONIOMPHALOS* (MORELET, 1866), FIRST INTERMEDIATE HOST OF THE LIVER FLUKE, *OPISTHORCHIS VIVERRINI* IN ENDEMIC AREAS IN NORTHEAST THAILAND

Apiporn Suwannatrai[a,*], Smarn Tesana[a], Kulwadee Suwannatrai[a], Kavin Thinkhamrop[b], Pairat Tarbsripair[c], Rasamee Suwanwerakamtorn[d], Thidarut Boonmars[a], Somsak Sukchan[e], John B. Malone[f], and Michael T. Kearney[f]

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A cross-sectional survey was conducted in northeast Thailand to determine the influence of salinity on *Opisthorchis viverrini* and other trematode infections in *Bithynia siamensis goniomphalos* snails from 95 water bodies located in areas representative of 6 classes of soil surface salt. Snails were randomly collected and water quality was measured. A total of 43,554 *B. s. goniomphalos* snails were found in 85 of 95 water bodies and were examined for trematode infection by a cercarial shedding method. Snails found to be negative (30-50%) were further examined by a crushing method to morphologically identify intramolluscan stages. Morphological identification was subsequently confirmed by a specific *O. viverrini* PCR primer. Snails from 70 of 85 water bodies were found to be infected with one or more of 12 types of cercariae (*O. viverrini,* mutabile, armatae, amphistome, monostome, xiphidio, virgulate, cystophorous, brevifurcate, longifurcate, unknown I, and unknown II cercariae). The prevalence of *O. viverrini* infection was found to range from 0.40-18.75% by both methods and the overall trematode infection was 3.53 %. Using the crushing method, unknown sporocysts and rediae were also found. The male: female sex ratio of snails infected with *O. viverrini* was 1: 1.75. The number of snails infected with *O. viverrini* significantly decreased with increasing salinity. The frequency of finding one or more of the 12 types of cercariae varied with different types of habitat and the prevalence of infection of each of the 12 trematodes was influenced, to a variable extent, by salinity levels in water bodies.

Keywords: *Bithynia siamensis goniomphalos*, *Opisthorchis viverrini*, cercariae, prevalence, trematode, northeast Thailand

# Wednesday 3rd December 2014

**Time:**
**15.30 – 17.00** **Room A**

## S29 Innovative discovery strategies for new chemotherapies to prevent relapse vivax malaria

**Chairperson:**


1. Thanat Chookajorn


2. Jeff Smith

Invited Speakers:

1 MMV portfolio for anti-hypnozoite and strategy to increase it
*Brice Campo*
Medicines for Malaria Ventures – MMV, Geneva Switzerland

2 The biology of hypnozoites
*Nicholas J. White*
Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand

3 Towards establishing an in vitro Pc liver-stage model for drug screening
*Pablo Bifani*
Novartis Institute for Tropical Diseases, Singapore

# MMV PORTFOLIO FOR ANTI-HYPNOZOITE AND STRATEGY TO INCREASE IT

Brice Campo, Medicines for Malaria Ventures – MMV, Geneva Switzerland.

Malaria remains a disease of devastating global impact, killing more than 600,000 people every year—the vast majority being children under the age of 5. *P. vivax* puts as many people at risk as *P. falciparum* and is more prevalent outside of sub-Saharan Africa. In 2007, the call for malaria eradicationwas made to researchers in the malaria community. To meet this objective, medicines that block the relapse of *P. vivax* and eliminate the asymptomatic and hepatic dormant forms (hypnozoites) of *P. vivax* need to be developed.Over the last decade there has been an increased investment in antimalarial research and development through the work of organizations such as Medicines for Malaria Venture (MMV), their partners, and others; new molecules with new modes of action are entering into preclinical development and beyond. Despite the relative abundance of projects targeting asexual stages very few drugs are directly targeting *P. vivax*hypnozoites.This is due to the lack of robust and reliable *in vitro*liver stage assays to, first, allow testingof large compound libraries (to find starting points – “hits”) and, second, to support medicinal chemistry (to optimize “hits” to candidate drugs). This presentation will review the current Global Malaria Portfolio focusing on drugs in development that target *P. vivax*liver stage and on the MMV strategy to increase the number of hypnozoiticidal drug candidates. As part of this,the strategy to deliver accessible *in vitro*decision making assays for *P. vivax*liver and blood stageswill be discussed.

# THE BIOLOGY OF HYPNOZOITES

NJ White

*Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand*

Relapse is a major cause of morbidity from vivax malaria. Frequent relapse, particularly in children, also contributes to mortality in areas of high transmission. Prevention of relapse requires treatment with 8-aminoquinoline antimalarials. The risks and the benefits of radical treatment will be reviewed.

# TOWARDS ESTABLISHING AN IN VITRO PC LIVER-STAGE MODEL FOR DRUG SCREENING

Pablo Bifani

*Novartis Institute of Tropical Diseases*

Malaria remains one of the most devastating infectious diseases of the 21st century responsible for over 600,000 death yearly and extensive morbidity. Existing and new anti-malarial drugs undergoing clinical trials have proven effective against *P. falciparum* but ineffective in eradicating a dormant form of malaria caused by *P. vivax* and *P. ovale*. These latter species are capable of developing a state of quiescence which is responsible for relapses which may occur weeks, months, or years following an infection. The asymptomatic dormant form of *P. vivax*, known as hypnozoites, can randomly reactivate and mature into liver-stage schizonts before being released into the blood stream leading to clinical symptoms and transmission.

This latent characteristic of *P. vivax* is also observed in *P. cynomolgi*, the simian malaria parasite equivalent, which has played a critical role in our current understanding of human malaria. Early investigation on *P. cynomolgi* has provided relapse models and facilitated the identification of the pre-erythrocytic liver stage phase. To date the *P. cynomolgi in vivo* model is currently the standard for identifying new chemical classes with potential radical curative activity. However due the complexity of the model and prohibitive nature of working with monkeys, an alternative yet predictive assay is needed.

In the present study, we discuss our efforts in establishing a liver-stage model to helps shed some light into the liver-stage biology of the parasite and potentially serve as a predictive assay for the identification of novel anti-hypnozoites drugs.

# Wednesday 3rd December 2014

**Time:**
**15.30 – 17.00** **Room B**

## S30 Highlight of bacterial infections and its epidemiology

**Chairpersons:**

1. Wirongrong Chierakul

2. Daniel Paris

Invited Speakers:

1 The global distribution and burden of melioidosis
*Direk Limmathurotsakul*
Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Thailand

2 Clinical and molecular epidemiology of *Staphylococcus argenteus* infections in Thailand
*Janjira Thaipadungpanit*
Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand

3 Characterization of *Staphylococcus aureusisolates* causing community-acquired sepsis in Northeast Thailand
*Narisara Chantratita*
Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University,Thailand

4 Tuberculosis in Papua New Guinea: A Public Health Emergency
*Suparat Phuanukoonnon*
Papua New Guinea Institute of Medical Research, and The Walter and Eliza Hall Institute of Medical Research, Australia

# THE GLOBAL DISTRIBUTION AND BURDEN OF MELIOIDOSIS

Direk Limmathurotsakul

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

# CLINICAL AND MOLECULAR EPIDEMIOLOGY OF *STAPHYLOCOCCUS ARGENTEUS* INFECTIONS IN THAILAND

Janjira Thaipadungpanit[1], Premjit Amornchai[1], Emma K Nickerson[2], Gumphol Wongsuvan[1], Vanaporn Wuthiekanun[1], Direk Limmathurotsakul[1,3], Sharon J Peacock[1,2,4,5]

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[5] *Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, United Kingdom*

*Staphylococcus argenteus* is a newly described species of Staphylococci. This was first recognised in indigenous communities in the Northern Territory of Australia, but has since been isolated from Cambodia, Indonesia, French Guinea, Fiji, Trinidad and Tobago. *S. argenteus* has not been reported from Thailand, but based on isolation elsewhere in southeast Asia we presumed that it was present. *S. argenteus* isolates characteristically lack staphyloxanthin, a carotenoid pigment responsible for the characteristic golden colony colour. Staphyloxanthin confers resistance against oxidative stress and neutrophil killing, leading to the hypothesis that *S. argenteus* may be less virulent.

We performed molecular evaluation of *S. aureus* isolated during a prospective study of 246 patients with invasive infection presenting to Sappasithiprasong Hospital, Northeast Thailand. Of these, 10 (4.1%) were *S. argenteus*, methicillin-susceptible, *mecA* negative and *pvl* negative. Eight patients had community-acquired infection but two were healthcare-associated, indicating that *S. argenteus* may cause nosocomial infection. Two patients infected with *S. argenteus* died, one as a direct result of the infection. In general, disease characteristics and outcome were not different between patients infected with *S. argenteus* and MSSA. However, the median age of patients infected with *S. argenteus* was higher than that of patients infected with MSSA, and patients infected with *S. argenteus* were more likely to have diabetes and renal disease than patients infected with MSSA. Contrary to the suggestion that this is less virulent compared with *S. aureus*, we demonstrated comparable morbidity, death and rates of healthcare-associated infection in patients infected with the two species.

Keywords: *Staphylococcus*, *argenteus*, *aureus*, Thailand

# CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUSISOLATES* CAUSING COMMUNITY-ACQUIRED SEPSIS IN NORTHEAST THAILAND

Narisara Chantratita

*Department of Microbiology and Immunology, and Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

*Staphylococcus aureus* is a leading cause of sepsis from bacterial infection. Few data on *S. aureus*infection from patients with community-acquired infection have been reported in Asia. The objective of this study was to characterizeinvasive *S. aureus* isolatescausing community-acquired sepsisand their association with clinical factors. Prospective study of *S. aureus* sepsis in four hospitals in northeast Thailand during March 2010-December 2013 was conducted. Isolates were analyzed using pulse-field gel electrophoresis (PFGE) and multilocus-sequence typing (MLST). The presence of virulence genes and *mecA* was determined by PCR. Antibiotic susceptibility was performed using disk diffusion test. The association between isolate characteristics and clinical data were analyzed by uni- and multivariate regression.313 isolates were obtained from 329 enrolled subjects. Thirty-eight sequence types (ST) were obtained including 7 new ST. The common ST wereST121 (35%) and ST2250 (18%). Phylogenetic analysis based on the MLST separated all isolates into twodistinct groups. One group,identified as *S. argenteus* isolates, was much less likely to cause organ failure than prototypical *S. aureus* infection. Genes coding for PVL (*luk*) and enterotoxin B (*seb*)were commonly detected (44% and 39%). Virulence genes *luk, sea, seb, sec,* and *see* were significantly less frequent in *S. argenteus* isolates. Seven isolates (2.2%) were methicillin-resistant but none were vancomycin- ortigecycline-resistant. Defined phylogenetic groups of community acquired *S. aureus* elaborating specific virulence factors. The association with baseline host factors and differential clinical presentations and outcomes will be discussed.

Keywords: *S. aureus*, *S. argenteus*, community-acquired infection, sepsis, outcome, Thailand

# TUBERCULOSIS IN PAPUA NEW GUINEA: A PUBLIC HEALTH EMERGENCY

Phuanukoonnon S, (on behalf of TB research group at PNGIMR)

*Papua New Guinea Institute of Medical Research, Goroka, Eastern Highlands Province, PNG*
*The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia*
*James Cook University, Townsville, Australia*

**Background:** Papua New Guinea (PNG) is a tuberculosis (TB) high burden country of the WHO Western Pacific Region, but so far only little data on TB epidemiology, drug resistance and genotyping is available. PNG Institute of Medical Research has conducted a number of TB epidemiology research since 2007-2013. The research aims to investigate the burden of TB in different locations, levels of drug resistance, genotyping patterns and health seeking behaviour. Here is the summary of the TB research in PNG.

**Methods:** The TB research project takes place in Madang, Gulf, Central provinces. The research methods were 1) Active case detection surveys, 2) Treatment cohort study, 3) qualitative study, and 4) Laboratory- culture, drug resistance and genotyping.

**Results:** High rates of TB were detected in all study locations with the highest TB incidence of 1290/100,000 pop in Kikori. A significant number of drug resistance TB infections are present (mono-resistance 10% and MDR 2.8%). HIV/TB co-infection was 1.9%. TB patterns (Pulmonary TB and EPTB) varied from locations to locations in PNG. Genotyping results also varied, lineage 4 (European-American lineage) is dominant in the Madang part, and lineage 2 (East-Asian lineage) or Beijing was a dominant type in the Gulf and central provinces. The qualitative study showed the reasons for delay health seeking and non-adherence to treatment.

**Conclusions:** PNG has the highest TB rate in the world and it is not driven by HIV. The high incidence and the presence of MDR warrant urgent attention to mitigate substantial morbidity, and strengthen the DOTS interventions.

**Keyword:** TB, Papua New Guinea, genotyping, active case detection

# Wednesday 3rd December 2014

**Time:**
**15.30 – 17.00** **Room C**

## S31 Parasitic diseases

**Chairpersons:**

1. Yanin Limpanont

2. Saovanee Leelayoova

Invited Speakers:

1 Evaluation of the immune response and protective efficacy of *Schistosoma mansoni* Cathepsin B in mice using CpG dinucleotides as adjuvant
*Momar Ndao*
Research Institute of the McGill University Health Centre, Canada

2 Evaluation of synthetic peptides for diagnosis of *Visceral leishmaniasis*
*Hanspeter Marti*
Swiss Tropical and Public Health Institute, Switzerland

3 Differentiation of two species of Leishmania infection in Thailand
*Saovanee Leelayoova*
Department of Parasitology, Phramongkutklao College of Medicine, Thailand

# EVALUATION OF THE IMMUNE RESPONSE AND PROTECTIVE EFFICACY OF SCHISTOSOMA MANSONI CATHEPSIN B IN MICE USING CpG DINUCLEOTIDES AS ADJUVANT

Dr. Momar Ndao

Schistosomiasis is the most important human helminth infection due to its impact on public health. Worldwide, schistosomiasis is estimated to infect at least 200 million individuals while 700 million are at risk. The clinical manifestations are chronic and significantly decrease an individual's quality of life. Infected individuals suffer from long-term organ pathologies including fibrosis which eventually leads to organ failure. The development of a vaccine against this parasitic disease would contribute to a long-lasting decrease in disease spectrum and transmission. Our group has chosen to target *Schistosoma mansoni* Cathepsin B as a prospective vaccine candidate. The recombinant protein was tested in the presence of synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides, which are Toll-like receptor 9 agonists known to stimulate a Th1 response. This formulation conferred a 59% decrease in worm burden as well as a reduction in egg burden. Hepatic egg burden and intestinal egg burden were decreased by 56% and 54% respectively. Immunizations with the formulation elicited robust production of Sm-Cathepsin B specific antibodies, both IgG1 and IgG2c but with the latter predominating. Furthermore, splenocytes isolated from the immunized animals, compared to control animals, had increased secretion levels of key Th1 cytokines, IFN-γ and TNF-α, as well as the chemokine CCL5 when stimulated with recombinant Sm-Cathepsin B. These results highlight the potential of Sm-Cathepsin B/CpG as a vaccine candidate against schistosomiasis.

Keyword: Schistosomiasis; vaccine; Cathepsin B; CpG dinucleotides; antigen-specific antibodies; cytokine production

# EVALUATION OF SYNTHETIC PEPTIDES FOR DIAGNOSIS OF VISCERAL LEISHMANIASIS

Beatrice Nickel, Claudia List, Fabienne Ahmarani, Pascal Mäser, Hanspeter Marti

*Swiss Tropical and Public Health Institute, Switzerland*

ELISAs for the diagnosis of visceral leishmaniasis mostly rely on crude antigen preparations, which are prone to batch to batch variation and nonspecific cross reactivity. In contrast, synthetic peptides can be synthesized in large amounts, high purity and highly reproducible quality. Recently, a recombinant protein, rK39 has found its way into routine diagnosis with good success. We evaluated several synthetic peptides derived from the kinesin-like protein K39 sequence of Leishmania chagasi for their suitability for serodiagnosis of visceral leishmaniasis.

K39 is expressed in different variants by the L. donovani complex. Several sequences from the C-terminal region were selected for synthesis of biotinylated peptides of 21 to 39 AA in length. Peptides were coated on streptavidin coated microtiter plates and evaluated with sera from patients with leishmaniasis, patients with other protozoan or helminth infections, and negative blood donors.

The sequence with the best sensitivity and specificity was the basis for creation of artificial peptide-8, consisting of three identical short repeats. This peptide was evaluated under routine conditions to determine its diagnostic performance.

The synthetic peptides show high reactivity with visceral leishmaniasis sera and reduced reactivity with cutaneous/mucocutaneous leishmaniasis sera. Peptide-8 shows a sensitivity of 96% (n=48) and a specificity of 99% (n=386) for visceral leishmaniasis.

Our findings suggest that synthetic peptides are very suitable antigens for sensitive and specific serological diagnosis of visceral leishmaniasis.

Keywords: Leishmaniasis, diagnostic peptides, serology

# DIFFERENTIATION OF TWO SPECIES OF LEISHMANIA INFECTION IN THAILAND

Saovanee Leelayoova[1], Suradej Siripattanapipong[1], Atitaya Hitakarun[2], Peerapan Tan-Ariya[2], Tawee Naaglor[1], Mathirut Mungthin[1]

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Autochthonous cutaneous and visceral leishmaniasis in Thailand was recently described as an emerging infectious disease. Using molecular and isoenzymatic techniques, parasite identification revealed two distinct species which were *Leishmania siamensis,* zymodeme; MON-324 (previously described as *L. siamensis*, lineage TR) and *Leishmania (Leishmania) martiniquensis,* zymodeme; MON-229 (previously described as *L. siamensis*, lineage PG). We determined the pattern of PCR-RFLP based on the ITS1 and *hsp*70 sequences which are well-suited for predicting the RFLP patterns of the two species, compared with those of *L. donovani*. Using only one restriction enzyme, *Hae*III, successful RFLP patterns of both ITS1 and *hsp*70 sequences were established.

Keywords: *Leishmania siamensis*, *Leishmania martiniquensis*, species differentiation, PCR-RFLP

# Wednesday 3rd December 2014

**Time:**
**15.30 – 17.00** **Room D**

## S32 Barcoding of pathogens, vectors and reservoirs

**Chairpersons:**

1. Jiraporn Ruangsittichai

2. Aurélie Binot

3.Serge Morand

Invited Speakers:

1 Barcoding procedures within a One Health approach
*Aurélie Binot* (Please see Abstract on P. 287)
Cirad and Faculty of Veterinary Medicine, Kasetsart University

2 Half decade on DNA barcoding researches of tropical arthropod vectors
*Jiraporn Ruangsittichai*
Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Thailand

3 The barcoding of parasitic helminthes
*Urusa Thaenkham*
Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand

4 The barcoding of parasitic protists: the example of Trypanosomatidae
*Marc Desquesnes,*
*Stéphane Herder,*
*Sathaporn Jittapalapong*

5 Barcoding of rodents and rodent-borne parasites: database and web tool
*Nicolas Clairon*, *Serge Morand*

6 Barcoding of pathogens, vectors and reservoir
*Claire Lajaunie*

# BARCODING OF PATHOGENS, VECTORS AND RESERVOIR

Aurélie Binot, Serge Morand, Jiraporn Ruangsittichai, Urusa Thaenkham, Marc Desquesnes, Stéphane Herder, Sathaporn Jittapalapong, Nicolas Claìron , Claire Lajaunie.

Assessing and managing the related risks of parasitic and infectious diseases, particularly at the human-animal interface, imply to take into account ecological and social environmental dynamics at play, in link with epidemiological patterns. In this framework, biological data referring to the emerging pathogens and their reservoir, vectors and hosts are key to better understand some patterns related to the emergence of zoonotic diseases. As these data need to be managed and shared within interdisciplinary and cross-sectoral collaboration processes, the utilization of molecular markers for barcoding appears nowadays to be necessary to fit quality standards in terms of biological data management and epidemiological assessment.

Barcoding procedures include 4 components: collection of voucher (or reference) specimens and/or biological materials and their management; molecular protocols (tools to get DNA barcode sequences or other molecular data); database (open library of geo-referenced traceable vouchers and their DNA barcode sequences); phylogenetic and molecular epidemiological analyses (identification of taxa and/or strains and their relationships). In the framework of most of the "One Health" research actions, barcoding tools and facilities are developed for pathogens' investigations (including viruses, bacteria, parasites, …), biodiversity monitoring or food safety. Barcoding appears to be a key step to improve data management procedures and methods, multidisciplinary databases set-up, data sharing and traceability.

This session aims at providing a general overview of barcoding procedures applied to vectors, reservoirs and pathogens in the field of One Health research. A debate about the possible implementation of bio banking procedures in the framework of cross-sectorial and interdisciplinary research collaborations will also be raised during the session, with a particular focus on linked ethical issues and web based barcoding identification procedures and virtual museum.

As an output of the session, we propose the launching of a "barcoding" group at the interface of medical/veterinary/wildlife health, in close collaboration with *ComAcross EuropeAid "One Health in Asia" project*".

# BARCODING OF PATHOGENS, VECTORS AND RESERVOIR

Claire Lajaunie

The ethical issues linked to research in infectious diseases are diverse and depend notably on: the source of material studied (animal, human), the storage of sample for future uses such as in the case of predictive medicine, or the activity of research itself. They can also widen to environmental ethics or information ethics (data sharing, distribution).

There is a constant interaction between ethics and law (Delmas-Marty, 2008), the law revealing sometimes ethical values.

There is a multi-scale approach of law and ethical norms going from international agreements (CBD, IHR) to the implementation of norms deriving from these agreements at the regional, national or local level.

Nevertheless we can observe differences regarding the integration of ethical and legal norms from human health to medicine conservation and from the global to the local level.

As the States are organizing regionally to address common issues such as the protection and control regarding infectious emerging diseases, the necessity to develop ethical standards regarding human and animal health as well as wildlife and to regulate research activities implying barcoding, biobanking and e-banking is arising.

An effective implementation of international law at the national level (CBD, CITES, CMS, IHR) could help to define the appropriate way, adapted to the local context, to regulate those activities.

# Thursday 4th December 2014

**Time:**
**9.00 – 10.30** **Convention**

## S33 Cercaria and snail hosts of fish-borne trematodes

**Chairperson:**

Urusa Thaenkham

Invited Speakers:

1 Human intestinal flukes Haplorchis taichui and Haplorchis pumilio in their intermediate hosts, freshwater snails of the families Thiaridae and Pachychilidae, in Thailand
*Duangduen Krailas*
Department of Biology, Faculty of Science, Silpakorn University, Thailand

2 Update biology and life cycle of *Opisthorchis viverrini*
*Smarn Tesana*
Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand

# HUMAN INTESTINAL FLUKES *HAPLORCHIS TAICHUI* AND *HAPLORCHIS PUMILIO* IN THEIR INTERMEDIATE HOSTS, FRESHWATER SNAILS OF THE FAMILIES THIARIDAE AND PACHYCHILIDAE, IN THAILAND

Duangduen Krailas[1*], Suluck Namchote[1], Wivitchuta Dechruksa[1] and Tunyarut Koonchornboon[2]

[1] *Department of Biology Faculty of Science, Silpakorn University, Nakhonpathom 73000, Thailand*
[2] *Department of Anatomy, Pramongkhutklao College of Medicine, Bangkok 10400, Thailand*
[*] *E-mail kduang @ su.ac.th, kduang @ gmail.com*

Intestinal flukes, *Haplorchis taichui* and *Haplorchis pumilio* are one of the most important food-borne parasitic zoonoses found in Southeast Asia. They were detected from freshwater snails Family Thiaridae and Pachychilidae in Thailand. The snails were collected by using handpicking and scoop methods every two months for one year for each location which started and ended at varying periods between December 2004 and September 2009 at 141 sampling sites from every region. Counts per unit of time method was used in this study, and the samples of snails were collected every 10 minutes per sampling by five collectors. The collected thiarid and pachychilid snails were examined for parasitic infections by shedding and crushing methods. The parasites were found at 66 locations. Six species of thiarid snails and two species of pachychilid snails were found *H. taichui* and *H. pumilio* infections. The infection rates of *H. taichui* and *H. pumilio* were 0.16% (136/82,816) and 0.74% (615/82,816), respectively. Five species of thiarid snails were found *H. taichui* infection; they were *M. tuberculata*, *M. jugicostis*, *T. scabra*, *S. riqueti* and *N. prasongi*. The infection rates were 0.11% (92/82,816), 0.002% (2/82,816), 0.001% (1/82,816), 0.01% (5/82,816) and 0.04 % (36/82,816), respectively. Four species of thiarid snails were found *H. pumilio* infection; they were *M. tuberculata*, *T. scabra*, *S. riqueti* and *T. granifera,* The infection rates were 0.44% (367/82816), 0.02% (18/82,816), *0.07%* (59/82,816) and 0.20% (169/82,816), respectively. And two species of pachychilid snails were found *H. pumilio* infection; they were *B. costula costula* and *B. citrine.* The infection rates were 0.001% (1/82816) and 0.001% (1/82816), respectively.

Keywords: Intestinal Fluke, Cercarial infection, Freshwater snail, Thailand.

This work was supported by the Research and Development Institute, Silpakorn University, Thailand.

# UPDATE BIOLOGY AND LIFE CYCLE OF *OPISTHORCHIS VIVERRINI*

Smarn Tesana

*Food-Borne Parasite Research Group, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen Province 40002, Thailand*

The liver fluke, *Opisthorchis viverrini* is high prevalence in Greater Mekong subregion and big health problem in Thailand especially northeastern region. Syrian golden hamster usually uses as experimental animal, the parasites rapidly develop for maturation within 14-21 days by determination of uterine eggs. Shape of ootype is a mold for egg characteristics. Eggs in proximal uterine tube were bigger then gradually decreased their size to distal end also larval development progressed to mature towards the distal uterus. The eggs accumulated in uterus afterwards they were laid in prepatent period of 24 days. The eggs which were ingested by snail host of *Bithynia siamensis goniomphalos* they hatched in snail digestive tract by help aids of leucine aminopeptidase. The infection in snail host was temperature dependent with the optimal temperature of 28-34°C. The development of intramolluskan stage in snail hosts was depended on environmental temperature with accumulated degree days to produce the cercaria of 893.81 and 951.36 degree days. Down regulation protein expression in infected snails was proteases and up regulation proteins were heat-shock proteins and actins. *B. siamensis goniomphalos* distribution and prevalence of opisthorchiasis correlated with soil-salinity. Cercariae were shed from snail by stimulation of light and light intensity of 1000 lux for 2 h gave the optimal shedding. Wavelength of orange light was the most attractive for *O. viverrini* cercariae. When cercariae penetrated cyprinid fish to be mature metacercariae required accumulated energy of 443.9-463.7 degree-days. Alcohol consumption enhances excystation and development of *O. viverrini* to become adult.

Keywords: *Opisthorchis viverrini*, *Bithynia siamensis goniomphalos*, life cycle, biology

# Thursday 4th December 2014

**Time:**
**9.00 – 10.30** **Room A**

## S34 Elimination of Malaria in Great Mekong Subregion Part I: Is elimination of malaria from Greater Mekong Region technically feasible by 2020?

**Chairpersons:**

1. Ivo Mueller

2. Wirichada Pan-Ngum

Invited Speakers:

1 Modelling malaria elimination in Great Mekong Subregion
*Lisa White*
Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand

2 Strategies for *P. vivax* elimination: Lessons from *P. vivax* transmission models
*Michael White*
Department of Infectious Disease Epidemiology, Imperial College London, United Kingdom

3 Modeling the impact of cross-border migration on malaria transmission and elimination strategies
*Guiyun Yan*
Program in Public Health, College of Health Sciences, California, USA

4 Malaria and population mobility: Towards a '3D' solution
*Maxine Whittaker*
Asia Pacific Malaria Elimination Network (APMEN)

# MODELLING MALARIA ELIMINATION IN GREAT MEKONG SUBREGION

Lisa White

*Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Thailand*

# STRATEGIES FOR *P. VIVAX* ELIMINATION: LESSONS FROM *P. VIVAX* TRANSMISSION MODELS

Michael White

*Department of Infectious Disease Epidemiology in Imperial College London*

*Plasmodium vivax* relapse infections occur following activation of latent liver-stages parasites (hypnozoites) causing new blood-stage infections weeks to months after the initial infection. We develop a within-host mathematical model of liver-stage hypnozoites, and validate it against data from tropical strains of *P. vivax*. The within-host model is embedded in a *P. vivax* transmission model to demonstrate the build-up of the hypnozoite reservoir following new infections and its depletion through hypnozoite activation and death. The hypnozoite reservoir is predicted to be over-dispersed with many individuals having few or no hypnozoites, and some having intensely infected livers. Individuals with more hypnozoites are predicted to experience more relapses and contribute more to onwards *P. vivax* transmission. Incorporating hypnozoite killing drugs such as primaquine into first-line treatment regimens is predicted to cause substantial reductions in P. vivax transmission as individuals with the most hypnozoites are more likely to relapse and be targeted for treatment.

# MODELING THE IMPACT OF CROSS-BORDER MIGRATION ON MALARIA TRANSMISSION AND ELIMINATION STRATEGIES

Guiyun Yan

*Program in Public Health, College of Health Sciences*

In order to better understand vector-borne disease dynamics in countries or regions with contrasting difference in transmission intensity, a critical evaluation of the contribution of human movement to the perpetuation of the disease is warranted. This information is critical to developing effective malaria elimination strategies. This presentation will define the migration patterns from Myanmar to its neighboring countries (Thailand and China), model the malaria risk and elimination strategies associated with malaria re-introduction due to human movement. The preliminary results from the Thai resident surveys indicate that there was a significant amount of cross-border travel into Myanmar in this region of Thailand. On average, our participants traveled across the border 1.6 times per week in the course of one year. Most were employed in the agricultural sector or as casual laborers, suggesting a propensity for seasonally defined border crossings. The EMOD simulations found that cross-border movement have an impact on malaria intervention efficacy.

