Supporting Information

Table S1 Sequences of oligonucleotide primers used to amplify the cDNA full-length of ALDHs for in vitro protein expression.

Table S2 Sequences of oligonucleotide primers used to amplify the fragment of *Ae. aegypti* ALDHs for quantitative PCR.

Table S3 Quantitative PCR results of *Ae. aegypti* ALDH.

Figure S1 Chromatograms of PBald oxidation to PBacid by recombinant aldehyde dehydrogenase.

Author Contributions

NL, PS and WC conceived and designed the experiments. NL, JW and PL performed the lab experiments. NL analysed the data. NL and PS interpreted the results. NL wrote the first draft of the paper. All authors read and approved the final manuscript.

Abbreviations

ALDH; aldehyde dehydrogenase; PBacid, 3-phenoxybenzoic acid; PBalc, 3-phenoxybenzyl alcohol; PBald, 3-phenoxybenzaldehyde

Figure 1

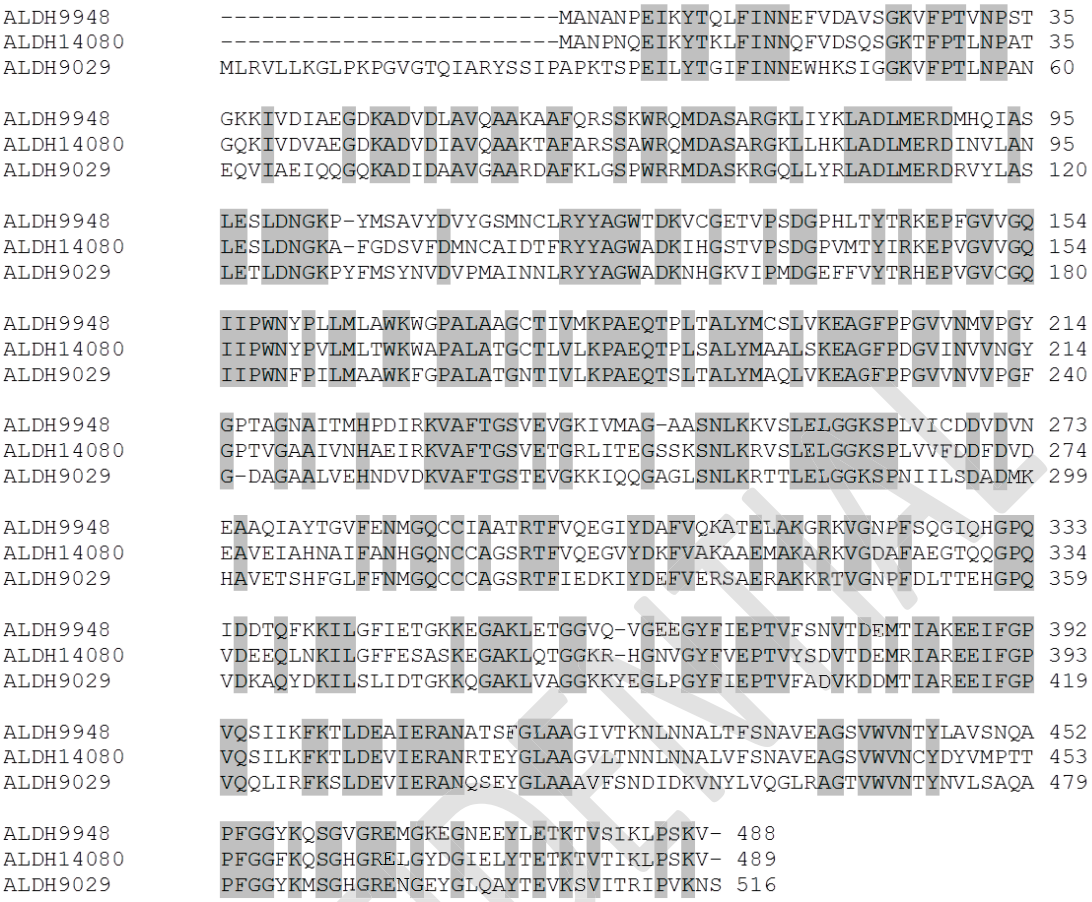


Figure 2

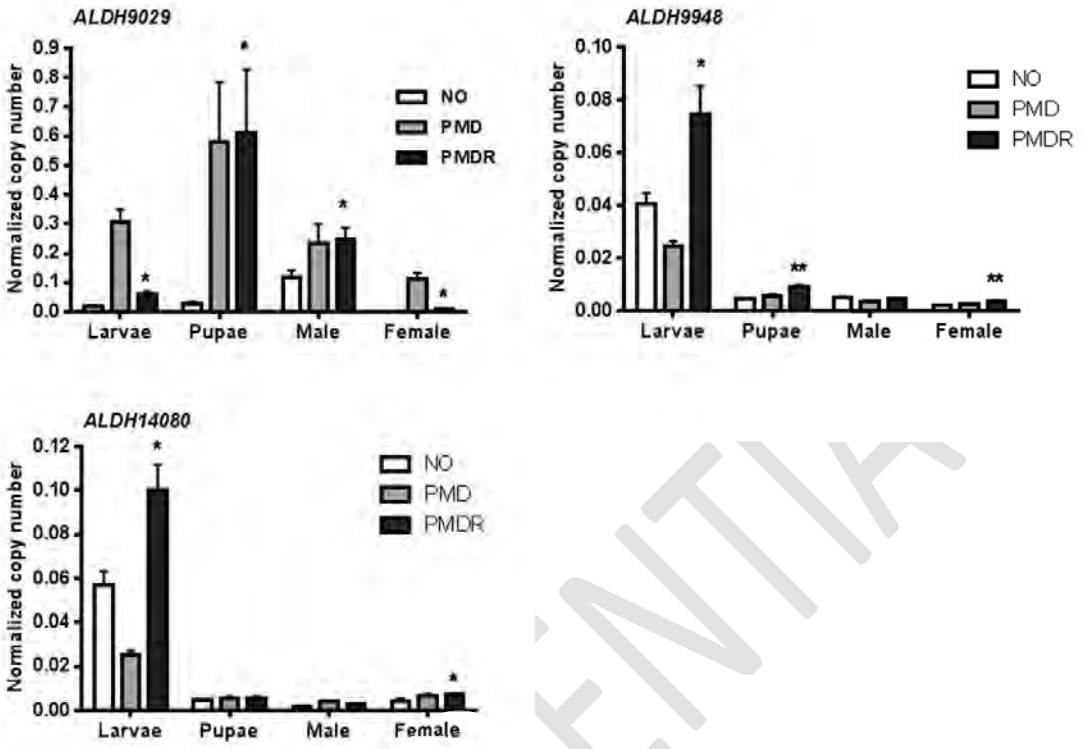
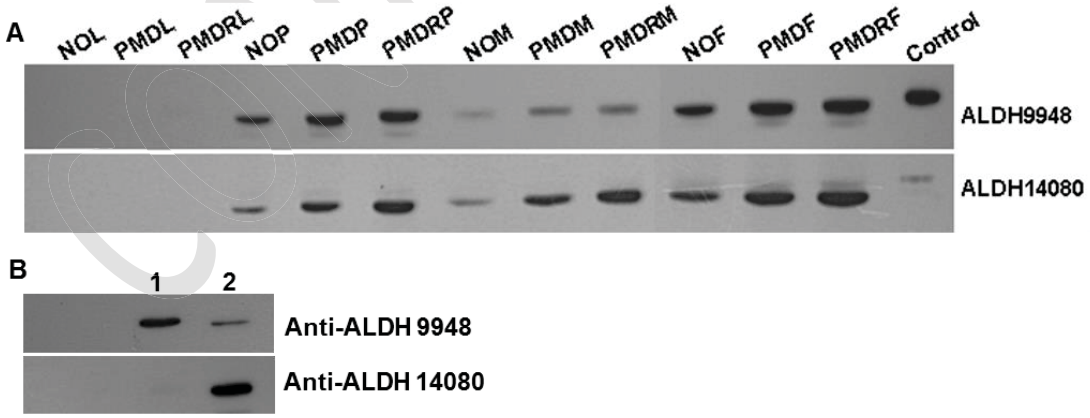


Figure 3



Supporting Information

Table S1. Sequences of oligonucleotide primers used to amplify the full-length of *Ae. aegypti* ALDHs for *in vitro* protein expression.

Gene	Primer name	Primer sequence (5'-3')
ALDH9029	ALDH9029F1	CACCATGTTGCGCGTTTTG
	ALDH9029R1	TTATGAATTTTTGACTGGAATACG
ALDH9948	ALDH9948F1	CACCATGGCTAACGCAAACC
	ALDH9948R1	CTAGACCTTAGACGGTAGCTTGATG
ALDH14080	AaALDHF	CACCATGGCCAATCCCAATC
	AaALDHR	TCAGACCTTCGATGGCAGC

Table S2. Sequences of oligonucleotide primers used to amplify the fragment of *Ae. aegypti* ALDHs for quantitative PCR.

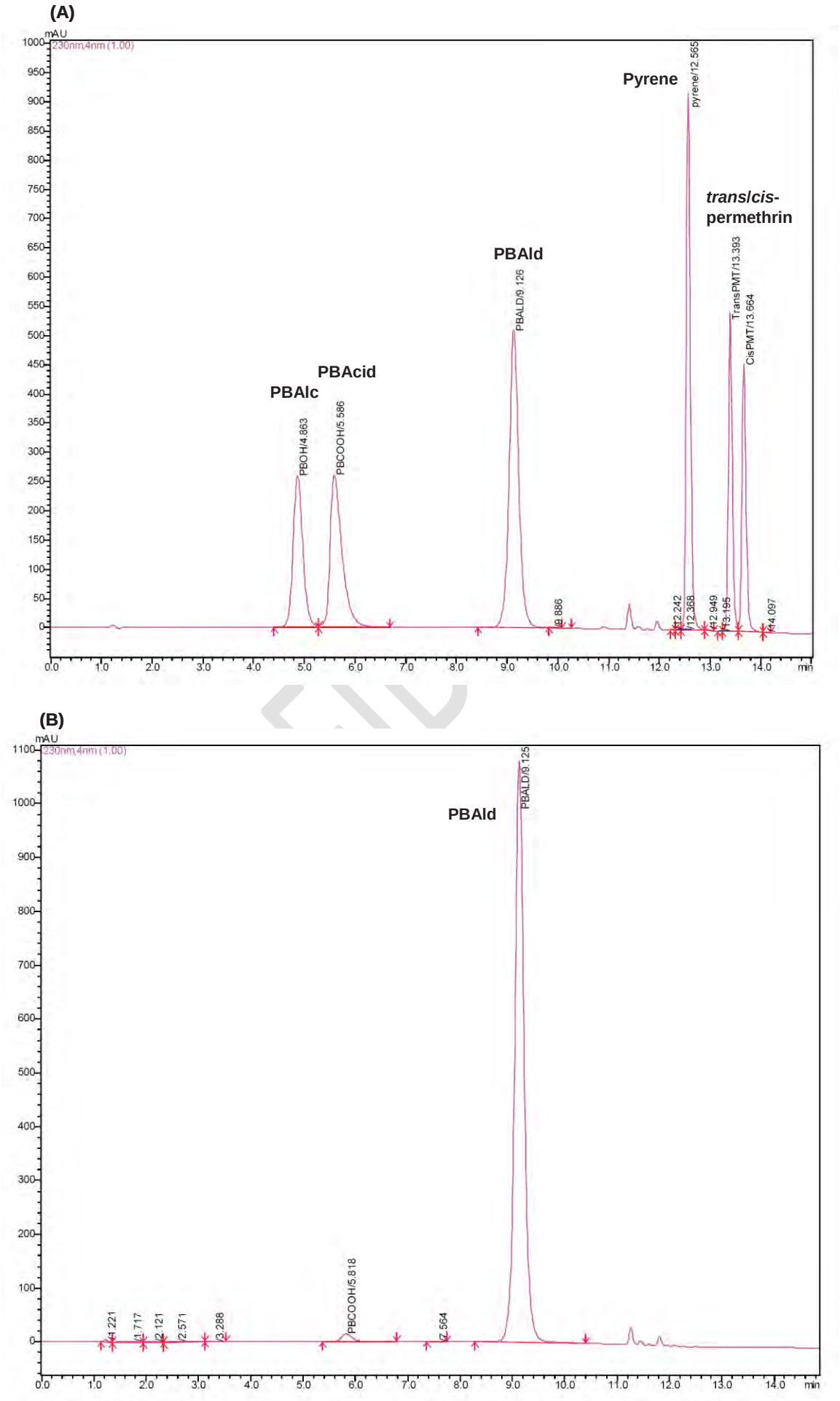
Gene	Primer name	Primer sequence (5'-3')
ALDH9029	ALDH9029F	TCCCTATGGCCATCAACAAT
	ALDH9029R	TTCATGACGGGTGTAAACGA
ALDH9948	ALDH9948F	GGTGGGAAAAATTGTGATGG
	ALDH9948R	TCGTAGATTCCCTCCTGCAC
ALDH14080	ALDH14080F1	GCTGGACAATGGCAAGGC
	ALDH14080R	TTCCAGGGAATGATTTGACC
SP7	AaSP7F1	GTACATCACCCGCGCTCGTG
	AaSP7R1	CTTGTCCAGGTGCACCTTG

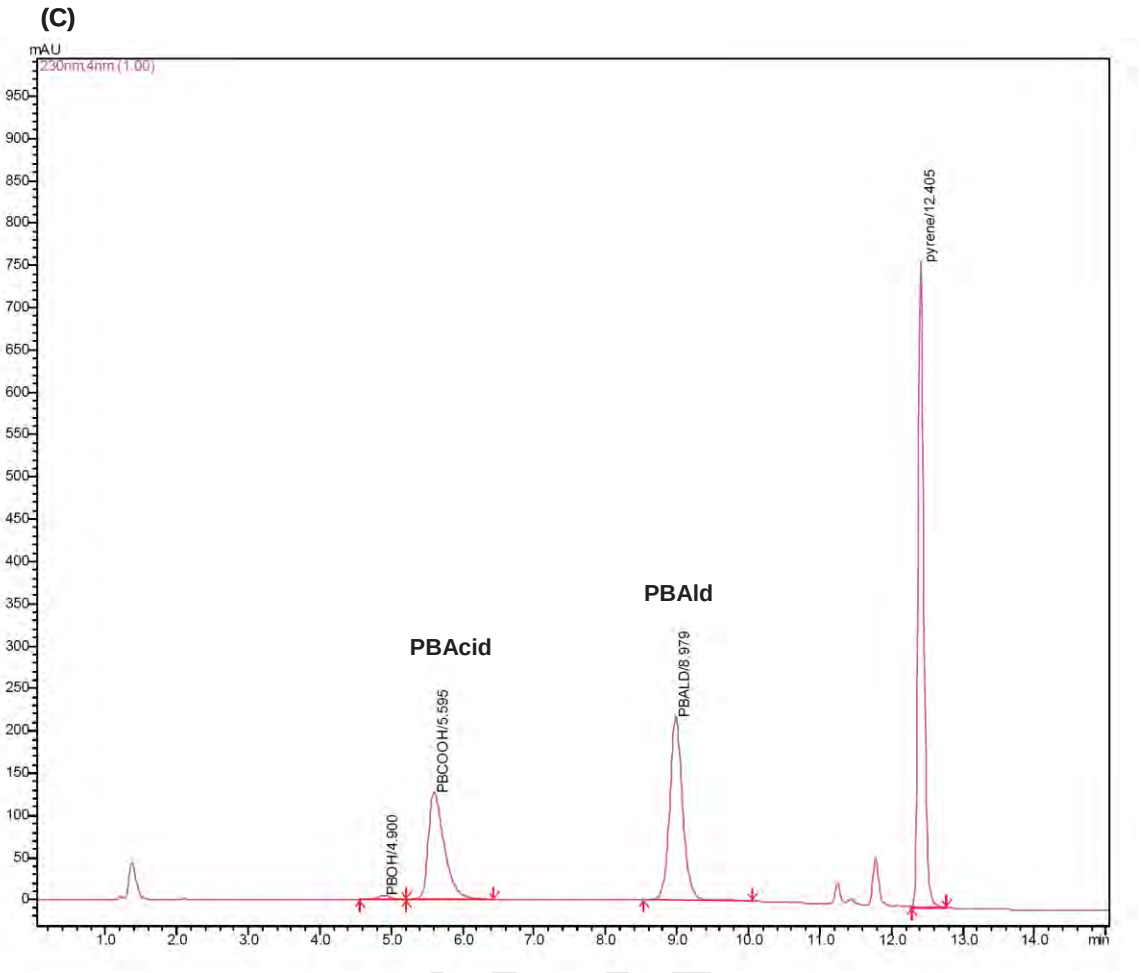
Table S3. Quantitative PCR results of *Ae. aegypti* ALDH

Gene	Life stage	Ratio of copy number		
		PMD/NO	PMDR/NO	PMDR/PMD
ALDH9029	Larva	19.1[a]	3.7[b]	0.2[a]
	Pupa	23.2[c]	24.5[c]	1.1
	Male	2.0	2.1[c]	1.1
	Female	2432.3[a]	170.4[c]	0.1[a]
ALDH9948	Larva	0.6[b]	1.8[b]	3.0[a]
	Pupa	1.2	2.0[a]	1.7[c]
	Male	0.7[c]	0.9	1.3
	Female	1.1	1.8[a]	1.6[a]
ALDH14080	Larva	0.4 [a]	1.8 [b]	4.0 [a]
	Pupa	1.1	1.2	1.0
	Male	2.1[a]	1.5	0.7[c]
	Female	1.5[c]	1.6[c]	1.1

The gene transcript copy number was determined by normalising with the transcript copy number of ribosomal S7 transcript. The ratio of the average copy number was calculated by comparison with the average copy number of the New Orleans or PMD transcript. Statistically significant differences were evaluated with ANOVA followed by a pair-wise t-test (a, $p<0.001$; b, $p<0.01$; c, $p<0.05$ relative to New Orleans (NO) or PMD strain.

Figure S1


(A)
mAU
230nm,4nm (1.00)
Pyrene
pyrene/12.565
transl*cis*-permethrin
PBAld
PBALD/9.126
TransPMT/13.393
CisPMT/13.664
PBAcid
PBAlc
PBOH/4.863
PBCOOH/5.586
(B)
mAU
230nm,4nm (1.00)
PBAld
PBALD/9.125
PBCOOH/5.818
min



FigureS1 Chromatograms of phenoxybenzylaldehyde (PBald) oxidation to phenoxybenzoic acid (PBacid) by recombinant aldehyde dehydrogenase. **(A)** Mixed standards containing 5 nmole of each standard per 10 μl injection of cis/trans-permethrin, phenoxybenzyl alcohol (PBalc), PBald and PBacid. Pyrene was spiked as an internal control. **(B)** Substrate PBald 10 nmole per 10 μl injection**(C)** Recombinant ALDH 14080 (20 μg) was incubated with 0.4 mM PBald in the presence of 3 mM NAD^+ in 0.1 M Tris-Cl buffer pH 7.4 at 37ºC for 10 min. PBacid formation was determined by HPLC as described.

<u>โครงการย่อยที่ 7</u> [Impact of knockdown resistance (kdr) genes in *Aedes aegypti* on the efficacy of vector control by using thermal fogging spray with pyrethroids: รศ. ดร.ปรัชญา สมบูรณ์]

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Parasites & Vectors

RESEARCH Open Access

Detection of the V1016G mutation in the voltage-gated sodium channel gene of *Aedes aegypti* (Diptera: Culicidae) by allele-specific PCR assay, and its distribution and effect on deltamethrin resistance in Thailand

Steven A Stenhouse[1], Suriya Plernsub[1], Jintana Yanola[2], Nongkran Lumjuan[3], Anchalee Dantrakool[1], Wej Choochote[1] and Pradya Somboon[1*]

Abstract

Background: Resistance to pyrethroid insecticides is widespread among populations of *Aedes aegypti*, the main vector for the dengue virus. Several different point mutations within the voltage-gated sodium channel (VGSC) gene contribute to such resistance. A mutation at position 1016 in domain II, segment 6 of the VGSC gene in *Ae. aegypti* leads to a valine to glycine substitution (V1016G) that confers resistance to deltamethrin.

Methods: This study developed and utilized an allele-specific PCR (AS-PCR) assay that could be used to detect the V1016G mutation. The assay was validated against a number of sequenced DNA samples of known genotype and was determined to be in complete agreement. Larvae and pupae were collected from various localities throughout Thailand. Samples were reared to adulthood and their resistance status against deltamethrin was determined by standard WHO susceptibility bioassays. Deltamethrin-resistant and susceptible insects were then genotyped for the V1016G mutation. Additionally, some samples were genotyped for a second mutation at position 1534 in domain III (F1534C) which is also known to confer pyrethroid resistance.

Results: The bioassay results revealed an overall mortality of 77.6%. Homozygous 1016G individuals survived at higher rates than either heterozygous or wild-type (1016 V) mosquitoes. The 1016G mutation was significantly and positively associated with deltamethrin resistance and was widely distributed throughout Thailand. Interestingly, wild-type 1016 V mosquitoes tested were homozygous for the 1534C mutation, and all heterozygous mosquitoes were also heterozygous for 1534C. Mutant homozygous (G/G) mosquitoes expressed the wild-type (F/F) at position 1534. However, the presence of the 1534C mutation was not associated with deltamethrin resistance.

Conclusions: Our bioassay results indicate that all populations sampled display some degree of resistance to deltamethrin. Homozygous 1016G mosquitoes were far likelier to survive such exposure. However, resistance in some populations cannot be explained due to *kdr* mutations and indicates that other resistance mechanisms are operating. The presence of this mutation alone does not fully explain the resistance phenotype we see among Thai *Ae. aegypti* populations.

Keywords: *Aedes aegypti*, *Kdr*, Pyrethroid, Deltamethrin resistance, AS-PCR, Thailand

* Correspondence: psomboon@med.cmu.ac.th
[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
Full list of author information is available at the end of the article

Background

Aedes aegypti is an important disease vector and nuisance throughout its range. In Thailand, as in many other regions, the species is incriminated as the major vector for dengue virus. Dengue fever, as well as its hemorrhagic manifestations, presents major public health problems in Thailand [1] and millions of people are at continuous risk of this disease. Currently no vaccines or specific anti-viral medications are available. In the event of an outbreak, disease control efforts must resort to vector control. Reducing vector populations below thresholds capable of sustaining viral transmission requires the heavy use of space sprays of insecticides, usually pyrethroids. These insecticides are also widely used outside of an outbreak control context, in that they are used for ongoing, seasonal control efforts as well as being used in numerous households for personal protection against mosquitoes. Pyrethroid compounds are thus the primary insecticides used for the control of *Aedes* in Thailand. However, a number of reports from throughout the country show widespread and varying resistance to a variety of insecticides, including DDT, organophosphate compounds and pyrethroids [1-4].

Resistance in *Ae. aegypti*, as well as in other vector and pest species, may arise through two major mechanisms. The first mechanism consists of metabolic or enzymatic resistance. In this case resistance is achieved through the up-regulation or constitutive overproduction of detoxifying enzymes. They work by rapidly metabolizing and detoxifying the insecticide or by sequestration, therefore inhibiting or preventing the insecticide from binding its target site [5]. The second mechanism is knockdown resistance or *kdr*, which is resistance resulting from insecticide selection that is not overcome by metabolic inhibitors, such as piperonyl butoxide (PBO) [6]. This frequently consists of single point mutations within the genes coding for proteins that are targeted by insecticide compounds. Pyrethroid insecticides work by binding to voltage-gated sodium channels (VGSC) of neurons. They bind preferentially to open channels. Bound sodium channels then remain in the open, activated state which leads to repetitive nerve firing, which in turn leads to a loss of control and uncontrolled activity. The target insect experiences convulsions and is unable to maintain normal flight behavior [7]. However if certain point mutations within the VGSC gene are present, the resulting amino acid transversion may greatly decrease the sensitivity of the sodium channel to pyrethroid binding. It may also alter the conformation of the sodium channel to an extent that it remains closed and inactivated.

In Thailand, two common *kdr* mutations within the *Ae. aegypti* VGSC gene are known to be involved in pyrethroid resistance. A phenylalanine to cysteine substitution at position 1534 within the third domain of the VGSC (F1534C) is associated with resistance to permethrin. It has been previously shown to be widely distributed throughout Thailand [8]. Other studies have indicated that this mutation is widely distributed, having since been detected within the Caribbean [9], and in Vietnam [10]. A second mutation, involving a valine to glycine transversion in domain II (V1016G) is associated with resistance to the type II pyrethroid, deltamethrin. At present it appears to be restricted to Southeast Asia, including Thailand [11,12], Indonesia [13], Vietnam [10] and Taiwan [14]. The 1016G allele frequency was found to be 0.23 in a previous study [11]. A similar mutation at the same position (V1016I) occurs among *Ae. aegypti* populations from Latin America [15]. Additionally, *Ae. aegypti* from Thailand are known to express various enzymatic resistance mechanisms. Increased expression of mixed function oxidases relative to a susceptible strain has also been seen in various pyrethroid-resistant populations originating from Thailand [16,17]. Such metabolic mechanisms can contribute to resistance along with *kdr* mutations.

A number of PCR-based techniques exist for detecting this and other nucleotide polymorphisms in *Ae. aegypti*. For the V1016G mutation, an assay has been developed, but it is optimized for use in a real time PCR machine, although amplified products could be determined on an agarose gel [15]. Another technique recently developed utilizes a heated oligotide ligation assay for detection [11]. Although requiring only a thermal cycler, that assay also requires additional reagents that can contribute to increased costs. The assay we utilized here is simpler although genotyping results can only be determined by gel electrophoresis. The purpose of this study was to develop a simple allele-specific PCR-based assay (AS-PCR) to detect the V1016G mutation and then determine its role in deltamethrin resistance in Thailand. Such information is useful to vector control operations in determining the effects and distribution of one of the major mechanisms underpinning deltamethrin resistance.

Methods

Larval collection and rearing

Larvae and pupae were collected from artificial containers situated within domestic and peridomestic areas from selected urban locales. Sampled locations consisted of detached housing, temples, schools, as well as higher-density residential areas including alleys and walkways near apartments. Larvae were collected from a total of 14 provinces from throughout Thailand (Figure 1). Additionally, a number of larvae collected from Lahore, Pakistan were given to our department for testing. Larvae obtained from 6 Thai provinces, as well as those from Pakistan, were submitted preserved in absolute ethanol. Those collected from the other provinces were brought back to our insectary and reared under standard insectary conditions. Larvae were maintained on a diet

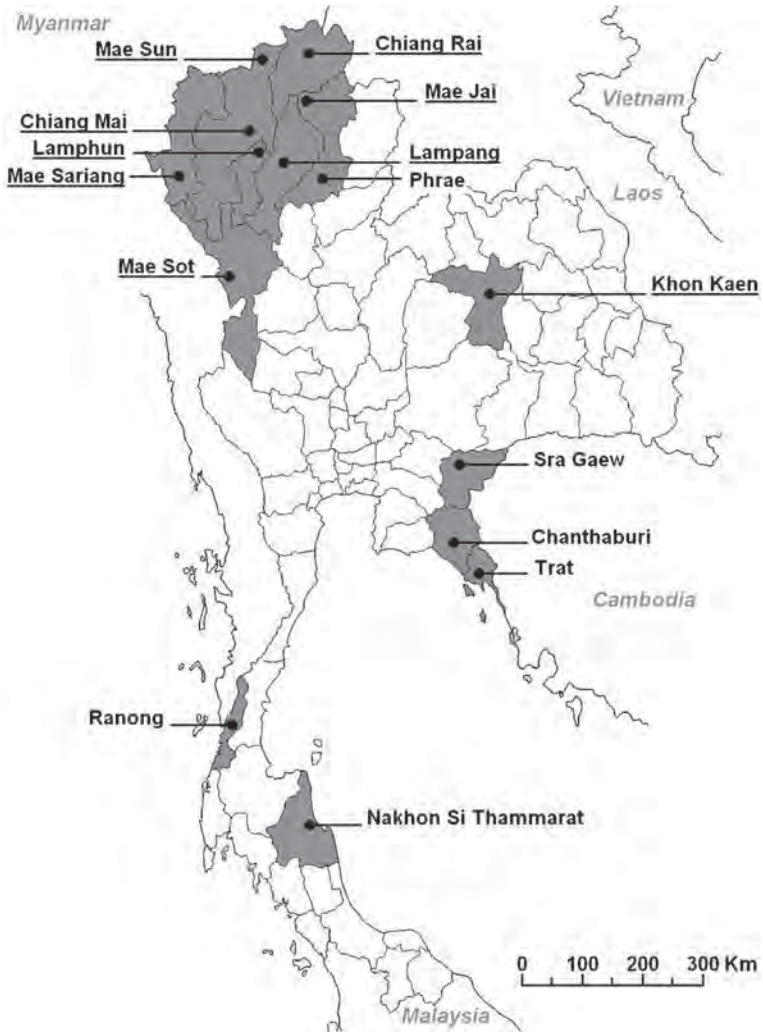

Figure 1 Larval collection map. Map showing locations of *Aedes aegypti* larval collections in Thailand. Underlined location names indicate sites from which collected larvae were reared to adulthood and tested with deltamethrin. Larvae from other provinces were killed and stored in absolute ethanol. No insecticide testing was performed on those samples.

of finely ground fish food until pupation, at which time pupae were separated according to sex. Upon emergence, female mosquitoes were maintained in small holding containers and provided a 10% w/v solution of sucrose absorbed onto a ball of cotton. To prevent injury or overcrowding, approximately 25–30 mosquitoes were kept in each holding container. Females were maintained under a 12:12 light: dark cycle under 80% RH. No blood meals were provided. No research involving vertebrates or regulated invertebrates was conducted during this study, therefore approval by the Chiang Mai University ethics committee was not required.

Deltamethrin bioassays

One to three day old female mosquitoes were used for deltamethrin susceptibility testing. Test procedures followed standard WHO protocols [18]. At least 100 females obtained from each location were used for testing, if available. This provided four replicates of 25 mosquitoes. Following the procedure, each replicate group was placed into a holding tube lined with filter paper and initially observed to determine if injured or otherwise unsuitable mosquitoes were present. Thereafter they were transferred into exposure tubes, each lined with 0.05% deltamethrin-impregnated papers (WHO, Malaysia). A control group, not exposed to insecticide, but transferred to another holding tube, was also used. Insecticide exposure lasted one hour. Thereafter, knockdown individuals were scored. Following exposure, the mosquitoes were reintroduced into their respective holding tubes and again provided a 10% w/v sucrose solution. After 24 hours dead mosquitoes, as well as those alive but incapable of coordinated movement, were scored as susceptible (S). Remaining survivors were

scored as resistant (R). All samples were subsequently stored in absolute ethanol.

V1016G AS-PCR assay development and usage

From each test of 100 mosquitoes, a total of 40 were processed for genotyping, 20 each from among susceptible and resistant mosquitoes, if available. Genomic DNA was obtained by using DNAzol® DNA extraction reagent (Invitrogen, USA). Extraction was performed according to the manufacturer's instructions, except that homogenized mosquito samples were incubated for 24 hours prior to further processing. DNA concentration was measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA) at 260 nm. Stock solutions were prepared at a concentration of 25 ng/μl and used for AS-PCR genotyping. For our study, we sought an AS-PCR assay which would utilize a standard PCR thermal cycler and the products of which could be visualized by gel electrophoresis. Our assay utilizes allele-specific primers previously developed for an assay optimized for RT-PCR use [15]. Each reaction was performed in a 10 μl volume consisting of 1.5 mM $MgCl_2$, 1x PCR buffer (Invitrogen, USA), 0.25 μM forward primer (5′-ACCGA CAAATTGTTTCCC-3′), 0.125 μM each reverse primer specific for either Gly (5′-GCGGGCAGGGCGGCGGG GGCGGGGCCAGCAAGGCTAAGAAAAGGTTAACTC-3′) or Val (5′-GCGGGCAGCAAGGCTAAGAAAAGGT TAATTA-3′), 200 μM dNTP mixture (New England Biolabs, USA), 0.2 units Taq polymerase (Invitrogen, USA) and 25 ng genomic DNA. The thermal cycling condition begins with an initial DNA denaturation step for two minutes at 94°C, followed by 35 cycles of 30 sec at 94°C (denature), 30 sec at 55°C (anneal), and 30 sec at 72°C (extension). This is then followed by two minutes at 72°C for final extension. Since the primers used had GC-rich tails of varying lengths, amplified products could be differentiated by size (60 bp for Val, and 80 bp for Gly) (Figure 2). PCR amplification products were loaded onto a 4% agarose gel and run for 50 min at 100 V in TBE buffer.

Figure 2 Gel electrophoresis results. Each of the three genotypes is shown from left to right: wild-type homozygous (V/V), heterozygous (V/G) and mutant homozygous (G/G). The lane to the far left contains low-molecular weight DNA ladder (L).

F1534C AS-PCR genotyping

In order to determine the contribution of both the V1016G and the F1534C mutations towards deltamethrin resistance, a number of samples including larvae, and susceptible and resistant adults were tested for the F1534C mutation which is known to confer resistance to permethrin. Testing for this mutation was conducted after testing for V1016G. The AS-PCR assay follows the protocol of one of our previous studies [8]. Each reaction was performed in a 10 μl volume with final concentrations of 1.5 mM $MgCl_2$, 1x PCR buffer, 0.5 μM Phe forward primer (5′-GCGGGCTCTACTTT GTGTTCTTCATCATATT-3′), 0.165 μM Cys forward primer (5′-GCGGGCAGGGCGGCGGGGGCGGGGCCTCT ACTTTGTGTTCTTCATCATGTG-3′), 0.5 μM common reverse primer(5′-TCTGCTCGTTGAAGTTGTCGAT-3′), 200 μM dNTP mix, 0.2 units Platinum *Taq* DNA polymerase, and 25 ng template DNA. Reactions were run at 95°C for 2 min initial activation stage and followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, in turn followed by a final extension at 72°C for 2 min. PCR products were loaded onto 3% agarose gels and electrophoresis was conducted ay 100 V for 45 min.

DNA sequencing

To validate the results obtained from our AS-PCR test specific for the V1016G mutation, we sequenced some of the genotyped samples in order to determine the accuracy of the assay. We obtained 90 previously tested samples, 30 from each genotype, and used these samples

for sequencing. We began by amplifying a fragment of domain II in the sodium channel gene that encompasses the V1016G mutation. The method has been described previously [8]. Each reaction was performed in a 20 μl reaction volume. Reagents are added to final concentrations of 1.5 mM $MgCl_2$, 1x PCR buffer, and 0.5 μM each of forward (5′-GGTGGAACTTCAC-CGACTTC-3′) and reverse (5′-GGACGCAATCTGGCTTGTTA-3′) primers, 200 μM dNTP mix, and 0.4 units of Platinum *Taq* DNA polymerase. PCR amplification begins with 2 min at 95°C, followed by 35 cycles of 95°C for 30 s, 63°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 2 min. Amplified products were purified using ExoStar DNA purification reagent (GE Illumina, USA). Purified samples were then sent to Macrogen, Inc. (Seoul, Korea) for sequencing. Sequencing reactions were performed on an ABI 3730XL DNA analyzer (Applied Biosystems Inc., USA). Resultant data were analyzed using Geneious software, version 5.3.6 (Biomatters Ltd., UK). A set of sequenced DNA samples representing each of the three genotypes was serially diluted from stock concentration and tested to determine the detection limit for the assay.

Statistical analysis

Pearson's X^2 test was used to compare the genotype and allele frequencies between susceptible and resistant mosquito groups, as well as to compare differences between the combined adult samples and larvae. Fisher's exact test was used to compare allele frequencies of dead and surviving mosquitoes at each sampled location. All tests and calculations were performed in R 2.15.1 [19].

Results

Deltamethrin bioassays and adult genotyping

A total of 1465 female *Ae. aegypti* mosquitoes collected from urban areas in 8 provinces were tested for deltamethrin susceptibility and resistance (Table 1). Overall susceptibility was 77.6%, with mortality rates varying widely from 50.0 % to 89.9%. Samples obtained from Chiang Rai, Mae Hong Son, Phayao Provinces show varying degrees of incipient resistance (80-97% mortality). There was no mortality among control mosquitoes. Genotype and allele frequencies were determined from 451 susceptible and 301 resistant mosquitoes selected at random. Genotype frequencies between resistant and susceptible mosquitoes were significantly different ($X^2 = 101.24$, df = 2, $P < 0.0001$).

Table 1 Deltamethrin bioassay and AS-PCR results

Province	Location	Total	Mortality	Status	n	Total	Genotype			G Allele	G Allele	Fisher's exact test
		Tested	(%)			PCR	V/V	V/G	G/G		95% CI	P value (α = 0.05)
Chiang Rai	Chiang Rai City	120	82.5	R	21	21	10	10	1	0.286	[0.172, 0.436]	0.00684
				S	99	20	18	2	0	0.050	[0.138, 0.165]	
Chiang Mai	Mae Sun	36	50.0	R	18	17	1	11	5	0.618	[0.450, 0.761]	0.02803
				S	18	17	6	11	0	0.324	[0.191, 0.492]	
Phayao	Mae Jai	109	85.3	R	16	16	15	1	0	0.031	[0.055, 0.157]	0.45710
				S	93	19	19	0	0	0.000	[0.000, 0.918]	
Chiang Mai	Chiang Mai City	538	75.7	R	131	126	16	72	38	0.587	[0.526, 0.646]	0.00000
				S	407	255	128	120	7	0.263	[0.226, 0.303]	
Lamphun	Lamphun City	99	66.7	R	33	20	11	9	0	0.225	[0.123, 0.375]	0.04763
				S	66	20	18	2	0	0.050	[0.014, 0.165]	
Lampang	Lampang City	100	79.0	R	21	20	15	5	0	0.125	[0.055, 0.261]	0.43150
				S	79	20	18	2	0	0.050	[0.014, 0.165]	
Mae Hong Son	Mae Sariang	99	89.9	R	10	10	0	9	1	0.550	[0.342, 0.742]	0.04266
				S	89	20	10	10	0	0.250	[0.142, 0.402]	
Tak	Mae Sot	159	80.5	R	31	31	19	12	0	0.194	[0.114, 0.309]	0.00448
				S	128	40	37	3	0	0.038	[0.013, 0.105]	
Khon Kaen	Khon Kaen City	205	77.1	R	47	40	10	19	11	0.513	[0.405, 0.619]	0.00000
				S	158	40	25	15	0	0.188	[0.117, 0.287]	
Total		1465	77.6	R	328	301	97	148	56	0.432	[0.393, 0.472]	
				S	1137	451	279	165	7	0.198	[0.174, 0.226]	

A sample of the total number of deltamethrin tested mosquitoes (n) was used for PCR testing. Samples of susceptible (S) and resistant (R) mosquitoes were genotyped and mutant allele (G allele) frequencies calculated. Fisher's exact test was used to test G allele frequency differences between S and R mosquitoes from each location. For X^2 results on the overall population genotype and allele frequencies, see results section. Locations are listed in order from north to south.

The frequencies of the 1016G allele were significantly different (X^2 = 95.19, df = 1, P < 0.0001) between dead and surviving mosquitoes as well (0.198 and 0.432, respectively).

Fisher's exact test was used to compare differences in 1016G allele frequencies between susceptible and resistant mosquitoes from each location. In most locations, the allele frequencies were highly significantly different between dead and surviving individuals; however, in those samples obtained from Lampang City (Lampang) and Mae Jai (Phayao), the differences in allele frequencies between susceptible and resistant groups were not significant (Table 1). These populations also had the lowest total mutant allele frequencies among those sampled (0.088 and 0.015, respectively). Furthermore we found that there was very little correlation when comparing 1016G frequencies directly against deltamethrin resistance at all locations (data not shown). We instead looked at the resistance phenotype of each genotype under selection with deltamethrin (Figure 3). Mutant homozygous mosquitoes survive at much higher rates. By multiplying the total number of insecticide tested mosquitoes against the genotype frequencies of the AS-PCR tested samples, we deduced the absolute phenotypic frequencies. Mutant homozygous mosquitoes had a resistance phenotype frequency of 0.772, whereas heterozygous and wild-type homozygous individuals show much reduced rates (0.279 and 0.131, respectively).

Larval genotyping

In addition to our adult testing, larvae from six provinces were also genotyped. (Figure 1, non-underlined location names). Samples represent areas from the north (Phrae), south (Ranong and Nakhon Si Thammarat) and the southeast of Thailand (Chanthaburi, Sra Gaew and Trat). A total of 128 larvae were genotyped. The frequency of the 1016G allele was 0.305 (Table 2). The frequency of mutant homozygous individuals was also found to be higher among larvae than that seen from the adult data (0.125 and 0.084, respectively), however this difference was not significant (X^2 = 1.85, df = 1, P = 0.174).

AS-PCR for the F1534C mutation

In order to determine the role that another common *kdr* mutation might play in contributing to deltamethrin resistance, we took 170 individuals previously genotyped for the V1016G mutation and tested them for the presence of the F1534C mutation (Table 3). A total of 62 larvae, as well as 47 susceptible and 61 resistant adult females, with representatives from all provinces, were tested. All three genotypes at position 1016 were represented (74 V/V, 66 V/G and 30 G/G). In our testing we found no individuals that expressed the wild-type at both positions simultaneously, nor were any found that expressed both mutations (double homozygous mutant).

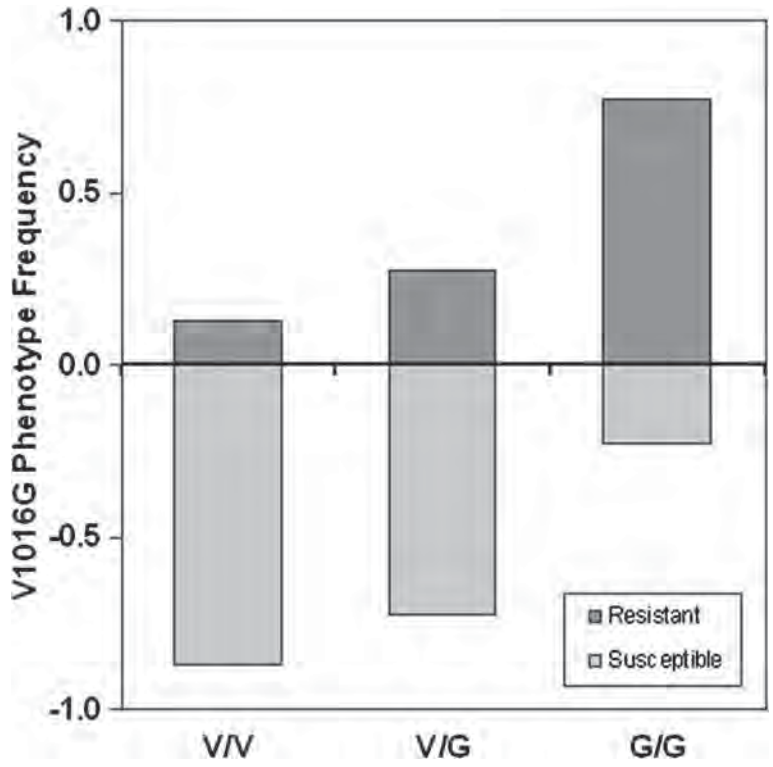

Figure 3 Correlation between V1016G genotypes and the deltamethrin resistance phenotype. The survival of individual genotypes was compared to determine the contribution of the V1016G mutation to deltamethrin resistance.

Table 2 Larval genotyping results

Province	Location	PCR	V/V	V/G	G/G	G Allele	G Allele 95% CI
Phrae	City	18	15	3	0	0.083	[0.029, 0.218]
Sra Gaew	City	22	17	5	0	0.114	[0.049, 0.239]
Chanthaburi	City	12	4	7	1	0.375	[0.212, 0.573]
Trat	City	16	8	7	1	0.281	[0.156, 0.454]
Ranong	City	30	9	11	10	0.517	[0.393, 0.638]
Nakhon Si Thammarat	City	30	13	13	4	0.350	[0.242, 0.476]
Total		128	66	46	16	0.305	[0.252, 0.364]

Locations refer to capitol cities named after their respective provinces with the same name. Due to the collection method, no insecticide testing could be performed. Locations are listed from north to south.

Double heterozygous samples were common; however, no samples expressed the homozygous type of one mutation combined with a heterozygous type for the other. Among the samples tested were larvae obtained from Lahore, Pakistan. Initial testing of 50 such larvae indicated they were all wild homozygous (V/V) at position 1016 (data not shown); but upon further testing of 23 of these samples, we found that all were mutant homozygous (C/C) at position 1534 (Table 3).

Sequencing of domain II of the sodium channel gene

Ninety samples, each distributed evenly among the different genotypes (30 each), were sequenced. All sequenced samples were in agreement with results previously obtained by AS-PCR testing. Sequencing also revealed the presence of the serine to phenylalanine transversion at position 989 within domain II (S989P) alongside V1016G. A single heterozygous resistant female from Chiang Rai also expressed the homozygous I1011V mutation. From three of these sequenced samples, a set of serial dilutions were made for each genotype and tested in order to determine the limit of detection. Homozygous samples of either genotype could be detected at 0.5 ng/μl, however, reliable detection of heterozygous individuals occurred down to 1.0 ng/μl of genomic DNA. Thus a concentration of 1.0 ng/μl represents the limit of detection for this assay.

Discussion

In this study, we have successfully developed a simple AS-PCR technique to detect the V1016G mutation in Thai populations of *Ae. aegypti*. The high level of agreement between this assay and sequenced samples should encourage the use of this assay in order to determine the full extent of this mutation throughout Southeast Asia and elsewhere. This mutation is evidently widespread throughout Thailand, although individuals homozygous for the 1016G allele are still relatively uncommon. The mutant allele was found, to some extent, in material from all locations. Unfortunately, all of these populations display some degree of resistance to deltamethrin, with an overall 77.6% mortality rate. This resistance level is considered to be an underestimate due to our use of 0.05% deltamethrin paper which is at a higher concentration than the discriminating dose (0.025%) for adult *Ae. aegypti* recommended by WHO [18]. Deltamethrin resistance rates and V1016G allele frequencies varied widely between locations and no strong correlation could be seen between deltamethrin survival and mutant allele frequencies. However, most homozygous mutant females were resistant and most wild-type homozygous were susceptible, whereas heterozygous mosquitoes displayed intermediate resistance to deltamethrin. This may be explained because the 1016G allele is recessive for the *kdr* characteristic [8,9]. The survival of approximately a third of the heterozygous mosquitoes also likely indicates that the 1016G mutation is not the only mechanism involved and that other *kdr* or enzymatic mechanisms may confer cross resistance or enhance resistance.

Interestingly, no homozygous 1016G mutants were ever found that also expressed the homozygous form of

Table 3 AS-PCR genotyping for F1534C

		Larvae n = 62				Susceptible n = 47				Resistant n = 61		
V1016G		V/V	V/G	G/G		V/V	V/G	G/G		V/V	V/G	G/G
	F/F	0	0	10	F/F	0	0	7	F/F	0	0	13
F1534C	F/C	0	10	0	F/C	0	20	0	F/C	0	36	0
	C/C	42	0	0	C/C	20	0	0	C/C	12	0	0

Samples of larval and adult mosquitoes previously genotyped by V1016G AS-PCR were selected for testing to detect the presence of the F1534C mutation. A total of 170 samples were tested.

the 1534C mutation, regardless of deltamethrin exposure status. Of 170 mosquitoes checked, these double mutants were never found, either in resistant or susceptible insects. Similarly, no double wild-type mosquitoes were found, indicating that in Thailand at least, *Ae. aegypti* harbor either the 1016G mutation, or the more common and widespread 1534C mutation. This is not to say double wild-type mosquitoes do not exist, but such specimens are likely quite rare in the wild as a result of both extensive and intensive pyrethroid usage throughout Thailand. This indicates that the ability to control *Ae. aegypti*, especially using pyrethroids such as permethrin, has been severely compromised. An exception to this is the pyrethroid-susceptible PMD strain that has been maintained in our insectary and was originally collected from a rural area of Chiang Mai province [20]. This strain harbors neither of the aforementioned *kdr* mutations [21]. Currently the 1016G mutation has thus far only been found within Southeast Asia including Bhutan. Our testing of *Ae. aegypti* larvae from Lahore, Pakistan revealed that the mutation was not present there. However, upon further examination, all larvae were homozygous for the 1534C mutation. This is therefore the first report of that mutation in Pakistan and likely indicates a population that is highly resistant to type I pyrethroid insecticides.

The 1534C mutation is far more common than the 1016G mutation. An earlier study revealed an allele frequency of 0.77 for the 1534C mutation in Thailand [8]. Mosquitoes with the homozygous 1534C mutation are generally susceptible to deltamethrin. The PMD-R strain maintained in our insectary is homozygous 1534C and exhibits 100% mortality after 1 h exposure to 0.05% deltamethrin paper (unpublished data). Conversely, specimens homozygous for the 1016G allele would likely be protected. The finding that these two mutations are apparently never found together in the same individual, other than in heterozygous forms, contrasts with results found in other regions. In the Cayman Islands, for example, a number of mosquitoes were found that were homozygous for two mutations, F1534C and V1016I [9]. A small number of samples were also wild-type homozygous at both positions. In Vietnam, many larvae were found to lack both mutations, and only two larvae were found to be 1016G heterozygous [10].

One limitation of this study is that we did not conduct insecticide testing in combination with the synergist PBO which suppresses the activity of detoxifying enzymes such as P450s and non-specific esterases, and thus may modify or reduce metabolic resistance to pyrethroids. Although the V1016G mutation is associated with deltamethrin resistance, as found in previous studies [12,13], and further corroborated in this study, it may not explain the resistance phenotype completely [22]. Clearly, individuals expressing the homozygous form of the mutation have much higher chances of survival under pyrethroid selection, but it should be noted that a greater number of homozygous wild-type individuals survived exposure (56 G/G and 97 V/V, Table 1), albeit at much lower frequencies than their mutant counterparts. There are likely a number of metabolic resistance mechanisms at work. It is also possible that these 'wild-type' mosquitoes harbor the homozygous 1534C mutation, as seen by our testing (Table 3). And although this mutation is correlated with resistance to type I pyrethroids, it does not contribute to resistance to type II pyrethroids, as indicated by the low numbers of 1016 V homozygous (and thus 1534C mutant homozygous) individuals that survived. This has been previously confirmed by the insertion of the 1534C equivalent mutation into the VGSC gene of the cockroach [23]. The channel remained sensitive to the action of deltamethrin, yet showed decreased sensitivity to permethrin.

In one study, low levels of deltamethrin resistance were found among *Ae. aegypti* populations sampled in central Thailand [24]. Sequencing of the partial sodium channel gene encoding segment domain II indicated that the 1016G mutation was not present, although two other uncharacterized polymorphisms were discovered. It was also determined that mixed function oxidases were elevated in all field collected material relative to a susceptible strain. The survival of such wild-type individuals after deltamethrin exposure is therefore likely due to metabolic mechanisms and warrants further investigation. Our previous study revealed that besides the F1534C mutation, oxidative enzyme systems also play a role in pyrethroid resistance in *Ae. aegypti* in Thailand [17].

From this study alone we cannot determine the contributions of metabolic mechanisms or other *kdr* mutations towards deltamethrin resistance. In many of our sampling locations we found that there are highly significant differences between the allele frequencies of susceptible and resistant mosquito groups. This provides strong evidence that the mutation contributes to deltamethrin resistance. However, in two populations, one of which displays incipient resistance (Mae Jai, 85.3% mortality) while the other appears resistant (Lampang City, 79.0% mortality), there were no significant differences between the mutant allele frequencies of susceptible and resistant groups (Fisher's exact test, $P = 0.4517$ and $P = 0.4315$, respectively). These two populations also had the lowest 1016G allele frequencies among all sampled groups. There is the possibility that other *kdr* mutations are involved in deltamethrin resistance in Thailand. The V1016I mutation, for example, is more commonly found in Central and South America, and has been correlated to resistance to deltamethrin [25]. Nevertheless, both the V1016I and I1011V mutations were recently found in a deltamethrin-

resistant strain from Vietnam [26]. The V1016I mutation has not been detected among Thai populations to date, however, I1011V has previously been found throughout Thailand at an allele frequency of 0.14 [11]. Based on our sequencing data, only one deltamethrin-resistant V1016G heterozygote was found to have the homozygous form of the I1011V mutation. At this time, little is known about the contribution of this polymorphism to insecticide resistance, and it has yet to be confirmed to reduce sodium-channel sensitivity to pyrethroids in *Xenopus* oocytes [27].

A similar mutation at the same position, I1011M, known from Latin American *Ae. aegypti* populations, was significantly associated with resistance to cypermethrin in a population from Brazil [28]. Mutations at this location may be important in conferring resistance to various pyrethroids and future studies should address this issue. Furthermore, the 1016G mutation is usually found with a 989P mutation as well [12]. Yet not all strains expressing the 1016G mutation simultaneously express 989P. A previously mentioned strain from Taiwan lacks 989P, but harbors a D1763Y polymorphism instead [14]. The 989P substitution was also lacking in some Thai and Indonesian strains that were homozygous for 1016G [13]. However, this mutation was apparent in our samples. More detailed studies are needed to determine what other polymorphisms may accompany this mutation and how they may contribute to the resistance phenotype. The fact that several different *kdr* mutations can be found within Southeast Asia underscores the need to further develop simple AS-PCR assays, as well as the need for multiplex PCR reactions to efficiently screen for multiple mutant alleles within a population.

Conclusions

The assay we used proved to be highly reliable and this should aid future studies aimed at further determining the extent of this *kdr* mutation, particularly within Southeast Asia. The presence of the 1016G mutation was associated with resistance to deltamethrin and was found to be common among populations of *Ae. aegypti* throughout Thailand. No mosquitoes from the Thai populations sampled have yet been found to harbor both the 1016G and 1534C mutations. Wild type individuals without any *kdr* mutations are now probably very rare in Thailand. This is also the first report of the 1534C mutation in Pakistan and increases the known range of that mutation. The 1016G mutation appears to be confined to Southeast Asia at the present time.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NL, WC and PS conceived the study. AD and NL supervised the study. SAS and SP collected and tested mosquitoes. SAS, SP and JY designed and performed lab experiments. SAS, SP and JY analyzed the data. SAS, JY and PS interpreted the results. SAS wrote the draft manuscript. All authors read and approved the final manuscript.

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Author details

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. [2]Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand. [3]Research Institute for Health Sciences, Chiang Mai University, Chiang Mai 50200, Thailand.

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Relative developmental and reproductive fitness associated with F1534C homozygous knockdown resistant gene in *Aedes aegypti* from Thailand

Plernsub, S.[1], Stenhouse, S.A.[1], Tippawangkosol, P.[1], Lumjuan, N.[2], Yanola, J.[3], Choochote, W.[1] and Somboon, P.[1*]

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
[2]Research Institute for Health Sciences, Chiang Mai University, Chiang Mai 50200, Thailand
[3]Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand
[*]Corresponding author email: pradaya.somboon@med.cmu.ac.th
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Abstract. The effect of permethrin resistance, conferred by a homozygous mutation (F1534C) in the voltage-gated sodium channel protein, upon the reproductive fitness of *Aedes aegypti* (PMD-R strain) from Thailand was evaluated by comparing with a pyrethroid-susceptible sub-colony (PMD strain). The parameters evaluated included larval development time, pupation success, adult emergence, egg production and hatchability, mating ability, female wing length and adult longevity. Larval development times were similar with very low mortality of larvae, pupae and emerging adults among either strain. However, PMD produced significantly fewer females than PMD-R. The mean numbers of eggs laid by PMD (54.2±15.9) and PMD-R (54.6±14.5) strains were not significantly different but the hatchability of PMD eggs (53.7%) was lower than PMD-R eggs (71.2%). The mean wing length of PMD females (2.85±0.15 mm) was longer than PMD-R females (2.74±0.09 mm). The insemination rates for both strains were 100%. The longevity of both strains was mostly not significantly different, over 90% of both sexes surviving at day 30. Our results suggest that the presence of the homozygous F1534C mutation does not lead to fitness reductions. This is in accordance with the high frequency of this allele found among wild populations of *Ae. aegypti* in many countries. These results also suggest that the removal of pyrethroid insecticide selection pressure may not lead to a regression of 1534C alleles in pyrethroid resistant *Ae. aegypti*.

INTRODUCTION

Aedes aegypti is the major vector of viral diseases including dengue and dengue hemorrhagic fever, and chikungunya which are serious public health problems in Thailand as well as many countries in tropical and subtropical areas. Because a dengue vaccine and specific treatment are not available, control of transmission is basically based on management of breeding places, or the application of larvicidal (e.g. temephos sand granules) and adulticidal chemicals (e.g. fogging and ultra-low-volume sprays). Many insecticides including Dichlorodiphenyltrichloroethane (DDT), the organophosphates (e.g., malathion, fenitrothion, and temephos), and carbamate (e.g., propoxur) were heavily used for mosquito control for over 50 years in Thailand before being replaced (except temephos) by pyrethroids in the early 1990s (Chareon-viriyaphap *et al.*, 1999). The adverse effect of the heavy and long-term use of insecticides is resistance of *Ae. aegypti* worldwide. In Thailand, resistance to DDT was first reported in *Ae. aegypti* in the mid 1960s (Neely, 1966). At present, it is known to be resistant to several insecticides, particularly pyrethroids (i.e., permethrin and delta-methrin), organophosphate compounds (i.e., temephos and fenitrothion), and carbamate

compounds (i.e., propoxur) (Somboon *et al.*, 2003; Ponlawat *et al.*, 2005; Jirakanjanakit *et al.*, 2007). This problem has severely hampered the control of vectors by insecticides.

There are two broad classes of resistance mechanisms that play an important role in mosquito resistance to insecticides: target site insensitivity and metabolic enzyme-based resistance (Hemingway & Ranson, 2000). Target site insensitivity to pyrethroids and DDT in mosquitoes and other insects is associated with single or multiple mutations, commonly referred to as knockdown resistance (*kdr*). These mutations modify the voltage-gated sodium channel protein, making it less susceptible to the binding of pyrethroids and DDT (Soderlund & Knipple, 2003). Metabolic enzyme-based resistance is principally associated with three enzyme groups: cytochrome P450 monooxygenases (P450s), esterases, and glutathione-S-transferases, depending on the insect species/strain and the insecticide (Hemingway & Ranson, 2000).

