

# JOINT INTERNATIONAL TROPICAL MEDICINE MEETING 2016 (JITMM 2016)

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The biggest Tropical Medicine conference in Southeast Asia  
Hosted by the Faculty of Tropical Medicine, Mahidol University

## Abstracts

Poster PRESENTATIONS  
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- Faculty of Tropical Medicine, Mahidol University
- SEAMEO TROPMED Network
- TROPMED Alumni Association
- The Parasitology and Tropical Medicine Association of Thailand

### **CO-ORGANIZERS :**

- Department of Disease Control Ministry of Public Health (MOPH)
- Mahidol - Oxford Tropical Medicine Research Unit (MORU)

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**Poster No. 1**

**EFFECTS OF FREEZER STORAGE TEMPERATURES ON THE VIABILITY AND INFECTIVITY OF *OPISTHORCHIS VIVERRINI* METACERCARIA**

**O**pisthorchiasis caused by *Opisthorchis viverrini* is an important food-borne parasitic zoonosis in South East Asian countries. Infection in humans occurs through consumption of raw or undercooked dishes made from cyprinid fish containing the viable metacercaria, infective stage of *O. viverrini*. Cooking processes to reduce viability and infectivity of *O. viverrini* metacercaria in the fish are required for prevention of the infection in humans. In this study, we investigated the effects of freezer storage temperature on the viability and infectivity of *O. viverrini* metacercaria. Cyprinid fish containing *O. viverrini* metacercaria were kept at 4°C, 0°C, -20°C for 12 hr, 48 hr. Then *O. viverrini* metacercaria were isolated and examined for viability under a stereomicroscope and infectivity using hamsters. Results showed that complete and viable metacercaria were not found in the fish stored at 0°C and -20°C for 24 hr. Infectivity of *O. viverrini* metacercaria stored at 0°C and -20°C for at least 24 hr was 0%. Results from this study can be used for cyprinid fish preparation for traditional raw or undercooked dishes in endemic areas to reduce *O. viverrini* infection. 🗨️

**Keywords:** *Opisthorchis viverrini*, metacercariae, freezing temperature



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**Poster no. 4**

**PRIMER DEVELOPMENT FOR DISCRIMINATION OF LIVER FLUKES FAMILY OPISTHORCHIIDAE, USING POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM (PCR-RFLP)**

Liver flukes in family Opisthorchiidae were a major public health problem in Southeast Asia. There were 2 species, *Opisthorchis viverrini* and *Clonorchis sinensis*, which were reported as the human pathogens. In the recent report, *Opisthorchis lobatus* was discovered in Lao PDR and had characteristics similar to *O. viverrini*. It was hardly to identify by morphological study. In this study, species-specific primers were developed for discriminate between species of liver flukes in family Opisthorchiidae. Adult worm of *O. viverrini*, *O. lobatus* and *C. sinensis* were extracting total genomic DNA. Designed primers (N-Ov-Cs-COI-F&R primers) amplified partial cytochrome c oxidase subunit I (COI) fragments of three worms. The genetic characters among of *O. viverrini*, *O. lobatus* and *C. sinensis* were distinguished by PCR-RFLP method. Polymerase chain reaction (PCR) amplicons were generated with low genomic DNA concentration ( $\approx 10^{-6}$ ng) at 50 °C annealing temperatures. Restriction site in COI sequences *O. viverrini*, *O. lobatus* and *C. sinensis* can be distinguished by enzyme BsrI. The BsrI restriction site profile obtained three fragment of 43, 162 and 219 bp from *O. viverrini*, two fragments of 43 and 381 bp from *O. lobatus*, two fragments of 102 and 322 bp from *C. sinensis*. The PCR-RFLP profile can use for diagnosing mixed of *O. viverrini*, *O. lobatus* and *C. sinensis* infections in endemic areas and can be applied to epidemiological studies. 🗨

**Keywords:** Opisthorchiidae, *Opisthorchis viverrini*, *Opisthorchis lobatus*, *Clonorchis sinensis*, PCR-RFLP.



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**Poster no. 5**

**NA<sup>+</sup>-TAUROCHOLATE CO-TRANSPORTING  
POLYPEPTIDE OF CLONORCHIS SINENSIS: 3D  
STRUCTURE AND FUNCTIONALITY**

**N**a<sup>+</sup>-Taurocholate Co-transporting Polypeptide (NTCP, SLC10A1) plays a key role in the bile acid recycling. The bile acids uptake activity of NTCP is electrogenically coupled with co-transport of sodium ion. When *Clonorchis sinensis* live in the bile duct, *C. sinensis* NTCP (CsNTCP) could play an important role for its physico-metabolism. Its cDNA 1,641 bp long encoded a putative polypeptide of 546 amino residues. Tertiary structure of CsNTCP was generated by homology modeling in region of 185-492 aa using a template, *Neisseria meningitidis* (PDB ID: 1zuy\_A). Remaining regions were predicted to be disordered. The modeled structure was further refined and validated as a reliable model. CsNTCP had 10 transmembrane (TM) regions forming two domains: core domain consisted of TM3-5 and TM8-10 and panel domain formed with TM1, 2, 6 and 7. In the CsNTCP, substrate-binding sites for sodium and taurocholate were conserved. Compounds selectively docking to active pocket were selected through structure-based virtual screening. A chimeric polypeptide (24 kDa) of two antigenic and soluble was bacterially produced and used to immune mice. CsNTCP was localized in basement of tegument, basal membrane of intestine of epithelium, excretory bladder wall and mesenchymal tissues by using mouse-immune sera, contributing to bile acid transport. Taken together, these results provide information for deeper understanding on structural and functional characteristics of CsNTCP and homologues of flukes. Two compounds are proposed as putative inhibitors to CsNTCP and deserve further studies for anthelminthics. 🗨

**Keywords:** Na<sup>+</sup>-Taurocholate



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**Poster no. 6**

**MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF *FASCIOLA* SPP. ADULT WORMS COLLECTED FROM CATTLE SLAUGHTERED IN HANOI, NORTH VIETNAM**

A total of 360 gall bladders from cows and buffaloes from slaughtered in Hanoi were examined for the presence of adults and/or eggs of *Fasciola* spp. The second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) and mitochondrial genes (Cox1) were amplified from specimens belonging to 4 distinct phenotypes by polymerase chain reaction (PCR), and the representative amplicons were sequenced. A significant higher prevalence of *Fasciola* spp. infection was observed in cattle (63.53%; 216/340) in comparison with buffaloes (30%; 6/20) (Chi2=8.98; P-value=0.003). In a high proportion of gall bladders (38.3%; 138/360), only eggs were observed. Four different phenotypes were observed: type 1 (long-wide; 38.95±2.83 – 13.4±0.71), type 2 (long-narrow; 43.07±4.41– 11.01-1.15), type 3 (medium-narrow; 30.39±1.45– 7.86±0.70), and type 4 (medium-wide; 25.43±2.08– 11.7±1.4). Among them, type 4 presented a phenotype compatible with *Fasciola hepatica* while the others were compatible with *F. gigantica*. Molecular characterization based on Cox1 sequences indicated that all *Fasciola* types fall into *F. gigantica* cluster. However, ITS-2 sequences of *Fasciola* type 1, 2, 3 fall into *F. gigantica* cluster while *Fasciola* type 4 and Belgian isolate fall into *F. hepatica* cluster. *Fasciola* type 4 sequence showed two peaks at 207, 327 nucleotide positions. In addition, 7 different nucleotide positions between *Fasciola* type 4 and other types were observed. By morphometric and molecular analysis, we identified *Fasciola* type 4 as being a hybrid form of *F. gigantica* and *F. hepatica*. 🗨

**Keywords:** *Fasciola gigantica*, hybrid, phenotype, ITS-2, Cox1, slaughterhouse, Vietnam





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**Poster no. 7**

## **MOLECULAR IDENTIFICATION OF *FASCIOLA* SPP. FROM THAILAND BASED ON PCR-RFLP**

**F**asciola spp. is a large liver fluke in family Fasciolidae (Railliet, 1895), subfamily Fasciolinae (Stiles et Hassall, 1898). These parasites have been known as a pathogen of human and livestock. *Fasciola hepatica* Linnaeus, 1758 and *F. gigantica* Cobbold, 1855 are the cause of human fascioliasis. The infection of *Fasciola* has become increasingly important because of the recent widespread emergence related to climate change and human activity, while the disease has been overlooked. Currently, there were many reports of *Fasciola* intermediate form, genetically mixed between *F. gigantica* and *F. hepatica*. Although, many research areas to study in *Fasciola* spp. has been conducted, the accuracy to identify the *Fasciola* based on morphology has still been problematic. To solve this problem, many genetic markers have been applied to molecular identification such as the ribosomal internal transcribed spacer 1 and 2 (ITS1 and ITS2 rDNA) and mitochondrial gene sequences. Among the molecular markers, which used to apply for the molecular identification of *Fasciola* species, there is no information that what kind of markers are appropriate for. In this study, we aimed to evaluate the utility of such molecular markers, which have been used to identify the *Fasciola* spp. The PCR amplicons of ITS1, ITS2 and COX1 sequences were conducted and then studied based on PCR-RFLP method. The results indicated that digested ITS1 and ITS2 amplicon could show clearer band patterns than the digested COX1 amplicon. Therefore, this study concluded that the mitochondrial COX1 gene may not be the good genetic marker for molecular identification of *Fasciola* spp. 🗨

**Keywords:** *Fasciola* spp., Molecular identification, Molecular genetic marker, PCR-RFLP



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**Poster no. 8**

**IDENTIFICATION OF SCHISTOSOMA MEKONGI EGG  
IMMUNOGENS BY AN IMMUNOPROTEOMICS  
APPROACH**

Schistosomiasis is one of the most important human parasitic diseases caused by parasites in *Schistosoma* species. In South-east Asia, *Schistosoma mekongi* is endemic in the lower Mekong river basin in Laos, Cambodia and northern Thailand. The major of symptoms of the disease is caused by the parasite eggs trapped in host tissues and formed granuloma formation resulted from the host induced immune response around the parasite eggs. The interaction between egg proteins and host immune system is important to be explored for further development of schistosomiasis diagnosis. In this study, crude egg antigens called soluble egg antigens (SEAs) were separated by one-dimension (1D) gel electrophoresis and analyzed by immunoblotting. Immunoglobulin IgG and IgM from uninfected and infected mouse sera at 2, 4 and 8 weeks after infection were used for schistosoma antigen detection. The evidence from this study suggested that only IgG at 4 and 8 weeks and IgM at 4 and 8 weeks were detected the interaction between mouse immunoglobulin and parasite proteins using immunoblotting technique. Subsequently, tryptic digestion and LC-MS/MS analysis were performed for protein identification. This study has found that mouse antibodies reacted with several *S. mekongi* egg proteins for example antioxidant proteins (thioredoxin peroxidase, 26kDa glutathione S-transferase (GST)), chaperone (70kDa heat shock protein) and major egg antigen. These findings enhance our understanding of host-parasite interaction. The obtained results are potential candidate proteins for *S. mekongi* infection diagnosis. 🗨️

**Keywords:** Schistosoma mekongi, proteomic





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**Poster no. 9**

## **CRISPR/CAS9-BASED GENE EDITING IN SCHISTOSOMES**

Schistosomiasis is the most important of the neglected tropical diseases caused by helminth parasites. Schistosomes live within the blood vessels of the human host. The hepatointestinal form of this debilitating disease results from a chronic infection with *Schistosoma mansoni*, *S. japonicum* and *S. mekongi* whereas infection with *S. haematobium* causes urogenital schistosomiasis. Events that lead to egg granuloma formation (the major cause of pathology) and other aspects pathogenesis remain poorly understood. We investigated the potential of the newly described CRISPR (clustered regularly interspaced short palindromic repeat)-Cas phenomena to edit the schistosome genome. Thus far we have targeted gene loci encoding major egg proteins, including omega-1, kappa 5 and Sm16, by introducing *Streptococcus pyogenes* Cas9-guide RNA ribonuclease complexes by square wave electroporation into developmental stages of *S. mansoni* in vitro. Quantitative PCRs used to estimate the insertion-deletion (INDEL) in the schistosome genome revealed gene knockout mutation rates ranging from 2-15% compared to control worms exposed to the Cas9 nuclease alone. Levels of messenger RNAs from the mutated genes were markedly reduced. In addition, using a donor single stranded oligoDNA that included homologous arms of ~50 nucleotides in length on both the 5' and the 3' side immediately adjacent the target double stranded break (DSB) site of the chromosome, along with a short tag region of 25 nt included between the two homology arms, we successfully epitope tagged the *omega-1* locus as demonstrated by PCRs. Together the findings revealed that both gene knockout, presumably mediated by non-homologous end joining (NHEJ) and gene knock-in mediated by homology directed repair (HDR) were active in these plathelminth

**Keywords:** gene editing, CRISPR, Cas 9



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**Poster no. 11**

## **IDENTIFYING VEGF-460 GENE POLYMORPHISMS IN FILARIASIS PATIENTS IN SOUTH BORNEO, INDONESIA**

Secondary lymphoedema (LE) for filariasis are the most common and affects only a portion of the 120 million people infected with *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Vascular endothelial growth factor (VEGF) was originally described as vascular permeability factor and is a relatively specific endothelial cell mitogen and chemotactic factor that is important in a wide variety of angiogenesis processes. Immune responses that are triggered by the filarial excretory-secretory product result in the activation of VEGF, thus promoting lymph vessel hyperplasia as a first step to lymphoedema development. The objective of this research was to study single nuclear polymorphism of VEGF-460 gene in filariasis patients in three endemic lymphatic filariasis (LF) villages, Balangan district, South Borneo, Indonesia. Blood samples taken by finger prick test from 50 acute filarial subjects were used to perform PCR and continued by sequencing. The microfilaria density count from 16 to 500 mf/60 µl blood and microfilaria (MF) rate in that district is 9%. PCR result in 50 samples obtained prevalence of heterozygous (C/T) 42%, homozygous variant (C/T) 42%, and homozygous variant (C/C) 8%. According to previous studies, polymorphisms of VEGF-460 gene were known associated with lymphoedema development in lymphatic filariasis. The study showed that plasma levels of VEGF-460 were significantly higher in subjects with the C/C genotype. In our study, C/C genotype was found only 8% from 50 patients with filariasis. Despite lower prevalence of C/C genotype, prevention is still needed to avoid filariasis and decreased morbidity. 🗨

**Keywords:** Lymphatic filariasis, VEGF-460 SNP



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**Poster no. 12**

## **BRUGIA MALAYI MICROFILARIA PERIODICITY IN SOUTH BORNEO**

**B***rugia malayi*, with prevalence more than 70% in Indonesia that cause the morbidity in lymphatic filariasis. The periodicity of *Brugia malayi* microfilaria also plays a vital role in the disease transmission and is related to the mosquitoes' blood sucking habit. The aim of this study is to see the periodicity of *Brugia malayi*. An observational, cross-sectional study was carried out in October 2014. There were 4 people that have been identified microfilaria positive were asked to join the study voluntarily. The finger blood approximately 1 cc were taken in 3 times a day as in 6 am, 1 pm and 11 pm. The measured blood samples of 60 mm<sup>3</sup> (20 mm<sup>3</sup>/line) and stained with giemsa. The identification and counting of microfilaria was carried out. All of the volunteer were indigenous inhabitants who engaged at the rubber plantation site from 5 am to 12 pm. All of the subjects have physical contact directly with the vector as they did not have personal protective equipment. The microfilaria count in morning ranged from 9 mf to 121/60 mm<sup>3</sup> blood. The microfilaria count in noon ranged from 14 mf to 121 mf/60 mm<sup>3</sup> blood. The microfilaria count in night ranged from 20 mf to 180/60 mm<sup>3</sup> blood. The periodicity of *Brugia malayi* is non periodic. 🗨️