# MALARIA AND POPULATION MOBILITY: TOWARDS A '3D' SOLUTION

Prof. Dr. Maxine Whittaker and Dr. Catherine Smith

*Asia Pacific Malaria Elimination Network (APMEN)*

In recent years many countries have become concerned about the challenges that population mobility poses to national malaria elimination programs and to the prospects for achieving regional malaria elimination. Population mobility is a '3D problem' that requires 3D solutions. The Asia Pacific Malaria Elimination Network (APMEN) is committed to supporting countries to achieve a malaria free Asia Pacific, including by helping countries to identify innovative solutions to complex regional challenges. APMEN recently commissioned a review to help countries to identify possible ways forward in addressing the challenges of population mobility. This review found that a '3D approach' to addressing cross-border malaria transmission involves four key elements: 1. Approaching mobility as a system involving multiple stakeholders, rather than a '1D' focus on mobile populations alone; 2. Implementing interventions at strategic locations in mobility systems; 3. Strengthening community engagement to reach mobile populations and border communities and to work toward community ownership of the elimination agenda; and 4. Ensuring that the health architecture enables the cross-border and cross-sectoral collaboration that is needed to address complex issues that extend across borders.

# Thursday 4th December 2014

**Time:**
**9.00 – 10.30** **Room B**

## S35 Vector Biology and Control

**Chairpersons:**

1. Narumon Komalamisra
2. Rutcharin Potiwat

Speakers:

1 Avoid the bite through the study of the sensory perception in mosquitoes
*Takao Nakagawa*
Kao Corporation, Kansei Science Research Lab, Kao Consumer Products, Thailand

2 Field Studies of *Aedes aegypti* Control in Thailand
*Alongkot Ponlawat*
Department of Entomology, Armed Forces Research Institute of Medical Sciences (AFRIMS), Thailand

3 Effect of attractant variations and its storage time on eggs number and percentage of hatched eggs using local ovitrap
*Lilik Zuhriyah*
Department of Public Health, Faculty of Medicine, University of Brawijaya, Indonesia

4 Malaria: vectors, repellents and the hindrance for vector control
*Theeraphap Chareonviriyaphap*
Faculty of Agriculture, Kasetsart University, Thailand

# AVOID THE BITE THROUGH THE STUDY OF THE SENSORY PERCEPTION IN MOSQUITOES

Takao Nakagawa

*(Kao Corporation, Kansei Science Research Lab)*

Host-seeking and blood-feeding are critical behaviors for the transmission of insect-borne diseases in humans. Female mosquitoes locate their host animals to get sufficient blood meal using their sensory system. Multiple sensory cues emanating from host animals: CO2, body odors, heat, are received by the specific receptors expressed in sensory neurons in the antenna and maxillary palps, resulting in the host-seeking and blood-feeding behaviors. Therefore, it could be possible to prevent human from being bitten through the control of the sensory perception in mosquitoes. For studying the sensory perception in insects, an electrophysiological recording has been useful tool to see chemical-evoked neural activation directly from live animal. In this talk, I will introduce the screening method using SSR(single sensillum recording) for a development of novel insect repellents.

# FIELD STUDIES OF *AEDES AEGYPTI* CONTROL IN THAILAND

Alongkot Ponlawat

*Vector Biology and Control section, Department of Entomology, AFRIMS*

Dengue fever is the most medically important arthropod-borne viral disease of humans, which infects millions of people annually worldwide. Thailand suffers from one of the highest rates of dengue in the world since the first outbreak in 1958.Dengue vaccines have been under development since 1940s. However, there is no vaccine to protect against dengue available yet. The only effective way to control these important diseases is the well-organized vector control. In the current study, we evaluated the impact of spatial repellents and different types of attractants for their ability to reduce *Ae. aegypti*populations under the field conditions. The overall aim is to develop an integrated push-pull strategy that effectively targets and controls mosquitoes. We also determined the efficacy of the pyriproxyfen/spinosad treated device on *Ae. aegypti*egg deposition and population density under semi-field conditions. This study demonstrated that our prototype device treated with pyriproxyfen and spinosad is a promising tool for *Ae. aegypti*control.

# EFFECT OF ATTRACTANT VARIATIONS AND ITS STORAGE TIME ON EGGS NUMBER AND PERCENTAGE OF HATCHED EGGS USING LOCAL OVITRAP

Lilik Zuhriyah[1], Sudjari[2], Aswin Djoko Baskoro[2]

*Department of Public Health 2. Department of Parasitology*

Improvement of local ovitrap to be more effectice and efficien was needed. This study was to compare the eggs number and the percentage of hatched eggs according to attractants variation and storage time variation using local ovitrap (Kepanjen). Four attractants were rice hay infusion, weed grass hay infusion, well water, tap water. Four storage time variations of rice hay infusion were 0, 12, 34, 90 days. A number of 100 blood fed *Ae. aegypti* females mosquitoes were entered into 6 large cages. The eggs number were counted on days 3 and 6. Hatching eggs was done by using water from each of various attractants and of various storage time. Data analysis was performed by One Way ANOVA test, Kruskal Wallis test, and Tukey test. The eggs number of rice hay infusion was more than other attractants. Rice hay infusion differed significantly from tap water and well water. In contrast, the lowest percentage of hatched egg was from rice hay infusion and the differences was significant between rice hay infusion and weed grass hay infusion as well as rice hay infusion and well water. Storage time variations did not affect the eggs number and the percentage of hatched eggs. The local ovitrap can be more effective by using rice hay infusion regardless its storage time.

Keywords: local ovitrap, *Aedes aegypti*, eggs, attractants, storage time

# MALARIA: VECTORS, REPELLENTS AND THE HINDRANCE FOR VECTOR CONTROL

Theeraphap Chareonviriyaphap

Malaria remains prevalent along the undeveloped borders of eastern Myanmar, western Cambodia, western Laos and northern Malaysia. One of the principal methods of malaria abatement has been through various methods of vector control to reduce transmission risk. Among several techniques, adult control strategy using various insecticides has shown success and is commonly being used elsewhere. However, chemical control can not be successful without a clear understanding on the natural behavior of each sibling vector species. In addition, chemical actions (toxic or nontoxic properties) should be investigated in all possible arrays and need to clearly define prior to launching any insecticide into the disease control program. Nontoxic properties of insecticide can be categorized into two different forms including irritability and repellency. We believed that chemical actions should be part of any evaluation of an insecticide's full capacities and potential to abate disease transmission.

# Thursday 4th December 2014

**Time:**
**9.00 – 10.30** **Room C**

## S36 Global Health Security Agenda

**Chairperson:**

1. Teerasak Chuxnum

Invited Speakers: 1 Global health threats and IHR

*Pasakorn Akarasewi*

Department of Disease Control, Ministry of Public Health, Thailand

2 Addressing prevention, detection and response package for accelerating IHR 2005 in Thailand

*Soawapak Hinjoy*

Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand

3 Global Health Security

*Rungrueng Kitphati*

Bureau of General Communicable Diseases, Department of Disease Control, Ministry of Public Health,Thailand

# GLOBAL HEALTH THREATS AND IHR

Pasakorn Akarasewi,

*Department of Disease Control, MOPH, Thailand.*

Global Health threats, in 21st century, from emerging infectious diseases (Ebola, Avian-origins Influenza etc), extreme climate changes/diasters and food safety, are increasingly visible and became immediate health risks to every corners of the world. The Need for global cooperation/response for health protection and disease control has been inevitable. None of the nations are risks/threats-free. International Health Regulations 2005 (IHR) provides an innovation framework for international cooperation among member states, globally, to enhance health-related surveillance, detection and response in timely manners with legal binding status. All 8 core capacities and preparedness for 5 major health threats incliding Infectious diseases, Zoonosis, Food Safety, Chemical and Radiation are key components of IHR which serves as foundation for Global Health Securities. Biregional of the WHO/SEAR-WPRO adds Asia-Pacific Strategy as tool for preparedness to disaster-proned region. Mechanism of collaboration and response include exchange of informations, prompt investigation and report, network of laborarory and access to lab tests, and platform for all capacity buildings. Monitoring/assess the progress of IHR implementation are key to understand the level for responses and gaps. Countries can be benefit from IHR implementation as well as bordering countries, regional and global. Combine with other platform such as MBDS, ASEAN and regional cooperations, IHR could fit very well for health protection and health securities.

# ADDRESSING PREVENTION, DETECTION AND RESPONSE PACKAGE FOR ACCELERATING IHR 2005 IN THAILAND

Soawapak Hinjoy

*Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand*

The Global Health Security agenda (GHSA) is an international cooperation framework which discusses public health implications on security, and is to spur progress towards full implementation of the WHO International Health Regulations 2005 (IHR), the OIE Performance of Veterinary Services pathway and other relevant global health security frameworks. High-level and professional representatives from the Ministry of Public Health, Thailand participated in the GHSA Events in Helsinki, Indonesia and Washington D.C. There are essentially nine GHSA objectives which fall under three headings; prevent avoidable epidemics, detect threats early and respond rapidly and effectively. By building health and security communities, Department of Disease control is able to develop strategies through which coordinated action to prevent, detect and response to accelerate IHR 2005 in Thailand. In this fiscal year 2015, target and activities for prevention, detection and response are being implemented into five border provinces. There are six targets for prevention strategy including 1) prevention of drug-resistant 2) prevent emergence and spread of zoonotic diseases 3) promoting food safety 4) promoting chemical safety 5) preparedness for radiological and nuclear threats and 6) prevention of hospital-acquired infections. For detection strategy, there are three targets including 1) development laboratory network and shipment of samples 2) strengthening border security between the ports of entry and 3) improving situation awareness in disease surveillance. Response strategy includes two targets. The last target is fostering and expanding field epidemiologist that this target can address all capacities of prevention, detection and response. When the targets and activities are completed in the five border provinces, desired national impact is comprehensive action to enhance prevention and control activities to prevent the emergence and spread of health threats and expand this model to other regions of Thailand to enhance national health security.

Keywords: Global Health Security Agenda, Prevention, Detection, Respond, Strategies, IHR2005, Thailand

# GLOBAL HEALTH SECURITY

Rungrueng Kitphati

*Department of Disease Control, Ministry of Public Health*

As a community of nations, ASEAN has raised to the challenge of addressing collectively, with one vision: investing in sustainable prevention, control and eradication strategies at the animal level (at-source) to reduce the threat to humans and to the economic and social development of nations. Emerging diseases issues are important concerns to ASEAN as they have serious impact to our regional growth and development. The number of EDs is expected to increase, creating a constant threat especially in countries with weak animal health and public health services. Addressing serious cross-border health threats is one of the major health issues to be dealt with by ASEAN members. Experiences from previous years show that collaboration mechanisms and strong capacity development at all level are necessary to cover all types of serious cross-border health threats. In order to strengthening ASEAN's regional coordination capacity in controlling and eradicating emerging diseases and other disease threats, ASEAN should address the challenge of implementation of the International Health Regulation (2005), and emphasize on the importance of working with regional partners, understanding their strengths, mapping the existed resources and defining the responsibilities of each partner. To ensure a safer and a healthier for all ASEAN citizens, it is thus essential to strengthen capacities of public health services at all level including the capacity for Prevent, Detect, and Respond.

# Thursday 4th December 2014

**Time:**
**9.00 – 10.30** **Room D**

## S37 Turbo Talks

**Chairpersons:**


1. Karunee Kwanbunjan


2. Yaowapa Maneerat

Speakers:

1 Factors influence the shedding of Blastocystis cysts in an Irritable bowel syndrome (IBS) patient- An evidence based case study
*Nanthiney Devi Ragavan* *(Please see Abstract Poster No. 31 P. 236)*
Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia

2 Factors related to rodent contact indoors and outdoors in Bolikhamxay Province, Lao PDR
*Kanokwan Suwannarong* *(Please see Abstract Poster No. 60 P. 265)*
FHI360, Asia-Pacific Regional Office (APRO), Bangkok, Thailand

3 Persistence of *P. falciparum* diagnostic antigens after treatment with artemisinins: Association with parasite stage and mechanism of clearance
*Charles J Woodrow* *(Please see Abstract Poster No. 22 P. 227)*
Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Thailand

4 Focal screening and treatment at households around passively-detected malaria cases in Pailin Province, Cambodia: a feasible and effective tool to target asymptomatic infections?
*Ruth Ashton* *(Please see Abstract Poster No. 18 P. 223)*
Malaria Consortium, Thailand

5 Folate and vitamin b12 deficiencies in relation to cardiovascular disease in Thai elderly
*Chirawat Paratthakonkun* *(Please see Abstract Poster No. 57 P. 262)*
Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Thailand

Speakers:

6 Potential Pharmacokinetic Interactions of Primaquine and Blood Schizontocides in Healthy Thai Volunteers

*Borimas Hanboonkunupakarn* *(Please see Abstract Poster No. 27 P. 232)*

Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand

7 Improving reliability of malaria research through a comprehensive quality assurance programme

*Christiaan Lourens* *(Please see Abstract Poster No. 65 P. 270)*

Worldwide Antimalarial Resistance Network (WWARN), Faculty of Tropical Medicine, Mahidol University, Thailand

8 Metabolite extract of *Streptomyces hygroscopicus* subsp. hygroscopicus causes morphological damage, decrease of parasite degree, and decrease of DNA density of *Plasmodium falciparum* 3d7 in vitro

*Annisa Alkarimah* *(Please see Abstract Poster No. 25 P. 230)*

Faculty of Medicine, Universitas Brawijaya, Indonesia

9 One health short course: Pilot workshop

*Irwin Chavez* *(Please see Abstract Poster No. 58 P. 263)*

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

10 The use of respondent driven sampling methods to identify malaria prevention knowledge and behaviours by migrant and mobile populations in western Cambodia

*Sophal Uth* *(Please see Abstract Poster No. 66 P. 271)*

Malaria Consortium, Cambodia

11 Behavioural study to assess consumer preferences and barriers to use of long-lasting insecticidal nets in Myanmar

*Htwe Htwe Htet* *(Please see Abstract Poster No. 67 P. 272)*

Malaria Consortium, Myanmar

12 Malaria and migrants in Thailand: Treatment-seeking (and other) behaviours

*Jintana Chaiwan* *(Please see Abstract Poster No. 70 P. 275)*

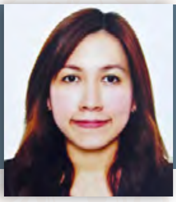
Malaria Consortium, Chaing Mai, Thailand

# Thursday 4th December 2014

**Time:**
**11.00 – 12.30** **Convention**

## S38 The situation of the enteric viral and bacterial infections in Thailand

**Chairpersons:**



1. Yuvadee Mahakunkijcharoen 2. Muthita Vanaporn

Invited Spaker:

1 Hand-foot-and-mouth disease in Thailand

*Yong Poovorawan*

Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Thailand

2 Simultaneous detection of enteric viruses associated with gastroenteritis in farm animals and humans by multiplex Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay

*James F. Kelley*

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

3 Update the situation of food and water borne bacterial infection in Thailand

*Nitaya Indrawattana*

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

# HAND-FOOT-AND-MOUTH DISEASE IN THAILAND

Yong Poovorawan, Jiratchaya Puenpa, Piyada Linsuwanon

*Center of Excellence in Clinical Virology, Department of Pediatrics,*
*Faculty of Medicine, Chulalongkorn University, Bangkok, 10330 Thailand*

Hand, foot and mouth disease (HFMD) is primarily caused by members of human enterovirus (HEV) group A. The disease has mostly been caused by enterovirus 71 (EV71) and coxsackievirus A16 (CA16) and coxsackievirus A6 (CAV6). During 2012, Thailand experienced a large scale outbreak of HFMD in several part of the country, resulting in more than 50,000 confirmed cases and 2 deaths. We found that the etiologic viruses were CAV6 (33.5%), followed by CAV16 (9.4%) and EV71 (8.8%) as the most frequent genotypes in HFMD, whereas herpangina was mainly caused by CAV8. Among those of children with influenza like illness without any skin and mouth lesions during HFMD outbreak, CAV6 was found only 1.5 percent. All CAV6 strains shared an isolated cluster and had high similarity in the phylogenetic analysis of VP1 region. Enterovirus infections were the most prevalent occurrence during the rainy season (June to September). The higher enterovirus infection associated with HFMD and herpangina occurred in infants between one and five-year-old. To gain insight into the role of population immunity against the major causative pathogen of HFMD, we also investigated seroprevalence of the neutralizing antibody (NAb) against EV71 genotype B5 among Thai population. Total of 161 sera samples was collected from healthy individuals among different age groups. The NAb titer was measured by using neutralization-ELISA assay. The cutoff value for data interpretation of detectable antibody was diluted titer as the following; Nab < 1:8 was scored as no immunity, 1:8 to 1:64 was low, 1:128 to 1:256 was medium, and ≥1:512 was high NAb titer. Overall, detectable NAb against EV71-B5 seropositive rate was 65.8% with 51.6 geometric mean titer. Of those, 20.5%, 41.6% and 3.7% had low, medium and high Nab titer against EV71-B5, respectively. The immunity level in relation with the age of children showed that the NAb seroprevalence was the highest in children between 5 and 12 years of age, followed by infants with the age less than 6 months, while detectable antibody was the lowest in children between 3 and 5 years of age. Passive maternal antibodies were responsible for increasing proportion of seropositive rate in the infant aged less than 6 months. Surveillance of epidemiology, immunity and monitoring of disease severity should be investigated, and as a prevention measure, sanitation and hygiene should be strictly concerned among Thai children and adults.

# SIMULTANEOUS DETECTION OF ENTERIC VIRUSES ASSOCIATED WITH GASTROENTERITIS IN FARM ANIMALS AND HUMANS BY MULTIPLEX REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR) ASSAY

Dr. Pornsawan Leaungwutiwong[1], Dr. Nathamon Kosoltanapiwat[1],
Dr. James F. Kelley[1,2,3], Mr. Narin Thippornchai[1], Ms. Kriszel Guiang[3],
Dr. Ruangrat Buddhirongawatr[4], Dr. Tanasak Changbunjong[4],
Mr. Thekhawet Weluwanarak[4], Ms. Poonyapat Sedwisai[4], Dr. Chalit Komalamisara[5]

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*[3] Fogarty Global Health Fellowship Program, NIH, USA;*
*[4] The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Thailand;*
*[5] Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand*

Simultaneous detection of enteric viruses is crucial to determine which virus is most prevalent in animals and humans in areas for gastroenteritis transmission. In this study, a multiplex reverse transcription polymerase chain reaction (RT-PCR) assay was used to simultaneously detect five different enteric viruses: norovirus GI, norovirus GII, enteric adenovirus, group A rotavirus and astrovirus. Stool specimen of pigs, chickens, and humans were collected in possible high gastroenteritis transmission areas from animals to humans in Thailand, Nakhon Pathom province. Of the 268 pig specimens screened, 14.5% were positive for enteric viruses, of which 89.8% were norovirus GII infection, 5.1% norovirus GI and GII coinfection and 5.1% enteric adenovirus or astrovirus. Of the 30 chicken specimens screened, 63.3% were positive for enteric viruses, of which 79% were norovirus GII infection and 21% norovirus GI and GII coinfection, Of the 25 human specimens screened, 24% were positive for enteric viruses, all of which were norovirus GII infection. In all specimens, norovirus GII was the primary enteric virus detected and a considerably large proportion of specimens had multiple viruses present. Both pigs and chickens appear to be principal reservoirs for enteric viruses in Nakhon Pathom province. The multiplex RT-PCR assay provides an efficient means to simultaneously detect gastroenteritis causing viruses, assisting to pinpoint high transmission areas and improve prevention and response to future outbreaks in Thailand.

# UPDATE THE SITUATION OF FOOD AND WATER BORNE BACTERIAL INFECTION IN THAILAND

Nitaya Indrawattana

*Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University*

Microbiological contamination of food and water is a major cause of deaths and illnesses due to diarrhea. In Thailand, food- and water borne diseases from bacteria are major public health problem for many years. The Bureau of Epidemiology, Ministry of Public Health, estimated nearly 1 million cases every year (in the period 2001–2005: 1,020,377, 1,055,393, 966,760, 1,161,877 and 1,142,581 respectively. The most important bacterial diseases transmitted through food- and water was caused by *Shigella* spp., *Salmonella* spp., *Escherichia coli* and *Vibrio cholera*. Food-related disease surveillance activities are also inconsistent. All countries of the South East Asian Region have implemented programs to collect illness and mortality data.

In this presentation, the situation of food- and water borne disease in Thailand are introduced and to be discussed for prevention and control of diarrheal diseases in the country.

Keyword: food- and water borne diseases, diarrheal diseases in Thailand, Shigella, Salmonella, Vibrio

# Thursday 4th December 2014

**Time:**
**11.00 – 12.30** **Room A**

## S39 Elimination of Malaria in Great Mekong Subregion Part II: Panel discussion: Are GMR countries ready for elimination?

**Moderators:**

1. Srivicha Krudsood

2. Jeff Smith

Invited Spaker:

1 Malaria elimination: Health systems readiness and surveillance as an intervention
*Marcel Tanner*
Swiss Tropical and Public Health Institute, Switzerland

2 The biology of hypnozoites
*Nicholas J. White* *(Please see Abstract on P. 288)*
MORU, Faculty of Tropical Medicine, Mahidol University

Panel Member:

1 *Jeerapat Sirichaisinthop*
Ministry of Public Health, Thailand
2 *Siv Sovannaroth*
Ministry of Health, Cambodia
3 *Myat Phone Yhaw*
Ministry of Health, Myanmar
4 *Arjen Dondorp*
MORU, Faculty of Tropical Medicine, Mahidol University

# MALARIA ELIMINATION: HEALTH SYSTEMS READINESS AND SURVEILLANCE AS AN INTERVENTION

Marcel Tanner

*Swiss Tropical and Public Health Institute, Switzerland*

# Thursday 4th December 2014

**Time:**
**11.00 – 12.30** **Room B**

## S40 Regional Diagnosis & Surveillance

**Chairperson:**

1. Jaranit Kaewkungwal

Speakers:

1 Utilizing industry assets and proactive partnering to stimulate neglected disease product development
*Ujwal Sheth (on behalf of* Katy M. Graef)
BIO Ventures for Global Health (BVGH), Seattle, USA

2 Pneumonia Diagnostics: the search for new tools for frontline health workers in resource poor settings
*Alexa Wharton-Smith*
Malaria Consortium, Cambodia

3 Existing situation of occupational and environmental disease surveillance system in community hospital of North-eastern Thailand
*Kesorn Thaewnongiew*
The office of Disease Prevention and Control, Khon Kaen, Thailand

# UTILIZING INDUSTRY ASSETS AND PROACTIVE PARTNERING TO STIMULATE NEGLECTED DISEASE PRODUCT DEVELOPMENT

Roopa Ramamoorthi[1], Katy M. Graef[1], Jennifer Dent[1]

*[1] BIO Ventures for Global Health (BVGH), 401 Terry Avenue North, Seattle, WA 98109, USA*

Neglected tropical diseases, malaria, and tuberculosis have a devastating effect on over 1.6 billion people worldwide. The World Intellectual Property Organization (WIPO) Re:Search consortium accelerates the development of new drugs, vaccines, and diagnostics for these diseases by connecting the resources of biopharmaceutical companies to academic researchers with novel product discovery or development ideas. Over 90 institutions are Members of WIPO Re:Search including 11 private industry, 36 academic, and 46 nonprofit or government research institutions across the globe. As the WIPO Re:Search Partnership Hub Administrator, BIO Ventures for Global Health (BVGH) proactively examines scientists' current neglected disease research and proposes novel collaboration opportunities with other Members. BVGH also fields requests from researchers, identifies Member organizations able to fulfill these requests, and helps forge mutually beneficial collaborations with clearly-defined responsibilities and expectations. BVGH has facilitated over 77 diverse research partnerships between Members including 33 compound/compound library requests, three agreements for assistance with computational chemistry or structure-activity relationship assays, and three projects to improve the formulation of promising compounds. Members have also agreed to share confidential data and expertise through 14 separate agreements. These partnerships have resulted in candidate products advancing along the development pipeline. Screens have identified compound hits for malaria, schistosomiasis, Chagas disease, soil-transmitted helminthiases, and Buruli ulcer. Several of these hits have advanced to dose response assays. Other partnerships are stimulating diagnostic development for diseases including dengue, tuberculosis, and malaria. This presentation will describe WIPO Re:Search's novel approach to product development, the collaborations BVGH has facilitated, and these collaborations' successes.

# PNEUMONIA DIAGNOSTICS: THE SEARCH FOR NEW TOOLS FOR FRONTLINE HEALTH WORKERS IN RESOURCE POOR SETTINGS

Alexa Wharton-Smith[1], Thany Thol[1], Chandary Rang[1], Chhuoy Meng[2], Kheng Sim[3], Po Ly[4], Siv Sovannaroth[4], Char Meng Chuor[4], Kevin Baker[5], Karin Kallander[5]

[1] *Malaria Consortium, Cambodia,*
[2] *Calmette Hospital, Cambodia,*
[3] *Ministry of Health, Cambodia,*
[4] *National Centre for Parasitology, Entomology and Malaria, Cambodia,*
[5] *Malaria Consortium, United Kingdom*

**Background:** Globally, pneumonia remains the leading cause of death in children under five. Diagnosis primarily involves respiratory rate assessment, which can be challenging with available tools used by frontline health workers in resource-poor settings. A large-scale evaluation project is being conducted to identify the most accurate, acceptable, scalable and user-friendly diagnostic tools for the respiratory rate measurement and oxygen saturation for diagnosis of pneumonia in children by first-level health facility workers and volunteer extended Village Malaria Workers (eVMW).

**Methods:** The study involves quantitative and qualitative methodologies. After a comprehensive landscape review, a panel of experts select twelve diagnostic tools to be analysed: from simple counting devices to sophisticated mobile phone applications and pulse oximeters. Qualitative stages of the study will document eVMW experiences of using the different devices, and perceptions of the parents of children assessed using the new tools. The quantitative elements measure the devices' accuracy and compare this to agreed gold standard measures. Data analysis will focus on measuring positive and negative percent agreement, as well as agreement in respiratory rate measurement and oxygen levels.

**Results:** The findings will highlight the most appropriate and accurate diagnostic tools for assessing children for uncomplicated and severe pneumonia in rural Cambodia, as well as document the perceptions of frontline health workers and caregivers/parents on the acceptability and usability of the devices.

**Discussion:** Through the identification of new diagnostic tools for pneumonia, the research findings will help to improve the quality of care for children with pneumonia in resource-poor settings. Furthermore, through improved diagnostics, the study will strengthen the rational use of antibiotics by providers.

**Keyword:** Pneumonia, child health, diagnostics, community case management

# EXISTING SITUATION OF OCCUPATIONAL AND ENVIRONMENTAL DISEASE SURVEILLANCE SYSTEM IN COMMUNITY HOSPITAL OF NORTH-EASTERN THAILAND

Kesorn Thaewnongiew PhD[1], Kesarawan Nilvarangkul PhD[2], Araya Junkung B.Sc[1], Jintawat Boonkapim M.Sc.[3], Kitiphitch Junte M.Sc[1]

[1] *The office of Disease Prevention and Control, Khon Kaen*
[2] *Research and Training Center for Enhancing Quality of Life of Working- Age People, Faculty of nursing, Khon Kaen University*
[3] *Roiet Provincial Health Office*

This study was descriptive, assessed the existing occupational and environmental disease surveillance system in the community hospitals of three provinces in Thailand. The subjects composed of: the first source was the patients who received medical records and were claimed by doctors or health officers that they got surveillance diseases. The second source was the data providers who report data to surveillance system, 60 subjects were selected by purposive sampling method. Quantitative data were collected from medical records, and qualitative data were collected from focus groups and in-depth interviews.

The results revealed that percentage of suspected cases reported was only 50.8%. Sensitivity was low for common diseases because the doctors did not report the diseases. Data quality for the diagnosis and signs and symptom was also low. Reporting was considered timely in only 50% of the cases. The health officers perceived that the system is not important, and they collected the data for the province without analysis. The information provided by the system was not the representative of the true epidemiological situation, and it could not be used to help monitor disease patterns or trends. The officers involved in the system lacked knowledge, and they perceived that the system created a high workload. This study indicated low levels of sensitivity and representativeness can occur in disease surveillance data and highlights the essential need to encourage accuracy in the reporting of occupational and environmental diseases in public hospitals.

Key word: Occupational and Environmental Disease, Surveillance System, Community Hospital

# Thursday 4th December 2014

**Time:**
**11.00 – 12.30** **Room C**

## S41 The Royal Project of Parasite Control Program in Thailand

**Chairperson:**

1. Yaowalark Sukthana

Invited Speakers:

1 The Royal Project of Parasite Control in Thailand 2014

*Thitima Wongsaroj* *(Please see Abstract on P. 284-285)*

Department of Disease Control, Ministry of Public Health, Thailand

2

*Suwich Thammapalo*

The Office of Disease Prevention and Control 12 Songkhla

3.

*Piya Sirilak*

Nan Provincial Public Health Office

4. The Seven Given Models : The prevention and control of Soil Transmitted Helminthes Infections among Primary School Students at PHU FA Development Area, Nan Province, Thailand

*Thanusin Saleeon* *(Please see Abstract on P. 286)*

Nan Provincial Public Health Office

# Thursday 4th December 2014

**Time:**

**11.00 – 12.30** **Room D**

## S42 Ebola Virus Infection & Other Hemorrhagic Fever Viruses

**Chairperson:**

Pornsawan Luengwutiwong

Invited Speakers:

1 Ebola outbreak in West Africa 2014 – epidemiologic response

*Michael Martin*

HIV Treatment and Care, Thailand MOPH - US CDC Collaboration, GAP Thailand/ Asia Regional Program, Ministry of Public Health, Thailand

2 Description of the new biosafety level 4 (BSL-4) laboratory and handling of viral haemorrhagic fever viruses at Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

*Petra Emmerich*

Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

3 Detection and Management of Ebola virus in Thailand

*Somchai Sangkitporn*

Department of Medical Sciences, Ministry of Public Health, Thailand

# EBOLA OUTBREAK IN WEST AFRICA 2014 – EPIDEMIOLOGIC RESPONSE

Michael Martin, MD, MPH

*CAPT USPHS | Senior Technical Advisor, HIV Treatment and Care | Thailand MOPH - US CDC Collaboration, GAP Thailand/Asia Regional Program | DDC 7 Building, 4th floor | Ministry of Public Health*

Ebola virus,a memberof the Filoviridae family, was first recognized in 1976 when two unrelated outbreaks occurred: one in northern Zaire and another in southern Sudan. Ebola outbreakstypically begin with introduction ofthe virus into humans from a wild animal reservoir,with subsequent person-to-person transmission. The infection is characterized by fever, headache, nausea, vomiting, and abdominal pain; signs and symptoms common to other viral and bacterial infections and malaria. In March 2014, the World Health Organization(WHO) was notified of a rapidlyevolving Ebola outbreak in Guéckédou, Guinea.The outbreak spread to Liberia and Sierra Leone and has evolved into the largest outbreak of Ebola ever reported with over 9000 cases and 4500 deaths. In this presentation, the speaker will briefly review the virology and epidemiology of Ebola virus and describe his activities in Guinea as part of the U.S. Centers for Disease Control and Prevention Epidemiologic Response Team.