The heavy use of insecticides in mosquito control programmes may cause a dramatic increase in the frequency of resistant alleles (Garcia *et al.*, 2009; Martins *et al.*, 2009). In the absence of insecticide pressure, however, reduced fitness of resistant insects is frequently reported, resulting in a reproductive disadvantage, which would probably decrease resistant allele frequencies in the field over time (Lenormand *et al.*, 1999). Several studies have shown that resistance to insecticides reduces, to some degree, the reproductive fitness of *Ae. aegypti* (Mebrahtu *et al.*, 1997; Kumar *et al.*, 2009; Belinato *et al.*, 2012; Martins *et al.*, 2010) as well as some *Culex* and *Anopheles* mosquito species (e.g. Amin & White, 1984; Rowland, 1991; Wang *et al.*, 1998; Hardstone *et al.*, 2009; Kumar & Pillai, 2011). The fitness reductions observed in insecticide-selected strains is generally considered due to pleiotropy of the resistant alleles or to a hitch-hiking effect (Smith & Haigh, 1974). By contrast, Okoye *et al.* (2007) reported that pyrethroid resistance in southern African *Anopheles funestus* does not incur any loss of fitness under laboratory conditions. In addition, other insect species, including boll weevils, houseflies and cockroaches, do not show differences in fitness between resistant and susceptible strains in the absence of insecticide treatment (Varzandeh *et al.*,, 1954; Perkins & Grayson, 1961; Thomas & Brazzel, 1961; Roush & Hoy, 1981). These studies have suggested that resistance and fitness may evolve independently (Heather, 1982) and differences in insecticide resistant mechanisms or alleles may affect fitness positively or negatively (Berticat *et al.*, 2008; Rivero *et al.*, 2010). It is, however, difficult to associate fitness disadvantages specifically with resistance in field populations. Estimates of relative reproductive and survival rates of resistant and susceptible genotypes obtained from laboratory studies are useful when considering the influence of resistance alone on biological fitness. As most previous studies concerning reproductive fitness of resistant *Ae. aegypti* are associated with metabolic resistance, little is known about the effect of knockdown resistance genes in regards to fitness and survival. The aim of this study was to determine whether pyrethroid resistance in *Ae. aegypti* conferred mainly by knockdown resistant mechanism affects certain fitness components when compared to the susceptible sub-colony. Understanding these aspects is essential for improving resistance monitoring, detection and management in vector control programmes in Thailand.

MATERIALS AND METHODS

Mosquito strains

Two laboratory strains of *Ae. aegypti*, PMD and PMD-R, were used in this study. Both originated from Ban Pang Mai Daeng, Mae Tang District, Chiang Mai Province, Thailand (Prapanthadara *et al.*, 2002). They have been maintained over 10 years in our insectary at 25±2°C, 70-80% RH and 14 h illumination. The PMD-R strain is resistant to both DDT and permethrin while PMD is resistant to DDT but susceptible to permethrin. Permethrin resistance in the PMD-R strain is mainly due to the homozygous mutation in codon F1552

of the *kdr* gene of *Ae. aegypti* (equivalent to F1534 in the house fly *Vssc1* sequence) resulting in the replacement of phenylalanine with cysteine in segment six domain III of the voltage-gated sodium channel protein (Somwang *et al.*, 2011; Yanola *et al.*, 2010). This mutation (F1534C) is common throughout Thailand (~0.8 allele frequency, Yanola *et al.*, 2011) and is widespread in Vietnam (up to ~0.87) (Kawada *et al.*, 2009) and Grand Cayman (0.68) (Harris *et al.*, 2010). In the PMD strain, no *kdr* mutation has been observed in domains II or III of the voltage-gated sodium channel protein (Yanola *et al.*, 2010, 2011). Our previous biochemical characterization revealed that the levels of total P450s, DDTase, esterase and glutathione-S-transferase (GST) were similar in both strains. However, there was a tenfold increase in DDTase activity and a fourfold increase in P450 activity compared to the susceptible Rockefeller strain, whereas the esterase and glutathione-S-transferase (GST) activities were only slightly increased (Prapanthadara *et al.*, 2002; Somwang *et al.*, 2011). The PMD-R adult mosquitoes have been maintained under regular insecticide pressure (0.75% permethrin) using standard WHO kits (WHO, 1975).

Exp. 1: Larval and pupal development and adult emergence

Stocks of previously dried eggs were submerged in distilled water for 1-2 days and newly hatched first instar larvae were randomly collected. For each strain, triplicates of one hundred first instar larvae were reared in 0.5 liter cups containing 480 ml of distilled water. On the first day, 0.15 g of ground fish food was offered and added every other day until the end of the experiment. The first day of pupation, mean pupation time, pupation rate and emergence rate were recorded. Newly emerged male and female mosquitoes were placed in 30 cm^3 cages covered with moist towel and used for Exp. 2.

Exp. 2: Insemination, fecundity, hatchability of eggs, size of females

Adult mosquitoes from Exp. 1 were kept in the cage for a week. A blood meal was then offered and the fully engorged females were randomly aspirated and kept individually in 50 ml cups lined with filter paper. They were provided with a 10% sugar solution. On day 5 post feeding a small amount of distilled water was put into the cup for oviposition. The resulting eggs were counted and air dried for a week. To determine the hatchability of the eggs, they were submerged in distilled water for 3 days and hatched larvae were counted. After oviposition, the females were dissected for spermathecae to determine the presence of spermatozoa. The degree of insemination was scored as 4+, 3+, 2+, 1+ or 0. A score of 4+ indicates the presence of spermatozoa filling all three lobes of the spermathecae, 3+ indicates the presence of spermatozoa in 2 lobes and only scanty spermatozoa in the other, 2+ signifies the presence of spermatozoa filling one lobe and scanty in the others, 1+ denotes the presence of only scanty spermatozoa in one or more lobes, and a score of 0 was given if no insemination occurred.

After the dissection of spermathecae, female mosquito size was determined by measuring the length of the right wing from the axillary incision to the wing tip excluding fringe. The measurement was performed under a stereomicroscope with calibrated ocular micrometer.

Exp. 3: Longevity

The longevity of adult mosquitoes fed on sugar solution was determined. Triplicates consisting of one hundred pairs of one day old male and female mosquitoes reared from larvae as described in Exp. 1 were placed in 30 cm^3 cages covered with moist towel. Cage floors were lined with white paper so that dead mosquitoes were easily seen. They were provided with a 10% sugar solution soaked onto cotton wool which was changed every other day. Dead mosquitoes were counted and removed daily. Similarly, the longevity of adult mosquitoes under starvation conditions was investigated by rearing under similar conditions but providing only water instead of a sugar solution.

Data were analyzed by using SPSS version 12.0.1. Student *t*-test was employed for comparing mean values of larval and

pupal development, adult emergence, egg production, hatchability, longevity and wing length. Chi-square test was performed for sex ratio and insemination rate.

RESULTS

Exp. 1: Larval and pupal development and adult emergence

Developmental times from first instar larvae to pupae were similar for both strains (Table 1). Pupation began on the tenth day, with mean pupation times (L1 to 50% pupation) being 14.3±0.9 and 15.2±0.6 days for PMD and PMD-R strains respectively. A total of 282 and 293 pupae were obtained from the PMD and PMD-R respectively. Percent pupation success and adult emergence success of the two strains were not significantly different. The observed sex ratios (M:F) of emerging adults of PMD was 165:102 (P = 0.007) and of PMD-R 126:159 (P =0.178), indicating that PMD produced 17.6% fewer females than PMD-R.

Exp. 2: Insemination, fecundity, hatchability of eggs, female size

There was no significant difference between the PMD and PMD-R strains regarding mean numbers of eggs laid (Table 2). Dissection of spermathecae revealed that all females from both strains were inseminated. The quantity of spermatozoa in PMD showed 11.4% of 4+, 84.1% of 3+, and 4.5% of 2+, and in PMD-R 5.4% of 4+, 91.9% of 3+, and 2.7% of 2+, which were not significantly different (X^2=1.23, d.f. =2, P >0.5). However, the mean number of hatching larvae per PMD female (average 53.7%) was significantly lower than PMD-R (average 71.2%). In addition, the mean wing length of PMD females was significantly longer than PMD-R females.

Exp. 3: Longevity

Provided with a 10% sugar solution, the average survival rates of males and females from both strains determined at 30 and 45 days post-emergence were not significantly different (Table 3). However, at day 60 when the experiment was concluded, more PMD-R

Table 1. Mean (±SD) developmental time (days), pupation success, adult emergence success and sex ratio of *Ae. aegypti* permethrin susceptible (PMD) and resistant (PMD-R) strains. Ranges (min–max) are given in parentheses

	PMD	PMD-R	P
L1 to first day of pupation (days)	10.7±0.6 (10-11)	10.0±0.0 (10)	0.374
L1 to 50% pupation (days)	14.3±0.9 (13.6-15.3)	15.2±0.6 (14.5-15.7)	0.249
L1 to pupation success (%)	97.2±1.5 (96.9-98.9)	98.8±1.1 (97.9-100)	0.349
Pupae to emerging adults (%)	98.2±0.6 (97.9-99.0)	97.9±1.1 (96.8-98.9)	0.408
Sex ratio (Male: Female)	165:102		0.007
Sex ratio (Male: Female)		126:159	0.178

Table 2. Mean (±SD) egg production, hatchability and wing length of the *Ae. aegypti* permethrin susceptible (PMD) and resistant (PMD-R) strains. Ranges (min–max) are given in parentheses

	PMD (n=43)	PMD-R (n=37)	P
number of eggs per female	54.2±15.9 (15-82)	54.6±14.5(12-78)	0.910
number of hatching larvae per female	29.1±17.1 (0-59)	38.9±12.6 (7-66)	0.005
wing length of females (mm)	2.85±0.15 (2.53-3.13)	2.74±0.09 (2.53-2.93)	<0.001

Table 3. Longevity of the *Ae. aegypti* permethrin susceptible (PMD) and resistant (PMD-R) strains provided with 10% sugar solution and water. Mean (±SD) and ranges (min–max) are given in parentheses

Day		% survival		P
		PMD	PMD-R	
With sugar				
30:	Male	91.0±3.6 (87-94)	95.0±2.6 (93-98)	0.217
	Female	95.7±3.8 (93-100)	97.7±1.5 (96-99)	0.444
45:	Male	78.7±6.4 (74-86)	89.3±2.5 (87-92)	0.055
	Female	92.7±3.1 (90-96)	95.3±2.1 (93-97)	0.280
60:	Male	66.3±9.1 (58-76)	81.0±4.0 (81-85)	0.063
	Female	82.0±1.0 (81-83)	88.3±2.5 (86-91)	0.015
With water				
3:	Male	99.0±1.0 (98-100)	99.3±0.58 (99-100)	0.643
	Female	98.3±1.2 (97-99)	96.7±5.8 (90-100)	0.649
6:	Male	25.0±16.1 (12-43)	33.7±14.6 (17-44)	0.527
	Female	15.3±8.5 (9-25)	18.0±13.9 (9-34)	0.791
9:	Male	11.7±9.5 (1-19)	2.0±1.7 (0-3)	0.216
	Female	12.0±11.1 (2-24)	3.0±2.0 (1-5)	0.240

females survived. When only water was provided, no significant differences of the average survival rates determined at 3, 6 and 9 days post-emergence were observed. Few individual males and females of both strains died in the first 3 days. About a half of the males and females from both strains died on day 4 and none survived after 12 days.

DISCUSSION

This study compared the reproductive fitness between the permethrin susceptible PMD and resistant PMD-R strains of *Ae. aegypti* in laboratory conditions. Both strains share a close genetic background since they originated from the same area and are resistant to DDT with similar levels of the major metabolic detoxification enzymes (Prapanthadara *et al*, 2002; Somwang *et al.*, 2011). Unfortunately, *Ae. aegypti* populations in Thailand are widely resistant to DDT (Somboon *et al.*, 2003; Ponlawat *et al.*, 2005) and hence a DDT-susceptible strain was unavailable for comparison. Nevertheless, the main difference between the two strains is the presence of the F1534C homozygous mutation in the PMD-R strain which allowed us to determine the relative fitness cost associated with *kdr* gene.

Larval development, pupation success, adult emergence and mating ability were considered normal and not significantly different between both strains. These results suggest that the presence of F1534C homozygous mutation does not have a negative impact on these parameters. Significant differences were observed between the number of females produced (PMD < PMD-R), the mean wing length of females (PMD > PMD-R), the mean number of hatching larvae per female (PMD < PMD-R) and the female survival rate (PMD < PMD-R) at day 60. PMD-R females, as determined by the mean wing length, were smaller than PMD females (Table 2), suggesting that the *kdr* gene may also affect the body size of mosquitoes. Bourguet *et al.* (2004) reported that overproduced acetylcholinesterase *Culex pipiens* showed shorter wing length. Also, Harstone *et al.* (2009) revealed that *Culex quinquefasciatus* mosquitoes resistant to pyrethroid due to elevated P450

were smaller than those of the susceptible strain. Larger female mosquitoes are generally considered to produce more eggs (Clements, 1992), but this was not observed in the present study.

Compared with the Rockefeller strain and the susceptible group of *Ae. aegypti* reported in Martins *et al.* (2010), the mean numbers of eggs and the hatchability of PMD-R and PMD strains were, respectively, about 60-70% and 40-70% lower. Although a direct comparison with these strains cannot be fully made due to different laboratory conditions and genetic background, these results suggest a relatively low reproductive fitness in both PMD and PMD-R strains, with a lesser extent in the latter. Although the amount of ingested blood, which is directly related to the number of eggs deposited, was not investigated, it is likely that insecticide resistant females consumed lower amounts of blood (Li *et al.*, 2002; Belinato *et al.*, 2012; Martins *et al.*, 2010) or required a higher number of blood meals to lay eggs (Okoye *et al.*, 2007).

The reduced egg hatchability in PMD could not be explained due to the reduced mating ability (as reported in Belinato *et al.*, 2012) since the insemination rates and the quantity of spermatozoa in the spermathecae were similar in both strains. The egg hatchability of PMD was 17.5% lower than PMD-R (Table 2). This was almost equal to the difference between the reduced number of females produced by PMD compared to PMD-R (17.6%) (Table 1). We expect that a number of female eggs of PMD were not hatchable or their hatchability was delayed or required re-submerging to hatch, resulting in significantly fewer adult females produced by PMD in Exp. 1. The PMD strain has been maintained in our laboratory for many years without insecticide pressure but the level of DDT resistance was only slightly decreased (unpublished data). By contrast the PMD-R strain has been regularly exposed to permethrin. It is possible that the F1534C mutation is favoured in insecticide treated environments. Thus, the reduced egg hatchability and diverted sex ratio in PMD are likely associated with a fitness cost (Rivero *et al.*, 2010). Relative reductions in the number of eggs laid and hatchability have often been reported in insecticide-resistant *Ae. aegypti* compared against susceptible strains (e.g. Mebrahtu *et al.*, 1997; Kumar *et al.*, 2009; Belinato *et al.*, 2012; Martins *et al.*, 2012). In *An. funestus*, however, Okoye *et al.* (2007) reported that the mean numbers of laid eggs between the pyrethroid resistant and susceptible strains were not significantly different, but the mean number of larvae per female and the mean number of females produced were significantly higher in the resistant strain as found in the present study.

In the experimental conditions of this study, the longevity of PMD and PMD-R adults provided with the sugar solution was almost similar, except for PMD-R females that survived slightly longer at day 60, suggesting again a slightly relatively low fitness of PMD in longevity (Table 3). Hardstone *et al.* (2009) also reported that when provided with sugar *Cx. quinquefasciatus* females resistant to permethrin survived longer than susceptible ones. However, there was no significant difference when they were provided with water as observed in the present study. Over 66% and 82% of male and female mosquitoes, respectively, survived up to 60 days which was slightly longer than *Ae. aegypti* strains in other reports (e.g. Belinato *et al.*, 2012; Martins *et al.*, 2010). This may be explained in part because their mosquito materials were newly selected or introduced whereas our colonies were well-adapted to laboratory conditions for many years. Braks *et al.* (2006) also reported that the medium survival time of laboratory adapted *Ae. aegypti* females was 57.18 days. This high survival, however, cannot reflect the longevity of wild *Ae. aegypti* mosquitoes which usually live for a few weeks (Scott *et al.*, 1997). Therefore, the present study does not necessarily suggest that the F1534C mutation shows greater fitness on longevity than wild population, but suggests that the mutation does not have negative impact on longevity of *Ae. aegypti* adults. Belinato *et al.* (2012) also revealed that the longevity of temephos and deltamethrin resistant *Ae. aegypti* with the V1016I mutation was not significantly different from the Rockefeller strain.

In nature, although female *Ae. aegypti* fed predominantly on humans and seldom fed on plant sugar (Edman *et al.*, 1992; Scott *et al.*, 1993), addition of sugar solution in rearing increased longevity of blood-fed mosquitoes (Braks *et al.*, 2006). The tolerance to starvation, with deprivation of energy resources derived from the immature stages, was evaluated by providing PMD and PMD-R adults with only water. Almost all males and females survived for 3 days without sugar which is considered long enough for the males to mate and for the females to find a blood meal. Paris *et al.* (2011) found no difference in adult starvation tolerance in an *Ae. aegypti* strain selected with *Bacillus thuringiensis* var. *israelensis* toxins. Similarly, Hardstone *et al.* (2009) reported that the median longevity of susceptible and resistant *Cx. quinquefasciatus* mosquitoes with only water was 4 days. However, Agnew *et al.* (2004) reported greater vulnerability to starvation for three *Cx. quinquefasciatus* strains homozygous for the *ester*1, *ester*4 or *Ace-1*R allele, all of which are related to resistance to organophosphate insecticides.

Reductions in reproductive fitness, including the size of insecticide-resistant insects are generally explained due to pleiotropic effects (Rivero *et al.*, 2011), reducing the energy and nutrition available for other biological functions and generating energetic trade-offs between insecticide resistance and key life history traits. However, large variations in fitness or even the absence of fitness costs have been reported, depending on insect species, genetic background and resistance mechanisms. For example, Belinato *et al.* (2012) reported that Brazilian *Ae. aegypti* highly resistant to temephos exhibited decreases in blood meal acceptance, amount of ingested blood, egg production and frequency of inseminated females, whereas the strain with a lower temephos resistance level presented impairment in only blood meal acceptance and frequency of inseminated females. Similarly, Martins *et al.* (2010) revealed that *Ae. aegypti* obtained from wild populations in Brazil, when selected with deltamethrin in the laboratory, displayed numerous fitness costs among the resistant groups including delayed larval development, low pupation, longevity reduction and reduced number of laid eggs and hatchability. In a strain resistant only to temephos, only delayed larval development was observed.

Inbreeding depression can also reduce the fitness of mosquito colonies under laboratory conditions (Munstermann, 1994; Armbruster *et al.*, 2000), but this effect, if any, is considered small in PMD and PMD-R strains because they have been maintained in our laboratory for many years without serious deteriorative signs, e.g. high mortality of immature stages and reduced longevity of adults.

In conclusion, the overall parameters evaluated suggest that the presence of the F1534C homozygous mutation is unlikely to have a serious negative effect on reproductive fitness of mutant *Ae. aegypti* compared to non-mutant populations. This finding is in accordance with the widespread distribution of the F1534C mutation in southeast Asia (so far detected in Thailand, Vietnam, Myanmar and Cambodia) and Grand Cayman (Harris *et al.*, 2010) with high mutant allele frequencies (0.68-0.87) whereas the wild type, like the permethrin-susceptible PMD strain, is found in only <1% of the wild population of *Ae. aegypti* in Thailand (Yanola *et al.*, 2011). More recently in 2012, examination of *Ae. aegypti* larvae collected from several containers from Lahore, Pakistan, where pyrethroids have been heavily used for dengue control, revealed 100% (n = 23) were 1534C homozygous mutant (unpublished data). In addition, previous reports have revealed that another *kdr* mutation (V1016I) has spread rapidly among field populations of *Ae. aegypti* in Brazil and Mexico (Garcia *et al.*, 2009; Martins *et al.*, 2009). These studies suggest that current insecticide-based control programmes may not be able to reduce *kdr* alleles in wild populations. With the relaxation of insecticide selection pressure, however, Chang *et al.* (2012) demonstrated in the laboratory that the *kdr* allele frequencies (V1016G and D1763Y) in permethrin resistant *Ae. aegypti* declined to about 20% after 15 generations. Whether this reversal would occur for F1534C and other

point mutations in the laboratory or in the field requires further study.

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A high resolution melting real time PCR for mapping of filaria infection in domestic cats living in brugian filariosis-endemic areas

Sirichit Wongkamchai[a,*], Hathai Nochote[a,c], Suporn Foongladda[b], Paron Dekumyoy[c], Suvit Thammapalo[d], John J. Boitano[a], Wej Choochote[e]

[a] Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[b] Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[c] Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
[d] The Office of Disease Prevention and Control, 12 Songkhla, Ministry of Public Health, Thailand
[e] Department of Parasitology, Faculty of Medicine, Chiangmai University, Chiangmai, Thailand

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ABSTRACT

We present here a real time PCR with high resolution melting (HRM) analysis for determining the prevalence and distribution of filarial species in domestic cats residing in brugian filariosis endemic areas of Narathiwat province, Thailand. Filarial species can be clearly distinguished in a single well using a single pair of primers. Blood samples were taken from a total of 2039 domestic cats living in endemic areas. Microfilariae were detected in 5.7% of the sample, while the overall prevalence of filaria infection by HRM analysis was 6.6%. The filariae species found in the infected cats were *Brugia malayi*, *Dirofilaria immitis*, *D. repens* as well as *Acanthocheilonema* (*Dipetalonema*) *reconditum*. This is the first report of *A. reconditum* infection from Thailand. The study also observed an overlapping of the distribution areas of animal and human filariae. From a public health perspective, the distribution and prevalence of these nematodes warrant an appropriate drug-based prophylaxis to be administered to cats in the endemic areas to reduce the number of diseased carriers. Furthermore, this molecular approach is more sensitive than microfilariae detection, enables species identification and greatly facilitates the collection of epidemiological data. Thus, the present study may help to bridge human–animal interface by coordinating research outcomes with the control of zoonoses that is vitally important for human and veterinary public health.

1. Introduction

In Thailand, endemic areas of brugian filariosis, caused by nocturnally subperiodic *Brugia malayi*, are located in the southern part of the country (Zielke et al., 1993). Several wild and domestic animals in endemic areas of Thailand, Indonesia, Malaysia, and Pacific island have been reported to be naturally infected with subperiodic *B. malayi* (Mak et al., 1982; Mak, 1984; Zielke et al., 1993). In domestic cats from Indonesia, two studies recorded infectivity rates at 7% (Masbar et al., 1981) and 6.1% (Palmeiri et al., 1985) while in Peninsular of Malaysia, the infectivity rate was 6.9% (Mak et al., 1980).

A survey of 66 domestic cats and 98 stray dogs in brugian filariosis endemic area in Chumphon province, southern Thailand, *B. malayi-like* microfilariae were observed in one cat while *Dirofilaria repens* and *D. immitis* were found in 2 cats and 34 dogs, respectively (Guptavanij et al., 1971a).

* Corresponding author. Tel.: +66 24196468; fax: +66 24112084.
E-mail address: sirichit.won@mahidol.ac.th (S. Wongkamchai).

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Further south in Narathiwat province, a blood sample from 93 cats showed the presence of *B. malayi*-like mf with an infectivity rate of 4.3% (Guptavanij et al., 1971b).

Brugian filariosis in Narathiwat province appears to be a particular problem of the eastern part of the province which includes Mueang, Bacho, Ra-ngae, Su-ngai Kolok, Su-ngai Padi, Tak Bai and Joh Ai Rong Districts. It is speculated that the prevalence of brugian filariosis may be due to the presence of a large peat-swamp forest (Phru Toh Daeng) encompassing approximately 303 km^2 (Chaipattana Network, 1996) which may serve as a breeding place for *Mansonia*, the main vector of the nocturnally subperiodic *B. malayi* (Sucharit et al., 1975). The majority of the households in the endemic areas prefer cats as a family pet, thereby facilitating a ready opportunity for filarial transmission with humans due to co-habitation. Human infectivity with filariae of animals, referred to as zoonotic filariasis, occurs worldwide. Since the first report of zoonotic filariasis in the modern literature occurred more than 100 years ago (Babes, 1880; Addario, 1885), the numbers of cases and parasitic species has steadily increased (Orihel and Eberhard, 1998).

This emphasizes the urgent need to focus on these reservoir/infective hosts. However, the current state of knowledge about the filarial species, their prevalence and distribution in domestic cats living in acknowledged endemic areas is obviously limited due to the marked morphological similarities of these parasites (Harbut et al., 1995). In the case of *B. malayi* and *B. pahangi*, it is extremely difficult to differentiate the microfilaria of filarial species by Giemsa staining (Yen and Mak, 1978).

Diagnosis is essential in the management of disease, both at the level of individual animals and at the level of disease control in populations. Either microscopic detection or serodiagnosis that are widely used for diagnostic purposes, confer little guarantee of species identification in areas where both the human and animal filarial species coexist. The application of molecular technology could have a marked impact in distinguishing closely related species or subspecies.

We present here a real time PCR with high resolution melting (HRM) analysis for the screening of the status of endemicity in the acknowledged endemic areas. Filarial species can be clearly distinguished in a single well using a single pair of primers. Moreover, a large number of cat samples can be rapidly performed in a single run. This method offers the advantage of detecting at least 5 species of the filarial parasites in a single step PCR, which, in turn, supports the continued prophylactic treatment of the infected cats as well as the current state of knowledge about the extent and frequency of occurrence of the filarial species in domestic cats staying in recognized endemic areas.

2. Materials and methods

2.1. Animal ethical approval

The study protocol was approved by the Animal Ethics Committee of Faculty of Medicine Siriraj Hospital, Mahidol University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand. The Certificate of Approval number is 004/2555.

2.2. Source of positive and negative control

The microfilariae (mf) of *B. malayi*, *B. pahangi*, *D. immitis*, *D. repens* and *Acanthocheilonema* (*Dipetalonema*) *reconditum* whose species were confirmed by the acid phosphatase staining method and HRM analysis, were used as positive controls. A parasite-free cat blood sample served as the negative control.

2.3. Study areas

This study surveyed filarial infection in the occasional feral cat and mostly domestic cats residing in households located in the brugian filariosis endemic areas of Narathiwat province. This data was obtained from the Filariasis Project, Pikhunthong Royal Development Study Center, Narathiwart province, Thailand. A total of 2039 domestic cats were randomly selected from 44 villages surrounding the central peat swamp forest, located in 4 districts, i.e. Su-ngai Kolok, Su-ngai Padi, Tak Bai and Mueng.

2.4. Blood samples

Using an ear-pricking procedure, blood samples were collected from the study population. Three thick blood smear slides were prepared from each cat. One slide was used in Giemsa staining, a second slide was used for DNA extraction and the last slide was used as a spare. The individual characteristics of each cat, including name, owner's name and address, date of extraction, gender and age were cataloged and entered into a standard record form.

Study samples consisted of stray and domestic cats from 8 sub-districts; i.e. 377 from Kaluwor Nuea, 458 from Kaluwor, 73 from Phraiwan, 293 from Bang Khuntong, 218 from Kosit, 310 from Sugnai Padi, 205 from Puyo and 105 from Prasemat.

2.5. Microscopic detection of microfilariae in the study samples using Giemsa staining

Giemsa staining was performed according to the standard WHO procedure (World Health Organization, 1991). Briefly, the dehaemoglobinized thick blood smear slides were immersed in freshly prepared working Giemsa stain for 45–60 min. Then, it was removed and rinsed by dipping 3–4 times in the Giemsa buffer. After air-drying, the slides were examined under a microscope (40×) for the detection of mf.

2.6. Extraction of filarial DNA from thick blood smear slides

Filarial DNA was extracted from the samples as follows: 100 μL of Tris–EDTA buffer solution was added onto a dehaemoglobinized thick blood smear slide and left for 5 min. Then the blood was scraped off the smear, transferred into a 1.5 mL microcentrifuge tube, and was centrifuged for 10 min at 15,520 × *g*. The Tris–EDTA buffer

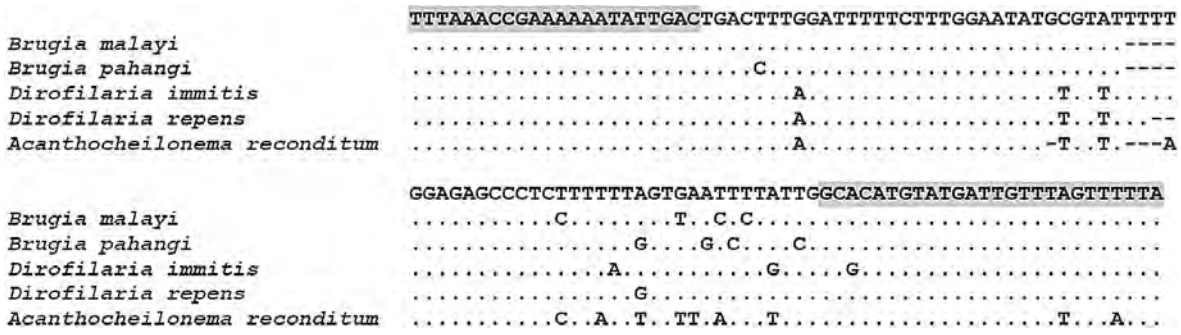

Fig. 1. Alignment of partial mitochondrial 12S rRNA gene sequences of *B. malayi* (GenBank accession number AJ544843; positions 177–287), *B. pahangi* (GenBank accession number AM779851; positions 154–264), *D. immitis* (GenBank accession number FN391554, positions 161–275), *D. repens* (GenBank accession number AM779776; position 145–258), and *A. reconditum* (GenBank accession number AJ544853, position 180–291). Dots indicate identity; dashes indicate deletion from the above consensus sequence; and gray areas indicate primers.

was discarded. The remaining sediment was kept for further DNA extraction. A high pure PCR template preparation kit (Roche Germany) was used for DNA extraction according to the manufacturer's instructions. The concentration of the extracted DNA was measured by the Nanodrop spectrophotometric procedure (Thermo Fisher Scientific) according to the manufacturer's instructions. The extracted DNA was used as a template in HRM real time PCR.

2.7. HRM real time PCR analysis for B. malayi, D. immitis, D. repens and Acanthocheilonema (Dipetalonema) reconditum

Previously designed primers (Wongkamchai et al., 2013) used for identifying the 3 filaria species, i.e. *B. malayi*, *B. pahangi* and *D. immitis* was used in this study. It was found that the sequences of the primers previously designed also recognized a 115-bp region of the mitochondrial partial 12S rRNA gene of *D. repens* and *A.* (*Dipetalonema*) *reconditum*. A BLAST search (www.ncbi.nlm.nih.gov/BLAST/) was performed to check the specificity of these primers to their respective DNA targets. The presence of the 115-bp amplification products was verified by 2% agarose gel electrophoresis (data not shown).

Fig. 1 shows the primers that are based on alignments of the mitochondrial partial 12S rRNA genes of 5 Filaria spp., i.e. *B. malayi* (GenBank accession number AJ544843; positions 177–287), *B. pahangi* (GenBank accession number AM779851; positions 154–264), *D. immitis* (GenBank accession number FN391554; positions 161–275), *D. repens* (GenBank accession number AM779776; position 145–258); and *A. reconditum* (GenBank accession number AJ544853; position 180–291).

The HRM were performed in a single run on a LightCycler LC480 instrument (Roche Diagnostics, Penzberg, Germany) as previously described (Wongkamchai et al., 2013). Briefly the 10 μL reaction mixture contained 3 μL of DNA, 0.25 pmol of each primer, and 3 mM $MgCl_2$ in 5 μL of the LightCycler 480 High-Resolution Melting Master mixture containing ResoLight dye (Roche Diagnostics). Nuclease-free water replaced the DNA template for negative controls. Positive controls were included in each run. The reaction conditions included an activation step at 95 °C for 5 min followed by a 40-step amplification of 10 s at 95 °C, 10 s at 58 °C, and 10 s at 72 °C. Subsequently, the products were heated to 95.8 °C for 1 min and then cooled to 40 °C for 1 min. HRM was performed from 65 °C to 95 °C, rising at 1 °C/s with 25 acquisitions per degree. The final cooling step was 40 °C for 10 s.

2.8. HRM real time PCR analysis for D. repens

Since the two species of *Dirofilaria* (*D. immitis* and *D. repens*) gave little different melting temperature (Tms) (<0.5 °C), they had to be further distinguished using a COXI gene primer pair designed by Rishniw et al. (2006).

3. Results

Of the total number of cats surveyed over the 4 districts, the gender comparison was significant ($p < 0.01$) as 58% were females and 42% were males. The number of younger cats between 1 and 3 years of age were significantly ($p < 0.000$) more prevalent (85%) than all the other cats combined. Young cats were evenly distributed over all the 4 districts with percentages ranging from an 80% to 89%.

The HRM result of the amplified product of the positive controls (Bm, *B. malayi*; Bp, *B. pahangi*; Di, *D. immitis*, Dr, *D. repens* and Ar, *A. reconditum*) and the study samples, as well as the HRM results of mix infection are shown in Fig. 2(A–C). Using the pair of primers which amplified the partial mitochondrial 12S rRNA gene of the filariae, the amplified products of the positive controls (Bm, *B. malayi*; Bp, *B. pahangi*; Di, *D. immitis*, Dr, *D. repens* and Ar, *A. reconditum*) obtained from the LightCycler 480 software, were recognized by the HRM assay at melting peaks (Tms) of 75.8 ± 0.3 °C, 77.46 ± 0.24 °C, 74.20 ± 0.42 °C, 74.10 ± 0.28 °C and 72 ± 0.25 °C, respectively. In the mix infection, two separate fluorescence peaks at different melting temperatures of species and heteroduplex melting peaks were observed at 68.5 °C and 71 °C (Fig. 2).

The DNA extract from 59 cat samples recognized by the HRM assay at melting peaks (Tms) of 74.20 ± 0.42 °C or 74.10 ± 0.28 °C, when using the pair of primers which amplified partial mitochondrial 12S rRNA gene of the filariae, were furthered amplified using the primer specific for *D. repens* COX I gene. The amplified product of the *D. repens* positive control was recognized by the HRM assay

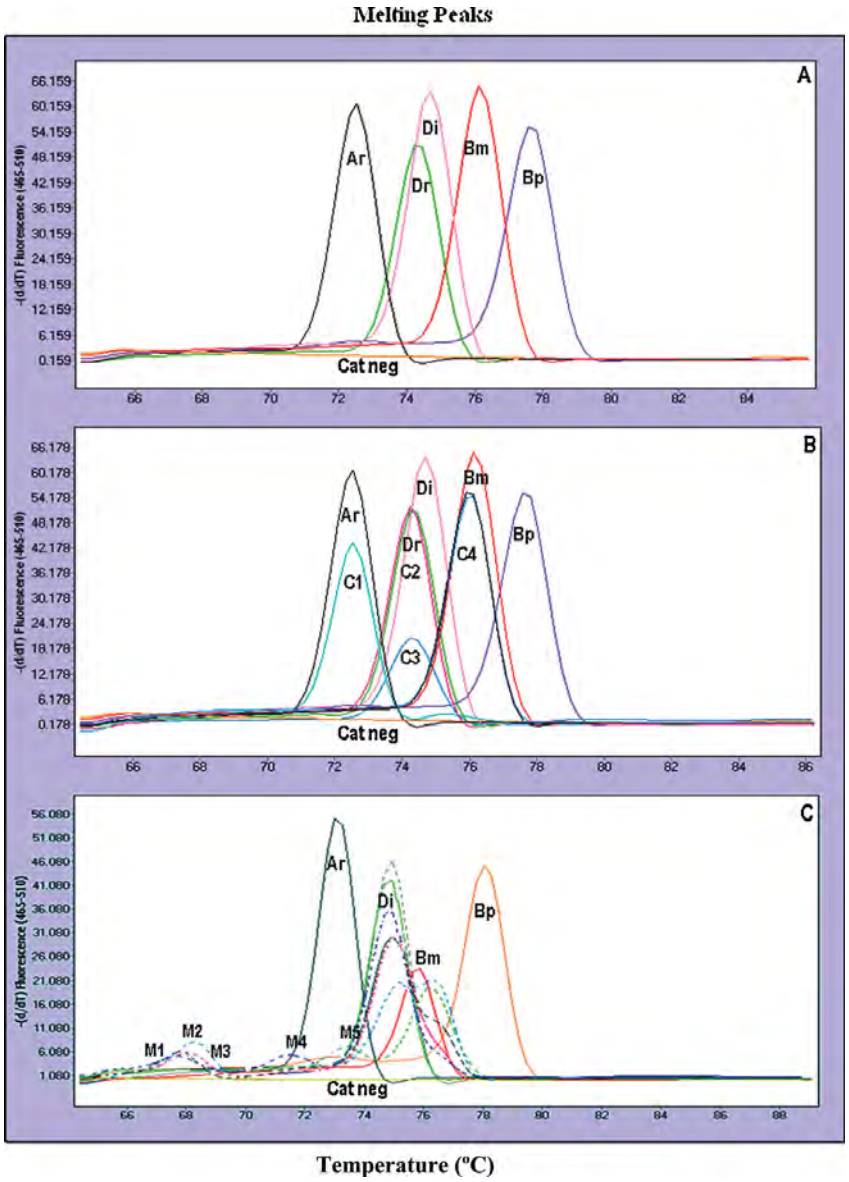

Fig. 2. Melting peaks (A) of the amplified product of the positive controls (Bm, *B. malayi*; Bp, *B. pahangi*; Di, *D. immitis*, Dr, *D. repens* and Ar, *A. reconditum*) as obtained from the LightCycler 480 software; (B) melting peaks of the HRM results of the study cat samples (C1–C4), and control; (C) melting peaks of the HRM results of mix infection (M1–M5, dotted lines). Two separate fluorescence peaks at different melting temperatures of species and heteroduplex melting peaks are present at 68.5 °C and 71 °C.

Table 1
The prevalence of filaria species identified in domestic cats in Narathiwat province, Thailand by HRM real time PCR and microscopy.

Species detected by HRM analysis	Microfilaria detection by microscopy (Giemsa staining)						HRM detection
	Brugia spp.	*D. immitis*	*D. repens*	*A. reconditum*	*Brugia* spp. and *D. immitis*	Mf negative	
B. malayi	57	0	0	0	0	10	67 (3.3%)
D. immitis	0	41	0	0	0	6	47 (2.3%)
D. repens	0	0	12	0	0	0	12 (0.6%)
A. reconditum	0	0	0	2	0	1	3 (0.15%)
B. malayi and *D. immitis*	0	0	0	0	4	1	5 (0.25%)
Negative	0	0	0	0	0	1905	1905 (93.43%)
Total	57 (2.8%)	41 (2%)	12 (0.59%)	2 (0.098%)	4 (0.19%)	1923 (94.3%)	2039 (100%)

Mf, microfilaria.

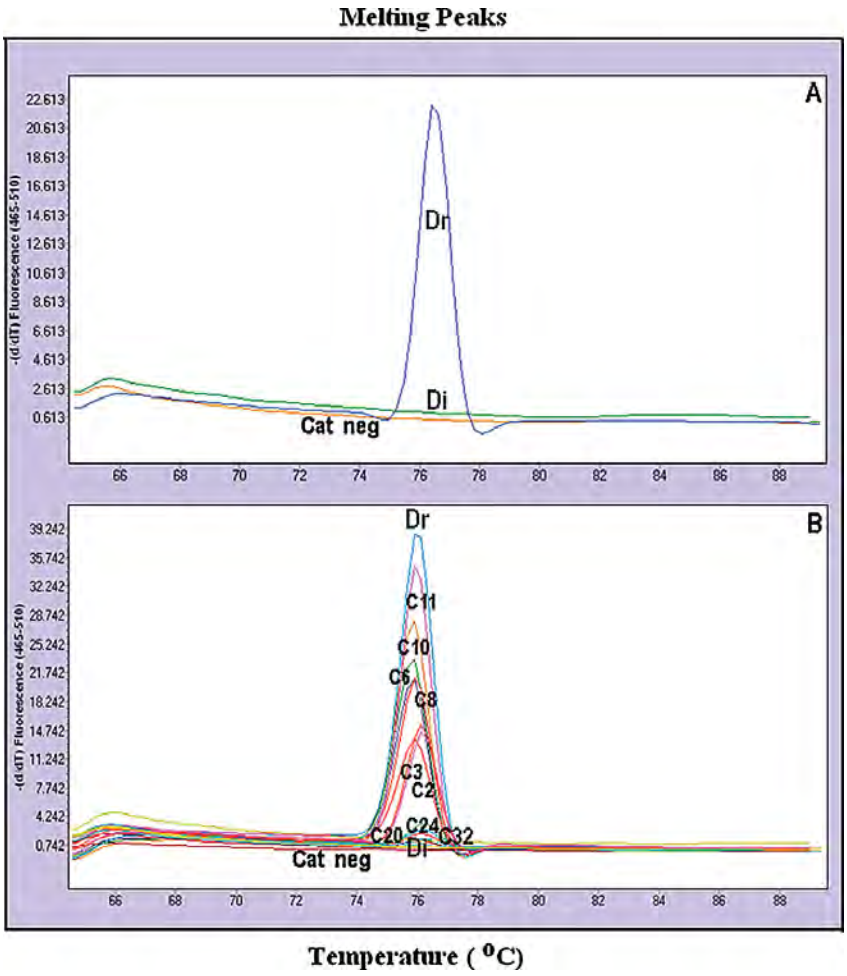

Fig. 3. Melting peaks (A) of the amplified product of the positive controls (Dr, *D. repens*) as obtained from the LightCycler 480 software using COX I gene primer; no amplicons were obtained when using the DNA of Di, *D. immitis*; (B) melting peaks of the HRM results of the study cat samples identified as *D. repens* (C2, C3, C6, C8, C10 and C11); no amplicons were obtained for the DNA of *D. immitis* (C20, C24, and C32).

at melting peak (Tms) of 75.98 + 0.3 °C, whereas the results were negative when tested with *D. immitis* or with negative cat blood samples (Fig. 3). Twelve out of the 59 samples were positive for *D. repens* (Table 1).

The prevalence of Filaria species identified in domestic cats in Narathiwat province, by HRM real time PCR and microscopy are summarized in Table 1. The overall prevalence of Filaria infection was 6.6% by HRM analysis, whereas microfilariae were detected via Giemsa staining in only 5.7%. Eighteen positive cats by HRM were found to be negative by Giemsa staining whereas no microfilariae positive cats which found to be negative by HRM. Thus, it is suggested that HRM-PCR analysis showed more positive rates, and therefore, may be considered a more sensitive measure. *B. malayi* was the most dominant filarial species detected in the infected cats, followed by *D. immitis*. The *B. malayi* infected cats were distributed in 3 districts indicated as high risk areas for brugian filariosis, i.e. Sugaipadi, Sugai kolok and Takbai (Fig. 4).

The map of Narathiwat province depicts the location of the study sites and the distribution of cats infected with Filarial parasites in each of the sub-districts (Fig. 4). The overall prevalence of filarial infection in study cats in each sub-district 0.8% (3/377); 6.3% (29/458); 15% (11/73); 3.1% (9/293); 9.2% (20/218); 15.8% (49/310); 7.3% (15/205) and 0% (0/105) in Kaluwor Nuea, Kaluwor, Phraiwan, Bang Khuntong, Kosit, Sugnai Padi, Puyo and Prasemat, respectively. This results in an overall prevalence rate of 6.6% (136/2039).

4. Discussion

In the present study, filarial infected cats were found to be clustered in households. Both human and animal Filariae were detected and their distribution areas were found to overlap. The highest prevalence of *B. malayi* infected cats (3.3%) was reported from villages surrounding the Phru Toh Daeng swamp forest, which coincided with the distribution of *B. malayi* infected people at the village level. The animal filaria, *D. immitis*, was the predominant filaria species detected in domestic cats inhabiting North Kaluvor in the Mueng district, which is an urban area of this province. Previous research has observed that *Culex* spp., the main vector of *Dirofilaria* is a common mosquito species in urban areas since *Culex* "thrives and proliferates excessively in crowed city areas" (Simonsen and Mwakitalu, 2013, p. 35). It is noted that the cats surveyed from Tak Bai district were found to be infected with all species of animal and human filariae identified in this study. This can be explained by the location of this district. Tak-Bai is located between

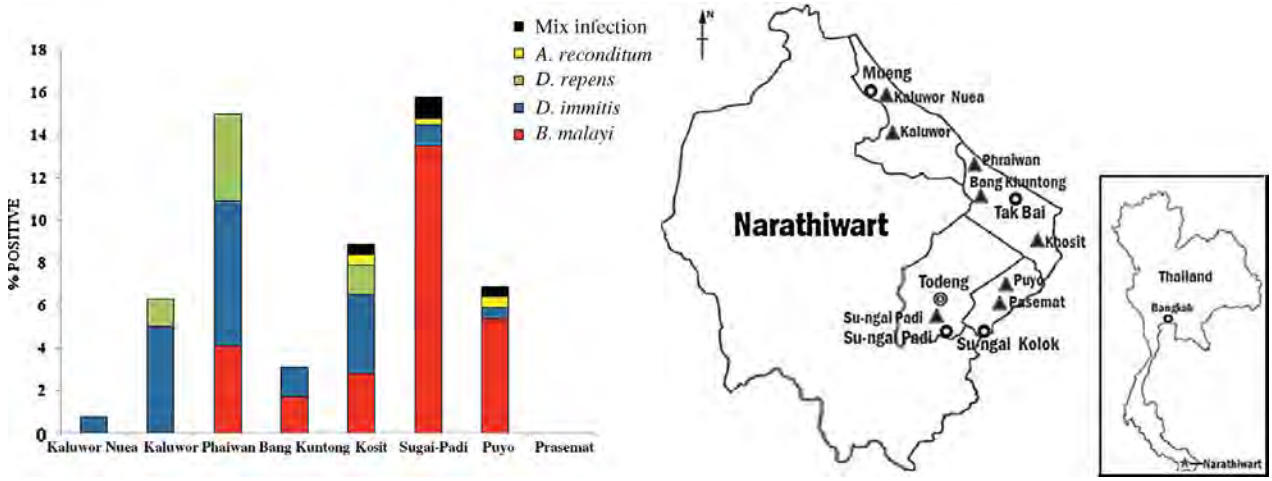

Fig. 4. The map of Narathiwat province showing the location of the study sites; areas outlined in rectangles indicate sampling locations; the circle indicate study districts; double circle indicate Todeng swamp forest and graph shows distribution of cats infected with Filarial parasites (% positive = number of filarial infected cats/number of study samples of each sub-district).

the Mueang district where animal filariae are predominant and the Su-gnai Padi district where *B. malayi* is the major filarial species. This finding suggests that the transmission cycle of the filaria has the potential to generate infection in (a) active endemic areas, (b) in adjacent areas due to movements of microfilaraemic cats between the two districts and (c) through an inadequate control of animal reservoir hosts. Moreover, no *B. pahangi* infected cats were identified in the present survey suggesting the absence of host-resources for this parasite in this province.

A. reconditum was also identified, which to our knowledge is the first reported incidence in Thailand. *A. reconditum* is mostly specific to canines and its infective stage found in the subcutis of intermediate hosts as fleas, ticks and chewing lice (Albrechtová et al., 2011; Pantchev et al., 2011). Usually *A. reconditum* is non-pathogenic but Hargis found a filariasis evidently due to an *Acanthocheilonema*-like parasite in 10 dogs exhibiting plaques and pruritic papules concurrent with scarring, alopecia, erythema in the head neck and shoulders region (Hargis et al., 1999), thus, emphasizing the need to treat *A. reconditum* infected cats as well as to eradicate fleas, ticks and chewing lice.

By using a molecular technique, the prevalence of filarial infection obtained from the present study was greater than the prevalence of microfilaria detected by the microscopic procedure. A similar observation has been demonstrated by Albrechtová et al. (2011). The reason may due to the ability of the real time PCR to capture positive cases even amongst those individuals diagnosed as amicrofilariaemic in which the parasitological method cannot help identify the parasite. Rawlings et al. (1982) enumerated four different types of occult infections: (a) infection with adult filarial worm in the absent of circulating microfilariae, i.e. prepatency which is evident up to 6 months after infection; (b) unisexual infections; (c) drug-induced sterility of adult worms; a condition which could be due to macrocyclic lactone or doxycycline treatment; or (d) an immune-mediated clearance of microfilariae by means of antibody-dependent cellular cytotoxicity (Gray and Lawrence, 2002). DNA released from adults or remnants of dead microfilaria present in the blood may potentially lead to a PCR-positive diagnosis even before or after the stage of microfilariaemia (Albrechtová et al., 2011).

In the samples studied here, mix infections were detected. Two separate fluorescence peaks at different melting temperatures of species and heteroduplex melting peaks were present as shown in Fig. 2C. However, mix infections of the two species of *Dirofilaria* (*D. immitis* and *D. repens*) may not be detected by the chosen primer due to the small temperature differential (Tm < 0.5 °C) of the HRM peaks.

Surveys of filarial infections in animals using blood smear staining methods might mistakenly lead to incorrect diagnoses due to the similarity of the morphology of microfilariae of filariae and when fatigue occurs due to the huge number of slides to be examined under the microscope. In 1995, a survey of filarial infection in cats in Narathiwat province reported that 4.13% (104 of 2515) cats were positive for *B. malayi*-like microfilariae (Phantana et al., 1995). In 2007, another survey of cats, dogs and monkeys from Su-ngai Padi and Su-ngai Kolok, in Narathiwat province reported that *B. malayi*-like microfilariae were detected in 4.05% (10/247) of cats, 3.1% (4/129) of monkeys and mixed infections of *B. malayi*-like and *D. immitis* in 1.05% (3/286) dogs. There were less species of microfilariae detected in these surveys compared to the present study which was undoubtedly due to reliance on morphological (microscopic) assessment only. The unavailability of reliable techniques has hampered epidemiologic surveys that attempt to ascertain the true prevalence of these zoonotic infections. Subsequently, prophylaxis practices of the Filariasis Division, Ministry of Public Health of Thailand which treating all *Brugia* spp. positive cats with a single dose of 100 μg/kg of Ivermectin by subcutaneous injection (Nuchprayoon et al., 2006). If recruitment of the infected cats is based on the microfilarae detection, a number of infected cats with densities of microfilariae below microscopic detection limit may be missing.

The real time PCR with HRM analysis is much more sensitive than blood smear methods for identifying, especially, the microfilarial carriers with low parasite densities.

Moreover, this procedure can be performed in a single tube (or well) using a single pair of primers without the necessity of specific probes. The assay can be processed rapidly with large sets, such as 96 or 384 samples, in a single run of the PCR procedure. The cost effectiveness of HRM is comparable to or cheaper than conventional PCR (Zhou et al., 2005; Ririe et al., 1997). In addition, by using a single primer pair, in a single well, the assay can identify the PCR product by differentiation of the melting peak based on 3–5 different bases in the amplicon. Each double-stranded DNA (dsDNA) fragment has its characteristic melting behavior, which depends mainly on its GC content, length, and sequence compositions (Dobrowolski et al., 2009). Thus, the filaria species can be clearly distinguished by the different melting temperatures of each species dsDNA. Only *D. immitis* and *D. repens* produce very similar Tms and have to be distinguished using additional primers.

Beside human filarial worms, human also reported to be infected with zoonotic filariae and the number of cases has steadily increased worldwide (Orihel and Eberhard, 1998). Since the first report of dirofilariasis in Hungary in 1879 (Babes, 1880), prevalence of human dirofilariasis is increasing (Kramer et al., 2007). Numerous human dirofilariasis (pulmonary, subcutaneous and ocular cases) have been reported from the European Union, Russia, Sri Lanka, Taiwan, north and south Americas (Orihel and Eberhard, 1998; Tsung and Liu, 2003; Kramer et al., 2007; Genehi et al., 2011; Simón et al., 2012).

The other zoonotic filariae are *Brugia* spp. which dwells in the lymph system. Thailand started a program to eliminate lymphatic filariasis in the year 2003. Annually mass drug treatment had been given to people living in the endemic areas. At present, Thailand is move to the post-MDA surveillance and certification process for elimination (World Health Organization, 2010). The fact that endemic diseases could re-emerge at any time, even in thoroughly controlled endemic regions, where the environmental factors provide suitable conditions for the re-emergence of the transmission-cycle, emphasizes the urgent need to focus on controlling of filarial infection in reservoir hosts.

In conclusion, this study can be used to bridge this human–animal interface by linking research knowledge to the control of zoonoses of human public health and veterinary public health importance. These findings might help in the determination of the public health value of its control efforts in targeting feral and domestic cats important for the successful prevention and control of filariasis in the nocturnally subperiodic *B. malayi* endemic areas. A large number of cat blood samples can be handled in a relatively short time by the real time PCR with HRM analysis. Moreover, the method offers greater sensitivity and validity in species identification than microscopic analysis of Giemsa stained mf slides. This eventually will facilitate the distribution and determination of filarial disease in specified populations, which in turn, supports a continued prophylactic treatment of infected cats in endemic areas.

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Chapter 2

Systematic Techniques for the Recognition of *Anopheles* Species Complexes

Wej Choochote and Atiporn Saeung

Additional information is available at the end of the chapter

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1. Introduction

Throughout the world, 528 species of *Anopheles* mosquitoes have been discovered, and approximately 80 of them play an important role as vectors of malaria, filarial nematode and encephalitis virus. Among these, at least 20 taxa represent species complexes, which comprise about 115 sibling species members. The existence of species complexes in *Anopheles* vectors leads to difficulty in precisely identifying sibling species (isomorphic species) and/or subspecies (morphologically/cytologically polymorphic races) members that possess identical morphology or minimal morphological distinction. In addition, those members may differ in biological characteristics (e.g., microhabitats, resting and biting behavior, sensitivity or resistance to insecticides, susceptible or refractory to malaria parasites, etc.), which can be used to determine their potential for transmitting disease agents. Incorrect identification of individual members in *Anopheles* species complexes may result in failure to distinguish between a vector and non-vector, and lead to complications and/or unsuccessful vector control [1-5].

So far, at least 1 and 2 traditional techniques have been used widely for the recognition of sibling species and/or subspecies members at post- and pre-mating barriers. For post-mating barriers; the hybridization or crossing experiment, using the artificial mating technique to determine hybrid non-viability, sterility or breakdown, is still a useful tool for recognizing *Anopheles* species complexes. Detailed genetic incompatibility, including lack of insemination, embryonation, hatchability, larval survival, pupation, emergence, adult sex distortion, abnormal reproductive system and complete or incomplete (some cases only at the inversion heterozygote regions) asynaptic salivary gland polytene chromosomes are useful criteria for elucidating sibling species and subspecies status. However, a point worth noting is that an isofemale line (isoline) colony established from the combinative characters of morphological and/or cytological markers has to be considered seriously. A laboratory raised colony established

from a naturally mixed population should be omitted, since it may be a mixture of cryptic species [6-10]. In addition, many *Anopheles* species do not reproduce in captivity. As for pre-mating barriers; examination of the polytene chromosomes in wild-caught adult females, and/or progenies of iso-female lines, provides clear evidence that different specific mate recognition systems (SMRS) exist. The total absence or significantly deficient number of heterozygotes for an inversion in a sympatric population entirely indicates the presence of reproductive isolation within a taxon [10-12]. Nonetheless, at least 4 problems have been raised regarding this matter, i.e., (1) a skilled person is needed to prepare a perfect chromosome and make an identification, (2) homosequential banding species cannot be employed, e.g., *An. maculipennis* complex [13] and *An. barbirostris* complex [14-17], (3) a relatively large amount of sample materials are required to perform the Hardy-Weinberg equilibrium, which cannot be applied to small numbers of rare species specimens that are caught during specific seasons, and (4) it cannot be performed in allopatric anopheline populations. Electrophoretic variations at enzyme loci are not only useful for identification of sibling species, but also for the correct identification of morphologically cryptic *Anopheles* species. Variations at a locus thus enable detection of reproductive isolation within populations, resulting from positive assortative (preferential) mating [10-11, 18]. Nevertheless, at least 2 problems have been raised regarding this technique, i.e., (1) specimens must be fresh or frozen until analysis, and (2) its use must be similar to that of the polytene chromosome, as it requires a relatively large amount of sample materials to perform the Hardy-Weinberg equilibrium and cannot be performed in allopatric anopheline populations, as previously described.

Regarding the modernized technique; molecular investigation of some specific genomic markers, e.g., ribosomal DNA (ITS2, D2, D3, IGS) and mitochondrial DNA (COI, COII, Cyt b, ND5), has been used extensively as a tool to characterize and/or diagnose cryptic members in the intra-taxa of *Anopheles* mosquitoes, and the advantage of this PCR-based technique is that few nanograms of DNA are required from preserved specimens [19]. Nonetheless, controversy arose when only comparative DNA sequence analyses of some specific genomic regions were used as first hand criteria to differentiate between the status of specific species, sibling species and subspecies within the taxon *Anopheles*. For example, based on a comparison of the D3 domain of 28S (28S-D3), *An. fluviatilis* S has been considered as synonymous to the *An. minimus* species C [20-22]. However, subsequent investigation of the conspecificity of these two species, based on ITS2 and D2-D3 domains of 28S rDNA regions, suggests that *An. fluviatilis* S and *An. minimus* C, do not deserve to be synonymous [23]. Similar results were also obtained in the determining on specific species status between *An. lesteri* and *An. paraliae* [unpublished data]. The comparative DNA sequence analyses between *An. lesteri* strain from Korea and *An. paraliae* strain from Thailand revealed low pairwise genetic distance for COI (0.007-0.017) and COII (0.008-0.011) regions with 4-9 and 5-7 base substitutions, respectively, whereas a considerable genetic distance (0.040) was obtained in ITS2 region with 16 base substitutions. Supportively, the phylogenetic trees demonstrated that these two species were separated from each other with a 74-100% bootstrap value for 3 regions. It was interesting to note that *An. lesteri* and *An. paraliae* were distinguished appreciably by DNA sequence data, however, were confirmed to be genetically compatible by the crossing experiments. Remarkably, prior to reaching a definite conclusion of specific species, sibling species and subspecies

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status within the taxon *Anopheles*, crossing experiments need to be carried out intensively using iso-female lines established from sympatric and/or allopatric populations, which relate to morphological variants, cytogenetic forms and/or comparative DNA sequence analyses of some specific regions.