**Keywords:** *Brugia malayi* microfilaria, periodicity



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**Poster no. 13**

**SELENIUM DEFICIENCY AMONG LYMPHATIC  
FILARIASIS IN INDONESIA**

In Indonesia, *Brugia malayi* is the major species cause of filariasis and remains as a major public health issue. Based on Health National report (2015), microfilaria rate (mf) was high, 1-20%. Mass drug administration (MDA) annually for 5 years if mf rate in one district more than 1%. Infection of variety species of helminths in human host always induce the common pathway in immune response. Selenium (Se) as a micronutrient plays important role in immune system to fight against parasite. The aim of study was to explore selenium level among 65 Lymphatic filariasis (LF) subjects from October 2014 to May 2015. They were from 3 villages of Balangan district, South Borneo, with mf rate 9% and have not performed MDA until now. Selenium level was measured by ICP-MS procedure for hair sample. The median age were 45 years (range 16 to 65) and majority (71%) were mf positive. Among them, whose positive mf and Se deficiency were 63%,  $0.3 \pm 0.05 \mu\text{g/g}$  compared to 26% deficiency ( $0.24 \pm 0.045 \mu\text{g/g}$ ) but mf negative. Good sources of selenium are seafood, organ and muscle meats, however rarely consumed by all of the subjects. On the contrary, they frequently have vegetables as a daily food, as fruits and vegetables are low in selenium, due to the poverty. Almost 90 % of all subjects were labor in rubber plantation with low income. Selenium supplementation can be considered to enhance selenium level to support the body's natural defence system due to filariasis infection. 🗨️

**Keywords:** indonesia, lymphatic filariasis, selenium deficiency





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**Poster no. 14**

**DETECTION OF SINGLE NUCLEOTIDE  
POLYMORPHISM (SNP) CODON 200 BETA TUBULIN  
GENE IN ASCARIS LUMBRICOIDES AND TRICHURIS  
TRICHIURA FROM EAST NUSA TENGGARA,  
INDONESIA**

**S**oil transmitted *Helminth* (STH) infection is still highly prevalent in Indonesia. The current national elimination strategy is mass administration of broad-spectrum anthelmintics as recommended by WHO. There is a concern that the repeated use of benzimidazole drugs in long term may cause resistance or decreasing of drug efficiency. Among the contributing factors is the presence of single nucleotide polymorphism (SNP) codon 200 beta tubulin gene of the worm which substitutes phenylalanine to tyrosine (TTC to TAC). This is the first study in Indonesia aiming to determine the profile of beta tubulin gene codon 200 in *Ascaris lumbricoides* (*A. lumbricoides*) and *Trichuris trichiura* (*T. trichiura*) from a treatment naïve human population in East Nusa Tenggara, Indonesia. DNA from stool samples of infected individuals or from worm tissues were isolated, and subsequently amplified by PCR. Sanger sequencing was performed only on the successful PCR result (two *A. lumbricoides* and one *T. trichiura* worms). Next the alignment of sequences with available references was done to obtain the bases of beta tubulin around codon 200. The bases in codon 200 beta tubulin gene from two *A. lumbricoides* and one *T. trichiura* worms were TTC. The result from this study showed that no SNP was detected from the two species of worms; however larger sample size will be needed to confirm the finding in the same worm population in East Nusa Tenggara, Indonesia. 🗨️

**Keywords:** beta tubulin, SNP codon 200



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**Poster no. 15**

**LONGITUDINAL STUDY OF SOIL-TRANSMITTED  
HELMINTH INFECTIONS AMONG PRIMARY SCHOOL  
CHILDREN AT THA-PYAY-CHAUNG VILLAGE, NAY  
PYI TAW**

A longitudinal study of soil-transmitted helminth infections in primary school children at Tha-Pyay-Chaung village was conducted with the objectives to find out its prevalence, intensity, health and developmental consequences and effects of health knowledge, practice and preventive chemotherapy towards the burden. The study has been carrying out for more than four years upon 170 to 200 children. Modified Kato-Katz technique was used for parasitological analysis and hemoglobin level and blood film were examined for hematological analysis. Deworming with albendazole 400 mg was used as intervention. Children had high STH infection rate during initial assessment. Among various helminthes targeted in this study, *Ascaris lumbricoides* was found predominantly with the prevalence of 17.5% initially which fell to 2.02% at 48 months after deworming. Hookworm was found only in one child during 36<sup>th</sup> month assessment and *Trichuris trichiura* was also found only in one child during final assessment. Reinfection rate after preventive chemotherapy was found to be high within first 24 months but it declined sharply after 30 months to reach 15.15% in final assessment. Present study showed only light intensity of helminth infection. Mean hemoglobin level of the children ranged between 9.2 g/dL and 14.1 g/dL during study period and found no effect due to deworming. However prevalence of children with anemia decreased from 48.67% to 11.61% within 48 months' assessment period. At initial assessment, underweight and stunting were found in more than 20% of children while wasting only in 10.6%. Prevalence decreased after deworming until it reached below 5% in all parameters. Socio-demographic factors including age, gender, school grade, family size, parents' occupation and income did not have any relationship with reinfection. 🗨️

**Keywords:** Soil-transmitted helminth infection, Anemia, Growth, Primary school children





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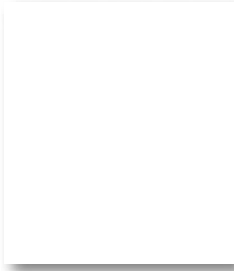
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**Poster no. 18**

## **TREMATODE INFECTIONS IN THE ASSASSIN SNAIL, CLEA (ANENTOME) HELENA (NEOGASTROPODA; BUCCINIDAE) IN SURAT THANI PROVINCE OF THAILAND**

**C**lea (*Anentome*) *helena* Philippi 1847 is freshwater snail in the family Buccinidae. It was found in Cambodia, Indonesia, Lao PDR, Malaysia and Thailand. This gastropod is the predator of other snails. In Thailand, it distributes in every province. It can live in both running and stagnant water such as pond or reservoir. Although the previous studies showed that trematode infections in this snail was rarely observed, but it also had the medical importance. It could be the intermediate host of *Echinostoma revolutum*, *Apatemon gracilis* and *M. appendiculatus*. The aim of this study was to investigate the trematode infections of *C. helena* in Surat Thani Province, South of Thailand. The snails were collected from eight localities by hand picking and scooping method. The parasitic infections were examined by shedding and crushing methods. The trematode larvae were stained with 0.5% neutral red and studied the light microscope. In this study, cercariae were not obtained from shedding method. But in crushing method, sporocysts of *Podocotyle lepomis* and metacercariae of *Plagioporus (Caudotestis) sinizini* were observed. The overall infection rate of *C. helena* was 1.74% (3/172). The infection rates of *P. lepomis* and *P. sinizini* were 0.58 % (1/172) and 1.16% (2/172), respectively. This study showed the more species of parasite in *C. helena* than the previous studies. So, the susceptibility of *C. helena* to the trematodes should be more studied further. 🗨

**Keywords:** trematode, infection, Clea (*Anentome*) *helena*



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**Poster no. 19**

## **TREMATODE INFECTIONS OF FRESHWATER SNAILS GENUS *CLEA* A. ADAMS, 1855 IN THE RESERVOIR OF LOWER NORTHEAST THAILAND.**

**T**rematode infections of freshwater snails genus *Clea* A. Adams, 1855 in the reservoir of lower northeast Thailand, were studied at 11 locations. The aim of this study was to evaluate the natural trematode infections of freshwater snail genus *Clea*. The snails were collected from four provinces (Nakhon Ratchasima, Buri Ram, Surin and Si Sa Ket) in March 2016. Five collectors picked the snails by hand for 10 minutes each station. The collected snails were observed for trematode infections by shedding and crushing methods. The infection rates were 1.92% (6/312). Cercariae were categorized by their morphological characteristics. Three types of cercariae were found. They were *Furcocercous* cercariae (tail forked), *Cotylomicrocercous* cercariae (cup-shaped tail with adhesive organ), and *Cercariaeum* cercariae (tail absent). The infection rates of these parasites were 0.64%, 0.96%, and 0.32%, respectively. Double infections were found with *Cotylomicrocercous* cercariae and *Cercariaeum* cercariae. 🗨

**Keywords:** Cercaria, Infection, Trematode, Genus *Clea*



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**Poster no. 20**

**DISTRIBUTION OF NEOTRICULA APERTA, SNAIL INTERMEDIATE HOST OF BLOOD FLUKE SCHISTOSOMA MEKONGI, IN MEKONG RIVER, THAILAND**

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The freshwater snail, *Neotricula aperta*, intermediate host for human blood fluke *Schistosoma mekongi*, was found in lower Mekong River and some of its tributaries in Lao, Cambodia and Thailand. The aim of this project is to study the distribution pattern and densities of *Neotricula aperta* along Mekong River of Thailand and to investigate ecological condition of *N.aperta* habitat in Mekong River. The study sites cover 8 provinces in Northern and Northeastern Thailand including Chiang Rai, Loei, Nong Khai, Bueng Kan, Nakhon Panom, Mukdahan, Amnat Charoen and Ubon Ratchathani. The survey was carried out 2 times during November – December (after rainy season) and April-May (dry season). Total 44 sampling sites were selected to survey for the snail *N. aperta*. Eighteen *N.aperta* habitats were identify along Mekong River in Ubon Ratchathani (10 sites), Amnat Charoen (1 site), Nakhon Panom (1 site), Nong Khai (5 sites) and Loei (1 site). *N.aperta* densities range from 0.2-1577.2 snails/m<sup>2</sup>. The snail distribution was mapping by GIS. The ecological conditions of each snail habitat and water quality parameters (water temperature, turbidity, dissolved oxygen, pH, hardness, salinity, ammonia, nitrate, nitrite, benthic diatom density and velocity) were recorded. The habitats of *N.aperta* in most sampling sites were in the islet of Mekong River. The bottom of the river or the islet type included bedrock, rock, pebble, sand, sandy soil, silt and muddy bottom. The substrates that *N.aperta* attached were natural rocks and also the rock that use for making river bank protection. 🗨️

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**Keywords:** Neotricular aperta, Distribution, Mekong River



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**Poster no. 21**

**CLONING AND SEQUENCING OF *PLASMODIUM FALCIPARUM* PHOSPHOETHANOLAMINE METHYLTRANSFERASE GENE AND VIRTUAL SCREENING TO IDENTIFY NOVEL INHIBITORS**

The growing resistance to current antimalarial drugs is a major concern for global public health and pressing need for new potent antimalarial. The biological processes that regulate gametocytogenesis remain unknown. Thorough understanding of these processes is crucial to the development of a new generation of dual activity antimalarials that can inhibit both infection and transmission. SAM dependent *Plasmodium falciparum* Phosphoethanolamine methyltransferase (PfPMT) catalyzes three step methylation of phosphoethanolamine to synthesize Phosphatidylcholine (PC) from host seine, for rapid development of membranes in *Plasmodium* species at both asexual and sexual stages, is a promising target for research on a novel class of antimalarials.

Gene PfPMT was amplified and sequenced. Phylogenetic was done with orthologues of *Plasmodium* spp. as well other species. PfPMT protein was used for structure based inhibitor identification.

PfPMT gene Amplification, sequencing and phylogenetic analysis demonstrated that PfPMT (Indian) isolate is 100% conserved with PfPMT 3D7 and also domain is also found significantly conserved with other *Plasmodium* spp. and very closely related. Compounds inhibitors of PfPMT have been identified through virtual screening based on Lipinski rule of five, ADMET and docking of compounds with PfPMT. Selected compounds showed potential effect on cell viability by SYBR green 1 dye method and showed good inhibitory activity against PfPMT protein. Based on computational ADMET analysis selected compounds found noncarcinogenic, nonmutagenic, non heptotoxicity and have good ADME physicochemical properties. Hence the identified compound inhibitors may lead to potent anaimalarial and as a lead for ligand based drug designing to overcome the problem of multidrug resistance. 🗨

**Keywords:** Pfpmt, SAM, SDPM, ADMET, Virtual Screening





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Poster no. 22

## ***P. FALCIPARUM* K-13 MUTATIONS AND TREATMENT RESPONSE IN PATIENTS IN HPA-PUN DISTRICT, NORTHERN KAYIN STATE, MYANMAR**

**BACKGROUND:** Artemisinin resistance has outpaced containment efforts in South East Asia. For national malaria control programs in the region, it is important to monitor the progression of K-13 polymorphisms and the allele diversity associated with the clinical phenotype.

**METHODS:** Parasite clearance half-lives were assessed in 35 patients with uncomplicated *P. falciparum* treated with artesunate monotherapy followed by 3-day ACT in an isolated area on the Thai-Myanmar border with relatively low artemisinin pressure. Molecular testing for K-13 mutations was performed on admission samples using dry blood spots.

**RESULTS:** The proportion of K-13 mutations in these patients was 41.7%, and only 5 alleles were detected: C580Y, I205T, M476I, R561H, and F446I. Of these, F446I was the most common, and was associated with a longer parasite clearance half-life (median) 4.1 (min-max 2.3-6.7) hours compared to 2.5 (min max 1.6-8.7) in wildtype ( $p=0.01$ ). By contrast the proportions of K-13 mutants in another area of the border, 200 km south was 84% and 15 different alleles were detected, C580Y being the most common one.

**CONCLUSIONS:** This study provides evidence of artemisinin resistance in a remote part of eastern Myanmar. The prevalence of K-13 propeller mutations as well as allele diversity varies considerably across short distances, presumably related to historical patterns of artemisinin use and population movements. 🗨️

**Keywords:** *Plasmodium falciparum* malaria, artemisinin, parasite clearance, K-13 mutation, drug resistance



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Poster no. 24

## GENETIC DIVERSITY OF MSP3 BETA GENE IN PLASMODIUM VIVAX FROM TWO REGIONS IN SOUTHERN INDIA BY PCR/RFLP ANALYSIS

**Introduction:** *Plasmodium vivax*, the most widespread malaria parasite species across the globe and equally competes with *Plasmodium falciparum* as a cause of human malaria. As compared to *P. falciparum*, *P. vivax* is not much studied and least understood. Knowledge about PvMSP3 $\beta$  as an epidemiological marker is not available in the Indian context.

**Objectives:** This study aimed to genotype the PvMSP3 $\beta$  gene in populations from two regions of Southern India- Mangalore and Pondicherry.

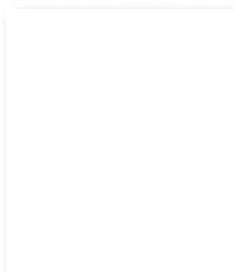
**Materials and Methods:** A total of 100 *P. vivax* positive samples were detected by microscopy, QBC, and RDT. Further, it was confirmed by nested PCR. For genetic diversity study PCR-RFLP method was employed which targeted MSP3 $\beta$  gene.

**Results:** Out of 100 samples, Pondicherry contributed 31/100(31%) and Mangalore 69/100(69%). The alleles of the amplified DNA fragments were classified into Type A (1.5-2.0 kb) & Type B (<1.5 kb to 1 kb). Of the 100 *P. vivax*-positive samples, 49 (49%) belonged to Type A, 46(46%) to Type B and 5 (5%) showed mixed infections. Type A was predominant in Mangalore and Type B in Pondicherry.. Further, PvMSP3 $\beta$  gene was analysed using Pst-1 restriction enzyme and suballelic variants were found in both Type A (A1-A17) and Type B (B1-B12).

**Conclusion:** In consensus to existing studies, our findings also suggest that PCR/RFLP of PvMSP3 $\beta$  is a reliable, cost effective epidemiological marker for the genotyping. 🗨️

**Keywords:** *Plasmodium vivax*, MSP3 $\beta$  gene, Genetic diversity, PCR-RFLP






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Poster no. 25

## IDENTIFICATION OF SMALL-MOLECULE INHIBITORS OF PLASMODIUM N-MYRISTOYLTRANSFERASE

There is a desperate need for new antimalarial drugs due to the rise in resistance to current therapies which are small in number and have undesirable side effects. The enzyme *N*-myristoyltransferase (NMT) is a validated drug target in the causative agent of malaria, the single-celled eukaryote *Plasmodium*. NMT catalyzes the myristoylation of protein substrates, which is an essential lipid modification with roles in membrane targeting and cell signaling. As NMT is conserved among eukaryotes, it is crucial to identify inhibitors that act specifically against *Plasmodium* NMT without impacting the human homolog. Here, we used a biochemical assay in a 1536-well format to screen ~2 million small molecules from the GSK compound library against NMT of the most widespread human malaria parasite *P. vivax*. Confirmed hits were triaged based on potency and preferred physicochemical properties and were tested in a dose-dependent manner against *P. vivax* and *P. falciparum* NMT for their cross-species activity and against human NMTs to discard compounds with a selectivity index lower than 10. Chemical clustering of the most potent compounds resulted in 9 series and 22 singletons. Several of these inhibited the growth of the pathogenic blood stages of *P. falciparum* by more than 50% at a 2  $\mu$ M compound concentration *in vitro*, and showed no to limited toxicity against the mammalian HepG2 cell line. These compounds represent good starting points for the development of new antimalarials. 