# DESCRIPTION OF THE NEW BIOSAFETY LEVEL 4 (BSL-4) LABORATORY AND HANDLING OF VIRAL HAEMORRHAGIC FEVER VIRUSES AT BERNHARD NOCHT INSTITUTE FOR TROPICAL MEDICINE, HAMBURG, GERMANY

Petra Emmerich, Stephan Günther

The high-security BSL-4 laboratory is designed as a suit laboratory. A nominal negative pressure of 130 Pa. is maintained in the laboratory interior. Intake and exhaust air is fed through class H14 high-performance airborne particulate filters. The BSL-4 containment envelope consists of two independent BSL-4 laboratories (main and supplementary laboratory) manufactured of stainless steel plate. No air can escape to the outside. The BSL-4 containment envelope including all of its associated systems has been approved by the authorities responsible (fire brigade, police, etc.). It guarantees the highest possible safety and security standards. A maximum of five persons are permitted to work simultaneously in the laboratory under regular operation. Granting of access authorisation to the BSL-4 Laboratory requires prior structured training (personal protective equipment, entering and exiting airlocks, conduct in the laboratory, pass-through airlock, autoclaving as required, emergencies, evacuation training). Work in the laboratory must be monitored by two external supervisors outside the laboratory in order to assure evacuation in case of danger. Work with infectious material must take place in class II biological safety cabinets.

Keywords: BSL-4 laboratory, training, practiceandevacuationtraining, VHF-Viruses.

# DETECTION AND MANAGEMENT OF EBOLA VIRUS IN THAILAND

Somchai Sangkitporn

*Department of Medical Sciences, Ministry of Public Health, Thailand*

# Thursday 4th December 2014

**Time:**

**13.30 – 15.00** **Convention**

## S43 Toward the national policy on "Elimination of the liver fluke to reduce cholangiocarcinoma in Northeast Thailand" is it achievable?

**Chairperson:**

1. Paiboon Sithithaworn

Invited Speakers:

1 Current epidemiology of *Opisthrorcist viverrinin* and current national policy for prevention and control in Thailand

*Sasithorn Tangsawad*
Office of Disease Prevention and Control, region 6 Khon Kaen, Thailand

2 A roadmap for control program is needed to eliminate the liver fluke and to reduce cholangiocarcinoma

*Paiboon Sithithaworn*
Department of Parasitology and Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Thailand

3 Update on carcinogenesis and biomarker recovery for periductal fibrosis and cholangiocarcinoma

*Puangrat Yongvanit*
Department of Biochemistry and Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Thailand

4 Infection with carcinogenic liver fluke *Opisthorchis viverrini* modifies biliary and intestinal microbiota

*Paul J Brindley*
Department of Microbiology, Immunology and Tropical Medicine, and Research Center for Neglected Tropical Diseases of Poverty, School of Medicine & Health Sciences, The George Washington University, USA

5 Cholangiocarcinoma Screening and Care Program (CASCAP) : health innovation for Esarn people

*Narong Khuntikeo*
Department of Surgery and Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Thailand

# CURRENT EPIDEMIOLOGY *OFOPISTHRORCIST VIVERRININ* AND CURRENT NATIONAL POLICY FOR PREVENTION AND CONTROL IN THAILAND

Dr. Sasithorn Tangsawad

*Office of Disease Prevention and Control, region 6 Khon Kaen, Thailand*

Ministry of Public Health ,Thailandtried to push the agenda of*Ophisthorchisviverrini*in to the office of the national economic and social development board since 1982 until then the current plan (the 11thnational economic and social development board) tries to decreasethe prevalence of *Ophisthorchisviverrini*and hookworm. Report from prevalence surveydiscovered that hookwormprevalence in the year 1957, 1981, 1991, 1996, 2009 and 2014 had decrease from 62.9 to 54.7, 41.7, 35.0, 22.5, 18.1 and 8.9respectively.But the total prevalence of *Ophisthorchisviverrini* in Thailand decrease slowly caused by the prevention and promotion of policy is not sustained so as local health centers.

According to the 8thhealth service region in 2013, *Ophisthorchisviverrini*prevalence in those who older than 15 year was survey. The 3,916 members of sample group live in 7 provinces which areUdonThani, Loei, NongbuaLamphu, Nongkai, Bungkan, Sakolnakonn and Nakonphanom. The group was recruited by 30 Clusters sampling technique. The total average Liver Fluke prevalence was 22.09 percent. The province which had highest prevalence by 40.90 percent was Nakonphanom. The ratio of female and male was 1:1.25. The group whose age between 40- 49 had highest prevalence by 49.65 percent. Every group had prevalence higher than 20 percent, and 4 out of 7 provinces had prevalence higher than 20 percent. This is similar to the 7thhealth serviceregion which 4 provinces investigated. Those were Roi-et, Kalasin, Mahasarakarm and KhonKaen. The sample group which were recruited by Clusters sampling technique were 2,336. The total Liver Fluke prevalence was 16.09percent . The province which had highest prevalence by 24.7 percent was Kalasin. The ratio of female and male was 1:125. The oldest group whose age at 50 -59 had prevalence at 27.77 percent. All 4 provinces had prevalence higher than 10 percent. When explored people behaviors, it revealed that 57.3 percent consume papaya salad with fermented fish. 10.8 percent consumeplasom, and 10.9 percent for raw fish. The reason were enjoying of taste and not being afraid of Liver Fluke. Those who infested with Liver Fluke may have 16 times risk chance to get cholangiocarcinoma and death in the next 20 – 30 years, and there are 20,000 of them per year. Therefore, their consume behaviors should be changed. They need a new belief for not eating raw fish which get the risk of Liver Fluke infestation. That will take time and service effort and the aspect of culteral and tradition are also need. So the national policyshould includeand support the curriculum in school for understanding along with the reducing risk of consume behaviors.

Key words: Epidemiology, *OpisthrocistViverrini*, National policy for prevention and control

# A ROADMAP FOR CONTROL PROGRAM IS NEEDED TO ELIMINATE THE LIVER FLUKE AND TO REDUCE CHOLANGIOCARCINOMA

Paiboon Sithithaworn

*Department of Parasitology, Faculty of Medicine, KhonKaen University, KhonKaen, 40002, Thailand; Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, KhonKaen University, KhonKaen, 40002, Thailand*

The national agenda for elimination of the liver fluke to reduce cholangiocarcinoma was launched by the Ministry of Public Health in 2012. The aim is to tackle the problem of high mortality of liver cancer in northeast Thailand. This policy has created series of strategic plans with emphasis on early detection, management and care of CCA. However, a detail road map is lacking and needed to be defined particularly those related to elimination of the liver fluke. A typical road map such as those recommended by World Health Organization (WHO) for elimination of filariasis composed of several modules mainly identification of target areas, eligibility, survey method, diagnostic tests and verification of elimination. The plan of actions toward liver fluke elimination should be considered and implemented soon if a long term outcome in reducing CCA is to be achieved in the next 20-30 years. An example will be illustrated regarding impact of diagnostic test on number of eligible people for treatment. When applying copro and urinary antigen detection for diagnosis of the liver fluke, 21-41% of positive were found in egg-negative group. Performances of antigen detections for diagnosis as well as evaluation of curative treatment will be discussed as one of the modules in the elimination program of the liver fluke.

# UPDATE ON CARCINOGENESIS AND BIOMARKER RECOVERY FOR PERIDUCTAL FIBROSIS AND CHOLANGIOCARCINOMA

Puangrat Yongvanit[1,3], Somchai Pinlaor[2,3], Watcharin Loilome[1,3], Paiboon Sithithaworn[2,3]

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*[2] Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand;*
*[3] Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand*

Human liver fluke, *Opisthorchis viverrini*, is the major risk factor of cholangiocarcinoma (CCA) in Northeastern Thailand. Our approach focuses on genetic progression and molecular changes in the carcinogenic pathway of liver fluke-associated CCA aimed at assessing patients at risk of CCA and using chemoprevention as the secondary cancer prevention to reduce the incidence of CCA. We summarizes altered gene expressions, biomolecules and their modification, i.e. DNA adducts, oxidized proteins, oxysterols and fibrotic markers in hamster- and human-CCA. Potential risk biomarker(s) and chemopreventive agent(s) criteria and selection were based on results from experimental and epidemiological studies identifying hepatobiliary disease, including CCA. Significant results reveal that oxidative stress induced by *O. viverrini* infection leads to biomolecule damage, tissue remodeling especially periductal fibrosis and alteration of gene expressions which could be involved in all steps of CCA carcinogenesis. Some of these molecules (i.e. oxidized alpha 1-antitrypsin) are reported to change their levels in opisthorchiasis, periductal fibrosis diagnosed by ultrasonography and CCA. These multiple risk biomarkers could now be explored for screening including chemopreventive intervention of subjects living in endemic areas where the prevalence of opisthorchiasis remains high.

# INFECTION WITH CARCINOGENIC LIVER FLUKE OPISTHORCHIS VIVERRINI MODIFIES BILIARY AND INTESTINAL MICROBIOTA

Paul Brindley

*Department of Microbiology, Immunology and Tropical Medicine, and Research Center for Neglected Tropical Diseases of Poverty, School of Medicine & Health Sciences, The George Washington University, USA*

Infection with Opisthorchis viverrini is classified by the WHO's International Agency for Research on Cancer as a Group 1 carcinogen, a biological agent that definitively causes cancer. Chronic opisthorchiasis frequently leads to cholangiocarcinoma (CCA), bile duct cancer. The mechanism(s) by which O. viverrini infection culminates in cancer of the biliary system remains unknown, although it is clear that liver fluke infection drives a smoldering and chronic inflammatory response. A little explored aspect is its influence on the host microbiome. Genomic DNAs of microbiota from colorectal contents and bile of hamsters and O. viverrini were examined in a hamster model of fluke-induced CCA. Microbial communities were characterized by high-throughput sequencing of variable regions 7, 8 and 9 of prokaryotic 16S ribosomal DNA. Of ~1,000,000 sequences, 536,009 with useable reads could be assigned to 29,776 operational taxonomy units (OTUs) and, in turn, to 20 phyla and 273 genera of bacteria or archaea. Microbial community analyses revealed that fluke infection perturbed the gastrointestinal tract microbiome, increasing Lachnospiraceae, Ruminococcaceae and Lactobacillaceae while decreasing Porphyromonadaceae, Erysipelotrichaceae and Eubacteriaceae ($p \leq 0.05$). > 60 OTUs were detected in the biliary system, which confirmed bacteriobilia and a noteworthy community of microbes associated with the parasites. The fluke-associated microorganisms included potential pathogens from the Enterobacteriaceae and Listeriaceae and others including cyanobacteria and Deinococci usually found in external environments. Similar changes and phenomena may take place in human populations in regions endemic for opisthorchiasis, and drive the distinctive inflammatory response of chronic opisthorchiasis, including the prominently elevated interleukin 6.

# CHOLANGIOCARCINOMA SCREENING AND CARE PROGRAM (CASCAP) : HEALTH INNOVATION FOR ESARN PEOPLE

Narong Khuntikeo[1,5], Bandit Thinkhamrop[4,6], Nittaya Chamadol[2,5], Watcharin Loilome[3,5], Puangrat Yongvanit[3,5]

*Departments of Surgery[1], Radiology[2], Biochemistry[3], Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand; Department of Biostatistics[4], Faculty of Public Health, Khon Kaen University, Khon Kaen, 40002, Thailand; Liver Fluke and Cholangiocarcinoma Research Center[5], Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand; Data Management and Statistic Analysis Center[6], Faculty of Public Health, Khon Kaen University, Khon Kaen, 40002, Thailand*

Cholangiocarcinoma (CCA) is bile duct cancer caused by liver fluke (*Opisthorchis viverrini*) infection. It is an extremely aggressive, usually fatal disease. There are estimated 14,000 cases annually in the northeast Thailand. Most patients are first seen during the late stage of the disease with five-year survival being less than 10%. Although early detection can substantially increase 5-year survival, there are currently no strategies in place to increase the success of early diagnosis. The Cholangiocarcinoma Screening and Care Program (CASCAP) aims at screening 150,000 individuals using ultrasonography in endemic area in order to provide a high quality database on CCA in the region, determine the optimal screening program for early diagnosis and maximize the success of surgical treatment thus increasing both the patient's quality of life and chances of long-term survival. Online consultation is part of the larger scheme called CASCAP Tools, which is accessible free of charge to public and private hospitals. The surveillance system stores both information regarding risk factors of CCA and ultrasonography imaging data. For the patient group, CASCAP Tools follows the treatment and success of the medical intervention. Both types of information are regularly updated over 6- and 12-month intervals for individuals whose ultrasonographic findings were positive and negative, respectively. Six months after the launch of CASCAP Tools, there were more than 75,000 individuals enrolled into the risk group for further screening. More than 40,000 have received an ultrasonography examination and there are more than 800 health care facilities that have activated CASCAP Tools.

Keyword: Cholangiocarcinoma; Opisthorchis viverrini; Cholangiocarcinoma Screening and Care Program

# Thursday 4th December 2014

**Time:**
**13.30 – 15.00** **Room A**

## S44 Innovative genetic strategies for antimalarial drug discovery strategies

**Chairpersons:**

1. Bill Baker

2. Porntip Petmitr

Invited Speakers:

1 A next generation genetically attenuated *Plasmodium falciparum* parasite created by triple gene deletion
*Stefan Kappe*
Seattle Biomedical Research Institute, USA

2 Structural insights into malaria parasite protein translation machinery and implications for drug discovery
*Amit Sharma*
Structural and Computational Biology Group, ICGEB, New Delhi, India

3 Chemical genomic profiling defines drug-drug and drug-gene interactions in *P. falciparum*
*John Adams*
University of South Florida, Tampa, USA

# A NEXT GENERATION GENETICALLY ATTENUATED *PLASMODIUM FALCIPARUM* PARASITE CREATED BY TRIPLE GENE DELETION

**Stefan H. I. Kappe**

*Seattle Biomedical Research Institute (Seattle BioMed), Seattle, WA 98109, USA; Department of Global Health, University of Washington, Seattle, WA 98195, USA*

The tremendous morbidity and mortality inflicted by *Plasmodium* parasites could be dramatically reduced by a malaria vaccine. Immunizations with live-attenuated sporozoites in animal models and humans have demonstrated the feasibility of a pre-erythrocytic stage-arresting malaria vaccine that confers complete and protracted protection against infection. Genetic engineering offers a versatile platform for controlled and consistent design of pre-erythrocyte stage-arresting genetically attenuated parasites (GAPs) as vaccine candidates. We previously generated a GAP by deleting the *P52* and *P36* genes in the NF54 wild-type (WT) strain of *P. falciparum* (*Pf p52*$^{-}$*/p36*$^{-}$ GAP). Preclinical assessment of the *Pf p52*$^{-}$*/p36*$^{-}$ GAP indicated an early and severe liver stage growth defect. However, human exposure to >200 *Pf p52*$^{-}$*/p36*$^{-}$ GAP-infected mosquito bites in a safety trial resulted in peripheral parasitemia in one of six volunteers, revealing that this GAP was severely but incompletely attenuated. We modeled this phenotype with rodent malaria *P. yoelii p52*$^{-}$*/p36*$^{-}$ GAP in highly susceptible Balb/cByJ mice. Encouragingly, the *Pf p52*$^{-}$*/p36*$^{-}$ GAP induced substantial immune responses in humans including functional antibody responses that could effectively block *in vitro* sporozoite infection of hepatoma cells. We have now created a next generation triple gene deleted GAP by additionally removing the *SAP1* gene (*Pf p52*$^{-}$*/p36*$^{-}$*/sap1*$^{-}$ GAP). *SAP1* deletion alone was sufficient to cause complete attenuation of *P. yoelii* in the highly susceptibility rodent model. Deletion of genes whose encoded proteins perform distinct biological functions (P52 and P36 in parasitophorous vacuole membrane formation and SAP1 in regulating RNA stability) should improve the robustness of complete attenuation and greatly reduce the possibility of loss of attenuation due to compensatory changes. This *Pf p52*$^{-}$*/p36*$^{-}$*/sap1*$^{-}$ GAP was indistinguishable from WT parasites in blood stage and mosquito stage development. Using an improved humanized mouse model transplanted with human hepatocytes and human erythrocytes, we demonstrate that despite a high dose sporozoite challenge, *Pf p52*$^{-}$*/p36*$^{-}$*/sap1*$^{-}$ GAP did not transition to blood stage infection and appeared to be completely attenuated. We have employed FLP/FRT-based recombination to remove all drug selectable marker cassettes from the *Pf p52*$^{-}$*/p36*$^{-}$*/sap1*$^{-}$ GAP. Preparations are underway to test this next generation GAP in a human phase I clinical trial.

# STRUCTURAL INSIGHTS INTO MALARIA PARASITE PROTEIN TRANSLATION MACHINERY AND IMPLICATIONS FOR DRUG DISCOVERY

Amit Sharma

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Malaria continues to be a major cause of morbidity and mortality throughout the world. Our laboratory uses multi-disciplinary techniques within modern biology - including biochemistry, bioinformatics, cell biology, parasitology and protein crystallography to unravel the mechanism of action of important parasite proteins. Over the past few years, we have made significant progress in understanding various parasite motors involved in protein translation machinery. We have also explored structure-based inhibitor discovery routines against several malaria parasite targets. We will explore examples from our studies that highlight attributes of malaria proteins that can be exploited for discovering new inhibitors.

# CHEMICAL GENOMIC PROFILING DEFINES DRUG-DRUG AND DRUG-GENE INTERACTIONS IN *P. FALCIPARUM*

Anupam Pradhan[1], Geoffrey Siwo[2†], Naresh Singh[1], Brian Martens[1] Bharath Balu[1*], Katrina Button-Simons[2], Asako Tan[2], Min Zhang[1], Kenneth O. Udenze[1], Rays H.Y. Jiang[1], Michael T. Ferdig[2], John H. Adams[1*] & Dennis E. Kyle[1*]

*[1] Department of Global Health, College of Public Health, University of South Florida, Tampa, FL 33612;*
*[2] Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, IN 46556*

The spread of *Plasmodium falciparum* multidrug resistance highlights the urgency to discover new targets and chemical scaffolds. Unfortunately, lack of experimentally validated functional information about most *P. falciparum* genes remains a strategic hurdle. In this study we conducted a forward, chemogenomic profiling screen of *P. falciparum piggyBac* mutants to identify and validate drug targets and resistance mechanisms. Mutants carrying a single transposon insertion were profiled for altered responses to antimalarial drugs and metabolic pathways inhibitors. We found that drugs targeting the same pathway have significantly higher network connectivity to each other than to drugs that inhibit other pathways. In addition, pairwise correlations between drugs in the chemogenomic profile revealed novel insights into artemisinin drugs and other antimalarial drugs. Our chemogenomic approach reveals unexpected drug relationships and connects them to gene functions. Importantly, a role of K13 in artemisinin sensitivity is confirmed and artemisinin mechanism of action is linked to regulation pathways.

# Thursday 4th December 2014

**Time:**
**13.30 – 15.00** **Room B**

## S45 Protozoan Infections

**Chairpersons:**


1. Ho-Woo Nam


2. Supaluk Popruk

Speakers:

1 Variation of protease among subtype – Evidence for subtype-dependent pathogenicity
*Arutchelvan Rajamanikam*
Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia

2 Role of hsp70 in *Blastocystis* sp.
*Gaythri Thergarajan*
Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia

3 Predominance of *Blastocystis* sp. infection among school children in peninsular Malaysia
*Nithyamathi Kalimuthu*
Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia

4 Tyrosine kinase inhibitors against HER4-like protein of *Toxoplasma gondii* inhibit the growth in ARPE-19 cells
*Ho-Woo Nam*
Department of Parasitology, College of Medicine, Catholic University of Korea, Korea

5 Presumptive evidence to associate *Blastocystis* sp. with colorectal cancer
*Vinoth Kumarasamy*
Department of Parasitology, Faculty of Medicine, University of Malaya, Malaysia

# VARIATION OF PROTEASE AMONG SUBTYPE – EVIDENCE FOR SUBTYPE-DEPENDENT PATHOGENICITY

Arutchelvan Rajamanikam[1], Suresh Kumar Govind[1]

[1] *University of Malaya, Kuala Lumpur, Malaysia*

*Blastocystis* sp. is the most frequently isolated parasite in any stool survey carried out globally. There are 17 subtypes (ST) reported with ST 3 incriminated to be the pathogenic one. Although protease activity of *Blastocystis* sp. was reported to contribute to pathogenesis, their correlation to different STs' and their respective specific type of protease remains to be elucidated. Both the lysates and excretory and secretory product (ES products) from ST1, ST3, ST 5 and ST 6 belonging to symptomatic and asymptomatic individuals were isolated and subjected for detection and quantification of protease. Inhibition assay involving different protease inhibitor was carried out to determine specific protease in each ST. The present study showed that protease activity from ST 3 to be 2 to 3 fold higher compared to other subtypes. Protease activity from all symptomatic isolates was significantly higher compared to asymptomatic isolates. All the STs showed highly significant ($p<0.05$) inhibition towards cysteine protease-specific inhibitor (E64) suggesting the predominance of cysteine protease in all STs with varying amount. This study for the first time has demonstrated a heightened protease activity with respect to different ST suggesting the genotypic variability in pathogenicity. Elevated protease activity in ST 3 explains the pathogenic potential of this ST as previously reported.

Keywords: *Blastocystis* sp., subtype, protease, cysteine protease, pathogenic potential

# ROLE OF HSP70 IN *BLASTOCYSTIS* SP.

Gaythri, T[1]., Suresh, K.G[*1]., Subha, B[2]., and Kalyani, R[1].

[1] *Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur*
[2] *Department of Genetics and Molecular Biology, Faculty of Science, University of Malaya, 50603 Kuala Lumpur*

Heat shock proteins (HSPs) are ubiquitous proteins found in both prokaryotic and eukaryotic cells. Heat shock protein 70 (HSP70) has been identified as a molecular chaperone that gets expressed in response to thermal stress. To date although the sequence of *Blastocystis* sp. subtype 7 heat shock protein 70 (HSP70) is available in the GenBank, there has been no further research carried out on this gene. Thus, the present study is aimed in identifying the presence of HSP70 in subtypes of *Blastocystis* sp. and understanding its role in *Blastocystis* sp. when thermal stress is induced. The designed primer set that amplified *Blastocystis sp*. subtype 7 HSP70 gene in subtypes 1, 3 and 5 was against a conserved region. Quantitative real-time PCR analysis of gene expression showed up regulation of HSP70 gene in thermal stressed *Blastocystis.* This observation was coupled with the growth analysis of thermal stressed *Blastocystis*. The parasite growth was reduced to a minimal with a majority of the organism showing granular forms when the cultures were subjected to 39 and 41°C. The growth however doubled compared to the control isolates when the parasites were re-cultured back at 37°C. Upon thermal stress at 41°C, subtype 3 and subtype 5 isolates' growth reached up to $2.97\times10^{6}$ and $3.05\times10^{6}$ cells/ml compared to their respective controlled culture tubes at 37°C which peaked only at $1.34\times10^{6}$ and $1.70\times10^{6}$ cells/ml respectively. These observations suggest that HSP70 does not only play its role in buffering the heat stress but also induce the formation of new progenies to ensure the viability of *Blastocystis* sp. in respond towards various environmental stressors.

Keyword: Blastocystis sp., HSP70, thermal stress

# PREDOMINANCE OF *BLASTOCYSTIS* SP. INFECTION AMONG SCHOOL CHILDREN IN PENINSULAR MALAYSIA

Nithyamathi K., Chandramathi S and Suresh K

*Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur*

One of the largest cross sectional study in recent years was carried out to investigate the prevalence of intestinal parasitic infections among urban and rural school children from five states namely Selangor, Perak, Pahang, Kedah and Johor in Peninsula Malaysia. This information would be vital for school authorities to influence strategies for providing better health especially in terms of reducing intestinal parasitism.A total of 3776 stool cups was distributed to 26 schools throughout the country. 1760 (46.61%) responded. The overall prevalence of intestinal parasitic infection was 13.4%, with *Blastocystis sp* (10.6%) being the most predominant, followed by *Trichuris trichiura* (3.4%), *Ascaris lumbricoides* (1.5%) and hook worm infection (0.9%). Only rural school children had helminthic infection. Prevalence of *Blastocystis* was similar in both urban and rural schools in Selangor implying that despite landscape differences in both settings similar modes of transmission must be existing in general Perak had the highest infection (37.2%, total, n=317), followed by Selangor (10.4%, total, n=729), Pahang (8.6%, total, n=221), Kedah (6.2%, total, n=195) and Johor (3.4%, total, n=298). School children from rural schools had higher infection (19.0%, total, n=922) than urban school children (7.2%, total, n=838). Subtype (ST) 3 (54.3%) is the most predominant ST with persons infected with only ST1 and ST3 showing symptoms. *Blastocystis* sp infection significantly associated with low household income, low parent's education and presence of symptoms ($p<0.05$). It is critical that we institute deworming and treatment to eradicate the parasite especially in rural school children.

Keyword: Intestinal parasitic infection, Blastocystis sp., prevalence, school children, Malaysia

# TYROSINE KINASE INHIBITORS AGAINST HER4-LIKE PROTEIN OF *TOXOPLASMA GONDII* INHIBIT THE GROWTH IN ARPE-19 CELLS

Ho-Woo Nam[1], Zhaoshou Yang[1], Hye-Jin Ahn[1], Chom-Kyu Chong[2],
Tong-Soo Kim[3] and Sung-Jong Hong[4]

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*[4] Dept of Medical Environmental Biology, Chung-Ang University, Seoul 156-756, Korea*

*Toxoplasma gondii* is a ubiquitous obligate intracellular parasite, which infects warm-blooded animals, including humans; it is a zoonotic pathogen widespread in nature. Approximately one-third of humans worldwide are chronically infected with *T. gondii*. To find a new drug for the treatment of toxoplasmosis, small molecules of tyrosine kinase inhibitor (TKI) against various kinds of growth factor receptors were tested the effect on the intracellular growth of *T. gondii* in the retinal cell line, ARPE-19. By counting the average number of tachyzoites per parasitophorous vacuole (PV) after treatment with TKIs, Nintedanib (blocks VEGFR1/2/3, PGFR1/2/3, PDGFRalpha/beta), AZD9291 (mutant EGFR), and Sunitinib (VEGFR2 and PDGFRbeta) did not affect the intracellular growth of tachyzoites up to the concentration of 10 microM, half the conc. of which destructed the host cell morphologically. On the while, the EGFR blocking TKIs, such as Lapatinib, Gefitinib, Erlotinib, AG-1478, Neratinib, Dacomitinib, Afatinib, and Pelitinib affected to inhibit the intracellular growth of tachyzoites of its own permissive conc. under the conc. of cytotoxicity. But for the former four TKIs which block the HER2 of EGFR family showed weak inhibitory effect with the treating conc. of 20, 30, 50, and 20-30 microM near to the conc. of cytotoxicity, whereas the latter four which block both HER2 and HER4 of EGFR family showed strong inhibitory effect with lower conc. of 0.5, 5, 5, and 5 microM. This suggests that those TKIs directly affect on the *T. gondii* molecule to inhibit the intracellular growth and that the target may be HER4-like protein of *T. gondii*.

Key words: *Toxoplasma gondii*, tyrosine kinase inhibitor, EGFR, HER2, HER4

# PRESUMPTIVE EVIDENCE TO ASSOCIATE *BLASTOCYSTIS* SP. WITH COLORECTAL CANCER

K Vinoth[1], UR Kuppusamy[2], K Suresh[1]

[1] *Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.*
[2] *Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.*

*Blastocystis* sp. is a commonly found intestinal microorganism and was reported to cause many non-specific gastrointestinal symptoms. Various subtypes have been previously reported and the pathogenicity of different subtypes of *Blastocystis* is unclear and remains as a controversial issue. A recent study has shown that the *Blastocystis* antigen isolated from an unknown subtype could facilitate the proliferation of colon cancer cells. Current study was conducted to determine the prevalence of *Blastocystis* genotypes among colorectal cancer (CRC) patients. We also compare the effect of solubilized antigen isolated from five different subtypes of *Blastocystis* on colon cancer cells, HCT116. The overall prevalence of *Blastocystis* infection among CRC patients was 20.1% (41/204) and 9.5% (21/221) in normal individuals. *Blastocystis* subtype 3 infection was significantly more prevalent, 7.35% ($P<0.05$) compared to other subtypes. A statistically significant proliferation of these cells was observed when exposed to 1.0µg/ml solubilized antigen isolated from subtype 3 *Blastocystis*. Real-time polymerase chain reaction (RT-PCR) demonstrated upregulation of Th1 cytokines especially transforming growth factor beta (TGF-β) in subtype 3 treated cancer cells. Of interest, subtype 3 *Blastocystis* antigen also caused a significantly higher upregulation of Cathepsin B (CTSB) which lead to the postulation that it may enhance the exacerbation of existing colon cancer cells by weakening the cellular immune response. Therefore, very likely subtype 3 *Blastocystis* to have higher pathogenic potential in CRC patients compared to other subtypes.