2. Formation of robust systematic procedures

In light of the advantages and disadvantages of the techniques mentioned above, 3 techniques, i.e., the crossing experiment, molecular investigation and cytogenetic markers (characteristics of metaphase karyotypes) were selected, and they formed the robust systematic procedures for the recognition of *Anopheles* species complexes [24] (Figure 1).

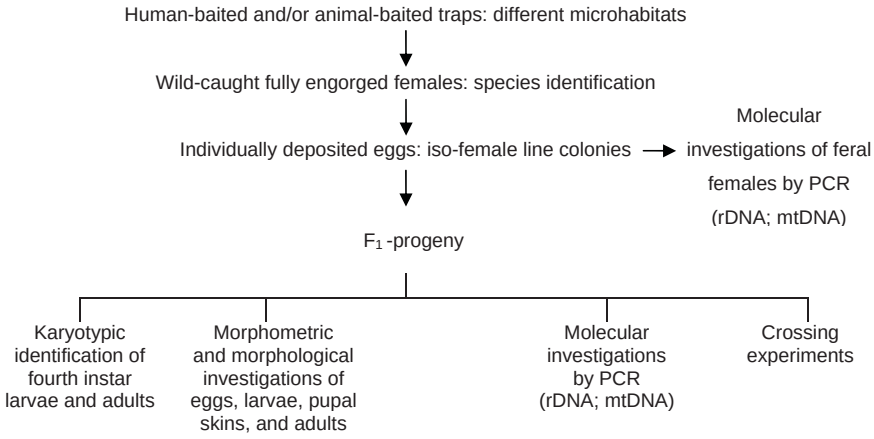

Figure 1. Summarized flow chart for robust systematic procedures

By following the flow chart: (1) try to collect anopheline mosquitoes that are distinct in their behavior (e.g., biting humans or animals with relation to different microhabitats and/or locations), (2) try to record morphological variation(s) as far as possible during the species identification process of wild-caught females, (3) establish an iso-female line colony by allowing gravid females to lay eggs individually, (4) conduct molecular investigation of laid-egg feral females to obtained a robust DNA marker, with this step usually taking about 1 week. Since development of the F_1-progeny usually takes about 2 weeks from first instar larvae to adults, the metaphase karyotype investigation of fourth instar larvae, newly emerged adult females and males is performed in order to (5) obtain a cytogenetic marker (karyotypic form), (6) if molecular investigation fails in the step of laid-egg feral female it will be performed in F_1-progeny, (7) carry out morphometric and morphological investigations of eggs, larvae, pupal skins and adults to confirm precise species identification, and (8) perform the important

step of crossing experiments among iso-female line colonies by using a karyotypic marker (or form) related to a DNA marker (large sequence divergence or very low intraspecific sequence variation) of each iso-female line colony.

Regarding techniques necessary for success in operating robust systematic procedure: 3 important techniques were developed by the authors, and they have been proven as efficient and necessary for the robust systematic recognition of sibling species and/or subspecies members within the taxon *Anopheles* species complex. They are: (1) the establishment of a healthy iso-female line colony that is the backbone of population-genetic study on *Anopheles* vectors, since it provides healthy larval and adult progenies for preparation of attractive metaphase and salivary gland polytene chromosomes, and potent adults for crossing experiments. The inability to establish a healthy iso-female line colony that can be colonized for many consecutive generations is the principle cause of failure in a population-genetic study of *Anopheles* vectors, (2) the technique for metaphase chromosome preparations in adult females and males by intrathoracic inoculation [25] and that for fourth instar larval brains [14] using extracted solution derived from dried seeds and rhizomes of a decoration plant (*Gloriosa superba* L.), instead of synthetic colchicine solution, and (3) modified technique for salivary gland polytene chromosome preparations in fourth instar larvae [26]. Detailed and important procedures regarding the 3 techniques are as follows:

3. Techniques for establishment of a healthy iso-female line colony of difficult-to-rear anophelines

An iso-female line colony of *An. campestris*-like Form E, Thai strain [14] was established from 1 wild-caught fully engorged adult female collected from a human-baited trap reared successfully under laboratory conditions for 98 consecutive generations and used as a role model for other fresh-water breeding anopheline species.

4. Procedures

4.1. Transportation of wild-caught anophelines

Wild-caught fully engorged adult females collected from human- and/or animal-baited traps in the field were kept in a plastic cup (8.5 cm in diameter and 11 cm in depth, lined inside with filter paper), with a pad of cotton wool soaked with 10% sucrose solution placed on top of the covering screen. It was covered with a translucent plastic bag in order to keep humid conditions in the cup and delay rapid drying of the soaked cotton wool (Figure 2a). It was stored in a humid chamber using a picnic foam-box (18 x 26 x 39 cm) to maintain humidity and temperature (Figure 2b). Then it was transported to the insectarium for colonization and biological studies. All of the experiments were performed in the insectarium at 27±2 ºC, 70-80% relative humidity, and illumination from a combination of natural daylight from a glass-window and fluorescent lighting was provided for approximately 12 hours a day.

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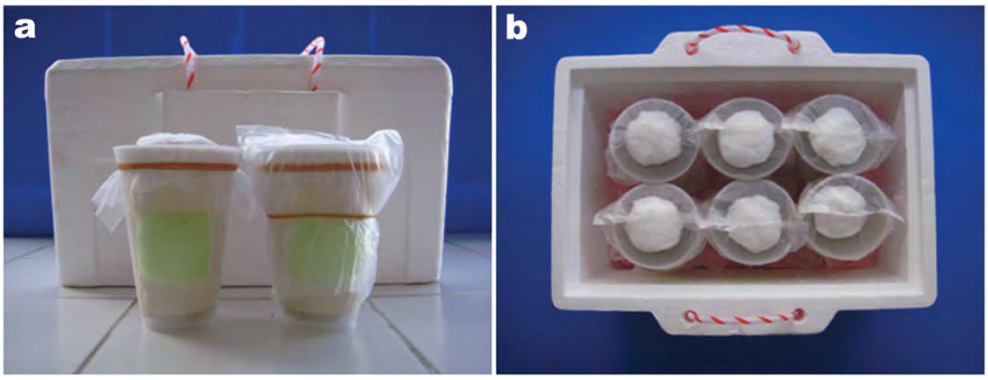

Figure 2. (a) A screen-topped plastic cup with a pad of soaked cotton wool placed on top of the covering screen (left), covered with a translucent plastic bag (right), and a humid chamber derived from a picnic foam-box (background). (b) Top view of the humid chamber showing 6 plastic cups placed on a wet towel lined the bottom (pink colour) and 10-15 ice cubes

4.2. Egg laying

After the engorged adult female was maintained for 4-5 days and/or until gravid in the insectarium, it was placed in a screen-topped oviposition plastic-cup (6 cm in diameter and 7 cm in depth) containing 25 ml of natural water (brought from a basin that was used for tap-water production). Wet filter paper lined the inside of the screen-topped was covered with a black plastic sheet (Figure 3a-c). The eggs attached to the moist side of the filter paper and/or floating on the water surface were rinsed and transferred to white plastic tray (25 x 36 x 6 cm) containing 1,500 ml rearing water (equal part of natural water and distilled water) with wet filter paper lining the inside. During the embryonation period, the eggs were exposed to a 40-watt light instead of sunlight, for warming the eggs until hatching (Figure 3d).

4.3. Rearing of larvae, pupae and adults

After egg hatching, first instar larvae were transferred daily from an ovipot to a white plastic tray (25 x 36 x 6 cm) containing 2,000 ml rearing water and approximately 15 stems of garden grass (*Axonopus compressus*), and 80 first instar larvae were reared in each tray. The rearing tray was covered with a transparent plastic sheet for reducing the need to change and/or re-fill the tray with rearing water during the larval development process (Figure 4a-b). An extra and/or a standard formula of fish food consisting approximately of protein 47.5%, oil 6.5%, fibre 2.0%, ash 10.5%, moister 6.0% and additives of vitamins A (29,770 IU/kg), D3 (1,860 IU/kg), E (200 mg/kg), L-ascorbyl-2-polyphosphate (138 mg/kg), lecithin, l-lysine monochlorhydrate, and citric acid was used as larval nutrient. Fine fish food was placed in a vial covered with a nylon screen (34 x 43 threads per cm^2) and sprinkled on the water until the food particles stopped spreading across the water's surface. First and second instar larvae were fed twice daily, and this schedule was increased to 3-5 times daily after most of the larvae reached third and fourth instars, respectively. Before each feeding, floating clumps of excess food were

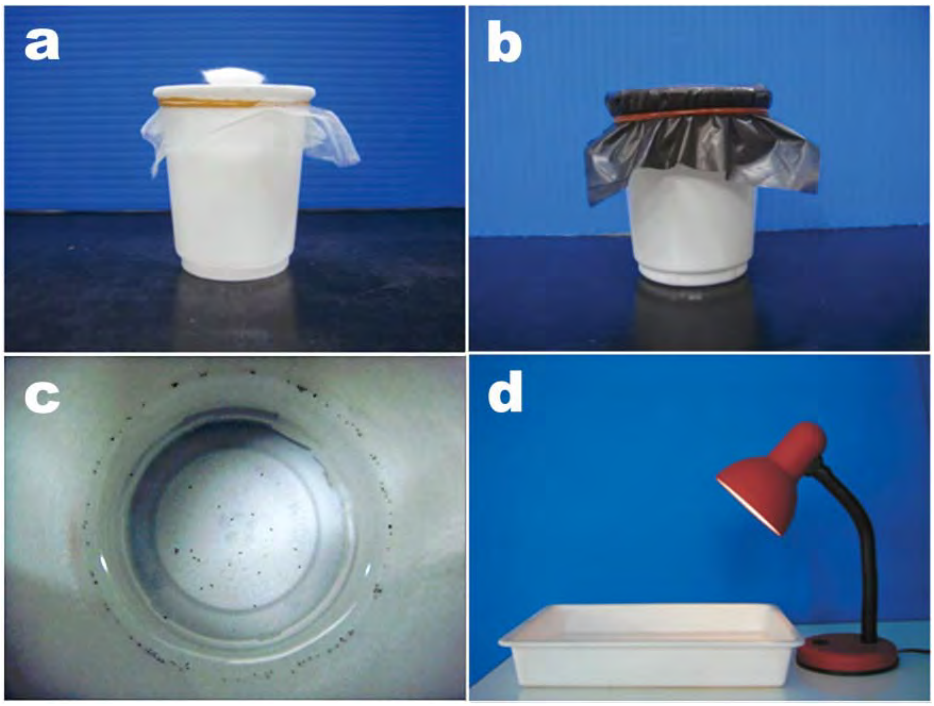

Figure 3. (a) A screen-topped oviposition plastic-cup, (b) covered with a black plastic sheet, and (c) top view of the plastic cup showing egg-batch after 12-hours-oviposition of a gravid adult female. (d) Eggs placed in a white plastic tray and exposed to a 40-watt light

removed by dragging a sheet of typing-paper across the water's surface. Any larvae trapped on the paper during the cleaning process were dislodged by rinsing the paper in a tray of rearing water and returning it to the rearing tray. After pupation, approximately 100 pupae placed in a plastic cup (14.5 cm in diameter and 6 cm in depth) containing 150 ml of distilled water were kept in a 30 x 30 x 30 cm cage, and the emerged adults were provided with both 10% sucrose solution and 5% multivitamin syrup solution (consisting approximately of vitamins A: 2,000 I.U., D: 200 I.U., E: 1.50 I.U., B1: 0.70 mg, B2: 0.85 mg, B6: 0.35 mg and C: 17.50 mg, nicotinamide: 9.00 mg, orange juice: 0.50 g and cod liver: 0.10 g per 100 ml solution) saturated in cotton wool coiled around a small piece of wood and placed in a small bottle. Increased humidity to promote adult survival was provided by covering the cage with a wet towel overlaid with a black plastic sheet (Figure 4c). One-day-old males were removed daily from the cage and kept in a screen-topped plastic cup (lined inside with filter paper), where they were provided with a 5% multivitamin syrup solution through a pad of soaked cotton wool, which was placed on top of the screen and changed daily. In order to keep humid conditions in the cup and delay rapid drying of the cotton wool soaked in 5% multivitamin syrup solution, the screen-top was covered with a translucent plastic bag (Figure 4d).

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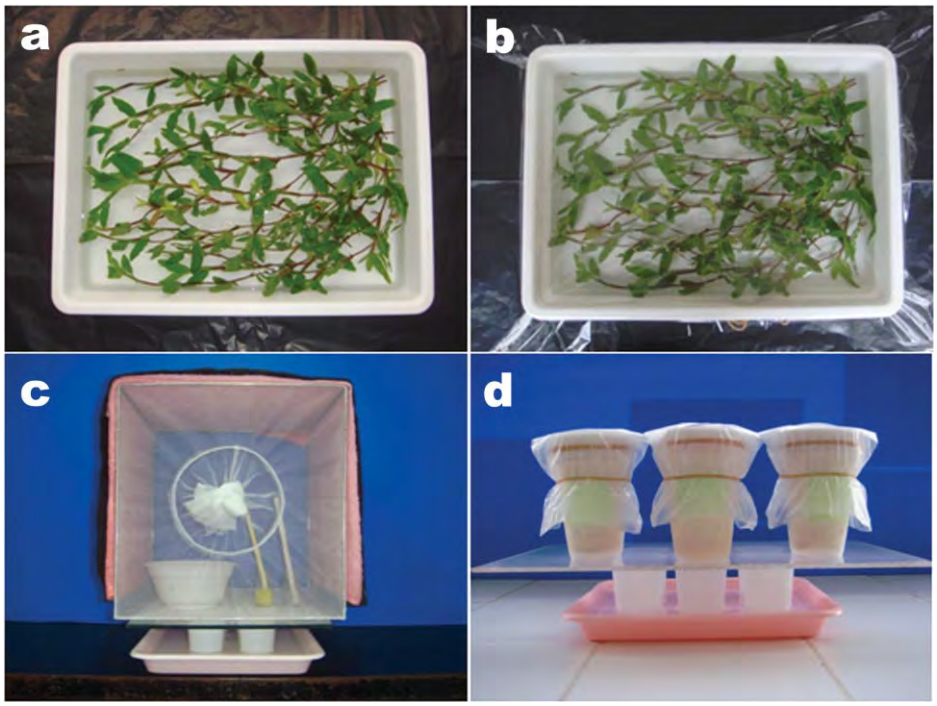

Figure 4. (a) Top view of a white plastic tray placed with 15 stems of garden grass, and (b) covered with a transparent plastic sheet. (c) Adult rearing cage partially covered with a wet towel (pink colour) and a black plastic-sheet with plastic container for holding pupae, and two bottles with cotton wicks, one containing 10% sucrose solution and another 5% multivitamin syrup solution. (d) Adult males being kept in a screen-topped plastic cup (lined inside with filter paper) with a pad of cotton wool soaked in 5% multivitamin syrup solution and the top covered with a translucent plastic bag to maintain humidity.

4.4. Suitable blood-feeding condition

Comparative direct feeding ability on white rat in a 30 x 30 x 30 cm cage, and artificial feeding ability on human heparinized-blood (obtained from human volunteers whom sign the consent form) in a plastic cup (8.5 cm in diameter and 11 cm in depth, lined inside with filter paper) (Figure 5), of female *An. campestris*-like Form E at different ages ranging from 1 to 10 days, demonstrated that in the cage, adult females aged of 3, 4, 5 and 6 days were successful in feeding on the blood of white rats, with feeding rates of 30%, 39%, 62% and 43%, respectively. Interestingly, the adult females aged 3, 4, 5, and 6 days succeeded in artificial feeding on human heparinized-blood in the plastic cup at higher rates than direct feeding on white rat in the cage in all experiments by yielding feeding rates of 62%, 68%, 78% and 61%, respectively. Nevertheless, the engorged females that derived from 2 feeding methods were used satisfactorily for the maintenance of an iso-female line laboratory-raised colony of *An. campestris*-like Form E. One difficulty and/or failure in rearing mosquitoes in the laboratory was the subsequent

generation's refusal to feed on blood, particularly from small laboratory animals such as guinea pig, white rat, golden hamster, etc. This leads to direct feeding from human volunteers, especially at the beginning of the first to fifth generations of the colony. However, to solve this problem, forced artificial feeding on human heparinized-blood by *An. campestris*-like Form E was successful in this study and has been used routinely up to this time. Nonetheless, a point to be kept in mind is that only the healthy progenies of laboratory-raised colonies could be used successfully. Additionally, the use of direct blood feeding of subsequent mosquito progenies from human volunteers is a potentially dangerous method and should be given up entirely, since at least 4 reports have declared that *An. peditaeniatus* [27], *An. subpictus* [28-29] and *An. barbirostris* [30] have been incriminated as secondary vectors of Japanese encephalitis virus, which is possibly transmitted vertically.

Figure 5. Artificial feeding system. A warm water-bath at 40ºC, with a water pump placed inside, is connected to glass inlet and outlet feeding-chambers by rubber tubes. Thin paraffin-membrane covers the bottom tip of the feeding chambers, which are filled with human heparinized-blood, and the bottom tip is in close contact with 50 fasted adult female *An. campestris*-like Form E that are inside a screen-topped paper cup.

4.5. Ability of free mating in a 30 cm cubed cage and male ability to mate artificially

One of the difficulties in the colonization of anopheline mosquitoes in the laboratory might be due to adults not being capable of copulation in a small and/or standard cage (30 x 30 x 30 cm). Thus, in order to determine the adaptive stenogamy of *An. campestris*-like Form E, the newly emerged females and males co-habitated at a ratio of 200/300, in a 30 x 30 x 30 cm cage for one week [31-32]. The results indicated that *An. campestris*-like Form E failed to mate freely in the cage at a 0% insemination rate (from experiments repeated 3 times), indicating strong eurygamy. Thus, the artificial mating methods as described by [33-34] were used. The best age for artificial mating in male *An. campestris*-like Form E was 5-days-old (100% mating rate, 86.67% insemination rate). Nonetheless, males aged 4 and 8 days old could be used satisfactorily (93.33-100% mating rates, 80-82.14% insemination rates) (Table 1).

Day after emergence*	No. successfully mated females (%)	No. insemination (%)
1	11 (36.67)	0 (0)
2	23 (76.67)	18 (78.26)
3	23 (76.67)	18 (78.26)
4	30 (100)	24 (80.00)
5	30 (100)	26 (86.67)
6	28 (93.33)	23 (82.14)
7	28 (93.33)	24 (85.71)
8	28 (93.33)	23 (82.14)
9	26 (86.67)	16 (61.54)
10	23 (76.67)	11 (47.83)

*Thirty males for each experiment.

Table 1. Artificial mating ability of *An. campestris*-like Form E males

4.6. Searching for a suitable oviposition-condition

Many anopheline colonies have been reported to adapt easily to oviposit eggs in the cage on various types of simple ovipots, e.g., petridish, crystallizing dish, terra-cotta bowl, white plastic cup, black cup, etc. [35-39]. In the case of using 20 gravid adult females of *An. campestris*-like Form E put in a 30 x 30 x 30 cm cage for 12 hours (starting from 18.00-06.00 hours), the results revealed that 0, 0, 279, 0 and 0 eggs per an oviposited-plastic cup (9 cm in diameter and 10.5 cm in depth, containing 80 ml of natural water) were found in experiments 1, 2, 3, 4 and 5, respectively; whereas the forced laying of eggs by placing 20 gravid adult females in an oviposited-plastic cup (details mentioned above in paragraph 2 "Egg laying") in the same size and conditions as used in the cage, a massive number of eggs, i.e., 1,273, 1,318, 1,705, 2,180 and 1,501 eggs per cup, were recovered for experiments 1, 2, 3, 4 and 5, respectively (Figure 6). The high yield of eggs recovered from the latter experiment appears to result in the fact that the close-system of an oviposited-plastic cup provided significantly higher relative humidity than a cage or open-system. The air-rich water molecules in high relative humidity are the important attractants to gravid female alfactometer, which indicates suitable or acceptable oviposition sites [40]. Thus, in oviposition of *An. campestris*-like Form E and other anopheline species in our laboratory, this method has been used routinely up until now.

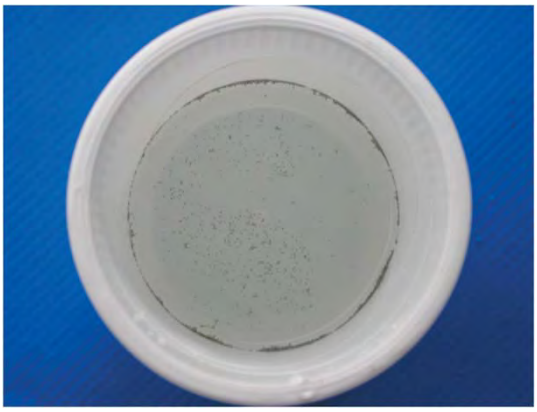

Figure 6. Top view of ovipot derived from a plastic cup showing massive egg-batches after 12-hours-oviposition of the 20 gravid adult females

4.7. Other important factors

Throughout the larval rearing period, the number of larvae, rearing conditions in the tray, and food were the most important factors, not only for routine rearing, but also special rearing in order to obtain a high yield of metaphase and polytene chromosomes, which were necessary for population-genetic study of anophelines. Stressful rearing-conditions, e.g., the overcrowding of larvae in a rearing tray (in this study, 80 larvae per 25 x 36 x 6 cm tray was an appropriate number for *An. campestris*-like Form E), and the use of inappropriate water medium and food would lead to a rapid drop in and/or loss of a colony. Also, this would result in low larval and pupal survival rates, adult F_1-progenies refusing to take blood meal, difficulty in artificial mating of adult females and males and/or failure to inseminate sperm into mated-female spermathecae, short life span of adult females and males, mated gravid adult females laying fewer numbers of eggs and/or failure to lay eggs, and low egg-hatchability. Thus, any rearing system, which is an important first step that leads to obtaining healthy larvae, would be a promising method for successfully establishing a colony, particularly an iso-female line colony, which is more difficult and complicated to establish than a mixed colony. As mentioned previously, food was one of the most important factors for obtaining healthy larvae, thus, several kinds of larval food were tested for use and comparison, e.g., mouse pellets, cat and dog biscuits and various formulas of fish food. The results indicated that the standard formula of fish food as mentioned in paragraph 3 ("Rearing of larvae, pupae and adults"), proved to be an excellent larval food for *An. campestris*-like Form E. It is expected that this fish food formula was also ideal for other anopheline species with rearing difficulties. The use of equal part of natural water and distilled water as the larval rearing medium also proved to be promising. Trials using boiled tap-water, filtered tap-water, polarized water and deionized water yielded unsatisfactory outcomes by providing low larval survival, particularly through subsequent progenies. The addition of garden grass to the larval rearing tray, as stated by [31], resulted in high larval survival for *An. campestris*-like Form E. Using few stems of garden grass,

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or withdrawing it, would lead to low larval survival and/or weak larvae for rearing subsequent generations. Using slightly more or less than 15 stems of garden grass, depending upon the size of the stems, and size and number of leaves, proved to improve conditions to a suitable level for larval rearing, since the grass provided a resting place for larvae, rendered shade as in natural breeding sites (rice paddy, ponds and swamps associated with water plants) [41-42], and aerated the medium. Its roots were also very important for maintaining clear and clean rearing medium by using larval waste products and unconsumed food as fertilizer, which determined the obvious active growth of grass in the rearing tray. Finally, we hope that the detailed information concerning rearing aspects of *An. campestris*-like Form E will prove to be important for the establishment of other anopheline species that have been previously difficult to rear.

Notes: By following the systematic rearing procedures as detail-mentioned above, at least 23 *Anopheles* species were successful reared in our insectarium, i.e., subgenus *Anopheles* [*An. argyropus* (F_{23}), *An. barbirostris* species A1 (F_{86}), *An. belenrae* (F_{26}), *An. campestris*-like Form E (F_{98}), *An. crawfordi* (F_{23}), *An. lesteri* (F_{60}), *An. nigerrimus* (F_{23}), *An. nitidus* (F_{28}), *An. paraliae* (F_{24}), *An. peditaeniatus* (F_{23}), *An. pullus* (F_{24}), *An. pursati* (F_{24}) and *An. sinensis* (F_{28})]; and *Cellia* [*An. harrisoni* (F_{51}), *An. jamesii* (F_{10}), *An. jeyporiensis* (F_5), *An. karwari* (F_{13}), *An. kochi* (F_{25}), *An. nivipes* (F_{12}), *An. pampanai* (F_{11}), *An. philippinensis* (F_{12}), *An. splendidus* (F_{10}) and *An. tessellatus* (F_{27})].

5. Techniques for metaphase and polytene chromosome preparations

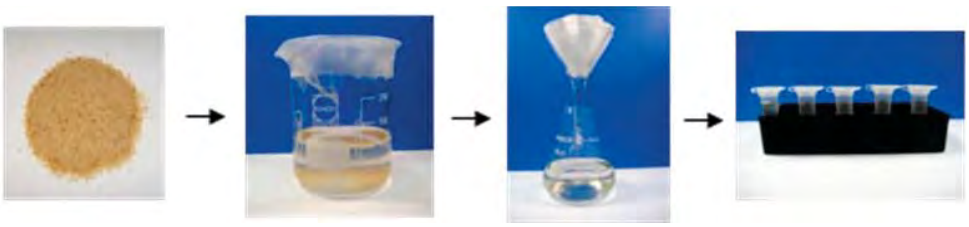
5.1. Rearing condition of mosquitoes for chromosome preparations

The methods for rearing conditions were generally routine as mentioned in paragraph 3, except, 10 first instar larvae per tray were used to obtain a high yield of metaphase chromosomes from larval brains, ovaries and testes, and polytene chromosomes from larval salivary glands. Comparative outcome rates of metaphase chromosomes from larval brains and polytene chromosomes from larval salivary glands between routine (80 larvae) and special (10 larvae) rearing revealed as follows: (1) metaphase chromosomes: experiment 1 [10 larvae (87.50%) vs. 80 larvae (33.33%)], 2 [10 larvae (75.00%) vs. 80 larvae (30.00%)] and 3 [10 larvae (77.78%) vs. 80 larvae (30.00%)]; and (2) salivary gland polytene chromosomes: experiment 1 [10 larvae (80.00%) vs. 80 larvae (50.00%)], 2 [10 larvae (66.67%) vs. 80 larvae (50.00%)] and 3 [10 larvae (100.00%) vs. 80 larvae (66.67%)]. Thus, a special rearing with 10 larvae was used routinely for chromosome preparation.

5.2. Preparation of metaphase chromosomes from adult females and males and fourth instar larvae

5.2.1. Preparation of 0.5% and 1% solutions of dried Gloriosa superba *seed and rhizome powders*

Summarized flow chart for normal saline-extracted *Gl. superba* seed and rhizome powders, as follows:



0.5 g of dried seed powder or 1 g of dried rhizome powder

Macerated in 100 ml of 0.85% sodium chloride solution for 6 hours at 27±2 °C, and kept at 4 °C for 14-16 hours (or in a refrigerator)

Filtrated

1 ml of filtrate filled in a 1.5-ml microcentrifuge tube, and kept at -20 °C*

*By keeping at this condition, the colchicine-like activity in the filtrate stays stable for at least 2 years.

Notes: colchicine solution has been used widely at a concentration of 0.05-1% for metaphase chromosome preparation in the cytogenetic study of eukaryotic organisms, e.g., protozoans [43], helminthes [44-45], snails [46], insects [8, 47-50], and plants [51-52]. Spindle formation or microtubule polymerization inhibits arresting mitosis at the metaphase [53-54]. The alkaloid colchicine was isolated from a plant named autumn crocus or meadow saffron (*Colchicum autumnale* L., Family Liliaceae) in 1820 by Pelletier and Caventou [53]. At present, the commercial products derived from this plant are merchandised extensively and used worldwide. Recently, systematic and continuous studies evaluated the colchicine-like activity of a common decorative plant found widely in tropical countries, Dong Deung (*Gl. superba*, Family Liliaceae) [55], which highlighted the benefits of this plant used for metaphase chromosome preparation in mosquitoes [14, 24, 56-58]. Various concentrations and/or extracted-fractions of dried *Gl. superba* seed and rhizome powders yielded similar metaphase rates and an average number of metaphase chromosomes per positive mosquito to synthetic colchicine solution, indicating that these extracts could be used to replace colchicine. In addition, the authors also mentioned that considerable budget savings could be realized by using their techniques.

Other benefits include a decorative plant that can be bought at many shops in Thailand's flower-markets, and it is hoped elsewhere in tropical countries. It can be grown easily in small-spaced land and outdoors with general fertilizers (e.g., simple formula chemical fertilizer, organic fertilizer and animal manure), which are necessary to promote its growth. It takes about 5-7 months to grow from small budding-rhizomes into mature tree with flowers and green pods (Figure 7a-d).

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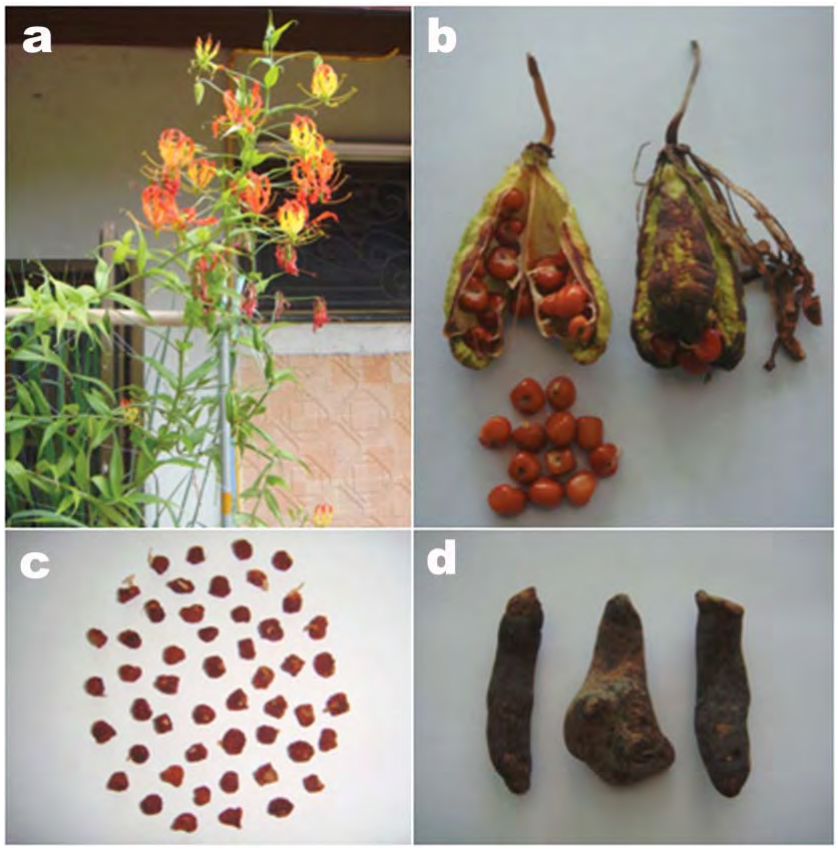

Figure 7. Showing a common decorative plant, Dong Deung (*Gl. superba*). (a) Dong Deung trees with beautiful flowers and green pods, (b) Ripe and broken Dong Deung pods with reddish-orange seeds, (c) Dried Dong Deung seeds and (d) Dried Dong Deung rhizomes

5.2.2. Preparation of the metaphase chromosomes from adult females and males and fourth instar larvae

5.2.2.1. Procedures

Metaphase chromosomes for adult females and males were prepared using the modified techniques described by [25]. The newly emerged adult females and males aged up to about 6-12 hours were starved, anaesthetized with ether and placed on their side on a slide under a binocular microscope. A needle was made by drawing out a glass capillary tube in a flame until the pointed end was approximately 80-100 μm in diameter; the shorter the needle the easier it was to handle. An inoculation was made into the post-spiracular area of the mesothorax, and a filtrate of 0.5% solution of dried *Gl. superba* seed powder was introduced into each mosquito by gently blowing down the attached rubber tube. The volume of inoculums

could be controlled by observing the extension of abdomen until it was similar in size to the fully-engorged mosquitoes post fed on 10% sucrose solution. A few minutes after inoculation, most of the mosquitoes had recovered completely. Five inoculated mosquitoes were then kept in a 10-ml test tube (1.5 cm in diameter and 10 cm in length), with cotton wool soaked by 3 drops of distilled water closing the opened-side in order to provide adequate moisture. Then, the cotton wool was sealed with paraffin and the test tube held in an insectarium at 27±2 °C and 70-80% relative humidity for 3 hours (Figure 8a-c).

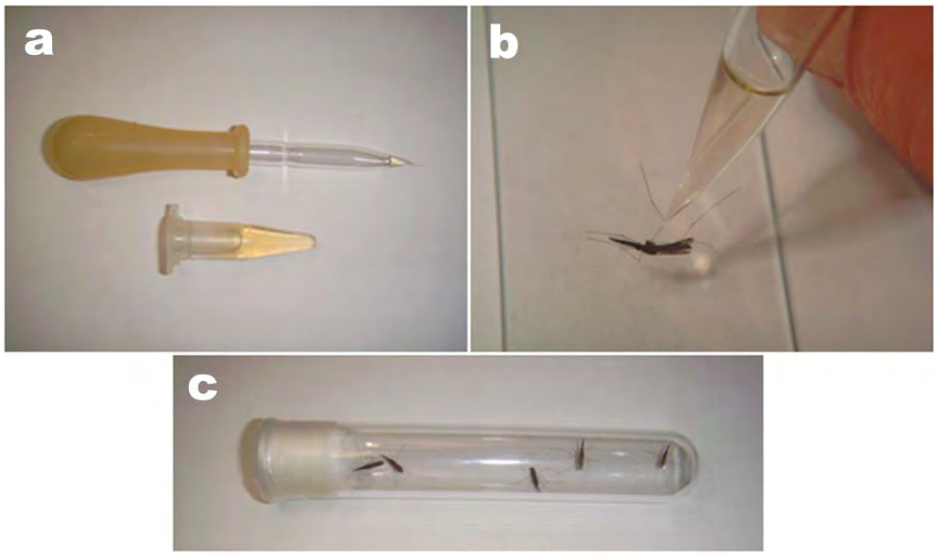

Figure 8. (a) Lower row: 1 ml filtrate of 0.5% solution of dried *Gl. superba* seed powder filled in a 1.5-ml microcentrifuge tube, and upper row: an inoculation glass-needle filled with a filtrate. (b) Intra-thoracic inoculation of a filtrate into the post-spiracular area of the mesothorax. (c) Five inoculated mosquitoes kept in a 10-ml test tube

The inoculated mosquitoes were dissected in a small drop of 1% hypotonic sodium citrate solution on a siliconized slide by pulling out the last abdominal segment to obtain the ovaries or testes under a binocular microscope. The organs obtained were left in 1% hypotonic sodium citrate solution for 10 minutes, and then transferred to a small drop of Carnoy's fixative on a siliconized slide for at least 2 minutes. Then, a drop of 60% acetic acid was added, and the organs were torn and mixed well with dissecting needles. A drop of cell suspension was placed on a clean microscopic slide on a warming plate at about 45–50°C. Droplets of cells were released slowly from a Pasteur pipette to form a circular trail of monolayer cells. The dried slides were stained with 20% Giemsa in phosphate buffer pH 7.2 for 1 hour, rinsed with deionized water, air-dried at room temperature, mounted in Permount® (Fisher, Fairlawn, NJ, USA) and examined under a green filter compound microscope. Metaphase karyotypes were identified by following the standard descriptions (Figure 9) [59-60].

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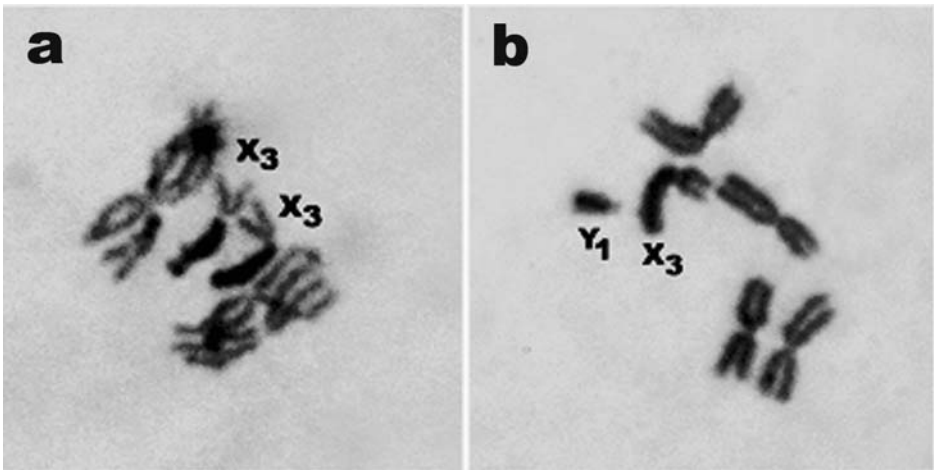

Figure 9. Metaphase chromosomes of *An. paraliae* Form A. (a) Ovary chromosomes, showing homozygous large submetacentric X_3 chromosomes. (b) Testis chromosomes, showing large submetacentric X_3 and small telocentric Y_1 chromosomes

The techniques for metaphase chromosome preparations in fourth instar larvae mainly followed those described above, except for the 5 fourth instar larvae that were incubated with a 1 ml filtrate of 0.5% dried *Gl. superba* seed powder solution in a 10-ml test tube for two hours. Then, the larval brains were excised, fixed, smeared, stained with Giemsa, mounted and examined under a green filter compound microscope (Figure 10).

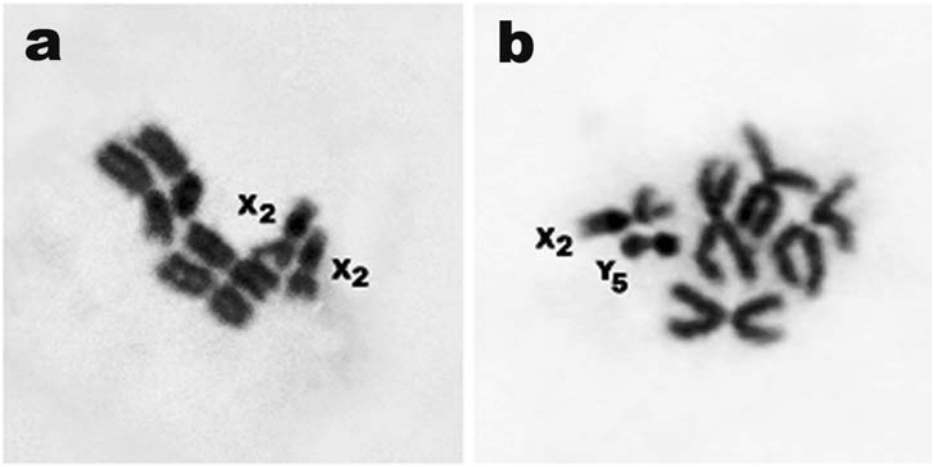

Figure 10. Metaphase chromosomes from brains of *An. campestris*-like Form E. (a) Showing homozygous submetacentric X_2 chromosomes. (b) Showing submetacentric X_2 and small metacentric Y_5 chromosomes

5.3. Preparation of the polytene chromosome from larval salivary glands

5.3.1. Procedures

Salivary gland polytene chromosomes were prepared using the slightly modified published techniques [26, 61]. The early fourth instar larvae were removed from the rearing tray by a dropper and rinsed in clean distilled water. A healthy larva with flared-thorax in appearance was picked up with forceps, attached to filter paper to remove excess water, placed on a siliconized slide filled with a drop of 1% hypotonic sodium citrate solution, and then dissected under a binocular microscope. The head was cut off, and one dissecting needle was inserted through the anterior end of thorax to posterior end. Then, another dissecting needle was scratched along the line of the inserted needle to tear the thorax integument, open the thorax and take out the internal organs before the thorax and abdomen were transferred into a drop of 15% acetic acid on a siliconized slide. The bilobed salivary glands were removed from the thorax using dissecting needles, and only the whitish anterior lobe of each salivary gland was transferred into a small drop of 45% acetic acid on a siliconized slide and left for 1 minute. After that, one drop of 2% aceto-lactic orcein stain was added. After 15 minutes of staining, a grease-free 22 mm^2 coverslip was placed on the stained salivary glands. The preparation was wrapped firmly in filter paper and gently pressed with a thumb to squash and spread the chromosomes. Then, the coverslip edges were sealed with transparent nail varnish. The prepared chromosomes were scrutinized under a green filter compound microscope. The arm of the polytene chromosomes was identified by following the standard map (Figure 11) [61].

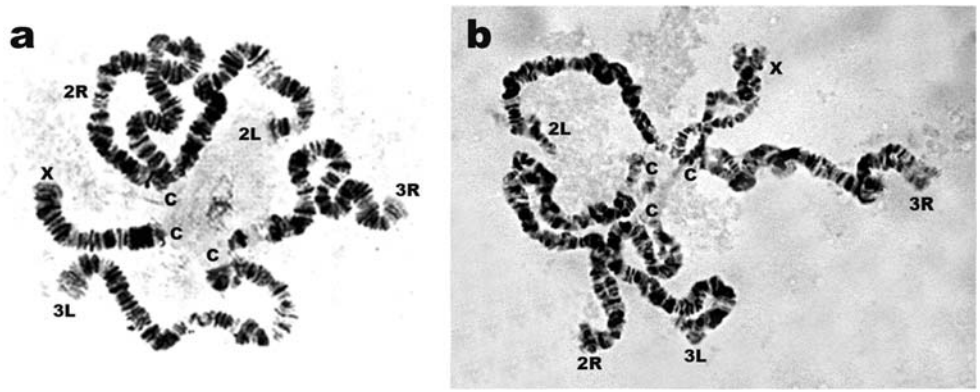

Figure 11. (a) Complete synaptic salivary gland polytene chromosome of *An.campestris*-like Form E. (b) Homosequential asynapsis in all autosomes and the X chromosome from crosses between *An.campestris*-like Form E and *An. barbirostris* species A1

Notes: by application of this robust systematic procedure, 5 sibling species members have recently been recognized in the taxon *An. barbirostris* complex within 2 years [14-16]. In addition, 8 species comprising a total of 26 subspecies (cytological forms) have been recognized during the past decade, i.e., *An. vagus* Forms A and B [62], *An. pullus* Forms A and B (= *An.*

yatsushiroensis) [62], *An. sinensis* Forms A and B [64-66], *An. aconitus* Forms B and C [67], *An. barbirostris* species A1 (Forms A, B, C and D) and A2 (Forms A and B) [14-16], *An. campestris*-like Forms B, E, and F [68], *An. peditaeniatus* Forms B, C, D, E [69], and *An. paraliae* Forms A, B, C, D and E [unpublished data].

6. Conclusion

The formation of robust systematic procedures is highly anticipated, based on the crossing experiments between iso-female lines using cytological markers (characteristics of metaphase chromosomes/karyotypic forms). Together with this information, the data on comparative sequence analyses of some specific genomic regions (rDNA and mtDNA) would bring success in recognizing and reliably identifying sibling species and/or subspecies members within the taxon of other *Anopheles* species complexes. In addition, the detailed techniques necessary for the establishment of difficult-to-rear anopheline species, which yield high rates of attractive metaphase and polytene chromosomes and potent adults for crossing experiments, would be main keys leading to successful study on the population-genetic structure of *Anopheles* vectors. These factors are important for studying the biology, behavior of *Anopheles* species, as well as for an epidemiology and a control approach of the targeted vector species.

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Author details

Wej Choochote* and Atiporn Saeung

*Address all correspondence to: wchoocho@mail.med.cmu.ac.th

Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

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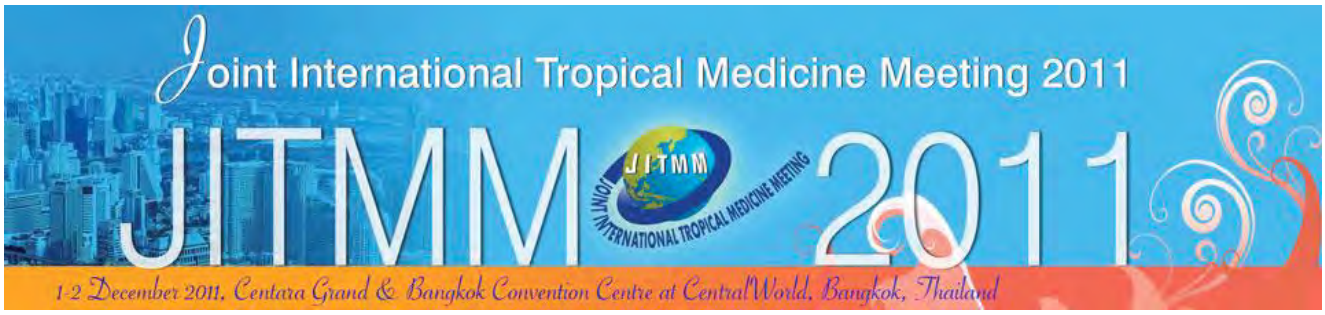


No. JITMM2011/SC043

22 September 2011

Prof. Wej Choochote
The Faculty of Medicine, ChiangMai University
E-mail: wchoocho@med.cmu.ac.th

Confirmation of your Presentation and Invitation to be an Invited Speaker at JITMM 2011, in Bangkok, Thailand

Dear Prof. Wej Choochote,

The Faculty of Tropical Medicine, Mahidol University, is currently organizing the Joint International Tropical Medicine Meeting 2011 (JITMM 2011), which will be held 1-2 December 2011 at Centara Grand & Bangkok Convention Centre at CentralWorld, Bangkok, Thailand.

The main theme of the JITMM is **"One World – One Health"**. Topics will include malaria, dengue, HIV/AIDS, vaccine updates, H1N1, H5N1, disease vectors, drug resistance, and other topics related to the tropical diseases.

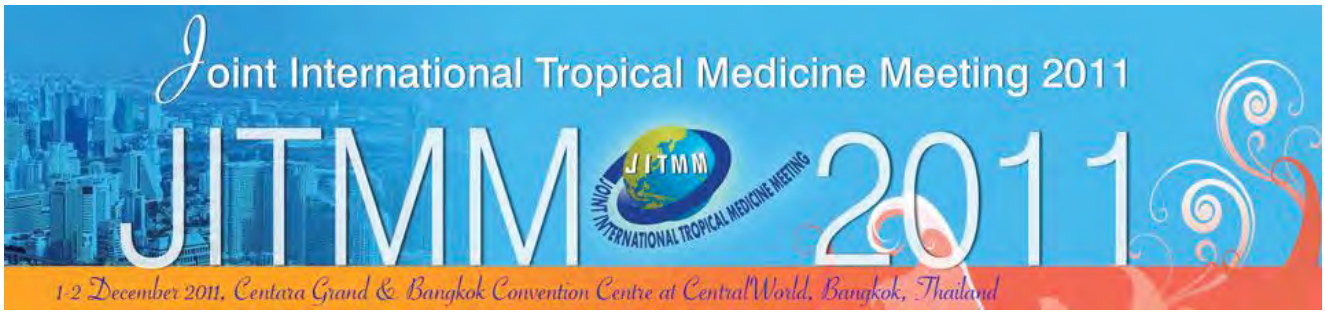
I am very pleased to learn that you have accepted Assoc. Prof. Chamnarn Apiwathnasorn's invitation to be an Invited Speaker, to speak in the session **Vectors on the changing environment**. May I now take this opportunity to invite you formally in this regard.

The Session is scheduled as follows:

Session:	**S20: Vectors on the changing environment**
Date:	**Friday 2 December 2011**
Between:	**11.00-12.30 hr** (You have been allocated 20 minutes for your presentation, including questions and answers)
Place:	Room D, Centara Grand & Bangkok Convention Centre at CentralWorld, Bangkok Thailand
Chairpersons:	1. Assoc. Prof. Narumon Komalamisra 2. Assoc. Prof. Supatra Thongrungkiat

Speakers and their topics

- Black flies plague hits in the northern Thailand
  Prof. Wej Choochote
- Dust mite allergens in the urban environment of Thailand
  Asst. Prof. Nat Malainual



- Morphometric phylogeography of Aedes albopictus: An observation on species diversification
  Dr. Ronald E.Morales Vargas
- From wheel rut to man-made container: Anopheles dirus in a changing environment
  Dr. Sungsit Sungvornyothin

In recognition of your ongoing support, we are honored to offer you complimentary registration for JITMM 2011.

To register online, please visit the unique URL generated for you at: http://www.jitmm.com/client/signUp.php?vip=1, which will allow you to waive the registration fee. Please do not share this special URL with anyone.

Kindly login to www.jitmm.com and send all of the following documents by 30 September 2011:
(1) The abstract of your paper
(2) A recent portrait photograph
(3) A short CV (please use the Short CV form attached)

Attached please find:
1. Second Announcement
2. Tentative Scientific Program (JITMM2011)
3. Short CV Form

Your very important contribution to the Meeting is greatly appreciated.

Looking forward to welcoming you to JITMM 2011, 1-2 December 2011.

Yours sincerely,

Pongrama Ramasoota

Asst. Prof. Pongrama Ramasoota
Chair, Scientific Committee, JITMM 2011
E-mail: jitmm@mahidol.ac.th
Tel: 66 (0) 2354 9100-4, ext 1524, 1525
Website www.jitmm.com

cc: Assoc. Prof. Chamnarn Apiwathnasorn<tmcaw@mahidol.ac.th>

BLACK-FLY FAUNA OF THAILAND: SPECIES DIVERSITY AND MEDICAL IMPORTANCE

Wej Choochote

Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

Objectives: 1) To search for new black-fly species and new records, 2) To identify man-biting black-fly species, and 3) To incriminate filarial transmission black-fly species.

Methods: A search for new species was performed by comparative morphological investigations of the collected larvae, pupae, and adult associated pupal skins with previously known species. Those with markedly morphological distinction were established as holotype specimens for description and illustration of new species. Comparative DNA sequencing accomplished of the mitochondrial 16S ribosomal RNA gene to confirm new species status. Seasonal abundance and diurnal flying activity studies of black flies attracted to humans were carried out at 3 localities in northern Thailand in order to determine man-biting black-fly species. Wild-caught female black-fly species, which were positive for third stage larvae of the filarial nematodes after dissections, were incriminated as natural vectors.

Results: During 2001-2010, 2 new records and 32 new species of black flies in the genus *Simulium* Latreille s.l. were discovered in areas of 34 provinces of Thailand. At least 13 black-fly species that preferred biting humans were reported, and only 4 species were ranked as main human-biting species, which seemed to exist in species-specific localities; determined by seasonal abundance and intensity of biting related to altitude, i.e.; *Simulium rufibasis; S. doipuiense; S. nigrogilvum* and *S. nodosum* at altitudes of 1,800-2,565 m; 1,300-1,800 m; 600-1,300 m; and < 600 m, respectively. In addition, 2 species, i.e., *S. nigrogilvum* and *S. nodosum*, were incriminated as natural vectors of *Onchocerca* spp., which can probably cause zoonotic filarial infection in the Thai population, and this was reported for the first time in Thailand and/or the Southeast Asian region. ♦

Keywords: Black fly, *Simulium*, man-biting species, filaria, *Onchocerca*, Thailand

DUST-MITE ALLERGENS IN THE URBAN ENVIRONMENT IN THAILAND

Nat Malainual

Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Rd., Bangkoknoi, Bangkok 10700 Thailand.

Allergens of dust mites have been known as the triggering factor for allergic exacerbation in patients worldwide including Thailand. Most important and abunadant dust mite allergens are of *Dermatophagoids pteronyssinus* and *Dermatophagoides farinae*.mite species. The WHO suggested threshold levels for both alllergens are 2 µg per gram dust for causing allergic sensitization and 10 µg per gram dust for causing acute attack in asthmatic patients. In Thailand, higher levels of either Der p or Der f allergens had been found in about 98% of dwellings, therefore most Thais can be sensitive to these dust mite allergens. However, only about 30% of population suffers from allergic diseases. Some other mite species in house dust are storage mites such as *Blomia tropicalis*, of which its allergen is named Blo t. This storage mite species encounters less than 1% of house infestation. However, this species can predominate over other dust mites in some houses. Major factors that influence dust mite population are temperature, humidity and food. Although climatic changes might have effects on most animals, but indoor environments play the major role for dust mite bio-diversity in dwellings. During the past decades, living behavior of people in the cities has been changing from open-air environment to closed room. This causes less air ventilation and humidity change, which support the increase of mite population and then increases mite allergens. Indoor environmental control strategy such as humidity controller could be benefit, especially for mite allergen-sensitive individuals. ♦

Keywords: dust mites, climatic change, indoor environment

KARYOTYPIC VARIATION AND GEOGRAPHIC DISTRIBUTION OF *ANOPHELES PEDITAENIATUS* IN THAILAND

Atiporn Saeung[a], Visut Baimai[b], Sorawat Thongsahuan[a], Gi-Sik Min[c], Mi-Hyun Park[c], Yasushi Otsuka[d], Pewpan M. Intapan[e,f], Verapong Lulitanond[e], Wanchai Maleewong[e,f], Wej Choochote[a]

[a] *Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.*

[b] *Center for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.*

[c] *Department of Biological Sciences, Inha University, Incheon 402-751, South Korea*

[d] *Department of Infectious Disease Control, Faculty of Medicine, Oita University, Hasama, Oita 879-5593, Japan*

[e] *Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen 40002, Thailand*

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

Objectives: 1) To search for new karyotypic forms within the taxon *An. peditaeniatus*, 2) To investigate the role of karyotypic forms that generate post-mating barriers by means of crossing experiment, and 3) To search for karyotypic form-specific sequence variation (rDNA: ITS2; mtDNA: COI, COII)

Methods: Wild, fully engorged female *An. peditaeniatus* were collected from buffalo-baited traps at 17 localities in Thailand. Fifty-five isolines were successfully established in an insectary and used for studies on metaphase karyotypes, crossing experiments, and molecular analyses.

Results: Fifty-five isolines showed six different karyotypes based on the amount of extra heterochromatin in the sex chromosomes, namely Forms A (X_3,Y_1), B (X_1, X_2, X_3, Y_2), C (X_2, X_3, Y_3), D (X_1, X_2, X_3, Y_4), E (X_1, X_2, X_3, Y_5), and a new karyotypic Form F (X_1, X_2, X_3, Y_6). Crossing studies among the 11 isolines, which were representative of 6 karyotypic forms of *An. peditaeniatus*, revealed genetic compatibility in providing viable progenies and synaptic salivary gland polytene chromosomes through F_2-generations, thus suggesting the conspecific nature of these karyotypic forms. These results were supported by the very low intraspecific sequence variations of the nucleotide sequences in ribosomal DNA (ITS2) and mitochondrial DNA (COI and COII) of the 6 forms. ♦

Keywords: *Anopheles peditaeniatus*, metaphase karyotype, crossing experiment, ITS2, COI, COII

RISK OF DRUG RESISTANT MALARIA CAN BE PREDICTED BY NESTED PCR ANALYSIS OF WILD *ANOPHELES* IN ENDEMIC AREA

Prapa Sorosjinda-Nunthawarasilp[a], Benjawan Wihokhern[b], Noojaree Suparach[b], Pongruj Rattaprasert[c]

[a] *Faculty of Public Health, Burapha University*

[b] *Faculty of Allied Health Sciences, Burapha University*

[c] *Faculty of Tropical Medicine, Mahidol University*

Objective: To assess the prevalence of drug resistant malaria using sporozoites found in wild anopheles.

Method: Anopheles mosquitoes were caught during 2010 in high prevalence areas of Thailand's border with Myanmar and Cambodian. Catches (3 nights each) yielded mosquitoes with sporozoites which were preserved in 95% alcohol. Nested PCR was used to detect genii of malaria, and later *plasmodium* species specific tests identified *P. vivax*. Using methods developed to test suspected resistance from human blood samples, antifolate was investigated. Primers designed as described by Malika et al. (2002) identified the dihydrofolate reductase gene as well as the *mdr1* gene in the sporozoites.

Results: PCR showed one *An.dirus* of the 12 anopheles caught in Borai forest (Trat) in Feburary, and two *An. aconitus* of 9 anopheles caught outside a village in ThongPhaPhum (Kanchanaburi) in December were positive for *P. vivax*. A previously unreported mutation was found in one of the samples from ThongPhaPhum which showed variation at codons 105 (Asp-> Asn) and 144 (Val -> Leu). A point mutation may also be occurring in the *mdr1* gene at codon 1076 (Phe->Leu).