**Keywords:** Malaria, drug discovery, high-throughput screening



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**Poster no. 26**

**ACTIVE SURVEILLANCE MALARIA IN MILITARY ALONG THAI – MYANMAR AND THAI – CAMBODIA BORDERS**

**T**hailand is still one of burden countries that impacted by malaria. In malaria endemic areas, especially along Thai borders, the border people including vigilant soldiers are the vulnerable groups take risk for malaria infection and transmission across Thai borders. This disease non-battle injury (DNBI) has been concerned and conducted an active surveillance among Thai military by Epidemiology section from RTA-AFRIMS for more than 20 years.

To survey and monitor the epidemiology evidence of malaria, active case detection along Thai-Myanmar and Thai-Cambodia borders has been performed using microscopic examination method and/or RDT. We also provided support for early detection and treatment at the troop level. In addition, monthly malaria case data from local public healthcare providers was integrated.

From October 2015 to June 2016, there is no malaria positive case in Thai-Myanmar border. In Thai-Cambodia AO, approximately 4,500 soldiers were screened for malaria infection and 151 cases (3.36%) were identified. The prevalence shown 1.22% *P. vivax*, 0.96% *P. falciparum* and 1.18% for mix infection of PV and PF. The annual prevalence was decreased for last few years; 2013-2016 (12.67, 7.45, 5.23 and 3.36 respectively). Interestingly, the prevalence of mix infection was increasing, meanwhile *P. vivax* infection prevalence was decreasing among malaria infected troop.

A trend of decreased prevalence implied that active surveillance strategy for reducing the burden of border malaria is substantial for malaria prevention and control among Thai military troops. The surveillance system would be further improved the coverage and expansion at the military operating base to the policy level to sustain malaria control toward progressive elimination. 🗨️

**Keywords:** border malaria, active surveillance, military area of operation



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**Poster no. 27**

## **CURRENT MALARIA SITUATION IN FIVE SOUTHERN PROVINCES, LAO PDR – A STUDY BY THE SATREPS PROJECT**

**M**orbidity and mortality of malaria in Lao PDR have decreased due to the continuous efforts of Lao Ministry of Health (MoH) with support of WHO, Global Fund and other developing partners. Presently, the Lao MoH has set a goal to eliminate malaria by 2030 in the country. To achieve this goal, we should know precise malaria situation in such high endemic Southern provinces such as, Savannakhet, Salavanh, Sekong, Champasak and Attapeu. In this study, 156 public health facilities in these provinces were selected and collected data on malaria suspected cases and positive cases. From June-October 2015, a total of 4,439 malaria suspected cases were recorded in the 156 public health facilities and 587 cases (13.2%) were diagnosed as malaria (133 *Plasmodium falciparum*, 196 *P. vivax*, 6 mixed-infection and 252 unknown species) by microscopy or rapid diagnostic test (RDT). When we analyzed the patients' data in each province, malaria positivity rate of male was significantly higher than that of female in the all provinces ( $P < 0.01$ ) except Savannakhet. Moreover, 15-39 years old male population tended to be malaria positive, and most of their occupations were agriculture. These results suggest that malaria high-risk population in the 4 provinces was adult male population, and seemed to be associated with their working style, i.e. they routinely work in their farms, as well as in forests for collecting foods and woods. Current malaria elimination program is basically household-based approach. Our result suggests that job-based approach is also needed for malaria elimination in Lao PDR. 🗨️

**Keywords:** SATREPS project, Lao PDR, Malaria



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**Poster no. 28**

## **COMPARATIVE STUDY OF WHEATLEY'S TRICHROME STAIN AND IN-VITRO CULTURE FOR THE DIAGNOSIS OF *BLASTOCYSTIS* SP. IN STOOL SAMPLES**

**M**ethod of detection of *Blastocystis* sp. either by permanent stain or short-term *in-vitro* cultivation techniques remains controversial in term of their effectiveness. Hence, we evaluate the performance of routine permanent stain and cultivation method in comparison with PCR assay as the reference technique to detect *Blastocystis* sp. A total of 359 stool samples were examined using Wheatley's trichrome stain, *in-vitro* culture and PCR assay. Fifty-six (15.6%) samples were detected positive with *Blastocystis* sp. by Wheatley's trichrome stain and 73 (20.3%) by *in-vitro* culture, while PCR assay detected 71 (19.8%) positive samples. Our results confirmed that 28 microscopy-negative samples were positive by PCR assay. Detection rate of *Blastocystis* sp. was highest in combination techniques (27.9%). The sensitivity and specificity of Wheatley's trichrome staining and *in-vitro* culture techniques compared to PCR assay (reference technique) were 49.3% (95% CI: 37.2-61.4) and 92.7% (95% CI: 89.1-95.4) and 39.4% (95% CI: 28.0-51.8) and 84.4% (95% CI: 79.7-88.4), respectively. However, the sensitivity [60.6% (95% CI: 48.3-71.9)] of the method increased when both techniques were performed together but the specificity slightly decreased to 80.2% (95% CI: 75.1-84.7). The agreement between Wheatley's trichrome stain, *in-vitro* culture and combination techniques with PCR assay were statistically significant by Kappa statistics (Wheatley's trichrome stain:  $K = 0.456$ ,  $p < 0.001$ ; *in-vitro* culture:  $K = 0.236$ ,  $p < 0.001$  and combination techniques:  $K = 0.353$ ,  $p < 0.001$ ). Hence, our results suggest combination techniques as a screening method in the diagnosis of *Blastocystis* infection either for clinical or epidemiological study to ensure better and accurate diagnosis. 🗨️

**Keywords:** Trichrome, culture, PCR, *Blastocystis*





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**Poster no. 29**

## **MOLECULAR DETECTION OF *BLASTOCYSTIS*, *CRYPTOSPORIDIUM* SPP. AND *ENTEROCYTOZOON BIENEUSI* IN HUMANS AND PIGS IN NAKHON PATHOM PROVINCE, THAILAND**

**B**lastocystis, *Cryptosporidium* spp. and *Enterocytozoon bieneusi* are enteric protozoa in a wide range of vertebrate hosts, causing diarrheal diseases. In this study, a total of 142 human and 102 pig stool samples were collected from pig farms in Nakhon Pathom Province. Presence of the parasites was done using nested PCR. In human, 4/142 (2.8%) of *Blastocystis* and 13/142 (9.2%) of *E. bieneusi* were found while 32/102 (31.4%) of *Blastocystis* and 17/102 (16.7%) of *E. bieneusi* were found in pigs. No *Cryptosporidium* spp. (0%) was detected in both pig and human samples. In pigs, four *Blastocystis* subtypes, ST14, ST1, ST12, ST3, and eight *E. bieneusi* genotypes, EbpC, H, 6 new genotypes, were identified. In humans, *Blastocystis* ST1 and ST3 and 10 *E. bieneusi* genotypes were identified. Our study indicated the zoonotic potential transmission of *Blastocystis* and *E. bieneusi* in Nakhon Pathom, Thailand. 🗨

**Keywords:** Blastocystis/*Cryptosporidium*/*Enterocytozoon*/pig/human



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**Poster no. 32**

## **ANTIPROTOZOAL ACTIVITY OF ESSENTIAL OIL FROM THAI MEDICINAL PLANTS AGAINST *GIARDIA DUODENALIS***

**G***iardia duodenalis* (synonyms: "*G. lamblia*" or "*G. intestinalis*") is a common flagellate protozoan that globally causes the diarrheal disease giardiasis. Most infections result from fecal-oral route. In Thailand, several herbs are used for medicinal purposes and in cooking to promote health and reduce antibiotic resistance. However, information on the effects of Thai herbs on intestinal protozoa are lacking. This study determined the effectiveness of Thai herbs-extracted essential oils (clove, ginger, galangal, litsea, star anise, indian ivy-rue, lemon, bergamot, orange, holy basil, and sweet basil) for inhibiting or killing *G. duodenalis*. The results showed that only 5 essential oils from lemon, litsea, galangal, clove and orange showed a significant inhibiting activity against *G. duodenalis* and IC<sub>50</sub> values were 6.96±0.13, 60.66±0.82, 118.95±1.93, 138.10±3.21 and 650.73±6.25 µg/ml, respectively. In addition, IC<sub>50</sub> value of metronidazole was 63.21±1.56 µg/ml, while the rest did not show inhibitive property. This is a pilot study to identify antiprotozoal essential oils, in which a further study will isolate the medicinal components for the development of herbal medication or products that will be used to treat giardiasis. 🗨️





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**Poster no. 33**

**GENOTYPE AND SUBGENOTYPE ANALYSES OF  
CRYPTOSPORIDIUM PARVUM AND CRYPTOSPORIDIUM  
HOMINIS ISOLATED FROM PHILIPPINE EDIBLE  
BIVALVES**

In the Philippines, raw or lightly-cooked bivalve dishes are very common. Being filter feeders, these bivalves might be contaminated with pathogens which can cause diseases to humans. One common pathogen found in bivalves is the protozoan parasite of the genus *Cryptosporidium* (Phylum Apicomplexa) which causes human gastroenteritis in both developed and developing countries. In this study, oocysts of *C. parvum* and *C. hominis* were isolated from common edible bivalves using combination of sucrose flotation and immunomagnetic separation (IMS). They were identified by 18s rRNA gene sequence analysis and were subjected to genotype and subgenotype analyses using the 60 kDa glycoprotein gene. For *C. parvum*, genotype IIa was found in 30 out of 31 isolates with subgenotype IIaA15G2R1 as the dominant subgenotype (n=24) followed by subgenotype IIaA15G2R2 (n=3). One *C. parvum* isolate had very low BLAST matches and may indicate possibility of obtaining a new genotype. While for *C. hominis*, two genotypes were detected in nine isolates (1b and 1f), eight of which belonged to subgenotype 1fA19G1 and one isolate belonged to 1bA10G2R2. All of these identified subgenotypes had been known to be involved in human cryptosporidiosis cases. Therefore, this study confirmed the presence of *C. parvum* and *C. hominis* genotypes and subgenotypes which are listed as common human pathogens. This study showed that humans ingesting contaminated shellfish are exposed to different *Cryptosporidium* genotypes and subgenotypes of public health significance. 🗨️

**Keywords:** *Cryptosporidium parvum*, *Cryptosporidium hominis*, bivalves



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**Poster no. 34**

## **SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION IN REFUGEE AND MIGRANT PREGNANT WOMEN ALONG THE THAILAND-MYANMAR BORDER**

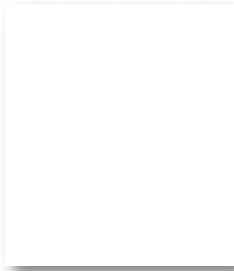
**Background:** Primary infection with *Toxoplasma gondii* in pregnancy is associated with poor obstetric outcomes and congenital infection. Prevalence rates in pregnant women in Thailand are well documented (rates vary between 5.3-53.7%) but there is only one study from Myanmar reporting 30.7% (95%CI 27.9-37.2). This study aimed to determine the seroprevalence of *Toxoplasma* infection in pregnant migrant and refugee women on the Thailand-Myanmar border.

**Material and methods:** A random selection of 199 stored blood samples of women attending antenatal clinics over an 18 month period (Jan-14 to Jul-15) were tested for *Toxoplasma* IgG and IgM antibodies. Avidity testing discriminated between acute or past exposure to the parasite if IgM results were positive.

**Results:** Seroprevalence of *Toxoplasma* infection was 31.7 % (63/199) (95%CI 25.6-38.4). No acute infection was detected in the 3 positive IgM cases. A significant positive linear trend was seen with *Toxoplasma* exposure and increasing maternal age ( $p=0.036$ ). Multiparity ( $\geq 3$  children) was associated with a 2.46 aOR(95%CI 1.23-4.90,  $p=0.011$ ) fold higher *Toxoplasma* seropositivity rate; while literacy, history of poor obstetric outcome, refugee status and ethnic group showed no significant associations. As expected, there was no association with poor outcomes in the current pregnancy as all results revealed past exposure.

**Conclusion:** Seroprevalence of *T. gondii* infection in pregnant migrants and refugees is similar to previously reported data in Myanmar and Thailand, and was only significantly associated with multiparity. *Toxoplasma* infection is important in pregnant women and is probably related to socioeconomic conditions. Nevertheless, in this marginalized population, this parasite infection may be given less priority, due to resource constraints in providing even the most basic components of safe motherhood programs. 🗨️

**Keywords:** *Toxoplasma gondii*, TORCH, seroprevalence, congenital



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**Poster no. 36**

## **TEMPORAL INCREASE IN PYRETHROID RESISTANCE AND FREQUENCY OF KDR MUTATIONS IN *AEDES AEGYPTI* FROM KOLKATA AN INDIAN METROPOLITAN CITY**

**E**mergence of pyrethroid resistance in *Aedes aegypti* is the main threat to worldwide dengue control programs. Knockdown resistance (*kdr*) is one of the mechanisms of resistance against DDT and pyrethroids resulting from non synonymous mutation(s) in voltage gated sodium channel (VGSC) gene-the target site of action for these insecticides. Emergence of pyrethroid resistance is reported worldwide but the status in Indian *Ae. aegypti* is limited. Thus, a pilot study for surveillance of insecticide susceptibility was conducted in *Ae. aegypti* from Kolkata city, India, which is a hot spot for dengue epidemic. Insecticide susceptibility was evaluated against DDT (4%), permethrin (0.75%) and deltamethrin (0.05%). Adult bioassays carried out in year 2012 and 2014 revealed increasing resistance against all three insecticides. Percentage mortalities against DDT, permethrin and deltamethrin in 2012 were 24%, 75%, 84% respectively which further reduced to 4%, 33%, 25% in 2014. DNA-sequencing of partial domain II, III and IV of the VGSC revealed two *kdr* mutations; first novel T1520I and another F1534C in domain III of VGSC whereas no *kdr* mutation was observed in other two domains. Further, PCR-RFLP assays were designed for genotyping of these mutations. Genotyping revealed increase in frequencies of both mutations in year 2014 as compared to year 2012. In 2012, no sample has T1520I mutation, whereas in 2014 its frequency increased to 0.6. Similarly, frequency of F1534C mutation increased from 0.25 in 2012 to 0.75 in 2014. This increase in *kdr* mutations with reduced insecticide susceptibility is alarming for vector control efforts. 🗨️

**Keywords:** *Aedes aegypti*, insecticide resistance, *kdr*



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Poster no. 37

## EFFECT OF TEMPERATURE ON THREE PYRETHROID SUSCEPTIBILITY OF *Aedes aegypti* IN NAKHONSAWAN AND RAYONG PROVINCES, THAILAND

**Background:** Dengue fever is a mosquito-borne viral disease that found in tropical and sub-tropical areas of the world, mostly in urban and semi-urban areas. The bite of infected female *Aedes aegypti* is the effective route of dengue transmission. The use of the chemical insecticide is the common control measure of *Ae. aegypti*. Currently, In Thailand it had reported insecticide resistance of *Ae. aegypti*. Temperature is the external factor of insecticide susceptibility. Therefore, effects of temperature on insecticide susceptibility were studied in field population of *Ae. aegypti*.