Keyword: Blastocystis sp., colorectal cancer, subtypes

# Thursday 4th December 2014

**Time:**
**13.30 – 15.00** **Room C**

## S46 Preventing HIV and ensuring quality HIV care in Thailand

**Chairperson:**

Michael Martin

Invited Speakers:

1 The quality of HIV treatment and care in Thailand: Past-Present-Future
*Cheewanan Lertpiriyasuwat*
Bureau of AIDS, TB and STIs, Ministry of Public Health, Thailand

2 HIV cascade in Thailand
*Achara Teeraratkul*
Thailand MOPH - U.S. CDC Collaboration, Thailand

3 Patching the leaks to improve HIV prevention among MSM and Transgender
*Chomnad Manopaiboon*
Thailand MOPH - U.S. CDC Collaboration, Thailand

4 Projects to promote quality care services by HIV-infected patients, the collaboration of the multidisciplinary team, municipality Chai Burin
*Thananya Asakit*
National Health Security Office (NHSO) ,Bureau of Quality Development and Good Governance

# THE QUALITY OF HIV TREATMENT AND CARE IN THAILAND: PAST-PRESENT-FUTURE

Dr.Cheewanan Lertpiriyasuwat,

*Head of HIV Care Development Cluster, Bureau of AIDS, TB and STIs, MoPH, Thailand*
*(e-mail: cheewananl@gmail.com)*

The development of HIV treatment with more advanced antiretroviral regimenshas transformed HIV into a chronic disease allowing infected individuals to live longer and healthier-but only if they receive adequate treatment as early as possible and adhere to drugs. In 2013, Thailand has almost 500,000 people living with HIV (PLHIV); out of that number, 388,833 registered in a care system, 256,391 alive and receiving treatment, and 227,451 on ART. Today more than 1,000 hospitals provide antiretroviral treatment(ART) services scattered in every province and district. The death rate and retention rate of PLHIV are challenging. How to patch the leaks to improve HIV quality of life, can only happen if the challenges of HIV can be met and overcame on the quality.

HIVQUAL-Tis a development HIV quality model for hospitals to enhance their capacity and capability to improve and manage for quality in HIV and AIDS care. In 2003, Thailand's Bureau of AIDS, TB and STIs (BATS), in technical collaboration with Global AIDS Programme, the U.S. Centers for Disease Control and Prevention (CDC), adapted the model to fit Thailand's HIV and AIDS Programme. BATS and CDC initiated the HIVQUAL-T model starting with only 12 government hospitals and participation grew nationwide. HIVQUAL-T has successfully improved the quality of ART services in more than 700 hospitals in Thailand. Its three component: performance measurement, quality improvement activity/program, and infrastructure support for quality improvement focuses on building capacity of care providers at all levels – national, regional, provincial, and hospital – to strengthen the local care and treatment network, and develop a coaching system for quality improvement.

Although HIVQUAL-T has been in operation for over 10 years, it's linked to the hospital accreditation system in the recent timeto ensure sustainability of continuous development of HIV service quality. In addition, it'sexpanding to other HIV related issues such as STI service, HIV prevention by targeted on quality of comprehensive HIV services.

# HIV CASCADE IN THAILAND

Achara Teeraratkul

*Thailand MOPH - U.S. CDC Collaboration, Thailand*

# PATCHING THE LEAKS TO IMPROVE HIV PREVENTION AMONG MSM AND TRANSGENDER

Chomnad Manopaiboon

*Thailand MOPH - U.S. CDC Collaboration, Thailand*

# PROJECTS TO PROMOTE QUALITY CARE SERVICES BY HIV-INFECTED PATIENTS, THE COLLABORATION OF THE MULTIDISCIPLINARY TEAM. MUNICIPALITY CHAI BURIN

Thananya Asakit

*National Health Security Office (NHSO) ,Bureau of Quality Development and Good Governance*

**Background:** This project to promote quality care services among HIV infected patients by the collaborator of the multidisciplinary team, Municipality of Chai Burin. The purposes of the study were to develop ability mentor team for empowerment monitoring visit the service system and search for good practice. The target groups included 40 improving the quality of mentors and consultants in 12 hospitals were recruited by voluntary. The Studies conducted in 4 Provinces includes of the Nakhonrachasima,Chaiyaphum,Buriram and Surin, Thailand from May - September 2012.

**Method:** Project included of four activities

1. Workshop training quality system involved HIV infection for mentors team and quality consultant
2. To empowerment and Monitoring visit activities
3. To take the successful lessons learned
4. To search good practices to improve the quality of care HIV infection

**Results:** The results showed that first, forty eighty mentors for training quality care service system. Second, Eleventh hospital receive empowerment and monitoring visit included Sikhiu ChokChai Prathai Phukieo Kasetsoomboon Bamnetnarong Nangrong Phlapphlachai Burirum Thatoom and Prasath hospitals and Finally, the fourteen good practices(CQI/Clinical tracer/CQI story) for quality care HIV infection. 9 in 14 works selected for posters presentation in HACC-Forum 6th conference. Paticipants'satisfaction level were 4.57 of 5 points showed high level that use integration routine and compliance the requirements.

**Conclusions:**

LESSONS LEARNED

Key success for sustainability quality care HIV infection

1. Leadership team support for development quality system.
2. Multidisciplinary team driven the patient care process.
3. Collaboration of internal and external organization.

Policy suggestion

1. To concrete policy for sustainability quality care HIV infection.
2. Quality structure HIV infection system integrated quality steering team
3. To promote multidisciplinary team both internal and external.
4. To extend network other disease.

This project is inspiration of the service system for HIV infection patients care. We would like thank you to the National Health Security Office Thailand as support the budget. Also would like to thank you Suranaree University of Technology, school of nursing that supported the academic Nurse consultant teams. The result is able to develop continuously for the optimal health of patients.

**Keywords:** 1. Quality care service, 2. Collaboration, 3. Multidisciplinary

# Thursday 4th December 2014

**Time:**

**13.30 – 15.00** **Room D**

## S47 Dengue Virus Infections

**Chairpersons:**

1. Pornsawan Luengwutiwong 2. Giauddin Ahsan

Speakers:

1 Increase of thrombin-cleaved form of OPN in recovery phase of dengue virus infection

*Toshio Hattori*

Division of Emerging Infectious Diseases, Department of Internal Medicine, Graduate School of Medicine, Tohoku University, Japan

2 Spatial pattern and variability of entomological parameters of the dengue vector populations after space spraying in an urban area of lower southern Thailand

*Napadol Sudsom*

Faculty of Environmental Management, Prince of Songkla University, Thailand

3 Ecology and disease dynamics in Bangladesh: Vector larval indices and risk for dengue epidemics in Dhaka

*Giasuddin Ahsan*

Department of Public Health, North South University, Dhaka, Bangladesh

# INCREASE OF THROMBIN-CLEAVED FORM OF OPN IN RECOVERY PHASE OF DENGUE VIRUS INFECTION

Toshio Hattori[a,b], Talitha Lacuesta[c], Susan Lerano[d], Efren Dimmano[c], Elizabeth Telan[d], and Haorile Chagan-Yasutan [a, b]

[a] *Disaster-related Infectious Diseases, International Research Institute of Disaster Science, Tohoku University, Sendai, Japan*
[b] *Division of Emerging Infectious Diseases, Department of Internal Medicine, Graduate School of Medicine, Tohoku University, Sendai, Japan*
[c] *Department of Blood Borne Diseases, San Lazaro Hospital, Manila, Philippines*
[d] *National Reference Laboratory for HIV/AIDS, Hepatitis, and other STDs, STD/AIDS Cooperative Central Laboratory, Manila, Philippines*

Background and aim: The activation of coagulation pathways and fibrinolysis have been reported in DENV-infection. Full-length osteopontin (FL-OPN), a glycoprotein, and its activated thrombin-cleaved product, trOPN, integrate multiple immunological signals through the induction of pro-inflammatory cytokines. Here we examined kinetics of full-length and thrombin-cleaved form of OPN in DENV infection. Method: we assessed circulating levels of FL-OPN, trOPN in blood obtained from 65 DENV infected patients in the critical and recovery phases of DF and DHF during a dengue epidemic in the Philippines in 2010. Several coagulation markers (D-dimer, thrombin-antithrombin complex [TAT], thrombomodulin [TM], and ferritin were also measured Results: In both the DF and DHF groups, the increased levels of FL-OPN, trOPN, d-dimer, TAT, and TM were observed in the critical phase. During the recovery phase, FL-OPN levels declined while trOPN levels increased dramatically. FL-OPN levels were directly correlated with D-dimer and ferritin levels, while the generation of trOPN was associated with TAT levels, platelet counts, and viral RNA load. Conclusion: Our study demonstrated the marked elevation of plasma levels of FL-OPN in critical phase and trOPN in recovery phase. Solution of the increase of trOPN in recovery phase would clarify the novel mechanism of inflammation-coagulation cross link network in DENV infection.

Keyword: Dengue fever, Dengue hemorrhagic fever,osetopontin, trOPN, thrombin

# SPATIAL PATTERN AND VARIABILITY OF ENTOMOLOGICAL PARAMETERS OF THE DENGUE VECTOR POPULATIONS AFTER SPACE SPRAYING IN AN URBAN AREA OF LOWER SOUTHERN THAILAND

Napadol Sudsom[1], Kuaanan Techato[2*], Suwich Thammapalo[3], Theerakamol Pengsakul[4], and Virasakdi Chongsuvivatwong[5]

*[1,2] Faculty of Environmental Management, Prince of Songkla University, Thailand*
*[3] The office of Disease Prevention and Control 12 Songkhla, Thailand*
*[4] Faculty of Medical Technology, Prince of Songkla University, Thailand*
*[5] Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Thailand*

Dengue fever is well known of mosquito born viral fever, which causes significant public health problem in Thailand. Here we illustrated a spatial interpolation approach to analyze the spatial pattern of important mosquito vector *Aedes aegypti.* Entomological parameters of *Ae. aegypti* populations after spray with standard indoor ultra low volume (SID-ULV) in Songkhla province done between March and September 2014 were evaluated by running a cluster-randomized investigations. Eight of house in clusters divided in two randomized spraying groups are intervention (SID-ULV 4 clusters) and control (regular space spraying 4 clusters). Parameters including ovitrap index, parous rate and resting rate were monitored in pre- and post-space spraying. Housing pattern data were collected, and urban environmental factors of forty houses within 100 meter radius of space spraying areas based on the guidelines and recommendations adopted from WHO were recorded with geographic coordinates. The spatial pattern analysis and the dynamics of *Ae. aegypti* populations were determined using application of poisson kriging methods based on epidemiological, entomological and environmental variables. Data gathered during the present study add to the significantly new knowledge useful in an improvement of space spraying in an urban area, and leads to further research on current dengue policy in Thailand with the aim of controlling the disease more effectively.

Keywords: spatial pattern, *Aedes aegypti*, dengue, space spraying, urban area

# ECOLOGY AND DISEASE DYNAMICS IN BANGLADESH: VECTOR LARVAL INDICES AND RISK FOR DENGUE EPIDEMICS IN DHAKA

G.U. Ahsan[1], K. Bashar[2],
O. Rahman[1], C.E. Haque[3], R. Lindsay[4], P.D-Chowdhury[3], M. Jahan[1] M Hasanuzzaman[1]

[1] *Department of Public Health, North South University, Dhaka, Bangladesh,*
[2] *Department of Zoology, Jahangirnagar University, Savar, Bangladesh*
[3] *Natural Resources Institute, University of Manitoba, Winnipeg, Canada*
[4] *National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada*

Dengue fever and dengue hemorrhagic fever are now endemic in Bangladesh. Since the outbreak of dengue in 2000, dengue disease has become an emerging major public health concern. Although, it is well known that transmission of dengue virus is maintained among the population by *Aedes* mosquitoes.

A multi-stage entomological survey was conducted in 844 households in 12 randomly selected wards of Dhaka city during July-August 2011. A total of 3,050 larvae and pupae of mosquitoes were collected in 467 positive containers distributed among 263 of 844 houses visited. The density of *Ae.aegypti* mosquitoes was higher than *Ae. Albopictus* in each ward. Among the indoor wet containers, the highest relative frequency was observed in water tanks(507/687; 73.8%) and the lowest was in clay pots & metal drums (0.15%). Despite their relatively low frequency of infestation, all clay pots & metal drums that were within households were infested (100%) with *Ae.aegypti*.

The mean *Aedes* larval house indices was 31.16 (range 5.1 to 62.3), the mean container indices was 37.78 (range 5.16 to 95.45); and the mean Breteau indices was 55.33 (range 18.57 to 90.16). Water type (p=0.05) and presence of vegetation (p=0.03) were both significant factors associated with the abundance of *Ae.aegypti*. In contrast, container location, presence of shade and whether containers were sheltered from rain did not significantly (p>0.05) influence their use by dengue vectors. The results of this study suggest that reducing the availability of selected, highly productive container types could have significant positive impact on dengue epidemics in Dhaka city.

Keyword: Ecology, Disease Dynamics, Dengue Fever, Dengue Hemorrhagic Fever.

# Thursday 4th December 2014

**Time:**
**15.30 – 16.15**

## S48 Sornchai Looareesuwan Medal

**Chairperson:**

Dean Yaowalark Sukthana

Keynote Speakers: The function of proteins involved in invasion of human erythrocytes by *P. falciparum* and their potential as vaccine candidates

*Alan F. Cowman*
Walter & Eliza Hall Institute in Melbourne, Australia

# THE FUNCTION OF PROTEINS INVOLVED IN INVASION OF HUMAN ERYTHROCYTES BY *P. FALCIPARUM* AND THEIR POTENTIAL AS VACCINE CANDIDATES

Lin Chen, Alan Yap, Julie Healer, Jenny K. Thompson, Clara Lin, Wai-Hong Tham, Anthony N. Hodder, and Alan F. Cowman

*Walter & Eliza Hall Institute in Melbourne, Australia*

In humans the most severe form of malaria is caused by *Plasmodium falciparum* and at least a third of the world's population is at risk of infection, with over three hundred million people developing clinical disease each year and over 700,000 deaths. *P. falciparum* invades human erythrocytes using several specific and high affinity ligand-receptor interactions that define invasion pathways.These parasite ligands bind to red blood cell receptors that include glycophorin A, B, C, complement receptor 1 and basigin and current evidence suggests that some of these interactions can provide a signal for activation of downstream processes, which commits the parasite to invasion of the host cell. We are addressing the function of these interactions in merozoite invasion and determining the complexes that form during this process. Antibodies to these ligands can inhibit erythrocyte invasion and may be important for acquired immunity. Analysis of these antigens has suggested them as potential vaccine candidates and has also provided functional insights.

NOTES:

JITMM
JOINT INTERNATIONAL TROPICAL MEDICINE MEETING

# JITMM2014 & FBPZ8

Joint International Tropical Medicine Meeting 2014
“3D Perspectives on Tropical Medicine:
Drivers, Diversity, and Determination”
The 8th Seminar on Food- and Water-Borne Parasitic Zoonoses
2 - 4 December 2014

FBPZ
Food-Borne
Parasitic
Zoonoses

## ABSTRACTS

### POSTER Presentations

Centara Grand & Bangkok Convention Centre at CentralWorld,
Bangkok Thailand

*POSTER No. 001*

# CARRION DISEASE: DIAGNOSTIC AND ANTIBODY LEVELS IN A NORTHERN ENDEMIC AREA OF PERU

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*Bartonella bacilliformis* is the causative agent of Carrion disease, a neglected illness, which in absence of treatment achieves mortalities of 44-88%. A relevant problem is the lack of an effective diagnostic to overcome misdiagnosis and treat asymptomatic carriers. The objective was to compare 2 different diagnostic techniques used in Peru and evaluate the antibody titters for *B.bacilliformis* in inhabitants of a northern endemic area.

Blood and serum samples from 177 people were collected in March 2014 in 5 different localities. Clinical data were recorded. Bacterial culture and specific 16SrRNA amplification were performed. ELISA for IgM/IgG with whole cell as antigen was also done.

The presence of at least one symptom compatible with Carrion disease was reported by 34.5%. Only 1.7% of samples had positive result by PCR. All were from Tunal where an outbreak was reported in late 2013. No growth was observed in cultures. In absence of an established breakpoint for ELISA we have considered as evidence of infection the samples with at least 2 times the level of a commercial negative control (X0939-DAKO). Fifty percent of volunteers' present levels of IgM equivalent with evidence of infection. For IgG this only happens for 2.83%. From these people with high titers of antibodies (51.4% for IgM and IgG), only 15.2% reported symptoms, suggesting that 36.2% are asymptomatic.

Both PCR and culture seems not to be efficient diagnostic tools. Meanwhile, ELISA showed the presence of a high percentage of apparently healthy population with higher levels of antibodies against *B.bacilliformis*. Present data highlight the need to develop a diagnostic tool.

Keywords: *Bartonella bacilliformis*, Carrion Disease, Neglected disease, Diagnostic, ELISA.

*POSTER No. 002*

# DETECTION OF COMMENSAL *ESCHERICHIA COLI* HARBOURING ESBL AND PLASMIDIC AMPC BETALACTAMASES IN PERUVIAN POULTRY

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The use of antimicrobial agents in food animal production is controversial and may contribute to increase the prevalence of antibiotic-resistant bacteria. The lack of control in the different stages involved from farm to fork is leading to this phenomenon. Commensal *Escherichia coli* strains, well-known inhabitants of gut microbiota of animals and humans, may act as a reservoir for transmissible antimicrobial resistance genes, being of relevance those conferring resistance to third-generation cephalosporines, mainly the ones encoding AmpC and extended-spectrum betalactamases (ESBL). The aim of the study was to detect the presence of ESBL and AmpC betalactamases in commensal foodborne *E. coli* strains from Peru.

Ninety-eight *E. coli* commensal strains were isolated from two types of meat, poultry (66) and beef (32), obtained from six markets of different areas of Lima. Clonality of the isolates was assessed by repetitive-sequence-based PCR (rep-PCR). Phenotypic detection of ESBL and plasmidic AmpC betalactamases was determined by double synergy test.

The presence of ESBL and plasmidic AmpC were detected in 26,9% and 5,1% of *E. coli* strains, respectively, and all of them found in poultry samples.

Further characterization of the ESBL and AmpC molecular mechanisms involved is necessary. The high presence of ESBL represents a major risk of transmission to humans, leading in some cases to therapeutic failure when treating an infection with antimicrobial agents. The present results highlight the need to strengthen measures to control the use of antimicrobials in veterinary and in food production.

*POSTER No. 003*

# DETECTION AND CHARACTERIZATION OF ESBL AND PLASMIDIC AMPC BETALACTAMASES IN *SALMONELLA* SPP. FOODBORNE ISOLATES FROM PERU

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The role of food as carrier of pathogens is a major public health problem being *Salmonella enterica* infections, mostly isolated in chickens, the leading cause of foodborne human infections. The problem is exacerbated by the increasing levels of antibacterial resistance, including resistance to cephalosporins by the presence of extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases. The aim of this study was to analyse the presence of ESBL and AmpC, the general susceptibility and the mechanisms of resistance to beta-lactams in *Salmonella* spp. isolated from meat samples from Peru. Twenty-one strains of *Salmonella* spp. isolated were serotyped by microarray. Antimicrobial susceptibility was tested by disk diffusion. The presence of ESBL and AmpC was determined by double synergy test. The presence of blaSHV, blaTEM, blaCARB, blaOXA1-like, blaOXA2-3-like, blaOXA5-7-like, blaCTX-M and class I integron was analysed by PCR and sequencing. The serotyping resulted in 17 *S.* Infantis, 2 *S.* Enteritidis, 1 *S.* Kentucky and 1 *S.* Anatum. The isolates were completely or highly resistant to rifampicin, ampicillin, cotrimoxazole and amoxicillin amongst others. The ESBL phenotype was found in 3 isolates, identified as blaCTX-M-65. The presence of AmpC beta-lactamases was confirmed in 7 isolates. Class I integron carrying the aadA1 gene was found in 18 isolates. The most prevalent serotype found was *S.* Infantis. The presence of multiresistance and these mechanisms involve a high risk of transmission to humans, already being reported outbreaks caused by *S.* Infantis ESBL-producers. All this shows the importance of adequate sanitary control in animal production to prevent foodborne human infections.

Keywords: food safety, betalactamases, Salmonella enterica

*POSTER No. 004*

# EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING BACTERIA ISOLATED FROM BLOOD CULTURE IN THAI HOSPITALS FROM 2007-2012

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**Background:** Antibiotic resistance in bacteria is a major public health problem. The resistance rates increase every year due to the evolution of bacteria and misuse of antibiotics. The objective of this study was to determine ESBL strains of the most common 2 gram-negative bacteria isolated from blood culture, *Escherichia coli* and *Klebsiella pneumoniae.*

**Methods:** We conducted blood stream infection surveillance in Sa Kaeo and Nakhon Phanom by using automated blood culture system. We performed disks diffusion assay following the criteria of the Clinical Laboratory Standards Institute (CLSI) for positive culture. Blood specimens were collected from all hospitals as clinical indicated sepsis.

**Results:** From 2007-2012, a total 11,846 blood specimens were collected. Gram-negative bacteria were mostly found; 2660 (22.5%) isolates of *E. coli* and 877 (7.4%) isolates of *K. pneumoniae.* In 6 years, ESBL producing strains were found in 487 isolates of *E. coli* (18.3%) and 166 isolates of *K. pneumoniae* (18.9%). The percentage of *E.coli* ESBL producing bacteria increased from 5.5% in 2007 to 25.0% in 2012, similar to those of *K. pneumoniae* which rose from 13.6% in 2007 to 22.4% in 2012. ESBL producing strains showed more than 90% susceptibility to Ertapenem, Imipenem, Meropenem and Amikacin.

**Conclusions:** The percentage of antibiotic resistance *E. coli* and *K. pneumoniae* increased from 2007 to 2012 which lead to problems in treatment and management in hospitals. The drug of choice for ESBL producing strains are carbapenem and aminoglycoside (only Amikacin). This study emphasizes the urgency to control and monitor antibiotic resistance bacterial pathogens.

POSTER No. 005

# FACTORS ASSOCIATED WITH CLINICAL OUTCOME BY VANCOMYCIN MIC CREEP IN PATIENTS INFECTED WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AT THAMMASAT UNIVERSITY HOSPITAL

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main causes of hospital-acquired infections. MRSA with reduced susceptibility to vancomycin, a phenomenon recognized as Minimum Inhibitory Concentration (MIC) creep, causes high mortality worldwide. This increase in vancomycin MIC creep strains is extremely challenging to physicians. The factors associated with clinical failure by vancomycin MIC creep in MRSA-infected patients at Thammasat University Hospital have not yet been intensively investigated. This study imparts insightful information as a basis for future research in the assessment, medical treatment, monitoring and prevention of drug-resistant bacteria spread. The association of clinical and microbiological characteristics, with clinical success and failure of MRSA-infected patients, was scrutinized by multivariable analysis. Patients' clinical data were collected from medical records; in addition, MIC and virulence genes' distribution were analyzed for associated microbiological factors in clinical outcomes. The results indicated that acute kidney injury (OR=8.77; 95% CI: 1.29-59.55; $p$ = 0.02) and anemia (OR=11.68; 95% CI: 1.07-126.71; $p$=0.04) were significant factors associated with clinical failure in infected MRSA patients. All isolates were positive for *mec*A and *ica*A genes, whereas all isolates were negative for *tst* and PVL genes. Thirty-two clinical isolates were susceptible to vancomycin with MIC values of 0.5 µg/ml (18.7%), 1 µg/ml (65.6%), 1.5 µg/ml (12.5%), and 2µg/ml (3.1%). However, there may be strains with unexpected resistant subpopulations resulting in treatment failures. Further studies are required to examine heteroresistant vancomycin intermediate *Staphylococcus aureus* (hVISA) and other genes associated with vancomycin MIC creep.

Keywords: Vancomycin, Minimal Inhibitory Concentration creep (MIC creep), Methicillin-resistant *Staphylococcus aureus* (MRSA)

*POSTER No. 006*

# THE ROLE OF CYCLE INHIBITING FACTOR IN *BURKHOLDERIA PSEUDOMALLEI*

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*Burkholderia pseudomallei*, a causative agent of melioidosis, harbor several virulence factors crucial for its pathogenesis. A cycle-inhibiting factor (Cif) is one of bacterial effector molecules produced by *B. pseudomallei*, however the role of this protein is still unclear. In our previous study, we constructed a *B. pseudomallei* cif mutant to explore the function of Cif in *B. pseudomallei* infection. Infection of U937 macrophage with *B. pseudomallei* revealed that host cell contact was required for the secretion of Cif. Interestingly, Cif was secreted in the host cell in a manner dependent on the Bsa type III secretion system. Moreover, the responsibility of Cif is likely important for *B. pseudomallei* pathogenesis as we found *B. pseudomallei cif* mutant showed significant defects in abilities to induce plaque formation and cytotoxicity of HeLa cells when compared to the wild type. We further investigated the host protein expression profiles of infected HeLa cells using mass spectrometry. Investigation by this technique identified 97 up-regulated and 166 down-regulated proteins that significantly altered in HeLa cells infected with *chbP* mutant compared with that of *B. pseudomallei* wild type strain. Among these, some are associated with metabolic process, catalytic activity and signaling pathways. Thus, based upon our findings, we indicate a requirement of Cif that deliver via Bsa apparatus in *B. pseudomallei* infection, and the role of Cif might be involved with regulation of the host cell expression to alter the host cell functions which benefit for the pathogenesis of *B. pseudomallei*.

Keywords: *B. pseudomallei*, cycle-inhibiting factor, pathogenesis

POSTER No. 007

# MOLECULAR EPIDEMIOLOGY OF ENVIRONMENTAL *LEPTOSPIRA* IN UDON THANI, THAILAND, AND IN VIENTIANE, LAO PEOPLES DEMOCRATIC REPUBLIC

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Leptospirosis is an acute febrile illness caused by pathogenic bacteria of the genus *Leptospira*. The bacteria are regularly excreted into the environment by carrier animals, and infection in man mainly results from indirect contact with contaminated water or soil, for example during rice farming. A single clone of bacteria called *Leptospira interrogans* sequence type (ST) 34 caused an outbreak of human leptospirosis in northeast Thailand and also frequently caused leptospirosis in the Lao PDR (Laos). Little is known about the presence and molecular epidemiology of *Leptospira* in these two endemic areas. This is mainly due to the difficulty of isolating pathogenic *Leptospira* species from the environment. With a new bacteria culture medium called *Leptospira* VW agar, isolation is more robust.

A total of 103 environmental water samples were collected from Udon Thani, Thailand (n=86) and Vientiane, Laos (n=17) in the dry and wet seasons. 19/86 (22%) samples from Udon Thani and 2/17 (12%) from Vientiane contained *Leptospira* species.

Single colonies of *Leptospira* were screened for pathogenic and intermediate group markers. Pathogenic (n=1, novel species) and intermediate (n=3, *Leptospira licerasiae* and *Leptospira wolffii*) isolates were identified only from Thai samples. *Leptospira interrogans* ST 34 or other pathogenic *Leptospira* species were not identified in samples from water in Thailand or Laos.

Keywords: *Leptospira*, Thailand, Laos, Environmental water

POSTER No. 008

# IDENTIFICATION OF ENTERIC BACTERIA IN DEEP WELLS AND GROUNDWATER WITHIN THE PHILIPPINES AND THE EFFECT OF ANTIBACTERIAL VARIANTS AGAINST THE ISOLATES

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The quality of a water source for consumption is affected by the microbial community present. Presence of fecal coliforms, especially multi-drug resistant strains in sources of freshwater for human consumption can lead to infections in the community. The National Water Regulatory Board has banned deep wells in the Philippines since 2008 due to their contribution to metal contamination of water sources. However, these water sources are still used for bathing, crops, and laundry. Thus, detection of fecal coliforms is an important step in ensuring clean water for communities that still rely on these water sources. Moreover, direct use of antibacterial soaps and detergents at these groundwater sites for laundry can also lead to exposure of fecal coliforms to antibacterial agents at low levels, potentially leading to selection and emergence of resistant strains. This study aims to detect the presence of fecal coliforms – in particular, *Escherichia coli, Salmonella* sp., and *Enterococcus* sp. – in deep wells and creek water in Quezon City, Philippines. Additionally, the minimum inhibitory concentration (MIC) of different regular and antibacterial variants of locally available laundry and dishwashing detergents were tested against these fecal coliforms. Results show that both *E. coli* and *Enterococcus* were found in groundwater. Further, *Enterococcus* isolates were found to be resistant to antibacterial variants and dishwashing reagents. Local government units that implement bans on using deep wells and other groundwater sources in Metropolitan Manila should also consider microbial infection and possible emergence of resistant strains due to the potential health risk.

POSTER No. 009

# KNOWLEDGE ON TUBERCULOSIS AND OCCURRENCE OF ADVERSE DRUG REACTIONS ARE FACTORS ASSOCIATED WITH ADHERENCE TO TREATMENT: LESSONS FROM A CASE-CONTROL STUDY AMONG MULTIDRUG-RESISTANT TUBERCULOSIS PATIENTS IN THE PHILIPPINES

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The World Health Organization (WHO) has identified 22 countries with the highest Tuberculosis-burden in the world, with the Philippines ranking as ninth overall. Multidrug-resistant tuberculosis (MDR-TB) significantly contributes to this burden, making the sixth Millennium Development Goal difficult to attain. The WHO has advocated for the use of Tuberculosis-Direct Observed Treatment Shortcourse plus (TB-DOTS plus) as a strategy for the management of MDR-TB. However, compliance to TB-DOTS plus remains a challenge in a developing country like the Philippines.

Using a case-control design, this study examined selected factors associated with non-adherence to TB-DOTS plus among MDR-TB patients at the Lung Center of the Philippines from June to August 2014. 83 patients were selected as subjects based on age, MDR-TB diagnosis, and treatment duration. Cases (40) were defined as patients who missed >25% of treatment or defaulted for >1 month, while controls (43) were patients who missed <25% of treatment.