The results coincide with reported monthly incidence of *P. vivax* malaria in Trat and the high prevalence of asymptomatic cases determined by screening to exist in ThongPhaPhum. A high proportion of infected mosquitoes during the dry season maintains the risk of humans getting *P. vivax* throughout the year. Similar PCR methods could be used to detect drug resistance whether mosquitoes are carrying the sporozoites of *P. falciparum* or *P. vivax* and could be used to estimate risk of infection with specific malaria types and monitor drug resistant strains. ♦

Keywords: Drug resistant gene, anopheles, Nested PCR, malaria, endemic

Section 6. Medical Entomology

August 23-24 (Thu-Fri)

P 6

Code No.	Presentation Title *Authors_**Presenting author***
	Predictors of Dengue Fever and Dengue Haemorrhagic Fever in Punjab, Pakistan ***Saleem Muhammad Rana**, Asma Abdul Latif, Tanveer Akhtar*
	Daily survival , anthropophily and the role of major Anopheline vectors in malaria transmission in South west Nigeria ***Kolade Tahiru Ibrahim,** Tonye Grace Okorie*
	The effect of oxidative stress on the DDT insecticide resistance phenotype and life history of the major Malaria vector Anopheles arabiensis ***Shune Oliver**, Basil Brooke*
,	COI gene information of medicinal insect Catharsius molossus ***Cheng-Ye Wang,** Ying Feng*
	Seasonal monitoring of dengue infection in Aedes aegypti and serological feature of patients with suspected dengue in 4 central provinces, Thailand ***Jakkrawarn Chompoosri**, Usavadee Thavara, Apiwat Tawatsin, Surapee Anantapreecha, Padet Siriyasatien*
	High throughput transcriptomic analysis to identify Ixodes ricinus ticks factors involved in Bartonella henselae transmission *Xiang Ye Liu, Martine Cote, Muriel Vayssier-Taussat , **Sarah Irene Bonnet***
	Effect of Mohlolo bait on age- related Blatella germanica population levels ***Clarah Mbowane**, Maboko Samuel Mphosi, Phatu William Phatu*
	VectorMap: leveraging spatial data on agents of febrile vector-borne infections in support of disease risk assessments ***Desmond Foley**, Richard Wilkerson, Lewis s long, Jason Richardson, Leopoldo Rueda*
	Feeding efficiency of Odonate species and Poecilia reticulata against 3 mosquitoes species in Malaysia ***Sitinurhafizah Saleeza Ramlee***
	Carbamate insecticides bioassays and resistance monitoring in field strains of adult German cockroach, Blattella germanica (L.), from southern Iran ***Mohammad Reza Fakoorziba**, Mohammad Djaefar Moemenbellah-Fard, Mohsen Mohebbi-Nodezh, Kourosh Azizi*
	Role of the conserved BBA68-BBA73 operon in the life cycle of Lyme spirochete, Borrelia bugdorferi ***Xuechao Zhang**, Haijun Xu*
	The first molecular identification of Borrelia spp. in Rhipicephalus sanguineus (Acari: Ixodidae) from domestic dogs in Taiwan *Chin-Gi Huang, Chi-Chun Fang, Lu Yun, **Kun-Hsien Tsai**, Wen-Jer Wu*
	Periungual tungiasis and Rickettsia felis in cat fleas in the Democratic Republic of Sao Tome and Principe, West Africa ***Kun-Hsien Tsai**, Chi-Tai Fang, Chin-Gi Huang, Lu Yun, Jih-Chin Lien, Wen-Jer Wu*
	New approaches to physical mapping of mosquito genomes ***Maria V.Sharakhova**, Vladimir A.Timoshevskiy, Becky S.deBruyn, David W.Severson , Igor V.Sharakhov*
	Insecticide resistance in bed bugs in Thailand and laboratory evaluation of insecticides for the control of Cimex hemipterus and Cimex lectularius (Hemiptera: Cimicidae) ***Apiwat Tawatsin**, Usavadee Thavara, Jakkawarn Chompoosri, ,Yutthana Phusup, Nisarat Jonjang, Chavada Khumsawads, Payu Bhkdeenuan, Pathom Sawapanyalert, ,Preecha Asavadachanukorn, Mir S. Mulla ,Padet Siriyasatien, Mustapha Debboun*
	Scabies disease prevalence and social factors affecting referrals to health centers in Hamadan and Kermanshah provinces during the years 2006 -2011 ***Mansour Nazari***
	Morphological and molecular phylogenetic studies on a novel anopheline species collected in Kushiro wetland, northern Japan ***Nozomi Imanishi**, Kenji Takai , Kyeong Soon Kim, Yoshio Tsuda, Mutsuo Kobayashi ,Kyo Itoyama*
	Silkworms in National Bio-Resource Project (NBRP) of Japan *Yutaka Banno, Toru Shimada, Zenta Kajiura, Hideki Sezutsu, **Hideaki Maekawa***
	Odor-based contagious transmission of pathogen by Drosophila melanogaster ***Kiyoshi Okado**, Hirotaka Kanuka*
	Distribution of Biting Midges Leptoconops spinosifrons (Carter) (Diptera: Ceratopogonidae) in Thailand and Field Efficacy of Herbal Repellent against L. spinosifrons ***Usavadee Thavara** , Apiwat Tawatsin , Jakkrawarn Chompoosr , Somchai Saengkitporn, Preecha Asvadachanukorn , Padet Siriyasatien , Arunrat Thepparat, Mir S. Mulla*

Code No.	Presentation Title *Authors_**Presenting author***
	Comparative detection of chikungunya and dengue virus in Aedes albopictus Skuse collected from Surat Thani province, Thailand by multiplex real-time RT-PCR and conventional RT-PCR ***Usavadee Thavara**, Apiwat Tawatsin , Jakkrawarn Chompoosr, Surapee Anatapreecha, Atchara Phumee, Veerayuth Kittichai, Padet Siriyasatien*
	Molecular identification of forensically important fresh flies (Diptera: Sarcophigidae) in Thailand by using the second internal transcribed spacer (ITS2) gene *Payu Bhakdeenuan , Kanok Preativatanyou, Ssunchai Payungporn, Payungporn, Usayadee Thavara,* ***Apiwat Tawatsin**, Kom Sukontason, Kabkaew L Sukotason, Wej Choochote, Siriyasatien ,Padet Siriyasatien*
	The bacterial and fungal florai associated with digestive system of Blattella germanica L. collected in spring and autumn from dormitories and dwellings in Mashhad, Iran ***Gholamhossein Moravvej**, Mahboobeh Naderi Nasab, Sahar Chitsazi*
	Field evaluation of BG-Sentinel and BG-Mosquitito traps for filarial vector Aedes polynesiensis in French Polynesia ***Limb Hapairai**, Hayley Joseph, Michel Cheong Sang, Wayne Melrose, Scott Ritchie,* *Tom Burkot Steven Sinkins, Herve Bossin*
	Chromosomal evolution in Anopheles mosquitoes ***Ai Xia**, Maria Sharakhova, Scotland Leman , Zhijian Tu, Igor Sharakhov*
	Abundance and Dominance of Sand fly Species (Diptera: Psychodidae: Phlebotominae) in Zahedan County, Sistan - Baluchistan Province, Southeastern Iran ***Hamid Kassiri**, Ezat-Aldin Javadian*
	Cockroaches [Periplaneta americana (L.), Dictyoptera; Blattidae] as Carriers of Bacterial Pathogens , Khorramshahr County, Southwestern Iran ***Hamid Kassiri,** Shahnaz Kazemi*
	Data on Sand fly Fauna (Diptera, Psychodidae, Phlebotominae) in Khash County, Sistan - Baluchistan Province, Southeastern Iran ***Hamid Kassiri**, Ezat-Aldin Javadian*
	American Cockroaches [Periplaneta americana (L.), Dictyoptera, Blattidae) as Potential Vectors of Nosocomial Infections, Ahwaz City , Khuzistan Province , Southwestern Iran ***Hamid Kassiri,** Mohammad-Reza Sepand*
	A Survey on Species of Isolated Bacterial Agents from American Cockroaches (Periplaneta americana) in Residential Areas of Ahwaz City , Khuzistan Province , Southwestern Iran ***Hamid Kassiri**, Anvar Ghaderi, Mahmod-Reza Shiravand*
	Efficacy of lethal ovitraps against Aedes aegypti (L.) and Aedes albopictus Skuse (Diptera: Culicidae) ***Apiwat Tawatsin**, Usavadee Thavara, Jakkrawarn, Jakkawarn Chompoosri, Chayada Khumsawads,* *Nisarat Jonjang, Somchai Saengkitporn, Preecha Asavadachanukorn, Padet Siriyasatien*
	Insecticidal efficacy of plant extracts against common bed bug Cimex lectularius L. (Hemiptera: Cimicidae) ***Apiwat Tawatsin,** Usavadee Thavara, Yutthana Phusup, Chompoosri, Jakkarawarn, Somchai Saengkiporn, Wongsinkongman, Somchit Niumsakul, Nalinphat Saktiyasunthorn, Padet Siriyasatien*
	An Epidemiological Study of Pediculus capitis Infestation in the Cases of Attending to the Azna Health Centers, Lorestan Province,Western Iran ***Hamid Kassiri**, Hamid Amani*
	Epidemiological and Clinical Characteristics of Scorpion Sting in Bagh-e-Malek County , Khuzistan province, Southwestern Iran ***Hamid Kassiri**, Ali Teimori*
	Entomological characteristics after five years of malaria control on Bioko Island, Equatorial Guinea ***Hans Jorgen Overgaard,** Simon Abaga, Abrahan Matias, Michael R.Reddy, Michel A.Slotman*
	Functional analysis of Wnt signaling pathway in the regulation of mosquito vitellogenesis ***Shin-Hong Shiao***
	Metarhizium anisopliae as a Potential Biopesticide for the Brown-banded Cockroach, Supella longipalpa (Blattaria: Blattellidae) ***Yu-Ting Ni,** Li-Cheng Tang*
	Breeding sites of main Bluetongue virus vectors in Belgian cowshed *Jean-Yves Zimmer , Claude Saegerman , Bertrand Losson , Eric Haubruge, **Frederic Francis***
	Insecticidal activity of Moringa oleifera seed extract against a dengue vector, Aedes aegypti L. (Diptera: Culicidae) in Faisalabad, Pakistan ***Muhammad Ashfaq**, Waqas Wakil, Umair Ashfaq*
	Natural product of Yartsa Gumba an entomo-fungal complex of Indian Himalayas ***Shahid sami siddique***
	Field efficacy of peridomestic insecticide space spraying against two Aedes populations in Tahiti, French Polynesia ***Herve C. Bossin**, Charles Jeannin, Jerome Marie, Stephane Loncke*

Code No.	Presentation Title *Authors_**Presenting author***
	Laboratory Evaluation of Efficacy of Bacillus thuringiensis Crude toxin (Bt.Ct) and Bt.Ct synthesized silver Nanoparticles (Bt.Ct-AgNps) against Human vector Mosquitoes ***Asanmoohamed Najitha banu**, Chelliah Balasubramanian, Puthamohan Vinayaga Moorthi, Thangavel kubendran*
	Prey preference of aquatic insects: implications in regulation of wetland mosquitoes ***Nabaneeta Saha**, Gautam Aditya , Goutam Saha*
	Insecticide resistance in An. arabiensis from Sudan: temporal trends and underlying mechanisms. ***Hiba Abdalla**, Lizette Koekemoer, Craig Wilding, Hilary Ranson, Maureen Coetzee*
	Evaluation of two storage mites life tables on dried oyster mushroom and their harmful effects at different constant temperatures ***Nevin ahmed** , Mo Wang*
	Molecular Detection of tick-borne pathogens in ixodid ticks from Pakistan ***Zafar Iqbal**, Nabanita Mukherjee, Steven Adamson, Zia-ud-Din Sindhu Shafiq Ullah, Arijo Abdullah, Dmitry Apanaskevich, Shahid Karim*
	A Long-Term Pattern of Prevalence of Dragged Ticks Captured in the Korean Peninsula from 2001 to 2010 ***Baek Jun Kim**, Sungjin Ko, Jun-gu Kang, Heung Chul Kim, Sung Tae Chong, Jeongmi Yoo A. Klein, Joon-Seok Chae*
	Influence of Antimicrobial Peptides Over-expressed in Damaged Gut Cells of Drosophila melanogaster ***Eun-Young Yun**, Young-Il Yoon, Jae-Sam Hwang, Tae-Won Goo, Eun-Young Kwon, Jeongmi Yoo*
	Contact and fumigant toxicity of Pinus densiflora needle hydrodistillate constituents and related compounds and efficacy of spray formulations containing the oil to Dermatophagoides farinae ***Ju-Hee Lee**, Jun-Ran Kim, Young Yull Koh, Young-Joon Ahn*
	Fumigant Toxicity of Plant Essential Oils Against Camptomyia corticalis (Diptera: Cecidomyiidae) ***Jun-Ran Kim**, Perumalsamy Haribalan , Bong-Ki Son, Young-Joon Ahn*
	Transovarial transmission of Orientia tsutsugamushi in Leptotrombidium palpale (Acari: Trombiculidae) ***Eun Hee Shin**, Jong Yul Roh, Won Il Park, Bong Gu Song,Chan Park, Mi-Yeoun Park, E-hyun Shin*
	Seasonal distribution of chigger mites surveillance using new chigger mite collecting traps in Korea (2010~2011) ***Won Il Park**, Jong Yul Roh, Eun Hee Shin, Bong Gu Song, Chan Park, Mi-Yeoun Park, Jin Suk Han, Hyeon Joo Lee, E-hyun Shin*
	Insecticide resistance monitoring in the principal Japanese encephalitis vector, Culex tritaeniorhynchus using bioassay and biochemical assays ***Hyun Kyung Kim**, Dae Hyun Yoo, Seong Yoon Kim, E Hyun Shin , Mi Yeoun Park, Kyu Sik Chang, Young Joon Ahn, Dong-Kyu Lee*
	Population density and Plasmodium vivax infection rates of Anopheles mosquitoes in the malaria epidemic areas, Incheon-si, Gyeonggi-do and Gangwon-do, in the Republic of Korea ***Tae Joon Lee**, Sun Jae Bang, Myung-Deok Kim, E Hyun Shin, Kyu Sik Chang*
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	Functional Genomics of Yellow-g and Yellow-g2 of The Asian Tiger Mosquito, Aedes albopictus ***Sung Hyun Kim**, Mi Young Noh, Yeon Soo Han, Yasuyuki Arakane*
	Repellent efficacy and safety evaluation of IR3535 derivative against Aedes albopictus, Culex pipiens pallens and Aedes togoi ***Sung Jin Park**, Mi Hee Yu, Ji Eun Kim, Min Ju Park, In Seon Lee, Jihoon Lee, Jinho Lee, Bae Hwan Kim, Dong Kyu Lee, Sam Pin Lee*

Development of a facile system for mass-production of *Brugia malayi* in a small-space laboratory

Prof. Wej Choochote, Ph.D.

Senior Researcher, Thailand Research Fund, Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand

E-mail: wchoocho@mail.med.cmu.ac.th

ABSTRACT

Brugia malayi is one of the important lymphatic filarial nematodes that cause elephantiasis in humans in the Asian region. Mass production at any stage of this nematode in both small laboratory animal hosts and mosquito vectors is still necessary in order to continue various research aspects. This has led to the search for reliable laboratory animal hosts and mosquito vectors for *B. malayi*. Thus, this study has developed a facile system for the mass-production of *B. malayi* in a small-space laboratory by combining benefits of the autogenous *Ochlerotatus togoi* colony (Thailand strain) with HBBS-rich-*B. malayi* microfilariae obtained from peritoneal withdrawal of *B. malayi*-infected jirds. The 2+ ml of HBBS-rich-*B. malayi* microfilariae could be prepared for at least 100+ ml of heparinized-blood containing *B. malayi* microfilariae, which is the main key for performing massive artificial feeding of autogenous *Oc. togoi* on blood containing *B. malayi* microfilariae. The high susceptibility rate of autogenous *Oc. togoi* to *B. malayi* has led to the mass production of L_3 larvae. This is an important key in performing massive subcutaneous and/or intraperitoneal inoculation of L_3 larvae into jirds in order to mass produce *B. malayi* developing stages and adults. Successful rearing of an autogenous *Oc. togoi* colony for many consecutive generations (F_{100+}) has led to the maximum reduction of animal-house space for maintaining blood-food animals, which are necessary for producing blood-feeding mosquito eggs, e.g., *Stegomyia aegypti* (Liverpool strain) and *Oc. togoi* (Taiwan strain). These aedines species have been used widely as efficient laboratory vectors for *B. malayi*.

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50th Anniversary Golden Jubilee
1964 - 2014
MALAYSIAN SOCIETY OF PARASITOLOGY AND TROPICAL MEDICINE

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GLOBAL CHALLENGES IN TROPICAL DISEASES:

Bridging Gaps and Building Partnerships

ABSTRACT BOOK

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KEYNOTE ABSTRACTS

Keynote Address - Future Threats and Global Challenges in Tropical Diseases

Pratap Singhasivanon
Mahidol University, Thailand

More than 2 billion people in 150 countries and territories suffer from tropical infectious and parasitic diseases, which kill several million people worldwide every year. The impact on worker productivity adds up to a loss of billions of dollars annually. For a very long time, tropical diseases have received little or no attention at all, despite their magnitude and their impact on both economic development and quality of life. Currently, the spread of tropical diseases is not only confined to the so-called tropical regions, which are traditionally associated with poor environmental hygiene and sanitation and limited access to basic healthcare.

With global climate change, air travel, urbanization, and extensive population movements, vectors of tropical diseases can thrive and expand beyond the formally defined regions to other latitudes with a new dimension of transmission affecting newly susceptible populations. The resurgence of dengue fever over the past decade to new geographical areas previously thought free of this disease is an example to this phenomenon. Rising trends of other vector-borne and food-borne parasitic zoonotic diseases will also likely become emerging global rather than regional problems. The high prevalence of intestinal parasitic infections among the urban poor, living in the informal settlements and shanty towns will also become increasingly challenging health problems in developing countries.

Recent global collaborations and substantial financial contributions from governments and the private sector have led to significant reductions in morbidity, disability and mortality of several important tropical diseases in many endemic countries. However, major challenges and threats will undoubtedly confront us in the future.

Keynote Address - Translational Research in Infectious Diseases

Shahnaz Murad
Institute for Medical Research, Malaysia

Although there is a wealth of knowledge in basic science, only a small fraction of promising discoveries are translated into new treatments and diagnostics. Despite massive R&D expenditure, the major medical needs are still unfulfilled and this is particularly true for infectious diseases. Translational Research is currently defined as the process of transforming research innovations into new health products and diagnostic and therapeutic methods. There are 4 to 5 phases of translational research and these form barriers.

The complexity of the translational process makes it unlikely that a single individual can be an expert in all the entire process, as it requires an overwhelming breadth and depth of expertise. Therefore the model of translational research requires a multidisciplinary team made up of basic researchers, clinicians, laboratory scientists and industry.

Translational research is challenging. Only a very small proportion of basic research makes it to therapies. The process is long, tedious and costly. The gap between research and clinical practice is often called the ‘valley of death’. This gap can be bridged with various innovative strategies.

Keynote Address - Tackling neglected tropical disease pathogens using next-generation technologies: prospects and need for public-private partnerships

Robin B. Gasser
The University of Melbourne, Australia

Many parasitic diseases have a devastating, long-term impact on human and animal health and welfare worldwide. Unlocking the molecular biology of these neglected pathogens, employing a raft of high throughput -omic and computer technologies will lead to entirely new ways of controlling them and will have substantial outcomes through the development of new drugs, vaccines and/or diagnostic tests. This talk will provide a perspective on breakthroughs made in this exciting field and will discuss biotechnological prospects that lie ahead.

Keynote session - Emerging Infectious Diseases in Southeast Asia

Paul Anantharajah Tambyah
National University of Singapore

Emerging Infectious Diseases were defined in a landmark report by the Institute of Medicine more than 20 years ago as diseases whose incidence had been increasing or threatened to increase in the coming years. For some reason, SE Asia has been the epicenter of a number of emerging and re-emerging infectious diseases from SARS to the Nipah virus to knowlesi malaria and a number of other pathogens. The key to understanding and controlling these pathogens is a comprehensive multi-disciplinary approach involving scientists, clinicians, epidemiologists as well as some we do not often work with including mathematicians, policy makers, evolutionary biologists, geographers and the like. The emergence of these diseases has provided tremendous opportunities for improving our understanding of endemic diseases as well as the health of the people of Asia.

Keynote Session - Our helminth infections: the bad and the good

Maria Yazdanbakhsh
Leiden University Medical Center, **Netherlands**

Helminth infections are highly prevalent in the world and can be associated with immunopathological conditions. However, majority of these infections appear clinically silent, which has been attributed to their ability to modulate the immune system. Indeed, helminth infections have evolved with their human host and are masters of immune manipulation. An important hallmark of helminth infections is the skewing of immune responses toward TH2. Population studies have shown increased intracellular IL-4 production and GATA-3 expression in CD4+ cells in areas where these parasites are highly endemic. Combining field studies in tropical areas where helminthes are endemic with molecular work in our laboratory in Leiden, it has been possible to show that helminths carry molecules that, in assays involving dendritic cell and T cell co cultures, lead to TH2 polarization. Characterization of one of these molecules, Omega-1 from *Schistosoma mansoni*, has helped to dissect the signals that are needed for TH2 polarization. It is becoming clear that TH2 responses can be involved in tissue repair and in glucose homeostasis. With respect to the latter, population studies have shown that in individuals carrying helminthes, the HOMA-IR is significantly improved, opening a new area for the use of immune modulators from helminth parasites in controlling Th1 and inflammatory diseases.

Sandosham Memorial Lecture - Tango with the parasites: Parasitic Diseases in the Disadvantaged community

Khairul Anuar B. Abdullah
MAHSA University, Malaysia

Prof Dr A.A. Sandosham, membership to MSPTM 0001 and elected a founder member on 10th Jan 1964. Professor Dr Sandosham was the first Professor and Head of the Department of Parasitology in the University Malaya, Singapore. He was an active researcher. He was a man who was highly intellectual and easily approachable. He was never tired of training young doctors to become a good Parasitologist. His dedication towards Medical training should always be an example to us.

The concept of neglected community traditionally cited: race, ethnic group or sex. Rather, the concept of neglected was focused on 'denial access to the tools needed for self-sufficiency.' People see themselves as disadvantaged to the extent they are denied access to and use of the same tools found useful by the majority of society. In this regard I would like to refer to two important disadvantaged communities. Migration is a phenomenon largely associated with economic and human resource needs or, at the extreme, survival. Although considerably less substantial than in North America and Western Europe at present, migrant labour is deemed to play an increasingly prominent role in the socio-economic development of the Asia Pacific region. In fact, 'importer' countries such as Malaysia and Singapore will depend on migrant workers to sustain their economic development while enabling 'exporter' countries, namely Indonesia, Philippines, Thailand, Bangladesh, Pakistan and Myanmar, to support its growing population to earn a much needed income. Up to 1990 there has been only one study on the effectiveness of a screening program for intestinal parasites among refugee populations. The Orang asli (aborigines) are the first people of the Melayu-Thai Peninsula. Various editions have been written about this community. Some officials in West Malaysia have chosen to ignore the problem, other has denied it exists, and still others have said that Orang Asli are negligent and thus justify their poor health. It is becoming increasingly clear, however, that the effects of helminthiasis are worse than they were assumed to be in the past. Current evidence points to a more significant role of helminthiasis in causing growth impairment and reduced cognitive and learning abilities in children. Both pre-school and school-aged children living in the disadvantaged communities are vulnerable to the problem of malnutrition and helminthiasis. While Malaysia has made great economic progress in the past few decades enabling a better standard of living for the people, there are still groups living in disadvantaged communities without basic health needs. They have a poor socio-economic background and lack proper sanitation and safe water supply. The Abrogine community in resettlement villages is an example of such disadvantaged community in Malaysia.

GOLDEN JUBILEE CONGRESS MSPTM

❖ History of MSPTM and the way forward

Speech by: CP Ramachandran

Malaysian Society of Parasitology and Tropical Medicine

❖ Investing In the Elimination and Control of Neglected Tropical Diseases in the Western Pacific Region: A Case for Support.

Speech by: Vicente Belizario

World Health Organization (WHO), WPRO

Parasitology: From Microscopy to Molecular

Fong Mun Yik
Molecular Parasitology Laboratory, Department of Parasitology,
Faculty of Medicine, University of Malaya,
50603 Kuala Lumpur, Malaysia

Microscopy is the gold standard method for the diagnosis of most parasitic infections. Malaria, filaria, and intestinal parasitic infections are some examples of diseases diagnosed through microscopic examination of specimens such as blood and stool. Microscopy, however, has disadvantages such as limited sensitivity and specificity, and it relies on competent and experienced microscopists. The era of molecular diagnosis began in the 80s with the introduction of DNA probes and hybridization technology. This technology promised more sensitive and specific detection of parasitic infections. However, in the early 90s hybridization technology was superceded by polynerase chain reaction (PCR) based methods. Since then, the advancement of PCR-based diagnosis has been very rapid, particularly for malaria. This paper highlights several novel findings on parasitic infections from our laboratory using PCR-based approaches.

Issues and Challenges in Vector Control: A Never Ending Journey

Indra Vythilingam
Parasitology Department, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

Vector borne diseases continue to plague the Southeast Asia region. Success in controlling some of these diseases over the years has been good. However, we now face the daunting task of controlling these diseases due to the changing landscape and environment. Mosquitoes have adapted themselves very well to the environment and now it is difficult to control these vectors with old tools. For instance malaria vectors are more exophagic and thus current control measures will not be effective. In addition malaria is now a zoonosis especially in Southeast Asia making the situation more complex. Cases of dengue is on the increase and without a vaccine and drugs, vector control is the only method to prevent dengue outbreaks. However, control methods for dengue vectors have not changed for the past forty years. *Aedes* mosquitoes are becoming more cryptic in their breeding habits making control difficult. For vector borne diseases as a whole the situation is exacerbated by unplanned urbanization, deforestation, rapid movement of pathogens, vectors and human by cheap jet travel. New tools will be needed to forestall epidemics and proactive approaches will be required for the prevention of vector borne diseases in this borderless world. Past achievements and future directions will be unveiled in the presentation.

Blastocystis - of paradigms and paradoxes

Suresh Kumar
Parasitology Department, Faculty of Medicine,
University of Malaya, Kuala Lumpur, Malaysia

The talk will trace the developments related to the biology, prevalence, biochemistry, pathology and chemotherapeutic approaches as well as discovering the paradoxes enshrouding this enigmatic organism called Blastocystis. The 20 year elucidation trails on many enigmatic aspects of this organism have generated us to rethink and shift our paradigms to question many fundamental aspects of the organism. The association and the role of his parasite in causing irritable bowel syndrome and colorectal cancer patients will be discussed.

MALARIA PLENARY

PfSPZ Vaccine for Malaria Elimination: Vision to Deployment

B. Kim Lee Sim,
Protein Potential LLC
Process Development and Manufacturing, Sanaria Inc.
Rockville, MD, USA

Radiation attenuated *Plasmodium falciparum* (Pf) sporozoites (SPZ) administered by mosquito bite to humans have been the gold standard for malaria vaccine development for four decades (Hoffman SL et al., *J Infect Dis* 185:1155-1164, 2002). They have been used as a model to identify the immune mechanisms and targets of protective immunity so as to develop highly effective, modern subunit malaria vaccines. However, thus far there is no such a vaccine. In 2003 it was proposed that because of the poor progress on subunit malaria vaccines, a whole PfSPZ vaccine should be developed. This proposal was greeted with skepticism and disparagement by virtually the entire malaria research community, because it was felt to be impossible to produce in compliance with current Good Manufacturing Practices (cGMPs) a live, attenuated vaccine in mosquitoes that met regulatory standards of purity, sterility, safety, and potency, would stand up to quality control release and stability assay specifications, and could be stored and shipped worldwide. In 2013 we reported complete protection against malaria in a clinical trial of our metabolically active, non-replicating whole PfSPZ vaccine, the PfSPZ Vaccine (Seder RA et al., *Science* 341:1359-65, 2013). We have overcome these challenges by careful, systematic scientific and empirical studies in parasitology, entomology, and cell biology, biochemistry including pharmaceutical chemistry, cryobiology, and immunology. This has included designing, building, and utilizing the world's only clinical manufacturing facility for such a vaccine. The progress we have made in each of these fields, and our plans for scale-up and manufacture for pivotal Phase 3 clinical trials and vaccine launch and deployment will be presented.

The road from concept to proof of principle to deployment of the PfSPZ vaccine for elimination of *Plasmodium falciparum* malaria

Stephen L. Hoffman,
Sanaria Inc, Rockville, MD, USA

The term, Vaccine that Interrupts Malaria Transmission (VIMT) was introduced by the Malaria Eradication Research Agenda (malERA) initiative [malERA Consultative Group on Vaccines. A research agenda for malaria eradication: vaccines. *PLoS Med.* **8**, e1000398 (2011)]. An ideal VIMT would induce protective immune responses against all stages of the parasite life cycle. However, the ideal single stage VIMT would prevent infection at the pre-erythrocytic stage of the parasite life cycle, thereby preventing erythrocytic stage infection and all parasite-caused disease and transmission of the parasite from humans to mosquitoes. The PfSPZ Vaccine, composed of radiation attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) is a pre-erythrocytic stage vaccine and the malaria vaccine closest to being able to be used as a VIMT. We recently reported the PfSPZ Vaccine to be safe and to completely protect from Pf infection six of six volunteers who received the highest dosage administered in a clinical trial (Seder RA et al., *Science* 341:1359-65, 2013). Clinical trials at 8 sites in Africa, Europe, and the United States will begin in late 2013 and the first half of 2014. These trials are designed to establish reproducibility of the safety and efficacy results of the first clinical trial, establish an immunization regimen that provides durable protection against all Pf strains with the least number of doses and least quantity of vaccine, identify an immunological test that predicts protection, and begin the implementation research needed to pave the way for mass administration campaigns. We have set an ambitious 4-year timeline for moving through the stages of clinical development to pivotal phase 3 clinical trials and then to licensure and demonstration of the capacity of the vaccine to eliminate Pf malaria from populations greater than 200,000 individuals. The plans for doing so, including the challenges we face, our strategies for overcoming them, and the roles of the numerous international partners involved in the process will be discussed.

SYMPOSIUM ABSTRACTS

Symposium Neglected Tropical Diseases I

S1.1- Chaotic situation of taeniases and cysticercosis as neglected tropical or zoonotic diseases in Asia

Akira Ito[1] and Munehiro Okamoto[2] and the working group in Asia*
[1]Department of Parasitology, NTD Research Laboratory, Asahikawa Medical University, Asahikawa, Japan, [2]Center for Human Evolution Modeling Research, Primate Research Institute, Kyoto University, Inuyama, Japan

Neurocysticercosis due to accidental uptake of eggs of *Taenia solium* is common in developing countries where people eat pork. It is transmitted from humans (taeniasis carriers) to both humans and pigs (cysticercosis), and emerging and reemerging worldwide. The life cycle of this parasite is completed between humans (tapeworm carriers contaminating environment with eggs after consumption of uncooked pork contaminated with cysticerci of *T. solium*) and pigs (cysticercosis caused after ingestion of eggs in human feces). Therefore, this disease is based on consumption of pork under poverty and expected to be a local disease in rural areas of developing countries where meat-inspection was not introduced, and was rare or not distributed in Muslim or Jewish societies in the 20 century. However, globalization with huge number of immigrants or refugees as labor and tourisms introduce cysticercosis everywhere even in Muslim or Jewish societies or developed countries including Japan. Nonetheless, we have almost no data on the real situation in any countries due to the lack of reliable tools to detect cysticercosis or identify the parasite. In Asia-Pacific, we have many other parasitic diseases including schistosomiasis, food- or fish-borne trematodiases, soil-transmitted helminthiases and fish- or meat-borne cestodiases. In any areas where we are facing these parasitic diseases, we are simultaneously facing unexpected outbreaks of cysticercosis. In this presentation, I will talk about the present situation of taeniases caused by three species and cysticercosis of *T. solium* in Asia and stress the background information of the reason why it is neglected.

S1.2 - Addressing key issues of soil transmitted helminthiasis in Orang Asli (indigenous) communities with modern technologies

Yvonne AL Lim
Department of Parasitology, Faculty of Medicine,
University of Malaya

As Malaysia aspires to become a fully developed nation, neglected tropical diseases such as soil transmitted helminthiasis (STH) is expected to abate. However, pockets of infections still linger in the country, for instance, in rural, suburban and minority communities such as the Orang Asli (indigenous). Most recent statistics have shown that infection rates are still remarkably high in some of these communities. In essence, it is challenging to craft and implement effective preventive and control strategies given the uniqueness and diversity of these indigenous communities. One of the key component in tackling this problem is to have a heighten understanding of these infections, taking into consideration the inter- and intra-diversity of these indigenous communities. Unfortunately, traditional tools which depended mostly on microscopy are not technically powerful in deciphering comprehensive information and accerelating accumulation of data. This presentation highlights the coupling usage of conventional and modern technologies such as high resolution melt (HRM) analysis, next-generation sequencing and geographic information system (GIS) and remote sensing (RS) approaches in bridging the gaps of knowledge in addressing key issues of soil transmitted helminthiasis in the Orang Asli. These valuable data will assist in tailoring control and preventive programmes in order that pragmatic customised strategies which have good synergistic effects can be formulated for the benefit of these communities.

S1.3 - Zoonotic infections of dogs and cats in the developing world and their Control

Dwight D. Bowman
Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY

Around the world, dogs and cats share their lives and often the housing of their human companions. Unfortunately, they often also share parasites with the human population in the area where they live. The two most common human parasites on the earth, *Toxoplasma gondii* and *Toxocara canis*/*Toxocara cati*, would not be with us if there were no cats and dogs. They are also, of course, the source of numerous other parasites which can sometimes be more localized and immediately troubling to the human host. These include parasites ranging from *Leishmania* species, *Schistosoma japonicum*, *Echinococcus granulosus* and *Echinococcus multilocularis*, *Dirofilaria repens*, *Ancylostoma braziliensis*; and a numerous list of other species that can be focal and local in their distribution. Dogs and cats have several types of relationships with humans, they can be well-cared for pets, dogs and cats that share companionship working dogs, strays, or feral. All can provide risks to the humans in the areas where they are found. This presentation will review some of the more common parasites of dogs and cats, and it will discuss means by which these agents can be minimized by treatment and control.

S1.4 - Eosinophilic meningitis caused by *Angiostrongylus cantonensis*: A neglected disease with escalating importance

Praphathip Eamsobhana
Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok Thailand

The rat lungworm *Angiostrongylus cantonensis*, a food-borne zoonotic parasite, has been recognized as the common pathogen associated with human eosinophilic meningitis or eosinophilic meningoencephalitis. This neurotropic nematode has a definitive rodent host and a snail intermediate host. The adult worms live in the pulmonary arteries of rats. Human is a non-permissive, accidental host. Transmission to humans is by eating of raw or undercooked snails, contaminated vegetables and paratenic hosts such as freshwater prawns, crabs, frogs or monitor lizards. Thousands of cases of human angiostrongyliasis have been documented worldwide. It is an infection of increasing public health importance as globalization contributes to the geographic spread of the disease. The parasite is on the move. It has spread from its traditional endemic regions of the Pacific islands and Southeast Asia to the American continent including the USA, Caribbean islands and Brazil. Recently, cases of angiostrongyliasis have increased rapidly. Most reports of the disease are from Thailand and Taiwan with increasing reports from mainland China. The emerging occurrence of the disease is due to modern food consumption trends and global transportation of food products. The infections posed challenges in clinical and laboratory diagnosis and in epidemiology and basic biology. Control of the parasite in nature is impossible and changes in eating habits can be difficult. Enhanced understanding of angiostrongyliasis epidemiology, increased public awareness about the risks associated with eating raw or undercooked food, and enhanced food safety measures are needed. The talk will put together the current knowledge on various aspects of the parasite and the disease it causes. More recent epidemiological data together with highlights on significant progress in laboratory investigation of *A. cantonensis* infection will be presented. Moreover, an international collaborative network for evaluation of diagnostic methods that provide opportunity for multi-centre blind testing and to share/distribute tasks to avoid repetitive efforts to improve the pace for developing well standardized diagnostic systems for human angiostrongyliasis remains to be discussed.

S1.5 - Current status and future trend of giardiasis and cryptosporidiosis in the Arabian peninsular

Mohammed A.K. Mahdy
Univisity of Science and Technology, Sana'a, Yemen

Giardiasis and cryptosporidiosis are waterborne diseases causing protozoal diarrhoea worldwide leading to significant morbidity and mortality in developing and industrialized nations. *Cryptosporidium* may also cause life-threatening diarrhea in immunocompromised patients. The two protozoa have been classified as neglected tropical diseases by WHO. The epidemiology of *Giardia* and *Cryptosporidium* is changing in parallel with the development in genotyping and subtyping technology. The prevalence of these protozoa among human in Arabian Peninsula (Arab Saudi, Kuwait, Bahrain, United Arab Emirates, Oman and Yemen) ranges from 1% to 35% for *Giardia* and from 1% to 70% for *Cryptosporidium*, depending on the country, study subjects and study design. Taking to consideration that these data are coming from research and that there is no surveillance system in place and that *Cryptosporidium* is not included in the routine examination, the scenario may be worse. *Giardia* and *Cryptosporidium* have also been isolated from animals. Genotyping identified both *Giardia* assemblages AI and AII to be prevalent in the region. Among *Cryptosporidium* species *C. parvum* is the most common species in the countries of Arabic Peninsula with IIa and IId subtype families being the dominant *C. parvum*, suggesting zoonotic transmission of *Cryptosporidium* may be common in these countries.

S1.6 - Mass drug administration: potential for reversing subclinical lymphatic pathology in *W. bancrofti* infection

[1]Bhagirathi Dwibedi ,[2] Birendra Kumar Das,[1] Shantanu Kumar Kar*
[1]Regional Medical Research Centre (ICMR), Nalco Square, Chandrasekharpur, Bhubaneswar, Odisha, India
[2]Utkal Institute of Medical Sciences and Imaging, Nayapalli, Bhubaneswar,Odisha

Natural course of lymphatic filarial infection in children accompanies prolonged asymptomatic phase till they attain adult hood. We investigated *W. bancrofti* infected (Og4C3 antigen or microfilarial positive) children from villages of Khurdha district of Odisha, India between 5-18 years age group with or without symptoms to elucidate any evidence of sub clinical lymphatic pathology and the effect of single dose DEC plus Albendazole currently used in mass drug administration programme . 102 enrolled children (52 asymptomatic and 50 symptomatic) were subjected to lymphoscintigraphy T_{99} sulphur colloid and ultrasonography to look for lymphatic pathology in the lower limbs and filarial dance sign (FDS) of adult worm respectively. Lymphoscintigraphy revealed lymphatic pathology in 73(71.5%) and ultrasonography detected FDS in 9(8.9%) out of 102 subjects. The children were randomized to receive either annual (n=51) or biannual dose (n=51) of DEC plus Albendazole. 102 enrolled children were followed 6 & 12 monthly while 90 and 50 subjects completed 18 & 24 months respectively. Repeat investigation at 6 & 12 months (n=72) with lymphatic pathology at baseline shown improvement in lymphatic flow in 66 (91.6%) & 70 (98.5%) cases respectively. Follow up at 18 (n=54) and 24 months (n=35) shown improvement in 53(98.1%) and 35 (100%) cases. The interim study indicated possible reversal of the lymphatic pathology with DEC plus Albendazole. This opportunity can be utilized for intervention to prevent further progress of disease. This evidence will be useful as a strong tool to improve compliance to MDA program especially in children benefiting lymphatic filariasis elimination programme.

S1.7 - The effect of retinoic acid levels against interferon gamma (IFNγ) and transforming growth factor beta (TGF-β) plasma induced by *Mycobacterium tuberculosis* antigen in the elementary school-age children with ascariasis.

[1]Lia Faridah, [2]Sunarjati Sudigdoadi, [1]Ajeng Pratiwi, [1]Ridad Agoes
1 Department of Parasitology, Universitas Padjadjaran Bandung
2 Department of Microbiology, Universitas Padjadjaran Bandung

Soil-transmitted helminth (STH) is an infectious disease that remains as a health problem in the world, especially in developing countries. In Indonesia, especially in urban areas, STH prevalence is still ranged from 7.7% -70% and mainly infect children aged 6-9 years. The most common worms that cause infection are *Ascaris lumbricoides*. Animal studies indicates that helminth infections may increase susceptibility to infectious diseases, such as reduction in the protective effect against infection with *Mycobacterium tuberculosis* (Mtb), and a decrease in the potential of the BCG vaccine. One reason is the decrease in IFNγ and TGFβ as protector in plasma. Retinoic acid is a form of vitamin A metabolite in the human body. Several studies have shown that retinoic acid can serve as imunomedulator, but the effect of retinoic acid against worm infections and Mtb infection is still unknown. This is an ongoing research which is aimed to find immunomodulatory substances that may help prevention of tuberculosis infection, both of preventive and curative. By using a cross-sectional study, the quantity of IFNγ and TGFβ were analyzed using Real Time PCR. The samples taken from PBMC culture that derived from peripheral blood positive children whom suffering from ascariasis and has been induced by Mtb antigen and treated with retinoic acid. Expected results are significantly increasing levels of IFNγ and TGFβ in PBMC cultures that had been treated with retinoic acid compared to control.

S1.8 - Soil-transmitted helminthes and *Mycobacterium tuberculosis* co-infection in aborigines of Malaysia

Lim Boon Huat[*1], *Wong Weng Kin*[1], *Rahmah Noordin*[2], *Norazmi Mohd. Nor*[2], *Mohd Akmal Dato' Dahlan*[3], *Puvaneswari, S.*[4], *Jalaluddin Ashraful Haq*[5]

[1]*School of Health Sciences, Health Campus Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.*
[2]*Institute for Molecular Medicine, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia.*
[3]*Hospital Orang Asli Gombak, 53100 Gombak, Malaysia.*
[4]*Pejabat Kesihatan Perak, 30590 Ipoh, Malaysia.*
[5]*Bangladesh Institute of Research in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh.*

Soil-transmitted helminths (STH) prevail in developing regions of Asia. *Ascaris lumbricoides* and *Trichuris trichiura* infect humans via oral-faecal route; whereas filariform of *Necator americanus* and *Ancylostoma duodenale* as well as *Strongyloides stercoralis* penetrate intact human skin. Similar to the intestinal helminths, *Mycobacterium tuberculosis* also infects an estimated 2 billion humans worldwide. Tuberculosis (TB) starts when the bacteria enter a susceptible host through inhalation of droplets released by active TB individuals. The biology and preventive measures for STH and *M. tuberculosis* are well understood, yet these ancient diseases still continue to thrive among impoverished humans in the developing world. There have been several reports to suggest that a positive association exists between these seemingly unrelated infections. This preliminary study aims to ascertain the relationship between soil-transmitted helminthiasis and TB in Orang Asli settlements in Malaysia. A total of 117 stool samples were collected from Orang Asli TB patients (n=69) and their healthy contacts (HC, n=48). Formalin-ether sedimentation technique was performed to detect the presence of helminth ova/larvae. Chi-squared tests were used to determine any significant differences between TB and HC in terms of each type of helminth infection or total STH. Interestingly, no significant differences were found between (i) TB/HC and total STH (p = 0.070); (ii) TB/HC and ascariasis (p=0.268); (iii) TB/HC and trichuriasis (p=0.073); (iv) TB/HC and hookworm infections (p=0.073). In conclusion, although 46.4% (32/69) of the Orang Asli TB patients sampled were co-infected with ascariasis (20%), trichuriasis (33%) and/or hookworm infections (26%), there were no statistical significant association between TB and any STH or total STH.

Dengue Symposium

S2.1 - The global strategy and challenges for dengue control

Raman Velayudhan
Department of Control of Neglected Tropical Diseases (HTM/NTD)
World Health Organization
20 Avenue Appia, CH-1211 Geneva 27. SWITZERLAND

Dengue is fast emerging pandemic-prone viral disease in many parts of the world. Dengue flourishes in urban poor areas, suburbs and the countryside but also affects affluent neighborhoods in tropical and subtropical countries. Reversing the increasing trend requires commitment and sustained support from partners, organizations and countries, as well as leadership and increased resources. A combined effort with focus on dengue as a public health problem in countries with substantial local and national resources must be effectively channeled through sound technical support. Dengue prevention and management can now exploit opportunities presented by promising advances in vector control technology interventions, diagnostics, and prognostic systems for triage, evidence-based clinical interventions and candidate vaccines under development. In order to realize these opportunities, we need to ensure they are implemented, coordinated and adequately resourced. The goal of the WHO global strategy is to reduce the burden of dengue. The specific objectives are to reduce mortality and morbidity from dengue by 2020 by at least 50% and 25% respectively (using 2010 as the baseline). These objectives can be achieved by applying existing knowledge.

S2.2 - Dengue challenge in Singapore

Ng Lee Ching,
Environmental Health Institute, Singapore

Despite Singapore's comprehensive and largely effective dengue surveillance and control programme, the country remains sensitive to dengue outbreaks, as evident by the 2013 outbreak which has yet to completely resolve. Low herd immunity, continuous importation of multiple variants of dengue viruses and presence of *Aedes aegypti* in the highly urbanised environment provide a delicate landscape for high transmission of the disease. Singapore's 2013 outbreak is driven by a new strain of DENV1, which was first detected last year. This has replaced DENV2 as the predominant serotype since early 2013. As a result of the strain replacement, there has been a record number (>22000) of confirmed dengue cases in 2013. Nevertheless, taking into account the low herd immunity, Singapore is likely to continue to face the dengue threat for an extended period of time. The talk will give an overview of Singapore's dengue situation and control strategy; and the potential of novel vector control tools available in recent years.

S2.3 - Dengue: Does age matter?

Sazaly AbuBakar
Tropical Infectious Diseases Research and Education Center (TIDREC), Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Dengue is a zoonotic mosquito-borne disease endemic in over 100 countries. World Health Organization (WHO) estimated that over 40% of the world's population is at risk of contracting the infection annually. In many endemic countries, dengue is a serious childhood disease affecting children as young as 4 months old. The demographic pattern of those who contract dengue however, is rapidly changing. The distribution of dengue among the different age groups nowadays, varies between countries and regions. In countries, such as Malaysia and Singapore, dengue is becoming more common amongst the young adults (ages 15 to 35). Dengue however, is relatively rarely reported amongst the elderly of 60 years old and above. Yet, according to the United Nations, by the year 2050, it is estimated that the population of elderly in the SEA are expected to reach 25% of the total regional population. Despite other complexities of dengue, age could be an important factor to consider in determining risk for severe manifestations of dengue.

S2.4 - The revised dengue case classification

Lucy Lam
Department of Pediatrics, faculty of medicine,
University of Malaya.

The revised dengue case classification (DCC) was introduced by World Health Organization's (WHO) Special Programme for Research and Training in Tropical Diseases (TDR) in 2009. Since the early 1970s, dengue has been classified into dengue fever, dengue hemorrhagic fever grades I and II and dengue shock syndrome grades III and IV (DF/DHF/DSS). The spread of dengue to other areas of the tropics and the adult population led to more diversity in clinical manifestations and modifications of the original DCC. Thus clinicians have questioned the shortcomings of this scheme. The issues revolved around the complexity of confirming DHF in clinical practice, misclassifying severe cases as DF, and the emphasis on hemorrhage rather than plasma leakage as the underlying problem in most severe dengue cases. A prospective cohort study in seven countries confirmed the difficulties in applying the DF/DHF/DSS criteria even in tertiary care hospitals, that DF/DHF/DSS do not represent levels of disease severity and that a clear distinction between severe dengue (defined by plasma leakage and/or severe hemorrhage, and/or organ failure) and (non-severe) dengue can be made using highly sensitive and specific criteria. Regional expert consensus groups in the Americas and Asia concluded that 'dengue is one disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome'. A revised scheme was developed distinguishing: dengue with or without warning signs and severe dengue. A study in 18 countries comparing the usefulness and applicability of the revised DCC to the DF/DHF/DSS showed that the former is better able to standardize clinical management, raise awareness about unnecessary interventions, match patient categories with specific treatment instructions, and make the key messages of patient management understandable for all health care staff dealing with dengue patients. Studies are under way on the predictive value of warning signs for severe dengue and on criteria for the clinical diagnosis of dengue.

S2.5 - Dengue virus disrupts the barrier functions of microvascular endothelial cell responses

Shamala Devi Sekaran[1], Anusyah Rathakrishnan[1], Yin-Quan Tang[1],Lee Sau Har[1], and Dennis J. Grab[2]
[1]Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur (Malaysia); [2]Department of Pathology, Division of Medical Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland (USA).

Dengue infections are characterized by increased vascular permeability leading to microvascular leakage and bleeding. The immunopathogenesis of dengue is often debated to be caused by host immune-mediated response (eg. cytokine storms) rather than a virus-mediated response leading to endothelium glycocalyx dysfunction. In this study, we aimed to evaluate the effect on dengue viruses (DENV) on endothelial cells (ECs) by application of the electrical cell-substrate impedance sensing (ECIS) system to monitor possible disruption of the ECs in a non-labeled real time manner. Following this, tight juctional proteins (ESAM, Claudin, Connexin, ZO-1, JAM-1, E-cadherin, Occludin, PECAM and Nectin) of these cells were evaluated using confocal microscopy. These tight juctional proteins were differentially expressed upon dengue infection. Lastly, oxidative stress caused by DENV was tested by the levels of total nitric oxide (NO), reactive oxygen species (ROS) and eNOS. For the first time, we show that infection of all 4 DENV serotypes (MOI 5) on the 2 ECsdisplayed an immediate response using the ECIS. Both THBMEC and HPMEC had reduced resistance (R) in the first 24 hours. However, while the HPMEC remained below the controls up to 72 hours, the THBMEC increased its barrier tightness after 24 hours. Upon ECIS modelling, the changes in DENV-infected ECs were mainly attributed by loss of barrier resistance (Rb) in both THMBEC and HPMEC. Due to the immediate response by Ecs against DENV, we then evaluated the tight junction proteins using confocal microscopy with focus on the first 24 hours of infection where pertubations to various proteins were observed. As oxidative stress have implied by vascular diseases, we noted differential response in the levels of NO, ROS and eNOS in DENV-infected Ecs across the same 24 hour period. Our findings may indicate that the microvascular ECs were activated upon DENV-infection. This activation seems to have occured within minutes and they behaved differently in different target organs. These findings provide the first support to show DENV are likely to disrupt the barrier functions of ECs immediately upon infection.

S2.6 - Innovative dengue vector control

Lee, H.L.
Medical Entomology Unit/Infectious Disease Research Centre, WHO Collaborating Centre for Vectors, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

Dengue is a serious mosquito-borne disease common in tropical and sub-tropical countries including Malaysia since its first description in 1901 by Skae in Penang. There is at present a lack of specific treatment and effective vaccines against dengue and the control of dengue depends solely on the suppression of the two most important vectors namely, *Aedes aegypti* and *Ae albopictus*. Despite intensive and extensive control efforts by health agencies, the disease continues to spread. This paper updates various innovations on control and surveillance of dengue vectors. Gene-based sterile insect technique using the RIDL technology for both *Aedes aegypti* & *Ae albopictus* control has now been actively researched and field trials are pursed to evaluate the effectiveness of the technology. The release of *Wolbachia*-infected *Ae aegypti* is another dengue control innovation. The infected mosquito cannot support development of dengue virus and has shorter life span. Other innovations include: autodissemination of insect control agents using ovitrap, autocidal adult and larva trap, outdoor residual spraying, insecticidal paint and biocontrol agent. In other innovations, outbreak prediction capability is enhanced by developing model based on environmental data and analysis utilising neural network. A lapse period of 3 weeks prior to outbreak can be predicted which gives ample time for remedial action. The detection of dengue virus in the vectors is another important tool in forestalling outbreak and molecular-based rapid detection method is available now. Such techniques are capable of detecting dengue virus infection in both adult and larval stage, thereby providing early warning of outbreak.

S2.7 - Mosquito population reduction using RIDL® technology

Renaud Lacroix, Richard Adey, Andrew McKemey, Luke Alphey
Oxitec Limited, 71 Innovation Drive, Milton Park, Oxford OX14 4RQ, United Kingdom.

The rise in dengue over the past 50 years has highlighted the lack of sustainable and efficient control of its primary vector *Aedes aegypti*. The development of genetic control has enabled the creation of possible alternative tools to traditional methods against mosquitoes either through population replacement or population suppression. RIDL® technology is one method that is well advanced and has completed field trials recently. Its principle is based on an improvement of the Sterile Insect Technique (SIT) which has been adopted operationally for over 50 years for the suppression or elimination of economically important insect species, including the New World Screwworm and Medfly. After over 10 years of development and testing in the laboratory and contained conditions, the first open releases and suppression trials using RIDL® SIT have been successfully conducted. In 2010, the first releases of RIDL® male *Ae. aegypti* achieved 80% reduction of the target wild *Ae. aegypti* population in the Cayman Islands. In Brazil in 2012, sustained releases over a year similarly resulted in 85% reduction in Itaberaba, Bahia State. In 2013, Mandacaru, in Bahia State, was treated successfully over 6 months with a 95% reduction in the target *Ae. aegypti* population which was maintained with lower rate of releases for a further 6 months. With the smaller scale experiment conducted in Malaysia in 2010, releases in these three countries have confirmed the suitability of the modified RIDL® male *Ae. aegypti* in terms of survival, dispersal, mating competitiveness against their wild counterparts and overall improved fitness compared to irradiated mosquitoes. These trials have also developed our knowledge of mass rearing, maintenance and release of the genetically sterile males and opened the door to larger scale programmes such as that currently underway in Jacobina in Brazil, which aims to cover a human population of 50,000.

Symposium Veterinary Parasitology

S3.1 - Emerging and re-emerging diseases – Let's get out of the comfort zone

Ramlan M. & Chandrawathani P.
Veterinary Research Institute,
59 Jalan Sultan Azlan Shah, 31400 Ipoh, Malaysia

Since the early 1900's there are several high profile emerging and re-emerging infectious disease outbreaks in this country causing severe economic losses and public health impact. A continuous effort by the relevant authorities to combat and control these infectious diseases has shown that we have won in each battle as evidenced in our records. Experience with a few zoonotic disease outbreaks in the last 10 to 15 years such as Nipah, H5N1, Q-fever, Lepto, Brucella or Sarcocystis has been a valuable learning curve for the DVS with increased awareness among related government agencies and also livestock industry players in the issues of Biosafety and Biosecurity. With the concept of One Health, collaboration between the various agencies is essential to effectively control the introduction of new emerging diseases and prevent them from spreading. This has created confidence among the public on food safety and security and resulted in a disease free country namely, from many infectious diseases for many years. However, animal disease is still considered as an economic and public health threat in this country. It is mostly due to the rapid population growth, unplanned urbanization, antimicrobial usage, social change and mass movement of people especially in the region. Malaysia is also subjected to high disease risk because we are surrounded by the countries where most of the infectious diseases are endemic. Previous records have shown that emerging and re-emerging infectious diseases occur every three years and major outbreaks will take place in a 5 to 10 year cycle periodically. Can we predict what will happen in next 3 to 5 years?

S3.2 - Veterinary parasitology – Resistance, R&D and regulation – the new 3 Rs

Peter Holdsworth,
Holdsworth Research Ltd,
United Kingdom

Twenty first century usage of parasiticides is challenged by the phenomenon of resistance development within parasites. This phenomenon affects both production and companion animals traversing ecto, endo and endectoparasiticides usage. The emergence of resistance has been documented ever since exposure of parasites to parasiticides occurred: a chronological record exists through the last two centuries of parasiticide discovery, development, usage and resistance emerging in parasites. Parasiticide resistant parasites is now an ever present challenge however it is not unique as similar phenomena occur elsewhere such as with bacteria and resistance to antimicrobial pharmaceuticals. A “One Health” strategy is advocated to manage and prevent further emergence and spread of parasiticide resistance in parasites. Initiatives to remove regulatory inefficiencies and duplication in data requirements linked to authorising usage of parasiticides by national and international regulators are underway so to offer indirect incentives for investment in discovery and development of new parasiticides plus alternatives such as vaccines. Industry supported incentives such as VICH and WAAVP along with government initiatives such as Codex are leading the way here. Such initiatives aimed at harmonisation of regulatory requirements for authorising the usage of new parasiticides, along with national government initiatives such as R&D business taxation credits plus data protection/intellectual property rights legislation should encourage further investment in the discovery and development of products. For the foreseeable future parasiticides will remain a cornerstone for parasite management. Their availability and judicious usage will support food production, food security, environmental protection, public health and animal welfare plus assist producers generate an income from agriculture.
*Research, Development and Extension

S3.3 - Biozoonosea: A regional biotechnology platform for research and training on zoonosis in Southeast Asia

Stéphane Herder[1], Marc Desquesnes[1], Serge Morand[2]
and Sathaporn Jittapalapong[3]
[1]UMR Intertryp IRD/CIRAD, Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand
[2]Institut des Sciences de l'Evolution, CNRS-UM2, Université Montpellier 2, 34095 Montpellier, France
[3]Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand

Most of the emerging infectious diseases are zoonotic; thus, most of the new arising human diseases are derived from animal pathogens. By nature, the fields of human and veterinary medicine are complementary and synergistic in confronting, controlling, and preventing zoonotic diseases from infecting across species. These facts were converted into the "One health" concept that is nowadays generally spreading into the scientific community. Southeast Asia (SEA) is characterized by a tropical location and a high biodiversity, threatened by rapid environmental changes under the pressure of the economic development and insertion into the global economy, thus, it is known to be a hotspot for infectious emerging diseases spreading, regarding the high human and domestic animal density, globalization and intensification of trade, combined to drastic land use changes and biodiversity erosion. The Faculty of Veterinary Medicine (FVM), Kasetsart University decides to create a biotechnologic platform for research and training, devoted to epidemiological studies of zoonotic diseases, from field to laboratory, including reservoir and vector studies, specialized laboratory, geographic information system (GIS) and web resources. This platform will host together projects, technicians, students (Masters and PhD) and confirmed scientists. The main goal of this platform would be to generate and deliver workshops / trainings (for technicians, Master and PhD students or scientists) and student supervising in order to acquire specific conceptual, technical and/or methodological skills related to zoonotic diseases and to implement specific research programs in the field of zoonoses.

S3.4 - Expression regulation mechanisms of epimastigote stage-specific genes in African trypanosome

Noboru Inoue and Keisuke Suganuma
National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan.

Many gene expressions in Kinetoplastida are regulated post-transcriptionally. Although previous studies have revealed that stage specific gene expression in trypanosomes was regulated by *cis*-elements locating at 3'-untranslated region (UTR) of mRNA and RNA binding proteins using bloodstream and procyclic forms, no studies have been done in epimastigote form (EMF) of African trypanosomes. Our study revealed that gene expression of *congolense* epimastigote specific protein (*cesp*) was regulated by *cis*-elements located in the 3'UTR. Using transgenic trypanosome cell lines with different *egfp* expression cassettes, we showed that an adenosine and uridine rich region is one of the regulatory elements for epimastigote form (EMF) stage-specific gene expression via the regulatory *cis*-element of the eukaryotic AU rich element (ARE). Therefore this required element within the *cesp* 3'UTR was designated as *T. congolense* ARE. This required *cis*-element might selectively stabilize mRNA in the EMF stage and destabilize mRNA in other stages. By RNA electro mobility shift assay, unknown stage-specific RNA binding proteins (RBPs) whose sequences specifically interacted with the required *cis*-element were found. These results indicate that EMF stage specific *cis*-element and RBP complexes might specifically stabilize *cesp* mRNA in EMF.