**Methodology:** *Ae. aegypti* larvae were collected from Nakhonsawan and Rayong for two times during December 2015-January 2016 and April-May 2016. The unfed females of F1 progeny aged 3-5 days were used in the experiment. They were exposed at three temperatures ( $20\pm 1^\circ\text{C}$ ,  $25\pm 1^\circ\text{C}$ , and  $30\pm 1^\circ\text{C}$ ) for 4 hours prior to insecticide susceptibility test with three pyrethroids (Cyfluthrin, Deltamethrin, and Lambda-cyhalothrin). Mortality was examined after 24 hr exposure.

**Results:** The mortality of  $20^\circ\text{C}$  pre-exposed groups were higher than  $30^\circ\text{C}$  pre-exposed ( $P < 0.05$ ). The relationship between temperature and pyrethroid susceptibility was found negative relationship.

**Conclusion:** Temperature affected to pyrethroid susceptibility and exhibited a negative relationship between temperature and mortality of *Ae. aegypti* strains from Nakhonsawan and Rayong, Thailand. 🗨️

**Keywords:** *Aedes aegypti*/ Temperature/ Insecticide susceptibility/ Thailand/ Pyrethroid





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**Poster no. 38**

## **COMPARATIVE REPELLENT ACTIVITY OF FIVE HERBAL ESSENTIAL OILS AGAINST AEDES AEGYPTI**

The repellent activity of five herbal essential oils from bergamot (*Citrus bergamia*), basil (*Ocimum basilicum*), patchouli (*Pogostemon cablin*), anise (*Pimpinella anisum*) and coriander (*Coriandrum sativum*) against the *Aedes aegypti* mosquito was investigated. The undiluted essential oil was tested individually under laboratory conditions by arm-in-cage method. A sample of 0.1 mL of essential oil was applied to 3 x 10 cm area of exposed forearm skin of the volunteer. Subsequently, a test was conducted every 15 minutes. This involved exposing the treated arm to 250 *Aedes aegypti* female mosquitoes for 3 minutes. The experimental endpoint was at least 2 mosquitoes landing or biting on treated arm during testing period, which indicated the stop point of the protection time of the essential oil. Of the five essential oils tested, patchouli gave the longest protection time at 67.5 minutes, followed by anise, bergamot, coriander and basil at 51, 48, 36 and 16.5 minutes, respectively. Due to their potent activity, patchouli, anise and bergamot oil could be considered for development as an alternative mosquito repellent formulation. 🗨️

**Keywords:** volatile oil, plant-based repellent, mosquitoes




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**Poster no. 41**

### **EFFECTS OF CONTAINER VARIATIONS ON PREDATION BY *TOXORHYNCHITES SPLENDENS* (DIPTERA: CULICIDAE)**

**T**he effect of container size on predation by fourth instar larvae of *Toxorhynchites splendens* was investigated in the laboratory. Experiments were conducted using containers with equal water volumes (500 ml) but different surface areas; small bowl (103.8 cm<sup>2</sup>), big bowl (188.6 cm<sup>2</sup>), small tray (300 cm<sup>2</sup>) and big tray (638 cm<sup>2</sup>). Also, three different water volumes were tested at 100, 300 and 500 ml. There were significant differences in prey consumed and killed among containers with different surface areas but there were no significant differences in prey consumed among containers of different volumes. The results showed that more prey were consumed in small bowls (615.5 larvae) than big bowls (486.9 larvae), small trays (442.1 larvae), or big trays (363.5 larvae). Fewer prey larvae were killed in the big trays than other containers. The daily mean consumption rate decreased over time but rates of killing (without consumption) increased as pupation approached. This study suggests that containers with small surface areas are most suitable for increasing consumption and killing by *Tx. splendens*. 

**Keywords:** Toxorhynchites, prey, container, consumed, killed



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Poster no. 42

## EFFECT OF PREY DENSITY ON THE DEVELOPMENT TIME AND BODY SIZE OF MALE AND FEMALE PREDATORY MOSQUITOES *TOXORHYNCHITES SPLENDENS* (DIPTERA: CULICIDAE)

The purpose of this study was to determine the effect of different prey densities on *Toxorhynchites splendens* development time and body size. Individual larvae were reared from 4<sup>th</sup> instars to adults in small plastic bowls and provided 4<sup>th</sup> instar *Aedes aegypti* larvae as prey at three different densities; 100, 200 and 300 larvae. The wing lengths of *Tx. splendens* adults were measured as an estimate of body size and the date of emergence and gender were recorded. There was a significant difference in the development times of *Tx. splendens* larvae and pupae when reared at different prey densities. The development times of larval and pupal stages were greater when reared at high prey densities (300 preys) compared to lower prey densities. The 4<sup>th</sup> instar predators consumed and killed significantly more prey at higher prey densities (300 preys) than low densities (200 and 100 preys). There were no significant differences between either gender in the amount of prey consumed and killed. However, prey density had a significant effect on male and female body size with larger adults emerging from tubs containing 300 preys (7.3 and 5.9 mm) compared to 200 preys (7.1 and 5.7 mm) and 100 preys (6.6 and 5.4 mm), respectively ( $p$ -value 0.000). The results show that high prey densities promote large adults that consume more prey, although they emerge slightly later. 🗨

**Keywords:** Toxorhynchites, prey, density, development, gender



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**Poster no. 43**

### **THE MORPHOLOGICAL STUDY OF *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE) IN THIRD-INSTAR LARVA STAGE RECEIVING *STEMONA COLLINSIAE* ROOT EXTRACT**

**M***usca domestica* (House fly) is a synanthropic fly which is easily found in human community, restaurant including cattle. In Thailand, the population of *M. domestica* is found about 80-90 percent of all population of flies. *M. domestica* is a vector of pathogenic bacteria, virus, parasite and fungi which these pathogens are transmitted to human and animal via mechanical and propagating transmission. Synthetic-chemical insecticides are used for eliminating and preventing human or animal from vector-borne diseases. Due to accumulation in environment including human and animal body and long half-life of these synthetic-chemical insecticides, bio-pesticide which produces from plant and microorganisms is considered as a friendly environment, low toxicity to mammal and short degradation time. Many insecticidal plants are found in Thailand including *Stemona* plant.

*Stemona collinsiae* has been traditionally used as pesticide for plantation and food protector from fly. In this experiment, third-instar larvae of *M. domestica* were tested with 70 percent ethanolic crude extract from root of *S. collinsiae* using topical application method and a development of these larvae was studied comparing with negative control groups which received water. Physical appearances were detected using visible and SEM techniques. The third-instar larvae were able to develop to be pupae but physical appearance as segmental and curved body occurred instead of a barrel-shaped which was a normal shape of *M. domestica* in pupal stage. The segmental body happened from a contractile of muscle and adult flies were not able to emerge from these abnormal pupae. 🗨️

**Keywords:** *Stemona collinsiae*, *Musca domestica*, Housefly





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**Poster no. 44**

**ACTION AGAINST DENGUE IN THE SIXTH ASEAN  
DENGUE DAY CAMPAIGN 2016 IN THAILAND**

**D**engue is a mosquito-borne viral disease problem among ASEAN countries which rapidly spread out to the other regions and become the global problem nowadays. Dengue is mostly found in tropical and subtropical climates worldwide and also in urban and semi-urban areas where they are the suitable places for spreading of the vectors (*Aedes aegypti* and *Aedes albopictus*). According to the report of the World Health Organization (WHO), 2015 was intense for dengue fever in many regions: the Philippines reported more than 169,000 cases, Malaysia had in excess of 111,000 cases and Vietnam reported some 80,000 where as Thailand reported more than 140,000 cases.

The Association of Southeast Asian Nations (ASEAN) designated 15<sup>th</sup> June in every year as the ASEAN Dengue Day to increase public awareness of dengue, to mobilize resources for its prevention and control and to demonstrate the region's commitment for tackling this disease. All ASEAN Member States agreed and supported the conduction of Annual Regional Observation of ASEAN Dengue Day as well as National ASEAN Dengue Day Campaign. ASEAN Dengue Day is an advocacy campaign that serves as a common platform for its 10 member-nations namely Brunei Darussalam, the Kingdom of Cambodia, the Republic of Indonesia, the Lao People's Democratic Republic, Malaysia, the Republic of the Union of Myanmar, the Republic of the Philippines, the Republic of Singapore, the Kingdom of Thailand and the Socialist Republic of Vietnam.

Action against *Dengue* in the *Sixth ASEAN Dengue Day Campaign* in 2016 was held on 14-15 June in Bangkok, Thailand under the theme "Community Empowerment: A Sustainable Success to Fight Dengue". This reflects the call for joint efforts from society to unite against the disease together as one community. The campaign has been organized in many community areas such as school, temple, houses and public spaces around community. Thailand has an outstanding profile in dengue prevention both the policy and implementation level. In 2015, the government stepped up efforts to reduce the number of dengue cases by convening the Memorandum of Understanding (MoU) signing ceremony among eight Ministries to work together to achieve the prevention goals. The morbidity rate is still high, but there are now very few deaths from the disease, with a mortality rate reduced to less than 0.1 percent.

Following the government officials' meeting, the Bangkok Call for Action in the Prevention and Control of Dengue has been launched and agreed upon by all member nations. Thailand would like to seek support from all ASEAN Member States for the proposed the conduction of Annual Regional Observation of ASEAN Dengue Day as well as National ASEAN Dengue Campaign and hope the implementation of the action plans in each ASEAN Member States will strengthen the community empowerment, social mobilization, public and private sectors engagement and participation for ensuring the effective dengue prevention and control as "Dengue Free Community" in this region. 🗨️

**Keywords:** ASEAN, Dengue, Campaign



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Poster no. 45

## ASSOCIATION OF CLINICAL MANIFESTATION AND LABORATORY CHARACTERISTICS WITH SEVERITY OF DENGUE VIRAL INFECTION IN CHILDREN IN DR. HASAN SADIKIN GENERAL HOSPITAL BANDUNG INDONESIA

**Background:** Dengue viral infection (DVI) is a major cause of morbidity and mortality in children. Indonesia has large amount of cases of dengue infection with 0.88% mortality rate. Unusual manifestation is commonly contributed to increasing mortality.

**Objective:** To describe association of clinical manifestation and laboratory characteristics of the confirmed DVI cases in children admitted to Dr. Hasan Sadikin General Hospital, Bandung, Indonesia with severity of disease.

**Methods:** All of the confirmed dengue viral infection cases from January 2014 to December 2015 were retrospectively studied. Data including patient demographic, initial symptoms, laboratory results, were collected. Severity of DVI is categorize as dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Association between initial symptoms, blood count, and severity of disease was analyzed using Chi-square and one-way ANOVA ( $p < 0.05$  = significant).

**Results:** There were 192 confirmed cases of DVI, 89.58% using dengue IgG-IgM antibodies, while 10.42% using NS-1 antigen. The median age was 6.46 years. Initial symptoms of diaphoresis, epigastric pain, cold extremity, seizure were associated with more severe infection ( $p < 0.005$ ). Epistaxis was the most frequent hemorrhagic manifestation (19.79%). The mean leukocyte count in DF, DHF, DSS patients were respectively 6,069  $\text{mm}^3$ , 4,196  $\text{mm}^3$ , and 4,392  $\text{mm}^3$ . While secondary infection in DF, DHF, DSS patients were respectively 22.8%, 58.8%, and 18.4%. The lower leukocyte count and secondary infection were associated with more severe infection ( $p < 0.001$ ).

**Conclusion:** Initial symptoms, leukocyte count, primary and secondary infection are associated with severity of DVI

**Keywords:** dengue, initial symptoms, leukocyte count, secondary infection, children



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**Poster no. 46**

## **CHARACTERIZATION OF ANTI-DENGUE VIRUS NS1 HUMABS AGAINST DENGUE VIRUS**

**Background:** Dengue fever (DF) and its severe form, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are one of the most important mosquito-borne tropical disease of public health throughout the world. The severe form of dengue disease was frequently hypothesized to be correlated with high viral titer and secondary infection with heterologous serotypes, so called ADE, that caused mostly by anti-E and anti-prM antibodies. Also, anti-NS1 antibodies were recently interested as one factor for severe dengue infection because of their cross-reactivity with human molecules, causing some severe symptoms like vascular leakage. However, information of anti-NS1 antibody, especially antibodies generated from human was still limited.

**Methods:** In this study, 5 anti-NS1 HuMAbs generated from our previous study were characterized. The hybridomas were cultured and re-cloned to obtain individual cells secreting HuMAb before proceeded to antibody gene cloning. Human variable heavy and light chain (VH and VL) were isolated from those hybridoma cells and analyzed by IMGT/V-QUEST database. HuMAbs were purified from collected culture supernatant for neutralizing activity test (NT).

**Results:** It was shown that most of the clones used the same parental germline sequence. As expected, for infection inhibition NT assay, studied on Vero cells, most of them showed moderate to weak neutralizing activities against DENV-2. Further studies on viral replication inhibition will be further performed. The results obtained from this study can be used as fundamental genetic information and neutralizing activity of the anti-NS1 HuMAbs, which can be used for further characterization of its enhancement mechanisms, epitope characterization, and their cross-reactivity. 🗨️

**Keywords:** Human monoclonal antibody, Dengue virus Non- structural protein 1 (NS1), Germline, Neutralization activity





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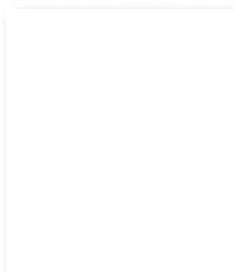
**Poster no. 47**

## **LARGE SCALE PRODUCTION OF HUMAN THERAPEUTIC MABS AGAINST DENGUE VIRUS USING STABLE CHO CELL EXPRESSION**

Using SPYMEG hybridoma cells, neutralizing human monoclonal antibodies (NhuMAbs) against 4 serotypes of Dengue virus (DENV) has been the world's first time established at CEAR. Two candidates NhuMAb (1B3B9 and 1G7C2). were successfully pre-clinically tested *in vitro* by 95-100 % neutralized all 20 clinical isolates DENV and *in vivo* by significantly decreased mortality of Dengue intra-cranially mice and eliminated DENV (from 10<sup>10</sup> to be 0) in blood of Marmosets within two days. For further used in human, large scale production of NhuMAb using the Food and drug administration (FDA) accepted method namely Stable Chinese Hamster Ovary (CHO) cell expression, is needed. This study reports the establishment of stably expressed CHO cells for large scale production of NhuMAbs against 4 serotypes of DENV. To express DENV IgG, two plasmids containing heavy and light chains of anti-DENV IgG were transfected into CHO-K1 cells using lipofectamine 2000. Two antibiotics, hygromycin and puromycin, were used to select for anti-DENV IgG producing cells. Optimal concentrations of the antibiotics were determined to create stable CHO cells. The transfected cells were trypsinized and cloning of single cell by limiting dilution method. The wells with one colony were marked. Cell growth was observed, culture supernatants were harvested and NhuMAb concentration was measured using ELISA to screen for positive clones. Stably expressed CHO cells were successfully established for two candidate anti-DENV IgG NhuMAb. These CHO cells could stably express IgG NhuMAbs with the same neutralizing activity against 4 serotypes of DENV, as same as those from hybridoma cells. These expressed NhuMAb will be further clinical trial test in human in the near future. 🗨️

**Keywords:** Stable Expression / Dengue Virus / Monoclonal Antibody / Mammalian Cell





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**Poster no. 48**

## **PERCEPTIONS TOWARDS DENGUE HEMORRHAGIC FEVER ETHNIC MINORITY GROUPS IN RATTANAKIRI AND MONDOLKIRI PROVINCE : A RAPID ASSESSMENT**

**Background:** The Cambodia-Thailand Malaria Control project, was launched in 2007 in two eastern Cambodian provinces, Ratanakiri and Mondolkiri. The project aimed at malaria control among indigenous people belonging to several ethnic minorities. Presently malaria problem has been significantly reduced over the past several years. However, the public health threat of dengue has found in the project areas. This study aimed to describe the perceptions towards Dengue Hemorrhagic Fever (DHF) among the Ethnic Minority Groups in both Provinces.

**Methodology:** A rapid assessment was organized. Community and household environment observation were conducted. Sixteen participants were in-depth interviews. Content analysis was used for data analysis.