Treatment adherence was not significantly associated with: (a) age; (b) living arrangement; (c) lifestyle factors; (d) travel time; (e) perceived attitude of healthcare workers; and (f) number of previous TB-DOTS treatment. On the other hand, knowledge on TB (OR=2.83; CI=0.99-8.04) and number of adverse drug reactions or ADRs (OR=1.27; CI=0.99-1.63) were found to be significantly associated with treatment adherence (p< 0.007). While further studies involving a bigger sample size are recommended, this study may provide a basis for the importance of pre-treatment briefing for effective patient education about TB and ADRs, among others, in order to increase patient compliance to TB-DOTS plus among MDR-TB patients.

Key words: Multidrug-Resistant Tuberculosis; Directly Observed Therapy; Medication Adherence; Knowledge; Adverse Drug Reaction; Philippines

*POSTER No. 010*

# MIDGUT MICROBIOTA OF THAILAND STRAIN OF *AEDES ALBOPICTUS* MOSQUITOES

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Mosquitoes are important vectors in the transmission of various diseases including malaria, dengue fever, and chikungunya. These particular diseases pose a great challenge to public health in many countries. Insects exhibit a broad range of relationships with symbiotic organisms. The mosquito and its microbiota are involved in complex mutual interactions such as host reproduction, survival, and protection against pathogens. This research aims to identify the microbiota present in the midgut of *Aedes albopictus*. The midgut bacteria in field caught and laboratory rearing of female *Aedes albopictus* mosquitoes were studied. The midgut from non-fed *Aedes albopictus* was dissected under aseptic conditions, homogenized and plated on blood agar. Microorganisms were first screened based on colony characteristics, morphology, arrangement of isolates, and Gram staining. The bacterial colonies were then identified by using polymerase chain reaction and 16S rRNA sequencing. The bacterial isolates found in field caught mosquitoes were *Bacillus* spp., *Bacillus subtilis*, *Serratia marcescens*, and *Staphylococcus hominis.* On the other hand, the bacterial isolates found in laboratory reared mosquitoes were *Klebsiella pneumonia*, *Micrococcus* spp., *Pandoraea* spp., *Staphylococcus* spp., and *Staphylococcus hominis.* It has also been observed that individual mosquitoes have a variety of bacteria in the midgut. These findings indicate that the variation in midgut microbiota may be one of the factors responsible for variation in disease transmission rates or vector competence within mosquito populations. However, further studies need to be undertaken to determine the mechanism of disease transmission.

Keywords: *Aedes albopictus*, midgut, microbiota, vector, transmission

POSTER No. 011

# INFLUENCE OF ORAL ADMINISTRATION OF MOSQUITO ISOLATED ENTEROCOCCUS BACTERIA ON DEXTRAN SODIUM SULFATE INDUCED MURINE COLITIS

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Objective - Infection of bacteria and viruses induces colitis in humans. However, *Enterococcus* bacteria seem to influence the process of inflammation in colitis. We isolated new *Enterococcus* bacteria (M-13) from the midgut of *Aedes albopictus* females, which were collected at Ohori Park (Fukuoka, Japan) in 2012. Here we investigated the influence of M-13 to dextran sodium sulfate (DSS) induced murine colitis.

Methods - Colitis was induced in five-week-old male mice by the addition of 3% DSS to the drinking water for 7 days. The mice were orally administered with saline, anti-inflammatory oligodeoxynucleotide 7F or M-13 every 3 days, starting 6 days before DSS treatment and for the 7 days of DSS treatment. Stool consistency, stool blood and body weight were recorded daily. Mice were sacrificed at the end of the experiment and the colon was collected. The length of colon was measured.

Results - 7F showed a significantly reduced stool consistency score when compared with saline or M-13, and there was no difference in stool consistency between saline and M-13. However, M-13 showed a decrease in stool blood on day 7. Moreover, in comparison with saline, M-13 showed a similar amount of bodyweight recovery as 7F. These results suggest that M-13 may have an anti-inflammatory effect on murine colitis.

Keywords: *Enterococcus*, mosquito, colitis

*POSTER No. 012*

# THE CHRONICLE OF CHIKUNGUNYA OUTBREAK IN THAILAND, 2009-2013

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Chikungunya fever is a dengue-like syndrome characterized by acute fever, arthralgia and maculopapular rash. Viral infections by mosquitoes continue to be a public health problem in Thailand. The occurrence of chikungunya epidemics in Thailand was revealed from a case investigation and disease surveillance report from the Bureau of Epidemiology, Ministry of Public Health, Thailand. The literature review revealed that there have been large chikungunya epidemics in the southern part of Thailand 2008-2009 affecting more than 30,000 people. Surveillance and clinical recognition will probably not prevent future outbreaks, but may give considerably help in organizing an early response to outbreaks and thus minimize unnecessare illness and death. This review seeks to provide the history of chikungunya epidemics in Thailand during 2009-2013 and the prospect for control of this disease which will be helpful for future research.

POSTER No. 013

# IMMUNOSUPPRESSION WITH DEXAMETHASONE AND CHIKUNGUNYA VIRUS INFECTION IN ICR MICE

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Chikungunya virus (CHIKV) infection is an emerging or re-emerging infectious disease in several countries including Thailand. The ecology of this virus involves human-mosquito-human and animal-mosquito-animal transmission cycles. CHIKV infection in ICR mice and that in immunosuppressed ICR mice were examined in this study. The first group or control group, 4-week-old mice were injected with 0.1 ml of normal saline by intraperitoneal route for 14 days. In the second group or immunosuppressed group, 4-week-old mice were injected with 0.1 ml or 100 mg of dexamethasone by intraperitoneal route for 14 days. All mice were then inoculated with 0.2 ml of CHIKV ($10^7$ CID$_{50}$/ml). Blood samples were collected and tested for CHIKV infection by using immunocytochemistry (ICC) and reverse transcription polymerase reaction (RT-PCR) for seven days post infection. For the control group, the average CHIKV levels in mice were $10^{2.0}$, $10^{1.0}$, $10^{2.4}$, $10^{3.5}$, $10^3$, $10^{2.7}$, and $10^{1.5}$ CID$_{50}$/ml of serum on day 1 to day 7 post infection (PI). However, only the serum samples of day 1, 2, and 4 PI were positive for CHIKV by using RT-PCR. For the immunosuppressed group, the average CHIKV levels in mice were $10^{2.7}$, $10^{3.0}$, $10^{4.7}$, $10^{3.5}$, $10^{2.8}$, $10^{3.0}$, and $10^{2.5}$ CID$_{50}$/ml of serum on day 1 to day 7 post infection (PI), respectively. However, only the serum samples of day 1 to day 6 PI were positive for CHIKV by using RT-PCR. Dexamethasone might affect CHIKV infection in ICR mice. Nevertheless, further studies are needed to confirm this finding.

Keywords: Chikungunya virus, Immunosuppression, ICR mice

POSTER No. 014

# TIMING OF B CELL AND T CELL RESPONSE DURING ACUTE DENGUE VIRAL INFECTION

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While B lymphocytes play important roles through antibody productions, CD8+ T cells release many types of inflammatory molecules. Both types of responses might play roles in the development of severe disease during the second infection with heterotypic serotype of dengue viral strain. Many studies showed high level of cross-reactive dengue specific antibody secreting cells (ASC), and generalized CD8+ T cell activation was also observed in dengue patients. The study of B cell and T cell response kinetics could provide an important clue on the association of these responses with disease severity. In this study, blood samples from dengue infected patients during febrile and afebrile stages were analyzed by polychromatic flow cytometry for B cell subsets based on the expression of CD21, CD27 and CD38. Activated CD8+ T cell subset was determined based on the expression of CD38 and HLA-DR. Results demonstrated that high levels of ASC and activated CD8+ T cells were observed in infected patients. However, while ASC was not measurable in infected patients until the last day of febrile phase, a significantly increased frequency of activated CD8+ T cell was observed on the same day. More importantly, significantly increased frequencies of ASC and activated CD8+ T cells were observed during afebrile phase. The result also showed a positive correlation between the frequencies of ASC and activated CD8+ T cells. This study suggests that the late appearances of B cell and T cell response might not be responsible for early triggering of a more sever disease of dengue infection.

Keywords: dengue, antibody secreting cell, activated CD8+ T cell, kinetic

POSTER No. 015

# MOLECULAR CHARACTERIZATION OF 4-DIPHOSPHOCYTIDYL-2C-METHYL-D-ERYTHRITOL KINASE (ISPE) GENE FROM *PLASMODIUM VIVAX*: LIGAND RECOGNITION IN A TEMPLATE FOR ANTIMALARIAL DRUG DISCOVERY

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The IspE gene is absent from humans but encoded in the *Plasmodium* genome. It is actively expressed throughout the intraerythrocytic stages of parasite.The stage responsible for the clinical symptoms of malaria. IspE is the only kinase in MEP pathway. It is a unique pathway through which plasmodium synthesis isoprenoid precursors. Isoprenoids support numerous important biological functions including respiration, photosynthesis, hormone-based signaling, protein cleavage and degradation, meiosis, apoptosis, etc.

The characterization and relative role of IspE have not yet been determined in any of the *Plasmodium species.* The main aim of our study is to characterize this enzyme in *Plasmodium vivax* at the molecular and biochemical level and Computer aided drug designing (CADD) against this enzyme. For that we have collected parasite (*P. vivax*) samples from five different geographical regions of India. Sequence analysis of the PvIspE gene have shown that it is conserved throughout India and has also shown a high degree of homology with all other sequences of Plasmodium species. A 3D model for the sequenced *PvIspE* has been constructed and we performed comparative modeling with multiple alignments with *P. falciparum* that shows high structural similarity. We performed docking studies using a number of known inhibitors and have designed some novel inhibitors against *Pv*IspE enzyme using Discovery studio3. Synthesis of novel compounds and their *in-vitro* testing is in process. This study may set the stage for structure-based design of novel small molecule inhibitors as antimalarial drugs. Moreover, the absence of *Pv*IspE in human hosts provides the opportunity to use it for diagnosis of malaria.

POSTER No. 016

# NEW PCR ASSAYS TO MEASURE EXPRESSION OF *PLASMODIUM VIVAX* RETICULOCYTE BINDING PROTEINS

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Invasion of red blood cells is a crucial step in malaria infection. Different malaria parasites have different preference for host cells. *Plasmodium falciparum* infects both reticulocytes and fully mature erythrocytes; *Plasmodium vivax* only infects reticulocytes. This difference is explained by the different ligand-receptor interactions used by these parasites. Two invasion ligand families have been implicated in host cell sensing during invasion: I. the Erythrocyte Binding Antigens (EBA) and II. the Reticulocyte Binding Proteins (RBP). The latter are believed to mediate specific binding of *P. vivax* merozoites to reticulocytes.

Protective immunity for malaria has been linked to the presence of antibodies against parasite invasion ligands. These proteins thus represent prime targets for vaccine development. While extensive studies have been performed on *P. falciparum* proteins and *P. vivax* Duffy Binding protein, a member of the EBA family, little has been done on *P. vivax* RBPs which comprise the vast majority of invasion ligands of this parasite. In this study, we aim to use quantitative polymerase chain reaction (qPCR) to measure the expression of the 9 different RBPs identified in the reference genome of *P. vivax.* The repertoire of RBP expression across different parasite isolates will provide valuable insights into *P. vivax* invasion and vaccine development. Here, we report our qPCR assays which have been optimized for robust quantification of RBP transcripts as well as the transcripts of house-keeping and schizont-specific genes. Preliminary measurements of a few clinical isolates are presented.

Keywords: qPCR / GENE Expression / *Plasmodium vivax* / Malaria Invasion / Reticulocyte Binding Proteins

*POSTER No. 017*

# INSIGHTS INTO THE DETECTION OF MALARIA PARASITES DNA FROM NON-INVASIVE BIOLOGICAL SAMPLES

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To study the progress towards malaria elimination and eradication, repeated blood samples by finger pick or venipuncture is needed for large-scale epidemiological investigations and for post treatment follow-up. Drawing blood increases the risk of contracting infectious diseases and is poorly accepted in some communities with blood taboos. Thus, noninvasive approaches are needed. We therefore evaluated the usefulness of saliva and urine samples in detecting human malaria infection. Saliva and urine samples were collected simultaneously with blood samples from 51 patients with microscopically confirmed *Plasmodium falciparum* (21) and *Plasmodium vivax* (30) infection. The 18S rRNA gene of *P. falciparum* and *P.vivax* was targeted in saliva, urine, and blood samples using nested PCR. The nested PCR of blood were positive in 20 and 26 patients for *P. falciparum* and *P.vivax* respectively. Of these, 4 patients were positive for both species (initially diagnosed as *P. falciparum* (1) and *P. vivax* (3)). The nested PCR of saliva and urine were 14% and 19% positive for *P. falciparum* respectively, while 10% were positive for *P. vivax* in saliva and urine. Both nested PCR results of saliva and urine samples had a specificity of 100% for identification of *P. falciparum* and *P. vivax* when compared with nested PCR results from blood samples. The sensitivity of non-invasively collected samples makes them (saliva, urine) unsuitable for the detection of parasite DNA using this pair of primers. Therefore, primers amplifying either smaller fragments or different target genes may increase the sensitivity of the protocol. Further studies are needed to validate these findings.

Keywords: Malaria, noninvasive, PCR, urine and saliva

POSTER No. 018

# FOCAL SCREENING AND TREATMENT AT HOUSEHOLDS AROUND PASSIVELY-DETECTED MALARIA CASES IN PAILIN PROVINCE, CAMBODIA: A FEASIBLE AND EFFECTIVE TOOL TO TARGET ASYMPTOMATIC INFECTIONS?

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Background: Malaria elimination in Pailin is a priority, due to the confirmed presence of artemisinin-resistant parasites. This study evaluated the feasibility of focal screening and treatment (FSAT) around passively-detected *Plasmodium* infections, as well as to assess the impact of FSAT in identifying and eliminating the asymptomatic reservoirs of *Plasmodium* in the population.

Methods: Following identification by RDT of malaria cases passively presenting to health facilities (the index case), targeted screening was conducted within three days among either i) residents of the index case household (nHH=255); ii) residents of the five households closest to the index (nHH=45); or iii) all residents of the closest 10 households (nHH=80). In addition, 60 households within the health centre catchment population were randomly selected for screening. Individuals provided a blood sample for microscopy, RDT, and PCR analysis.

Results: A total of 270 index cases were identified over nine months, prompting additional screening activities in the community. Of a total 1869 non-index case samples tested by PCR, 43 (2.3%) were positive for *Plasmodium* infection; 25 *P. falciparum*, 16 *P. vivax* and two mixed infections. We present these PCR data stratified by household selection criteria (index, nearby and randomly selected) to explore if or at what level FSAT around passively-detected symptomatic infections is useful in this context. Full details of risk factors for symptomatic and asymptomatic infection in this population will be presented. These data provide additional evidence to assess the usefulness of focal screening around symptomatic malaria cases as a strategy to identify further asymptomatic infections in the population.

POSTER No. 019

# QUANTITATIVE PROTEOMIC ANALYSIS OF *PLASMODIUM VIVAX* UIS3-INDUCED PROTEOME BY LABEL-FREE LC-MS

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Apicomplexans lack the ability to synthesize fatty acids *de novo* because it is different from human fatty acid synthesis. Plasmodium has an essential unique protein on its parasitovacuole membrane (PVM) called UIS3 (up-regulated infective sporozoite 3). UIS3 binds to host L-FABP (liver-fatty binding protein) in order to convey essential lipids through PVM. L-FABP plays an important role in lipid synthesis, it has been hypothesized that interaction between UIS3 and L-FABP interfere in the overall fatty acid metabolism of hepatocytes. Quantitative proteomics have been used for analysis of the protein expression level of hepatocytes. Mass spectrometry data were analyzed by Skyline software with MS1 filtering label free technique. It has been found that the protein involving in glycolytic pathway and fatty acid synthesis pathway such as citrate synthase, fatty acid synthase are up-regulated. This will be analyzed in a further study.

POSTER No. 020

# POTENTIAL ASSOCIATION OF BLOOD MONOCYTE PHENOTYPES AND PATHOLOGY IN *PLASMODIUM VIVAX* INFECTION

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Monocyte subpopulations display distinct phenotypes and functions which can drastically change during inflammatory states. Recently, the increased number of a pro-inflammatory monocytes subpopulation ($CD14^{lo}CD16^{hi}$) during *P. falciparum* infection was observed, with remarkable levels of CX3CR1, HLA-DR, TREM-1 and CD56 presented in this monocyte subset. However, the role of human blood monocytes in vivax malaria has never been explored. This study aimed to determine any potential association between blood monocyte phenotypes and clinical manifestation in patients with *P. vivax* infection. Monocyte phenotypes were characterized by immunophenotyping, followed by FACSCalibur, the 4 colors detected cytometer. The investigation was performed as a blind study. The result showed that on admission (D0), both percentage and absolute number of blood monocytes in patients with *P. vivax* infection are significantly increased (p= 0.0033 and p=0.0073, respectively). Interestingly, the numbers of $CX3CR1+CD14^{lo}CD16^{hi}$ blood monocytes are different among the 3 groups of thrombocytopenic patients with severe, moderate and mild thrombocytopenia, which is associated with blood platelet levels. In addition, a positive correlation between $CX3CR1+CD14^{lo}CD16^{hi}$ blood monocytes and percentage of hematocrit was observed (r=0.3168, p=0.0436). The results suggested that $CX3CR1+CD14^{lo}CD16^{hi}$ blood monocytes are induced during *P. vivax* infection, which may associate with pathology of the disease.

Keywords: *Plasmodium vivax,* human malaria, monocyte, pathology, thrombocytopenia

POSTER No. 021

# IDENTIFICATION OF NOVEL *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN COMPLEXES, FOR THE DEVELOPMENT OF A MULTI-COMPONENT MALARIA VACCINE

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Despite concerted efforts to control malaria, the disease is accountable for millions of deaths annually. A malaria vaccine would be a crucial component of anti-malarial programs as they are the most cost-effective tools for public health. Current attempts on malaria vaccine development are primarily focused on a complex, multi-stage and multicomponent vaccine. Using complementary approaches such as protein-protein interactions, yeast/bacterial two hybrid analyses and immune-precipitation followed by mass spectrometry, a large protein complex on the merozoite surface has been identified. The complex consists of merozoite surface proteins, MSP-1, -3, -6, -7, -9, PfRhopH3, PfRhopH1, PfRAP-1, PfRAP-2 and two RAP domain containing proteins. The present study carried out detailed *in silico* domain analysis of these proteins. Based on *in silico* domain analysis, RAP1, RAP2, RhopH3, Clag9, MSP-6 and MSP-7 protein fragments have been cloned and expressed in a bacterial expression system for protein-protein interaction studies. Eleven candidate proteins have been identified on the merozoite surface as having high molecular weight complex, probably having role in RBC invasion so the parasite can continue the life cycle. The antibodies generated from merozoite surface proteins will be used for blocking the invasion process of merozoites using In-vitro Growth Inhibition Assay (GIA). This study will lead to the development of a subunit multicomponent vaccine based on the virtual complex that was identified using various biochemical studies.

Keywords: malaria, vaccine, *Plasmodium falciparum*, Disease

POSTER No. 022

# PERSISTENCE OF *P. FALCIPARUM* DIAGNOSTIC ANTIGENS AFTER TREATMENT WITH ARTEMISININS : ASSOCIATION WITH PARASITE STAGE AND MECHANISM OF CLEARANCE

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The most sensitive rapid diagnostic tests of *P. falciparum* are based on detection of histidine-rich protein 2 (HRP2), but HRP2 persists in the blood after treatment and cannot be used to assess treatment response. We measured antigen levels over a 4-week period in ACT-treated hyperparasitaemic patients with *P. falciparum* in Uganda and Thailand (prior to the emergence of artemisinin resistance). Antigen clearance was quantified by calculation of area-under-curve (AUC) after normalization to baseline antigen level. Whole blood HRP2 levels showed a wide range of AUCs: Uganda median 415 d·% (range 48–2588); Thailand 590 d·% (33–1534). The primary determinant of AUC was admission stage; in patients presenting with >75% tiny/small rings (32/40 in Uganda, 24/38 in Thailand), median HRP2 AUCs were respectively 6.4 and 10.7-fold higher than cases with later stages (p=0.0005, <0.0001). Stage was also a key determinant of the time for the Paracheckä RDT to become negative during follow-up. These data support the hypothesis that the mechanism for HRP2 persistence is pitting in the spleen since once-infected erythrocytes returning to the circulation can provide a source for persistently high levels of HRP2 and ongoing positivity of HRP2-based RDTs.

Keywords: malaria falciparum RDT HRP2 persistence

POSTER No. 023

# EPITOPE MAPPING OF INHIBITORY ANTIBODIES TARGETING THE LOOP REGION OF *PLASMODIUM FALCIPARUM* ENOLASE USING SYNTHETIC PEPTIDE LIBRARIES

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**Objective -** Our sero-epidemiological analysis in Japan and endemic regions suggested that the 47 kD antigen of *Plasmodium falciparum*, enolase, provokes some immune responses which may be crucial for the host to overcome the acute symptomatic stage of falciparum malaria. Other synthetic studies have shown that a series of partial peptides of *P.f.* enolase have antigenic reactivities against patients' sera and they can produce inhibitory antibodies against in vitro parasite growth. Among several sequences, we have especially focused on a loop region of the *P.f.* enolase sequence, $\mathrm{A}^{256}$SEFYNSENKTYDLDFKTPNND$^{277}$ (AD22), which would be a suitable target for an inhibitory antibody. In this poster, we will discuss the epitope mapping studies using synthetic peptide libraries for monoclonal antibodies (mAbs) raised against AD22. The reactivities were also compared with malaria patients' sera.

**Methods -** mAbs were produced by a conjugate of AD22 peptide and maleimide-activated hemocyanin as the immunogen following the standard hybridoma technique.

**Result -** The first screening was done for a library of the 35-amino-acids sequence at the loop region of *P.f.* enolase. It was divided into six overlapping peptides of 10 amino acids each with an offset of five amino acids, and analyzed with mAbs. Interestingly, all the mAbs strongly reacted with one specific sequence, $\mathrm{N}^{264}$KTYDLDFKT$^{273}$. To precisely map the sequence of the binding domain, another library with an offset of 1 amino acid and a peptide length of 6-10 amino acids was prepared. The screening results identified an 8-amino-acid-peptide with the sequence of $\mathrm{N}^{264}$KTYDLDF$^{271}$ as the minimal binding motif of mAbs.

Keywords: Synthetic peptide, Epitope mapping, Anti-enolase antibodies, Malaria.

*POSTER No. 024*

# ANALYSIS OF EPONEMYCIN (α'-β EPOXYKETONE) ANALOGOUS COMPOUNDS FROM *STREPTOMYCES HYGROSCOPICUS* HYGROSCOPICUS EXTRACTS AND ITS ANTI-PLASMODIAL ACTIVITY

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**Background:** The greatest challenge in reducing the high mortality and morbidity of malaria is due to the emerging incidence of artemisinin resistance. *Streptomyces hygrocopicus* contains eponemycin that demonstrates anti-plasmodial activity by inhibiting the activity of 20S proteasome in Ubiquitin-Proteasome System (UPS).

**Aim:** To analyze the eponemycin analog contained in crude metabolite extract of *S. hygrocopicus* Hygroscopicus and to reveal its capability as anti-plasmodial in BALB/C mice infected with *Plasmodium berghei.*

**Method:** Isolates of *S. hygroscopicus* Hygroscopicus were made by the maceration extraction method using ethyl acetate: ISP4 (1:1 v/v). Analysis of Dihydroeponemycin was conducted by Thin Layer Chromatography and High Performance Liquid Chromatography. Mice were divided into control (infected mice without treatment) and 3 treatment groups (infected mice treated with crude metabolite extract dose 130, 580 and 2600 μg/kgBW for 5 days). The degree of parasitemia and spleen morphological changes were observed.

**Result:** TLC analysis showed a spot with Refractory factor (Rf) 0.7. HPLC result showed that the level of Dyhidroeponemycin on ABAR sample was 3.768% and on BAR sample was 5.796%. The degree of parasitemia of all treatment groups decreased significantly ($p < 0.05$). The highest inhibition demonstrated by 2600 μg/kgBW compared to 130 and 580 μg/kgBW dose ($p=0.002$ and $p=0.018$). A strong inverse correlation was shown between extract dose and the degree of parasitemia on day-3 till day-6 of treatment ($p<0.05$). Spleen histological examination showed morphological improvement in all treatment groups.

**Conclusion:** Eponemycin analog in crude metabolite extracts of *S. hygroscopicus* Hygroscopicus is a potential candidate for a new antimalarial drug.

**Keywords:** TLC, HPLC, S. *hygroscopicus*, malaria, eponemycin, UPS

POSTER No. 025

# METABOLITE EXTRACT OF *STREPTOMYCES HYGROSCOPICUS* SUBSP. HYGROSCOPICUS CAUSES MORPHOLOGICAL DAMAGE, DECREASE OF PARASITE DEGREE, AND DECREASE OF DNA DENSITY OF *PLASMODIUM FALCIPARUM* 3D7 IN VITRO

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**Background:** *Plasmodium falciparum* resistance to antimalarial drugs increases the burden of malaria worldwide. Recently many researches have placed 20S proteasome within ubiquitin – proteasome system (UPS) as a novel therapeutic target against this resistance. Secondary metabolite of *Streptomyces hygrocopicus* subsp. Hygroscopicus contains eponemycin that is known for having 20s proteasome inhibitory activities in UPS.

**Aim:** To reveal if metabolite extract of *Streptomyces hygrocopicus* subsp. Hygroscopicus can decrease the parasite degree and DNA density of *Plasmodium falciparum* 3D7 through its 20S proteasome inhibition activity.

**Method:** This was an experimental study using *Plasmodium falciparum* 3D7 culture which treated with 0.02, 0.104, 0.52, 2.6, 13 mg/ml of crude metabolite extract of *Streptomyces hygrocopicus* subsp. Hygroscopicus. DNA parasite density was measured using flow cytometry after 8 hours of treatment and the degree of parasite as well as parasite morphology were observed after 12 hours of treatment.

**Result:** Treatment with crude metabolite extract of *Streptomyces hygrocopicus* subsp. Hygroscopicus resulted in damage of parasite asexual stages which turned into crisis form. DNA parasite density significantly decreased in the group treated with compound doses of 2.6 mg/ml and 13 mg/ml ($p= 0.002$; $p=0,024$) as well as in the degree of parasite in all groups compared to control ($p<0.05$). A higher dose of extract led to a decrease of DNA density and the degree of parasite ($p= 0.001$, $r = -0.629$; $p= 0.000$, $r = -0.772$).

**Conclusion:** Metabolite Extract of *Streptomyces hygroscopicus* subsp. Hygroscopicus causes morphological damage and decrease of parasite and DNA density of *Plasmodium falciparum.*

**Keywords:** *Streptomyces hygroscopicus* subsp. *Hygroscopicus*, *Plasmodium falciparum* 3D7, Morphology, DNA Parasite Density, Parasite Degree

POSTER No. 026

# LOW MONOCYTE TO NEUTROPHIL RATIO IN PERIPHERAL BLOOD ASSOCIATED WITH DISEASE COMPLICATION IN PRIMARY *PLASMODIUM FALCIPARUM* INFECTION

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Immunity to malaria can be acquired but only after repeat exposures to polymorphic *Plasmodium*. However, the development of clinical outcomes during *P. falciparum* infection is not clearly understood. This study elucidated whether monocytes, neutrophils and pro/anti-inflammatory cytokines were associated with clinical outcomes in single infection and prior repeated malaria infections. Two hundred and seventy-nine patients with complicated and uncomplicated malaria were investigated. Peripheral blood IFN-γ, TNF-αand IL-10 levels were measured by ELISA, and monocytes and neutrophils by an automated cell counter. On admission, in patients with uncomplicated malaria prior repeated infections, absolute neutrophil counts were positively and monocyte to neutrophil ratio negatively correlated significantly with parasitemia ($r = 0.358$, $p = 0.000$; $r = -0.356$, $p = 0.000$, respectively), while those with single infection absolute monocyte counts and monocyte to neutrophil ratio were significantly correlated negatively with IFN-γ ($r = -0.381$, $p = 0.001$; $r = -0.393$, $p = 0.000$), and positively with TNF-α levels ($r = 0.310$, $p = 0.007$; $r = 0.227$, $p = 0.017$). In sharp contrast, in complicated malaria with single infection extremely high IFN-γ and IL-10 levels but significantly low percent monocyte counts and monocyte to neutrophil ratio were seen. After 7 days of treatment, absolute monocyte counts and monocyte to neutrophil ratio were significantly increased, while absolute neutrophil counts significantly decreased ($p = 0.000$, 0.000, and 0.001), similarly after 28 days of treatment ($p = 0.008$, 0.000 and 0.000). These results suggest different functions of monocytes, neutrophils and pro/anti-inflammatory cytokines in complicated and uncomplicated malaria with single *P. falciparum* infection or prior repeated infections in the context of disease severity. Low monocyte to neutrophil ratio may be regarded as a risk factor in developing complication in primary malaria infection.

POSTER No. 027

# POTENTIAL PHARMACOKINETIC INTERACTIONS OF PRIMAQUINE AND BLOOD SCHIZONTOCIDES IN HEALTHY THAI VOLUNTEERS

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Primaquine is a drug widely used with other schizontocides in treating malaria. It has been used with chloroquine for radical treatment of vivax malaria, and with artemisinin-based combination therapy (ACT) as a gametocytocide to contain the spread of artemisinin-resistant *Plasmodium falciparum*. Since primaquine and many other antimalarials are metabolized by cytochrome P450, drug-drug interactions are likely to occur. We conducted 3 crossover studies in healthy Thai adult volunteers to determine potential pharmacokinetic interactions between primaquine and three other important blood schizontocides, namely chloroquine, dihydroartemisinin-piperaquine, and pyronaridine-artesunate. In each study, all volunteers were randomised into two groups of three sequential hospital admissions to receive 30 mg base primaquine, the blood schizontocide under study (i.e., 600 mg base chloroquine, 120-960 mg dihydroartemisinin-piperaquine or 540-180 mg pyronaridine-artesunate) 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. All treatment regimens were well-tolerated and none was associated with significant electrocardiographic QT prolongation. The single oral dose of primaquine did not result in any significant pharmacokinetic alterations of the studied schizontocides. In contrast, the co-administration with these long half-life schizontocides significantly increased plasma primaquine concentrations. These results suggest a synergistic effect of chloroquine and primaquine for curative treatment in vivax malaria. They also confirm the recent WHO guideline that primaquine is to be given in addition to ACT on the first day of treatment of *falciparum* malaria.