S3.5 - Mechanical transmission of blood pathogens by biting insects: From experimentation to mathematical model; news & perspectives

*Marc Desquesnes[1&2 *], Jérémy Bouyer[1&3], Theeraphap Charoenwiryapaph[4], Gérard Duvallet[5] & Sathaporn Jittapalapong[2].*
[1] Centre de Coopération Internationale en Recherche Agronomique pours le Développement (CIRAD), Montpellier, France
[2] Departments of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand
[3] Institut Sénégalais de Recherche Agricole (ISRA), Laboratoire National d'Elevage et de Recherches Vétérinaires (LNERV), Service de Parasitologie, Dakar, Sénégal,
[4] Departments of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
[5] Centre d'Ecologie fonctionnelle et évolutive (CEFE) (UMR 5175), Université de Montpellier, Montpellier, France

Biting insects such as tabanids and stomoxes are present worldwide in all kinds of landscapes and climates. In humans and animals, these insects are not only blood feeders and annoying pest, they are also acting as mechanical vectors of a number of pathogens, some of them being zoonotic agents, including bacteria, viruses and parasites. To assess the importance and main parameters of mechanical transmission, we performed a series of experiments on mechanical transmission of African trypanosomes by *Atylotus* sp. (Diptera: Tabanidae). The main parameters were either quantified (parasitaemia, insect burden, daily prevalence) or estimated (unknown parameters), and a mathematical model was developed. Within 3 weeks of exposure to insect bites, the incidence of the infection was above 60%, which demonstrated the efficiency of mechanical transmission. Number of insects and level of pathogens in the blood were the main parameters of transmission, which proved to occur when the pathogenemia was above 100.000 infective doses per ml of blood. *Stomoxys* would not only act as immediate transmitter, such as tabanids, they are also suspected of delayed transmission by regurgitation of blood from crop or gut, which may considerably impact their role in the epidemiology of the diseases they transmit. Mathematical models could be adapted to various pathogens, providing their specific parameters be established in experimental conditions. Based on a better knowledge of their nuisances and their biology, new means of control of tabanids and stomoxes are currently under study to specifically attract these insects to traps or toxic targets.

S3.6 - ESCCAP: A model to combat global parasitic zoonoses?

M A Fisher
Affiliation: Shernacre Enterprise Ltd, The Mews Studio, Portland Road, Malvern, Worcs., WR14 1UX

The global cost of human disease caused by parasitic zoonoses is high. For example, cystic echinococcosis, identified by the World Health Organisation (WHO) as one of seven neglected parasitic zoonoses in 2006, is estimated to cost in excess of 1 billion dollars per year. Those most affected are often in developing communities where treatment may be problematic. There are numerous organisations that share the aim of reducing human disease from parasitic zoonoses, ranging from the WHO to charities; however the continuing scale of human disease indicates that there is scope for more to be done. Resourcing education and control is costly and so initiatives must be targeted to obtain the greatest impact for the available resource.

ESCCAP (European Scientific Counsel Companion Animal Parasites) is a not-for-profit company with the aim of controlling parasitic diseases of companion animals so that they are no longer a cause of disease in humans or animals in Europe. It provides a recognisable, standard brand to accompany parasite control messages targeted at both the veterinarian and pet owner. The messages are produced through Europe-wide consensus guidelines, published in English, however made available for customisation and translation into other languages. The company shares resources on a Europe-wide basis, and uses websites and other web-based media thereby reducing costs. The ESCCAP network of veterinary parasitologists and public health experts support the “One Health” initiative by organising joint meetings of veterinarians, medical professionals and public health organisations, such as Echinococcus2014.

ESCCAP has been operating for over 6 years during which time its guidelines and materials have been translated into over 8 languages, and it is widely referred to and cited in the literature. It provides a model which can be adapted for other regions of the world where zoonoses are an ongoing threat to public health.

S3.7 - Three decades of zoo and wild animal parasites in peninsular Malaysia

Vellayan Subramaniam[1] and John Jeffery[2]
[1]Faculty of Pharmacy, Universiti Teknologi MARA, Campus Puncak Alam, 42300 Selangor.
[2] Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur.

The Malaysian National Zoo is home to numerous animals both exotic and local. There are about 5446 animals including mammals, birds, reptiles and fishes. They are held in captive situation as free – ranging or in cages or aquarium. Although adequate medical care is directed towards their well – being, they however, are susceptible to parasitic and other infestations / infections. Organisms, namely parasites obtained from them over three decades are presented here. In addition ectoparasites recovered are also discussed. Some organisms obtained during this period include:1.Nematodes: *Trichuris trichiura*, hookworm, *Dirofilaria immitis, Dirofilaria repens, Oxyspirura mansoni, Loxodontofilaria asiatica, Setaria cervi,* and *Syngamus trachea*;2.Trematode: *Eurythrema pancreaticum, Fasciola hepatica, Paramphistomum epiclitum* and *Pfenderius papillatus*; 3.Cestode: *Variolepis* sp., *Raillietina celebensis, Raillietina echinobothrida* and *Raillietina misroscolecina;*4.Protozoa: *Sarcocystis* sp*., Blastocystis hominis, Balantidium coli, Entamoeba histolytica, Entamoeba coli, Giardia lamblia* and *Chilomestix mesnilii*;5.Arthropods: *Armillifer moniliformis* (pentastomid), *Cephalopina titillator* (camel bot), *Gyrostigma pavesii* (rhino bot*), Chrysomya bezziana* (myiasis causing fly), *Haematomyzus elephantis* (elephant lice), *Haemaphysalis nadchatrami* (tick), *Rhipicephalus* sp. (tick). Infestation / infection of many the above organisms results in fatal outcome to important exhibits.

S3.8 - *In vitro* assessment of the viability of *Dicrocoelium dendriticum* Metacercariae

Douglas D. Colwell, Cameron P. Goater, Melissa A. Beck, Dawn Gray, Alycia Ivarson.
Department of Biological Sciences, University of Lethbridge

Dicrocoelium dendriticum is a parasite of the liver of a wide variety of mammals. This trematode has achieved iconic status for its effect on the behaviour of ant second intermediate hosts. Ants can harbour up to 300 metacercariae, but whether they are all viable is unclear. A combination of trypsin and bile salts in saline at 37^{o}C was used to assess the viability of metacercariae recovered from *Formica fusca* collected from several sites in Cypress Hills Interprovincial Park, Alberta, Canada. Metacercariae from each site were pooled and placed in 24 well plates to which combinations of excystation medium was added. Saline with no trypsin or bile salts or distilled water were included as controls. Between 20% to 90% of metacercariae excysted after exposure to trypsin and bile salts, while no excystation was observed in the saline or distilled water. Significant variation in percent excystation between ant-collection sites in the Park and between collection dates was observed. The reason for variability among sites and collection dates remains a matter of speculation, but could be the result of age of the infection which may have resulted in an increase rates of metacercariae mortality.

S3.9 - Filarioids found in birds, bats, rodents and a treeshrew in Krau wildlife reserve, Pahang, Malaysia

Alwyn Cabil Bundong[1], Saharul Kasim[1], Rasul A. Halim[1], Masako Fukuda[2], Shigehiko Uni[1] and Rosli Ramli[1]*
[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Research Promotion Project, Oita University, Oita 879-5593, Japan.

Some filarioids can cause zoonotic infection in humans. Wild animals in Kuala Lompat, Sungai Teris and Kuala Serluk in Krau Wildlife Reserve, Pahang, were examined for filarial parasites and other helminthes between March and September 2013. A total of 90 wild animals consisting of 30 birds, 39 bats, 20 rodents and one treeshrew were examined with the use of skin snips and blood smears for microfilariae. Adult filarioids were searched in the animals by dissection under a stereomicrosope. The infection rates of filarioids in birds, bats and rodents was 53%, 8% and 10%, respectively; one treeshrew was infected with filarioids. Three species of birds (*Trichastoma bicolor*, *Copsychus malabaricus* and *Malacocincla sepiaria*) showed coexistence with two or three filarioid species based on microfilariae. The co-occurring of more than one species of filarioids may affect the structures and behavior of host-parasite communities. In *Copsychus malabaricus*, 10 filarioids of *Pelecitus* sp. were found in a swollen footpad of the right leg. This finding appears to be important because *Pelecitus* sp. has caused zoonotic filariasis in humans. Three bats (one *Balionycteris maculata* and two *Hipposideros bicolor*) were infected with filarial parasites. *Tupaia glis* and *Maxomys rajah* showed the coexistence of two different species of filarioids. The phylogenetic tree was constructed to show the relationship of the species found in the birds with other filarioids. The results of this study indicated the current status of filarial infection in wild animals in the Krau Wildlife Reserve, Pahang, Malaysia.

Symposium Sigma-Tau

Advancing And Optimizing Treatment For *P. falciparum* & *P. vivax* Uncomplicated Malaria: Which Role For Eurartesim® (DHA-PQP)

S4.1 - From Abele Sola to Ho Chi Minh: Malaria historical references & treatment

Marco Corsi
Senior Consultant Tropical Diseases,
Rome, Italy

Intermittent fevers have accompanied the path of humanity for many centuries. In particular, that, at the end of the Middle Ages, were defined “mal'aria”, were believed to be caused by the miasma exhaled from stagnant waters and marshes. Even when the first effective treatment, the powder of the Countess of Cinchòn, became available, the cause of these fevers remained unknown. It was only at the end of the XIX century that most of the information on the cause and transmission of malaria were discovered. There are many paths of scientific knowledge that have crossed the brilliant insights of Hippocrates and Varrone, the therapeutic approaches of Talbor, Sydenham and Torti, the devotion to the cause of Manuel Incra Mamani and the discoveries of Laveran, Ross and Grassi.

With the aim to be a little bit unconventional (this should intrigue the audience), we have chosen to include in the title the fil rouge that binds the almost completely unknown Abele Sola with the very well-known Ho Chi Min.

S4.2 - Resistance to artemisinins and/or to the partner drugs: Update and what if it will spread

Nicholas J White
Oxford-Mahidol University,
Bangkok, Thailand

Considerable increases in the availability and use of artemisinin-based combination therapies (ACTs) together with increased deployment of insecticide treated bed-nets (ITN) have resulted in a substantial fall in global malaria morbidity and mortality. These gains and the prospects for malaria elimination are now threatened by the emergence of artemisinin resistance in Plasmodium falciparum. It is essential that all possible measures are taken to prevent spread of resistance genes to African malaria parasite populations.

Artemisinin resistance was reported first from Western Cambodia, where resistance to previous antimalarial drugs also first emerged, but has since either spread or emerged in other areas in mainland SouthEast Asia. There is now evidence for a decline in the efficacy of ACTs in foci of artemisinin resistance. This reflects the reduced contribution of the artemisinin to treatment efficacy, and inevitably places greater selection pressure on the partner drugs. In most of South-East Asia malaria transmission is low and seasonal, although the epidemiology there has certainly been underestimated. Plasmodium vivax comprises approximately half the malaria infections and is more difficult to eliminate than P. falciparum. ITN benefits are often small in settings with exophilic early evening biting vectors and a highly mobile adult population. Early diagnosis and effective treatment is the mainstay of malaria control. While the incidence of falciparum malaria continues to fall in this region there is still an opportunity to eliminate malaria, but if treatment efficacy falls further, and incidence begins to rise again, elimination of P. falciparum will not be possible without new medicines. Radical approaches may be necessary to achieve elimination of artemisinin resistance before it spreads to infect Africa. It is not clear whether there is an appetite for such radical action.

S4.3 - DHA-PQP in combination with primaquine for vivax malaria radical treatment

Kevin Baird
Eijkman Oxford Clinical Research Unit, Jakarta, Indonesia

Primaquine kills the liver dormant liver stages of Plasmodium vivax that cause multiple clinical attacks over a year or two. Chloroquine and primaquine have been used for radical cure of vivax malaria since 1952. Worsening resistance to chloroquine by P. vivax requires new partner blood schizontocides with primaquine for radical cure.

That task is complex and difficult because drug-drug interactions may impact the safety, tolerability, and efficacy of primaquine against relapse. These features of primaquine therapy may not be presumed with any given new partner drug(s). They must be ascertained.

Reliably estimating the efficacy of primaquine is notoriously difficult. Relapse occurs over months, as does reinfection, and these events cannot be distinguished by laboratory techniques. Moreover, relapse occurs at highly variable rates and intervals.

Studies in Indonesia have addressed these problems by treating heavily exposed soldiers returned to an area where reinfection is highly improbable, and by measuring the natural relapse rates among them in the absence of primaquine. These studies confirmed the good safety and efficacy of primaquine against relapse of P. vivax when partnered with dihydroartemisinin-piperquine for radical cure.

S4.4 - Can the efficacy of DHA-PQP be increased in children ?

Francois Nosten
Shoko Malaria Research Unit, Mae Sot, Thailand

DHA-piperaquine is one one the major ACT commercialised in endemic countries. It is safe and effective in the treatment of P.falciparum and P.vivax malaria. Recent studies in South East Asia and in Africa have focused on the pharmacokinetics and the pharmacodynamics of piperaquine, the partner drug that is crucial for cure.

These studies indicate that the dose of piperaquine recommended in young children is suboptimal, because the kinetics of this drug are different in young children compared to adults. These results and the modelling of the PK/PD relationship suggest that the dosage of DHA-piperaquine should be modified for the youngest groups of patients.

S4.5 - The role of the MMV-Sigma Tau partnership in developing eurartesim as a quality act for malaria endemic countries

George Jagoe (Switzerland)
MMV Executive Vice President-Access
Product Management, Geneva, Switzerland

By the late 1990s, it was evident that “market dynamics” had failed to create the proper incentives for pharmaceutical companies to invest beginning-to-end in the discovery and development of new treatments for malaria. In the face of widespread resistance to existing medicines, malaria endemic countries were defenseless in the battle to treat the most lethal form of this disease.

A medicine for Malaria Venture, a non-profit Swiss foundation, was created in 1999 to help address the gap in the development of new effective treatments for malaria. In the past 15 years, MMV has catalyzed the largest drug development pipeline for malaria prevention & treatment, working with committed pharmaceutical partners, research institutions, and independent expert advisers. It has partnered with industry in the development and launch of three new prequalified artemisinin combination therapies, one new prequalified treatment for severe malaria, and one prequalified medicine for seasonal malaria chemoprevention.

This presentation will describe the principles and practicalities of MMV are pioneering work as a public-private drug development partnership (PDP). It will then review the highlights of its strong collaboration with sigma-tau in the development of Eurartesim and in the drug’s introduction into malaria endemic countries.

Symposium Omics in Parasitology

S5.1 - Metagenomic approach to identify tick-borne pathogens by using ultra high throughput DNA sequencing and data analyzing technologies

*Yongjin Qiu, Ryo Nakao and Chihiro Sugimoto**
Research Center for Zoonosis Control, Hokkaido University
Sapporo 001-0020, Hokkaido, Japan

Tick can transmit a variety of viral, bacterial and protozoal pathogens, which are often zoonotic. The fact that there are up to 9 rickettsial diseases caused by spotted fever rickettsia newly reported between 1984 and 2001 indicates there are many potential pathogens which have not been unidentified or found in ticks. Regarding emerging tick-borne viral diseases, several fatal cases, severe fevers, thrmbocytopemia syndrome (SFTS virus) which had been originally reported in China were reported in Japan in 2013. Since metagenomic analysis provide a powerful tool to detect unculturable microbes from environmental samples such as sea water, hot spring and soil, and analyze microbial floras in mammals, we used similar approach to analyze tick "microbiome". In order to analyze tick mirobiome, we use several approaches. Firstly, we prepared bacteria-enriched fraction form the lysates of tick whole body, and purified genomic DNA samples. Data obtained by the second generation sequencer (Roche FLX454) were analyzed by using Batch Learning Self-organizing Map (BLSOM) and BLAST. As the results, sequences derived from Chlamydiae were frequently found in *I. persulcatus* and *H. flava*, and those from Rickettsiales were dominant in *I. ovatus*. In addition, gene sequences related to bacterial virulence were found. Secondly, we have analyzed microbes in ticks by analyzing amplicon obtained by PCR-amplification by using primers specific for the V1-3 domains of bacterial 16S rRNA genes. From Ixodes ovatus, we could detect bacteria of over 100 different genera including *Rickettsia*, *Ehrlichia* and *Coxiella*. In salivary gland preparations of *Ixodes ovatus* and *I. persulcatus*, limited ranges of microbial population including *Rickettsia*, *Ehrlichia* were detected. These results help us to construct a database of tick microbes which may contain unknown zoonotic pathogens. We are currently trying to develop methods to detect RNA viruses which have potential to cause human and animal diseases. Our efforts to develop the tick pathogen database may lead to the empowerment to predict emerging tick-borne diseases.

S5.2 - Metabonomics: Concept and application in parasitology

Ivan Yap Kok Seng
International Medical University,
Kuala Lumpur. Malaysia

Metabonomics was introduced in the late 90s and has since proven invaluable in uncovering and decoding metabolic signatures of health, disease and various other biological challenges. The most widely used definition for metabonomics is "… the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification". Metabonomics enabled assessment of global metabolic responses of the organism in response to a stressor and, coupled with advanced statistical pattern recognition analyses, offer an unbiased untargeted approach to identifying and understanding metabolic pathways that are perturbed in a disease state. A range of analytical tools such as high-resolution nuclear magnetic resonance spectroscopy and mass spectrometry combined with multivariate statistical analysis can be employed to create comprehensive metabolic signatures of various biological samples. Metabonomics strategy was first employed in parasitology with the study of *Schistosoma mansoni*-infected murine model. This initial study revealed a plethora of metabolic effects by the parasite on both the host and the commensal gut microbiota. It showed that parasitic infection not only affect host endogenous metabolism but host gut microbiome as well. The ability to obtain metabolic information from multiple compartments and analyse the data simultaneously allows metabonomics to play an important role in bridging the gap in understanding parasitic infection in multi compartmentalised 'superorganism'. Such fingerprinting strategy may prove useful in future for early diagnosis and monitoring host-parasite response to therapeutic intervention.

S5.3 - Discovery of novel targets for coccidiosis control via transcriptome analysis

Kiew-Lian Wan
School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor DE, Malaysia

Coccidiosis is an intestinal disease caused by the protozoan parasites *Eimeria* species. This disease has most impact in the poultry industry where almost all of the over 50 billion chickens reared worldwide annually are exposed to and thus must be either medicated with prophylactic anticoccidial drugs or vaccinated to prevent the disease. However, both methods of control have their limitations that confine their effective and widespread application. Persistent use of anticoccidial drugs reduces their effectiveness due to the emergence of drug-resistant strains, whilst vaccination with live vaccines is hampered by constraints and high cost associated with the production of live parasites. These underpin the importance of transcriptome analysis as an approach to the discovery of gene products that may be useful biological drug targets or vaccine candidates. In recent years, a number of parasite transcriptomics efforts have been initiated, and various transcriptomic data including expressed sequence tags (ESTs), open reading frame expressed sequence tags (ORESTES), full-length cDNAs and RNAseq data, have been generated. This valuable resource allows for the identification of novel molecules in these parasites, and provide an opportunity to generate valuable information underlying their biology, which will be crucial for the design of future, more effective anticoccidial agents. Identification and characterisation of such molecules will be highlighted.

Symposium Vector Control and Insecticide Resistance

S6.1 - Urban pests revisited – What comes next

Ridad Agoes
Padajaran University,
Bandung, Indonesia

The 20th century and the early 21st century have witnessed important changes in urban development influenced by booming economy, and changes in the urban environment, climate change and communication that favour urban pest. Currently, Asia and the rest of the world are facing dramatic development of urban megapolitan where cities expanded into area of the natural habitat of mosquitoes, ticks, termites and rodents. All these developments create directly or indirectly an impact by urban pests on human health. Vertical or hanging garden, wood paneling, wall-to-wall carpeting can all present opportunity and encourage pests and pest-borne diseases into urban communities. The seriousness of public health significance of urban pests and the medical condition, as well as the economic burden they create, force us to reconsider our future perspective in dealing with the ever growing situation.

S6.2 - Field evaluation of DEET and picaridin repellents reveals differences in repellent sensitivity between Southeast Asian vectors of malaria and arboviruses

Durnez Lies[1], Mao Sokny[2], Karel Van Roey[1], Vincent Sluydts[1], Tho Sochantha[2], Marc Coosemans[1,3]
[1] Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[2] National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia
[3] Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

Scaling up of insecticide treated nets has contributed significantly to a substantial malaria decline. However, some malaria vectors, and most vectors of arboviruses, bite outdoors and in the early evening. Therefore, topically applied insect repellents may provide a crucial additional protection against mosquito-borne pathogens. Among topical repellents, DEET is the most commonly used, followed by others such as PMD or picaridin (icaridin/KBR 3023/Saltidin®). A study was carried out in Cambodia to determine the entomological efficacy of DEET and picaridin repellents on wild populations of several mosquito genera, including vectors of arboviruses (*Aedes aegypti* and *Ae. albopictus*) and malaria (*Anopheles dirus*, *An. minimus*, *An. maculatus* and *An. barbirostris*). During 230 survey days in two consecutive years, the lower limbs of 5 persons were treated with repellents ('DEET 20%', 'picaridin 20%', or 'picaridin 10%') or ethanol (2 negative controls), followed by mosquito collections on the treated limbs during 5 consecutive hours. The treatments were grouped following a 5x5x5 Graeco-latin square to equalize the effects of treatment days, collection sites, and test persons. Protection rates were high (91-99.2%), with significant differences between treatments, genera, and species. For malaria vectors, 'DEET 20%' performed better than 'picaridin 20%' or 'picaridin 10%'. The protection rate against *An. barbirostris* was significantly lower as compared to the other vectors, especially for the picaridin repellents. As malaria endemic areas often differ in their vector species composition, this heterogeneity in repellent sensitivity between vector species might result in a geographically heterogeneous epidemiological impact of repellent use for malaria control.

S6.3 - Studies on *kdr* mutations associated with insecticide resistance in *Aedes ageypti* in Singapore

Koou Sin Ying[1,2], Tan Cheong Huat[1], Pang Sook Cheng[1],
Lee Kim Sung[1], Chong Chee Seng[1], Lee Chow Yang[2], Ng Lee Ching[1]

[1]Environmental Health Institute, National Environment Agency,
11 Biopolis Way #06-05/08, Helios Block, Singapore 138667.
[2]Urban Entomology Laboratory, Vector Control Research Unit,
School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang Malaysia.

Aedes aegypti in Singapore has been found to be highly resistant to pyrethroids. Failure of synergists to enhance the toxicity of these insecticides suggests that knockdown resistance (*kdr*) plays a significant role in conferring pyrethroids resistance in local *Ae. aegypti* population. Knockdown resistance phenotype occurs due to mutations in the *para* type voltage-gated sodium channel (vgsc), which causes reduce sensitivity of insect nervous system towards pyrethroid and DDT insecticides. A preliminary study based on the analysis of the domain II of vgsc gene revealed that majority of *Ae. aegypti* population in Singapore possessed V1016G and F1269C mutations, suggesting a *kdr*-associated pyrethroid resistance. However, in order to fully understand the high level of pyrethroid resistance seen in local *Ae. aegypti* population, there is a need to characterize the complete vgsc gene. In this study, the complete vgsc gene of *Ae. aegypti* mosquitoes collected from seven locations in Singapore were analyzed for *kdr* associated mutations. In addition to the previously identified mutations, two novel mutations, L219M and V929G were also detected in domain I and II, respectively. Together with the analyses of bioassay, synergist and biochemical data, the relationship between *kdr* genotypes and pyrethroid resistance will be discussed.

S6.4 - The detection of insecticide synthetic pyrethroid resistance on dengue vector, *Aedes aegypti* in Palembang, using polymerase chain reaction technique

Ahmad Ghiffari[1], Humairo Fatimi[2], Chairil Anwar[1]
[1]Department of Parasitology, Faculty of Medicine, Sriwijaya University
[2] Balai Besar Laboratorium Kesehatan Palembang

Aedes aegypti is a vector of several pathogens including dengue fever/dengue hemorrhagic fever virus. Five hundred thousand dengue hemorrhagic fever new cases occur every year throughout the world. Vector control is a way to break the chain of transmission. The use of one type of insecticide intensively for a long time to control the vector ultimately causes resistance. Insecticides resistance in *Ae. aegypti* was first discovered on trichloroetane diphenyl dichloro (DDT), followed by temephos and synthetic pyrethroid. Insecticide resistance can be detected by two ways: the enzymatic detection and mutation detection. The enzymatic detection means detecting the raise of the detoxifiying enzymes quantity. Second way is detection of the mutation of voltage gated sodium channel gene (VGSC). The purpose of this research was to identify the *Val1016Ile* and *Val1016Gly* point mutation in the VGSC gene of *Ae. aegypti* in Palembang. Population were all 3rd and 4th instar larvae of *Ae. aegypti* derived from breeding eggs obtained from villages of Bukit Kecil, Ilir timur I and Sukarami sub district. Identification took place in BBLK Palembang while molecular test took place both in BBLK Palembang and Clinical Microbiology Department of Muhammad Hoesin Hospital Palembang. Results showed that there was *Val1016Ile* point mutation and there was no *Val1016Gly* point mutation of voltage gated sodium channel gene. PCR methods that were used to identify *Val10616Ile* point mutation showed there was single 82 bp band (homozygous mutant) while PCR method that was used to identify *Val10616Gly* point mutation showed there was single 60 bp band (wild type). It can be concluded that there was *Val1016Ile* point mutation in the voltage gated sodium channel gene of *Ae. aegypti* as the marker of synthetic pyrethroid insecticides resistance in Palembang and and there was no *Val1016Gly* point mutation of voltage gated sodium channel gene of *Ae. aegypti*.

S6.5 - Larvicidal efficacy of the methanolic extracts of *Allium sativum*, *Cymbopogon citratus* and *Murraya koenigii* against *Aedes albopictus*

[1*]*Ho Lai Yee*, [1]*Lee Li Wen* and [1]*Heng Wee Vian*
[1]*Department of Biomedical Science, Faculty of Science,*
Universiti Tunku Abdul Rahman,
Kampar, Perak, Malaysia.

In recent years, environmental friendly and biodegradable insecticides of plant origin have become the new alternatives as agents for vector control. In this study, larvicidal properties of the bulbs of *Allium sativum* (garlic), and the leaves of *Cymbopogon citratus* (lemongrass) and *Murraya koenigii* (curry) were examined. The dried plant materials were powdered then extracted using organic solvent methanol. Subsequently, the crude extracts were concentrated on rotary evaporator. Each plant residue was then dissolved with methanol prior to larvicidal testing. These stock solutions were diluted with distilled water into five different concentrations and tested against 20 late third instar *Aedes albopictus* larvae. Results revealed all three plant extracts showed no larvicidal activities for the first 2 hours of exposure, and minimal to moderate at 24 hours and 48 hours of exposure. The methanolic extracts of *M. koenigii* had the highest larvicidal potential with the LC_{50} and LC_{95} values of 457.69ppm and 3189.88ppm. Methanolic extracts of *A. sativum* were the least effective with the LC_{50} and LC_{95} values at 7613.49ppm and 53805.38ppm respectively. On the other hand, methanolic extracts of *C. citratus* showed moderate larvicidal activity with LC_{50} and LC_{95} values of 1272.10ppm and 5599.52ppm. In conclusion, all three plant extracts exhibited some level of larvicidal potential; with *M. koenigii* shown to have the most significant larvicidal properties amongst the three plants.

S6.6 - Residual larvicidal activity of fenitrothion (sumithion® 50 Ec) against *Aedes aegypti* (l.) and *Culex quinquefasciatus* Say applied using a compression sprayer, Jacto® hd400

Teoh Guat Ney[1,3], Loke Seau Rong[2], Norazizah Ali[1], Chandru Angamuthu[1], and Lee Han Lim[1]
[1]Unit of Medical Entomology, Institute for Medical Research, 50588 Kuala Lumpur,Malaysia.
[2]Agricultural Chemicals (M) Sdn. Bhd. 962, Lrg Perusahaan 8, Taman PerindustrianPerai, 13600 Perai, Pulau Pinang, Malaysia.
[3]Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Larviciding is one of the effective mosquito control measures by preventing the emergence of adult mosquitoes. Residual larvicidal effectiveness of a fenitrothion formulation, Sumithion® 50EC via target spray using a hand-operated compression sprayer; Jacto® HD400 was evaluated in a field simulation trial to determine the optimum dosage of Sumithion® 50 EC to be applied in field applications. Residual larvicidal activity of Sumithion® 50EC was tested on laboratory bred *Aedes aegypti* and *Culex. quinquefasciatus* with 5 test dosages, namely 0.025, 0.05, 0.1, 0.5 and 1.0 gm ai/m^2. Test dosage of 0.05 gm ai/m^2 induced 100.00±0.00% mortality on *Ae. aegypti* larvae and 50.22±5.46% to 100.00±0.00% mortality on *Cx. quinquefasciatus* larvae 3 hours post exposure throughout the evaluation period of 4 weeks. For 24 hours post exposure mortality, 0.025 gm ai/m^2 gave near complete larval mortality on *Ae. aegypti* (98.67±0.67% to 100.00±0.00%), while 0.05 gm ai/m^2 gave complete mortality of *Cx. quinquefasciatus* larvae until 28 days post treatment. In view of the real life field situation such as the occurrence of rain soon after application, the dosage used should exhibit early larvicidal activity, e.g. within 3 hours of application. The dosage of 0.05 gm ai/m^2 of fenitrothion seems to fulfill this requirement.

S6.7 - Effect of the seaweed extracts towards the larvicidal activity, growth, and development of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae

Yu KeXin[1], Ibrahim Jantan[1], Rohani Ahmad[2] and Wong Ching Lee[3]
[1]Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia.
[2] Entomology Unit, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia.
[3] School of Biosciences, Taylor's University, Taylor's Lakeside Campus, 47500 Subang Jaya, Selangor, Malaysia.

Dengue fever is a common urban disease in Southeast Asia. Generally, the control of dengue fever depends mainly on the preventive measures in eradicating the mosquito vector. The present study aims to investigate the effect of methanol seaweeds extracts (*Bryopsis pennata*, *Padina australis* and *Sargassum binderi*) towards the larvicidal activity, growth and development of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). WHO larvicidal assay was conducted. In the sublethal assay, third instar larvae were treated at LC50 value and followed by daily observation until the emergence of adult mosquito. Dissecting stereo microscope and scanning electron microscope were used to investigate the morphological aberrations caused by the seaweed extracts. In the larvicidal assay, green seaweed *B. pennata* has showed the lowest LC50 values towards *Ae. aegypti* (156.97 mg/l) and *Ae. albopictus* (177.50 mg/l) as compared to the other two brown seaweeds (*P. australis* and *S. binderi*). This indicated that *B. pennata* has the strongest larvicidal properties. Of the three extracts tested, *S. binderi* gave the strongest prolongation effect against *Ae. aegypti* and *Ae. albopictus* larval period (11.50 and 11.01 days, respectively) as compared to control larvae (9.77 and 8.61 days, respectively). Among all the treated larvae, *S. binderi* treated *Ae. aegypti* and *Ae. albopictus* larvae have demonstrated the lowest adult emergence rate with 1.33 and 8.50%, respectively. This indicated that *S. binderi* has the strongest sublethal effect towards the growth and development of the tested larvae. The morbid larvae observed showed damaged anal gills, spiracle organ, darken body parts and distorted body shape. The results obtained suggest that the seaweeds studied have the potential as an alternative bioinsecticide.

S6.8 - Reception of visual stimuli: effects of ipomoea plant extract on *Culex quinquefasciatus* Say gravid females in choosing oviposition site

Wan Fatma Zuharah[1,2], Maniam Thiagaletchumi[1], Nik Fadzly[1]
[1]School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia
[2]Vector Control Research Unit, Universiti Sains Malaysia, Penang 11800, Malaysia

The interaction between plant and insect is dynamic which may favor either the plant or insect. Plant chemicals deeply implicated in this relationship and influence the insect behavior. These led us to examine the effects of acethonilic extract of *Ipomoea cairica* leaves on the oviposition behavior response of *Culex quinquefasciatus* gravid female mosquitoes. We also investigated the reception of vision stimuli due the tested plant extract using spectrographic analysis. In this study, two different set of oviposition choice experiment were conducted; (1) Single solution in a cage, (2) Multiple concentration solutions in one cage. In single solution experiment, only one available oviposition site was offered to five gravid females and in multiple concentration tests, four available oviposition sites were offered to twenty gravid females. The tested concentrations for the two different experiments were 100ml of (1) control (distilled water only), (2) 50ppm, (3) 150ppm and (4) 300ppm of *I. cairica* plant extracts. Data of drowned gravid female were recorded. No eggs were found in tested solutions except in control. The highest concentration, 300ppm of the studied plant extract appeared to show highest intensity with darker color followed by 150ppm and 50ppm concentrations. More gravid females were found drowned in the highest concentration, 300ppm of acethonilic leaves extract compared to 150ppm and 50ppm of the tested extract. The studied extract was found to effectively attract gravid *Cx quinquefasciatus* females and subsequently cause mortality of the gravid females, inhibiting egg deposition. The interference caused by the acethonilic extract of *I.cairica* on the oviposition activity of *Cx. quinquefasciatus* can result in better control of the insect vector.

Symposium Infectious Diseases

S7.1 - Sarcocystosis, an emerging muscle infection

Chong Tin Tan
Division of Neurology, Department of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Human muscular sarcocystosis is thought to be largely asymptomatic infection. There are recent reports of returning tourists from Pulau Tioman suffering from febrile myalgia illness. A large outbreak in 2012 involved 89/92 (97%) of campers returning from Pulau Pangkor. They developed relapsing fever and myalgia. About 10% had a distinctive myositis of jaw muscle with facial swelling. Muscle biopsy identified *Sarcocystis nesbitti as the etiology.* Thus, *Sarcocystis nesbitti* causes an acute, relapsing febrile myalgia with a high attack rate, with a distinctive myositis of the jaw muscle.

S7.2 - Prospective epidemiological and aetiological study of meningitis and encephalitis in Kota Kinabalu, Sabah, Malaysia

Heng Gee Lee[1], Timothy William[1], Jayaram Menon[1], Nicholas M. Anstey[2], and Tsin Wen Yeo[2]*
[1]Department of Medicine, Queen Elizabeth Hospital, 88586 Kota Kinabalu, Sabah
Menzies School of Health Research, Darwin, Australia

Central nervous system (CNS) infections in developing countries remain a common and life-threatening disease. A better understanding of the aetiological agents, epidemiology and clinical presentations of CNS infections in Sabah, Malaysia is essential to guide empiric therapy and to develop public health policy. We conducted a prospective observational cohort study in patients aged 12 years or older at Queen Elizabeth Hospital, Kota Kinabalu, Sabah between February 2012– March 2013. We recruited 84 patients with clinically suspected meningitis and encephalitis. 60/84 (71.4%) patients affected were young people between the ages of 12 and 44. An aetiological agent was identified in 46/84 (54.8%) of the patients. The most common pathogens were *Mycobacterium tuberculosis* (42/84, 50%) and *Cryptococcus neoformans* (13/84, 15.5%). *Mycobacterium tuberculosis* was confirmed in 11/42 (26.2%) patients by cerebrospinal fluid PCR and culture. The acute case fatality rate during hospital admission was 14/84 (16.7%). In conclusion, *Mycobacterium tuberculosis* is the most common cause of CNS infection in patients aged 12 years or older and is associated with high mortality. Further study is urgently needed to better understand the pathogenesis of this disease and to improve its clinical management and outcome.

S7.3 - A study on phylogenetic relationships among *Sarcocystis* spp. identified in rodents in peninsular Malaysia.

*Stephen Ambu[1] and Voon Gah Leong Kenny[1]
[1]International Medical University,
126 Jalan Jalil Perkasa 19, 57000 Kuala Lumpur, Malaysia

Rats were captured from four states, namely Kedah, Kelantan, Selangor and Johor in Malaysia. *Sarcocysitis sp*. were detected and identified in rats' muscle tissue by routine hematoxylin and eosin staining. This study was conducted to determine the phylogenetic relations among *Sarcocystis sp.* identified in rodents in Malaysia. The formalin fixed paraffin embedded (FFPE) rats' muscle blocks were subjected to DNA extraction and followed with semi nested PCR targeting 5' and 3' regions of 18S rRNA of *Sarcocysti s sp.* Phylogeny analysis showed two distinct groups *of Sarcocystis sp.* among the rats in Malaysia. Most of the identified *Sarcocystis sp.* were genetically closely related to *Sarcocystis rodentifelis* and *Sarcocystis muris*. The remaining identified *Sarcocystis sp.* were genetically closely related to *Sarcocystis sp.* ex Columba livia and *Sarcocystis sp.* cyst type I ex Anser albifrons .

S7.4 - Early stage diagnosis of cysticercosis in brain with the metacestode excretory-secretory peptide markers looks promising for large scale epidemiological screening and treatment monitoring in elimination programs

Priyadarshi Sahu[1], Subhash Parija[2]
[1]Division of Pathology, School of Medicine,
International Medical University, 57000 Kuala Lumpur, Malaysia.
[2]Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research,
Pondicherry-605006, India.

Neurocysticercosis (NCC) is a human to human disease and a major public health problem Worldwide. It is identified as the single most common cause of community acquired active epilepsy in tropical developing countries including Indian subcontinent and most of South East Asia. Majority of cases do not show typical imaging features. Moreover, in epidemiological studies imaging tools are not suitable due to cost and lack of availability. So, serology using specific antigens of the parasite is essential for confirming the diagnosis. The objective of the present study was to characterize the excretory-secretory (ES) antigens collected from *in-vitro* culture of the metacestode larvae, and to identify specific ES peptides as diagnostic markers. Serum and CSF IgG antibodies specific to the parasite ES antigens was detected by ELISA and the reactive ES antigenic peptides were identified by immunoblotting. ES peptides distributed at 67kDa, 43kDa and 32kDa were found diagnostic for NCC based on high sensitivity and specificity of their detection by antibodies either in serum or CSF. More remarkably, the 43kDa ES peptide was found reactive to the CSF and serum specimens from confirmed NCC patients with absolute specificity and a high sensitivity (88.23% in serum; 89.28% in CSF). This peptide was also detected by sera and CSF from clinically suspected NCC cases but with a decreased sensitivity correlating with the decreasing order of the certainty of diagnosis as per a criteria proposed earlier. The 43kDa ES peptide is suggested to be an important peptide of diagnostic utility in NCC especially for diagnosing the asymptomatic carriers at an early stage infection or in treatment monitoring during an elimination program. So development of an effective and definitive immunodiagnostic test for NCC is possible, but a series of considerations and evaluations need to be addressed.

S7.5 - Light microscopy and molecular identification of *Sarcocystis* spp. Of meat producing animals in Selangor- Malaysia

Latif, B.[1], Kannan Kutty, M.[1], Muslim, A.[1], Hussaini, J.[1], Omar, E.[1], Heo, C.C.[1], Rossle, N.F.[1], Abdullah, S.[1] Kamarudin, M.A.[1], Zulkarnain, M.A.[1], Tappe, D.[2]*

[1] Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Malaysia
[2] Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Germany

A total 199 samples were collected from sheep, goats and cattle slaughtered in Selangor abattoir from February to October, 2013. Samples include tongue, heart, diaphragm, esophagus and skeletal muscles. Each sample was split into three pieces about 2-3 mm^3, and examined microscopically at x10. Three to four positive samples were fixed in 10% buffered neutral formalin and embedded in paraffin blocks and stained with hematoxylin and eosin (H&E). Seven positive samples collected from each animal species were preserved in -80 °C and 90% ethanol for PCR. Out of 199 samples, 110 were positive (55.8%). The rate of infection for sheep, goats and cattle was 86%, 64.6% and 28.65% respectively. In all examined animal species, the cysts are microscopic; cysts, spindle to oval in shape, and the mean size was 172.96 µm x 53.64 µm (sheep), 131.68 µm x 49.49 µm (goats), and 151.66 µm x 75.83 (cattle). The wall was thin, with the thickness 2.85 µm in sheep, 1.56 µm in goats and 2.47 µm in cattle. The size of the bradyzoite was 10.43 µm x 2.24 µm for sheep, 9.34 µm x 2.88 µm for goats and 15.23 µm x 2.2 µm for cattle. Positive samples were subjected to parasite-specific 18S rRNA gene PCR and confirmed *S. tenella* for sheep, *S. capracanis* for goats and *S. cruzi* for cattle. Further EM study is in progress to compare the ultrastuctural features and differences between *Sarcocystis* spp. in meat producing animals in Malaysia.

S7.6 - Orange jessamine (Murraya Paniculata L., Jack) roots extract inhibit the development of *Plasmodium berghei* in mice through inhibition of hemozoin deposition

Kresna Septiandy[1], David Christianto[1], Tomson Kosasih[2], Vina Amelia Prajitno[1], Yunita Wibowo[1], Loeki Enggar Fitri[3]*

[1]Medical Study Program, Faculty of Medicine, University of Brawijaya, Jl Veteran, Malang, Indonesia
[2]Pharmacy Study Program, Faculty of Medicine, University of Brawijaya, Jl Veteran, Malang, Indonesia
[3]Department of Parasitology, Faculty of Medicine, University of Brawijaya, Jl Veteran, Malang, Indonesia

Orange jessamine (*Murraya paniculata-L*., *Jack*) roots is commonly used as a traditional medicine against bacterial infection. One of it compunds is acridone alkaloid, which can do harm to *Plasmodium falciparum* by inhibiting mitochondrial bc_1 complex and hemozoine production**.** This research model used one control group and three treatment groups of Balb/c mice that were infected by *Plasmodium berghei* and treated intraperitoneally daily with n-hexane fraction of the roots of orange jessamine. Dose for each treatment groups were 50 mg/kg b.w., 100 mg/kg b.w., and 150 mg/kg b.w respectively. After 6 days of treatment, the development of malaria parasites was suppressed ($p = 0.029$) and the hemozoine (end metabolites of hemoglobin digestion) on liver's Kupffer cells deposition levels was found to be lowered as well ($p = 0.004$), with 100 mg/kg b.w. as the most potent dose. Plasmodium's mitochondrial bc_1 complex activity was evaluated by spectrophotometer and found insignificant ($p = 0.871$), but there was still inhibition at a dose of 100 mg /kg b.w. shown with no decrease in absorbance values of decylubiquinole. There was no obvious acute toxic effects from the tested dose. It can be concluded that Orange jessamine (*Murraya paniculata-L*., *Jack*) roots can decrease the growth of *Plasmodium* by inhibiting the hemozoine production in certain dose.

S7.7 - Gender dimensions of TB susceptibility, detection, and outcomes: A comparative study in Myanmar & China

Samantha Watson,
Department of Global Health & Development, LSHTM,
UK

One notable aspect of TB prevalence worldwide is its general propensity[1] for disproportionate occurrence (or reported occurrence) in men. The most recent available figures for high TB prevalence countries report an average male:female ratio of new smear-positive cases of 1.85:1 (for the years 1998 to 2008), which tends to increase with age. This tendency has been widely and repeatedly identified, and a variety of explanations have been forwarded. The explanations for differential prevalence rates of recorded TB infection in men and women conflict regarding the extent to which it can be explained as an artefact of reporting. In this paper I report on recent (and ongoing) mixed-methods and trans-disciplinary research undertaken in Yangon Region, Myanmar and Yunnan Province, China. The research combines carefully designed survey data collection and analysis with detailed field to enable explanatory accounts of patterns observed in survey data. While the varied, and sometimes contradictory, findings reported to date suggest that different components and mechanisms perform different roles in different social configurations, meaning it may be unwise to overly generalise findings, I make a case for the wider international validity of the study's methods, reasoning, and approach.

Symposium Medicines For Malaria Venture II

S8.1 – 8.4 Drug Development Partnerships: Driving Innovations For Malaria Elimination

Despite great progress in reducing deaths from malaria between 2000 and 2013, significant commitment is still needed to achieve malaria elimination in countries and eventually disease eradication in the future. This will require improved delivery of existing interventions for the treatment and prevention of malaria, and concomitant focus on developing better medicines for elimination and eradication that will also prove effective against emerging drug resistance.

This symposium will review different areas of progress in the development of these critically needed medicines. Medicines for Malaria Venture (MMV, a leading product-development partnership) will describe the global pipeline for new malaria medicines, including progress towards a SERCAP (single exposure radical cure and prophylaxis) to support elimination and eradication. The Novartis Institute for Tropical Diseases (NITD), based in Singapore, will describe how Novartis, in collaboration with MMV and others, develops new approaches to identify the next generation of radical cures for malaria. GlaxoSmithKline, in partnership with MMV, is developing tafenoquine as a new single-dose radical cure for *P. vivax,* and will present an update on plans for Ph III studies after the recent announcement of encouraging Ph II results. Lastly, the Eijkman Oxford research unit in Indonesia will provide updates on collaborative research projects with MMV and other partners that are enhancing scientific knowledge about relapsing malaria and the role of improved drugs in treating *P. vivax*.

Speaker List and Topics
Each speaker's presentation will be limited to 15 minutes, thus allowing 30 minutes of discussion after all four presentations have been delivered.

The evolving malaria drug pipeline: new tools for elimination and eradication
Dr. Stephan Duparc, *Chief Medical Officer, Medicines for Malaria Venture (MMV), Switzerland*

Drug discovery for next-generation antimalarial drugs: supporting the elimination and eradication of malaria
Dr. Christophe Bodenreider, *Investigator, Novartis Institute for Tropical Diseases (NITD), Singapore*

Collaborative effort in malaria drug development: the development and delivery of tafenoquine, a single-dose radical cure for *P. vivax*
Dr. Justin Green, *GlaxoSmithKline, United Kingdom*

R&D capacity building in Asia – Indonesia case study
Dr. Kevin Baird, *Senior Investigator and Vice Director, Eijkman Oxford Clinical Research Unit, Indonesia*

Symposium Veterinary Parasitology II

S9.1 - Is there a role for Veterinary Parasitologists in the control of human soil-transmitted helminthiasis?

*Jozef Vercruysse[1] * and Bruno Levecke[2]*
Ghent University, Faculty of Veterinary Medicine,
Salisburylaan 133, 9820 Merelbeke, Belgium

Veterinary parasitologists (VPs) have a long experience in a wide range of aspects related to the control of gastro-intestinal nematodes. More than 50 different GI nematode species are found in animals both in temperate and tropical regions, and remain an important cause of loss in production in livestock. The current means to control these infections is the periodic administration of anthelminthic drugs. However, the increased use of these drugs over the last decade has resulted in the development of anthelminthic resistance against the major group of anthelminthic drugs. As a response to this, VPs have developed novel tools to determine impact of infections and to monitor the efficacy of anthelminthic drugs. Given the apparent similarities, these tools will also find applications in the control of human soil-transmitted helminthiasis (STH). We will illustrate how VPs have contributed to the field of STH in terms of diagnosis, monitoring of efficacy (new WHO guidelines on assessing the efficacy of anthelmintic drugs), STHhost reactions and identification of sources of contamination. In addition, we explore future contributions on how (i) to optimize the control of STH, (ii) to measure the exposure/morbidity and (iii) to monitor the development of anthelmintic resistance.

S9.2 - Neospora Caninum DNA detection in caprine and bovine milk

Hong-Kean Ooi[1], Ming-Yi Chien[2], Shih-Te Chuang[2],
Cheng–Hsiung Yang[2], Kensuke Taira[1],
[1]Department of Veterinary Medicine, Azabu University, Japan
[2]Department of Veterinary Medicine, National Chung Hsing University, Taiwan

Neosporosis causes abortion in cattle with serious economic impact worldwide. To elucidate the transmission of this parasite through milk, we examined goat and cattle milk, especially colostrum, for the presence of *Neospora caninum* DNA. A total of 582 goat milk samples and 55 bovine milk samples, were examined by conventional PCR and nested PCR for the presence of *N. caninum* DNA. Among the 582 goat milk, 3 were positive by the conventional PCR method and 130 positive by the nested PCR. Of 130 positive milk samples, 122 (93%) were colostrum. Among the 55 bovine milk, none was positive by the conventional PCR method but 10 were positive by the nested PCR. Of 10 positive milk samples, 9 (90%) were colostrum. All products amplified by conventional PCR and nested PCR were sequenced to confirm that the amplicons were those of *N. caninum*. This is the first finding of *N. caninum* DNA in goat milk. Loop-mediated isothermal Amplification PCR (LAMP) were also developed for the quick diagnosis of neosporosis using milk samples. A set of primers designated NCHU-5 was constructed for detecting *N. caninum* DNA by LAMP method. NCHU-5 showed negative reaction with *T. gondii, Cryptosporidium* and *Giardia* DNA, indicating good specificity to *N. caninum*. By adding SYBR green I to the LAMP products, the resulting color reaction can be determined visually. Thus, NCHU-5 can be used for the rapid diagnosis of *N. caninum* in the field.

S9.3 - Study on gliding motility of *Babesia bovis* merozoites using bioimaging analysis

Masahito Asada[1], Shin-ichiro Kawazu[2],
[1]Institute of Tropical Medicine, Nagasaki University,
Sakamoto, Nagasaki 852-8523, Japan
[2]National Research Center for Protozoan Diseases,
Obihiro University of Agricultural and Veterinary Medicine,
Inada, Obihiro 080-8555, Japan

Babesiosis is a zoonosis caused by tick-transmitted intraerythrocytic protozoa of the Phylum Apicomplexa. Invasive stages of apicomplexan parasites, such as sporozoites in *Plasmodium falciparum* and tachyzoites in *Toxoplasma gondii*, invade their target host cells using a unique, active process known as gliding motility. However, in case of the *Babesia* parasites, it is not thoroughly understood how the merozoites target and invade the host red blood cells (RBCs), and the gliding motility has not yet been observed in this parasite. In this study, we investigated the gliding motility of *B. bovis* merozoites. Gliding motility of *B. bovis* merozoites was observed and investigated by time-lapse video microscopy with the green fluorescent protein-expressing parasite strain. Formation and breakdown of the parasitophorous vacuole during the invasion of the parasite in the host RBCs were observed with membrane-stained bovine RBCs. The recorded images revealed that the processes included egress of the merozoites from the infected RBC, gliding motility, and succeeding invasion into new RBCs. The gliding motility was similar to the helical gliding of *Toxoplasma* tachyzoites. The trails left by the merozoites were detected by indirect immunofluorescence assay using antiserum against *B. bovis* merozoite surface antigen 1. Inhibition of gliding motility by actin filament polymerizer or depolymerizer indicated that this movement was driven by actomyosin-dependent process. This first report of gliding motility in *B. bovis* is notable and significant for the apicomplexan parasites since merozoites of *Plasmodium* parasites do not glide on the substrate. Also, we revealed the timing of breakdown of the parasitophorous vacuole that the formation and breakdown took place within ten minutes during the invasion. Currently, we are investigating the role of the thrombospondin-related anonymous/adhesive protein (TRAP) family in the gliding motility of *Babesia* merozoites by taking advantage of recently developed reverse genetics technologies.

S9.4 - Diet composition and prevalence of selected feline pathogens of Palawan leopard cat (*Prionailurus bengalensis heaneyi* Groves, 1997) in Barangay Cabigaan, Municipality of Aborlan, Palawan Island, Philippines

Carah Lyn G. Calawagan
University of the Philippines Los Baños,
Philippines

The status of Palawan leopard cat (*Prionailurus bengalensis heaneyi* Groves, 1997), a subspecies endemic to Palawan Island of the Philippines, has not yet been assessed and may fall under a threatened category due to its restricted geographical range and few sighting records which merits researches regarding its biology and ecology. This is a pioneer study on the diet and pathogens of *P. b. heaneyi* and the objectives are to determine the diet composition through analysis of hair present in scats, to compute for the prevalence of each pathogen present in the species, and to investigate possible pathogen transmission from domestic cats (*Felis catus* Linnaeus, 1758) to Palawan leopard cats and vice-versa. Twenty box-caged live traps placed one kilometer apart and left overnight for five consecutive nights yielded one capture and a blood sample was collected for the serological detection of pathogens using antibody and antigen test kits. A fecal sample was also collected and formalin-ether concentration technique was employed for the coprological detection of pathogens. Cuticular and medullary patterns of hair found from the fecal sample were observed for prey identification. Preliminary data reveals that the captured individual is highly positive for *Toxoplasma gondii* and *Chlamydophila felis* but negative for *Giardia duodenalis,* Feline Immunodeficiency Virus (FIV), Feline Coronavirus (FCoV), and Feline Infectious Peritonitis Virus (FIPV). Identification of prey and parasites of *P. b. heaneyi* is still ongoing and a longer duration of trapping and sampling are scheduled on November to December 2013. The manuscript is projected to be done by January 2014. Knowledge of the diet composition of the species is advantageous in formulating conservation strategies and emerging zoonotic diseases will be recognized if wildlife pathogens are carefully monitored. Habitat destruction remains to be the biggest threat to Palawan leopard cats followed by hunting for bushmeat, fur, and pet trades.

S9.5 - Cystic echinococcosis in cattle slaughtered at 4 selected abattoirs in Oman (2012-2013)

Fadya Al-Kitani[12], Derek Roberts[1], Senan Baqir[1] and Muhammad Hammad Hussain[2]*
[1]Department of Biology, College of Science, Sultan Qaboos University, Oman
[2]Animal Health Research Centre, Ministry of Agriculture and Fisheries, Oman

To determine the occurrence of cystic echinococcosis (CE) in cattle, 28269 cattle slaughtered in the 4 selected abattoirs (Salalah, Nizwa, Sohar and Boushar) belonging to different governorates of Oman were examined during March 2012 to April 2013. In total, 254 organs suspected for CE were removed and examined morphologically at Animal Health Research Center. Of these 203 (78.9%) samples were confirmed as CE. The highest percent of infection was found in the lungs (47.7%) followed by liver (39.9%) and multiple organs (12.3%), χ^2=13.1, p=0.001. Percent positivity was observed as 82.7% in females (n=86) and 76.5% in males (p=0.23). Highest percentage (88.6%) of positive samples belonged to cattle above 5 years old followed by those from 3-5 years old (80.8%) and under 3 years (54.8%), χ^2=13.7, p=0.001. Among breeds, 75.6% samples from imported breeds (from Somalia) and 80.6% from local breeds of cattle were found positive (p=0.36). Furthermore, (56.4%) of the cysts were sterile, (14.8%) fertile, (14.8%) degenerated, (11.4%) calcified while only (2.5%) were undeveloped. A significant difference (χ^2=13.9, p<0.01) was observed regarding the cyst size, 63.3% cysts were of small size (<5cm), 36.1% of medium (5-10cm) and 0.5% of large size (>10cm). Highest percentage of positive samples belonged to abattoir in Salalah (87%) followed by Nizwa (76.7%), Bousher (73.5%) and Sohar (60%), χ^2=10.5, p=0.014. However, the highest incidence (per 1000) of CE was found in cattle of Nizwa (98.6) followed by Bousher (8.9), Salalah (6.2) and Sohar (1.6). Further studies based upon molecular techniques are recommended to contemplate control measures.

S9.6 - *Toxocara canis* infection of household dogs in Calamba, Laguna, Philippines

Ma. Angelica Tujan, Rachell Gallano, and Vachel Gay Paller,
Animal Biology Division, Institute of Biological Sciences, College of Arts and Sciences,
University of the Philippines Los Baños, 4031 Laguna, Philippines

Toxocara canis is an emerging zoonotic disease in humans causing ocular and visceral Toxocariasis. This study was conducted to determine the extent of *T. canis* infection among household dogs in Calamba, Laguna, Philippines. Fecal samples were collected from household dogs using stratified random sampling and were subjected to Formalin-Ether Concentration Technique. The sex, condition (leashed or unleashed) and age of household dogs were recorded. Results revealed that the pattern of *T. canis* distribution among household dogs was highly aggregated (k=0.043), i.e., some dogs had high intensity of infection while others remain with low or no infection at all. Results also showed that 29 out of 129 (22%) household dogs examined were positive for *T. canis* with a mean intensity of 3eggs/g stool. The prevalence of infection in male and female household dogs were 23% and 22%, respectively (not significant: $x2$=0.001, P≥0.05). Meanwhile, the prevalence of leashed and unleashed household dogs were 21% and 24%, respectively (not significant: $x2$=0.241, P≥0.05). Finally, prevalence of *T. canis* among varying age groups showed a significant difference (P≤0.001), i.e., dogs belonging to ≤6 months (61%) were commonly infected with *T. canis* than those of 7-11 months (27%) and ≥12 months (7%). This study revealed that prevalence of *T. canis* infection among household dogs in Calamba, Laguna was relatively high. Also, younger dogs revealed higher infection rate than older ones. Hence, these data can be used in promoting awareness regarding proper care and management of pets that could serve as source of parasitic infection to humans.

S9.7 - Ectoparasitiasis, a challenge in sheep and goat production in Uli, Anambra State, Nigeria

Obi, Zita C[1]., Anyaegbunam Lucy[2] ; Orji MaryKate N[2] , Ikeh, Abunike J[2].
[1]Department of Science Education, Anambra State University Uli, Nigeria
[2]Department of Biological Sciences, Anambra State University Uli, Nigeria

Sheep and goat farming is one of the main animal husbandry activities in Eastern and Northern Nigeria. These animals are reared for meat, milk and ritual sacrifices. A total of 68 sheep and 92 goats were randomly sampled in Uli for the study between August – October 2011. The sampled animals were pre-numbered for clarity and examination for ectoparasites. The animals were brushed and the parasites picked up using forceps with the aid of a hand len. The ectoparasites recovered were ticks, lice, mites and fleas which were found to be common among the ruminants. The overall infestation were; sheep (69.8%) and goat (70.7%). The prevalence of these ectoparasites were; in sheep, ticks (17.0%), lice (25.8%), mites (15.0%) and fleas (42.2%), while in goats were; ticks(12.7%), lice (28.5%), mites (15.4%) and fleas (43.4%). The age related infestation was more among 1 – 5years sheep and goat with percentage infestation being 58.5% and 54.0% respectively. However, ectoparasite infestation in the studied animals was observed to be age related. The predilection sites were mostly; ears (67), neck (57), back (51) and limb (48) in sheep, while in goats, they were; ears (83), neck (76), back (69), limbs (61), abdomen (56) and chest(52). Following the results obtained, regular treatment of these small ruminants is strongly advocated to increase their economic values.