**Results:** Most of them (13/16) had ever heard about DHF. Among those, one half of them unable to describe about causes, signs/symptoms, prevention and control of DHF. More than half stated that headache and fever were main signs and symptoms of DHF. Less than half had known how to prevent and control DHF in the community. Most of participants perceived that their villages and they themselves were DHF at risk, "... there are a lot of mosquitoes in the villages and DHF cases were found in the villages". The observations found that very few households covering water jars/water containers and sleeping in mosquito nets in the day time.

**Conclusion:** The study, which is still underway, was undertaken to develop an improved, community-led socio-culturally sensitive approach to DHF prevention and control among the ethnic groups. 🗨️

**Keywords:** perception, Dengue Hemorrhagic Fever, ethnic group



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**Poster no. 53**

## **MAN-MADE CONTAINER THE RISK OF DENGUE EPIDEMIC IN MONDULKIRI AND RATTANAKIRI, CAMBODIA**

Entomological surveys were carried out from September 2014-January 2016 to investigate the presence of dengue vectors in man-made containers, discarded tires and artificial water containers in houses and peridomestic areas. A cross-sectional immature stage survey was done indoors and outdoors in 2-3 villages in Mondulkiri and Rattanakiri, Cambodia respectively. Larvae were identified morphologically, larval indices were also calculated, and key container were defined separately at each location. Mosquito larval sampling was conducted using pipette or dipper depending on container types.

Results showed *Aedes albopictus* was the predominant species found higher than *Aedes aegypti* in Rattanakiri and Mondulkiri province. Larval Indices were seasonally different among localities. HI in localities showed significant difference ( $p < 0.05$ ) between seasons: Dry (Dec) and Wet (May–October) of  $X^2 = 80.45$  ( $df = 4$ ) and  $4.98$  ( $df = 3$ ) in Rattanakiri and Mondulkiri respectively. Both *Aedes aegypti* and *Aedes albopictus* are breeding in a wide range of artificial containers. However the key positive containers differed among each province, with 4 and 5 main breeding types in Mondulkiri and Rattanakiri respectively. Used oil drums, water containers, used tires, and discarded containers were indicated as 'Key Containers' in Mondulkiri. While in Rattanakiri survey showed the same Key container, plus one additional type, large water jars. We suggest to achieve controlling these mosquitoes, the integration of different methods with community participatory should be taken into consideration. 🗨️

**Keywords:** Dengue, *Aedes* vectors, key container



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**Poster no. 55**

## **RISK FACTORS FOR CHIKUNGUNYA INFECTION DURING A RE-EMERGED CHIKUNGUNYA OUTBREAK IN TAKBAI DISTRICT, NARATHIWAT PROVINCE DURING 2014-2015**

In late November 2014, an outbreak of the Central East African chikungunya virus re-emerged in Takbai district, Narathiwat province. A serosurvey was conducted to investigate the clinical features and risk factors of infection. Sera from 208 volunteers aged more than 17 years had chikungunya IgM antibodies by Haemagglutination inhibition test and interviewed with structured questionnaire. Of 161 infected cases, 122 (58.6 %) had chikungunya-like-symptoms. The dominant symptoms reported were joint pain (99.2%), headache (94.3%), fever (87.7%) and rash (73.1%). Chikungunya infection was associated with no outdoor mosquito biting protection (adjusted odds ratio=3.13, 95%CI =1.27-7.67), having outdoor garbage pile (adjusted odds ratio=2.79, 95%CI =1.26-6.17) and illiteracy or lower primary school education (adjusted odds ratio=2.79, 95%CI =1.01-7.67). Therefore, self protection from outdoor mosquito biting and clearing of garbage piles should be promoted to residents during chikungunya outbreak. 🗨️

**Keywords:** chikungunya, outbreak, risk factor



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**Poster no. 57**

**DIFFERENTIAL VACCINIA VIRUS ENTRY AND  
INNATE IMMUNE SIGNALING IN MACROPHAGES**

Previously, we showed that Vaccinia virus Western Reserve (WT-WR) contains A26 protein and enters HeLa cells through fluid phase endocytosis whereas a mutant, A26 deletion virus (WRDA26) enters through plasma membrane fusion. Now we extended this finding into the primary innate immune cells particularly macrophages to understand whether WT-WR and WRDA26 also enter immune cells through different pathways. Literatures showed that infection of WR in the human or mouse macrophages is not permissive, causing apoptosis of infected cells; however, the intracellular mechanism of restriction is unclear. We infected bone marrow derived macrophages (BMDM) with WT-WR and WRDA26 viruses and utilized microarray to analyze RNA harvested at 1, 2, 4 and 8 hours post-infection times. We found that WT-WR induced higher interferon and antiviral signaling compared to WRDA26. In addition, we generated recombinant viruses expressing fluorescence early Venus and late mCherry marker proteins to analyze for viral gene expression kinetics by live imaging and flow cytometry. We found that WRDA26 indeed expressed robust early and late proteins while WT-WR only expressed limited amounts of early proteins in BMDM. Further experiments to understand the molecular basis that contributes to differential viral gene expression due to different virus entry routes are currently underway and the results will be discussed. 🗨️

**Keywords:** Vaccinia virus entry and interferon





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**Poster no. 58**

## **INCIDENCE OF SALMONELLA AND SEROTYPES WELTEVREDEN AND INFANTIS IN PORK PRODUCTS COLLECTED FROM SELECTED PUBLIC WET MARKETS IN METRO MANILA, PHILIPPINES**

**S**almonella is one of the most significant pathogens that cause food-borne diseases in the world. Although there are 2,659 identified *Salmonella* serovars, most human infections are caused by limited number of serotypes, which include Weltevreden and Infantis. Majority of *Salmonella* infections in humans are associated with the consumption of contaminated meat products, such as pork. Pork is the most widely produced and consumed meat products in the Philippines. The objectives of this study were to determine the incidence of *Salmonella*, its common O-serogroups, and serotypes Weltevreden and Infantis in pork. Five types of pork products, including ground pork (n=50), meatloaf (*embotido*) (n=50), ham (n=50), sausage (*longganisa*) (n=56), and cured pork meat (*tocino*) (n=50), were collected from five selected public markets in Metro Manila. Multi-step PCR assays were performed to determine the presence of *Salmonella*, and characterize the isolates according to their respective serogroups and serotypes. One hundred thirty-two out of 256 samples were found to be *Salmonella*-contaminated. All of the ground pork samples were found to be *Salmonella*-contaminated, while *embotido* and ham were the least contaminated (22.00%). Majority of *Salmonella* contaminants in pork samples were categorized to serogroup O:3,10 (66.67%), followed by O:4 (46.97%), O:6,7 (44.70%), O:8 (22.73%), others (6.06%), and O:9 (3.03). The low incidences of *S. Weltevreden* (14.39%) and *S. Infantis* (2.27%) detected in pork products were perhaps indicative of their low reports in clinical samples. This study provides baseline information regarding incidence of *Salmonella* and the distribution of its serogroups and serotypes in food, which is currently lacking in the Philippines. 🗨️

**Keywords:** Salmonella Weltevreden Infantis Pork Philippines



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**Poster no. 59**

**MOLECULAR DETECTION, CHARACTERIZATION, AND  
ANTIMICROBIAL PROFILING OF SALMONELLA SPECIES ISOLATED  
FROM SLAUGHTERED SWINE**

Pork is one of the most commonly consumed meats worldwide. It is also considered as one of the main sources of salmonellosis which affects 93.8 million humans per year globally. In the Philippines, the swine industry contributes to the largest volume of livestock production. In the present study, we aimed to detect and characterize *Salmonella* spp. from slaughtered swine and screen the isolates for resistance genes. Tonsil and jejunum samples of freshly slaughtered swine were collected in three locally regulated meat establishments in Metro Manila. *Salmonella* spp. were isolated using conventional culture methods and confirmed by PCR amplification of the *invA* gene, a gene unique to *Salmonella* spp. which encodes for the protein involved in the invasion of the intestinal epithelium. Isolates were further characterized based on somatic antigen and presence of *bla* genes by multiplex PCR assays. Our findings reveal that out of 180 carcasses of slaughtered swine, 76 (42.22%) were contaminated with *Salmonella* spp. Majority (31.58%) of the contaminated carcasses carried *Salmonella* spp. under serogroup E1, and 21.05% carried multiple types of *Salmonella* spp. Twelve (15.79%) carcasses were contaminated with *Salmonella* spp. of unknown serogroup. Out of 132 *Salmonella* spp. isolates, 4 (3.03%) and 78 (59.09%) carried *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> respectively. Co-carriage of the *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> genes was observed in three (2.27%) isolates. The gene *bla*<sub>SHV</sub> was not found in any of the isolates. We report here the incidence of *Salmonella* spp. in Philippine swine and the characterization of the somatic antigen and resistance genes of the isolates. 🗨️

**Keywords:** Antimicrobial resistance, pork, *Salmonella*, swine



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Poster no. 60

## DEEP TUBE-WELL WATER USE AND MODERATE-TO-SEVERE CHILDHOOD DIARRHEA EPISODE DUE TO *S. SONNEI* INFECTIONS: A CROSS-SECTIONAL STUDY IN KUMUDINI WOMEN'S MEDICAL COLLEGE AND HOSPITAL, MIRZAPUR, BANGLADESH

**Background and aim:** The present study aimed to determine any association between drinking deep tube-well water and childhood diarrheal illness due to *S. sonnei* in rural Bangladesh.

**Methods:** A total of 1,394 children aged 0-59 months who presented with moderate-to-severe diarrhea in Kumudini Women's Medical College and Hospital for care from December 2007 to March 2011 were enrolled into the study.

**Results:** *Shigella* was the leading pathogen causing childhood moderate-to-severe diarrhea episodes. The isolation rates of *Shigella* spp. were similar from children belonging to those families who are using deep tube-well water as well as those from shallow tube-well water user families (41% vs. 44%). However, 13% of the children from deep tube-well water using families had *S. Sonnei* infection compared to 8% of the children from shallow tube-well water user families (OR 1.81, 95% CI 1.26-2.60,  $p < 0.001$ ). Usage of electricity was higher in deep tube well water users (OR ratio 1.38, 95% CI 1.10-1.71;  $p < 0.004$ ). Higher proportion of shallow tube-well water users treated drinking water compared to deep tube-well user families (7% vs. 2%, adjusted OR 0.26, 95% CI 0.14-0.47,  $p < 0.000$ ) and treatment method of filtering was through a cloth (OR 0.208, 95% CI 0.09-0.45,  $p < 0.000$ ).

**Conclusion:** It is well accepted that exposure to *P. shigelloides*, through contaminated drinking water, may immunize people to *S. sonnei* in developing countries. Increased and sustained access to safe drinking water and less exposure to contaminated water with presence of *P. shigelloides* that shares antigen with *S. sonnei* may have played a role in excess detection of *S. sonnei* in stool specimens of childhood moderate-to-severe illnesses as well as who are the users of deep tube-well water in this context. 🗨️

**Keywords:** Bangladesh, diarrhea, tube well, Under-five children



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**Poster no. 61**

## **UNRAVELLING OF HOST SPECIFICITY OF SALMONELLA INFECTION BY COMPARATIVE SECRETOME PROFILING**

**T**yphoid is an acute and life threatening febrile illness caused by *Salmonella enterica* serovar *typhi*. It is a major cause of morbidity and mortality worldwide and highly endemic in south Asian sub-continent. Despite ~90% genome similarity with *S. Typhimurium*, *S. Typhi* exclusively infects humans and causes systemic illness while *S. Typhimurium* does so in mice but only self-limiting gastroenteritis in humans. The exact mechanisms contributing to this host specificity are still unclear and the existing knowledge of *S. typhi* pathogenesis is based upon the *S. Typhimurium* infection in mice. Therefore, it is imperative to study *S. Typhi* pathogenesis through alternate models that could mimic *in vivo* *S. Typhi* infection in humans. We have utilized an *in vitro* model consisting of polarized intestinal epithelial cells (IEC) mimicking the gut to study *S. typhi* infection. To obtain a comprehensive overview of the contrast in the host secretory signatures post-infection amongst two closely related serovars, *S. typhi* or *S. Typhimurium*, we have used this IEC culture system wherein the Caco-2 cells were grown on porous inserts of trans-well plates. The monolayer integrity and polarity was determined through trans-epithelial electrical resistance measurement and the bacterial infection was carried out at 100 MOI. A comparative proteomic profiling of the basolateral secretome was carried out by LC-MS/MS and a total of 175 host proteins were identified of which 18 proteins were unique to *S. Typhi* and 12 proteins unique to *S. Typhimurium* infection with 14 common proteins. Identification of unique molecules and elucidation of their role would enable us to gain deeper insights and develop a better understanding of *Salmonella* pathogenesis. The study has important translational implications through identification and utilization of novel targets for rational design of typhoid diagnostics and therapeutics. 🗨️

**Keywords:** Typhoid, proteomics, *Salmonella typhi*, *typhimurium*





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**Poster no. 62**

## **MOLECULAR CHARACTERIZATION OF SALMONELLA ENTERICA ISOLATES FROM MEAT PRODUCTS THROUGH ANTIMICROBIAL RESISTANCE AND VIRULENCE GENE PROFILING**

This study aimed to examine the antimicrobial resistance and virulence profiles of *Salmonella enterica* isolates from raw and processed meat products. A total of 95 *S. enterica* isolates were characterized for virulence genes encoding for fimbriae, and beta-lactamase resistance genes. Antimicrobial susceptibility tests were performed for aztreonam, ceftazidime and cefotaxime. The results showed that 39 isolates were shown to be resistant to at least one antibiotic. Resistance to third generation cephalosporins ceftazidime (23%) and cefotaxime (15.8%) was most frequent. Among the three resistance genes examined, the *bla*<sub>TEM</sub> gene was most common (67%). The long polar fimbriae (*lpfA*) gene was the most common virulence gene found in *Salmonella* isolates (34%). A strong positive correlation was observed between the *sefA* and *pefA* fimbrial genes, which were each found in 12% of the isolates. The results show that raw and processed meat products in Philippine wet markets act as salient reservoirs of *Salmonella* harboring virulent and resistance genes. Strong statistical associations were observed between the  $\beta$ -lactamase gene *bla*<sub>TEM</sub> and the long polar fimbriae gene *lpfA*. This demonstrates that antimicrobial resistance genes and fimbrial genes can coexist in *Salmonella* isolates, and are selected through antimicrobial selective pressures. These findings have significant implication with regard to the evolution and distribution of resistance and virulence genes, because selective pressures conferred by inconsistent use of antimicrobials lead to the emergence of new pathogens with increased virulence and resistance potential. 🗨️

**Keywords:** *Salmonella enterica*, antimicrobial resistance, virulence



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**Poster no. 63**

**DETECTION OF *SALMONELLA ENTERICA* FROM PORK SAMPLES USING LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)**

In developing countries such as the Philippines, the risk of food poisoning due to the ingestion of food contaminated with pathogens such as *Salmonella enterica* has been a constant concern. Contamination may occur due to improper handling anywhere from the slaughter of the animals to delivery to handling for sale to consumers. Standards have been set in the Philippines that consider detection of 10<sup>4</sup> viable cells of *S. enterica* in 25 g of meat as unfit for consumption although this is not as stringent as in other countries. Turnover time between the slaughter of the animals and their sale to the general public is also short making efficient detection necessary. Loop-mediated isothermal amplification (LAMP) is an easy-to-use method with potential use for rapid and accurate detection of *S. enterica* in meat and meat products. In this study, LAMP was optimized and used to determine the efficiency of the method in detecting *S. enterica* of different serotypes and serogroups from meat products, specifically pork. The assay was found to be specific for different serogroups and serotypes of *S. enterica*. The sensitivity of the assay was found to be in the order of 2000 copies, the minimum tested amount of DNA. The assay was also found to be able to detect *S. enterica* from pork samples within 4 hours of pre-enrichment. LAMP was found to be an effective method for the detection of *S. enterica* from pork samples and it could present an efficient solution to increase health safety for the public. 🗨️