Keywords: Antimalarial drugs, phase I studies

*POSTER No. 028*

# ASSESSMENT OF ADHERENCE TO A THREE DAY COURSE OF ARTEMETHER-LUMEFANTRINE TREATMENT IN RAKHINE STATE, MYANMAR

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**Background:** The development and spread of drug-resistant strains of malaria parasites has been identified as a key factor in malaria resurgence and is one of the greatest challenges to malaria control today. It is important to prevent or delay significantly the spread of artemisinin resistant parasites within the country and beyond its borders. Efforts to prevent deterioration of this problem are, therefore, at present directed towards finding novel ways of intervening to improve adherence and prevent the irrational use of currently available multi-dose anti-malarial drugs. The primary objective of this study was to access the level of patient adherence to artemether-lumefantrine under routine conditions of prescription.

**Method and Technique:** Patients who consulted the fixed clinic or mobile clinic were routinely examined and screened for eligibility of inclusion in the study. The malaria positive *P. falciparum* malaria cases were treated with six doses of artemether-lumefantrine. Patient adherence was conducted by questionnaire and counting remaining pills on day 3 of anti-malaria treatment at their home. The adherence level was classified into three categories: definitely non-adherent, probably non-adherent and probably adherent. Reasons for being definitely non-adherent were also assessed.

**Results:** Out of the total initially enrolled 175 patients, 161 (92%) patients completed the follow-up. Out of these, 138 (85.7%) were classified as probably adherent, 8 (5%) as probably non-adherent, and 15(9.3%) were definitely non-adherent. 40% of patients among definitely non-adherent group were age more than 15 years of age while 2-4 years of age compromised 70% of probably non-adherent group.

*POSTER No. 029*

# INVESTIGATION OF REGULATORY SNPS IN THE PROMOTER REGION OF TNF-α, INF-γ AND IL-10 GENES AND THEIR ASSOCIATION WITH MALARIA CLINICAL OUTCOMES IN A BRAZILIAN AMAZONIAN POPULATION

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**Introduction:** Host genetic factors contribute to the variability of malaria phenotypes and thus should help to determine some of the mechanisms involved in the susceptibility to *Plasmodium* infection. In order to identify polymorphisms that might be associated with malaria clinical outcomes in Brazil, we sequenced the promoter regions of cytokines genes related to malaria response to investigate the impact of these genetic variations with susceptibility/resistance to *Plasmodium* infection.

**Methodology:** DNA samples were isolated from 702 individuals and the association tests included four groups: 1) *never_malaria* group as controls; 2) *clinical_malaria* group; 3) *asymptomatic_Plasmodium infection* group and 4) *any_malaria* group (individuals with history of previous malaria, clinical malaria and asymptomatic *Plasmodium* infection).

**Results:** After data analysis we have identified a significant association ($P<0.05$) between the IL10 -1117GG genotype with a reduced risk to malaria ($p=0,0351$), haplotypes ACC and GCC (-1117/-854/-627) associated with increased ($p=0,037$) and reduced risk ($p=0,001$) of *Plasmodium* infection respectively; an association of the TNF-a -308AA genotype with susceptibility to malaria ($p=0,0213$)and identified the haplotype AGAGA (-572/-376/-308/-238/-122) associated with increased susceptibility to malaria ($p=0,028$).

**Discussion and Conclusion:** Further studies should include the functional analysis of the TNF/INF SNPs and genetic association studies of others pro and anti-inflamatory cytokines to identify additional functional variants most relevant for malaria in Brazil.

**Funding:** CNPq/DECIT-Brazil.

POSTER No. 030

# EXPERIMENTAL STUDY OF *PLASMODIUM GALLINACEUM* INFECTION IN RHODE ISLAND RED AND ISA BABCOCK B 380 CHICKENS

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*Plasmodium gallinaceum* is an apicomplexan intracellular protozoan that causes avian malaria disease, especially in domestic chickens. *P. gallinaceum* is transmitted by mosquito vectors, and the development of this parasite in the erythrocytic stage has two forms; asexual and gametocyte forms. The objective of this study was to investigate *P. gallinaceum* levels in chickens which were inoculated with $10^6$, $10^4$ and $10^2$ *P. gallinaceum* infected red blood cells (iRBCs). In this study, two breeds of chicken were included. The first group, 14–day–old Rhode Island Red chickens (n=15), were divided into three subgroups (n=5), each of which was intravenously inoculated with $10^6$, $10^4$, and $10^2$ iRBCs. In the second group, 7–day–old Isa Babcock B 380 chickens (n=30), were divided into three subgroups (n=10), each of which was intravenously inoculated with $10^6$, $10^4$, and $10^2$ iRBCs. The percentage of parasite and gametocyte were examined from day 3 to day 21 post inoculation (PI). $10^6$ iRBCs dose can infect both breeds of chicken, but $10^4$ and $10^2$ iRBCs dose could only infect Isa Babcock chickens. The percentages of parasite and gametocyte were related to the duration of infection in Rhode Island Red chickens. The comparison of infection levels between $10^6$ and $10^4$ iRBCs in Isa Babcock chickens indicated that parasite levels in infected chickens with $10^6$ iRBCs were significantly higher than the other level on day 5 to day 10 PI ($p<0.05$). The results suggest that chicken breed, chicken age, and infective dose affected parasite infection as well as its level of infection.

Keywords: *Plasmodium gallinaceum* Infection, Rhode Island Red, Isa Babcock B 380 chickens

*POSTER No. 031*

# FACTORS INFLUENCE THE SHEDDING OF *BLASTOCYSTIS* CYSTS IN AN IRRITABLE BOWEL SYNDROME (IBS) PATIENT-AN EVIDENCE BASED CASE STUDY

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*Blastocystis*, a common gut parasites found in the intestinal tract of humans and animals. Many studies have been carried out to associate *Blastocystis* and Irritable bowel syndrome (IBS). In this study, we assessed the factors influencing the shedding pattern of cysts from a *Blastocystis* ST3-infected IBS patient who volunteered her stool samples for 30 days. A questionnaire on the factors was designed to assess the following information a) the frequency of toilet visits in a day, b) the timing of frequenting the toilet, c) stool forms, d) type of mood the patient was in whilst frequenting the toilet and e) food intake. The highest average cyst count recorded was 22.2X$10^6$ cysts/g, recordedwhen the patient visited the toilet 3 times a day. Frequenting the toilet between 6 am to 12 noon showed the highest average number of cyst count i.e. 21.7 X $10^6$ cysts/g and semi-solid forms showed the highest cyst count i.e, 2.00X$10^6$cysts/g. Irregular shedding of cysts within a day can be observed in 10 out of 30 days where the widest range was recorded on day 17, 0 to 1.2X$10^6$ cysts/g. The average daily cyst count on days of emotional fluctuations was from 0 to 5.13X$10^6$ cysts/g compared to 0.001X$10^6$ to 0.144X$10^6$ cysts/g on days when the patient was happy and unperturbed by events. In conclusion, the study confirms that there are factors influencing shedding patterns of *Blastocystis* and these have important implications when it comes to diagnosis as well as transmission of the parasite.-

Keywords: Blastocystis cyst, Phenotypic, Subtype 3, Pathogenicity, shedding pattern of cysts

POSTER No. 032

# CHARACTERIZATION OF MONOCLONAL ANTIBODY SPECIFIC TO *ENTAMOEBA HISTOLYTICA* CELLS AND ERYTHROPHAGOSOMES

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**Objective**: *Entamoeba histolytica* is a pathogenic protozoan which causes the third most deaths from parasitic diseases worldwide. Monoclonal antibodies (mAbs), which are specific to *E. histotytica* were successfully produced previously. In this study, *E. histolytica* specific mAbs were characterized for localization of mAb-binding partner in the live *E. histolytica* trophozoites.

**Methods:** Trophozoite cells were stained with a mAb and analyzed by fluorescent microscopy and flow cytometry, to investigate the localization of a mAb reacting protein in the pathogen cells. mAbs were further tested with proteins extracted from cellular fractions of *E. histolytica* using ELISA. Trophozoites were optimally challenged with red blood cells and proteins were solubilized from the erythrophagocytosed cells by detergents. Soluble proteins were incubated with protein G-beads linked mAb#32 or a control mAb#19. *E. histolytica* proteins pulled down by mAbs were analyzed with SDS-PAGE and visualized by silver stain.

**Results and Discussion**: The results showed that most of the mAbs bind to cytoplasmic proteins, with 4 clones interacting with the membrane proteins and two clones reacting with phagosomes located inside the cells. It was found that challenging *E. histolytica* cells with 0.25% human red blood cells for 1 hour at 37˚C resulted in the highest yield of the erythrophagosomes. SDS-PAGE revealed *E. histolytica* proteins with a molecular masses of about 60-65 kDa and more than 170 kDa, which are successfully pulled down by mAb #32. These proteins will be subjected to further protein identification by mass spectrometry and may play role erythrophagocytosis, which is a virulent factor of the parasite during tissue invasion.

**Keywords:** *Entamoeba histolytica*, erythrophagocytosis, monoclonal antibody

POSTER No. 033

# DETECTION AND MOLECULAR CHARACTERIZATION OF DOUBLE-STRANDED RNA VIRUSES IN PHILIPPINE *TRICHOMONAS VAGINALIS* ISOLATES

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The flagellated protozoon *Trichomonas vaginalis* that parasitizes the urogenital tract of humans was reported to harbour double-stranded RNA (dsRNA) viruses. These viruses, identified as *Trichomonas vaginalis* virus (TVV), belong to the genus *Trichomonasvirus* of the family *Totiviridae*. Four species, formally recognized by International Committee on Taxonomy of Viruses (ICTV), have been reported and distinguished by pairwise comparison of sequences of genes coding for major capsid protein (CP) and RNA-dependent RNA polymerase (RdRp). Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify the complimentary DNA of target virus genes coding for CP and RdRp. Pairwise comparison of the sequences confirmed the identity of the TVV isolates from *T. vaginalis* cultures. A total of 35 dsRNA viruses were identified from 18 *T. vaginalis* isolates. Single infections were found in isolates harbouring TVV1 and TVV2. Multiple infections were also observed in 6 of the 18 *T. vaginalis* cultures. Phylogenetic analyses using maximum likelihood show that TVV1 and TVV2 isolates have monophyletic group while TVV3 and TVV4 appear paraphyletic. Subgrouping was observed in TVV1 and non-distinct clustering of TVV2, TVV3 and TVV4 with the reference isolates was also observed. This is the first study to report on the detection of all known TVV and its multiple occurrence in *T. vaginalis* isolates.

Keywords: *Trichomonas vaginalis* virus (TVV), Reverse transcription polymerase chain reaction (RT-PCR), Capsid protein (CP) gene, RNA-dependent RNA polymerase (RdRp) gene, Philippines

POSTER No. 034

# FIRST REPORT ON MOLECULAR DETECTION OF *ENTEROCYTOZOON BIENEUSI* IN BLOOD FROM HIV/AIDS PATIENTS

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Human microsporidia parasite infections have been reported from all over the world, and the majority of cases involving immunocompromised patients. *Enterocytozoon bieneusi* is being increasingly recognized as one of the most common microsporidian species infecting HIV/AIDS patients. To date, routine diagnosis of microsporidiosis is limited to stool examination, which is unable to rule out systemic infection. There are few reports on disseminated cases of *E. bieneusi*. The purpose of this study is to detect the presence of disseminated *E. bieneusi* infection in the blood of HIV/AIDS patients via PCR assay. During July 2013 to August 2014, blood samples from 93 HIV-positive patients with CD4 count ≤ 200cell/mm$^3$ were collected from Sungai Buloh Hospital and Universiti Kebangsaan Malaysia Medical Centre (UKMMC), and subjected to PCR assay using species-specific primer EBIEF1/EBIER1. A total of 19 out of 93 HIV-positive patients (20.4%) were confirmed positive for *E. bieneusi* by PCR. A fragment of 607 bp was successfully amplified from all positive samples. To our knowledge, this is the first study to report on the molecular detection of disseminated *E. bieneusi* infection in HIV/AIDS patients. This study may aid in the diagnosis and treatment of patients with disseminated microsporidiosis.

Keywords: microsporidia, *E. bieneusi*, HIV patients, disseminated microsporidiosis, PCR

POSTER No. 035

# KNOWLEDGE, PERCEPTION AND HEALTH RISK BEHAVIOR OF *OPISTHORCHIS VIVERRINI* INFECTION IN UPPER NORTHEAST THAILAND, 2013

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*Opisthorchis viverrini* remains a major public health problem in many parts of Southeast Asia, including Thailand. *OV* infection is induced by eating raw or uncooked fish products. This study is a cross-sectional study aiming to study the risk behavior of *O. viverrini* infection. The subjects were chosen by using 30 clusters sampling technique from 7 Provinces which cover and represent the population in upper northeast Thailand. The data was collected by questionnaire and stool examination by using modified Kato Katz technique. A total of 3,916 subjects were obtained from 1,782 males and 2,134 females, mean age 43 years. The people knew that eating raw fish caused *OV.* They knew that eating raw freshwater fish was a cause of opisthorchiasis (91%), but did not know how to take praziquantal correctly, and did not know the side effects of medication (58.8% and 52.8% respectively). They perceived that it is their way of life since the time of their ancestors, and a delicacy. Currently, 67.1% reportedly continue to eat raw fish and 90.1% eat raw fermented fish. 10.3% of people have never thought about giving up raw fish salad (Koi pra), where as 10.5% of them have never considered given up raw fish (Lap pra). *O viverrini* is still a problem in some areas of the upper Northeast. The results of this study showed that, to prevent these diseases, peoples' behavior need to be adapted, and they should be educated about the dangers of the disease.

Keywords: *Opisthorchis viverrini*, Knowledge, Perception, Health risk behavior

POSTER No. 036

# PREVALENCE OF *OPISTHORCHIS VIVERRINI* INFECTION AND BEHAVIOR FOR PREVENTION AND CONTROL AMONG PEOPLE, 7th HEALTH SERVICETHAILAND, 2014

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A liver 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. The subjects were chosen by using 30 Clusters Sampling technique from 4 provinces which represent the whole population in 7th health service Thailand. The data collected by questionnaire, and stool examination by using method of Modified Kato Katz. A total of 2,336 subjects were obtained from males (43.7%) and females (56.3%), mean age 47 years. Stool examination showed that 24.7% were infected with *O. viverrini*. High endemic area (prevalence > 10 %) about 4 of them due to the low priority in national plan. The subjects knew that eating raw freshwater fish was a cause of opisthorchiasis (87.5%), not knowing how to take praziquantal correctly (49.2%). The people still enjoy eating raw fish 66.3%. 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 7th health service. The people were not aware of the severity of *O. viverrini* infection. 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: *Opisthorchis viverrini*, Prevalence, 7th Health service

POSTER No. 037

# PREVALENCE OF OV INFECTION IN REGION HEALTH SERVICE NO.7, NORTHEAST THAILAND, 2014

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Liver fluke, *Opisthorchis viverrini* (OV), is the major cause of the high incidence of cholangiocarcinoma (CCA) in Thailand. The prevalence of OV infection remains high in various parts of the country, especially in wetland rural areas where a large proportion of the community work in agriculture and continue the traditional practice of eating raw or uncooked cyprinoid fish products. This is a cross-sectional study aiming to study the prevalence of *O. viverrini* infection. The study was chosen by using 30 clusters sampling technique from 4 Provinces which cover and represent the population in regional Health service No. 7. The data was collected by questionnaire and stool examination by using a modified Kato Katz technique. The participant survey covered 120 villages, aged 15-70 years. Stool examinations showed that 17.7% were infected with OV. Male infections were slightly more common than female. A total of 3,068 samples were obtained from 1,782 males and 2,134 females, mean age 43 years. The mean egg per gram of feces (MEPG) was 300.81 eggs, and most intensity was of low level (94.08%). The study suggests that opisthorchiasis is still a problem in the study area, and infection is more common in the elder groups than the younger. Some areas had a high positive rate (>10%), most of them loaded in low level. Studying ways to reduce risky behavior that leads to infections is necessary to decrease morbidity of opisthorchiasis and CCA.

Keywords: Prevalence, *OV.* infection

*POSTER No. 038*

# CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF PROTEIN 14-3-3 IN CARCINOGENIC LIVERFLUKE *OPISTHORCHIS VIVERRINI*

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** Presenter*

*Opisthorchis viverrini* causes the food-borne disease opisthorchiasis that is involved in various hepatobiliary abnormalities including one of the most common forms of liver cancer sub-types- cholangiocarcinoma (CCA). One of the predicted mechanisms in causing bile duct cancer by these flukes is their capability to secrete carcinogenic excretory-secretory (ES) products. These products play pivotal roles in parasites survival. Many of OV-ES products have been uncovered and predicted to be responsible for causing cancer; one of them could be protein 14-3-3. 14-3-3 are phosphoserine/phosphothreonine binding proteins, which are involved in many biological processes including cell cycle regulation, protein trafficking, metabolic regulation, cell proliferation and apoptosis. Proteomic analysis found that there are two isoform of 14-3-3 protein (14-3-3 ζ and 14-3-3 ε) identified in its ES proteins. Their function in *O.viverrini* induced cancer has never been studied. In this study we characterize these proteins by expressing the recombinant protein in Escherichia coli system, mice immunization to get specific antibody for western blot and immunolocalization, recombinant protein will be co-cultured with cholangiocyte cell line to study functional study. Currently, we have recombinant proteins and specific antibodies. The specific antibody can recognize the native protein in O. viverrini protein extract. Moreover, the recombinant protein could be recognized by O. viverrini infected hamster serum. The immunolocalization and effect of protein 14-3-3s on cholangiocyte will be further investigated. The ultimate aim of this study is to elucidate the mechanism of host-parasite interaction and carcinogenesis of O.viverrini induced cholangiocarcinoma.

Keywords: OV-ES, Opisthorchiasis, 14-3-3 protein, Cholangiocarcinoma

POSTER No. 039

# EFFECTS OF PEROXIREDOXIN 6 EXPRESSION ON NF-KB-MEDIATED INFLAMMATION IN *CLONORCHIS SINENSIS* EXCRETORY-SECRETORY PRODUCTS TREATED HUMAN CHOLANGIOCARCINOMA CELLS

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*Clonorchis sinensis* is a carcinogenic human liver fluke by which chronic infection in strongly associated with the development of cholangiocarcinoma. Liver flukes induce chronic inflammation leading to oxidation DNA damage of the infected biliary epithelium and malignant transformation. Our previous report that free radicals enzymatically triggered by *C. sinensis* excretory–secretory products (ESPs) cause NF-κB-mediated inflammation in human cholangiocarcinoma cells (HuCCT1). In the present study, we further examined the effect of free radical modulation on NF-κB-mediated inflammation in ESP treated HuCCT1 cells. ESP-triggered intracellular free radical generation was decreased by the change in the expression of an antioxidant enzyme, Peroxiredoxin 6 (Prdx 6). Overexpression of Prdx 6 protein resulted in the attenuation of NF-κB-mediated inflammation by inhibiting the activation of NADPH oxidase and inducible nitric oxide synthase. Although NF-κB suppressed Prdx 6 transcriptional activation via binding to κB sites within Prdx 6 promoter, other redox sensitive transcription factors transactivated the Prdx 6 expression. Immunohistochemical analysis showed that increased expression of Prdx 6 was observed mainly in hyperplasia region of infected liver, whereas phospho-p65 subunit of NF-κB in the nucleus of inflammatory cells. These findings provide new insights into different pathophysiologic roles of Prdx 6 and NF-κB in hepatobiliary diseases caused by clonorchiasis.

POSTER No. 040

# CERCARIAL INFECTIONS OF BRACKISH WATER SNAILS ON THE EAST COAST OF THE SOUTH THAILAND

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Brackish water snails are suspected to be an intermediate host of trematode diseases in vertebrates. The aim of this study is to investigate cercarial infection of brackish water snails along the east coast of the southern Thailand. Snails were collected from forty one locations of mangrove forests and estuaries in six provinces (Phetchaburi, Phachuap Khiri Khan, Chumphon, Suratthani, Nakhonsithammarat and Songkhla) between June 2013 and March 2014. Five collectors picked the snails by hand and scoop for 10 minutes at each station. The cercariae that emerged were identified based on their morphological characteristics. A total of 8,729 brackish water snails were classified into ten families, namely Neritidae, Ellobiidae, Haminoeidae, Muricidae, Nassariidae, Littorinidae, Stenothyridae, Thiaridae, Potamididae and Assimineidae. Seven species were found to have trematode infections, specifically *Cerithidea cingulata*, *Cerithidea djadjariensis, Cerithidea alata*, *Cerithidea quadrata, Clithon pequensis, Sermyla riqueti* and *Assiminea brevicula*. The infection rates were 0.19% (17/8,729), 0.05% (4/8,729), 0.01% (1/8,729), 0.01% (1/8,729), 0.05% (4/8,729), 0.72% (63/8,729) and 0.07% (7/8,729),. The cercariae were categorized into nine species, *Cloacitrema philippinum, Parorchis acanthus, Haplorchis taichui*, *Metorchis intermedius*, *Stictodora tridactyla*, *Ascorhytis charadriformis*, Larvae of *Mesostephanus appendiculatoides*, *Hypoderaeum conoideam* and *Himasthla interrupta*.

Keywords: brackish water, snail, trematode infection, the South of Thailand

POSTER No. 041

# CO-ENDEMICITY OF *STRONGYLOIDES STERCORALIS* AND *OPISTHORCHIS VIVERRINI* IN NORTHEAST THAILAND

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Strongyloidiasis, caused by *Strongyloides stercoralis,* is a neglected tropical disease (NTD) that is distributed globally, including Thailand. This study was carried out to determine the prevalence of parasitic infections, especially *S. stercoralis,* in areas known to also be endemic for opisthorchiasis in Northeastern Thailand. A survey conducted in Khon Kaen Province, Northeast Thailand (n=1,280) revealed that the most common parasite was *Opisthorchis viverrini* (39.1%) followed by *S. stercoralis* (8.9%). Others parasites found in this area were minute intestinal flukes, *Taenia* spp., *Echinostoma* spp., Hookworm and *Trichuris trichiura*. The agar plate culture method (APCT) which is sensitive for strongyloidiasis performed simultaneously gave two folds higher prevalence (16.5%) of *S.stercoralis*. Concurrent infections between *S. stercoralis* with *O. viverrini* were in found in 7.89% of subjects. Seroprevalence based on serum-based enzyme-linked immunosorbant assay (ELISA) (n=73) yielded the prevalence of 73.97% compared with 53.42% by APCT and 28.77% by FECT. The results indicated that the prevalence of *S. stercoralis* is still high and is the second most common parasite after *O. viverrini.* The real values could be even higher if serological methods are employed. Therefore, further studies in larger areas in Northeast Thailand and other opisthorchiasis endemic areas are required for better prevention and control of these parasitic infections.

Keywords: *Strongyloides stercoralis*, *Opisthorchis viverrini*, parasitological method, prevalence, northeast Thailand

POSTER No. 042

# IMMUNOMICS ANALYSISOF EXCRETORY-SECRETORY ANTIGENS FROM 3RD STAGE LARVA *GNATHOSTOMA SPINIGERUM* FOR DEVELOPMENTOF IMMUNODIAGNOSIS

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The parasite *Gnathostoma spinigerum* is a causative agent of gnathostomiasis and can be found in several countries. Infection leads to permanent organ damage or death, therefore rapid and reliable diagnosis is necessary and a major priority for effective treatment. In our investigation we determined novel target proteins from excretory-secretory (ES) antigens of 3rd stage *G. spinigerum* larva using immunomics and mass spectrometry approaches. We prepared ES antigen of *G. spinigerum* for protein profiling analysis by 2D-gel electrophoresis and subsequently transferred it onto PVDF membrane. The membranes were reacted with *G. spinigerum* infected or other helminthic infected sera. Results show that approximately seven spots at the molecular size of 12, 20, and 50 kDa represented *G. spinigerum* infection. Next, these protein spots are going to be analyzed by mass spectrometry and available databases will be searched for annotation. Identifying the type of protein and/or tryptic peptide sequences will be useful for the development of future immunodiagnosis for gnathostomiasis.

Keywords: *Gnathostoma spinigerum*, excretory-secretory antigens, 2D-gel electrophoresis, immunomics, immunodiagnosis

POSTER No. 043

# EVALUATION OF RECOMBINANT CATHEPSIN L FOR IMMUNODIAGNOSIS OF GNATHOSTOMIASIS

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*Gnathostoma spinigerum* infection is a serious health problem occurring in several countries, including Thailand. The gold standard for diagnosis is currently detection of antibody- specific 24 kDa antigens with immunoblotting. However, this technique requires lengthy antigen preparation, and experience to interpret correctly. In this study, recombinant antigen, cathepsin L (GsCatL), of *G.spinigerum* was evaluated as an immunodiagnostic tool. Several previous studies suggested cathepsin L as a potential antigen for the diagnosis of helminthic infections. Initially, GsCatLcDNA was cloned into a prokaryotic expression vector and then expressed as recombinant GsCatL (rGsCatL) in *Escherichia coli*. The rGsCatL expressed at the molecular size of 48 kDa was purified and evaluated with gnathostomiasis-, other helminthic infected-and healthy sera using immunoblot and indirect ELISA. The results showed that rGsCatL cross-reacted with all sera by immunoblot with lowsensitivity (25.7%) and specificity (41%). The indirect ELISA also exhibited the same result as immunoblotting. These results suggest that rGsCatLusing a prokaryotic expression system is not suitable for diagnosis of gnathostomiasis. However, a eukaryotic expression system still needs to be verified for correct conformational structure through further evaluation.

Keywords: *Gnathostoma spinigerum*, Cathepsin L, recombinant antigen, immunoblot, indirect ELISA

POSTER No. 044

# THE GENETIC ANALYSIS OF ASCARID EGGS OF DOMESTICATED BEAR IN KOREA

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The bile juice of bears has been used by traditional medicine until recently in Korea, but the Korean government have taken steps to prohibit its use as a medicine, and to control domesticated bears due to animal welfare and preservation of the biological environment. In order to control domesticated bears, we surveyed the biology and parasites of bears, by investigating their intestinal parasites. The feces of one hundred bears were collected from bear farms and intestinal parasites were investigated by sedimentation and floatation techniques. *Baylisascaris* sp. was detected from nine of the 100 bear feces samples. The M±SD of egg size are 49.0±2.4 µm in length and 38.1±3.6 µm in width. The isolated ascarid eggs was identified by PCR and sequencing. The *Bayliascaris* sp was differ from other *Bayliascaris* spp in Gene Bank, so that we confirm more the genetic aspects.

Keywords: Bear, *Baylisascaris procioni*, PCR, sequencing

POSTER No. 045

# CORRELATION BETWEEN EOSINOPHIL COUNT AND SOIL-TRANSMITTED HELMINTH INFECTION IN CHILDREN

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**Background:** Soil-transmitted helminth (STH) infections in Indonesia are still a major health problem. They are often correlated with increased incidence of significant eosinophilia. Several studies have reported that elevated eosinophil counts could be used as a marker of STH infection.

**Objective:** To investigate possible correlations between eosinophil count and STH infection in children.

**Method** This is an observational analytic study with cross-sectional design in elementary children, run between February and March, 2014. It was held in Medan Belawan district, North Sumatera province. Feces samples were examined using the Kato-Katz method, and blood samples were examined by peripheral blood smears. Data was analysed using Pearson correlation (significance if $p<0.05$).

**Result** Ninety-nine subjects were involved in this study (Ascariasis n=40, Trichuriasis n=9, mixed infection n=50). STH prevalence was found to be 65.4%, with prevalence of Ascariasis, Trichuriasis, and mixed infections being 37.1%, 21.9%, and 40.8%. Mean eosinophil count in children with Ascariasis, Trichuriasis, and mixed infection were 7.18, 8.11, and 8.64/μL blood ($p=0.319$). Eosinophilia prevalence in Ascariasis, Trichuriasis, and mixed infection were 47.5%, 77.8%, and 70% ($p=0.054$). Eosinophil counts showed significant correlation with amount of egg per gram feces (EPG), where Ascariasis showed strong correlation ($p=0.000$, $r=0.622$) and Trichuriasis showed weak correlation ($p=0.038$, $r=0.208$).

**Conclusion:** Prevalence of STH infections was still high among school-aged children in North Sumatera province, Indonesia. Highest prevalence of eosinophilia was observed in mixed infection. The degree of intensity of infection has a significant correlation with the number of EPG. Eosinophil count can be used as a marker of STH infection.

**Keywords:** Soil-transmitted helminth infection, eosinophil count, eosinophilia

*POSTER No. 046*

# A COMPARATIVE EFFICACY STUDY BETWEEN ALBENDAZOLE VERSUS MEBENDAZOLE FOR 2 CONSECUTIVE DAYS AS THE TREATMENT OF *TRICHURIS TRICHIURA* IN PRIMARY SCHOOL CHILDREN AT BELAWAN, NORTH SUMATERA, INDONESIA

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**Background** Trichuriasis is a major health related problem for children in Indonesia. Single dose albendazole and mebendazole had showed many unsatisfactory results, making trichuriasis more difficult to eliminate. Antihelmintic drugs with repeated doses increased the drug efficacy for trichuriasis.