S9.8 - Development of a lateral flow dipstick test using *Echinococcus granulosus* native antigen B and comparison of GG and GG4 dipsticks for diagnosis of cystic hydatidosis

Akbar Khalilpour[1], Seyed Mahmoud Sadjjadi[2], Zohreh Kazemi Moghadam[1], Muhammad Hafiznur Yunus[1], Nor Dyana Zakaria[1] and Rahmah Noordin[1]
[1]Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Penang 11800, Malaysia;
[2]Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

Hydatid cyst, caused by infection with the larval stage of Echinococcus *granulosus*, is still a major concern for humans and livestock in many parts of the world. There are various serodiagnostic tests for the disease with different sensitivities and specificities, using protoscolex, hydatid fluid or recombinant antigens. The current tests are mostly in ELISA or Western blot formats which are time consuming and not suitable for low-resource settings. Thus, the present study was aimed at developing a lateral flow rapid test using native antigens B of the parasite. Based on Western blot analysis, selected batches of hydatid fluid antigen B were concentrated and buffer-exchanged into PBS. It was then used as the test line by jetting an optimized concentration of the antigen linearly onto a Hi-flow Plus 90 membrane card using IsoFlow™ Dispenser. The serum samples were from 21 hydatidosis patients, 17 individuals infected with other parasitic infections, and 15 healthy people. Optimized concentration of colloidal gold conjugated anti-human IgG and IgG4 were used as the antibody probes.IgG4 dipstick showed diagnostic sensitivity of 95 %(20/21) and specificity of 100% (32/32); while IgG dipstick showed diagnostic sensitivity of 100% (21/21) and specificity of 87.5% 28/32). In conclusion, both IgG and IgG4 dipsticks showed high sensitivity but the latter showed much higher specificity, thus IgG4 dipstick test has better potential for rapid diagnosis of cystic echinococcosis/hydatidosis.

Symposium Malaria

S10.1 - Global updates on malaria vector control priorities, policies and challenges

Abraham Mnzava,
Coordinator Malaria Vector Control, Global Malaria
Switzerland

Since 2000, a tremendous expansion in the financing and coverage of malaria control programmes has been mirrored by a wide-scale reduction in malaria incidence and mortality, reversing a decades-long trend of neglect and inaction that led to the deaths of tens of millions of people, most of them young children in Africa. Worldwide, between 2000 and 2012, estimated malaria mortality rates fell by 45% in all age groups and by 51% in children under 5 years of age. On the African continent, the estimated reductions were slightly greater: 49% and 54% respectively. The increase in access to vector control interventions over the past 12 years has been particularly notable. The percentage of households owning at least one ITN in sub-Saharan Africa is estimated to have risen from 3% in 2000 to 56% in 2012, before declining slightly to 54% in 2013. The proportion of the population with access to an ITN also increased markedly during this period, reaching 42% in 2013. The proportion of the population sleeping under an ITN was estimated to be 36% in 2013. While these gains are impressive, the glass remains only half full. For families in the part of the glass that is half empty, our efforts to date have not been good enough, especially given the low cost – about $5 for an ITN lasting 3 years – of this intervention. The main barrier to sleeping under an ITN is availability; 86% of the population with access to an ITN actually uses it. While not nearly as widely available as ITNs, IRS has also been made much more widely available over the past 12 years. In 2012, 135 million people (4% of the global population at risk of malaria) were protected by IRS worldwide. In the WHO African Region, the proportion of the population at risk protected by IRS rose from less than 5% in 2005 to 11% in 2010; this figure declined to 8% in 2012, when 58 million people benefitted from the intervention. In terms of policy, there has been no functional mechanism for malaria vector control. It is for this reason that WHO established two committees to address this problem following the recommendations of the Malaria Policy Advisory Committee (MPAC). The first is the Vector control Advisory Group (VCAG) jointly run by GMP and NTD and makes recommendations to MPAC and STAG on new forms/tools/technologies on vector control. The other committee – Technical Expert Group (VCTEG) is tasked with reviewing and providing guidance on the implementation of malaria vector control - including issues related to programme management. This presentation will describe outcomes of the recent VCTEG recommendations. On the other hand, the gains recorded in the past decade as a result of scaling up vector control interventions with the use of LLINs and IRS are very fragile. In addition to unstable resources, maintaining the effectiveness of these tools is threatened by the development and spread of insecticide resistance. The development and launching of the Global Plan for Insecticide Resistance in malaria vectors (GPIRM) in 2012 to address this challenge is not enough. Countries need to implement the approaches and recommendations proposed in GPIRM to pre-emptively manage insecticide resistance. Moreover, there is also a need to support industry to develop new and durable vector control products that allows procurement decisions based on years of protection rather than on unit cost.

S10.2 - Success and challenges in managing malaria in Malaysia

Timothy William
Infectious Disease Unit, Dept of Medicine, Queen Elizabeth Hospital,

Sabah, Malaysia

About 3.3 billion people around the world are at risk of getting malaria. An estimated 250 million cases occur per year with nearly 1 million deaths per year around the world. The majority of cases in Malaysia occur in Sabah and Sarawak. The incidence in Sabah is 0.7/1000 while in West Malaysia it is less than 0.1/1000. Nevertheless, there have been huge advances in malaria control over the past 5 to 10 years. The control strategies include the increased use of mosquito nets, increase in spraying and the increase in use of Artemesinin based therapy. Malaysia is aiming to eliminate malaria by the year 2020. The challenges we face currently in Malaysia is the difficulty in reducing the number of *P. vivax* cases and the emergence of *P. knowlesi*. Malaria deaths while few, unfortunately still occur in Malaysia. Early diagnosis, early treatment and the use of artemesinin based therapy is very important in preventing mortality. Supportive care and close monitoring is also essential. Comprehensive public health measures and optimal clinical treatment will hopefully help Malaysia achieve it target. Eliminating *P. knowlesi* will be very challenging if not impossible.

S10.3 - Knowing knowlesi malaria

Singh, B.
Malaria Research Centre, Faculty of Medicine and Health Sciences,
Universiti Malaysia Sarawak

Until recently, malaria in humans was thought to be caused by four species of *Plasmodium*: *P. falciparum, P. vivax, P. malariae* and *P. ovale*. Naturally acquired human infections with simian malaria parasites were thought to be extremely rare until a large focus of human *P. knowlesi* infections were reported in 2004 in the Kapit Division of Sarawak, Malaysian Borneo. Human knowlesi malaria cases have since been described in other parts of Malaysian Borneo and in Peninsular Malaysia, Indonesian Borneo, Brunei, Singapore, Thailand, Myanmar, Vietnam, Cambodia, Philippines and the Nicobar and Andaman Islands of India. This has resulted in *P. knowlesi* being recognized as the fifth species of *Plasmodium* causing malaria in humans. Long-tailed and pig-tailed macaques, the most common non-human primates in Southeast Asia, are the natural hosts of *P. knowlesi*. The molecular, entomological and epidemiological data indicate that human infections with *P. knowlesi* are not newly emergent in Southeast Asia and that knowlesi malaria is primarily a zoonosis, with wild macaques as the reservoir hosts. Current malaria control and elimination efforts are directed mainly at preventing human-to-human transmission of malaria, and involve methods that target vectors that are predominantly endophagic and endophilic. Therefore, the existence of zoonotic knowlesi malaria, caused by vectors that are exophagic and exophilic, and a huge population of the macaque reservoirs of *P. knowlesi* parasites, poses a challenge for the control and elimination of malaria.

S10.4 - Evaluation of additional vector control measures to control residual transmission in malaria pre-elimination areas

Marc Coosemans[1,2], Vincent Sluydts[1], Tho Sochantha[3], Siv Sovannaroath[3], Somony Heng[3] Lydie Canier[4], Nimol Khim[4], Saorin Kim[4], Mao Sokny[3], Charlotte Gryseels[1], Sambunny Uk[3], Karel Van Roey[1], Koen Peeters Grietens[1] , Didier Menard [4], Lies Durnez [1]
[1] Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[2] Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
[3] National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia
[4] Institut Pasteur du Cambodge, Phnom Penh, Cambodia

In Southeast Asia a substantial decrease of malaria has been achieved during the last decade and elimination is now becoming a realistic goal. However residual transmission due to outdoor and/ or early biting vectors is not tackled by wide coverage of insecticide treated nets (ITNs). This may compromise the elimination efforts. For this purpose we set up a study to evaluate the public health value of mass use of topical repellent in addition to ITNs. A randomized community based design has been adopted covering a population of 40,000 inhabitants in the province of Ratanakiri in Cambodia. The 98 clusters were randomly divided in two arms after a pre-trial survey: one intervention arm (ITN and repellent) and one control arm (only ITNs). Comparing to a randomized household trial, present design has the advantage to avoid the risk of the repellent aversion effect and the exchange of products between the households. The principal indicator of effectiveness is the prevalence of parasite carriers measured by PCR techniques using a mobile molecular lab in the field, and the measurement of malaria antibodies. The choice for molecular testing is fully justified as this will detect both symptomatic and asymptomatic individuals. While parasite-prevalence provides a snapshot of the exposure to malaria at a certain moment, sero-prevalence provides a picture of the “force of malaria infection” over a prolonged period. Moreover passive case detection based on an extensive network of Village Malaria Workers provides a measurement of malaria disease incidence in both arms. Beside individual protection, large scale use of ITNs in the community protects also the non-users from malaria transmission occurring during sleeping hours. Similarly we expect a community protection against residual transmission when a high adherence in the use of repellents is achieved. To address this working hypothesis of mass effect of repellents on the vector population entomological surveys are carried out in both arms. The effectiveness of the intervention is dependent on the efficacy of the repellents against vector bites and the effective use of repellents by the population. The first one is evaluated by using a protocol adapted from the WHOPES guidelines in which human landing collections are carried out on volunteers applying either a placebo or a test repellent on their exposed limbs. The second one, the effective repellent use, is based on socio anthropological studies, mixing qualitative and quantitative methods for assessing the acceptability, adherence and adequacy of topical repellents in the community. This information will be crosschecked with the effective distribution of repellent bottles to the households. Preliminary results will be presented. The outcome of this study will be crucial in the development of new strategies to control not only the indoor transmission during sleeping time (ITN) but also the increasing proportion of residual transmission which occurs mainly outdoors, and before and after sleeping time.

S10.5 - Using *Ex Vivo* studies to develop a mechanistic understanding of P. vivax pathobiology

Benoit Malleret[1], Rou Zhang[1], Wenn-Chyau Lee[2], Francois Nosten[3,4], Laurent Renia[5] and Bruce Russell[1,2]

[1]*Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, Singapore* [2]*Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia;* [3]*Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand,* [4]*Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, UK and* [5]*Singapore Immunology network (SIgN), A*STAR, Singapore*

Vivax malaria continues to burden the lives of millions who live in Asia. Unfortunately research into this serious infectious disease lags behind the other major cause of malaria (*P. falciparum*). Except for the seminal *in vivo* vivax studies using human syphilitics and primates, most of the work on *P. vivax* over the past three decades has been largely epidemiological or descriptive. More recently, a range of new or optimised *ex vivo* methods now provide for the first time, a mechanistic approach to understanding the pathobiology of vivax malaria. Here we discuss some of the latest developments *ex vivo* methods (extended culture, reinvasion assays, drug testing, biomechanical testing, microfluidics and cell sorting) to study of fresh and cryopreserved human *P. vivax* isolates. Importantly we provide information on the prerequisites and pitfalls of new *ex vivo* methods used to manipulate *P. vivax* in ways once not considered possible. Throughout the presentation we will showcase the latest data on the biology of *P. vivax* revealed by these *ex vivo* methods. This presentation emphasises that a better understanding of *P. vivax* pathobiology is not necessarily contingent on the continuous culture of this parasite species (which is still not achieved). *Ex vivo* studies are a cost effective solution to unravelling the real world problems associated with vivax malaria.

S10.6 - In pre-elimination settings a multiplex assay for detecting malaria antibodies: a necessary tool for assessing malaria transmission

Karen Kerkhof[1]*, Lydie Canier*[2]*, Somony Heng*[2]*, Tho Sochantha*[3]*,*

*Niel Hens[4], Didier Menard[2], Marc Coosemans[1,5], Vincent Sluydts[1], Lies Durnez[1]**

[1] Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
[2] Molecular Epidemiology Unit, Institut Pasteur du Cambodge, Phnom Penh, Cambodia
[3] National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia
[4] Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium
[5] Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

In countries achieving pre-elimination, malaria is concentrated in hotspots. Identifying these hotspots for targeted intervention and evaluating innovative control strategies are key for achieving malaria elimination. However, when malaria prevalence is low, detection of parasitological indicators is very insensitive. Seroprevalence offers the advantage that anti-*Plasmodium* antibodies can persist for months after infection and can serve as proxy of the force of malaria infection. A multiplex serological assay has been recently developed, allowing simultaneous detection of multiple antibodies. This assay was adapted to include 19 *Plasmodium* specific antigen (14 peptides, 5 recombinant proteins) and 1 antigen specific for the *Anopheles gambiae* saliva protein. The assay was applied on 1440 blood samples collected in the Ratanakiri province of Cambodia within the framework of a project that aims to evaluate changes in the force of infection due to the additional use of topical repellents (MalaResT). The assay performed equally well in monoplex and in multiplex formats for all Ags ($p<0.001$), and was highly repeatable. A comparison between the serological markers showed that high seropositivity and specific antibody levels were detected for the antibodies against Pf-LSA3-RE, Pf-CSP, Pf MSP1-19, Pf-GLURP, Pf-SALSA2 and Pf-GLURP-R2. Blood samples positive for malaria by PCR showed a higher response to some of the antigens as compared to PCR negative samples. An increase in seropositivity was observed with increasing age. Differences in seropositivity were observed between different districts, indicating the heterogeneity of malaria transmission within the most endemic province of Cambodia.

S10.7 - Challenges on the frontline: the influence of political and social forces on the roles and perceptions of barangay health workers in malaria control in Palawan, The Philippines

Alison Crawshaw
London School of Hygiene and Tropical Medicine
United Kingdom

The Philippines adopted primary health care (PHC) as a national strategy in 1978 post-Alma Ata, with *barangay,* or community, health workers (BHWs) conceived as frontliners in its delivery. Following the enactment of the Local Government Code in 1991 and devolution of health services, the malaria control programme was integrated into the PHC structure. Donor and NGO support helped overcome initial setbacks and contributed to significant reductions in malaria morbidity and mortality, however regional conflict, rural and indigent populations, bureaucratic inflexibility, and the recently documented zoonotic malaria infection (caused by the simian parasite, *Plasmodium knowlesi*), remain obstacles to malaria elimination by 2020. There is limited literature documenting the involvement of BHWs in PHC in the Philippines. This study seeks to explore the work of BHWs in the malaria control programme and their perceptions towards their roles and interactions, while considering the implications for the programme's future and transition to malaria elimination. Eleven BHWs (9 trained in malaria RDT or microscopy) were recruited from 2 barangays in Puerto Princesa City, Palawan, to participate in focus group discussions, in-depth interviews and observation. Grey literature and interviews with key informants from the health services and NGOs provided background information. Qualitative findings were encoded using NVivo10; malaria surveillance data was analysed using Microsoft Excel 2008. BHWs are highly valuable to, and valued by, their communities. They have good knowledge of malaria and vector control strategies, and proficiency in malaria microscopy, where trained. They also benefit from the programme in terms of personal development and other manifestations of empowerment. However, impeding factors of inadequate support, supervision and training, and potentially political forces, must be addressed in order to ensure the stability and effectiveness of the BHW programme and the accomplishment of health goals, including malaria elimination.

Symposium Neglected Tropical Diseases II

S11.1 - Helminths and their impact on co-infections

Maria Yazdanbakhsh[1], Linda Wammes[1], Sanne de Jong[1],
Ulysse Ateba Ngoa[1,2], Aprilianto Wiria[1,3], Akim Adegnika[2], Taniawati Supali[3]

[1]*Leiden University Medical Center, Center of Infectious Diseases, Department of Parasitology, Leiden, The Netherlands*
[2] *Centre de Recherches Médicales de Lambaréné, Albert Schweitzer Hospita, Lambarene, Gabon*
[3] *University of Indonesia, Medical Facluty, Department of Parasitology, Jakarta, Indonesia*

As helminths do not multiply in their human host, they have exploited their relatively large genome size to develop mechanisms that dampen effective parasite-specific immunity and allow their long-term survival and successful transmission. Helminths induce strong TH2 as well as regulatory responses. This modified immune system can have implications for responses to non-related antigens. One area of interest is responses to concurrent infections. For example, in many parts of the world, helminth infections and malaria are co endemic raising the question whether one infection influences immune responses to the other. Malaria parasites themselves are strong inducers of TH1 and pro-inflammatory cytokines and therefore one might expect that these responses are attenuated when a subject is co infected with a helminth. A number of studies have recently been completed that examine the effect of co infection at the population as well as cellular level. The results of these studies will be discussed during the presentation.

S11.2 - The epidemiology of neglected tropical diseases in P.R China

Shan Lv, Xiao-Nong Zhou
National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, People's Republic of China
WHO Collaborating Center of Malaria, Schistosomiasis and Filariasis, Shanghai 200025, People's Republic of China

The World Health Organization (WHO) lists 17 neglected tropical diseases (NTDs), which is dominated by helminth infections, such as lymphatic filariasis, schistosomiasis, and soil-transmitted helminthiasis (STH). Efforts to combat NTDs have been implemented along with social and economic developments in P.R. China. Progress not only depends on resource allocation and priority-setting, but also on the understanding that any activity directed towards disease control and elimination needs to recognize that socially embedded vulnerabilities can be as powerful as externally imposed infections. With the sustained political commitment, coupled with integrated control measures tailored to local settings, filariasis has recently been eliminated in 2007, and the burden of schistosomiasis and STH has been reduced significantly. Nevertheless, schistosomiasis prevalence is more than 5% in some endemic areas and 300,000 people are infected. The overall prevalence rate of STH infection due to *Ascaris*, *Trichuris* or hookworm is about 8% in endemic areas. Furthermore, P.R. China also harbours more than 90% of the world's burden of alveolar echinococcosis, and food-borne zoonoses are emerging owing to widespread consumption of raw or undercooked food. Dengue, leishmaniasis, leprosy, rabies, and trachoma exist in many places in P.R. China and these serious are not be overlooked even if they are less uncommon than other NTDs. It is conceivable that transmission of vector-borne diseases can be interrupted in many places; yet, epidemics occur in remote, mountainous areas due to viral (e.g., dengue) and bacterial infections (e.g., *Chlamydia*) creating a challenge for surveillance and control activities. To effectively improve the current situation, surveillance and response need to be shared and compared across different, endemic settings as well as various cultural and social systems.

S11.3 - Lateral flow dipstick test for extraintestinal amoebiasis utilizing recombinant *Entamoeba histolytica* pyruvate phosphate dikinase

[1]*Syazwan Saidin,* [1]*Muhammad Hafiznur Yunus,* [1]*Nor Dyana Zakaria,* [2]*Khairunisak Abdul Razak,* [3]*Lim Boon Huat,* [1]*Nurulhasanah Othaman and* [1]*Rahmah Noordin**

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

[2]*School of Materials and Mineral Resources Engineering & INFORMM, Universiti Sains Malaysia, Penang, Malaysia*
[3]*School of Health Sciences, Universiti Sains Malaysia, Kelantan, Malaysia*

Entamoeba histolytica infection is an important parasitic cause of morbidity and mortality in humans; the latter is mainly due to extraintestinal infection, particularly amoebic liver abscess (ALA). Accurate and early diagnosis is thus important. Previously, we have reported *E. histolytica* pyruvate phosphate dikinase (PPDK) as a potential diagnostic marker for ALA, hence it was exploited in the development of a rapid dipstick test. rPPDK was expressed, purified and first evaluated by Western blot. In parallel recombinant Gal/GaNAc lectin (rGal/GalNAc), as control antigen, was produced and tested similarly. The dipstick test using rPPDK was subsequently developed and evaluated. Western blots of rPPDK using sera from patients with ALA, healthy individuals and other diseases probed with anti-human IgG_4-HRP showed the highest sensitivity (93.3%) and specificity (100%); as compared to blots using IgG and IgG_1 as secondary antibodies. Additionally, rPPDK showed better specificity when compared to rGal/GalNAc. The initial optimized parameters of the dipstick test were rPPDK at 1.25 mg/ml and colloidal gold conjugated anti-human IgG_4 at OD5, with diagnostic sensitivity and specificity of 87% and 100% respectively. Subsequently the purity of the rPPDK was enhanced to increase the concentration of the lined rPPDK; thereby increasing the diagnostic sensitivity to at least 90%.The lateral flow dipstick developed using rPPDK showed good potential for rapid diagnosis of extraintestinal amoebiasis. Such 'point of need' format would enable the test to be accessible to a larger at risk population.

S11.4 - Neglected tropical diseases and human nutritional status: Malaysian scenario

Norhayati Moktar

Department of Parasitology, Faculty of Medicine,
Universiti Kebangsaan Malaysia,
Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia

Neglected tropical diseases (NTDs) are a group of poverty-associated chronic infectious diseases, which are endemic in poor and rural population of Africa, America and Asia. Thirteen parasitic infections are classified as core NTDs. Of these, soil-transmitted helminthiases (STH), giardiasis, schistosomiasis and amoebiasis are known to interfere with human nutritional status. There are many ways these NTDs can affect human nutritional status; ascariasis for example is associated with growth failure in children by imparing lactose digestion, decreasing food consumption and lowering plasma vitamin A. Chronic giardiasis can interfere the growth of children by impairing digestion and absorption of fat and vitamins and causing lactose intolerance. However, the impact of parasites on the nutritional status of a host is not only on nutrient requirement but it is mainly to the host's responses to these infections leading to loss of appetite, increased in metabolic rate, innitiate immune responses and produce pathological changes in human tissues. Chronic infection of NTDs cause undernourished especially in children with poor food intake. Furthermore, undernutrition itself increases individual susceptibility with infection, reduces resistant and become more prone to develop severe disease. These relationships are always synergistic. In Malaysia STH and giardiasis are considered endemic with large burden of infections seen in low-income communities living in the rural and suburban areas. Similarly, the distribution of protein-energy malnutrition and micronutrient deficiencies is prevalent where NTDs prevail. The impact of these NTDs on nutrition and development of children has been reported by many Malaysian researchers. Most of these findings were reported from cross sectional and interventional trial studies carried out among Orang Asli and rural communities. Recently few randomised controlled trials have been carried out to determine the association. However, as reported by many researchers the associations have always remained controversial.

S11.5 - Interleukin-6 is required for worm establishment early during filarial infection

Muhsin Muhsin, Katrin Gentil, Marc P. Hübner, Achim Hoerauf.*

Institute of Medical Microbiology, Immunology and Parasitology,
University Hospital Bonn, Sigmund Freud Str. 25, 53105 Bonn, Germany

Interleukin-6 has a wide range of biological activities that include anti- and pro-inflammatory aspects. In this study, we investigated the role of IL-6 in immune responses to the rodent filarial nematode *Litomosoides sigmodontis*, a murine model for human filariasis. IL-6 deficient mice had a significantly increased worm burden after natural infection compared to wild type controls at early time points post infection that was no longer observed after the molt to adult worms and during chronic infection. Given that the worm burden in IL-$6^{-/-}$ mice was already increased at the time point the infectious larvae reached the pleural cavity, the site where they molt and reside as adult worms, immune responses were analyzed that may facilitate the migration from the site of infection (skin) via the lymphatics to the pleural cavity. Increased vascular permeability may facilitate larval migration, but blocking of histamine receptors had no effect on worm burden and vascular permeability was similar between IL-$6^{-/-}$ mice and wild type controls. In contrast, blocking mast cell degranulation reduced the worm burden in IL-$6^{-/-}$ mice partially, suggesting that release of mast cell derived mediators impair larval migration to some degree. Additionally, bypassing the skin barrier by inoculating infectious L3 subcutaneously resulted in a worm recovery at 15 dpi that was comparable between the BALB/c and IL-$6^{-/-}$ mice. Those results indicate that protective immune responses in the skin impair larval migration in wild type animals. Eosinophils and neutrophils are recruited to the site of infection within the skin and we hypothesize that this recruitment is delayed in IL-$6^{-/-}$ mice. Currently we are performing eosinophil and neutrophil depletion experiments to test this hypothesis.

S11.6 - Disseminated cysticercosis: Uncommon manifestation of a common disease

Naveen Vandanapu[1], Vengamma Bhuma[1], Vijayalakshmi B[2], Lakshmi A[2] and Alladi Mohan[3]

[1]Department of Neurology,Sri Venkateswara Institute of Medical Sciences,

[2]Department of Radiology,Sri Venkateswara Institute of Medical Sciences,
[3]Department of Medicine, Sri Venkateswara Institute of Medical Sciences,
517507 Tirupati, India

[3]Department of Medicine, Sri Venkateswara Institute of Medical Sciences, 517507 Tirupati, India
We report our experience with an uncommon presentation of disseminated cysticercosis in an immunocompetent patient. This type of involvement is extremely rare and only few case reports have been reported till date. A 25-year-old, human immunodeficiency virus (HIV) sero-negative male patient presented with a history of recurrent seizures, headache and occurrence of multiple nodules over the body for the last 6 months. He was otherwise keeping good health and did not have any co-morbid conditions. Physical examination revealed multiple nodules measuring 1-2 cm in size over the anterior aspect of neck, both arms and over the back. Neurological examination was normal. Ultrasonography and magnetic resonance imaging (MRI) showed cysts in extraocular muscles, multiple cysts in different stages of evolution in brain, tongue and temporalis muscle. Excision biopsy of the nodule over the chest wall on the right side confirmed the diagnosis of cysticercosis. The patient was treated with oral albendazole 400 mg twice-daily for one month, oral prednisolone and anti-epileptic drugs. At 15 days follow-up, a significant decrease in the size of all the nodules was noted. The present case documents the uncommon occurrence of disseminated cysticercosis with simultaneous involvement of brain, orbits, tongue, skeletal muscles, subcutaneous tissues and salivary glands in an immunocompetent individual and highlights the importance of considering this condition in the differential diagnosis of multiple swellings all over the body in endemic areas.

S11.7 - Diversity of intestinal parasitic infections (IPIs) in HIV and non- HIV prison inmates in Selangor, Malaysia

Lorainne Angal[1], Yvonne AL Lim[1], Sajideh Samin[2], Nan-Jiun Yap[1],
Romano Ngui[1], Amirah Amir[1], Init Ithoi[1], Adeeba Kamarulzaman[3] and Rohela Mahmud[1]

[1]Department of Parasitology, Faculty of Medicine, University of Malaya,
50603 Kuala Lumpur, Malaysia
[2]Penjara Utama Kajang, 43000 Kajang, Selangor, Malaysia

[3]*Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

The present study (carried out from June 2012 to January 2013) highlights the diversity of intestinal parasitic infections (IPIs) in HIV infected and non-HIV infected inmates in a prison setup in Malaysia. A total of 300 stools and 303 blood samples were successfully collected from 314 consented inmates, involving 137 HIV and 177 non-HIV adult male inmates whose age ranged from 21 to 70 years old. Samples were subjected to microscopy examination and serology test (only for *Strongyloides*) for the presence of IPIs. Microscopy positive samples were confirmed via PCR thereafter. Overall prevalence via microscopy among all sampled inmates showed that 20.0% (60/300) of the stool samples were positive for various IPIs. Of these, *Blastocystis* spp. had the highest prevalence rate (14.3%; 43/300), followed by *Entamoeba* spp. (3.3%; 10/300), *Cryptosporidium* spp. (2.3%; 7/300) whilst both *Giardia* spp. and *Trichuris trichiura* had rates of 0.3% (1/300) respectively. Comparatively, rate of IPIs was higher among the HIV infected inmates (21.6%; 29/134) than non-HIV infected inmates (18.7%; 31/166). Similarly, there was higher diversity of parasitic infections in HIV infected compared to the non-HIV infected inmates. HIV infected inmates were observed to be harbouring all the species mentioned above, however, non-HIV inmates were only infected with *Blastocystis* spp., *Entamoeba* spp. and *Cryptosporidium* spp. As for *Strongyloides stercoralis*, the overall seroprevalence was 8.9% (27/303). Interestingly, seropositivity for *S. stercoralis* was more predominant in non-HIV inmates (10.6%; 18/170; age range: 21-70 years) compared to HIV infected inmates (6.8.0%; 9/133; age range: 31-60 years). Molecular analysis inferred the presence of *Blastocystis hominis, Entamoeba histolytica, Entamoeba dispar* and *Giardia duodenalis.*

S11.8 - Transmission aspects of faciolosis in Vietnam

[1,2*] *Bui Thi Dung,* [1]*Pham Ngoc Doanh,* [1]*Dang Tat The,*
[2]*Yannick Caron,* [2]*Claude Saegerman,* [2]*Bertrand Losson*

Institute of Ecology and Biological Resources,
Vietnam Academy of Science and Technology, Ha Noi, Vietnam.
[2)] *Department of Infectious and Parasitic Diseases,*

Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

Fasciolosis is a relatively serious problem and is distributed in many different provinces with a particularly high impact in central Vietnam. However, the fasciolosis transmission aspects to cattle and human are still on debate. The objective was to determine the current status of *Fasciola* spp. in snails and determine the risk factor transmit to human and cattle. Snails sampling was conducted in 2012. A questionnaire was developed and used during interviews involving local people and dealing with their fasciolosis knowledge and vegetable eating habits. Snails were identified based on morphology and molecular analysis and were examined regarding to their infection status through microscopy and multiplex PCR. Three snail species were identified as *Austropeplea viridis, Radix auricularia. A. viridis* appears to be the most abundant species with diverse distribution in Vietnam. Only *A. viridis* harboured *Fasciola* spp. with an infection rate of 1.17% (299/25,422) as assessed by microscopy. *Fasciola* spp. infection rate in *A. viridis* was significantly less by microscopy (1.14%; 57/5,000) than by multiplex PCR (1.82%; 91/5,000) (Chi2 = 7.93; 1df, *P*=0.005). The prevalence of *Fasciola* spp. in snails was significantly higher in central part (1.52%; 167/11,011) than in northern part (0.91%; 132/14,411) (Chi2 = 19.38; 1df; *P*<0.0001). *Fasciola* spp. infection rate was remarkably high in two periods linked to rice cultivation in north while it can take place through the year in central part. The contaminated submerge vegetable consuming are the potential risk factor transmit *Fasciola* to human in central.

ORAL ABSTRACTS

Extended Parasitology and Entomology I

O1.1 - Biodiversity: A key element for health in the ASEAN region

Ahmad Ghiffari[1,2], Chairil Anwar[1], Ulrich Kuch[2]

[1] Departement of Parasitology, Faculty of Medicine, Sriwijaya University
[2] Biodiversity and Climate Research Centre (BiK-F), Frankfurt, Germany

3.490 species of mosquito have been described worldwide, and new species are regularly found. Recent decades have seen a re-emerging interest in the description and understanding of mosquito biodiversity because of the risks that are associated with the invasive species and the emergence of vector-borne diseases in our globalized world. The first rank drivers of the emergence and re-emergence of human pathogens are land use changes and agricultural practices. Insular Southeast Asia experienced the highest level of deforestation among all humid tropical regions of the world during the 1990s. Deforestation in this region not only increases carbon emissions directly contributing to global warming, but also endangers the existence of numerous forest mosquitos' endemic species. Furthermore the areas will be suitable for the major endemic disease vectors and attracting others that are classified as invasive species. In the context of the ASEAN region, international collaboration in the field of biodiversity and health should therefore also consider mosquito biodiversity with special attention to species invasions, the risk of spreading vector-borne diseases and the underlying environmental and social-ecological factors. Finally, mosquito vector control strategies also need to integrate questions of biodiversity at various levels.

O1.2 - New cases of furuncular myiasis in Saudi Arabia: *Cordylobia anthropophaga* established an autochthonous transmission and gained more territories

Mohammed Afifi, Asif Jiman-Fatani[1]

Dept of Medical Microbiology and Parasitology, Faculty of Medicine,
King Abdul Aziz University, Jeddah, Saudi Arabia.

Cordylobia anthropophaga has long been limited to tropical Africa without any presence in ecologically similar regions. As a region, South Western Saudi Arabia became a possible focus for *Cordylobia anthropophaga* outside Africa where indigenous cases of myiasis were identified. We are the first to report indigenously acquired cases of *Cordylobia anthropophaga* myiasis, from Al Baha Province. It is distanced 320 km from the primary focus in Aseer. Larvae were extracted from multiple skin lesions, of two young sisters, and examined for species identification. Cephaloskeleton and posterior spiracles was dissected and examined microscopically after the standard procedures of fixation, dehydration and clearing. The larvae were identified as third-instar of *Cordylobia anthropophaga* by size, oval body, spine pattern and posterior spiracles morphology. Triggered by identification of these cases, study was continued to reconsider the epidemiology of *Cordylobia* myiasis, examining its spread and identifying the influencing factors in that region of Saudi Arabia. Al Baha ecology has some similarities to regions of subtropical Africa with a wide variety of indigenous potential hosts and climatic conditions conducive for the fly. It has a mostly rainy climate, high relative humidity and is surrounded by many forests with sylvan hosts. Our findings and observations suggest a re-evaluation of the understanding of distribution and frequency of *Cordylobia anthropophaga* myiasis in Saudi Arabia. The study confirms an autochthonous pattern of transmission and a slow but continuous gain of territories. Clinical staff will have to consider *Cordylobia* myiasis in furuncular skin lesions, even in individuals without travel history to Africa.

O1.3 - Genetic diversity studies on *Culex quinquefasciatus* from four different larval habitats using RAPD-PCR in Agra city

Shivani Gupta and Shabad Preet

Department of Zoology, Faculty of Science,
Dayalbagh Educational Institute, Dayalbagh, Agra-282005, India.

Prevalence of vector born diseases depends upon the vectorial capacity of vectors and their competence can be influenced by the genetic variations among vector populations. The objective of this study was to explore the presence of *Cx. quinquefasciatus* in various larval habitats and analysis of population genetic structure using random amplified polymorphic DNA (RAPD), which may provide insight into its dispersal patterns, behaviour and resistance to insecticides. The population differentiation parameters and genetic distances were calculated using POPGENE software. GenALEx package was used to compute molecular variance and the cluster analysis technique of unweighted pair-group method of arithmetic averages (UPGMA) was used to develop the phylogenetic tree. Out of 140 RAPD primers screened, four primers exhibited clear, concrete and distinct RAPD banding patterns showing up to 100% polymorphism. The results displayed moderate variation among larval populations which is evident from low value of G_{ST} indicating moderate genetic differentiation. Effective migration rates were observed to be depicting high gene flow. The AMOVA analysis revealed high intrapopulation genetic variation (92%) with only 8% variation among populations. The cluster analysis showed one main cluster which is divided into two subclusters, one between pond and waste water habitat and another cluster of cement tanks along with plastics as a separate branch. The study revealed establishment of *Cx. quinquefasciatus* in diverse larval habitats with excellent adaptability as indicated by high gene flow and polymorphism. Hence, for controlling this nuisance vector all habitats needs to be targeted for an effective mosquito eradication programme.

O1.4 - Differences in *Aedes albopictus* glutathione s-transferases (GSTs) expression in response to oxidative stress.

Siti Nasuha Hamzah, Zazali Alias

Department of Biochemistry, Institute of Biological Sciences,
Faculty of Sciences, University of Malaya, Kuala Lumpur, Malaysia.

Glutathione S-transferases (GSTs; EC 2.5.1.18) are multifunctional enzymes involved in detoxification of xenobiotics and endogenous compounds. In insect most studies have focused on their role in insecticide resistance. Our previous study has investigated the expression of the enzymes in three different life cycles of *Ae. albopictus* which are designated as AaGST1 to AaGST 12 according to the ascending order of their pI values. In this study, we manage to look at the relationship of the dengue vector's GST isoforms and response toward chemical challenges. Hydrogen peroxide and Paraquat were used to investigate the acute effect of oxidative stress on GSTs expression. By using 2-DE gel analysis, ten isoforms of Hydrogen peroxide-treated GSTs and seven isoforms of Paraquat-treated GSTs showed over expression compared to the non-treated GSTs, ($p<0.05$). From the probit analysis, the susceptibility level indicates that Paraquat (LC50=0.74mg/l) was less effective against *Ae. albopictus* larvae following by Hydrogen Peroxide (LC50=1.42mg/l). Comparison of the total protein content of non-treated, Hydrogen Peroxide-treated and Paraquat-treated of these dengue vector's GST batches of GSTrapTM(C12) isolated showed 45% increase in the total protein content after Hydrogen Peroxide treatment and 41% increase after Paraquat treatment. The higher total protein content in treated *Ae. albopictus*'s GSTs could mean that higher detoxification ability is needed for the protection of crucial and important biosynthetic pathway from inhibition by toxic substances.

O1.5 - Mite fauna and mite antigen detection in house dusts from residential areas in los Baños,Llaguna, Philippines

Vachel Gay V. Paller and Ronal Aldous M. Catanghal

Animal Biology Division, Institute of Biological Sciences,
University of the Philippines Los Banos

Dust mites are a medically important group of animals commonly found in carpets and mattresses in houses. Antigens in their feces cause allergic reactions such as asthma and contact dermatitis. Dust

samples were vacuum-collected in a special collecting bag from a one square meter area from living room floors of 100 randomly sampled houses in Los Baños, Laguna. Chromato-immunoassay ELISA (Mitey Checker) was used to detect mite antigenicity. Twenty-three species of mites were identified belonging to seven families. Of these, *Blomia tropicalis* (265 mites/g of dust in 87% of households) of Family Glycyphagidae and *Dermatophagoides farinae* (71 mites/g of dust in 58% of households) of Family Pyroglyphidae were the most prevalent and abundant species. Forty-eight percent (48%) of households were detected to have low levels of antigen (≤5 μg/m^2). There was a weak correlation between mean total mite intensity and antigen levels (r = 0.129). Mean *Dermatophagoides* intensity and antigen levels were also found to have weak correlation. More mites were found in carpeted living rooms (822 mites/g) compared to non-carpeted living rooms (645 mites/g). Different floor types did not show any difference in mean mite intensity. Likewise, mite intensity did not show correlation with household size.

O1.6 - Microbial detection of parasitic protozoa and free-living amoebae at various sites of two drinking water treatment plants in Kuching, Sarawak, Malaysia

Reena Leeba Richard, Init Ithoi, Mohamad Azlan Abd Majid,
Wan Yusoff Wan Sulaiman, Rohela Mahmud and *Yvonne Ai Lian Lim

Department of Parasitology, Faculty of Medicine,
University of Malaya, 50603 Kuala Lumpur, Malaysia.

Potable drinking water is vital to human life and its quality is of great public concern. However, there is a scarcity of information regarding drinking water quality in Malaysia, especially in Sarawak. The aim of this study was to determine the occurrence of parasitic protozoa (*Cryptosporidium* and *Giardia*) and free-living amoebae (*Acanthamoeba* and *Naegleria*) at various sites of two drinking water treatment plants in Kuching, Sarawak. Water samples were collected on three separate occasions from each site (i.e., raw, coagulation, flocculation, sedimentation, filtration, treated and distribution system) of the two plants between July 2012 and October 2013. The water samples were collected and filtered through nitrocellulose membrane, then purified using the immunomagnetic separation (IMS) technique prior to FITC and DAPI staining to detect the presence of *Cryptosporidium* and *Giardia* (oo)cysts. As for *Acanthamoeba* and *Naegleria*, samples were cultivated on non-nutrient agar (NNA) lawned with *Escherichia coli* and detected under light and inverted microscope. Of 91 samples taken, 17.6% (16/91) and 30.8% (28/91) samples were positive for *Cryptosporidium* and *Giardia* (oo)cysts, respectively. Meanwhile, out of 63 samples, 41.3% (26/63) and 30.2% (19/63) were shown to be positive of *Naegleria* and *Acanthamoeba*, respectively. Occurrence of these protozoa and FLA was higher at the raw water, coagulation, and flocculation sites. In providing knowledge of the presence of these parasites at the specific treatment sites, this study will assist key stakeholders in addressing contamination of these parasites in these treatment plants.

O1.7 - Pathogenic potential of parasitic interaction between *Acanthamoeba* spp. and *Legionella* sp.

Faizah Mohd Hanapiah, Anisah Nordin Norhayati Moktar,
Yusof Suboh, Noraina Ab Rahim and Adibah Mohd Roslan

Department of Parasitology and Medical Entomology,
Faculty of Medicine, Universiti Kebangsaan Malaysia,
Jln Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Acanthamoeba spp. is an opportunistic protist ubiquitously distributed in the natural environment and also known to be able to cause human infections. *The existence of this amoeba in engineered human habitats has been widely documented,* thus it is accepted that humans commonly encounter this organisms in our routine live. The ability of *Acanthamoeba* to act as a reservoir for other microbial agents has caused a great concern in the ability of this amoeba to indirectly transport a pathogen from the environment to humans. This paper intended to discuss the interaction between *Acanthamoeba* spp. and *Legionella* sp., an agent of Legionnaires disease causing pulmonary infection and the possible effects their interaction has on the virulence and pathogenicity of both organisms in human infections. By focusing on the interaction of these organism within environment that are closely related to human activities, we hope it will greatly benefit medical practitioners in understanding the disease process and the public health authorities in proposing a better control or educational to program reduce the risk of infection.

O1.8 - Mecsus, an advanced tool for finding new antiparasitic drugs from Malaysian fungal biodiversity

J.-F.F. Weber[1*], *Rasha Saad Abdelgadir Soliman*[2]

[1]*Atta-ur-Rahman Institute for Natural Product Discovery (RiND), Faculty of Pharmacy, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia*
[2]*Management and Science University, 40100 Shah Alam, Selangor, Malaysia*

Drug discovery for parasitic diseases is more than ever an endeavour that only the academia and public research institutes are willing to undertake. In our centre, we have conceived a protocol for generating libraries of natural products in order to find new antimalarial and antitrypanosomial entities from the ultra-rich fungal biodiversity of Malaysia. Only one compound out of x thousand tested will reach the patient. Therefore, any successful drug discovery approach will combine strategies that will 1) decrease the size of the library to be tested, and 2) shorten the time required to build that library. In our centre, we have conceived a novel protocol for generating libraries of natural products in order to find new antimalarial and antitrypanosomial entities from the ultra-rich fungal biodiversity of Malaysia. Is was named MECSUS (Microtiter plate, Elicitors, Combination, Solid phase extraction, UHPLC, Statistical analysis) and involves i) miniaturized parallel fermentations in 96-well microtiter plate with 12 standard media for maximum exploration of the biosynthetic potential; ii) parallel extraction; iii) chromatographic profile by LC-DAD-ELSD or –MS; and iv) computerised data analysis. This MECSUS protocol was developed in view of full automation of the various steps. It is ready now for large scale *in vitro* and *in vivo* screening

O.1.9 - Autism and *Toxoplasma*-induced metabolomic changes: Novel pathogenetic pathways and risk-predictor biomarkers

Mohammed Afifi[*1], Faten Al-Brikan[1], Mohammed D'Ajani[2]

[1] Dept of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdul Aziz University, Jeddah, Saudi Arabia.

[2] Dept of Pediatrics, King Fahad Specialist Hospital, Dammam, Saudi Arabia.

Autism is a serious neurodevelopmental disorder. It lacks definite laboratory diagnosis and specific lines of treatment mainly because of obsecure etiology/pathogenesis. Infection with *Toxoplasma gondii* is common among humans worlwide. The parasite has a special predilection to brain residing inside for life. It induces degradation of host's branched-chain amino acids and lipid metabolism alterations. This study was done to explore a possible role of *Toxoplasma* infection in the development of autism, utilizing the parasite-induced metabolomic changes as possible risk-predictor biomarkers. A total of 81 children, with an age range of 3-11 years, were enrolled. Aautistics and healthy children were subjected to thorough history taking and clinical evaluation. Collected blood samples were tested for anti-*Toxoplasma* IgG and IgM antibodies, branched-chain amino acids, adiponectin and lipoproteins profile. The mean age for autistic children (n=30) was; 6.23 ± 2.65 years while that for healthy controls (n=51) was 5.94 ± 2.64. Results showed higher positivity rate (50%) among autistics compared to7.4% positivity among healthy controls. The mean anti-Toxo IgG levels, in autistic children were significantly higher ($P<0.001$). All proposed biomarkers, except triglycerides, were significantly lower in autistics compared to healthy children. Logistic regression analysis showed that a set of 6 biomarkers had a predictive utility in autism.The results suggest an infectious basis of autism. *T. gondii* or other infectious agents that induce similar metabolomic changes might share in the development of autism. A novel set of biomarkers, with a predictive diagnostic ability for autism, was introduced.

O1.10 - Profile of hepatic involvement in dengue infections in adult Pakistani population.

Somia Iqtadar[1], Nabeel Akbar[2],Naeem Majeed[3],Numan Majeed[3]

[1] Department of Medicine, King Edward Medical University Lahore, Pakistan
[2] Department of Medicine,Sir Ganga Ram Hospital, Lahore ,Pakistan
[3]Services for Public Health and Research (Sphere), Lahore, Pakistan.

To estimate the range of hepatic involvement in dengue infections, clinical and biochemical profile of **s**erologically confirmed 220 adult cases of dengue infection were studied**.** Analysis of the biochemical profile showed that liver dysfunction was more common in DF compared to DHF (38.15 vs 18.6%). which indicated that the degree of liver impairment is not related to the severity of infection. Hyperbilirubinemia was noted in 40 (18.2%) patients, 28 (12.7%) in DF and 12(5.5%) in DHF. The mean serum bilirubin was higher in DHF [0.87±0.33] compared to DF [0.74±0.27].Bilirubin was higher in male patients and in younger (< 20 years) age group. Elevated levels of serum alanine aminotransferase was observed to be more frequently in male patients in age group of 31-40 years and was more elevated in DF patients as compared to DHF 72(32.7% vs 40(18.2%). The mean (range) serum ALT levels were 103.7 (41-344) U/l in DHF and 69.2(20-228) U/l in DF. AST levels were raised in all DHF patients as compared to DF in which 40% patients had normal AST levels. Alkaline Phosphate was high in all DHF patients Alkaline phosphatase was raised in most of the DF patients as well and majority of patients were in age group of 31-40 years. This study leads to a conclusion that liver involvement is very common in dengue infections .Therefore in adults with fever, jaundice, hepatomegaly and altered liver function tests, the diagnosis of dengue infection should be strongly considered in areas where dengue infection is endemic.

O1.11 - The quiescent cells of masenchymal stem cells (MSCs) by hypoxic preconditioning for therapy of testis degeneration caused by protein energy malnutrition

[1,2]*Erma Safitri,* [1]*Suzanita Utama,* [2,3]*Candra Bumi,*
[4]*R. Heru Prasetyo,* [2,5]*Purwati, and* [2,6]*Fedik Abdul Rantam*

[1] Department of Veterinary Reproduction Faculty of Veterinary Medicine, Airlangga University
[2] Stem Cells Research Divison, Institute of Tropical Disease (ITD), Airlangga University
[3] Department of Epidemiology and Biostatistics Public Health, Jember University
[4] Department of Parasitology, Faculty of Medicine, Airlangga University
[5] Department of Internal Medicine, RSUD Dr. Soetomo Surabaya
[6] Department of Vet. Microbiology and Virology Faculty of Vet. Med, Airlangga University

The aim of this research was to obtain the quiescent cells of MSCs that were viable and adaptive for the therapy of testis degeneration caused by protein energy malnutrition by a treatment of hypoxic precondition in vitro culture. In this research, hypoxic precondition was the use of 1% O_2 concentration which was compared to those of culture under hyperoxic (21 % O_2) condition. Flowcytometric analysis showed that in MSCs culture under 1% O_2 concentration, the level of $CD90^+$, $CD44^+$ and $CD45^-$ were not altered (remained undifferentiated), meanwhile under 21 % O_2 concentration, cells had experienced alteration (became differentiated), that was indicated by the down regulation of $CD90^+$, $CD44^+$ and up regulation of $CD45^-$. Immunocytochemical and immunofluorescence analysis showed that under 1% O_2 concentration, MSCs culture expressed G0 cells with p63 as marker of quiescent cells, meanwhile under 21 % O_2 concentration, p63 as quiescent cells marker (stemness function), were not expressed. In conclusion, hypoxic preconditioning with 1% O_2 concentration very supported MSCs to maintain stemness before transplantation for the therapy of testis degeneration caused by protein energy malnutrition, as shown by the remaining undifferentiated cells and self renewal capacity.

O1.12 - Rapid amplicon-based sequencing of multiple clinical isolates of chikungunya virus using the ion torrent PGM platform

Tan Kim Kee[1,2], Khoo Jing Jing[2], Ava Kristy Sy[3], Syuhaida Sulaiman[2], Amado III Tandoc[3], and Sazaly Abu Bakar[1,2]

[1]WHO collaborating centre for Arbovirus Reference & Research (Dengue/Dengue Haemorrhagic Fever), Department of Medical Microbiology, Faculty of Medicine,

University of Malaya, 50603 Kuala Lumpur, Malaysia
[2]Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, 50603 Kuala Lumpur, Malaysia
[3]Virology Department, Research Institute for Tropical Medicine, Department of Health, FCC Compound, Alabang, Muntinlupa City, Philippines

Before the advent of Next Generation Sequencing (NGS) technologies, the sequencing of viral genomes relied mostly on sequencing individual virus isolates based on the Sanger sequencing method. This approach may be cost-inhibitive and time-consuming for large numbers of virus isolates, which is frequently encountered in the clinical settings especially during viral outbreaks. NGS technologies have revolutionized the means of viral genome sequencing with the ability to generate large amount of data in a short time frame. NGS is particularly suitable for sequencing viral genomes, due their relatively small size. Hence, there is considerable interest in the application of NGS technologies in rapidly sequencing viruses isolated from large numbers of clinical samples. Here, we report an amplicon-based sequencing approach for sequencing virus using the Ion Torrent PGM platform. We demonstrate the effectiveness of this method by concurrently sequencing the complete coding sequences (full genomic RNA) of 17 isolates of Chikungunya virus (CHIKV), which were extracted from mosquito cell culture supernatants after a single round of propagation from patients' sera collected in a recent outbreak in the Philippines. This approach will enable for the rapid generation of viral sequences for future phylogenetic and comparative genomic analysis of the outbreak strains with known CHIKV sequences retrieved from Genbank. Therefore, the method described here will be of great utility to generate complete coding sequences of CHIKV from clinical samples within a short time frame, having a great potential to be extended to other viruses of medical importance.

O1.13 - Antimicrobial resistance of *Salmonella enterica* serovar Typhimurium from meat origin received in VRI

**Khoo, E., Roseliza, R., Khoo, L.L, Nafizah, M., Saifu, N.R., Hasnah, Y., Norazariyah, M.N., Rosnah, Y., Rosna, D.,Siti Hanani, R. and Ramlan, M.*

Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak

The antimicrobial drug resistance pattern of *Salmonella enterica* serovar Typhimurium of meat origin received in Veterinary Research Institute (VRI) was evaluated for the period of 2009 to 2012. A total of 64 strains of *Salmonella* Typhimurium were isolated from meat samples, where 32 strains (50%) isolated from beef and pork and 32 isolates (50%) were from poultry meat. All isolates were tested for resistance against 12 different antimicrobial agents. Antimicrobial susceptibility of the isolates was tested using the disk diffusion technique with commercially available antibiotic discs on Mueller-Hinton agar according to the standards of Clinical and Laboratory Standards Institute (CLSI, 2007). 49 isolates (76.5%) showed multidrug resistance (MDR) and 51% of them were from beef and pork origin. *Salmonella* Typhimurium isolates from beef and pork showed multiple antimicrobial resistant to ampicillin, kanamycin, streptomycin, tetracycline and nalidixic acid, while isolates from poultry origin showed common profile of multiple antimicrobial resistant to sulphamethoxazole, sulphonamides and tetracycline.

O1.14 - Phylogenetic analysis of Newcastle disease virus genotype vii isolated from Perak between 1999-2012

Syamsiah Aini Shohaimi. Faizul F.M.Y, Ong G.H.,
Leow B.L., M.Redzuwan.S., Basirah M.A., Nursyuwari N.,
M.Hasrul A.H., Zunaida B.,Mazaitul Z., M. Jihan R., Ramlan M.

Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak

Here we report the sequence and phylogenetic analysis of virulent strains of genotype VII Newcastle Disease Virus (NDV) that have been circulating in Perak. A total of 61 NDV genotype VII isolates recovered during 1999-2012 throughout Perak were characterized phylogenetically. The partial region of the matrix and fusion protein was amplified by reverse transcriptase PCR, directly sequenced and compared genetically to published sequences obtained from GenBank. The deduced amino acid sequence of the fusion protein cleavage site revealed the presence of two different motifs; ^{112}RRRKRF^{117} and ^{112}RRQKRF^{117} typical for velogenic strains. The phylogenetic analysis revealed that genotype VIIa, VIIb, VIId and new subgenotype VII have been circulating in Perak not only from backyard poultry but also commercial poultry. Based on the phylogenetic tree and geographical data, it is found that NDV genotype VIIb was isolated in 1999 while in year 2001 to 2009; most of the NDV isolates were NDV genotype VIId originated from China except for one isolate that was isolated in 2005. Interestingly in 2010 and 2011, NDV outbreaks were caused by new subgenotype VII in Perak which was the same strain as isolated in 2005. In 2012, NDV outbreaks were caused by genotype VIIa which is similar to isolate from Indonesia that was isolated in 2009. This information points to the existence of multiple subgenotypes of NDV genotype VII in Perak. It is suggested that more studies should be conducted from NDV isolates of other states in Malaysia as it is essential for improving the disease control strategies and epidemiology reference.

Extended Parasitology and Entomology II

O1.16 - Effect of *Equisetum arvense* and *Urtica piluifera* plant extract on viability of protoscolices hydatid cyst of *Echinococcus granulosus in vitro*, new approach

Balsam Qubais Saeed[1] , Hussain Fadhil Hassan[2] and Hssain Ismail Arteen[3]

[1] Department of Health and Medical Sciences, Community College, University of Sharjah, Sharjah, UAE

[2] Department of Biology, College of Science, University of Kirkuk, Kirkuk, Iraq
[3] Department of Biology, College of Science, University of Mosul, Mosul, Iraq

The study included the inhibition effect of each *Equisetum arvense* and *Urtica piluifera* extracts dissolved in cold and hot water, on protoscolecis hydatid cyst**.** The protoscolices viability decreased oppositely with increasing the concentration rate of extracts, that it decreased to (32.55%) by between (0.1) and (40) mg/cm^3 concentration rate of *Equisetum arvense* extract and (31.43%) and (36.22%) by between (0.1) to (30) mg/cm^3 concentration rate of *Urtica piluifera* extracts dissolved in hot and cold water during (24) hours of treatment. The concentration of the (IC50) for extracts determineded which were (40) mg/cm^3 for *E. arvense* and (30) mg/cm^3 for *U. piluifera* dissolved in hot and cold water during (24) hr.expossure. The concentration of the (IC50) for extracts determineded which were (40) mg/cm^3 for *E. arvense* and (30) mg/cm^3 for *U. piluifera* dissolved in hot and cold water during (24) hr.expossure.

O1.17 - *Setaria cervi*, a bovine filarial parasite activates macrophage through Toll like Receptor4 (TLR4) mediated signaling pathway: *In vitro* and *in vivo* approaches

Suprabhat Mukherjee[1], Soma Seal[2], Niladri Mukherjee[1], Samir Bhattacharya[2] and Santi P. Sinha Babu[1]*

[1]Parasitology Laboratory, [2]Cellular and Molecular Endocrinology, Department of Zoology (*Centre for Advanced Studies*), Visva-Bharati University, Santiniketan- 731 235, West Bengal, India

Lymphatic filariasis (LF) is a vector borne disease mainly caused by the infectious filarial parasites viz. *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori.* This has become a serious global problem constituting 120 million of infection in 81 tropical countries and includes morbidity, disabilities and deaths. Several failures in controlling LF prompted the emergence of effective strategies like pharmacological and immunotherapeutic approaches. In this context, Toll like receptors play crucial role by regulating the expression of different genes which in turn causes pro-inflammatory responses through the activation of innate immune system and also instructs the development of adaptive immunity. Stimulation of macrophages was studied *in vitro* and *in vivo* respectively with Raw macrophage (MΦ) cell line and in rat model. Raw MΦ cells were stimulated with the endotoxin free cuticular extract of *Setaria* cervi, a model filarial parasite. After 24 hours of treatment, TLR4 signaling was studied using immunoblotting of TLR4, CD14, MyD88, pTAK1, pNF-κB and pro-inflammatory cytokines (TNF-α and IL-1β). The expression of TLR4 and its downstream signaling intermediates were found significantly high in the treated MΦ compared to control. Up regulation of MyD88 at translational level suggested that the pathway is MyD88 dependent. For *in vivo* studies, peritoneal MΦs were isolated from adult rats (n=6) experimentally infected with *S. cervi* microfilariae and aforementioned parameters were tested. *In vivo* assessment of signaling molecules clearly supported the outcomes of *in vitro* experiments. Therefore, downstream signaling pathway originated from TLR4-parasite interface may involve in the recognition and induction pro-inflammatory response against filarial parasites.

O1.18 - Ethanolic extract of *Azadirachta indica* causing apoptosis by ROS enhancement in Dirofilaria immitis microfilaria: *In vitro* and *in vivo* studies

*Niladri Mukherjee, Prasanta Saini, Suprabhat Mukherjee, Priya Roy, *Santi P. Sinha Babu*

Parasitology Laboratory, Department of Zoology (Centre for Advanced Studies), Visva-Bharati University, Santiniketan- 731 235, West Bengal, India

Canine cardiopulmonary dirofilariasis is caused by the filarial nematode *Dirofilaria immitis*. Chemoprophylaxis is difficult as adulticide agent causes complications due to dead worm accumulation, whereas, available microfilaricidal agents like ivermectin can cause compliance failure due to overuse. Therefore, there is always a need to find better agents that can be effective against heartworms; and certainly plant extracts can provide ones. *Azadirachta indica* (A. Juss.), a common evergreen tree, found throughout the Indian subcontinent have huge pharmacological properties including anthelmintic action. In this study we prepared an ethanolic extract from the leaves of *A. indica* (EEA), validated its chemical properties through effective classes identification and HPTLC. We tested EEA against *D. immitis* microfilariae (mf) *in vitro* and found that motility reduction and death of mf with profound alterations of external morphology. The mechanisms underlying were investigated and an enhancement of ROS parameters was found. Occurrence of apoptosis was evident by fragmented DNA and TUNEL assay. In addition, we also found down-regulation of anti-apoptotic and up-regulation of pro-apoptotic protein expressions. Finally, we assessed the efficacy of EEA against *D. immitis in vivo*. We had selected two doses; 25 and 50 mg/kg body weight/twice a day; both for 15 days. The effect of EEA on circulating mf was continuous and persistent. The highest reduction was counted on day 60, showing reduction of mf count by 77.94 and 86.71%. Additionally no appreciable side effects in the treated dogs were observed. These results clearly indicate that EEA might be taken into consideration for heartworm chemoprophylaxis.