**Keywords:** Salmonella LAMP Detection



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**Poster no. 64**

## **THE PREVALENCE OF SALMONELLA SPP. AND ASSOCIATED GASTROINTESTINAL PATHOGENS IN POULTRY BASED FOOD PRODUCTS IN DHAKA CITY**

**N**owadays safety of food is our main concern which is hampered by the presence of gastrointestinal pathogens like *Salmonella*. Present study reveals the presence of *Salmonella* and other potential pathogens in poultry based food products collected from different places in Dhaka. For pre-enrichment, Buffered Peptone Water and for selective enrichment, Rappaport-Vassiliadis Soya broth was used. Pure colonies were identified presumptively based on colony morphology and biochemical test results. The percentages of *Salmonella* on egg shells were 15%, the egg content had 5%; in grilled chicken, pudding and in mayonnaise- it was 30% and 20% for each of the latter two samples respectively. Isolated *Salmonella* strains were mostly resistant against Streptomycin, Nalidixic acid, Rifampicin, Kanamycin, Trimethoprim-sulfamethoxazole and Erythromycin and susceptible to Cefotaxime, Ciprofloxacin and Chloramphenicol. Amongst other gastrointestinal pathogens, higher percentages of *Shigella* in raw food (in egg shell 60% and egg content 25%) had been observed whereas, in cooked food samples, the percentages were lower (in grilled chicken 10%, in pudding and mayonnaise 0%). Other pathogens like *E.coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Citrobacter*, *Pseudomonas* had also been isolated in these samples. Additionally, higher numbers of *Bacillus* and *Staphylococcus* had been observed nearly in all of these samples. The presence of multi-drug resistant *Salmonella* and other potential pathogens are alarming and draw our concerns as poultry based products constitute a major part of the diet of children in our country, adequate measures must be taken to reduce the microbial load. 🗨️

**Keywords:** Salmonella, poultry product, gastrointestinal pathogen



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Poster no. 65

## **SURVEY ON THE PRESENCE OF EXTENDED-SPECTRUM $\beta$ -LACTAMASE (ESBL) PRODUCTION BY IDENTIFYING THE BLATEM GENE AMONG ESCHERICHIA COLI ISOLATES FROM DIFFERENT VEGETABLE PRODUCE IN SELECTED OPEN AIR MARKETS AND SUPER MARKETS IN THE PHILIPPINES**

Foodborne illnesses may be accounted to the risk of microbial contamination that can be due to several factors such as irrigation waters, handling or processing. Bacterial contamination harbor antibiotic resistance conferred by enzymes like extended-spectrum  $\beta$ -lactamases (ESBLs), which can hydrolyze penicillins, cephalosporins, and aztreonam, limiting options for treatment. Considering the possibility of transmission of antibiotic resistant bacteria through different sources and consequently fresh produce, ESBL production was evaluated in 12 *Escherichia coli* isolates from 132 vegetable samples in different open air and supermarkets in the Philippines. Initial and confirmatory screenings for ESBL production were conducted through disk diffusion method with ceftazidime and cefotaxime and through double disk synergy test, respectively. Polymerase chain reaction (PCR) testing was carried out for *bla*<sub>TEM</sub> ESBL genes. PCR testing showed *bla*TEM to be present in most of the confirmed ESBL-producing isolates. Presence of ESBL-producing bacteria in different readily eaten fresh produce suggests increased risk for public health. Anthropogenic sources of antibiotic contaminants that promote the dissemination of antibiotic resistance accent the need for control of the release of contaminants into the environment. 🗨️

**Keywords:** *bla*TEM, *Escherichia coli*, fresh produce





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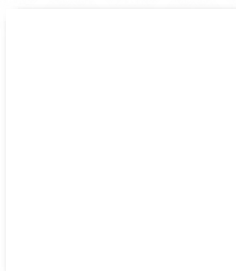
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**Poster no. 66**

## **DETECTION AND PREVALENCE OF ANTIMICROBIAL RESISTANCE PATTERNS OF *ESCHERICHIA COLI* ISOLATES FROM AGRICULTURAL IRRIGATION WATERS IN BULACAN, PHILIPPINES**

**E***scherichia coli* is an important cause of human illness in the Philippines, thus there have been numerous reports on water and food contamination which is associated with it. As *E. coli* is one of the reliable indicator of fecal pollution and an effective model for monitoring antimicrobial drug resistance, the study aims to detect and confirm the identity of the thermotolerant *E. coli* isolates in 10 different farming sites in Bulacan, Philippines using conventional and molecular methods and, to determine the patterns of antimicrobial resistance in confirmed *E. coli* isolates. From the 90 irrigation water samples, a total of 79 isolates were confirmed through PCR amplifying the *uidA* gene. Ten (10) antibiotics covering eight (8) different classes were used to determine the phenotypic resistance of the confirmed isolates. Forty-six out of 79 (58.23%) isolates were found to be multidrug resistant (MDR), which is the resistance to three or more classes of antibiotics tested. Highest resistance was observed in cephalothin (73.42%), followed by tetracycline (50.63%), streptomycin (49.37%), ampicillin (48.10%) trimethoprim (45.57%), nalidixic acid (35.44%), and chloramphenicol (25.32%). Least resistance, on the other hand, was observed in ciprofloxacin (5.06%) followed by cefotaxime (8.86%) and gentamicin (13.92%). Most common pattern observed was the combination of Chl-Nal-Tet-Amp-Cep-Strep-Tri (8.70%) and Tet-Amp-Tri (6.52%). Overall, the results imply that the agricultural water used in Bulacan is contaminated with fecal material of man or other animals present within the area and the MDR isolates detected in the study pose a potential threat to individuals exposed to the area. 🗨️

**Keywords:** Antibiotics, *E. coli*, Multidrug resistance, PCR, *uidA* gene



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**Poster no. 69**

## **PUTTING LIFE ON HOLD: A CLINIC BECOMES A COMMUNITY FOR TB PATIENTS**

**W**hile there has been some progress in case detection and treatment of tuberculosis (TB) and multi-drug resistant tuberculosis (MDR TB) globally, major challenges remain especially with regard to case holding and follow ups. These problems are exacerbated in highly mobile populations, such as those along the Thailand-Myanmar border. For example, treatment for MDR TB can take up to two years and is only provided by a limited number of centers, meaning a significant space-time commitment for people who are normally quite mobile. Conversely, persons with MDR TB who do not seek proper treatment and who are highly mobile may contribute to the geographic spread of the disease. MDR TB treatment therefore poses a difficult ethical conundrum. In 2009 Shoklo Malaria Research Unit (SMRU) established a TB detection and treatment program following an observed increase in TB cases presenting at clinics along the Thailand-Myanmar border. The program consists of two TB clinics, one on each side of the border, with a combined capacity of 220 patients and the provision of care and directly observed treatment for both drug sensitive and resistant TB. Most patients come from within a 30 kilometer radius of the clinics, while some are 100s of kilometers away from their home communities. Here we discuss the creation of a specific model of care in which TB infected migrants are given the opportunity to live on site for the duration of their treatment. We use patient and health provider stories and insights to highlight the challenges faced by both parties, together with obstacles and successes faced by the SMRU TB program, and some important ways forward for MDR TB treatment in the region. 🗨️

**Keywords:** putting life on hold



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**Poster no. 71**

## **NEISSERIA MENINGITIDIS CARRIAGE AMONG MARCHING SOLDIERS**

**N**eisseria meningitidis is the most deadly meningitis disease in the world. The disease is important because of its high case fatality rate, up to 50%, especially if the treatment is late. Different serotypes of meningococci are seen among various Asian countries although some countries do not give report and the others do not recently.

Meningococci is present in the throat of 10-20% of normal population. Although exact mechanism is unknown, meningitis occurs when the organism gains access into blood stream of the host. Carrier rate becomes higher in crowds and at the time of outbreaks. Transmission is through respiratory route and the crowded places like military camps and barrack become targets. Meningitis occurred at times with some incidences during 2006-2007 periods at Nay Pyi Taw military camp where troops were practicing for Armed Forces Day marching parade.

In this year 2016, in early March, meningococcal B disease outbreak occurred at Thazi township, 150 km north of Nay Pyi Taw, with 7 children hospitalized and 4 deaths. Therefore a study was done towards 200 marching soldiers at Nay Pyi Taw military camp practicing for 71st Arm Forces Day parade during March, 2016. Questionnaire concerning sociodemographic and behavioral factors were asked and oropharyngeal swabs were taken. Isolation of meningococci, bacterial identification and antimicrobial susceptibility tests were done using conventional solid culture, microscopy and Vitek 2 at Defence Services Medical Research Centre.

Among 200 soldiers tested, only 19 (9.5%) carried meningococci. Soldiers of age between 35 and 39 carried the most and over 40 the least. Association was seen with smoking and alcohol drinking but not with betel chewing. Resistance to Rifampicin and Penicillin showed 80% and almost 90% of isolates respectively. Isolates showed no resistance to Ciprofloxacin still keeping it as an effective chemoprophylaxis and treatment drug. 🗨

**Keywords:** Neisseria meningitidis, meningitis, soldiers, military camp, Myanmar



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**Poster no. 72**

## **MOLECULAR EPIDEMIOLOGY OF HUMAN LEPTOSPIROSIS WITH SEPSIS**

**Background:** Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira* species. The clinical manifestations of human leptospirosis range from sub-clinical symptoms to multi-organ involvement. Here, we aim to evaluate molecular epidemiology of *Leptospira* species causing sepsis in Southeast Asia.

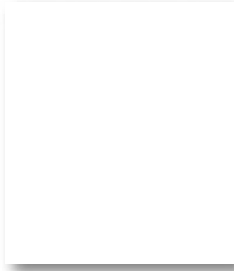
**Methods:** Blood samples were obtained from "An Observational Study of the Causes, Management, and Outcomes of Community-acquired Sepsis and Severe Sepsis in Southeast Asia" (NCT02157259) conducted during 2013 to 2016 in 15 hospitals in Thailand, Vietnam, and Indonesia. Among the sepsis cases, leptospirosis was identified at 5.9%, 6.0%, and 7.6% for Thailand, Vietnam, and Indonesia, respectively. All blood samples with real-time PCR *Leptospira* positive results were used for identifying *Leptospira* species. The partial *rrs* gene was amplified using the nested PCR as described previously by Boonsilp *et al.* The amplicons were purified and sequenced. A phylogenetic tree of the 443-nucleotide *rrs* fragments was re-constructed prior to inferring *Leptospira* species.

**Results:** A total of 34 real-time PCR positive blood samples collected from 34 sepsis patients from Thailand were included in the study. Of overall, 30 (88.2%) were positive for the nested PCR. The remaining 4/34 (11.8%) were negative. *L. interrogans* and *L. weillii* were identified in 29 and 1 samples, respectively. Of 29 patients with blood sample on admission with nested PCR positive for *L. interrogans*, 19 (65%) had MAT positive, 7 (25%) were MAT negative with paired serum samples and 3 (10%) were MAT negative but without convalescent serum samples. The single patient PCR positive with *L. weillii* had MAT negative.

**Conclusions:** *L. interrogans* is a major cause of human leptospirosis with sepsis in Thailand. 🗨️

**Keywords:** Leptospirosis, sepsis, *L. interrogans*, *L. weillii*, Southeast Asia





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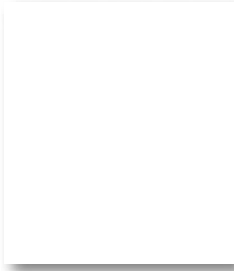
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**Poster no. 75**

## **TRYPTOPHOL (QUORUM SENSING MOLECULES) INDUCED APOPTOSIS IN CANDIDA ALBICANS**

Quorum sensing molecules (QSMs) is referred to as auto-induction, microbial cell-cell communication. Various studied the role of QSMs (farnesol and tyrosol) in *Candida albicans* morphogenesis and program cell death. The other QSMs i.e. tryptophol do not know the biological effect on *C. albicans*. Therefore, we investigated the effect of tryptophol on *C. albicans* apoptosis pathway by observing apoptosis-related gene expression levels (CARD9, NOXa and BCL-2) after treated with tryptophol and farnesol. *C. albicans* were treated with farnesol and tryptophol for 2, 6 and 24hr. The level of apoptosis signalling gene expression was evaluated by qPCR. We found that the expression of CARD9 level was increased at 2, 6, and 24 h comparing with untreated cells. The highest expression level of CARD9 was seen after treated with tryptophol at 2 and 6 h whereas the expression level of CARD9 treated with farnesol was lower than farnesol but increasing at 24h. In NOXa, the expression level was increased at 6 and 24 h compared with untreated cells. Moreover, NOXa expressed the highest level in tryptophol treated-cells at 6 h, whereas in farnesol treated-cells at 24 h. Moreover, we found BCL-2 expressed the highest level at 2 h after treated farnesol and tryptophol compared with untreated cells. Although, BCL-2 expression level was decreased at 24h in tryptophol treated-cells but BCL-2 expression level was increased at each time point in farnesol treated-cells. Therefore, tryptophol plays some key role in apoptosis pathway in *C. albicans* but different mechanisms from farnesol. These molecules will be valuable in research field of the signaling pathways regulating the apoptosis and also may serve as starting points for potential new antifungal therapeutics. 🗨️

**Keywords:** Tryptophol, Farnesol, Apoptosis, *Candida albicans*



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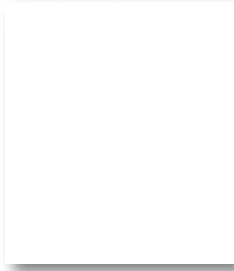
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**Poster no. 76**

## **APOPTOSIS OF LOMENTOSORA PROLIFICANS AND SCEDOSPORIUM BOYDII INDUCED BY FARNESOL**

**L**omentospora prolificans (formerly Scedosporium prolificans) and Scedosporium boydii are important emerging fungal pathogens. These two pathogenic fungi cause severe infection in immunosuppressed individuals. Moreover, antifungal resistant of *L. prolificans* has been reported. Farnesol, a quorum sensing molecule firstly identified in eukaryotic organism; *Candida albicans*, has been studied in several fungi in terms of the effect of fungal morphology, biofilm development, conidia germination and apoptosis. However, the effect of farnesol on these two fungi has not been performed. Thus, the aim of this study was to investigate the effect of farnesol on apoptosis of *S. boydii* and *L. prolificans*. *S. boydii* CBS 120157 and *L. prolificans* CM324 were grown on Sabouraud Dextrose Agar slants at 37°C for 7 days. Conidia were collected and suspended in phosphate-buffered saline (PBS). Apoptotic cell investigation was performed by staining *S. boydii* CBS 120157 and *L. prolificans* CM324 with ethidium bromide (EB) and acridine orange (AO) and observed under a fluorescent microscope. Farnesol-treated *S. boydii* and *L. prolificans* showed organelle condensation and orange staining of the hyphae. This study provides a starting point for further investigation of the mechanism of the farnesol-induced apoptosis of these two pathogenic fungi. 🗨

**Keywords:** Farnesol, Quorum sensing molecule



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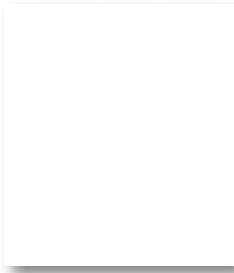
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**Poster no. 77**