**Objective** To determine the efficacy of albendazole versus mebendazole for two consecutive days against trichuriasis.

**Methods** A randomized double blind clinical trial was conducted in March 2014 to June 2014 among primary school children at Belawan subdistrict, North Sumatera. Stool samples were collected before treatment and after treatment at 7th and 14th day using Kato Katz methods. Group I received Albendazole 400 mg and group II received Mebendazole 500 mg for two consecutive days. Cure rate (CR) and egg reduction rate (ERR) were analyzed and compared using Chi square and Wilcoxon rank sum test.

**Results** 156 subjects were enrolled (group I n=78, group II n=78) in this study. The prevalence of trichuriasis was 37.7%, 62.2% of which were co-infected with Ascaris lumbricoides. On day 7 and 14, egg reduction rates showed Mebendazole slightly higher than Albendazole (99.97% & 99.8%, P=0.048; 99.41% & 95.64% P=0.014). The efficacy of both regimens showed high cure rate percentages, with Mebendazole higher than Albendazole on day 7 and 14 (96.15% & 91.03%, P=0.119; 89.74% & 79.49%, P=0.043).

**Conclusion.** Two day treatments with either albendazole or mebendazole show high efficacy against trichuriasis.

**Keywords:** albendazole, mebendazole, trichuriasis, repeated doses, cure rate, egg reduction rate

POSTER No. 047

# INTESTINAL HELMINTHIASIS AMONG STUDENTS AT THE PUBLIC ELEMENTARY SCHOOL (SDN) 020260 OF BINJAI, NORTH SUMATERA – INDONESIA

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Intestinal helminthiasis among children of school-age is commonly found in tropical countries such as Indonesia. Common types are of the species of soil transmitted helminthes such as *Ascaris lumbricoides* & *Trichuris trichiura*. *Enterobius vermicularis* infection, likewise, is often found. *Hymenolepis nana*, a member of cestodes, has also increased in numbers, alongside *Taenia saginata* & *Taenia solium*. Waris (2012) reported the prevalence of hymenolepiasis nana in 5 districts (kecamatan) at Nunukan regency, Indonesia is 4.5%.

This is a descriptive study aimed to determine intestinal helminthes infection rate among students at the public elementary school (SDN) 020260 of Binjai, North Sumatera. Stool samples were derived from students of the 4th, 5th, and 6th grade. Direct stool examination was done with lugol and Kato technique, in addition to scotch tape preparation to detect *Enterobius vermicularis*.

The study showed that of the 131 students included in the study, 12 were found positive for intestinal helminthiasis. 3 students were positive for *Ascaris lumbricoides* detection alone, 5 were positive for *Trichuris trichiura* alone, 1 was infected with *Hymenolepis nana* alone, and 3 were co-infection cases.

Based on this, we conclude that 9.16 % of students at SDN 020260 Binjai were infected with intestinal helminthes. Of those, *Hymenolepis nana* detection in the samples show rising cases of hymenolepiasis nana. It is suggested that care should be taken for the prevention & eradication of these helminthes, which includes targeting of *Hymenolepis nana*, particularly by the local department of health.

Keywords: soil transmitted helminthes, hymenolepiasis nana, student, elementary school

POSTER No. 048

# GREEN CHEMISTRY FOCUS ON OPTIMIZATION OF SILVER NANOPARTICLES USING RESPONSE SURFACE METHODOLOGY (RSM) AND MOSQUITOCIDAL ACTIVITY: *ANOPHELES STEPHENSI* (DIPTERA: CULICIDAE).

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There is an exigent necessity for development of environmentally friendly bio-control agent(s) for the elimination of mosquitos due to increased resistance resurgence against synthetic control agents. Mosquito control strategy will lay a strong foundation to malaria exclusion or it can be curbed to certain level especially in the developing nations. In this study, silver nanoparticles were synthesized by green chemistry approach using *Tridax procumbens* leaf extract as a reducing agent. The reaction medium involved in the synthesis process was optimized by statistical experimental design using response surface methodology to obtain better yield, uniform size, shape and stability. These synthesized nanoparticles were then confirmed through UV-Visible, FT-IR spectroscopy, PSA and SEM. Subsequently, the bioefficacy of these particles was investigated on *Anopheles stephensi* for larvicidal and pupicidal activity. Interestingly, for the time period of 90 min, temperature of 76±2°C, pH 7.2±2, 2 mM silver nitrate ($AgNO_3$), 3 mM PEG and 2 mM PVP showed excellent parameters for bioprocess design for large scale production of stabilized nanoparticles. A concentration of 5 ppm of PVP stabilized nanoparticles exhibited 100% mortality. Thus, the obtained results clearly suggest that silver nanoparticles stabilized by PEG and PVP may have an important function as stabilizers, dispersants as well as larvicides for mosquito control.

Keywords: *Anopheles stephensi*, *Tridax procumbens,* RSM, PEG, PVP, mosquitocidal.

POSTER No. 049

# MOSQUITOES BETWEEN DOUBLE LAYER NET AND CONVENTIONAL METHOD IN SEMI-FIELD CONDITON AT MAHIDOL CAMPUS, SAI YOK, KANCHANABURI PROVINCE, THAILAND

Sungsit Sungvornyothin

A double layer net (DLN) for sampling of malaria vectors was developed to compare the sampling efficacy of this method against the conventional method of human landing catch under semi-field conditions in a screen-walled corridor using laboratory reared *Anopheles dirus* KMK strain.

A total of 1300 *Anopheles dirus* mosquitoes were used for the study and we found that the collection ratio between the double layer net and human landing catch was 1:2. The ratio of double layer net catches to those of the human landing catch increased with decreasing mosquito density. The collection rate was higher for the conventional method upon the release of the mosquito but gradually decreased with decreasing density. Mosquito species did not affect the ratio of double layer net to human landing catches since the trial with *Aedes aegypti Aedes albopictus* showed a consistent ratio.

Two different colors of outer nets were used (black and white) to compare the sampling sensitivity between the two different colored materials. The results indicate that there was no difference in the number of host seeking mosquitoes caught with an equal ratio of number of catches.

The double layer nets were used to prevent collectors from potentially infectious mosquito bites and are designed to catch host-seeking mosquitoes only. Although the sensitivity is half that of conventional method, these traps could be further improved.

Keywords: Anopheline mosquitoes, double layer net, human landing catch

*POSTER No. 050*

# COMPARISION OF HOST SEEKING BEHAVIOUR OF *ANOPHELINE* MOSQUITOES BETWEEN DOUBLE LAYER NET AND CONVENTIONAL METHOD

Nima Wangdi Gyeltshen, Sungsit Sungvornyothin, Supatra Thongrungkiat, Jean Pierre Dujardin

A double layer net (DLN) for sampling of malaria vectors was developed to compare the sampling efficacy of this method against the conventional method of human landing catch (HLC) under semi-field settings in a screen-walled corridor using laboratory reared *Anopheles dirus* mosquitoes. Two different colours of outer nets were also used, namely, black and white, to compare the sampling sensitivity between two different coloured materials. The results indicated that white DLN sampled more mosquitoes than the black DLN, *p*-value=0.015, 95% C.I.: (-2.64,-0.33). A total of 26 replicate nights with 1300 *Anopheles dirus* mosquitoes were used to conduct competitive comparison between the DLN and HLC, and found that the double layer net caught 7.6 ± 1.1% and human landing catch caught 15.9±2% of the released mosquitoes, respectively and the proportion of collection by double layer net against human landing catch was 0.625 ± 0.12. The result showed that the collection ratio between DLN and HLC was 1:2.09.

Different genera of mosquito species showed lower collection of mosquitoes by DLN compared to HLC with the ratio of 1:1.94 for *Aedes aegypti* and 1:1.56 for *Aedes albopictus* but the comparison between the black and white DLN showed that there was no statistical significance difference on the number of *Aedes aegypti* sampled by the two types of nets, *p*=0.86, whereas, in the case of *Aedes albopictus*, black DLN was fond slightly more efficient that white DLN, *p*=0.006, 95% C.I.: (0.82, 4.36).

The double layer net does not expose people to potentially infectious mosquito bites and it is designed to catch host-seeking mosquitoes only. Although the sensitivity is half that of conventional method, yet, these traps could be further improved upon by conducting field trails and its sensitivities further increased.

Keywords: Anopheline Mosquitoes / *Anopheles Dirus* / Double Layer Net / Human Landing Catch / Thailand

POSTER No. 051

# INSECTICIDE RESISTANCE IN THE BANCROFTIAN FILARIASIS VECTOR, *CULEX QUINQUEFASCIATUS* IN THAILAND, AND POSSIBLE RESISTANT MECHANISMS

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The bancroftian filariasis vector *Culex quinquefasciatus* is known to be resistant to insecticides worldwide, including Thailand, but the mechanisms conferring resistance are poorly understood. This study was the first investigation of the insecticide resistance mechanisms, involving metabolic detoxification and target site insensitivity in *Cx. quinquefasciatus* from Thailand. The mosquitoes were collected from Chiang Mai city and maintained in the insectary as a colony, namely Cq_CM strain under deltamethrin selection pressure. The deltamethrin resistance level was determined by the WHO larval and adult bioassay tests. A synergist assay was performed to assess the detoxifying enzymes. A fragment of sodium channel gene was amplified and sequenced to identify the *kdr* mutation. The Cq_CM strain, after exposure to 0.05% deltamethrin, showed a mortality of 15.2% and the level of resistance rapidly increased after two generations of selection showing 0% mortality. Addition of piperonyl butoxide (PBO) and *S*, *S, S*-tributylphosphorotrithioate (DEF), inhibitors of cytochrome P450 monooxygenases and esterases, respectively, into the larval bioassay revealed a synergist effect suggesting that both enzyme families are conferring the deltamethrin resistance in this population. The L1014F mutation was observed in these mosquitoes, with the mutant F1014 allele frequency significantly higher in the survivor group (0.54) than the dead group (0.01) after exposure to 0.05% deltamethrin ($p<0.05$), suggesting that the L1014F mutation also plays a potential role in deltamethrin resistance. We conclude that the resistance to deltamethrin in the Cq_CM population is conferred by multiple insecticide resistance mechanisms, involving cytochrome P450 monooxygenases and esterases enzymes and the L1014F mutation.

Keywords: *Culex quinquefasciatus*, Insecticide resistance, Resistance mechanisms, deltamethrin

*POSTER No. 052*

# EFFECTS OF HUMAN BLOOD AGE ON THE FEEDING RESPONSE & FECUNDITY OF *ANOPHELES CAMPESTRIS, AN. KLEINI* AND *AN. SINENSIS*

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Membrane feeding is the standard for mosquito colony maintenance by the Department of Entomology, USAMC-AFRIMS. Preservatives, such as citrate-phosphate-dextrose (CPD), are used to extend the shelf-life of whole blood up to 21 days. To date, the effect of blood age on mosquito feeding or fecundity in our colonies has not been investigated. We fed *Anopheles campestris, An. kleini* and *An. sinensis* using 5, 10, 15, & 20 day old blood. Both *An. campestris* and *An. sinensis* showed significantly higher engorgement rates of 64.8 and 71.2% with 5-day old blood compared to older blood (*An. campestris* 15.6, 19.6 and 49.2%) and (*An. sinensis* 57.6, 58.4 and 61.2%), at 10, 15, & 20 days respectively. However, blood age did not impact the feeding rate of *An. kleini* (22.0-29.6%). For comparison, direct feeding on live mice resulted in rates of 67.6, 40.4 and 16.4% for fully engorged *An. campestris, An. kleini* and *An. sinensis*, respectively. Blood age also negatively impacted fecundity rates of these three species, with rates falling from 191.6 to 138.2 eggs per *An. campestris* female, 154.2 to 105.0 eggs per *An. kleini* female and 146.6 to 93.0 eggs per *An. kleini* female as blood age increased from 5 days to 20 days.

Keywords: *Anopheles campestris, An. kleini, An. sinensis*, human blood age, feeding, fecundity

*POSTER No. 053*

# INVESTIGATION OF ARTHROPOD VECTORS AND RESERVOIR HOSTS FOR *ORIENTIA TSUTSUGAMUSHI* IN ARMY TRAINING GROUND, SRIRACHA DISTRICT, CHONBURI PROVINCE, THAILAND

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Scrub typhus is an important health problem in the tropics. The disease is caused by the gram negative intracellular bacterium, *Orientia tsutsugamushi*. Transmission occurs when humans interact in its natural life cycle via its vectors, which include arthropods, chiggers (mite larvae), and small mammals, such as rodents. In September 2013, an outbreak of scrub typhus was reported in Thai Army troops who joined annual field training in the Sriracha district, Chonburi province. Outbreak investigation found that 29% (32/110) of trained soldiers were sick and 9.1% (10/110) had significant seroconversion against *O. tsutsugamushi*. To understand the transmission cycle of scrub typhus in this area, we further investigated the presence of reservoirs and vectors of the disease. Rodents were trapped to collect blood, liver, and spleen specimens for evaluation of scrub typhus infection using IFA method. Ectoparasites on trapped rodents were also collected and identified. Seventeen rodents were captured in August 2014. A majority of them (14) were rats: *Bandicota indica* and *Rattus rattus*, another three were ground squirrels, *Menetes berdmorei* and *Tupaia glis*. Using IFA technique, 92.9% of rats revealed antibody titer against *O. tsutsugamushi*. Wild collected ectoparasites were chiggers, *Leptotrombidium deliense*, the specific species reported as scrub typhus vectors. These results suggest that this Army training ground in Sriracha district, Chonburi province, is an endemic area of scrub typhus. An effective disease prevention and control program for training troops, entitled "Nawaminthachini Model", established in 2005 was then activated for the 2014 annual training. Monitoring of scrub typhus after this training showed no disease occurrence.

Keywords: Scrub typhus, Orientia tsutsugamushi, Chiggers.

POSTER No. 054

# EFFICIENT *IN VITRO* REFOLDING AND FUNCTIONAL CHARACTERIZATION OF RECOMBINANT HUMAN LIVER CARBOXYLESTERASES (CES1) EXPRESSED IN *E. COLI*

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Human liver carboxylesterase 1 (CES1) plays a critical role in hydrolysis of various ester- and amide-containing molecules, including active metabolites, drugs and prodrugs. It has been problematic to express recombinant CES1 in bacterial expression system. Due to low solubility, CES1 protein was mainly expressed in inclusion bodies, with insufficient purity. In this study, we reported an efficient *in vitro* method for refolding recombinant CES1 from inclusion bodies. One-step purification using immobilized metal affinity column was utilized to purify his-tagged recombinant CES1. Conveniently, denaturant and imidazole can be removed while the enzyme is refolded via buffer exchanging, a dilution method. We showed that refolding recombinant CES1 was successful in Tris-HCl at pH 7.5 containing a combination of 1% glycerol and 2 mM b-mercaptoethanol. Mixtures of other additives (trehalose, sorbitol and sucrose) and b-mercaptoethanol failed to recover the functional protein.  After refolding, his-tagged recombinant CES1 retained its biological activity and can be used directly without removing fusion tag. Taken together, our results provide an alternative method for obtaining substantial amount of functionally active protein, which is advantageous for further investigations such as structural and functional studies.

Keywords: Carboxylesterase 1, refolding, enzyme activity

*POSTER No. 055*

# VALIDATION OF PLASMA MEMBRANE PROTEINS EXPRESSED IN INVADING HEPATOCELLULAR CARCINOMA

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**Introduction:** The current gold standards and most commonly available tests for hepatocellular carcinomar surveillance are assay for serum α-fetoprotein (AFP) and hepatic ultrasonography. Although widely used, AFP level has limited sensitivity and specificity for HCC. Newer methods such as biomarkers or radiological assays for early detection of HCC are urgently needed. This study identified and validated the potential membrane protein related to invading HCC, which may be of interest for discovering a novel potential membrane protein contributing to human HCC.

**Objective:** To identify and validate plasma membrane proteins expressed in invading HCC

**Methods:** Cell surface proteins of invading HepG2 were isolated and separated by SDS-PAGE. Protein bands were cut to be analyzed by mass spectrometry. Analysis by mass spectrometry of proteins was checked by RT-PCR and real time PCR, Western blot and immunolocalization in cell line, and validated in various grades of tumor tissues by immunohistochemistry. Correlations of these finding were investigated to gain insights into potential candidate proteins contributing to invasion and metastasis in human HCC.

**Results:** Protein spot named P2 and P3 were identified by mass spectrometry and validated by real time PCR, western blot and immunolocalization and showed in various grades of HCC tissues.

**Conclusion:** 2 and P3 are membrane proteins expressed in invading HCC cell line and HCC tissues that might be potential proteins contributing to invasion and metastasis in human HCC.

**Keywords:** hepatocellular carcinoma, membrane proteins, mass spectrometry, immunohistochemistry

*POSTER No. 056*

# ASSOCIATION OF LOW DENSITY LIPOPROTEIN RECEPTOR RELATED PROTEIN 5 GENETIC VARIATION, A1330V AND BMD IN THAI MENOPAUSAL WOMEN

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Postmenopausal osteoporosis and bone mass are influenced by multiple factors including genetics. Low density lipoprotein receptor related protein 5 (LRP5), a co-receptor of Wnt signaling, is an important regulator of bone development and maintenance. The presence of a LRP5 single nucleotide polymorphism (SNP), A1330V, was determined by PCR-RFLP method in 332 Thai menopausal women. AA genotype subjects had lower radial BMD (*p value* = 0.015). Furthermore, women with low BMI (≤ 25) had higher risk of radial BMD osteoporosis (Odd's ratio = 7.41, *p value = 0.007*) but aging (>50 years) and A1330V SNP had no effect. In conclusion, LRP5 A1330V SNP may contribute to osteoporosis susceptibility in Thai menopausal women.

Keyword: A1330V, LRP5, osteoporosis, postmenopausal women, BMD

POSTER No. 057

# FOLATE AND VITAMIN $B_{12}$ DEFICIENCIES IN RELATION TO CARDIOVASCULAR DISEASE IN ELDERLY THAIS

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Thailand's aging population is leading to the increased prevalence of chronic diseases, especially cardiovascular disease (CVD). However, there have been few update reports on folate and vitamin $B_{12}$ deficiencies associated with homocysteine (Hcy). Elevated total homocysteine is an important risk factor for CVD. This study aimed to investigate the association of folate, vitamin $B_{12}$ and CVD in elderly Thai people (over 60 years old). Two-hundred elderly Thais, consisting of 43 male (21.5%) and 157 female (78.5%) subjects participated this study. Serum Hcy, folate, and vitamin $B_{12}$ levels were determined. High levels of Hcy were found, (Median (Min-Max) = 11.49 (6.63-30.11) µmol/L, cutoff point ≤ 10 µmol/L). However, folate (Median (Min-Max) = 7.20 (1.60-337) ng/mL, cutoff point < 3 ng/mL) and vitamin $B_{12}$ (Median (Min-Max) = 496.00 (37.30-4,405.00) pg/dL, cutoff point < 200 pg/dL) were within normal values. A significant negative correlation (r) between Hcy and folate (r = -0.154, *p*-value = 0.033) was evident, but not seen in Hcy and vitamin $B_{12}$ (r = 0.016, *p*-value = 0.828). Significantly different levels of Hcy (*p*-value = 0.003) and folate (*p*-value = 0.049) were also observed among different age groups (<65 years, 66-75 years, and >75 years). Additionally, males' CVD risk was four-times higher females, because of lower serum folate levels (OR = 4.30, 95%CI = 1.03-17.93, *p*-value = 0.031). These results indicate a relationship between CVD, Hcy and folate, but not vitamin $B_{12}$, among elderly Thais. These findings will be useful for further prevention policies for CVD in elderly Thais.

Keywords: Folate Deficiency / Vitamin $B_{12}$ Deficiency /Thai Elderly / Cardiovascular Disease

*POSTER No. 058*

# ONE HEALTH SHORT COURSE: PILOT WORKSHOP

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The Southeast Asia One Health University Network and partners designed modules to develop core competencies that focus on the One Health approach. A 3-day One Health Short Course (OHSC), aiming to 1) demonstrate innovative teaching techniques, 2) develop skills and knowledge with core and technical competencies, and 3) share ideas on the application and integration of OHSC modules into existing courses or programs, was attended by 55 lecturers from 9 faculties from six universities. Fourteen participants served as trainers previously trained on how to use the modules. Participants were evaluated through questionnaires to assess the workshop and their proficiency levels before and after the course.

The majority of the participants rated the course as “Good” (67%) and “Excellent” (29%). Participants indicated that they “Agree” that the training: met objectives (59%), met expectations (74%), new information presented (55%), informative trainers (69%), useful materials useful (55%), and well-managed (61%). While 59% “Strongly agree” that the course was relevant to their work.

The Communication domain was developed most with 4% “High proficiency” before which increased to 59% after the course. Other domains showed significant increase in the “High proficiency” level: Collaboration & Partnership (45%), Leadership (39%), Culture & Beliefs, Values & Ethics (39%), Management (29%), and Systems Thinking (25%).

The OHSC Pilot Workshop was successful in propagating the One Health approach among lecturers from Thai universities. Feedback from the participants is very encouraging where replicating and scaling up are logical next steps to advance the One Health approach in Thailand.

Keywords: One Health approach, Thailand One Health University Network, core competency

POSTER No. 059

# GEOHEALTH THAI PLATFORM (GEOHTP): TOWARDS A NETWORK TO GATHER EXPERTISE, KNOWLEDGE AND RESOURCES IN HEALTH GEOGRAPHY

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Driven by the recent awareness of the magnitude of climate and environmental changes and their impact on human health, interdisciplinary approaches are increasingly being implemented to understand health inequalities and the dynamics of diseases. Although the availability of data is growing, researchers are facing difficulties in identifying and accessing relevant data and, above all, in using these data, resulting in a paradoxically limited use of geographical information.

The GeoHealth Thai Platform project aims to promote geographical and environmental approaches in the understanding of health inequalities through the use of Geographic Information Systems and Remote Sensing techniques. It proposes to address the difficulties encountered by many individual researchers by:

- gathering experts and researchers together during workshops, in order to define the needs and identify the barriers to be solved;
- training and providing expertise to researchers for the use of Geographic Information Systems and Remote Sensing techniques;
- building an open geo-catalogue to facilitate the access to spatial data.

This project will be supported by a dedicated website, which will integrate the catalogue of geo-referenced data, together with online resources (documents, courses and tutorials). This poster will present the geo-catalogue, at the heart of the project, as well as current and future project activities.

GeoHealth Thai Platform is funded by Franco-Thai Cooperation Program in Higher Education and Research 2013-2014.

Keywords: Health, GIS, geo-catalog, remote sensing, environment

POSTER No. 060

# FACTORS RELATED TO RODENT CONTACT INDOORS AND OUTDOORS IN BOLIKHAMXAY PROVINCE, LAO PDR

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Zoonotic diseases from wildlife such as rodents have elicited increasing public health concern in Southeast Asian countries including Thailand and Lao PDR. A cross-sectional study was conducted to characterize human-rodent exposure in Bolikhamxay Province, Lao PDR, from March to May 2013, aiming to understand any common characteristics which might be associated with rodent contact. Five hundred and eighty four participants were interviewed, using a modified version of a questionnaire previously used by the research team in Thailand in 2011.

The results showed that about 85.3% of respondents reported finding rodents entering their homes, 99.0% reported encountering rodents while working around gardens/crops, 56.3% reported noticing rodents in the past year while gathering things in the forest and other places, while 35.6% reported finding dead rodents in the past year.

Two characteristics were related to reports of rodents coming into the homes: having drinking water from open natural sources and having a wooden floor in their dwelling. Two characteristics were related to reports of encountering rodents while working around gardens/crops: working as a farmer, the number of cultivation-related tasks undertaken, and the number of food crops grown.

In addition, the number of food crops grown was associated with reports of both noticing rodents in the past year while gathering things in the forest and other places, and finding dead rodents in the past year. However, having flush toilets was a protective factor related to reporting that they noticed rodents in the past year while gathering things in the forest and other places. While, having waste collected as part of waste disposal was also a protective factor related to reporting that they found dead rodents in the past year.

These findings suggested that proper environmental management, such as having proper sanitation and waste disposal systems, can help reducing the exposure to rodents, which indirectly would lead to a reduced risk of rodent-borne disease in the community.

Keywords: Rodent, Contact, Exposure, Interaction, Hmong, Lao-Tai, Lao PDR

*POSTER No. 061*

# FACTORS AFFECTING PERCEIVED STIGMA IN LEPROSY AFFECTED PERSONS IN WESTERN NEPAL

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Background: There are various factors which construct the perception of stigma in both leprosy affected persons and unaffected persons. The main purpose of this study was to determine the level of perceived stigma and the risk factors contributing to it among leprosy affected person attending the Green Pastures Hospital, Pokhara municipality of western Nepal.

Methods: A cross-sectional study was conducted among 135 people affected by leprosy at Green Pastures Hospital and Rehabilitation Centre. Persons above the age of 18 were interviewed. Each individual were asked using a set of questionnaire form and additional Explanatory Model Interview Catalogue (EMIC).

Results: Among 135 leprosy affected persons, the higher perceived stigma score was found in illiterate persons whose incomes were self-described as inadequate and who had changed their occupation due to leprosy. Patients who lacked knowledge about the causes and transmission of leprosy and had a perception that leprosy is a severe diseases which is difficult to treat had higher perceived stigma score. Patients with visible signs of leprosy and/ or ulcers had higher perceived stigma score.

Conclusion: There is an urgent need of stigma reduction strategies which should focus on health education and health awareness programs targeting leprosy affected people. In addition to the health education, leprosy affected persons should be provided with technical education, vocational training, empowerment, and active social participation. The current study indicates that patients with (visible) disabilities and ulcers bear both physical and psychosocial burdens. Programs that increase early case detection and health awareness, and prevent disabilities and ulcer development will reduce perceived stigma among leprosy affected people.

Keywords: Leprosy, perceived stigma, Nepal

POSTER No. 062

# PSYCHIATRIC CONDITIONS OF JAPANESE IMMIGRANTS IN BANGKOK, THAILAND

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Officially reported, approximately 30,000 Japanese are living in Bangkok. Generally speaking, immigrants tend to have many stresses and sometimes they have mental disorders. We used self-administrated questionnaires to investigate mental health condition for Japanese residents in Bangkok through the internet. A total of 48 residents answered. Male residents were 35(72.9%). Mean age is 35.8 (SD 9.25) years old. We used Insomnia score (IS) from Hamilton Rating Scale for Depression and K6 score for the evaluation of the depression. We defined IS more than 3 as serious insomnia and K6 more than 5 as possible depression from former studies. IS more than 3 was 4 (8.3%) people and K6 more than 5 was 13 (27.1%) people respectively. The characteristics that living period was less than 1 year and smoker are significantly higher for possible depression (K6≥5) people after adjustment for other factors.

According to our former research for the adults in Kagawa prefecture, we concluded 'loneliness' was the most important factor for depression. But we could not find any relationship about loneliness and depression. Although the sample size is small, our research results suggest that relatively high number of people may suffer from mental illness compare with original Japanese residents. To confirm our results, we will progress the research in the near future.

Keywords: Mental Health, Japanese immigrants, Bangkok

*POSTER No. 063*

# CAUSES OF ACUTE FEBRILE ILLNESS AMONG SCHOOL CHILDREN IN LADKRABANG DISTRICT, BANGKOK: COHORT STUDY

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Fever is a common clinical sign of most infectious diseases. School children are a population at risk, and also play an important role as a source of disease outbreak spreading in community. This study aimed to describe and investigate causes of acute febrile illness among school absentees

Study was conducted in seven primary schools containing 5000 students from Ladkrabang district, Bangkok between 1 November 2013 and 31 August 2014. School absentees were monitored by an electronic absenteeism system. A list of school children who were sick with fever for 2 consecutive days were extracted and sent to nurses who are responsible for home visits. During home visits, consenting children and their parents were asked to complete a questionnaire. Sick children were also tested for dengue infection using a dengue duo rapid test. Their parents were advised to take their children to see a doctor for further investigation and treatment. One week later, the parents were asked for a final diagnosis of their sick children via phone.

60 % of the 83 investigated sick children received self-medication and recovered without seeing a doctor. The most common clinical signs were headache (64%). No dengue infection results were found. The top cause of acute febrile illness was the common cold (36%).

Most parents preferred to give self-medication, maybe due to most students not having serious illnesses. No positive dengue test results were possibly due to the low incidence of infection. Understanding pattern of acute febrile illness in this population would greatly help to prevent disease outbreaks in schools and communities.

Keywords: Acute febrile illness, absent school children, dengue infection, cohort study

*POSTER No. 064*

# HYPERTENSION AND FATIGUE IN CORRUGATED BOX FACTORY WORKERS: A CASE STUDY IN A TROPICAL ENVIRONMENT

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Working in a tropical environment is an interesting topic in tropical occupational medicine. Due to the specific tropical climate, worker fatigue can be easily observed. To assess this fatigue is an important issue that is not widely mentioned in the literature. In this study, the authors perform a study to assess the problem of fatigue among corrugated box factory workers in Pathum Tani Province, Thailand. The setting is a factory located in a tropical environment about 50 km from Bangkok, Thailand. 105 workers (51 male and 54 female) were enrolled in this study. The authors measured 2 main parameters, blood pressure and fatigue score. This study found that hypertension can be seen in 24.07 % (35.29 % and 14.81 % of male and female workers, respectively). A strong correlation between the fatigue score and blood pressure can also be observed. Factory work in a tropical environment, requires careful management of the problem of hypertension and fatigue.

KeywordS: fatigue, hypertension, factory, worker, tropical

*POSTER No. 065*

# IMPROVING RELIABILITY OF MALARIA RESEARCH THROUGH A COMPREHENSIVE QUALITY ASSURANCE PROGRAMME

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**Background:** Antimalarial drug resistance in Southeast Asia is the greatest threat to global malaria control and elimination efforts. Current assessments rely on clinical monitoring of antimalarial drug efficacy and the use of molecular, pharmacological and in vitro analyses to confirm resistance. Effective and proficient use of these tools is required to produce reliable intelligence to inform on public health threats.