O1.19 - Epidemiological survey of gastrointestinal parasites in Cholistani Cattle

Musadiq Idris[1], Jahanzeb Malik[2], Touseef-ur-Rehman[1], Umer Farooq[1], Khalid Mehmood[1], Riaz Hussain[1], Haseeb Anwar[3], Tahir Riaz[1]

[1]*University College of Veterinary and Animal Sciences, Islamia University of Bahawalpur, 62300, Bahawalpur, Pakistan*
[2]*Livestock and Dairy Development, Punjab, Pakistan.*
3Governament College University, Faisalabad, Pakistan

Pakistan is an agriculture country and livestock contribute 11.9 percent of its total GDP in the year 2012-13. The Cattle population of Pakistan is estimated about 38.3 million. Among so many cattle breed the cholistani cow is famous for their good meat, milk and hide production. There are about 6, 67,000 heads of the cholistani cattle in cholistan region which are the only source of income for the residents of this area. Keeping in view the importance of the breed, an epidemiological survey was conducted to evaluate the major parasitic problems of Cholistani cattle of the Bahawalpur and adjascent Cholistan area. Data regarding 3530 cholistani cows was collected from the different regions of the Cholistan and adjascent area of the district Bahawalpur from January, 2012 to January 2013. The data obtained was statistically analyzed by chi square test. Out of the 3530 cholistanicattles, 2646 (74.96%) were positive for gastrointestinal parasites while 884 (25.04 %) were found negative for gastrointestinal parasites. The class wise helminths infestation of the nematodes (32.80 %) showed top prevalence followed by protozoans (26.29 %), trematodes (12.18 %) and cestodes (3.68 %). Among nematodes *ascarids* were found in 1076 samples (30.48 %) followed in order by *Nematodiuris* 58 (1.64 %), *Strongylus* 12 (0.34%), *Trichuris* 8 (0.23 %) and *Oesophagostomum* 4 (0.11 %) while among protozoans Oocysts of *Eimeria* were found in 928 (26.29 %) samples. Among trematodes, prevalence of *Fasciola hepatica* was found highest in 342 (9.68 %) samples followed in order by *Schistosoma* 84 (2.37 %) and *D. dendriticum* 4 (0.11 %). Among cestodes *Hymenolepis nana* were 118 (3.34 %) on the top followed by *T. radiatum* 12 (0.34 %). The gastrointestinal parasitic infestation is significant (P< 0.001) in the cholistani cattle population and proper control measures should be adopted to control the parasitic infestation.

O1.20 - Vector of *Plasmodium knowlesi* in peninsular Malaysia: Where are the breeding sites?

Rohani A., Zamree I., Aidil AA, Ariffin MM, Hanif MO, Zuhaizam H.Wan Najdah WMA and Lee HL

Medical Entomology Unit, WHO Collaborating Centre for Vectors,
Infectious Disease Research Center,
Institute for Medical Research, 50588 Kuala Lumpur

A major reason for the study of mosquito larval ecology is to gather information on environmental variables that may determine the species of mosquitoes and the distribution of larval in the breeding habitats. As part of a study to assess remote sensing data as a tool for vector mapping, we conducted entomological surveys to determine the type of mosquitoes, their characteristics and the abundance of habitats of the vector *An. cracens* in knowlesi malaria endemic areas in peninsular Malaysia. A total of 102 breeding habitats were sampled in *Anopheles* breeding sites. The study showed that *An. cracens* preferred to breed in ground pool, animal pool, animal track and tyre track in recreational park, fruit orchard, jungle fringe, rubber and palm oil plantations. The commonest larval habitats were shallow ground pools at a depth of 1.5 – 8.5 cm with clear water and mud substrate. The mosquito also preferred open and partially shaded habitats. Breeding habitats were generally located at 0.8-3.7 km from the nearest human settlement. Environmental variables influenced the suitability of aquatic habitats for anopheline and culicine larvae, but not significantly associated with the occurrence of both larvae genera ($p > 0.05$). This study provides information on mosquito ecology in relation to breeding habitat which will be useful in designing and implementing larval control operations. This study also amplifies the need for a broadening of the GIS approach which is emphasized with the aim of rejuvenating the dynamic aspect of entomological studies in Malaysia. In fact, the use of such basic GIS platforms promotes a more rational basis for strategic planning and management in the control of endemic diseases at the national level.

O1.21 - Using time allocation study to measure level of human risk and exposure to *Plasmodium knowlesi* infection

Paul Porodong, Kntayya Mariappan, Gaim James Lunkapis,
Maureen De Silva, Mohammad Tahir Mapa, Diana Peters,
Maine Suadik, Amy Lim Ping Ping

University Malaysia Sabah

Occupational status has long been regarded as “central dimension of social inequality”. Some put it “Occupational position neither does nor encompass all aspects of social class, but it is probably the best single indicator of it”. They further argued that occupational position can explain other social inequality and this include health status and risk particularly in developed countries. Although latter study found out that the correlations between indicators of occupational status are diminishing once other variables are taken factored in, it remains as important variable to consider in many health related studies. This paper however is an attempt to provide an alternative look on how health risks and exposures are better understood by using time allocation study with specific reference to *Plasmodium knowlesi* infection.

O1.22 - Enhance Th2 and suppress Th1 improvement of colitis by *Heligmosomoides polygyrus* larva 3rd extract

Agustina Tri Endharti[1], Aswin D. Baskoro[1], Eviana Norahmawati[2], Donabella F. Santosa[1],*

Department of Parasitology, Faculty of Medicine, University of Brawijaya[1], Department of Pathology Anatomy Faculty of Medicine, University of Brawijaya[2]

Ulcerative colitis is an ulcero inflammatory disorder that affects the colon and chronically caused by an imbalance of the immune system. The aim of this study was to determine the effects of *Heligmosomoides polygyrus* extract on the pathogenesis of DSS-induced colitis. *Heligmosomoides polygyrus* extract can be used as an alternative therapy to help the balance of the immune system. Extracts of *H. polygyrus's* colon can enhance Th2 and suppress Th1 improvement. This study showed the expression of TNF-α dan TGF-β derived from colon of Balb/c mouse with and without treatment of *H. polygyrus* extract. There are 5 groups, there were the negative control group, positive control and three groups with *H. polygyrus* extract at various doses (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg, respectively. Mice were induced colitis by 1-week oral exposure to 3% DSS in drinking water, then treated with *H. polygyrus* extract for 12 weeks and followed by RNA isolation to detected TNF-α and TGF-β expression. *Intracellular* expression of TNF-α on each group also were assessed by flow cytometer. One way ANOVA showed increased TNF- α and decreased TGF expression, at a dose of 0.4 mg/kg body weight. It can be concluded that gene expression alterations in regulation of counteracting TNF-α and TGF-β expression in colitis by *Heligmosomoides Polygyrus* Larva 3rd Extract.

O1.23 - Genetic diversity of the *Plasmodium vivax* Duffy-binding protein (PvDBP) gene among samples in Sabah, Malaysia

Muhammad Nor Farhan, S.[1], Myat, H. N.[2], Noor Rain, A.[1], Umi Rubiah, S.[1], Azrina, A.,[1] Prem Kumar, M.[1], Jenarun, J.[3], Khairul Faiz R.[4], Moizin, T.[5] and Zakiah, I.[1]

[1]Herbal Medicine Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Ministry of Health, Malaysia
[2]Parasitology Research Division, Department of Medical Research, Dagon PO 11191, Yangon, Myammar.
[3]Sabah State Health Department, Rumah Persekutuan, Jalan Mat Salleh, 88590 Kota Kinabalu, Sabah, Ministry of Health, Malaysia.
[4]Tawau Health Department, P.O box 990, 910080 Tawau, Sabah, Ministry of Health, Malaysia
[5]Vector Borne Disease Control Unit, Hospital Kota Marudu, P.O box 255, 89108 Kota Marudu, Sabah, Ministry of Health, Malaysia.

Plasmodium vivax Duffy Binding Protein (*Pv*DBP) is a member of the Duffy binding-like erythrocyte binding protein (DBL-EBP) family expressed in the micronemes on the surface of *P. vivax* merozoites. It consists of seven regions responsible for the invasion of the parasite into erythrocyte. DBP type II region is highly polymorphic and genetically diverse. A molecular study was conducted to determine the polymorphism pattern of the *P. vivax* DBP type II (*Pv*DBPII) on samples from Sabah. This study was conducted using *P. vivax* infected blood on filter paper collected from Kota Marudu and Kalabakan, Sabah during 2009 to 2010. There were 20 *P. vivax* samples and DNA was extracted and Nested Polymerase Chain Reaction was performed to amplify the *Pv*DBPII region. Twenty samples were selected for cloning, purification and sequencing. Sequence data were analysed using Seqman II software for sequence assembling. The sequences were aligned with Salvador-1 strain (Sal-1) as the standard *Pv*DBPII sequence using Clustar W algorithm method from Megalign software of DNAstar programme. The sequence data was then further analysed using Mega 5.2 software, for determination of mutation at *Pv*DBPII and phylogenetic tree was constructed from the aligned sequence. The findings showed that all samples were successfully amplified with PCR products of about 900bp. Our finding showed there are 288 amino acids within *Pv*DBPII gene, of which 36 are nonsynonymous mutation and 11 are synonymous silent mutation. Phylogenetic analysis of *Pv*DBPII showed that the phylogenic tree has 9 Groups and samples from Sabah are categorized into 4 groups mainly in Group 1 (6 samples) with Myanmar and Thailand, Group 6 (1 samples) with Thai, Myanmar and Sal-1, Group 8 (8 samples) and Group 9 (5 samples) with Thailand and Myammar. The function of the mutations are unknown, however both the nonsynonymous and synonymous mutation can affect *P. vivax* parasite sequence structure at Sabah. In conclusion, the results suggested that *Pv*DBPII gene among Malaysia isolates is genetically diverse. Sequence information of this *Pv*DBPII gene can provide useful information for development malaria vaccines and the implementation of malarial elimination programmes in Malaysia.

O1.24 - Molecular genotyping of *P. vivax* at *pvdhps* and *pvdhfr* genes among sample in Sabah

Noor Rain, A.[1], Umi Rubiah, S.[1], Nor Azrina, N.,[1] ,Muhammad Nor Farhan, S.[1],Prem Kumar, M.[1], Jenarun, J.[2], Moizin, T.[3] , Hasidah, M.[4] ,Zakiah, I.[1]

[1]Herbal Medicine Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Ministry of Health, Malaysia
[2]Sabah State Health Department, Rumah Persekutuan, Kota Kinabalu, Sabah, Ministry of Health, Malaysia.
[3]Vector Borne Disease Control Unit, Kota Marudu, Sabah, Ministry of Health, Malaysia.
[4]School of Biosciences and Biotechnology, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Malaysia

The combination of sulfadoxine and pyrimethamine (SP / Fansidar) has been used as a second line drug to treat cloroquine-resistant plasmodial parasite infections. Resistance to SP was determined by specific point mutations in two drug resistant marker genes, *P. vivax dihydrofolate reductase (pvdhfr)* and *P. vivax dihydropteroate synthase (pvdhps)*. Both genes can cause alterations in key amino acid residues at the active sites of these enzymes thus reducing affinity of the enzyme for the drug. Prevalence and pattern of mutations in *pvdhfr* and *pvdhps* genes were examined in 37 samples collected from patients with *P. vivax* infection. Those patients were screened in Kalabakan and Kota Marudu, Sabah during 2008 until 2012. The study was carried out to determine the profile of *pvdhfr* and *pvdhps* genes. Nested polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) showed that a single nucleotide polymorphism-haplotype at amino acid positions 13, 33, 57, 58, 61, 117 and 173 of *pvdhfr* gene and amino acid position 383 and 553 of *pvdhps* gene were detected. All isolates showed the presence 100% of the wild type for *pvdhps* gene at codon 553 (Ala553) while mutation 383Gly was detected in 64.9% of sample isolates. For *pvdhfr* gene, all (100%) isolates were found to carry the wild-type Ile13, Pro33 and Ile173, with 117Thr and 57Leu mutations. The 58Arg and 61Met mutations were detected in 40.5% and 43.2% of the isolates, respectively. The combination of *pvdhfr* and *pvdhps* haplotypes demonstrated four distinct haplotypes. The most prevalent haplotypes were the combination of 57Leu + 117Thr in *pvdhfr* gene and 383Gly in *pvdhps* gene (37.8%). The highest quintuple *pvdhfr/pvdhps* (27.1%), was four *pvdhfr* (57Leu, 58Arg, 61Met, 117Thr) and one *pvdhps* (383Gly) mutations. 117Thr mutation capable of conferring pyrimithamine resistance on its own and likely to be a precursor to further *dhfr* mutation. The single mutant with 58Arg was suggested as the second mutation that arose under pyrimethamine pressure. The information helps to understand how the SP resistance arises and spreads in natural *P. vivax* populations in Sabah. Our results provide basic information on the potential appearance of the mutations responsible for SP resistance. The paucity of *P. vivax* underlines the need to extend the molecular analyses to other areas where *P. vivax* is common.

Extended Parasitology and Entomology III

O1.25 - Photovoice technique as a tool to understand the level of risks to *Plasmodium knowlesi* (malaria) infections: A preliminary analysis of findings of a study in Sabah, Malaysia.

Kntayya Mariappan, Paul Porodong, Gaim Lunkapis, Mohd Tahir Maureen De Silva, Diana Peters, Maine Saudik Mapa,

School Of Social Sciences, University Malaysia Sabah

Photovoice method has now been used as yet another tool to community-based participatory research to document and understand the aspects of communities' lives and experience. In this study conducted by LSHTM (U.K) to identify risk factors that lead people to be exposed to malaria infection (*pk*) in Sabah, this method has been adopted as one of the components of methodology of social science to understand the communities' exposure to risk factors of malaria infection in some key areas that are prone to malarial infection in Sabah (Kudat). This paper examines the risk factors as disclosed by the participants through a series of interviews conducted by researchers based on the description of the participants of a collection of photos taken by the participants themselves. This paper tries to present some of the earlier findings of research work that is still ongoing.

O1.26 - Control of gastro-intestinal nematodes in ruminants using a mixtures herbal extracts

*Kimani, D.[1], Kareru, P.G.[1], Kutima, H.L.[3], Njonge, F.K. *[2], Nyagah, G.C.[1], Karanja, J.M.[1]*

[1] Chemistry Department, Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O Box 62000, Nairobi, Kenya
[2] Land Resource, Planning & Management Department, JKUAT, P.O Box 62000, Nairobi, Kenya
[3] Zoology Departments, JKUAT, P.O Box 62000, Nairobi, Kenya

Helminthes infestation is a major constraint to livestock production. Increasing anthelmintic resistance and the impact of conventional anthelmintics on the environment has led to increased interest on new novel plant-based compounds. In this study, *in vitro* anthelmintic activities of herbal mixtures of *Entada leptostachya* and *Prosopis juliflora* were investigated. Egg hatch inhibition tests were done using various ratios of aqueous extract mixtures of *Entada leptostachya* and *Prosopis juliflora.* Graduated doses of between 0.4 and 3.2mg/ml were used on fresh nematode eggs harvested using simple salt floatation method. The eggs were determined to be a mixture of nematode species, namely, *Haemonchus spp., Trichostrongyle spp.* and *Oesophagostomum spp.* The *in vitro* anthelmintic activities of the plant extract mixtures were compared to albendazole. The most active aqueous extract mixture was then subjected to acute oral toxicity tests using adult female Wistar Albino rats. The OECD 425 guidelines (Up-and-Down procedure) were followed. The ratio 1:7 (*E. leptotachya*:*P. juliflora*) was the most active (LC_{50}= 0.370 mg/ml) while the ratio 2:1 was the least active (LC_{50}= 2.052 mg/ml). The activity of the plants ratio 1:7 was comparable to albendazole (LC_{50}= 0.248 mg/ml) and their activities were not statistically different (P>0.05). The toxicity studies of the ratio 1:7 showed that the LD_{50} was above the upper limit of 5000mg/kg B.W. and no toxicity signs were observed for the entire period of the studies. In conclusion, the extract mixture ratio of 1:7 was therefore relatively active and safe and has potential as a novel anthelmintic drug for the treatment of gastro-intestinal nematodes.

O1.27 - A survey on seroprevalence of toxoplasmosis in sheep in Tabriz (Northwest of Iran) by ELISA

Ahmad Nematollahi

University Of Tabriz

Toxoplasmosis caused by Toxoplasma gondii, an intracellular parasite, is one of the important zoonotic diseases in the world. Cat is the definitive host and human and ruminants are intermediate hosts. One of the major aspects of this disease is annual economical losses. In addition, hygienic losses in the human societies, abortion in sheep are the other important aspects in economical losses. In this survey, 186 sera samples from Tabriz (Northwest of Iran) suburb sheep livestock were studied by using ELISA. Thirteen samples (6.98%) (Seven rams and six ewes) were positive according to the experiments. Data analysis by Chi Square and Fisher's methods showed no significant differences between sex of examined sheep and infection rate. In addition, the results of this study in comparison to other studies performed in this field in Iran, showed lower infection rate in sheep of Tabriz district to toxoplasmosis. High hygienic levels and advance prevention methods in this region against toxoplasmosis and using different serological method for diagnosing of disease can be this difference in infection rate between Tabriz sheep and sheep of other regions of Iran.

O1.28 - Epizootiology of tick-borne theileriosis in the selected water buffalo (Bos bubalus) population of district Khanewal, Punjab, Pakistan

Muhammad Sohail Sajid[1], Rao Muhammmad Siddique[1], Sohail Ahmad Khan[2], Muhammad Jawad-ul-Hassan[1], Zafar Iqbal[1],*

[1]Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan, [2]District Diagnostic Laboratory, Khanewal, Punjab, Pakistan

The purpose of this presentation is to identify the epidemiological determinants which are associated with the frequency distribution of tick-borne theileriosis in tropics, generally and in Pakistan specifically. Ticks (Acari:Ixodidae) are a sustained nuisance to the livestock and dairy sector round the globe. The signficance of ticks and tick-borne diseases is increased manifolds in tropical countries due to favorable enviromental conditions for the vector species. Theileriosis, caused by different members of family *Theileridae* (Apicomplexa: Piroplasmida) is transmitted through *Hyalomma* spp. ticks. This study describes results of a cross-sectional survey of theileriosis conducted in water buffaloes (*Bos bubalus*) of district Khanewal (Punjab, Pakistan) through conventional optical microscopy of Giemsa-stained blood films. Overall, 22.78% (339/1488) buffaloes were found infected with theileriosis. Buffalo calves, females and Kundi breed were at higher risk than adults, males and Nili Ravi breed, respectively. Summer was found optimum for the disease prevalence followed in order by autumn, spring and winter. Higher prevalence of theileriosis has been recorded in tethered animals than non-tethered, closed housing than semi-closed or open housing systems. Poor hygienic animals reared on un-cemented floor were more prone to theileriosis as compared to those having good hygienic measures and kept on partially cemented and cemented floors. The data will not only be helpful for the dairy farmers of tropical countries to modulate farming practices but also for the policy and decision makers of the continent to control the biological vectors and ultimately theileriosis in the livestock population.

O1.29 - Screening of Malaysian biodiversity for new candidates effective against leishmaniasis

[1]Roshan Jahn Mohd Salim*, [1]Getha Krishnasamy, [1]Hema Thopla, [1]Norhayati Ismail, [1]Muhammad Syamil Azahar, [1]Mohd Ilham Adenan, [2]Vanessa Yardley

[1]Drug Discovery Center, Natural Product Division, Forest Research Institute Malaysian (FRIM), 52109 Kepong, Selangor, Malaysia.
[2]Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom.

Leishmaniasis categorized as a neglected tropical disease is caused by a parasite known as Leishmania. The parasite is transmitted through the bite of female sandfly from the *Phlebotomus* genus. Though leishmaniasis is not endemic to our nation a recent outbreak in the southern province of Thailand should send an alarm to all neighboring countries. Hence, this project was carried out to screen our mega-diverse plant and microbial biodiversity for active compounds effective against the most prevalent form of the disease, cutaneous leishmaniasis. A total of 144 culture extracts prepared from 81 isolates of soil actinobacteria and 125 extracts obtained from 111 plant species were tested for anti-leishmania activity using the *Leishmania major* species. An extracellular promastigote assay was used with Amphotericin B as the positive control. The assay identified hit extracts from six actinobacteria isolates and three plant species which showed strong anti-leishmania activity (IC_{50} < 20 µg/ml). Out of these the actinobacteria TY031-010 and three plant samples DDC 126-1, DDC 134-1 and DDC 133 showed promising activity with high selectivity (SI>11) towards the leishmania pathogen.

Zoonosis

O2.1 - Helminth fauna of *Rattus tanezumi* and *Rattus norvegicus* in Muñoz Nueva Ecija, Philippines and its zoonotic potential

Diane Shiela Castillo[1], Gil Gabriel Villancio[1], Vachel Gay Paller[1]*

[1]Animal Biology Division, Institute of Biological Sciences, University of the Philippines Los Baños, Laguna, Philippines 4031

Rattus tanezumi and *Rattus norvegicus* are the most common rodent species in the Philippines, with the former mainly inhabiting urban areas and the latter in agricultural lands. Generating information on the zoonotic helminth species harboured by these rodents would be useful for public health. Samples of 100 rats were collected from traps placed each month from July to December in five towns of Muñoz, Nueva Ecija. A total of 600 rat samples, consisting of 300 *Rattus tanezumi* and 300 *Rattus norvegicus*, were collected and sacrificed within a span of six months. Parasitic helminths collected from the sacrificed rats were: Trematoda- *Echinostoma ilocanum* and *Echinostoma malayanum*; Cestoda- *Dipylidium sp*., *Hymenolepis sp.* and Strobilocercus fasciolaris (*Taenia taeniaformis*); Nematoda- *Angiostrongylus cantonensis*, *Nippostrongylus sp.*, *Rictularia sp.*, Meanwhile, helminths detected through fecalysis were: Trematoda- *Echinostoma sp.*; Cestoda- *Hymenolepis sp.* and *Taenia sp.*; Nematoda- *Trichuris muris* and eggs of strongylids. From all these, *Dipylidium sp.* was only found in *R. norvegicus.* Sex and weight was also considered parameters in the dynamics of parasite distribution. The difference in prevalence, intensity and abundance of all the other helminths between and among the places of collection as well as the date of collection was noted. Zoonotic potential of some of the helminths was also discussed in the paper. *Echinostoma* sp. and *Angiostrongylus* sp. on the other hand were the parasites found that pose an immediate zoonotic threat. Data on the encroachment of these rodent species in human settlements further increase the risk of exposure to such zoonotic species.

O2.2 - Fecal detection of *Cryptosporidium* oocysts from individuals with diarrhea and domestic animals in three costal districts of Odisha (India): Possible zoonotic transmission and concerns

Arpit Shrivastava[1], Miles Daniel[2], Woutrina Miller[2], Marion Jenkins[3], Mrutyunjay Suar[1], Priyadarshi Sahu[1]

[1]School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India.
[2] Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California, USA.
[3] Department of Civil and Environmental Engineering, University of California, Davis, California, USA.

Cryptosporidium is an important intestinal protozoan pathogen recognized to have zoonotic risk and an under-recognized public health problem in India. This is the first study in this region aiming towards exploring the burden of cryptosporidiosis in human and various domestic animals of regional economic importance with a transmission consideration in the community. Fecal samples were collected and tested from domestic animals (cows, buffalos, sheep, goats, dogs, cats, and chickens) and human cases presenting with acute diarrheal symptoms living in three districts on eastern coast of Odisha. *Cryptosporidium* oocysts were concentrated by immunomagnetic separation (IMS) technique and identified by direct fluorescent antibody (DFA) staining. Overall the test was found positive for *Cryptosporidium* oocysts in 10% of human and 17.11% of animal samples examined. Of the positive human cases majority were found in the age group of <5 years compared to >5 years age group ($P = 0.05$). Estimate of environmental loading of oocysts was found to be highest for sheep ($\approx 1 \times 10^8$ oocycts day-1 animal-1) followed by goat ($\approx 5 \times 10^5$ oocycts day-1 animal-1). The incidence was more clustered in the rural area compared to the urban cities. The present study revealed the potential of cryptosporidiosis in coastal Odisha, a province which was never explored before. Sheep and goat were identified to be major contributors in terms of environmental loading of infective oocysts thereby causing human infections in this region.

O2.3 - Molecular characterization of a porcine rotavirus detected in a Sri Lankan child with acute diarrhea

Minako Takaki[1], Nilmini Chandrasena[2], Shaman Rajindrajith[3], Kithsiri Gunawardena[2], Akira Nishizono[4], and Kamruddin Ahmed[1]*

[1]School of Medicine (Research Promotion Institute), Oita University, Yufu, Oita, Japan
[2]Department of Parasitology, and [3]Pediatrics, University of Kelaniya, Ragama, Sri Lanka
[4]Department of Microbiology, Faculty of Medicine, Oita University, Yufu, Oita, Japan

Globally, rotaviruses are important cause of acute childhood diarrhea. The rotavirus genome consists of 11 segments of double-stranded RNA encoding 6 structural (VP1 – VP4, VP6, and VP7) and 6 non-structural (NSP1 – NSP6) proteins. Interspecies transmission of rotaviruses is a major source of generation of new reassortants and genetic variants. Interspecies transmission of rotavirus or reassortants has not been reported from Sri Lanka. We detected a rotavirus strain of porcine origin from a 12 month-old boy with diarrhea and fever hospitalized in June 2009 at the Colombo North Teaching Hospital (CNTH), Ragama, Sri Lanka. He was treated with oral rehydration solutions and probiotics, and was discharged 4 days after hospitalization. Rotavirus was detected in the stool sample using commercial ELISA kit. By reverse transcription (RT) PCR, the genotype was determined as G4P[6]. Nucleotide sequencing of all genes identified the strain to be of porcine origin rotavirus containing at least two genes of rotavirus from human origin, thus indicating possibility of a porcine-human reassortant rotavirus. The child's neighbors have been raising pigs on a domestic scale for additional income. This report provides first evidence of interspecies transmission of rotaviruses in Sri Lanka. Due to close proximity of humans and pigs, zoonotic transmission generated this new reassortants and genetic variant of rotavirus. Surveillance should be strengthen to reveal the extent of spread of this type of reassortants in Sri Lankan children since reassortants are a challenge to the currently available rotavirus vaccine and is important for understanding the evolution of rotaviruses.

O2.4 - Filarial parasites infecting anurans and reptiles in Krau wildlife reserve, Pahang, Malaysia

Saharul Kasim[1], Alwyn Cabil Bundong[1], Daicus M. Belabut[1], Masako Fukuda[2], Shigehiko Uni[1] and Rosli Ramli[1]

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[2]Research Promotion Institute, Oita University, Oita 879-5593, Japan

We have studied morphologic and molecular characteristics of filarioids in anurans and reptiles to comprehend the phylogeny of filarioids. We examined filarioids in anurans and reptiles in Krau Wildlife Reserve, Pahang, Malaysia, between March and September 2013. Thirty-four anurans (three toads and 31 frogs) and seven reptiles (two lizards, two geckos and three snakes) collected were dissected under a stereomicroscope to detect adult filarioids. Blood smears and skin snips were made to detect microfilariae. The results showed that 15 (44%) of 34 anurans were infected with adult filarioids and microfilariae: the host anurans were three *Phrynoidis aspera*, eight *Hylarana glandulosa*, one *Hylarana labialis*, two *Limnonectes blythii* and one *Polypedates leucomystax*. One species of filarioids found in the body cavity of *H. glandulosa* was *Waltonella malayensis* Petit and Yen, 1979 and another species found in the subcutaneous connective tissues of *B. asper* was *Icosiella* sp. The adults and microfilariae of *Icosiella* sp. were different from those of *I. innominata* Yuen, 1962 and *I. laurenti* Bain and Purnomo, 1984, as described in Malaysian anurans. Out of seven reptiles, two species of lizards were infected with filarioids: adult worms that appear to be *Gonofilaria* sp. were found in a large forest gecko (*Gekko smithii*), and microfilariae were found in a skink, *Eutropis multifasciatus*. The phylogenetic tree was constructed to indicate the relationship between *Icosiella* sp. and *Waltonella* sp. found in anurans. Besides this, we found cestodes, trematodes, nematodes and acanthocephalans in the host animals.

O2.5 - Analysis of biomarkers in plasma and urine of leptospirosis patients

*Haorile Chagan-Yasutan[1, 2], Susan Leano[3], Elizabeth Telan[3], Hiroko Iwasaki[1], Toshio Hattori[1,2]**

[1]Laboratory of Disaster-related Infectious Disease, International Research Institute of Disaster Science, Tohoku University, Japan; [2]Emerging Infectious Diseases, Division of Internal Medicine, Graduate School of Medicine, Tohoku University, Japan; [3]National Reference Laboratory for HIV/AIDS, Hepatitis, and other STDs, STD/AIDS Cooperative Central Laboratory, Manila, Philippines

Leptospirosis is a zoonosis of worldwide distribution, endemic mainly in countries with humid subtropical or tropical climates and has epidemic potential. Early and accurate diagnosis of leptospirosis is important for proper and prompt treatment. In this study, we measured potential biomarkers in plasma and urine to assist in early detection, monitoring disease progression in response to the therapeutic interventions on clinical diagnosis of leptospirosis. A total of 135 leptospirosis suspected patients were enrolled in the study and 112 of them were confirmed to be leptospirosis. Biomarkers of osteopontin (OPN), N-half OPN, galection-9 (Gal-9) were measured by Elisa. Compared with controls, confirmed leptospirosis patients had significantly higher plasma levels of OPN ($P < 0.0001$), N-half OPN ($P < 0.0001$), Gal-9 ($P < 0.0001$), and urine levels of OPN N-half/creatinine ($P = 0.04$) and NAG/creatinine ($P = 0.004$) with no significant differences in OPN/creatinine ratio. Receiver operating characteristic curve analysis showed that the plasma Gal-9 levels exhibited the greatest ability to discriminate leptospirosis patients from healthy group based on the AUC (0.953), followed by plasma OPN (0.882) and plasma OPN N-half (0.632). In urine samples, the urine NAG/creatinine ratio levels exhibited the greatest ability to discriminate leptospirosis group from healthy group based on the AUC (0.848), followed by urine OPN N-half/creatinine ratio (0.667) and urine OPN/creatinine ratio (0.619). Our result showed that those biomarkers reflect disease activity of leptospirosis and urinary biomarker is also useful to monitoring the information status in the leptospirosis patients.

O2.6 - Past, present and future of Japanese Encephalitis in Sarawak

Ooi Choo Huck[1], Bibiana Krystal[1], Tatieng James[1], Richard Chares[1], Billy Sujang[1], Jamilah Hashim[2], Andrew Kiyu[3]

[1]Vector Borne Diseases Section, Sarawak Health Department, 93050 Kuching, Sarawak, Malaysia.
[2]Director's Office, Sarawak Health Department, 93050 Kuching, Sarawak, Malaysia.
[3]Public Health Research Section, Sarawak Health Department, 93050 Kuching, Sarawak, Malaysia.

Japanese encephalitis is endemic in Sarawak with an annual incidence rate of 1.18 cases per 100,000 populations in 1996-2000. Inactivated mouse-derived JE vaccine (Biken, Japan) was introduced into the Expanded Programme of Immunisation in Sarawak in July 2001. The recommended vaccination interval schedule was two primary doses at nine and ten months followed by booster at 18 months and 4.5 years. The objective of this study is to compare the epidemiological situation of Japanese encephalitis in Sarawak before and after the introduction of the Japanese encephalitis vaccination. A retrospective descriptive analysis was conducted on Japanese encephalitis epidemiological surveillance database of Sarawak State Health Department between January 1996 and December 2012. Significant seasonality was demonstrated for Japanese encephalitis cases in Sarawak in 1996–2001, with maximum cases occurring in October, November, December, January and February ($p < 0.001$). Significant seasonality was also seen for cases in 2002-2012, with maximum cases occurring in September, October, November and December ($p < 0.001$). Median age for cases in 1996-2001 was 10 years and was significantly higher than the cases in 2002-2012 (median age 7 years, $p < 0.001$). In the multivariable negative binomial regression analysis, the incidence rate of Japanese encephalitis in 1996-2001 was significantly higher than in 2002-2010 (incidence-rate ratios = 1.78, $p < 0.001$, 95% confidence interval 1.29–2.45). In conclusion, there was significant reduction of Japanese encephalitis cases in Sarawak and increase in the median age of cases following the introduction of Japanese encephalitis vaccination in 2001.

O2.7 - Prevalence and genetic characterization of hookworms in humans and dogs living in the same village in Cambodia

Tawin Inpankaew[1,2]*, Fabian Schär[3,4], K. Darwin Murrell[1], Anders Dalsgaard[1], Muth Sinuon[5], Peter Odermatt[3,4], Rebecca J Traub[6]

[1] Department of Veterinary Disease Biology, Faculty of Health and Medical Science, University of Copenhagen, Denmark
[2] Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University,Bangkok, Thailand
[3] Swiss Tropical and Public Health Institute, Basel, Switzerland
[4] University of Basel, Basel, Switzerland
[5] National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia
[6] School of Veterinary Science, University of Queensland, Gatton, Queensland, Australia

Hookworms are a common cause of iron deficiency anaemia in both humans and animals in tropical and sub-tropical regions of the world. In addition to anthroponotic species *Necator americanus* and *Ancylostoma duodenale,* humans are also susceptible to infection with the zoonotic species of hookworm, *Ancylostoma ceylanicum*. This study aimed to determine the prevalence, associated risk factors and potential transmission dynamics of *A. ceylanicum* in humans and dogs living in a hookworm endemic village in Cambodia. A total of 218 human and 94 dog faecal samples were collected from 67 households in Dong village, Preah Vihear province, Cambodia. Faecal samples were examined microscopically for hookworm eggs by sodium nitrate floatation and screened to a species level using PCR targeted against the internal transcribed spacer region. The prevalence of hookworm infection in humans was 26.6 % by microscopic examination and 56.9% by molecular diagnosis. In dogs 80.8% were positive for hookworm eggs by microscopic examination and 95.7% were positive by molecular diagnosis respectively. Molecular characterization of human hookworm revealed that 59/124 hookworm positive humans (47.6%) to be infected with *N. americanus*, 46.0% (57/124) with *Ancylostoma ceylanicum* and 0.8% (1/124) with *A. duodenale,* respectively with 95.2% (118/124) as single infection. In total 85/90 (94.4%) dogs were positive for *A. ceylanicum* and 8/90 (8.9%) for A. caninum, respectively with 96.7% (97/90) as single infection. Further characterization of *A. ceylanicum* isolates from dogs and humans at mitochondrial genes (cox-1) revealed A. ceylanicum divided into two clades, consisting of human isolates, and the other clade comprising of mixed human and dog isolates. This finding implies that dogs act as a significant source of zoonotic hookworm infection to human population in this study especially *A. ceylanicum*.

O2.8 - Clinical risk factors of severely dehydrated childhood diarrhea in Queen Sirikit national Institute of Child Health, Thailand

C Thu Win*[1], Kriengsak Limkittikul[2], Chukiat Sirivichayakul[2], Siriluck Jennuvat[3]

[1]Defence Service Medical Research Unit, Naypyidaw, Myanmar
[2]Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Thailand
[3]Queen Sirikit National Institute of Child Health, Thailand

Diarrheal disease is a leading cause of childhood morbidity and mortality in the developing world. This retrospective case-control study aimed to identify the clinical risk factors and clinical characteristics on admission day for severe-dehydration diarrhea among a study cohort of 140 children. The study group comprised 35 cases (severe dehydration) and 105 controls (mild and moderate dehydration), aged between 1 month and 5 years, and admitted to Queen Sirikit National Institute of Child Health from July 1, 2006 to June 30, 2009. The study showed that children with lower weight were more prone to severe-dehydration diarrhea (P=0.034). A common finding among the severely dehydrated patients was sunken eyeballs. Pre-admission administration of ORS was significantly higher among severe-dehydration group (P=0.024). For laboratory results, higher urine specific gravity (> 1.020) and lower bicarbonate were significant findings for the severe-dehydration group. Regarding hospital treatment, the severely dehydrated group underwent longer courses of intravenous fluid treatment, and was more commonly administered parenteral antibiotics. These findings are useful for the treatment of diarrheal diseases, especially those involving severe dehydration, in peripheral hospitals lacking laboratory facilities.

Malaria and Vectors

O3.1 - Household enumeration of a small highland malaria-endemic community in Puerto Princesa City, Palawan, Philippines

[1]*Pauline Joy M. Lorenzo,* [1]*Arianne Katrina Coballes,*
[2]*Gloria Luz M. Nelson, and* [1]**Fe Esperanza Espino*

[1]*Department of Parasitology, Research Institute for Tropical Medicine, FCC Alabang, Muntinlupa City, Philippines*
[2]*Department of Social Sciences, University of the Philippines Los Baños, College, Laguna, Philippines*

Total household enumeration was conducted in three selected sitios in Barangay Bacungan, Puerto Princesa City, Palawan. The enumeration, which was carried out in May-July 2013, was divided into two phases. All structures with roof and have at least 1x1 meter in dimension and areas that people usually sleep were identified during the household tagging. During the second phase, household interviews were conducted by trained staff to gather information about the household profile, household structures and basic services, and their observations of monkeys. A total of 169 identified main houses were geo-located and 152 households interviewed. The survey revealed that farming is their major occupation. A map of the site was also created. Statistical analysis was done with the aid of MS Excel and SPSS. Anecdotes of the respondents revealed that monkeys are observed in the land where they farm in two out of the three sitios. Majority of them also perceived monkeys as pest since it devour their crops especially during harvest season. Thus, others resorted in hunting monkeys to be eaten or kept as pets. This enumeration was a preliminary activity of a bigger project entitled: *Plasmodium knowlesi* ESEI Research Project. One of the objectives of the study is to identify the risk factors that put people at risk of having *P.knowlesi* malaria infection which is known to originate from monkeys. This was conducted to provide the project team the pertinent socio-demographic information about the people in the site and a detailed map for the project's future activities.

O3.2 - *Anopheles culicifacies* Giles s.l. sibling species E in Sri Lanka varies intra-specifically in amino acid sequence of the cytochrome oxidase ii gene of mitochondrial DNA

Iresha Harischandra[1], Ranil Dassanayaka[2] ,Nissanka De Silva[1]
[1]Department of Zoology, Faculty of Applied sciences,

University of Sri Jayewardenepura, Sri Lanka
[2]Department of Chemistry, Faculty of Science,
University of Colombo, Sri Lanka

Anopheles culicifacies comprises as a complex of five sibling species provisionally designated as A, B, C, D & E. The presence of these five sibling species was reported from India and while Sri Lanka was reported to have sibling species B and E and of these, sibling species E is the major vector. Precise identification of these sibling species is important in the malaria control programs. D3-PCR/ITS2-*Rsal* PCR assay followed by either AD-PCR or the BCE-PCR assay based on mitochondrial cytochrome oxydase subunit *II* (*CO II*) is the only available method to identify the sibling species in this complex. Therefore, the latter molecular method was implemented in this study for *An. culicifacies* isolated from Sri Lanka to ascertain its utility in identification of sibling species and to investigate changes in amino acids, if any in the coding region of the *CO II* gene. In doing so, *CO II* regions of sibling species B and E were amplified and sequenced, and corresponding amino acid sequences were obtained and compared. Amino acid sequences of *CO II* region showed three types of variation amongst sibling species E populations in Sri Lanka and the amino acid substitutions observed were amino acids that are reported to be involved in mutational pressure. Sibling species B showed an identical amino acid sequence for *CO II* gene although; there were variations in nucleotide sequences. In BCE-PCR, the banding pattern obtained for sibling species found in Sri Lanka was different from the banding pattern of Indian sibling species casting a doubt about its utility in differentiation of sibling species in Sri Lanka. Finally, amino acid sequence variations of *CO II* within the sibling species E suggest the possibility of speciation within the *An. culicifacies* population in Sri Lanka which may have some impact on the vector control programs.

O3.3 - Preliminary study of entomologic parameters in relation to simian malaria in Kudat Division, Sabah, Malaysia

Meng Li Wong[1], Indra Vythilingam[1], Cherng Shii Leong[1], Tock Hing Chua[2], Benny Obrain[2], Heather Ferguson[3], Loke Tim Khaw[1] and Chris Drakely[4]

[1]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Department of Pathobiology, School of Medicine, University of Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Malaysia.
[3] Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, United Kingdom .
4 London School of Hygiene and Tropical Medicine, London, United Kingdom

Plasmodium knowlesi is the predominant malaria species affecting humans in Sabah. The most number of cases is being reported from Kudat District. Thus a study was conducted in Kudat Division, Sabah, Malaysia from June to December 2013 to determine the vectors. A total of 900 anophelines was collected outdoor using human landing catch from two locations in Pulau Banggi and one in Kudat. Thirteen species of *Anopheles* were obtained, namely, *Anopheles balabacensis* (90.11%), *Anopheles donaldi* (3.11%), *Anopheles vagus* (2.11%), *Anopheles aconitus* (1.00%), *Anopheles flavirostris* (0.89%), *Anopheles umbrosus* groups (0.56%), *Anopheles vanus* (0.44%), *Anopheles macarthuri* (0.44%), *Anopheles maculatus* (0.44%), *Anopheles latens* (0.11%), *Anopheles barbirostris* groups (0.22%), *Anopheles tessellatus* (0.22%) and *Anopheles watsonii* (0.22%). The mosquitoes were dissected to extract salivary glands and midgut, and these were examined under light microscope for sporozoites and oocysts respectively. Out of 900 anopheline females, twenty *An. balabacensis* were positive for malaria parasites whereby seven were found infected with both sporozoites and oocyst; and five were infected with sporozoites and eight with only oocyst. One *An. vagus* and two *An. donaldi* were found positive for oocysts. From the study, it was found that *An. balabacensis* was the predominant mosquito in the study site, and it was biting in the early hours of the night. Molecular studies are being conducted and the results will be reported.

O3.4 - *Anopheles introlatus* vector of *Plasmodium knowlesi* in Hulu Selangor District, Selangor, Malaysia.

Leong, C.S.[1], Vythilingam, I[1]. Wong, M.L.[1],
Khaw, L.T.[1], Lau, Y.L[1]., Venugopal, B[2], Wan Yusof, W.S.[1]

[1]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia.
[2]Vector Borne Disease Control Programme, Selangor, Malaysia

A 24 month study was carried out in Hulu Selangor to identify the mosquito vectors and to determine their distribution and infection rates. A total of 532 mosquitoes belonging to 16 species of *Anopheles* were collected using bare leg catch method. The species collected were *An. maculatus* (49.81%), *An. letifer* (13.15%), *An. introlatus* (11.09%), *An. kawari* (7.33%), *An. hyrcanus gr.* (4.89%), *An. sinensis* (3.57%), *An. barbirostris gr.* (3.00%), *An. separatus* (2.82%), *An. philippinensis* (1.88%), *An. barbirostris* (0.75%), *An. donaldi* (0.38%), *An. peditaniatus* (0.38%), *An. tesselatus* (0.19%), *An. umbrosus* (0.19%) and *An. umbrosus gr.* (0.19%). All mosquitoes collected were dissected and mid-gut and salivary glands were screened under microscope. One *An. introlatus* out of 13 was found positive with 56 oocytes in the mid-gut. The results from the PCR showed that *An. introlatus* was positive for *P. knowlesi.* Although, *An. maculatus* was the predominant species in that area, it shows that it is not a vector for *P. knowlesi*. Perhaps due to the low numbers of *An. introlatus* the cases of knowlesi malaria in Hulu Selangor are low and sporadic. Further studies need to be conducted in that area to study the vector host parasite relationship.

O3.5 - Impacts of tropical deforestation and fragmentation on mosquito community dynamics

Hayley L. Brant[1], Robert M. Ewers[2], Indra Vythilingam[3], Chris Drakeley[4], Suzan Benedick[5] and John Mumford[1]

[1]Centre for Environmental Policy, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot, Berkshire SL5 7PY, UK
[2]Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot, Berkshire SL5 7PY, UK
[3]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia [4]Immunology and Infection Department, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK
[5]School of Sustainable Agriculture, Universiti Malaysia Sabah, Sekolah Pertanian Lestari, Beg Berkunci No.3, 90509 Sandakan, Malaysia.

Human modification of natural landscapes can influence the emergence and proliferation of zoonotic parasitic diseases. Land use change, such as deforestation, urbanisation and agricultural development, can affect the biology and ecology of mosquito vectors, and their disease transmission potential. The expansion of oil palm is the main driver of deforestation in Malaysia, which could result in a higher prevalence of mosquito-borne diseases. This study investigates the impact of deforestation and fragmentation on malaria, as well as investigating how land use change affects mosquito diversity, abundance and community composition of common mosquito genera. Mosquitoes were collected over six months (October-March 2013) using a variety of methods, including bare leg catches (BLC) and ovitraps from primary forest, secondary forest, oil palm plantations and housing estates. Further surveys during this study will assist in recognising the impacts of land use change on medically important vectors, and the implications it has on human development of these habitats.

O3.6 - The monkey bar project: Defining biomedical, environmental and social risk factors for human infection with *Plasmodium knowlesi*

[1]*KM Fornace, [2]NM Anstey, [3]WE Aure, [4]TH Chua, [5]J Dimalibot, [3]FE Espino, [6]H Ferguson, [7,8]B Goossens, [2]MJ Grigg, [6]RR Kao, [1,3]J Luchavez, [4]K Mariappan, [9]S Nathan, [4]P Porodong, [7,8]M Salgado-Lynn, [7,8]DJ Stark, [10]SJ Torr, [11]I Vythilingam, [12]T William, [2]TW Yeo, [1]J Cox, and [1]CJ Drakeley

[1]*London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom.*
[2]*Menzies School of Health Research, Darwin, Casuarina NT 0810, Australia.*
[3]*Research Institute for Tropical Medicine, Muntinlupa City 1781, Philippines.*
[4]*Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.*
[5]*University of the Philippines Los Baños, Laguna 4031, Philippines.*
[6]*University of Glasgow, Glasgow G12 8QQ, United Kingdom.*
[7]*Danau Girang Field Centre, c/o Sabah Wildlife Department, 88100 Kota Kinabalu, Sabah, Malaysia.*
[8]*Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3AX, United Kingdom.*
[9]*Sabah Wildlife Department, 88100 Kota Kinabalu, Sabah, Malaysia.*
[10]*Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom.*
[11]*University of Malaya, 50603 Kuala Lumpur, Malaysia.*
[12]*Hospital Queen Elizabeth, Kota Kinabalu, Sabah, Malaysia.*

Plasmodium knowlesi (Pk) is a malaria parasite endemic in long-tailed and pig-tailed macaques. Although long known to be transmissible to humans, naturally acquired cases were, until recently, considered rare. However, in 2004 a study using molecular methods demonstrated a high prevalence of *Pk* infections among malaria cases in Sarawak, most of which had been diagnosed microscopically as *P. malariae.* Since then, *Pk* has been reported in several countries in Southeast Asia and is now the predominant malaria species in parts of Sabah. Malaysian data indicate a large proportion of *Pk* infections occur in adult males, suggesting forest use a key determinant of exposure. However, substantial numbers of cases have also been detected in children and/or individuals not living in forest fringe areas, raising the possibility of peri-domestic transmission in the absence of primate hosts. To date, no detailed study of risk factors for *Pk* has been conducted and substantial knowledge gaps exist in relation to the transmission system and the role of ecological factors in determining spatial and temporal patterns of risk. Improving epidemiological knowledge and assessing future risks of infection requires an interdisciplinary approach to understand how dynamic interactions between ecological factors and vector/human/macaque populations affect disease transmission within and between populations. MONKEYBAR involves clinicians, epidemiologists, social scientists, entomologists, primatologists and medical geographers working together to address knowledge gaps around the epidemiology of human *Pk* in Malaysia and the Philippines. This paper provides an overview of the main activities within MONKEYBAR and presents preliminary data from different project elements.

O3.7 - A spatial model describing *Plasmodium knowlesi* malaria transmission in both simians and humans in Southeast Asia

Parmiter M., Ferguson H.M. and Kao R.R.

Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, United Kingdom.

A fifth species of human malaria, *Plasmodium knowlesi*, was identified by scientists in Malaysia in 2004 and subsequently found to be causing human infections throughout much of Southeast Asia. It is thought to be primarily epizootic, with most human cases occurring in proximity to the forest and associated exposure to the macaque monkey reservoir. However, the epidemiology of the disease appears to vary widely across small geographical areas, often with equally distinct ecologies. Here we use a spatial adaptation of the Ross-MacDonald framework to explicitly model the low human-host density typical of the forest-fringe areas where transmission has been reported. In doing so, we move away from the classic assumption of large, homogeneously mixed populations. This helps us better understand key drivers of simian-to-human transmission at the local scale, as well as key data gaps in the field. Additionally, through comparison of observed case reports with transmission-scenario simulations, a longer-term aim is to assess whether current incidence data are consistent with direct human-to-human transmission, or if they are more highly dependent on the primate reservoir. Such information is fundamental to intervention choice for disease control. This work is being done in parallel with the 'MONKEYBAR' consortium, so as to integrate field data and inform model parameters, as well as ensure that model development is refined according to the expertise of those working closely with the disease.

Dengue, Chikungunya and Vectors

O4.1 - The concordance of rapid diagnostic testing kits for dengue in a Malaysian District Hospital

[1]David Clement Batut, [2]Cameron Warren, [1]Chien Su Lin,
[1]Hon Siong Leng, [3]Ooi Choo Huck, [1,4*]Wong Jin Shyan

[1] Bintulu Hospital, Bintulu, Sarawak, Malaysia
[2] Graduate School of Medicine, University of Wollongong, New South Wales, Australia
[3] Vector Borne Diseases Section, Sarawak Health Department, Kuching, Sarawak, Malaysia
[4] Internal Medicine Department, Borneo Medical Centre, Kuching, Sarawak, Malaysia

Dengue is a common infection endemic to Malaysian Borneo. It presents with non-specific symptoms and is often diagnosed clinically. The high prevalence of dengue leads to overuse of vector control resources. This study assessed the concordance of rapid diagnostic testing kits in patients diagnosed as dengue based on WHO definition. Cases were identified from the Bintulu Hospital laboratory register between May and August 2013. Medical records of 153 suspected dengue patients were reviewed. We compared admission dengue serology results, using two rapid diagnostic kits (Panbio dengue kit and Combo-NS1 kit) against gold standard ELISA (Bio-Rad). We analyzed 110 patients who had all three investigations performed. Male constituted 73%, median age of 27 (IQR 15,43). Nineteen percent were paediatric patients. Fifty-six percent presented before day 5 from onset of fever, with a mean duration of 5 ± 2 days from onset to blood test. Dengue was confirmed serologically with ELISA method in 11, 9 and 14 cases (out of 110 patients) using IgM only, IgG only and using either test respectively. Panbio IgM was positive in 12 patients (specificity 96%, sensitivity 72.7%) and IgG was positive in 11 patients (specificity 94%, sensitivity 73%). Combo-NS1 IgM was positive in 5 patients (specificity 100%, sensitivity 45.5%) and IgG was positive in 7 patients (specificity 97%, sensitivity 47%). Diagnosing dengue fever based on clinical criteria leads to over diagnosis. Routine use of rapid diagnostic kits can improve diagnosis accuracy and potentially direct an efficient use of vector control resources.

O4.2 - Elevation of matricellular proteins in dengue virus infection

Toshio Hattori[1], Haorile Chagan-Yasutan[1], Susan Leano[2], Talitha Lacuesta[2] and Elizabeth Telan[2]*

[1]Laboratory of Disaster-related Infectious Diseases, IRIDeS, Tohoku University, Japan
[2]San Lazaro Hospital, Manila, Philippines

Two matricellular proteins are measured using plasma of dengue infected individuals to elucidate their pathogenesis. Galectin-9 (Gal-9) is an inducer of apoptosis and danger associated molecule, and osteopontin (OPN) is known to inhibit apoptosis. During the critical phase, Gal-9 levels were significantly higher in DENV infected patients compared to healthy or those with non-dengue febrile illness. The highest Gal-9 levels were observed in dengue hemorrhagic fever (DHF) and dengue fever (DF) patients. In the recovery phase, Gal-9 levels significantly declined from peak levels in DF and DHF patients. Gal-9 levels were associated with multiple cytokines and chemokines, and monocyte frequencies and hematologic variables of coagulation. Plasma levels of OPN as well as its thrombin cleaved product N-half OPN were significantly elevated in both DF and DHF patients as compared to healthy individuals. During the recovery phase, levels of N-half OPN were higher than that in the critical phase of both DF and DHF patients. In contrast, OPN levels significantly decreased. Thus, elevation of OPN followed by the increase of N-half OPN may represent a unique link between coagulation and inflammation in dengue virus infection. Thus, matricellular proteins can predict severity of dengue virus infection and inflammation.

O4.3 - Gravitraps for surveillance and control dengue in Selangor

Lau Sai Ming[1], Indra Vythilingam[2], B. Venugopalan[1], Wan Yusoff Wan Sulaiman[2], Karuthan Chinna[3], Yvonne Ai Lian Lim[2] and Ahmad Safri bin Mokhtar[1]*

[1]Selangor State Health Department, Wisma Sunwaymas, Lot 1, Jalan Persiaran Kayangan, 40100 Shah Alam, Selangor
[2]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur.
[3]Department of Preventive and Social Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur.

Malaysia experienced a tremendous increase of dengue cases in 2013 with 36,021 cases and 74 dengue death up to week 48, which is about 81% increase compared to year 2012. Selangor which is the most developed and populated state in Malaysia contributed to 53% of cases reported in the country. The objective of this study was to develop a new tool for vector surveillance and control of dengue in Selangor. The study site was Mentari Court Apartment, Petaling Jaya, Selangor which is a dengue hotspot locality in Selangor. The gravitrap is a container with hay infusion water and sticky inner surface which is used as an oviposition site for gravid female *Aedes* mosquitoes. A maximum of 441 gravitraps were set in all 7 blocks and 7 floors per block. At the same time, a maximum 49 conventional ovitraps were also set in every floor which had Gravitraps. The Gravitraps trapped 329 *Aedes aegypti*, 8 *Aedes albopictus* and 233 *Culex* sp. *Aedes* were caught from every block and every floor up to 17th floor, with highest percentage trapped on ground floor which is about 45.2%. Dengue virus was initially detected in *Ae. aegypti* on the same day as the first reported case and 10 days before dengue outbreak was declared. Six pools of *Ae. aegypti* were detected positive with dengue virus using NS1 rapid test kit. The highest number of infected *Ae. aegypti* was obtained during the peak of the outbreak which was from 1 July 2013 until 13 August 2013. A total of 17,552 *Aedes* eggs were collected by ovitrap. However, the correlation between eggs and mosquitoes is low (correlation coefficient=0.290310932). The study is ongoing. However, it shows potential as a tool that can be used for vector surveillance and control of dengue.

O4.4 - Genotypic variation of *Aedes aegypti* and its relation to dengue transmission in Malaysia

Nava Yugavathy Indra Vythilingam
Wan Yusof Wan Sulaiman

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

Dengue is an emerging arboviral infection which causes severe health burden especially in the tropical region of Asia. *Aedes aegypti* plays an important role as the primary vector and humans as the main host. Thus the measures taken focuses on vector control and surveillance. Experimenting the genetic flow pattern is important to carry out effective control measures and tracking vector competence. Studies have been carried out to observe the dispersal pattern of Ae. *aegypti* and its relation to dengue susceptibility in many other countries. However, almost no studies have been conducted in Selangor and Federal Territory of Kuala Lumpur region of Malaysia. Therefore this study focusses on 11 locations from Selangor and KL which are merged as Selangor samples and 2 locations from Perak. In contrast to Selangor, Perak was chosen due to its low dengue prevalence. Samples were collected from all 13 locations and DNA was extracted for PCR amplification of CO1 and ND5 gene. These mitochondrial genes were then sent for sequencing to analyse the dispersal pattern. Aligned sequences were used to build phylogenetic tree using the Neighbour Joining and Maximum Likelihood method to observe the distance topology. The result shows 3 clades; Gunung Rapat with the strongest bootstrap value, samples from Selangor regions forms another clade and Pasir Puteh, Perak falls under the third clade together with the reference sequences obtained from GenBank. This shows diversity among the Perak and Selangor samples proving dispersal of genotypes among the states in Malaysia. . Further analysis was done with Tajima's D test which gives a negative value, rejecting the null hypothesis of neutrality. Selangor and KL has recorded the highest number of dengue cases. The genotypic difference obtained might play an important role and it can be used to study the susceptibility rate of dengue in *Ae. aegypti.*

O4.5 - Distribution and dynamics of *Wolbachia* in Malaysian *Aedes albopictus*

Sylvia Joanne[1], Indra Vythilingam[*1], Zulqarnain Mohamed[2], Sazaly Abu Bakar[3]*

[1]Department of Parasitology, Faculty of Medicine, University of Malaya,
[2]Institute of Biological Science, University of Malaya, Kuala Lumpur, 50603, Malaysia
[3]Department of Microbiology, Faculty of Medicine, University of Malaya,
Kuala Lumpur, 50603, Malaysia

Wolbachia are intracellular bacteria found in most arthropods and nematodes. *Wolbachia* have shown a wide range of reproductive phenotypes in their hosts. *Aedes albopictus* is found to be naturally superinfected with *Wolbachia pipientis* supergroup A and B. The infectivity pattern of *Wolbachia* and the dynamics of their infection in Malaysian *Aedes albopictus* still remains unknown. In this study, we have extracted DNA from 286 *Aedes albopictus* from various locations in Malaysia. The samples were amplified using *wsp* specific primers. Besides, *Wolbachia* distribution in the organs of *Aedes albopictus* was also studied using PCR method. Dynamics of *Wolbachia* infection on *Aedes albopictus* was explored by studying the fecundity, longevity and egg viability of the mosquitoes infected and uninfected with *Wolbachia*. *Aedes albopictus* from all districts analysed had *Wolbachia* infection. The infectivity rate ranged from 60-100%. No diversity was found within *Wolbachia A* and *Wolbachia B* sequences. In addition, *Wolbachia* distributions in organs were resolved. Significant result was obtained for the fecundity, longevity and egg viability tests conducted. Infectivity pattern was not homogenous among districts indicating possible effect of geographical factors. *Wolbachia* A and B having identical sequence within supergroups may have been due to they were maternally transmitted, therefore no evolutionary differentiation have occurred. A significant *Wolbachia* infection was only found in midgut and gonads. Salivary glands were not infected in contrary to previous findings. The effect of *Wolbachia* infection on *Aedes albopictus* was able to be deduced with the fecundity, longevity and egg viability results.