## **DNA BARCODING FOR IDENTIFICATION OF SCEDOSPORIUM APIOSPERMUM SENSU STRICTO**

**S**cedosporium apiospermum sensu stricto is an important emerging fungal pathogens that have been frequently reported in immunocompromised patients and immunocompetent patient after near-drowning. Nowadays, *Scedosporium apiospermum* sensu stricto was classified as member of *Scedosporium apiospermum* complex (together with other 5 species; *Scedosporium apiospermum*, *Scedosporium boydii*, *Scedosporium angusta*, *Scedosporium minutisporum*, *Scedosporium dehoogii*, and *Scedosporium aurantiacum*) by genetically relationship. Here, we assessed the utility of DNA barcoding for identification of *Scedosporium apiospermum* sensu stricto in Thailand based on sequence comparison of the DNA barcode region. Thirty environmental strains of *Scedosporium apiospermum* sensu stricto were used for the DNA barcoding study. DNA was extracted and the partial of ITS region of the rDNA gene cluster,  $\beta$ -tubulin (TUB) and  $\beta$ -tubulin (BT2) were amplified. The sequences were edited and subjected to pairwise alignment using BioEdit software. Edited sequences were compared with existing sequences in GenBank using BLASTn. Phylogenetic trees were constructed in MEGA7 a bootstrap analysis was conducted with 1,000 replications by individual loci and concatenated sequences. ITS region remain the best performance for identification in species level almost fungi. Our study consistency indicated that  $\beta$ -tubulin (TUB) and  $\beta$ -tubulin (BT2) showed a high threshold value and suitable for distinguishable within the *Scedosporium apiospermum* complex lineages than ITS region. Additional data from phylogenetic analysis, we provided the basis information for future monitoring of their populations in the Southeast Asian region. 🗨️

**Keywords:** DNA barcoding, *Scedosporium apiospermum* sensu stricto



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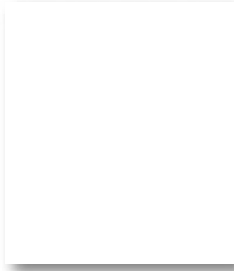
**Poster no. 78**

## **CLINICAL-MYCOLOGICAL STUDY OF DERMATOPHYTES FROM HOSPITAL FOR TROPICAL DISEASES, BANGKOK**

**D**ermatophytes comprising of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are also important cause of superficial mycoses. We not known the current situation of dermatophytes infections, precise size of infected patients and species distribution of these organisms. Therefore, the aim of this present study was to investigate the clinical pattern of dermatophytes infection patients and to identify the species of these dermatophytes from Hospital for Tropical Diseases, Bangkok. A total of 25 samples from 23 patients who diagnosis cases of dermatophytosis attending the skin out-patient clinic, Hospital for Tropical Diseases, Bangkok, period of September 2015-September 2016 were studied. Skin scrapings from the patients were direct examined the fungal element by 10% KOH. The other portion of the sample was used for the culture on sabourauds dextrose agar and dermasel (sabourauds dextrose agar with antibiotics and cycloheximide) and incubated at 25 °C. The agar were observed for 4 weeks. Any colony growth on the agar was observed morphology characteristic and did wet mount by using Lactophenol Cotton Blue, then examined under the light microscope for the looking of specific characteristic of dermatophytes species. Out of 23patients, Tinea corporis was the most common clinical type (52%) followed by tinea capitis, tinea faciei, tinea cruris (12%) and tinea manuum, tinea pedis, tinea unguium (4%). Overall positivity by culture was 28 %. We found *Microsporum canis* 4 isolates, *Trichophyton* spp. 3 isolates, *Trichophyton verrucosum* 1 isolate and also found other non-dermatophytes i.e. *Candida albicans* 2 isolates and Dematiaceous fungi 1 isolate. Therefore, both direct microscopy and culture are important tools of diagnosis for help dermatophytes infection patients. 🗨

**Keywords:** Dermatophytes, Trichophyton, Microsporum, Epidermophytions





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**Poster no. 79**

## **THE POTENTIAL ROLE OF FUNGAL QUORUM SENSING MOLECULES STUDY: *IN VIVO* PATHOGENESIS OF *CANDIDA ALBICANS* USING *GALLERIA MELLONELLA* MODEL**

The fungal quorum sensing molecules (QSMs) has been identified the role in various study, mostly for control the fungal biological activities and behaviors. Hence, the QSMs can caused programmed cell death and also play role in fungal virulence and pathogenicity. The potential role of fungal QSMs with pathogenicity has not been clearly understood in vivo. In this study we use the *Candida albicans* treated with tryptophol (QSMs) to identified the morphological and biological change and used the *Galleria mellonella* (wax worm) to study the pathogenesis and virulence of fungus after treated with QSMs. The results shown that the slow rate of hyphae development in tryptophol treated group rather than the without QSMs treated group significantly. In vivo study, the results shown the survival rate of *Galleria mellonella* in group of *C. albicans* infection without QSMs in day 1, 2, 3 after post infection are 66.66%, 66.66% and 0, respectively. In the group of *C. albicans* infection treated with QSMs, the survival rate of *Galleria mellonella* is 100% until 3 days post infection. The results suggested that the QSMs play the potential role in fungal biological activities and might be reduce the virulence of *C. albicans* in vivo. Further study the role of fungal QSMs need to determine to clarify the potential role of QSMs and further application development particularly in therapeutic and prevention. 🗨️

**Keywords:** fungal quorum sensing molecule, *Galleria mellonella*, *Candida albicans*



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**Poster no. 80**

**URETHRITIS IN MALE, YOUTH CLINIC, BANGRAK HOSPITAL**

Urethritis is inflammation of the urethra and is either gonococcal or non-gonococcal or of mixed etiology which are the most common reported STI in youth. The prevalence and incidence of urethritis are higher in developing countries. The usual presentations are discharge with dysuria and it is diagnosed by Gram stain. The complications are rare. WHO recommended syndromic management guidelines of men with urethral discharge should be treated with both gonorrhoea and chlamydial.

A retrospective descriptive study of male urethritis was conducted at Youth clinic in Bangrak Hospital during the two fiscal years 2008 and 2009. The prevalence of male urethritis and percentage of risk factors, clinical features, diagnosis, treatment and treatment outcomes of GU and NGU were determined. Univariate analyses were applied to identify the association between the characteristics of urethral discharge and GU-NGU.

There were 75 cases and 116 episodes studied and the prevalence of male urethritis was 21.9% in fiscal year 2008, 18.4% in fiscal year 2009 and 19.8% in the two fiscal years. Forty four percentages of patients were students and 89.3% were single. On Gram staining, 72 patients positive for GU and 44 positive for NGU. After doing cultures, 67 patients were positive for GU. Three patients positive for *chlamydia trachomatis* (CT) among 72 GU patients and 7 patients were positive CT among 44 NGU. Dysuria with urethral discharge was the most common presenting symptom in both GU (74.7) and NGU (66.7%). Fifty patients were presenting with yellow and green color discharge and 25 patients with white color. Eleven patients had got heavy discharge. First symptom was occurred mostly in 3-5 days (36.6%) in GU and <3days (25.9%) and 3-5 days and ≥ 1 months (14.8% each) in NGU. Majority of the cases were treated with cefixime based regimen (59.7%) for 2 weeks duration (67.7%). In which 48.8% were cured and 7% were recurrent/persistent. Doxycycline based regimen (20.7%) for 2 weeks duration (54.8%) was usually used for NGU, the outcomes were cured (70.5%) and recurrent (12.5%). Complications of urethritis were found in 12 cases.

Prevalence of male urethritis in Youth clinic was determined and syndromic approach to the management of urethritis should be implemented to improve the outcomes of patients in Youth Clinic, Bangrak hospital. 🗨️

**Keywords:** urethritis/male/youth/thailand/gonococcal/non-gonococcal



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**Poster no. 81**

**SYNTHESIS AND ANTIMALARIAL ACTIVITY STUDY  
OF A SERIES OF NOVEL HYBRID DERIVATIVES OF  
4-AMINOQUINOLINE AND MANNICH BASES**

The emergence of resistance in parasites towards currently available antimalarial drugs has become a major health concern of the developing world. This resistance has necessitated an urgent awareness to constantly look for new chemotherapeutic agents for the treatment of malaria. The purpose of the present study was to develop new potential antimalarial agents.

A new series of hybrid derivatives from 4-aminoquinoline and Mannich bases were synthesized [1] by modifications at the side chain of chloroquine with *m*-phenylenediamine and pendent amino group with Mannich bases of substituted acetophenone without making alteration in 7-chloro-4-aminoquinoline nucleus. The structure of all the synthesized compounds were confirmed and characterized by using various spectral technique like IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectroscopy. All the synthesized compounds were evaluated for *in vitro* antimalarial activity [2] against 3D7 strain of *P. falciparum* and all the tested compounds exhibit moderate antimalarial activity at the tested dose. The Compound (2c) with significant *in vitro* activity was selected for *in vivo* antimalarial activity [3] against CQ-resistant N-67 Strain of *P. yoelii* in swiss mice at 100 mg/kg by oral route. This compound exhibits significant activity against CQ-resistant N-67 Strain of *P. yoelii* infections in mice. 🗨

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**Keywords:** 4-Aminoquinoline, Mannich bases, antimalarials



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**Poster no. 82**

## **AN ASSESSMENT OF IRON FOLIC ACID SUPPLEMENTATION DURING PREGNANCY IN RURAL BANGLADESH**

**B**ackground: The World Health Organization (WHO) estimates that about half of the global maternal death due to anaemia occurs in South Asian countries, including Bangladesh. The most significant contributor to maternal mortality is iron deficiency anaemia (IDA). IDA during pregnancy is an important cause of restricted fetal growth leading to low-birth weight and preterm delivery as well as maternal illness and death. IDA has underlying significance for the achievement of Millennium Development Goals 4 & 5 to reduce child mortality and improve maternal health. In Bangladesh, 50% of pregnant women suffered IDA.

**Objectives:** To assess coverage and barriers of therapeutic supplementation of Iron-Folic Acid (IFA) tablet among women during pregnancy in a selected rural area of Bangladesh.

**Methods:** A combination of quantitative and qualitative methods was followed to address the objectives. A household survey was conducted with 114 women having at least one child  $\leq 2$  months of age, to capture their experience with IFA supplementation during their last pregnancy by using an adopted UNICEF questionnaire. The survey was supplemented by Focus Group Discussion (FGD) with selected mothers and was designed to explore the demand-side perspectives. Simple Random sampling techniques were used for the selection of the respondents. The facility assessment involved six health facilities in Kalai upazial of Joypurhat district. Key informant interviews (7), health facility inventory (6) and review of service statistics related to IFA supplementation during pregnancy over the year prior to the study, were conducted to reveal the supply-side perspectives. The Tanahasi framework was applied to identify coverage and barriers to coverage of IFA supplementation through its five tracer indicator e.g. availability, accessibility, utilization, adequate coverage and effective coverage.

**Results:** The IFA supplementation coverage in terms of availability, accessibility, utilization, adequate coverage and effective coverage was 100%, 80.7%, 67.4%, 13.3% and 2.1% respectively among the selected women during the reference period. The percentage of women who completed four ANC visits was only 37.7%. At about 37% of the respondents reported that they received more than 100 IFA tablet during their last pregnancy, while only 38% of mothers consumed more than 100 IFA tablets during their last pregnancy. A statistically significant relationship was found between the receipt of 100 IFA tablets and continuing use of ANC services. Supply-side barriers to IFA coverage revealed some important programmatic issues: lack of training, lack of job aid and IEC materials, shortage of workforce and high workload on the part of the service providers. Lack of consciousness, lack of proper knowledge about IFA, some side effects (bad smell, nausea) of the tablets and unpacked tablet were also identified as demand side barriers.

**Conclusions & recommendations:** The results of this study provided a better picture of coverage of IFA supplementation at different government service delivery points in Kalai upazila of Joypurhat district. At the same time, this study focused on understanding barriers of IFA supplementation and its determinants from both supply and demand sides. We strongly recommend to comprehensive training for service providers, a user friendly job aid, improved physical quality of IFA tablet and mass awareness raising campaign should be implemented. 🗨️

**Keywords:** Iron folic acid, Pregnancy, Bangladesh





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**Poster no. 83**

## **EVALUATION OF THE PERFORMANCE OF A NEW SIMPLE OVITRAP WITH DIFFERENT ATTRACTANTS, STORAGE DURATIONS, AND COVER MATERIALS**

**O**bjective: This study was to evaluate performance of simple ovitrap and identify the affecting factors.

**Methods:** Using different attractants (rice hay infusion, weed grass hay infusion, well water, and tap water) and storing rice attractant for different durations (0, 12, 34, and 90 days), we assessed the effectiveness by comparing the number of eggs trapped and the percentage of eggs hatched. We compared the percentage of mosquitoes trapped between plastic mesh and nylon covers to evaluate its security.

**Results:** Rice hay infusion trapped most eggs, and the differences reached statistical significance in comparison with tap water and well water, but not with weed grass hay infusion. Rice hay infusion also had the lowest percentage of hatched egg, and the differences reached statistical significance in comparison with weed grass hay infusion and well water. This is supported by bacterial profile. Storage duration did not appear to be a significant factor affecting the number of eggs trapped or the percentage of eggs hatched. The less support from chemical profile indicate the role of others chemical factor. Using larva instars III, we found nylon was better than plastic mesh in terms of security.

**Conclusions:** The simple ovitrap is as efficient as the more complicated ones that were previously reported. Rice hay infusion was more efficient in trapping eggs than weed grass hay infusion, well water, and tap water. With rice hay infusion, storage duration did not affect the efficiency up to 90 days, and nylon cover was more secure than plastic mesh. 🗨️

**Keywords:** Ovitrap, *Aedes aegypti*, egg, attractant, storage duration, hole size



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**Poster no. 84**

**PREVENTION OF SEXUALLY TRANSMITTED  
DISEASES JEOPARDIZED BY DRINKING ALCOHOL  
IN TATKON CANTONMENT**

A cross-sectional descriptive study was conducted among alcohol users in No.(4) Defence Industry and No.(2032) Air Defence Battalion in Tatkone Cantonment, Nay Pyi Taw Region. The main objective is to find out the prevention of Sexually Transmitted Diseases (STD) jeopardized by drinking alcohol among alcohol users. The study recruited 100 participants by using simple random sampling method. Pre-tested was done on 30 volunteers in Defence Services Medical Research Centre (DSMRC), Nay Pyin Taw. Study period was from September to October 2016. Data were collected by using the structured questionnaires. Each participant read and responded the survey questionnaire contained in an envelope except that the illiterates was aided by interviewer. The duration for each respondent was at least 30-45 minutes. After data collection, the data were cleaned, coded and entered by SPSS version 22. The entire data were maintained confidential. The participants were between 18 and 45 years. The

majority of participants were Bamar (99%) and all were Buddhist (100%). Regarding the educational status, there were illiterate (2%), read and write (1%), primary school level (3%), middle school level (40%), high school level (44%) and graduated (10%) respectively. The common reasons of initiation to alcohol use were social drinking (52%) and for pleasure (20%). In risky sexual behavior, there were oral sex (8%), anal sex(2%) and group sex (4%) respectively after alcohol drinking. According to results, we found that either increasing the frequency or the amount of alcohol drinking declined condom use with female sex workers. However, there was no significant association between the alcohol drinking and condom use for prevention of STD in this study ( $p=0.476$ ). 🗨️

**Keywords:** alcohol users, STD, risky sexual



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**Poster no. 85**

## **DATA QUALITY AND USERS' ATTITUDE TOWARD USING HIVINFO SOFTWARE IN VIET NAM**

**O**bjectives: The objective of this study are to (i) assess quality of data collected in HIVINFO 3.0 software used to manage HIV infected cases in Vietnam; and (ii) assess the users' attitude toward using this software. Methods: The study will be mixed design including retrospective study for the first objective and a descriptive cross sectional study for the second objective. The retrospective data from HIVINFO 3.0 will be collected to assess quality of data of HIVINFO 3.0 and questionnaire survey will be develop and send to HIVINFO 3.0 users to assess their attitude toward using this software. Results: Overall, the completeness of data was recorded highly in most variables (95.0% - 100%). The percentage of lacks information in mountain was higher than delta with 64.6% and 74.0% by region and in the large amount of patient was higher than small amount of patient with 59.8% and 79.8% by amount of patients. The timeliness of the data often delay in three lag time variables consist of HIV date; AIDS date; Dead date in the HIVINFO 3.0 and not satisfy the requirement as requests of Circular No 09/2012-BYT. The information collected from respondents on PEOU, PU, overall attitude, satisfaction also present high percentage of in all PCAs. Conclusion: This study provides an open platform of completeness and timeliness of data in the northern part of Vietnam. The level of data completeness and timeliness of software can confirm that the data quality is being used for these reports and research, and especially the reports to VAAC, Ministry of Health. Moreover, findings from a survey for HIVINFO 3.0 users' attitude was carried out in whole country to show the positive attitude and have high percentage to continue to use HIVINFO 3.0 software. 🗨️

**Keywords:** Hivinfor 3.0/ Hivinfor Software/ Completeness/ Timeliness/ Users' Attitude



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**Poster no. 86**

## **PROTECTIVE EFFICACY OF DOXYCYCLINE ON MALARIA PROPHYLAXIS IN THE SOLDIERS DEPLOYED TO THE THAI-CAMBODIA BORDER**

**Background:** Malaria has been a threatening disease to Royal Thai Army (RTA) troops who were deployed to borders, especially Thai-Cambodia border for decades. The incidence of malaria in the soldiers operating along Thai-Cambodia border in year 2010 was 25.36 %. Although there are supporting data from clinical trials that showed that daily 100 mg dose of doxycycline is an effective prophylactic agent for malaria but it has not been used for malaria prophylaxis in RTA Troops for around 10 years due to its gastrointestinal adverse symptoms. In the past, there was only hydrochloride form of doxycycline prescribed in Thailand. Now hyclate form of doxycycline is generally prescribed and there was evidence that it caused less gastrointestinal adverse symptoms than hydrochloride form. This brought to initiation to study protective efficacy, tolerability and compliance of doxycycline hyclate in RTA soldiers.