**Methods:** WWARN has developed a platform to support collaborative clinical research and drug efficacy assessments in malaria. One component is an External Quality Assurance (EQA) Programme to assess proficiency for malaria laboratory analyses and ensure quality for biological research samples. WWARN EQA includes provision of high-quality antimalarial reference materials to pharmacology, in vitro and drug quality laboratories and biannual proficiency testing (PT) for pharmacology and molecular laboratories. PT participants receive scores for accuracy of test results and quality of analytic assessments. Biological sample management for malaria clinical trials, including standard procedures and field staff training augments WWARN EQA.

**Results:** Since 2009, WWARN EQA has provided reference materials to 50 laboratories worldwide. The PT programme includes 28 participants from five continents. Pharmacology and molecular laboratories have shown 41% and 29% improvement in overall results, respectively. WWARN's Specimen Management Centre supports 3 multicenter malaria clinical trials involving 32 field sites and 90,000 clinical specimens. An unprecedented number of high-quality samples were collected for genomics, transcriptomics and cell culture studies.

**Conclusion:** WWARN EQA supports malaria clinical trials globally to facilitate collection of high-quality data. Programme results demonstrate improved malaria laboratory proficiency and sample quality.

**Keywords:** Malaria, quality assurance, clinical trials

POSTER No. 066

# THE USE OF RESPONDENT DRIVEN SAMPLING METHODS TO IDENTIFY MALARIA PREVENTION KNOWLEDGE AND BEHAVIOURS BY MIGRANT AND MOBILE POPULATIONS IN WESTERN CAMBODIA

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**Background:** Mobile and migrant populations (MMPs) along the Thai-Cambodian border are at high risk of malaria and have been found to carry artemisinin-resistant parasites. However, the mobile nature of this population makes it difficult to adequately measure malaria infection and risk behaviours, which is vital as we move towards malaria elimination in the region.

**Methods:** Utilising respondent driven sampling methods, MMPs residing within two villages in Pailin province (Pang Rolim and Sala Krau) were recruited in two independent rounds of sampling (602 in 2013 and 604 in 2014). All responses were adjusted for network size and recruitment patterns allowing for calculation of population-adjusted statistics.

**Results and conclusions:** While the prevalence of *P. vivax* is estimated to be 0.2% among the general population, this study found 2.0% and 1.3% of MMPs in these networks to be infected with *P. vivax* in 2013 and 2014, respectively, and an absence of *P. falciparum*. When comparing indicators in these two villages during 2013 and 2014 sampling rounds, in Pang Rolim there was no change in proportion of MMPs reporting seeing malaria messages in the previous three months and proportion not using a mosquito net, and an increase knowledge of malaria transmission, symptoms and prevention. Indicators were lower or declined from 2013 to 2014 in Sala Krau. These findings correlate with the fact that there were increased efforts on malaria prevention in Pang Rolim (for example, concerts and videos with malaria messaging) and not in Sala Krau; suggesting that as MMPs change frequently there is a need for sustained public health efforts to reach this population, especially within an elimination context.

**Keywords:** malaria, surveillance, mobile migrant population

POSTER No. 067

# BEHAVIOURAL STUDY TO ASSESS CONSUMER PREFERENCES AND BARRIERS TO USE OF LONG-LASTING INSECTICIDAL NETS IN MYANMAR

Htwe Htwe Htet

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**Background:** Previous assessments have highlighted a lack of data on consumer preferences towards a range of malaria prevention tools, particularly in high-risk groups such as mobile populations. A qualitative study was undertaken in mid-2014 across three regions in Myanmar with varying levels of artemisinin resistance, to explore preferences and barriers associated with using long-lasting insecticidal nets (LLINs).

**Methods:** A maximum variation sampling approach was followed to include a range of participant perspectives sufficient to reach theoretical saturation. Focus group discussions and key informant interviews were conducted in rural and urban sites across three regions with a total of 339 participants, including community members, migrant workers, forest goers, health facility, NGO, government staff and community health volunteers. Translated verbatim transcripts were analysed using a content analysis approach.

**Results:** Across the regions, community level participants weren't always able to correctly explain how malaria is transmitted and prevented. A common theme among participants was a dislike towards the hard, rough LLIN texture, strong odour, and reported adverse effects; influencing LLINs usage. Texture was the most consistent and important feature of nets reported, with participants preferring soft nets with small holes. Migrant workers and forest goers generally preferred a single size net, whilst community members, those with large families preferred larger nets. Most participants preferred LLINs over untreated nets due to the insecticidal action to kill mosquitoes and prevent malaria.

**Conclusion:** Knowledge gaps around malaria transmission amongst community members, migrant workers and forest goers highlight the need to improve health awareness to further encourage the use of effective prevention tools. Strong preferences were reported for particular net characteristics, with the potential to enhance usage.

POSTER No. 068

# SURVEILLANCE OF MALARIA INFECTION IN ROYAL THAI ARMY AREAS OF OPERATION (AOS) ALONG THAI-CAMBODIAN BORDERS IN FISCAL YEAR 2014

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MALARIA, a high priority tropical disease, is endemic along the Thai-Cambodian border, and has been found to be multidrug-resistant, especially in military areas of operation (AOs) (Suranaree Command). The annual case report of malaria suggests that the Thai-Cambodian border is a risk area of malaria transmissions. Since 2006, during the Phra Vi Han situation, many solidiers have been deployed in this area to maintain peace in spite of the risk for malaria infection. Malaria surveillance is crucial for effective malaria prophylaxis and to decrease disease non-battle injury (DNBI). We have conducted a continuous surveillance program to obtain epidemiological information of malaria in AO Thai-Cambodian border (Ubon Ratchathani, Srisaket, Surin, Burirum provinces). During the Fiscal Year 2014, monthly active surveillance has indicated that malaria infection rate was 1.32% *P. falciparum*, 5.42% *P. vivax* and 0.69% mix-infection of *P. falciparum* and *P. vivax* in the Thai-Cambodian border. The ratio of *P. falciparum* to *P. vivax* was 1:4.

However, prevalence of malaria infection in this area has decreased slightly from Fiscal Year 2013, since the portable inventory sets called “AFRIMS Anti-Malaria Rescue Pack” have been effectively implemented to protect troops deployed to military operating areas from disease and non-battle injury (DNBI).

POSTER No. 069

# COMMUNITY CAPACITY STRENGTHENING ON MALARIA PREVENTION AND CONTROL AMONG ETHNIC MINORITY GROUPS IN RATANAKIRI AND MONDOLKIRI PROVINCE: THE COMMUNITY LIFE COMPETENCE PROCESS TRAINING

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**Background:** The Cambodia-Thailand Malaria Control project had been implemented in the ethnic minority groups in Ratanakiri and Mondolkiri Province since 2007. In 2014, the Community Life Competence Process (CLCP) was introduced to the project to enhance ownership of malaria issues and response by the community.

**Objective:** This report aims to share the lessons learned of the CLCP training program.

**Findings:** Forty two participants from Mondolkiri and Ratanakiri Province were recruited for a week-long training course on Community Life Competence Process (CLCP). The participants consisted of health officials, community leaders, health volunteers, traditional birth attendants, and selected villagers.

The CLCP consists of 1) the Concept of CLCP: Ways of Thinking, Ways of Working, the Steps of working; 2) Introduction and practice of methods and tools: SALT visit SALT (S= support, stimulate, share; A= appreciate, analyze; L= listen, learn, link; T= transfer , Team), dream building, self-assessment, resource mapping and action planning, 3) Facilitation skills and 4) Planning for future action. The After Action Reviews (AAR) and Reflections were conducted after the SALT visit and the final session of the training.

In conclusion, most of the participants gained an understanding of the CLCP concept and their role as facilitators. Participants started to have a shift in mind-set. For instance, in their community action planning exercise they looked for existing resources in their community first before looking for outside support. Since all participants are used to the old ways of working (as service providers, e.g. public health officials, volunteer workers), they will need more practice to master their new roles as facilitators.

**Keywords:** Community Life Competence Process, Malaria, Ethnic Minority Groups

POSTER No. 070

# MALARIA AND MIGRANTS IN THAILAND: TREATMENT-SEEKING (AND OTHER) BEHAVIOURS

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Artemisinin-resistant malaria has emerged as an urgent concern in South East Asia. One strategy to address the problem is to improve the access of mobile and migrant populations (i.e. those considered at highest risk of malaria) to health services. This study sought to understand the knowledge, attitudes and practices (KAP) of different migrant groups in Thailand, in order to design strategies to increase migrant's health access. In September 2013, 386 migrants who visited selected health facilities along the Thai-Myanmar border participated in a KAP retrospective study. The results showed that net usage among migrants who crossed borders daily was lower than migrants who stayed in Thailand for longer periods. Less than half (48.3%) of fever cases in the past 3 months sought treatment at public health facilities, even though 36.4% of those who were tested for malaria were positive. Lower net usage, as well as delayed treatment seeking for fever, was also reported among rubber plantation workers. New intervention packages (such as insecticide-treated net delivery and behaviour change communication delivered at official and unofficial border points) must be devised to reach migrants as well as those who work in rubber plantations, as both groups are more likely to sleep without mosquito nets, and delay seeking treatment.

Keywords: Mobile populations ; Migrants ; Artemisinin-resistance

POSTER No. 071

# MODIFIED THAWING PROCESS YIELDED A HIGH NUMBER OF VIABLE-INTACT ERYTHROCYTIC STAGES OF THE LONG-TERM CRYOPRESERVED *PLASMODIUM VIVAX* MALARIA

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Cryopreservation is an important technique for long-term starage of malaria for use in the laboratory as well as preservation of field isolates. For decades, freezing medium containing glycerolyte has been the solution of choice for preservation of the parasites. The current technique available for the preservation of *P. falciparum* and *P. vivax* demonstrate successful cryopreservation of parasites in the early stages of the life cycle from both cultureadapted parasites and infected blood samples taken directly from the host. However, parasites in mature stages cannot be preserved by the standard thawing technique. Low recovery of parasites following the freezing and thawing process may be a selective event favoring mutant subpopulation; therefore, it is essential that the preservation technique used is capable of preserving parasites at all stages of its life cycle. In this study, we introduce a modified method of malaria parasite cryopreservation that results in significantly higher parasite recovery of all stages ($p<0.001$). Higher percentage of mature parasites was recovered from both malarial spices of *P. falciparum* and *P. vivax*, when compared the modified thawing method to the standard method ($p<0.001$). This modified thawing method will enhance opportunity for research in *P. vivax* strain allowing successful maintenance of long-term *in vitro* culture, assessment of drug effectiveness and vaccine development studies.

Keywords: Freeze and thaw, glycerolyte, *Plasmodium vivax*, fX PlasmoFreeze

POSTER No. 072

# MASS SPECTROMETRIC ANALYSIS OF PARTICULAR PROTEINS OF THAI *PARAGONIMUS HETEROTREMUS* WORMS

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*Paragonimus heterotremus* is the causative agent of human paragonimiasis in Thailand. The protein molecular weights (MW) of 32.5, 33 and 35 kDa were used as diagnosis bands, and were reacted with human paragonimiasis sera confirmed by the presence of eggs and parasites, for immunoblotting. To study biological systems of the immunodominant worm antigen, protein MWs at 25-50 kDa were analyzed by Mass Spectrometry, Scaffold Version 4 and Blast2go against NCBI. The Top-Hit species were *Clonorchissinensis, Schistosoma japonicum*, *Schistosoma mansoni*, *Paragonimus westermani*, *Fasciola hepatica* and other parasites. The sequence distribution of molecular function indicated that most functions were binding, catalytic activity or ion binding. Mass Spectrometry data showed that proteins at 32.5, 33 and 35 kDa were succinate dehydrogenase complex, subunit B, iron sulfur [*Schistosoma japonicum*], actin beta/gamma 1 [*Clonorchis sinensis*] and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein [*Clonorchis sinensis*]. This study aimed to understand more about the antigenic proteins of *P. heterotremus* in terms of protein identification, functions and their components. This data will also be used to develop and produce new types of antigen for immunodiagnostic applications.

Keywords: *Paragonimus heterotremus*, proteins, mass spectrometry

POSTER No. 073

# CDNA EXPRESSION LIBRARY AND IMMUNOSCREENING OF THAI *PARAGONIMUS HETEROTREMUS*

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A cDNA expression library of *Paragonimus heterotremus*, the main causative agent of human paragonimiasis in Thailand, was performed to produce recombinant antigens. To identify the antigenic clones, immunized sera were tested using immunoscreening. Ten positive clones were selected, and the inserted genes partially sequenced. The sequencing data were analyzed using BLASTp searches. Six clones were found to contain a gene insert that encoded a protein similar to that found in helminthic worms (*Clonorchis sinensis*, *Schistosoma mansoni*, *Schistosoma japonicum*, *Echinococcus multilocularis, Brugia malayi*), two clones similar to *Toxoplasma gondii*, while two more clones were shown to encode proteins similar to that of *Plasmodium chabaudi chabaudi*and *Microcystis* spp. (uncultured). The clones will be further studied in term of protein expression through recombinant antigen evaluation using human paragonimiasis and other parasitic infection sera.

Keywords: *Paragonimus heterotemus*, cDNA, immunoscreening

POSTER No. 074

# PREVALENCE OF *HYMENOLEPIS NANA* IN HAMSTER’S FECES BY DIRECT SMEAR METHOD FROM PET SHOPS AT CHATUCHAK WEEKEND MARKET, BANGKOK, THAILAND

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*Hymenolepis nana* (*H. nana*) is the most common cosmopolitan tapeworm infection. It is more prevalent in temperate zones where sanitation is poor, and is one of the most common cestodes of humans, especially children. *H. nana*found in mice can be transmitted to humans. Mice and humans are the definitive hostof this tapeworm. They are often caused by human accidentally eating the eggs of *H. nana,* which is in the infective stage, contaminated with food or drinking water. The symptoms resulting from the infection are abdominal pain, diarrhea and weight loss. *H. nana* infection rates found in Thailand are as follows: Children--Institutional care at 13-14%, about 20% in mice and 28% in garbage mice. The purposes of this study was to study the prevalence of *H. nana* in hamsters’s feces from pet shops at Chatuchak weekend market, Bangkok, Thailand by direct smear method.In a sample of 40 hamsters from the 4 pet shops at 10 hamsters per shop, the results of the research found were *H. nana* in 10 hamsters (25%) *Syphacia* spp.in 13 hamsters (32.5%) and *Nippostrongylus* spp. in 1 hamster (2.5%). However, the knowledge about the infection of *H. nana*, including how to prevent disease, should be made available to potential hamster owners and the general public.

Keywords: *Hymenolepis nana*, Hamster, Direct smear method

POSTER No. 075

# ACCIDENTAL FINDING OF ASYMPTOMATIC HUMAN PARAGONIMIASIS AMONG KAREN PEOPLE, MAE LAH VILLAGE, THA SONG YANG DISTRICT, TAK PROVINCE, THAILAND

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Parasite surveys were conducted at Ban Mae Lah, Tha Song Yang District, Tak Province, Thailand in December 2013 and February 2014. A total of 343 fecal samples were collected to identify parasite infections. We report an accidental finding of *Paragonimus* spp. eggs in the feces of an asymptomatic 74-year-old female Karen patient. Her stool was separated and preserved in 95% alcohol for species identification by PCRtechnique.Theresults showed a band reacting to *Paragonimus heterotremus*. She reported a history of eating semi-cooked or dry waterfall crabs while fishing in mountain streams far from the village a long time ago. 30 waterfall crabs were collected from this stream and examined for *Paragonimus* metacercariae; all were found negative. Paragonimiasis remains a prevalent health problem in some rural areas of Thailand. With asymptomatic patients, a careful interview about past eating habits is very important, together with laboratory investigations to identify the infective agent specifically. A health education program was implemented in the village to help prevent *Paragonimus* and other helminth infections.

*POSTER No. 076*

# PRE-CLINICAL EVALUATION OF GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) AS A POTENTIAL UNIVERSAL MALARIA VACCINE

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Malaria is a major global health burden, causing approximately 627, 000 deaths annually, especially in children under the age of five. There are five *Plasmodium* species that infects humans, of which *Plasmodium falciparum* (*P.f.*) and *Plasmodium vivax*(*P.v.*)are the two most prevalent. Currently, no effective vaccine against malaria exists, hence the global effort aim to develop a vaccine to prevent infection, limit disease and interrupt mosquito transmission for all *Plasmodium* species.Glycosylphosphatidylinositol (GPI) is a potential target as it isa conserved glycolipid anchor of many essentialparasite proteinsfound across mostdifferentiated stages and *Plasmodium* species. Additionally, GPI is also a toxin that causes immuno-pathological symptoms of malaria. Previously, we proved that vaccination against GPI protects mice against severe malarial disease. This study further investigates the potential of the synthetic anti-GPI vaccine in preventing infection and blocking parasite transmission into the mosquito vector in a pre-clinical rodent malaria model, *P. berghei* (*P.b.*). The vaccine showed significant efficacy in reducing liver, blood stage infection and parasite transmission to the mosquito vector independently. When mouse liver infection was left to progress through to blood stage infection and parasite uptake by the mosquitoes,remarkable reduction in oocyst numbers were observed in mosquitoes fed on vaccinated mice compared to those fed on control mice. This suggests cumulative anti-GPI antibodies generated from the vaccine have stronger parasite transmission blocking activity. This was further validated by passively immunizing mice with anti-GPI antibodies prior to mosquito infection and preliminary data showed similar reduction in oocyst burden in this group of mosquitoes.

POSTER No. 077

# MOLECULAR CLONING AND CHARACTERIZATION OF GROWTH FACTOR RECEPTOR BOUND-PROTEIN IN *CLONORCHIS SINENSIS*

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*Clonorchis sinensis* causes very serious clonorchiasis. Growth factor receptor bound-protein 2 (Grb2) is a very important protein that is located in cytoplasm. Grb2 is found to be conserved in many animals and plays very important roles in many cellular functions and pathological processes including meiosis, organgenosis and even in energy metabolism. It is not know whether there is a Grb2 in *C. sinesis* to control its development. This study was performed to identify a grow factor receptor bound protein in *C. sinesis* (CsGFBP) and to characterize its possible functions in organogenesis and development of *C. sinensis*. A *C. sinensis* cDNA clone (CsGFBP) encoding putative polypeptide of 311 amino acids with the predicted SH3-SH2-SH3 structure was retrieved from the *C. sinensis* transcriptome. Recombinant CsGFBP was expressed and purified bacterially. Native CsGFBP was recognized from *C. sinensis* adult extract by western blotting using a mouse immune serum raised against recombinant CsGFBP. CsGFBP was expressed higher in the metacercariae than in the adult determined by quantitative real time PCR. By immunohistochemical staining with CsGFBP immune mouse sera, CsGFBP was localized in oral sucker, mesenchymal tissues, sperms in seminal receptal and ovary of the adults and abundantly in most organs of the metacercariae. The CsGFBP was highly conserved and suggested to play roles in organogenesis, energy metabolism and sperm production of *C. sinensis*.

POSTER No. 078

# SUPPRESSION OF *OPISTHORCHIS VIVERRINI* GRANULIN GENE EXPRESSION INHIBITS PROLIFERATION OF HOST BILIARY EPITHELIAL CELLS

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Multistep processes likely underlie cholangiocarcinogenesis induced by chronic infection with the fish-borne liver fluke, *Opisthorchis viverrini*. One of these processes appears to be cellular proliferation of the host bile duct epithelia driven by excretory-secretory (ES) products of this pathogen. Specifically, the secreted growth factor *Ov*-GRN-1, a liver fluke granulin, is a prominent component of ES and driver of hyper-proliferation of cultured human and mouse cells. To explore the influence of *Ov*-GRN-1 on the flukes and the host cells, expression of *Ov-grn-1* was repressed using RNA interference. Expression of *Ov-grn-1* was suppressed by 95%by day 3 and by ~100% by day 7. Co-culture of *Ov-grn-1* suppressed flukes for three days with human primary cholangiocyte (H-69) or human cholangiocarcinoma (KKU-M-214) cell lines retarded cell proliferation by 25% and 92%, respectively. In addition, similar growth was stimulated by the ES and by soluble lysate of the adult worms. Flukes in which expression of *Ov-grn-1*was repressed were less viable in culture, suggesting that *Ov*-GRN-1 is an essential growth factor for survival of the adult stage of *O. viverrini*. To summarize, specific knock down of *Ov-grn-1* shortened the duration of *in vitro* survival and reduced the capacity of liver fluke ES to drive host cell proliferation. These findings contribute to a deeper understanding of cholangiocarcinogenesis induced by opisthorchiasis.

(S41)

# THE ROYAL PROJECT OF PARASITE CONTROL IN THAILAND 2014

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The Royal Project of Parasite Control in Thailand has initiated by Her Royal Highness Princess Maha Chakri Sirindhorn. The implementation of her initiative focused on children in remote areas which suffering from malnutrition, unhygienic, environment, and inadequate public health status. The initial stage of The Royal Project development plan goals to improve the quality of life and future outlooks. Since the beginning of The Royal Project, the education has become main concern and Her Royal Highness recognize that education is the process of learning and a total for human development.

Her Royal Highness Princess Maha Chakri Sirindhorn has initiated many development projects. Among those Royal Projects, The Royal Project of Parasite Control were facilitated by and working in coordination with various agencies, both of MOPH and private sector. The Royal Project set to implement in the remote areas and PHU FA development area at Nan province during 2002 to 2014. Currently, the grand total target of school children are 771 schools and 61 villages about 131,000 people. At Remote areas within 53 provinces are the project for quality of life and the target schools are as follows; 1) Border Patrol Police school, 2) Primary school , Ministry of Education, 3) Monastic school (Northern province), 4) Islamic Private school (Southern border province), and 5) Suksasongkroh school (welfare school) etc

In PHU FA Development Project, students /youth/villagers of people were 24,000. According to National Program for parasite control in Thailand have various program for parasite control are as follows; 1) Intestinal parasite control program (STH), 2) Liver fluke control program (*Opisthorchis viverrini*), and 3) Vector Borne Disease Control program (*Filariasis, Malaria* , Dengue fever etc.).

The aims of Royal project of Parasite Control in Thailand are mainly to minimize the prevalence of OV and STH to be at least 5% by the year 2015 and have the model of school and villages for parasite free. At The remote area including PHU FA area , Parasitic infections are mainly relate to socio – economics , development process of risk behaviors due to inadequate knowledge and sanitary facility awareness of parasite infection Among student/youth are villages in the remote area. The most prevalent of disease was soil transmitted disease (*Ascariasis, Trichuriasis*, Hook worm). The STH Control Program focuses on health behavior development practice were coverage of school examination and 100% treatment. STH control strategy focused on shoes

wearing , proper defecation, hands washing, clean vegetable before eating and case finding for treatment 100%. Liver fluke control strategy have crucial activities included 1) Organizing mobile examination and treatment teams. 2) Community empowerment and effective health education to change habit (eating of unwell-cooked fresh water fish and using defecation behavior).

The results of stool examination among students in remote area from 2002 to date 2013 revealed the declining trend of the overall helminthiasis infection from 26.8 % to 13.4% . It was noticed that the highest prevalence was 45.6% in Chiangmai province follow by 39.6 % in Tak province, 19.5% in Naratiwat province and 16.5% in Songkhla province. Whereas the parasite infection rate at Phu Fa area, Nan Province found in 2002 was 52.4% which declined to be 12.6% in 2014 and the highest was *Ascaris* species. Besides, the prevalence among the villagers also gave high *Opisthorchis* infection with the beginning survey was 56.8% to 20.9% in 2014.

In addition, to reach the target plan, since 2007 to 2016, the national policy has organized the Royal Project of Parasite Control project by offering the provincial Program Manager with strategies as follows 1) To emphasize on solving the problem in the high risk groups at the remote areas. By using 100% coverage of case finding and treated all students, youths, and villagers as specific target group. 2) To integrate in the prevention and health promotion including food sanitation, environmental, nutrition, exercise, mental health, spiritual, and social marketing for behavioral change. 3) School and Community based approach. 4) Teachers gained whole comprehensive about subject type of STH and other worms and empowerment process should be integrated the existing lesson. 5) Corroboration among local government, provincial health office, non – governmental and control have to be plan the suitable of Participatory learning school based intervention for changing risk behavior to prevent the diseases. 6) Participatory learning process to empower the community for sustainable Royal Project development Plan.

Keyword: Royal project, parasite control, remote area, Phu Fa development area, soil transmitted helminthes (STH), Liver fluke, Infection, Prevalence

(S41)

# THE SEVEN GIVEN MODELS : THE PREVENTION AND CONTROL OF SOIL TRANSMITTED HELMINTHES INFECTIONS AMONG PRIMARY SCHOOL STUDENTS AT PHU FA DEVELOPMENT AREA, NAN PROVINCE, THAILAND

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The objective of this study was to develop integrative approach model (Seven given models) for Soil Transmitted Helminthes (STH) prevention and control on primary school students at PHU FA Development Area (Development Areas under the project initiated by Princess Maha Chakri Sirindhorn) Nan Province, Thailand.This study was a Participatory Action Research (PAR). The participants involved stakeholder in Boklue Districts and Chalermprakiat District; Sheriff, Director of school, teachers, public health officer, health village volunteers(VHV), parents, local organization officer and NGOs (World Vision Foundation). Brain storming, focus group discussion and future search conference (F.S.C.) were conducted to drive a learning process of the stakeholders in those areas. The results of a study showed that the model for closing the gaps and had a possibility to enforce task of STH prevention and control of primary school students at PHU FA Development Areas. The Seven Given Models consists of 1).Given rewards by the governor or sheriff 2).Given participatory by local organization as support workers and equipments 3). Given the knowledge and results of stool examination with STH by public health officer 4).Given man power by health village volunteer as health promoting, public relation and collect stool specimen 5).Given an attention by parents dealing with personal hygiene 6). Given carefulness by friends and class mate (Buddy) 7). Given supporting from NGOs as scholarship, preliminary sanitation. Conclusion: Seven Given Model is an appropriated pattern to implement in school and community. Continuous revise and adjust this model, learning and share experience in these areas need to be recommended.

Keywords: Seven Give Model/ STH/Primary School Student/ PHU FA Development Area

(S32)

# BARCODING PROCEDURES WITHIN A ONE HEALTH APPROACH

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As SEA is characterized by high human and domestic animal densities, growing intensification of trade, drastic land use changes and biodiversity erosion, this region appears to be a hotspot to study complex dynamics of zoonoses emergence at the Animal-Human-Environmental interface. Zoonotic diseases have devastating socioeconomic and wellbeing impacts for the poorest communities. The management of such health related risks implies to take into account ecological and social environmental dynamics at play, in link with epidemiological patterns. In this framework, biological data referring to the emerging pathogens and their reservoir, vectors and hosts are key to better understand some patterns related to the emergence of zoonotic diseases, with a huge impact on the capacity to address such diseases, and thus an excepted impact on public health. These data need to be safely and properly managed and shared within interdisciplinary and cross-sectorial collaboration processes. The utilization of molecular markers for barcoding and the implementation of biobanking approaches appears nowadays to be necessary to fit quality standards in terms of biological data management. Furthermore, in the framework of One Health cross-sectorial and interdisciplinary projects and activities, such process could contribute to sustainable and secure data sharing in the framework of improved networking and synergies among laboratories managing human, wildlife or livestock samples.

This will be illustrated through a short presentation of the expected outputs of EU INNOVATE One Health in Asia program (including ComAcross project).

(S39)

# THE BIOLOGY OF HYPNOZOITES

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Dormancy is a feature of many pathogens. In vivax malaria persistent dormant liver stage parasites “hypnozoites” wake at intervals after the primary infection to cause relapses. The factors which determine periodicity in relapse have not been elucidated fully, but there is an increasing body of evidence that hypnozoites can be activated by external stimuli including malaria illness. This will be reviewed.

(S16)

# ARTEMETHER-LUMEFANTRINE IN VULNERABLE PATIENT POPULATIONS: GETTING THE DOSE RIGHT

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**Background:** Artemether-lumefantrine is the most widely used antimalarial treatment in the world and it is also safe for pregnant women. However, low cure rates have been reported in pregnant women with malaria at the Thai-Myanmar border. This has been explained partially by lower drug exposures during pregnancy [1-3]. Plasma concentrations at day 7 of lumefantrine and its metabolite, desbutyl-lumefantrine, are a commonly used pharmacokinetic endpoint to relate drug exposure to efficacy.

**Objective:** To evaluate the pharmacokinetic and pharmacodynamic properties of lumefantrine and desbutyl-lumefantrine in pregnant women in Thailand with uncomplicated *Plasmodium falciparum* malaria.

**Methods**: Dense venous [3] and sparse capillary [1] lumefantrine and desbutyl-lumefantrine plasma concentration samples from 116 patients were evaluated simultaneously in a drug-metabolite model using nonlinear mixed-effects modelling. Different absorption, distribution, variability, covariate and error models were assessed. The final pharmacokinetic drug-metabolite model was fixed and used to evaluate the efficacy of the treatment (pharmacodynamic properties) with a time-to-event approach.

**Results:** Population pharmacokinetic data were best described by a multiphasic distribution, and treatment outcome data (i.e. recrudescent malaria) were best described using a Gompertz-hazard model. Lumefantrine and desbutyl-lumefantrine had similar effects on treatment outcome, when implemented separately in the model as traditional maximum effect functions. However, a model containing a lumefantrine drug-effect did not support the further inclusion of a desbutyl-lumefantrine drug effect. *In-silico* simulations with the final model showed an increased treatment success rate after a prolonged treatment.

**Conclusions:** Lumefantrine and desbutyl-lumefantrine concentrations were highly correlated and could be used interchangeably to predict the time to recrudescent malaria. Confirming studies evaluating optimised dose regimens are required to ensure a high efficacy in pregnant women.

NOTES:

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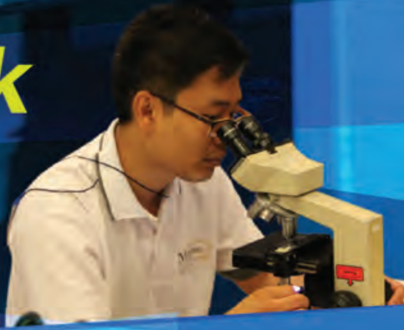

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