**Objective:** To study protective efficacy (PE) of doxycycline hyclate on prevention of malaria infection in RTA troops deployed to Thai-Cambodia border.

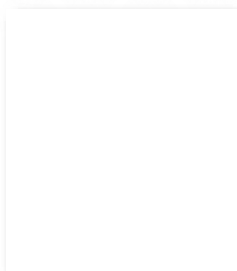
**Methodology:** A double-blind, placebo-controlled randomized field trial was designed. Eligible participants were male Royal Thai Army soldiers, 18-60 years old, deployed for at least 6 month period to the Thai-Cambodia border in Sisaket province of Thailand between October 2014 and September 2015. Blocked randomization with a block size of five was used to assign subjects to doxycycline hyclate capsule (DH, n=73), doxycycline hyclate film-coated tablet (DHFC, n=74) or placebo (n=37). Within each arm, subjects had to take daily, 100 mg of doxycycline hyclate or placebo for 3 months.

**Result:** Attack Rates (AR) of malaria in DH group, DHFC group and placebo group were 5.7%, 2.7 % and 15.0% respectively. Protective efficacy of DH was 62% and of DHFC was 82%. Results of survival analysis showed that Hazard Ratio (HR) in placebo group was 5.793 compared to DHFC group ( $p=0.031$ ) and HR in DH group was 2.137 compared to DHFC group ( $p=0.381$ ). Blood samples from two cases with *Plasmodium falciparum* were studied for immunomarkers. The results showed *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) gene, *Plasmodium falciparum* multidrug resistance-1 (pfmdr-1) gene and gene mutation at K13 Propeller domain (C447R in one case and 580Y in both cases).

**Conclusion:** Doxycycline hyclate film-coated tablet was more efficacious than doxycycline hyclate capsule and placebo on malaria prophylaxis in Royal Thai Army soldiers deployed to Thai-Cambodia Border.

**Keywords:** doxycycline hyclate protective efficacy malaria prophylaxis





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**Poster no. 87**

## **PRODUCT DEVELOPMENT : PASTEURIZED SUAEDA MARITIMA READY TO DRINK**

The research aimed to study *Suaeda maritima* extraction process, the standard formula for pasteurized. *Suaeda maritima* Ready To Drink and its shelf life based on physical quality, chemical composition, microorganism quantity, and sensory evaluation. It was found that 1,000 grams of *Suaeda maritima* fresh leaves when boiled and squeezed out the excess water, only 650 grams of the leaves were left. The leaves went through the extraction process in which the leaves were boiled in 105% of water weight per weight. The extract was then used as a raw material to create a standard formula for the RTD. One, 1 or 5 % of brown sugar was added into the extract. The extract with 5% brown sugar was most accepted in all aspects: appearance, color, flavor, taste, texture, and overall preference; the mean scores were 8.02, 7.95, 7.86, 8.07, 7.91, and 7.96, respectively. This indicated that the product was very much and most accepted. The lightness (L\*), the greenness (a\*), and the yellowness (b\*) were at 18.34, -9.95 and 19.51. The soluble solid Brix was 6.85; the pH-alkalinity (pH) was 6.91. The total energy for 100 ml. of the product was 24 k. cal.; the beta - carotene and the phenolic compound of Alice were 1056 and 360 micrograms. The consumers' test result showed that most consumers accepted the product at the moderate level; and, they were interested in buying the product. The best price was 20 baht per 180 ml. of the product in a clear glass bottle. The product was best kept at 7 °C for not longer than 5 days when there was still no physical or chemical change. All the microorganisms found did not exceed the FAD Standard or OTOP *Indian pennywort* Drink Standard (163/2552). These confirmed that the product was safe to consume. 🗨

**Keywords:** Suaeda maritima , beta -carotene ,Phenolic compound, pasteurization.



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**Poster no. 88**

## **FACTORS RELATED TO STRESSES OF FOREIGNER STUDENTS IN TRADITIONAL CHINESE MEDICINE STUDENTS PROGRAM, GUANGZHOU UNIVERSITY OF CHINESE MEDICINE**

This descriptive research aimed to study stresses and social supports of foreign students in Traditional Chinese Medicine program, Guangzhou University of Chinese Medicine, and to study factors related to the stresses. The subjects of this research were 193 1st–5th year foreigner students selected by stratified random sampling method. The research instrument to collect the data was questionnaires.

Data was analyzed by mean, percentages and chi-square test. The findings showed that the majority of the foreign students were female (58.5%); aged 20-24 (53.4 %); 3rd year students (28.0%); earned less than 1,001 CNY a month (30.1); single (95.3); resided in the university's dormitories (74.6%); domiciled in Hong Kong (52.8%); and gained social supports from friends in the medium level (54.4%); were highly stressed (51.2%). There was a relation between the foreigner students' stresses and their genders, ages, collage years, monthly earnings, marital statuses, domiciles and social supports with statistically significant ( $p < 0.05$ ).

**Keywords:** stresses, social supports of students, foreign students



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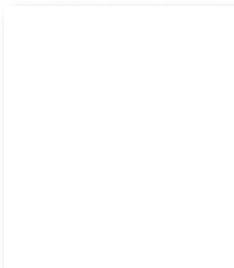
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**Poster no. 91**

## **HEMOZOIN CORRELATES TO MALARIA- ASSOCIATED ACUTE RESPIRATORY DISTRESS SYNDROME THROUGH PNEUMOCYTIC APOPTOSIS MECHANISM**

**M**alaria stills a major global health problem especially in Tropical region including Thailand. Severe malaria can cause vital organ dysfunction and death in patients who infected with *Plasmodium falciparum*. Lung complication, pulmonary edema (PE) and acute respiratory distress syndrome (ARDS), is one of the clinical features in severe malaria that can be life threatening, however its mechanism is not well understood. Generally, the pathogenesis of PE and ARDS is closely connected to the integrity of blood-gas barrier, the composition of endothelium, basement membrane, and pneumocyte. One postulated mechanism may associate with hemozoin-induced barrier injury. In this study, lung histopathological and ultrastructural studies were examined in severe malaria patients with and without ARDS. In vitro co-culture model of pneumocyte (A549) and *P. falciparum* hemozoin was also performed to determine cellular-apoptotic effect. Apoptotic cell was characterized by pneumocytic-membrane, -cytoplasm, and -nuclear chromatin using Ethidium-bromide/Acridine-orange (EB/AO) staining. Caspase-recruitment-domain-containing-protein (CARD)-9, apoptosis regulator gene, was quantified using real-time polymerase chain reaction technique to confirm the level of existing apoptosis. The results revealed that the occurrence of deposited hemozoin pigment in the lung had positive correlation to hyaline membrane formation ( $r=0.364$ ,  $p=0.007$ ) and blood-gas barrier damaged score ( $r=0.563$ ,  $p=0.002$ ). In pneumocyte/hemozoin co-culture, early-phase of cellular apoptosis was significantly observed in all periods of time post induction (1, 6, 12, and 24 hours) relevant to the level of CARD-9 gene expression ( $p<0.05$ ). The present study suggests that hemozoin relates to the pathogenesis of malaria-associated ARDS and damages lung epithelium verified by apoptotic gene expression. 🗨️

**Keywords:** Malaria, Acute-Respiratory-Distress-Syndrome, Pneumocyte, Apoptosis, Hemozoin



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**Poster no. 92**

**REVIEW OF COMPARATIVE EFFICACY AND ACCEPTANCE OF INTERVENTIONS OF MONTHLY PROPHYLAXIS VS. SCREENING AND TREATMENT IN HIGH-RISK, MILITARY MOBILE POPULATIONS TO SUPPORT MALARIA ELIMINATION IN CAMBODIA**

The effectiveness of malaria elimination strategies in hard-to-reach mobile populations, including the military, is largely unknown. We conducted a two-arm, controlled, cluster-randomized, open-label pilot study to determine the effectiveness of monthly malaria prophylaxis (MMP), using dihydroartemisinin-piperaquine (DP) and weekly primaquine (22.5 mg), compared to focused monthly screening with microscopy and PCR and treatment (FSAT) of malaria positive volunteers according to the Cambodian national treatment guidelines. After 3 months of interventions, both arms were followed for 3 more months to assess malaria incidence in the rainy season when malaria usually peaks. Of 1,114 active duty military and dependents screened in Oddar Meanchey province near the Thai-Cambodian border, 1,050 volunteers were enrolled into 8 clusters. At the time of enrollment, the baseline prevalence of malaria was 17% (45 cases of *Plasmodium falciparum*, 39 cases of *P. vivax*, 8 cases of mixed) and 10% (39 cases of *P. falciparum*, 13 cases of *P. vivax*, 1 case mixed) in the MMP and FSAT arms, respectively. Transmission of *P. falciparum* malaria was interrupted with the prevalence of *P. falciparum* malaria reduced from 9.17% at enrollment to 0.62% on month 6 follow up visit, and from 7.75% down to 0.67%, for MMP and FSAT arms, respectively. *P. vivax* parasite positive rate was 2.52% at enrollment in FSAT arm, and 0.9% at month 6 follow up, compared to a higher 7.87% parasite positive rate at enrollment and 4.97% rate of *P. vivax* at month 6 in the MMP arm. The majority of *P. vivax* cases in the MMP group were detected in the last 3 months of follow up, possibly representing *P. vivax* relapses. Investigation into CYP450 2D6 polymorphisms in the study population, responsible for primaquine metabolism, may provide additional evidence for the cause of *P. vivax* recurrences in the MMP arm. Both interventions under study received overall favorable ratings among military and civilian study participants despite concerns over mass drug administrations for malaria in other reports. 🗨️





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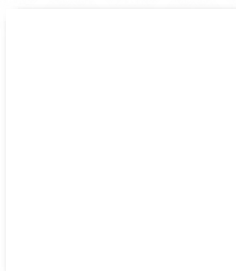
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**Poster no. 94**

## **BACTERIAL AEROSOLS AND OCCUPATIONAL RISK IN A LANDFILL SITE IN METRO MANILA, PHILIPPINES**

**P**ayatas landfill site is one of the major dumpsites in Metro Manila, Philippines. It was converted in 2004 to utilize methane gas as an energy source, following the tragedy and closure in 2000. In this study, the landfill was evaluated based on the presence and concentration of pathogenic bacterial aerosols through culture and molecular techniques. Bioaerosols are biological airborne particles that may or may not cause diseases, though some may stay virulent for a long time. MAS-100 Air Sampler was used for sampling from both indoor and outdoor locations. Culture technique employed general and selective media in order to identify the organisms. To test the accuracy of the said technique, those that were presumptively identified as *Staphylococcus aureus* and *Klebsiella pneumoniae* using selective media were subjected to molecular analysis using 16s rRNA sequencing. As a result, those that were presumptively recognized as *S. aureus* were identified as *S. saprophyticus*, *S. hominis*, *S. kloosi*, *S. arlettae*, *Bacillus licheniformis*, *B. safensis*, *Enterococcus casseliflavus*, and *E. faecium*; while those that were presumptively identified as *K. pneumoniae* were identified as *Pseudomonas stutzeri*, *Agrobacterium larrymorei*, *Acinetobacter baumannii*, and *Enterobacter* sp. The exposure assessment done by interview showed matches between prevalent diseases caused by the identified bacterial species. Passive and active sampling were also compared based on aerobic plate count and active sampling showed more accuracy and efficiency. The results of this study can be used as basis for creating standards and regulations for workers' and residents' safety and health in the landfill site. 🗨️

**Keywords:** bacterial aerosol, landfill site, active sampling, exposure assessment



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**Poster no. 95**

## **PREVALENCE AND ASSOCIATED FACTORS FOR CHRONIC KIDNEY DISEASE IN THE THAI ELDERLY POPULATION IN BANGKOK, THAILAND**

**B**ackground: Chronic kidney disease (CKD) is one of the major public health problems in Thailand and worldwide. CKD is also a prominent problem in the elderly and is associated with a higher risk of kidney failure, cardiovascular complication, and mortality. This study aimed to determine the prevalence and associated factors for CKD in the elderly.

**Methods:** A community-based, prospective study was conducted during 2014-2016 in 7 (out of 50) randomly selected districts in Bangkok. Thai participants aged  $\geq 60$  years living in Bangkok were included. Participants who had fever or acute illness one week before screening were excluded. Medical and social histories, physical examinations, venous blood, and urine samples were collected. The prevalence of CKD and its 95%CI were calculated. Factors associated with CKD stages 3-5 compared with no CKD were measured by adjusted prevalence ratio (aPR), using multivariate Poisson regression performed in SAS version 9.1.

**Results:** The study included 1,058 participants. The mean age was 69.2 years (SE=6.7), and the age range was 60-95 years. The overall prevalence of CKD in the Thai elderly was 30.6% (95%CI, 27.9-33.4%) while the prevalence in males was 35.6% (29.4-41.3%) and the prevalence in females was 29.2% (26.1-32.3%). The prevalence of CKD stages I, II, III, IV, and V were 4.9%, 7.9%, 16.5%, 0.9%, and 0.4%, respectively. Factors associated with CKD (stages 3-5) were older age comparing those aged 60-69 years (aPR =1.9 [1.4-2.8]) with those aged 70-79 and  $\geq 80$  years (aPR=3.2 [2.1-5.0]), hypertension (aPR=1.63 [1.2-2.3]), diabetes (aPR=1.5 [1.1-2.0]), and hyperuricemia (aPR=3.0 [2.2-4.2]).

**Conclusions:** The prevalence of CKD in the Thai elderly is still high. Appropriate monitoring and management of underlying diseases / associated factors will be beneficial in slowing the progression of CKD. 🗨️



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**Poster no. 96**

## **MULTIPLEX PCR ASSAY FOR IDENTIFYING FORENSIC RELATED BLOW FLIES**

**B**low flies are commonly found with dead animals including those of humans. Information of these insects can be used in forensic investigation such as time or place of death. Two forensically related blow flies mostly found in Thailand are *Chrysomya megacephala* and *C. ruffifacies*. Of which, the immature stages can be easily collected in the scene, however the identification of them by non-specialist is rather difficult and time consuming. Therefore, a PCR-based assay was developed in this study in order to overcome those disadvantages. After testing a number of primers, two sets of primer pairs which were designed from the genes *internal transcribed spacer 1* (ITS1) and ITS2 were selected and the PCR conditions were optimized. Finally, duplex PCR had been successfully developed, of which it can clearly identify DNA from those two blow fly species with the sensitivity and specificity at 90% and 100%, respectively. This assay is simpler than the morphological identification which requires microscope and it is more rapid and less laborious. It can be applied for the forensic investigation work relating to the estimation time of death and/or determination place of death. 🗨️

**Keywords:** Blow flies, molecular identification, ITS1, ITS2, Duplex PCR



# *First Announcement*



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