O4.6 - Discriminable roles of *Aedes aegypti* and *Aedes albopictus* in establishment of dengue outbreaks in Taiwan

[1,2*]*Wei-June Chen*

[1]*Graduate Institute of Biomedical Sciences, Chang Gung University, Kwei-San, Tao-Yu,an 33332, Taiwan*
[2]*Department of Public Health and Parasitology, College of Medicine, Chang Gung University, Kwei-San, Tao-Yuan 33332, Taiwan*

Aedes aegypti and *Aedes albopictus* were reported to be significant as vectors of dengue fever. In Taiwan, the latter is distributed throughout the island while the former appears only south of the Tropic of Cancer; *i.e.*, 23.5°N. In the past decade, there were five outbreaks with over 1000 cases of dengue fever in Taiwan. Without exception, these outbreaks all occurred in the south where the two *Aedes* mosquitoes are sympartic. According to the Center for Disease Control of Taiwan, imported cases are thought to provide the seeds of dengue outbreaks every year. Mostly, the number of imported cases is greater in northern island, probably due to a larger population of travelers and migrant workers from endemic countries. Looking at the example in 2002, northern, central, and southern parts of Taiwan reported 28, 11, and 13 imported cases, respectively. However, 54, 21, and 5309 total cases were confirmed in the corresponding regions over the entire year, indicating a significant skew of case distributions. A hypothesis is thus inspired that the existence of *Ae. aegypti* is a prerequisite to initiate a dengue outbreak, while participation of *Ae. albopictus* expands or maintains the scale until the de novo herd immunity reaches high level.

O4.7 - Human immune response of people living in endemic area DHF as indicator of immunogenic protein against protein salivary gland of *Aedes aegypti*

Rike Oktarianti[1,2], Huan Huu Nguyen[3] ,
Cecil Czerkinsky[3], Kartika Senjarini[1],

[1]Biology Department-Mathematics and
Natural Sciences Faculty-University of Jember
[2]Biology Department-Mathematics and Natural Sciences
Faculty-University of Brawijaya
[3]International Vaccine Institute (IVI), Seoul, South Korea

Aedes aegypti is the main vector of Dengue Haemorraghic Fever (DHF). The salivary glands of *Ae. aegypti* contain proteins that counteract vertebrate host hemostasis and have important role in viruses transmission to the vertebrate hosts. These proteins may induce both cellular immunity and the production of specific antibodies, thus human who after being bitten by arthropod or exposed to insect salivary proteins will be able to develop anti-salivary protein. The object of research is to examine whether *Ae. aegypti* saliva gland could elicit humoral immune response in humans under natural conditions and to identify immunogenic protein of salivary gland. We have collected sera from people living in endemic area (DHF patients and healthy people) and non endemic area. Identification of immunogenic proteins from Salivary Gland Extract (SGE) based on individual and pool sera response was carried out by using Western Blot Analysis. Two immunogenic proteins (bands) i.e. ~ 31 and 56 kDa were appeared only in samples from humans, who were living in endemic area, previously exposed to mosquitoes bites. Therefore, these immunogenic salivary proteins may serve as indicators for the immune response in humans against protein from salivary gland *Ae, aegypti*.

O4.8 - Early detection and serotyping of dengue virus by using one step multiplex real time RT-PCR assay

[1]Je-Hyoung Kim, [2]Chomkyu Chong, [1*]Hyun-Ok Song, [1*]Hyun Park

[1]Zoonosis Research Center, Department of Infection Biology, Wonkwang University School of Medicine, Iksan, Jeonbuk, Republic of Korea
[2]Genbody Inc., Cheonan-si, Chungchengnam-do, Republic of Korea

Dengue fever and Dengue hemorrhagic fever caused by dengue virus (DENV) are important global public health problems. In 2004, the WHO reported 414,785 cases worldwide. DENV has four different serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) and four serotypes of DENV give similar symptoms in clinics. For this reason, it is impossible to distinguish each serotype with symptoms only. However, secondary infection among different serotypes causes severe symptom in the host. Thus, differential diagnosis of four serotypes is important and necessary. Especially, it is necessary to detect DENV in the early stages of the disease because the detection and serotyping of DENV from patients with suspected dengue fever is important both for the diagnosis of the disease and for the implementation of epidemiologic control measures. We developed one step multiplex real time RT-PCR for the differential and simultaneous detection as well as serotyping of DENV from clinical samples. All primers and probes were targeted to the sequence of the NS1 gene. The method comprised of 4-plex assay and was validated for sensitivity, specificity and limit of detection. Commercial serum samples from dengue patients were evaluated by our one step real time RT-PCR. Our results showed that the developed one step real time RT-PCR could differentially diagnose DENV serotypes and co-infection of several serotypes from the early stage infection.

O4.9 - Dengue drug development

Cynthia Sung

Program in Emerging Infectious Diseases,
Duke-NUS Graduate Medical School
8 College Road, Singapore 169857

The soaring number of dengue cases in ASEAN countries and the unexpected difficulties of developing a dengue vaccine underscore an urgent need for an effective dengue drug. Knowledge of the structure and function of the various dengue proteins is guiding many dengue drug development programs. The history of the development of the neuraminidase inhibitor oseltamivir will be presented to illustrate the scope and timeline of a successful, structure-based drug development program for an acute viral illness. A potential shortcut to drug approval is to repurpose drugs from other indications, e.g. Hepatitis C, which is also a *Flaviviridae* virus. Clinical trials that have been undertaken in Vietnam and Singapore to evaluate repurposed drugs for dengue will be reviewed. Lessons learned from these trials are instructive for the design of future dengue drug trials. For regulatory approval, a drug development program must have data from multiple clinical trial sites because this increases the robustness and generalizability of the findings. As dengue drug candidates advance through the preclinical pipeline towards clinical testing, ASEAN countries should streamline the logistics of conducting clinical trials and establish infrastructure to perform trials to Good Clinical Practice standards so that dengue drug trials can be performed reliably and efficiently.

Blastocystis

O5.1 - *Blastocystis* molecular epidemiology and susceptibility patterns from Sydney, Australia

Tamalee Roberts[1,2], Damien Stark[1], John Harkness[1], John Ellis[2]*

[1] Department of Microbiology, SydPath, St. Vincent's Hospital, Darlinghurst, N.S.W, Australia
[2] i3 Institute, School of Medical and Molecular Sciences, University of Technology, Sydney, N.S.W, Australia

Blastocystis has a world-wide distribution and is the most common enteric parasite isolated from human stools. There has been much controversy over whether this protozoan is considered pathogenic in humans but many recent *in vivo* and *in vitro* studies strongly suggest that this organism is a pathogen. It has been suggested that the pathogenicity of this parasite could be directly related to subtype infection. Due to the lack of knowledge on pathogenicity, treatment for this parasite varies. In this study a total of 513 stool samples were tested for the presence of *Blastocystis.* There was a 19% incidence rate of *Blastocystis* seen in the Sydney population. There were six different subtypes identified by sequencing (1, 2, 3, 4, 6 and 8). Subtype 3 was the predominant subtype found in clinical samples with 45% of isolates belonging to this group. Metronidazole was found to be the least effective drug against all of the subtypes with paramomycin and ivermectin showing higher rates of sensitivity. This is the first large scale molecular epidemiological study on *Blastocystis* from Sydney, Australia.

O5.2 - *Dientamoeba fragilis* - *In vitro* growth studies

Anitamalar Devi Ragavan[1], Suresh Kumar Govind[2]

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

Dientamoeba fragilis, a trichomonad parasite is usually found in the gastrointestinal tract of humans and is known to be the cause of gastrointestinal disease. This parasite can be found in most parts of the world both in rural and urban areas. Although *Dientamoeba fragilis* have been discovered more than 87 years ago, very little is known about this parasite and it's life cycle and mode of transmission. Human stool samples from rural villages were collected fresh and in preservative using Sodium acetate formalin (SAF). Positive samples were confirmed using the polymerase chain reaction (PCR) with primer 400f and 1525r. A positive sample was cultured in four different media i.e. Loeffler, Jones', Hollander and IMDM medium. All culture medium was supplemented with approximately 3 -5 mg of rice starch. 1 x 10 ^{4} trophozoites per ml of *Dientamoeba fragilis* was inoculated into 4 sets of three culture tubes containing the 4 culture media respectively. Cell counting was done for 8 days using the heamocytometer chamber. Loeffler and Jones' medium showed a higher growth of *Dientamoeba fragilis* on day 2. Loeffler medium shows an average count of 23.86 x 10^4 while Jones medium is 3.3 x 10^4. Although, the number of trophozoites in Jones' medium was low compared to the parasite count in Loeffler medium, Jones' medium can be used to isolate *Dientamoeba fragilis* from stool samples and grown initially before transferring to Loeffler medium for long term maintenance. In the light that preparation of Loffler medium is time consuming and more tedious compared to Jones' medium, it is recommended that for rapid use especially in the field, Jones' medium be used especially so when it has been shown to be an ideal medium to isolate *Blastocystis* another common protozoan.

O5.3 - Amoebic form of *Blastocystis* spp. – Evidence for a pathogenic role

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

Department of Parasitology, Faculty of Medicine, University of Malaya

Blastocystis spp. is one of the most prevalent parasites isolated from patients suffering from diarrhea, flatulence, constipation and vomiting. It's pathogenicity and pathophysiology remains controversial to date. Protease activity and amoebic forms have been reported previously in symptomatic isolates but there has been no conclusive evidence provided to correlate the protease activity and any specific life cycle stage of the parasite thus far. Symptomatic isolates with amoebic form were tested for protease activity and compared with symptomatic and asymptomatic isolates without amoebic form for 10 days culture period. The present study demonstrates an elevated protease activity in cultures having a higher percentage of amoebic forms seen in symptomatic isolates. The growth curve demonstrated a significantly ($p < 0.05$) higher average number of parasites count in asymptomatic compared to symptomatic isolates. Symptomatic isolates showed amoebic forms with percentages ranging from 5% to 17%. Elevated protease activity was demonstrated in isolates that had higher percentages of amoebic forms with intense bands at higher molecular weight proteases (60 – 100 kDa). As days of culture proceeded, the protease quantification also showed a steady increase. This study elucidates a correlation between protease activity and percentage of amoebic forms. The finding implies that these forms could play a role in exacerbation of intestinal symptoms during *Blastocystis* spp. infection.

O5.4 - Molecular epidemiology of *Blastocystis* isolated from Malaysia and Libya

Awatif Mohamed Abdulsalam, Init Ithoi, Hesham M Al-Mekhlafi, Abdulhamid Ahmed and Johari Surin

Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

This study was conducted to investigate the molecular epidemiology of *Blastocystis* infection in Malaysia and Libya. Stool samples were collected from 300 primary schoolchildren in Pahang, Malaysia and 380 outpatients attending the Central Laboratory in Sebha, Libya. The samples were processed and subjected to *in vitro* cultivation in complete Jones' medium followed by PCR, cloning, sequencing and phylogenetic analyses. In Malaysia, the overall prevalence of *Blastocystis* infection among schoolchildren was 25·7%. Univariate and multivariate analyses showed that absence of a piped water supply (OR = 3·13; 95% $P <$ 0·001) and low levels of mothers' education (OR = 3·41; $P <$ 0·01) were the significant predictors of *Blastocystis* infection. Phylogenetic analysis revealed that *Blastocystis* isolates were classified into three distinct subtypes (ST); ST3 (39.4%) followed by ST1 (36.4%) and ST2 (18.2%). ST1 was more common among schoolchildren aged ≤10 years (P = 0.012), those who lack piped water supply (P = 0.026) and toilet facility (P = 0.037) in their households. In Libya, the overall prevalence of *Blastocystis* infection among outpatients was 22.1%. Univariate and multivariate analyses showed that the age of ≥ 18 years (OR = 5.7; P = 0.001) and occupational status (OR = 2.2; P = 0.045) as significant predictors of *Blastocystis* infection. *Blastocystis* ST1 (51.1%) was the most common among the outpatients followed by ST2 (24.4%) and ST3 (17.8%). ST1 infection was significantly associated with female gender (P = 0.009) and educational level (P = 0.034). ST3 was significantly associated with diarrhoea (P = 0.008). .

O5.5 - Identification and characterisation of heat shock proteins 70 in thermal stressed *Blastocystis* sp.

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

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

Protozoan parasites in order to ensure their viability and to demonstrate successful progression in their life cycle need to respond towards various environmental stressors. *Blastocystis sp*. is known to be the most commonly found intestinal protozoan parasite in any human stool surveys and has been incriminated to be responsible for diarrhea and bloating stomach. The present study demonstrates for the first time the presence of HSP70 in subtypes of *Blastocystis* sp. when the cultures were subjected to 39 and 41^{0}C. The parasite growth was reduced to a minimal with a majority of the organism showing granular forms. The growth however doubled compared to the control isolates when the parasites were re-cultured back at 37^{0}C. Upon thermal stress at 41^{0}C, subtype 3 and subtype 5 isolates' growth reached up to 2.97×10^6 and 3.05×10^6 cells/ml compared to their respective controlled culture tubes at 37^{0}C which peaked only at 1.34×10^6 and 1.70×10^6 cells/ml respectively. The designed primer set that amplified *Blastocystis sp*. subtype 7 HSP70 gene in subtypes 1, 3 and 5 was against a conserved region. The gene was amplified at 318bp. The multiple sequence alignment showed that the targeted sequence length ranges from 291-295bp. The pair wise alignment result showed that the sequence identity among the four sequence ranges from 88% to 96%. Higher number of granular forms was significantly found in thermal stressed isolates of subtype 3 and 5 which implicates that this life cycle stage has a role in responding to thermal stress.

O5.6 - Subtype 6 *Blastocystis* sp. in ostrich population in Malaysia

Hemalatha C[1]., Suresh Kumar G[1]., Chandrawathani P[2]., Premaalatha B[2]., 1Kalyani R[1]., Gaythri T1., Nanthiney D.R[1]

[1] Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur.
[2] Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak.

Blastocystis sp. is an anaerobic enteric protozoan organism found in the intestinal tract of a range of animals, including humans which can be symptomatic or asymptomatic. The possibility of zoonotic transmission to human from birds especially domestic ostriches led us to determine the morphological, ultrastructural and molecular differences of *Blastocystis* sp. isolated from the ostrich faeces. The growth profile of *Blastocystis* sp. in culture was distinct and different compared to the other animal and human isolates where parasites peaked on day 2 and 3 of culture. The parasite count dropped tremendously on day 7 where majority of organisms were viable granular forms. Cells were spherical in shape with a smooth surface under the scanning electron microscope. Transmission electron microscopy revealed two surface coats surrounding the *Blastocystis* sp. cells. High electron dense material was observed in the central vacuole. Out of 37 ostrich isolates, 14 were confirmed to be subtype 6 (37.8%) when amplified with PCR using sequence-tagged site (STS) primers. The infectivity of two *Blastocystis* isolates obtained from two domestic ostriches examined in a 2 weeks old male Sprague dawley indicates that *Blastocystis* sp. is subtype-specific when amplified with PCR using sequence-tagged site (STS) primers. This finding shows that there is need for consistent monitoring of the presence of *Blastocystis* sp. in the animals and to educate workers handling animals on personal hygiene, such as wearing of a mask and gloves to eliminate the risk of infection to humans. The present study is the first report on the presence of *Blastocystis* infection in the ostrich population in Malaysia.

O5.7 - Discovery of nim gene in *Blastocystis* sp.

Kalyani, R[1]., Tan, T.C[1]., Subha, B[2] ., Gaythri, T[1]., Suresh,K[1] .

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

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

O5.8 - Phenotypic variation in *Blastocystis* sp. St3

Nanthiney Devi Ragavan, Suresh Kumar Govind, Tan TianChye

Department of Parasitology, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia

Blastocystis has many conflicting reports on its pathogenic role. Gut conditions could play an important role in its pathogenicity. This is the first study to investigate both phenotypic and genotypic characteristics of *Blastocystis* isolated from asymptomatic, symptomatic and IBS isolates. A total of 8 *Blastocystis* isolates were obtained from four IBS patients (IBS1-4) and four symptomatic patients (S1-4) at a local gastroenterology clinic. Asymptomatic isolates (A1-4) were obtained from a field survey at a local village. A1-4 isolates showed the highest peak growth followed by IBS1-4 isolates and S1-4 isolates for the growth profile. Parasites from IBS isolates (IBS1-4) showed the largest diameter with a mean of 18.43±5.10µm compared to parasites of symptomatic isolates (isolates S1-4) 15.54±4.30µm and asymptomatic isolates (isolates A1-4) 11.76±3.37µm. The parasites isolated from IBS isolates showed strong aggregation and clumping, the intensity of which was markedly seen reduced in parasites of symptomatic isolates S1-4. Parasites from A1-4 isolates were seen to be distinct with no clumping. The outer surface of parasites in IBS isolates showed greater binding affinities towards FITC-labelled Concovalin A(Con A) than symptomatic isolates and asymptomatic isolates. Scanning electron microscopy showed that *Blastocystis* isolated from asymptomatic isolates possess a very smooth surface meanwhile the *Blastocystis* isolated from symptomatic isolates showed slightly rough surface with tiny pores. In IBS isolates, the surface of *Blastocystis* showed a very coarse and intensely folded surface. The IBS isolates also exhibited a dense material. In contrast, the dense material was not seen in any of the parasites from both the asymptomatic and symptomatic isolates. *Blastocystis* in IBS isolates showed a thicker layer of surface coat surrounding the parasites compared to a relatively thinner layer seen in both asymptomatic and symptomatic isolates. There have been no studies thus far providing evidence for phenotypic variation within a particular subtype. The present study is the first to demonstrate the phenomenon of gut environment facilitating adaptation of parasites possibly for survival leading to phenotypic differences for Blastocystis.

O5.9 - Predominance of *Blastocsytis* spp. among school children in peninsular Malaysia

Nithyamathi K. , Chandramathi S., Suresh K.

Department of Parasitology, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia

A cross sectional study was conducted among school children from urban and rural areas in five states namely Selangor, Perak, Pahang, Kedah and Johor. This study which obtained approval from the Ministry of Education was to assess the status of intestinal parasitic infection among school children living in rural and urban areas in Peninsular Malaya. Establishing such a data would enable school authorities to influence strategies for providing better health especially in terms of reducing intestinal parasitism. A total of 3776 stool cups was distributed to 26 schools throughout the country. 1760 (46.61%) responded. The overall prevalence of intestinal parasitic infection was 13.4% with *Blastocystis sp*.(10.6%) being the most predominant, followed by *Trichuris trichiura* (3.4%), *Ascaris lumbricoides* (1.5%) and hook worm infection (0.9%). In general Perak had the highest infection (37.2%, total, n=317), followed by Selangor (10.4%, total, n=729) , Pahang (8.6%, total, n=221), Kedah (6.2%, total, n=195) and Johor (3.4%, total, n=298) . School children from rural schools showed the highest infection (19.0%, total, n=922) while school children from urban areas (7.2%, total, n=838) had the lowest infection. The risk factors associated with intestinal parasitic infection in relation to socio-demographic and lifestyles among school children were analysed using a questionnaire. Age group, gender, socio-economic status and the level of education of parents, educational levels of students, symptoms, water source and lifestyle were assessed to establish a data to correlate these factors to intestinal parasitic infection.

O5.10 - *Blastocystis* sp. enhances oxidative stress and stimulates the formation of aberrant crypts in Aom-induced wistar rats

Vinoth Kumarasamy[1], Umah Rani Kuppusamy[2*], Suresh Kumar[1§]*

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

The association of *Blastocystis* sp., a common intestinal microorganism, with colorectal cancer (CRC) have been recently discussed. CRC have been linked with oxidative stress and has been attributed to the presence of Blastocystis. The main aim of this study was to investigate whether *Blastocystis* promote colon cancer by enhancing oxidative stress in azoxymethane (AOM)-treated rats. Male Wistar rats were injected with AOM and fed on a basal diet supplemented for 8 weeks. Colonic aberrant crypt foci (ACF) and adenomas were examined. The oxidative stress biomarker levels in the urine and serum samples were determined as well. The total number of ACF was significantly higher in rats infected with *Blastocystis* ($P < 0.01$, Student's *t*-test). High levels of oxidative indices including lipid hydroperoxide (LHP), advanced oxidative protein products (AOPP), hydrogen peroxide (H_2O_2) and high level of antioxidant power level via ferric reducing ability of plasma (FRAP) can be observed in *Blastocystis* infected rats. Our study demonstrated that *Blastocystis* has the ability to promote carcinogenesis in the intestine of rats which can be correlated to oxidative stress.

O5.11 - A microarray study to determine the role of *Blastocystis hominis* in enhancing colorectal cancer

Chandramathi S[1], Suresh K [1] and Kuppusamy UR[2]*

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

Colorectal cancer (CRC) incidences and deaths are on the rising trend. Epidemiological and scientific reports have evidenced that apart from the genetic inheritance, infectious organisms such as bacteria, virus and parasite do contribute to the pathophysiology of this devastating disease via inflammatory processes. *Blastocystis hominis*, the most common intestinal protozoan found in humans have been associated with CRC in our previous studies. Thus far, we have shown that *B. hominis* Subtype 3 possess the highest proliferative effect towards CRC cells. However a comprehensive study to understand the genes and pathways involved in the pathogenicity of *Blastocystis* sp. Subtype 3 in colorectal cancer are still lacking. Here, we carried out a microarray analysis to compare the effects of antigens from symptomatic and asymptomatic isolates of *Blastocystis* sp. Subtype 3 against colorectal normal and cancer cell lines. Our results showed that symptomatic isolate resulted in higher level of cell proliferation in both cancer and normal cell lines. However the level of proliferation is higher in cancer cells. This observation correlated with microarray clustering result showing 33 and 869 differentially expressed genes (DEG)s. Interestingly, venn diagram analysis revealed that symptomatic and asymptomatic isolates employ the same genes but in reciprocal manner in both normal and cancer cells. The functions of these genes in terms of cell proliferation, apoptosis and tumorigenesis have been well studied by previous researchers. However to further validate the results obtained from microarray study, the expressions of some selected genes were studied and the results will be discussed further.

O5.12 - Detection of *Blastocystis* sp. In companion animal, wild rat and chicken population

Farah Haziqah, M.T.[1], Mohd Zain, S.N.[1], Suresh, K.[2], Chandrawathani, P.[3], Hemalatha C.[2], & Premaalatha B.[3]*

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur.
[2]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur.
[3]Department of Parasitology and Hematology, Veterinary Research Institutes, Ipoh, Perak.

From October 2012 to October 2013, a total of 519 intestinal contents/faecal samples from different hosts were collected from two sampling sites; Selangor and Perak. The prevalence of *Blastocystis* sp. from cat, dog, wild rat and chicken population was investigated by screening and *in vitro* cultivation method. It was found that 73 (45.6%) out of 160 wild rats intestinal samples screened were positive for *Blastocystis* sp. Thirty three positive (41.8%) samples out of 79 wild rats from wet market in Ipoh, Perak while in Selangor and Kuala Lumpur, 40 (49.3%) samples out of 81 wild rats were positive for *Blastocystis* sp. Meanwhile, 36 (30.3%) out of 119 chicken faecal samples screened were positive for *Blastocystis* sp. with prevalence ranging from 33.3-100% between several free-range species compared to cage-reared chicken with lower infection (20.5%). However, no *Blastocystis* sp. was recovered from faeces and *in vitro* cultivation from cat and dog populations. *Blastocystis* sp. found in chicken isolates was generally larger than *B. hominis* whereas the cells were smaller in wild rat isolates. Otherwise, the general morphology of this organism appeared similar to *B. hominis*. In addition, animals that were infected were all asymptomatic. This study embarks in determining the presence of this organism in the companion animals, household pests and poultry population as no data is available on the nature and prevalence of *Blastocytis* infection in those animals in Malaysia. The importance of understanding this organism in the environment is important because they are in close contact with human everyday life and could be a source of human infection.

Water Borne and Soil Transmitted Parasitic and Viral Diseases

O6.1 - Contamination of soil with parasitic helminthes eggs among organic and conventional farms in selected towns of Laguna and Quezon Province, Philippines.

Babia Shiela A. and Paller Vachel Gay V.

University of the Philippines, Los Banos.

The study aimed to determine the extent of parasitic contamination of soils between organic and conventional farms in selected towns of Laguna and Quezon Province, Philippines. Soils were randomly sampled at different depth, i.e., depth 1 at 0-5 cm and depth 2 at 6-10 cm. Soil samples were processed by sugar floatation technique to isolate parasites eggs and protozoans. Also, fecal samples from human and animals were collected from nearby areas and were processed by formalin-ether concentration technique. Results revealed that 48% of soil samples were contaminated with parasites. Parasites detected were *Ascaris* (44.53%), *Trichiuris* (28.47%), Hookworm (18.25%), *Toxocora* (3.65%), *Taenia* (2.91%), *Isospora* (1.46%) and *Balantidium coli* (0.73%). However, the prevalence rates between depth 1 (72%) and depth 2 (60%) showed no significant difference (x^2=0.239). Meanwhile correlation analysis showed that prevalence of parasitic contamination of soil and the incidence of infection in human and animals showed a strong positive relationship (r= 0.998). The eggs detected from human were *Trichiuris trichiura* (45%), Hookworm (44%), and *Ascaris lumbricoides*(11%), while in animals *Ascaris sp.*(50%), Hookworm (28.33%), *Taenia sp.* (11.11%), *Paramphistomum* (5.56%) and *Isospora* (5%). Furthermore, this study revealed that organic farms had higher rate of contamination than conventional farms. Good sanitation practices, health education, and strict implementation of the guidelines for organic farming are recommended for control and prevention of parasite transmission.

O6.2 - Detection of T4 genotype in *Acanthamoeba* isolates from different sources

Nakisah Mat Amin, Sanggari Anamalai and Cha Thye San

Department of Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malayasia

Acanthamoeba spp are free-living amoebae that are ubiquitous in our environment and some of them have been reported as facultative pathogens to humans. Pathogenic isolates of *Acanthamoeba* are medically relevant since they can inflict severe infections in man such as sight-threatening *Acanthamoeba keratitis*. Previous work employing 18S rDNA revealed diversity of *Acanthamoeba* genotypes and the T4 genotype has been associated with their pathogenic properties. In this study, df3 region of four *Acanthamoeba* isolates including both clinical and environmental isolates was amplified using JDP1 and JDP2 primers to detect T4 genotype using specific PCR based technique. A positive strain of *Acanthamoeba* with T4 genotype was used as a positive reference. Detection and determination of the T4 genotype was done using multiple sequence alignment and phylogenetic context.. Results obtained in this study showed that both clinical isolates are of the T4 genotype confirming their pathogenic status. Only one environmental isolate can be grouped in this pathogenic genotype, therefore it can be considered as potential pathogenic *Acanthamoeba*. The conclusion of this study is, only *Acanthamoeba* with T4 genotype are potential to cause infections in man.

O6.3 - Parasitic contamination of vegetables from selected farms in Quezon and Laguna, Philippines

Ordoñez, Kathleen N.[1,2]*, and Paller, Vachel Gay V.*[1]

[1] *Institute of Biological Sciences, University of the Philippines, Los Banos Laguna, Philippines*

[2] *College of Arts and Sciences, Southern Luzon State University Lucban, Quezon, Philippines*

This is a study on parasitic contamination of vegetables from selected farms in Quezon and Laguna, Philippines. A total of 359 freshly harvested vegetable samples were collected by systematic random sampling from organic and conventional farms. The vegetable samples were examined by means of sedimentation technique. Also, fecal samples of farmers and farm animals from nearby areas were processed using formalin-ether concentration technique to determine their parasitic infections. A correlation analysis was done to determine the association between vegetable contamination and human and animal infections. Results showed that 210 out of 359 (58%) vegetable samples were contaminated with parasites. The parasites found were *Ascaris sp.,Toxocara sp., Trichuris sp., Balantidium coli and Isospora sp.* with higher number of positive samples from organic (50%) than conventional farms (30%), however, there was no significant difference between them ($p > 0.05$). Parasites in fecal samples from farmers and farm animals showed a strong positive relationship with parasite contamination of vegetables ($r = 0.996$). These results imply that vegetables from both organic and conventional farms have the potential to be contaminated with parasites. This could be due to farming systems as discussed in the paper. Also, as revealed in the results, human and farm animals could be a potential source of contamination. These results, although partial, showed high level of parasitic contamination of vegetables in the area, thus, proper hygiene and farming practices should be addressed for the prevention and control of parasitic infections in humans and animals.

O6.4 - Detection of parasites from environmental samples in the Philippines: A current situation.

Subashini Onichandran[1], Thulasi Kumar[1], Cristina C Salibay[2],

Julieta Z Dungca[3], Yvonne AL Lim[1], Nongyao Sawangjaroen[4], Hemah Andiappan[1], Tan Tian Chye[1], Init Ithoi[1], Lau Yee Ling[1] and Veeranoot Nissapatorn[1]

[1]Department of Parasitology (Southeast Asia Water Team), Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, [2]Biological Sciences Department, College of Science and Computer Studies, De La Salle University-Dasmariñas, Philippines, [3]School of Science and Technology, Centro Escolar University, Philippines and [4]Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand.

This study determined the occurrence of *Cryptosporidium*, *Giardia* and *Acanthamoeba* from various water samples collected from Pampanga, Manila and Cavite, Philippines. The types of samples collected comprised of well, river, tap water storage tank, spring, swimming pool, pond, mineral water, drinking water, tap, rain tank, and volcanic lake. *Cryptosporidium* and *Giardia* were purified using immunomagnetic separation method prior to enumeration via fluorescence microscope, while *Acanthamoeba* was cultured before microscopy examination. From a total of thirty three samples, 12 samples from river, swimming pool, pond, rain tank, and volcanic lake were positive for *Cryptosporidium* spp. (36.4%), 15 samples from river, pond, rain tank, and volcanic lake were positive for *Giardia* spp. (45.5%) while, 11 samples from river, swimming pool, pond, tap and volcanic lake were positive for *Acanthamoeba* spp. (33.3%). Physical parameters such as temperature, conductivity, total dissolved solid (TDS), salinity, dissolved oxygen (DO), pH, and turbidity and chemical parameters such as ammonia, chlorine, fluoride, nitrate and nitrite were also measured. The highest chemical contamination was observed at River 2. A good correlation was observed between *Giardia* and nitrite ($r = 0.736$, $p<0.01$) and *Giardia* and nitrate ($r = 0.502$, $p<0.01$). This study was aimed to create greater awareness of parasitic contamination in the environment of the Philippines and also to act as a platform of current scenario for policymakers as water pollution is a key health issue there.

O6.5 - Prevalence of IGA antibody against *Toxoplasma gondii* in newborns in Ipoh, Malaysia

Cheah, T. S., Syed Rahim, S. H.,

Muhammad Hadhrami, M, Maizun Ishak and. Ong, B. G
Faculty of Medicine, Universiti Kuala Lumpur Royal College of Medicine Perak
Ipoh, Malaysia

An investigation was conducted to determine the prevalence of IgA antibodies in the cord blood of newborns as there is little information available in Malaysia. A total of 450 cord blood were collected into a tube without anticoagulant from newborns by trained nurses at two medical centres in Ipoh. Approximately 8 mL of cord blood were collected from each newborn. Blood samples were centrifuged at 3,000 rpm for 10 minutes, and the sera were stored at – 20^0 C until use. Information on the age, occupation, number of parity, consumption of undercooked vegetables and keeping of cat as pet were also collected from the mother. The serum samples were tested for IgA antibody using Platelia Toxo IgA TMB (Bio-RAD, France). One cord blood sample was positive for the IgA antibody. The finding will be discussed in relation to the sociodemographic characteristics.

O6.6 - *Toxoplasma gondii* clonal type and its relation with clinical disease in humans and animals

Adibah Mohd Roslan, Anisah Nordin, Norhayati Moktar, Yusof Suboh, Noraina Ab Rahim and Faizah Mohd Hanapiah.

Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jln Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Toxoplasma gondii, a zoonotic parasite causing toxoplasmosis is a widely prevalent obligate intracellular parasite that has no host specificity and infects all warm-blooded vertebrates including mammals and birds. It has been studied for years and has come to an extent where the clonal type has been identified to up to 11 strains. Recent interest on *T. gondii* is on the association of host species with its strains and the effect each strain has on the clinical spectrum or manifestations in affected animals and humans host. This is important because by understanding the population biology of *T. gondii*, researchers could enable prediction of the outcome of an infection based on the genotype of the infecting organism. At the same time, understanding the genetic factors that influence *T. gondii* virulence and the mechanism of genotype selection according to host species could contribute to the development of therapeutics designed to eliminate transmission or cure disease. This review paper aim to broaden our knowledge on the diversity of *T. gondii* strains found worldwide and in this region and to discuss the virulence and clinical spectrum of diseases associated with each strains.

O6.7 - Helminth colonization is associated with increased diversity of the gut microbiota of indigenous communities in Malaysia

[1]Soo Ching Lee, [2]Mei San Tang, [1]*Yvonne A.L. Lim,
[1]Seow Huey Choy, [3,4]Zachary D. Kurtz, [3,4]Laura M. Cox,
[4]Uma Mahesh Gundra, [3]Ilseung Cho, [3,]
[4]Martin J. Blaser, [5]Kek Heng Chua and [4]* P'ng Loke

[1]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur,
[2]Jeffrey Cheah School of Medicine and Health Sciences,
Monash University Malaysia, Johor Bahru, Johor, Malaysia.
[3]Department of Medicine, New York University School of Medicine, New York, NY, USA.
[4]Department of Microbiology, New York University School of Medicine, New York, NY, USA.
[5]Department of Biomedical Science, Faculty of Medicine,
University of Malaya, Kuala Lumpur,

Soil-transmitted helminths colonize more than 1.5 billion people worldwide yet little is known about how they interact with bacterial communities in the gut microbiota. Differences in the gut microbiota between individuals living in developed and developing countries may be partly due to the presence of helminths, since they predominantly infect individuals from developing countries, such as the indigenous communities in Malaysia. We compared the composition and diversity of bacterial communities from the fecal microbiota of 51 people from two villages in Malaysia, of which 36 (70.6%) were infected by helminths. The 16S rRNA V4 region was sequenced at an average of 19,002 ± 6,451 sequences/sample. Helminth-colonized individuals had greater species richness in bacterial communities that were significantly different from uninfected individuals. When we compared all the Malaysian samples with samples from USA residents, we confirmed the increased bacterial diversity in inhabitants from rural regions of developing countries, with distinct bacterial communities. Indigenous Malaysians had microbiota enriched in Ruminococcaceae, *Prevotella*, *Faecalibacterium* and Gammaproteobacteria and reduced Firmicutes abundance. These results suggest that helminths may have an impact on the diversity of the gut microbiota.

O6.8 - Molecular epidemiology of group a rotaviruses from children in the Philippines

Takashi Mastumoto[1], Lindsay Sydney N. Fajardo[2], Takaaki Yahiro[1], Maria Luisa G[2]. Daroy, Ma. Victoria C. Ribaya, [3] Filipinas F. Natividad[2], Akira Nishizono[1], Kamruddin Ahmed[1,4]

[1]Department of Microbiology, Faculty of Medicine, Oita University, Yufu, Oita, Japan
[2]Research and Biotechnology, St. Luke's Medical Center, Quezon City, Philippines
[3]Department of Pediatrics, St. Luke's Medical Center, Quezon City, Philippines
[4]Research Promotion Institute, Oita University, Yufu, Oita, Japan

Globally, rotavirus diarrhea is an important cause of child mortality. Most of these deaths occur in the developing countries of Asia and Africa. Recently two rotavirus vaccines have been introduced. Therefore prior studies are needed for the introduction of vaccine to determine whether the circulating genotypes are compatible with the vaccine strains and to monitor the post-vaccine effects. This study was carried out to determine the prevalence of rotavirus diarrhea and genotypes circulating in the Philippines. Stool samples were collected from children under 5 years of age attending at the Philippine Children Medical Center and St. Luke's Medical Center in Quezon City, the Philippines. Genomic dsRNA was extracted from stool samples for electropherotyping by polyacrylamide gel electrophoresis (PAGE), and G and P typing by RT-PCR. Phylogenetic analysis of the VP7 gene was performed by neighbor-joining method. From January 2010 through April 2013, a total of 103 samples were collected, among the 34 (33%) were positive for rotavirus by ELISA. All samples were subjected to PAGE which revealed 7 different electropherotypes (E1–E7). Among them E1 type was 65.4%, followed by E4 (11.5%) and E5 (7.7%). Each of the E2, 3, 6, and 7 type was 3.8%. G1P[8] was the dominant genotype 22 (64.7%), followed by G2P[4]: 6 (17.6%), G3P[8]: 3 (8.8%), G9P[8]: 2 (5.88%) and G4P[8]: 1 (2.94%). Further surveillance and genetic analyses of circulating rotavirus strains are needed for the introduction of universal rotavirus vaccine in the Philippines.

O6.9 - Cysteine proteases inhibitors (phenyl vinyl sulfone and valproic acid) in treatment of *Schistosomiasis mansoni*-infected mice: An experimental study to evaluate their role in comparison to praziquantel

Sherif M. Abaza, Amal A. Al-Moamly, Ola A. Ismail, Maha M. Alabbassy

Medical Parasitology Department, Faculty of Medicine, Suez Canal University

The emerged resistance to praziquantel (PZQ) in treatment of schistosomiasis necessitates the search for novel drugs. Cysteine proteases inhibitors (CPIs) have shown promising results in either parasitic or non-parasitic infections. The study aimed to evaluate the therapeutic effects of two CPIs: phenyl vinyl sulfone (PVS) and valproic acid (VA) in comparison to PZQ in *S. mansoni*-experimentally infected mice. Swiss albino mice were experimentally infected with *S. mansoni* cercariae. The mice were divided into 4 groups (25 mice each), and G1 mice were not treated and used as control group. Mice of G2, G3 and G4 were treated by the evaluated drugs (PVS, VA and PZQ, respectively) at the end of the 6th week post infection (PI). The evaluating parameters were 1) fecal egg count, 2) worm burden, 3) oogram pattern, 4) tissue egg count and 5) hepatic granuloma number and size. The results showed that by the end of 10th week PI, PZQ was the most effective drug resulting in decrease worm burden in the portal vein, increase proportion of dead eggs in the oogram pattern, decrease in the hepatic egg count and decrease in granuloma numbers. On the other hand, the granuloma diameter was smallest in PVS treated group compared to the other groups. CPIs have a relative fair favorable therapeutic outcome on *S. mansoni* with the advantage of being novel drugs with no therapeutic resistance, especially PVS which showed an added specific anti-immunopathological effect reflected by the small hepatic granuloma size.

O6.10 - Tropical animal parasites in the Arctic: How did they arrive?

Antti Oksanen

Finnish Food Safety Authority Evira,
Production Animal and Wildlife Health Research Unit (FINPAR),
Elektroniikkatie 3, FI-90590 Oulu, Finland.

There are a number of parasite genera regarded as tropical but also found in the arctic and boreal regions of the world. What is perhaps even more surprising is that they may lack counterparts in temperate areas. Homoeothermic animals have very similar body temperatures around the world but free-living stages or those residing in arthropod vectors are exposed to the ambient temperature, which may be very different in the tropics and subtropics compared to arctic and boreal regions. Some tropical parasites have obviously spread into the arctic or boreal regions with human activity. Those include e.g. the sheep abomasal nematode *Haemonchus contortus*, which may only have one generation yearly in the northern pastures, but there are also indications of the possibility of indoor life cycle taking place in sheep barns. However, there are also typical tropical parasites naturally infecting arctic or boreal animal species, such as the reindeer, which harbours e.g. the protozoan *Besnoitia tarandi*, the closest known relative of which is *B. besnoiti*, a parasite of bovines typical of tropical Africa. Another example is the pentastomid *Linguatula arctica* related to hyena parasites in Africa and *L. serrata* of canids in tropics and subtropics. Assuming that these arctic parasites are derived from their closest relatives in the tropics, they demonstrate that parasites can adapt to even extreme climate changes.

O6.11 - Genetic diversity analysis of *Schistosoma japonicum* isolated from endemic provinces in the Philippines

Ian Tabios[1], James Chua[2], an Fontanilla[3], Elena Villacorte[2], Mihoko Kikuchi[4], Yuichi Chigusa[5] and Lydia Leonardo[2],

[1]College of Medicine, University of the Philippines Manila, 1000 Manila, Philippines
[2] College of Public Health, University of the Philippines Manila, 1000 Manila, Philippines
[3]Institute of Biology, University of the Philippines Diliman, 1100 Quezon City, Philippines
[4]Department of Immunogenetics, Nagasaki University, 8521 Nagasaki, Japan
[5]Laboratory of Tropical Medicine and Parasitology, Dokkyo Medical University, Tochigi, Japan

Schistosomiasis has always been a major public health problem in the Philippines. Despite its implication in disease epidemiology and control strategies, knowledge regarding the species diversity of *Schistosoma japonicum* geographical isolates from the Philippines is limited. This study aimed to investigate genetic variation among *S. japonicum* isolated from endemic provinces in the Philippines using complete ITS1 and ITS2 and partial COI DNA sequences. Individual adult parasites were obtained from mice infected with cercariae from *Oncomelania hupensis quadrasi* taken from schistosomiasis endemic localities in the islands of Luzon and Visayas, Philippines. Schistosomes from the different provinces formed separate clades in both Neighbour-joining (NJ) and Maximum Likelihood (ML) phylogenetic trees. Each geographical origin had single non-overlapping haplotypes. Moreover, there was relatively high degree of genetic variation based on nucleotide and haplotype diversity. These suggest the effect of geographic isolation in the genetic structure of schistosomes in the Philippines. Further studies are warranted to determine the potential insinuations in disease morbidity of the observed genetic diversity among Philippine schistosomes.

O6.12 - Asian clams (*Corbicula fluminea*) as bioindicators of *Cryptosporidium* contamination in Laguna De Bay, Philippines

Vachel Gay V. Paller[1] , Renz L. Salumbre[1], Christian Paul P. de la Cruz[2]

[1]Animal Biology Division, Institute of Biological Sciences, UPLB
[2]Microbiology Division, Institute of Biological Sciences, UPLB

Members of the genus *Cryptosporidium* are ubiquitous waterborne parasites causing diarrhea and have resulted in considerable morbidity among human and animal hosts. Freshwater Asian clams (*Corbicula fluminea*) collected from Laguna Lake were examined for the presence of *Cryptosporidium* oocysts employing sucrose-phenol flotation and Kinyoun acid fast staining procedure. This study revealed the presence of viable forms (morphotype-1a) of *Cryptosporidium* oocysts in clams, with potential to infect humans upon ingestion. Out of the total 45 pooled-clam samples, nine pools (20%) were found positive for the presence of oocysts. The mean abundance was four oocysts per gram. In terms of temporal variability, both prevalence and abundance were higher during rainy month periods (26% and six oocysts/g) relative to drier months (11% and one oocyst/g). Results of exact logistic regression analysis showed that both rainfall and water clarity appeared to have significant association with the occurrence of oocysts in clams. The chance of a pooled-clam sample being contaminated with *Cryptosporidium* oocysts increased by 8% when heavy rainfall occurred several days prior to clam collection and decreased by 7% as water clarity increased. Dissolved oxygen, water temperature, and water pH were not significantly associated with the occurrence of oocysts in clams. Nevertheless, it appeared that increasing water temperature and water pH decreased the chance by 22% and 48%, respectively; whereas, increasing dissolved oxygen increased the odds by twenty-nine percent (29%). Results of this study have indicated *Cryptosporidium* contamination in Laguna Lake using *C. fluminea* as potential bioindicators.

Malaria: Diagnosis, Treatment, Pathology

O7.1 - Flow cytometry antimalarial sensitivity assay for *Plasmodium vivax* and *Plasmodium falciparum* in field conditions

Benoit Malleret[1,2], Rossarin Suwanarusk[2], Alice S. M. Ong[2], Claudia Anthony[2], Charles J Woodrow[4,5]., Kanlaya Sriprawat[6] François Noten[4,5,6], Laurent Rénia[2,3], Bruce Russell[1,3]

[1]Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, 117597 Singapore, Singapore.
*[2]Laboratory of Malaria Immunobiology, Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Biopolis, 138648 Singapore, Singapore.*
[3]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[4]Mahidol-Oxford-University Research Unit, 10400 Bangkok, Thailand.
[5]Centre for Tropical Medicine, University of Oxford, Churchill Hospital, OX3 7LJ Oxford, United Kingdom.
[6]Mahidol–Oxford University Tropical Medicine Research Programme, Shoklo Malaria Research Unit, 63110 Mae Sot, Thailand

Microscopic examination of *ex vivo* matured malaria parasites, remains the gold standard method used for antimalarial sensitivity assay of *Plasmodium vivax* and *Plasmodium falciparum*. However, the microscopic examination of Giemsa stained thick films is a tedious, time-consuming method and requires skilled microscopists. Moreover, large inter-observer variations of parasite staging are frequently recorded. The recent development of portable, low cost cytometers has allowed us to develop and validate a simple, field optimized protocol using only 2 different dyes (Sybr-Green/Dihydroethidium or Hoechst/Dihydroethidium). Forty eight isolates of *P. vivax* and 15 isolates of *P. falciparum* were tested with Sybr-Green/Dihydroethidum for Artesumate (AS) and Chloroquine (CQ) sensitivity. Our data support the use of flow cytometry method as a precise and more objective alternative to the microscopic determination of antimalarial drug sensitivity in both species. Furthermore, 12 cryopreserved isolates of *P. vivax* and *P. falciparum* were used with Hoechst/Dihydroethidium and a portable flow cytometer with near UV laser to show the potential of Methylene Blue (MB) as an effective therapeutic agent against *Plasmodium vivax*. *Plasmodium vivax* and *P. falciparum* are equally sensitive to MB treatment at physiologically relevant levels. Importantly the late stage trophozoites of *P. vivax* are sensitive to MB. In conclusion, the 2 methods are able to give accurate and quick results for *ex vivo* drug testing in endemic regions for *Plasmodium falciparum* and *Plasmodium vivax*.

O7.2 - Proteasome polyclonal antibodies influence the density of *Plasmodium falciparum* DNA in vitro through inhibition of ubiquitin – proteasome system (UPS)

[1]Loeki Enggar Fitri, [2*]Alfian Wika Cahyono, [2]Wiji Astuti, [2]Dyah Ayu Laksmi, [2]Stefan Bram Kanigia and [2]Lazuardiah Anandi

[1]Department of Parasitology, Faculty of Medicine, University of Brawijaya, 65145 Malang, Indonesia
[2]Medical Study Program, Faculty of Medicine, University of Brawijaya, 65145 Malang, Indonesia

The Ubiquitin – Proteasome System (UPS) has an important role in protein degradation pathway of eukaryotes and is involved in many cellular processes such as cell cycle control. Previous study showed that inhibition of UPS function on *Plasmodium berghei* caused stress and death of the parasite. This research was done to reveal if Proteasome polyclonal antibodies that produced from rabbit can inhibit UPS function of *P. falciparum* and cause the DNA proliferation inhibition of the parasite in vitro. Samples of *P. falciparum* cultures were divided into 1 positive control group (*P. falciparum* culture without any exposure), 3 treatment groups (*P. falciparum* culture exposed by proteasome polyclonal antibodies with dose of 100µg/mL, 200µg/mL, 300µg/mL respectively) and 1 placebo group (*P. falciparum* culture exposed by 200µg/mL Tris-HCL and adjuvant). The density of Plasmodium DNA was measured using *Flowcytometry* and the levels of *polyubiquitin* accumulation were measured using *ELISA* method 48 hours after exposure of the antibody. The density of Plasmodium DNA 48 hours after exposure showed significant differences among control, treatment and placebo groups (p=0,000). Using ELISA method, there was an accumulation of *polyubiquitin* with higher density in the group exposed by antibody proteasome 300µg/mL than control group (p=0,0079). It can be concluded that polyclonal antibody proteasome is a potential vaccine candidate to prevent proliferation of the parasite by inhibition of UPS function.

O7.3 - Sequestration of infected erythrocytes in the placenta of malarial pregnant mice induces the expression of hypoxia inducible factor-1α (Hif-1α) and causes fetal low birth weight

Teguh Wahju Sardjono[1], Loeki Enggar Fitri[1], Zainabur Rahmah[2], Budi Siswanto[3]

[1]Department of Parasitology Faculty of Medicine University of Brawijaya, Malang
[2]Reproductive Biology Doctoral Program in Medical Science Faculty of Medicine, University of Brawijaya
[3]Departement of Obstetrics and Gynecology RSSA/Faculty of Medicine University of Brawijaya Malang

Placental malaria would lead to sequestration of infected erythrocytes in placental intervillous space, infiltration of monocytes, proliferation of cytotrophoblastic cells and thickening of the trophoblastic basement membrane. Those can lead to mechanical obstruction and impaired delivery of nutrients and oxygen, causing hypoxia and placental insufficiency. Hypoxic placenta will produce hypoxia inducible factor-1α (HIF-1α), a transcription factor that may effect fetus to have low birth weight (LBW). This study was conducted to prove whether the sequestration of infected erythrocytes in the placenta increases expression of HIF-1α and causes fetal LBW. Seventeen of Balb/c strain mice consist of nine pregnant mice which infected with *Plasmodium berghei* on day 9th post mating used as treatment group and eight normal or non infected pregnant mice used as control group. The mice were scarified on day 18th post mating; the fetus were weighed individually and the placentas were isolated separately. Sequestration of erythrocytes in the placental intervillous space were detected from histopathologic slides using hematoxylin and eosin (HE) staining. Expressions of HIF-1α were detected from slides with immunohisto-chemistry staining using anti-HIF-1α antibody (H1α67) ChIP Grade from Abcam. Fetal body weights in the treatment group were significantly lower than control group (p=0.02; T-test). Expression of HIF-1α in treatment group was also significantly higher to that of control group (p=0,01, T-test). Pearson Correlation analysis showed that there were positive correlation between the level of erythrocytes sequestration and the HIF-1α expression in the placenta (r=0.730; p=0.02), and negative correlation between the sequestration and fetal body weights (r=-0.693; p=0.03); but no correlation between the expression of HIF-1α and fetal body weights (r=0.104; p=0,791). It can be concluded that placental sequestration of erythrocytes in pregnancy infected by malaria induces expression of HIF-1α and causes fetal LBW, but the increase of HIF-1α expression does not cause fetal LBW.

O7.4 - Conserved domains and phylogenetic relationships of the circumsporozoite protein of *Plasmodium vivax* in Sri Lanka

[1]*Lakma N Gunarathne*, [2]**Preethi V Udagama*

[1]*Bioinformatics Special Degree Programme, Department of Plant Sciences, Faculty of Science, University of Colombo, Colombo 03, Sri Lanka.*
[2]*Department of Zoology, Faculty of Science, University of Colombo, Colombo 03, Sri Lanka.*

The present study focused on bridging a few existing knowledge gaps in a Sri Lankan context on vaccine informatics of *Plasmodium vivax* Circumsporozoite Protein (*PvCSP*), a leading vaccine candidate. Conserved domains of *PvCSP* were determined using entropy measures of protein. Phylogenetic relationships of *PvCSP* among Sri Lankan and global isolates were analyzed (NCBI GenBank database: Sri Lanka, N=60, China N=37, India N=70, Iran N=113, Latin America N=7, South Korea N=62 and Thailand N=2). A 36-base post repeat insertion followed by the terminal motif GGQAA at the end of the central repeat region of *PvCSP* with a 12 amino acid insert GGNAANKKAEDA was first observed in a reemerged Korean strain and recently recorded in *P. vivax* parasites collected in Thailand and Iran. The insert was followed by a single copy of GGNA, a tetra peptide repeat. During the current study, for the first time, the same sequence pattern was observed in the Sri Lankan, Chinese, Thai, Indian and Iranian isolates examined. Five prominent conserved regions were observed for *PvCSP* by analyzing 60 Sri Lankan single clone isolates, spanning nucleotide positions 129-143, 156-170, 210-224, 379-395 and 577-654. Significant low entropy was observed approximately in nucleotide position 577-654 and the nucleotide diversity (π) for that region was approximately 0.005 indicating a highly conserved region which coincided with the terminal motif followed by the insert region. The Maximum Likelihood phylogenetic tree showed an adjacent clustering of isolates from Sri Lanka, India and China. This was confirmed by the F_{ST} values between 0 - 0.05 representing no significant differentiation among these populations. This may provide a clue that parasites circulating in Sri Lanka may be derived from strains introduced from South-East Asia. Rather limited diversity in *PvCSP* within Sri Lanka, and phylogenetic alliance to South East Asian origin implies that a CSP based vaccine against *P. vivax* may be effective in the region.

O7.5 - Apoptosis in alveolar epithelium of severe falciparum malaria with pulmonary edema

[1]*Chuchard Punsawad, [2]Chayanee Setthapramote, [1]Sarawoot Palipoch and [3]Parnpen Viriyavejakul

[1]School of Medicine, Walailak University, Nakhon Si Thammarat 80161, Thailand
[2]Department of Clinical Pathology, Faculty of Medicine, Vajira Hospital, Navamindradhiraj University, Bangkok 10300, Thailand
[3]Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

The exact mechanism of pulmonary edema in severe malaria patients is unknown. This work aimed to study in detail the histopathological changes in the lung of severe *Plasmodium falciparum malaria* and to investigate the occurrence of apoptosis in the alveolar epithelium. (n = 10). The immunohistochemistry method was used to determine the apoptosis in lung tissues using antibodies specific to cleaved caspase-3. The normal lung tissues (n = 10) were used as control group to compare the differences in number of positive staining cells. Results of histopathology demonstrated degree of pulmonary edema, diffuse alveolar damage, pulmonary hemorrhages, activation of alveolar macrophages laden with hemosiderin pigments and parasitized red blood cells in the blood vessels. The expression of cleaved caspase-3 was significantly increased in the alveolar epithelium of severe falciparum malaria with pulmonary edema, compared with the control lung tissues. This finding documented the occurrence of apoptosis in alveolar epithelium in severe falciparum malaria with pulmonary edema. Apoptosis of alveolar epithelium was associated with pulmonary edema and can be one of the cause that contributes to cell junctional alteration, fluid leakage and eventually pulmonary edema in severe malaria.

O7.6 - Mangosteen (*Garcinia mangostana* L) as antioxidant, antimalaria, and its synergistic activity with artemisinin *in vitro*

Susy Tjahjani[1], Wahyu Widowati[2], Khie Khiong[2], Adrian Suhendra[3]

[1]Department of Parasitology, Medical Fakulty of Maranatha Christian University,
[2]Department of Biology, Medical Fakulty of Maranatha Christian University,
[3]Department of Clinical Pathology, Medical Fakulty of Maranatha Christian University,
Bandung, Indonesia

Malaria especially falciparum malaria causes high morbidity and mortality because of multidrug resistant cases. On the other side, malaria pathogenesis is closely correlated to free radical overproduction. Artemisin, used in ACT (artemisinin based combination therapy) to overcome the drug resistance, is a free radical producing antimalaria. That's why antioxidant must be put into consideration to be added in ACT therapy regimen. Indonesia has plenty of mangosteen. Its rind, as a waste product, besides has antioxidant properties, can inhibit heme polymerization in vitro. The aim of this study was to evaluate mangosteen rind ethanolic extract as antioxidant in DPPH (diphenyl picryl hidrazyl) scavenging activity, as antimalaria, and its sygergism potency with artemisinin in vitro. Antioxidant test was done spectrofotometrically according to Unlu *et al*, while its IC50, indicating its antimalaria activity, was determined by parasitemia counting in 3D7 Plasmodium culture in vitro. Synergism potency of this extract with artemisinin was evaluated by IC50 determination in this culture incubated with various concentration of this extract and artemisinin in 1:1 ratio. This study showed that mangosteen rind ethanolic extract had high antioxidant activity, high antimalaria activity, and also had sinergistic activity with artemisinin as antimalaria and it might be closely correlated with its high phenolic content.

O7.7 - A clinical trial of the efficacy of extract of Sambiloto (*Andrographis paniculata*) for falciparum malaria

[1]*Umar Zein**, [2]*Awaluddin Saragih*, [2]*Sumadio Hadisahputra*, [3]*Loeki Enggar Fitri*
[4]*Abdul Halim Mubin*, [5]*RHH Nelwan*

[1]Department of Internal Medicine, Faculty of Medicine, Prima Indonesia University/Royal Prima Hospital- Medan, Indonesia
[2]Faculty of Pharmacy, Sumatera Utara University, Medan, Indonesia
[3]Department of Parasitology, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[4]Division of Tropical Diseases and Infections, Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.
[5]Division of Tropical Diseases and Infections, Department of Internal Medicine, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

The increasing resistance of *Plasmodium falciparum* against antimalarial drugs inspires us to search for some new drugs, particularly those available in this country which presumably potential as antimalaria, hoping that overtime it can be further developed. Actually, Sambiloto, (*Andrographis paniculata*) is a kind of plant found all over the country. For this reason this study on the efficacy of the extract of sambiloto against falciparum malaria patients is worth conducting. A clinical trial was conducted in the District of Mandailing Natal in the North Sumatera Province with a design of randomized clinical trial, double-blind control with four treatment groups of adult falciparum malaria patients without complication. They were, those with single sambiloto of 250 mg, those with single sambiloto of 500 mg thrice a day for 5 days, a combination of 250 mg of sambiloto extract with chloroquine 1,000 mg for Day I and II and 500 mg on Day III, and a combination of sambiloto extract with artesunate 200 mg per day for 3 days. An observation of the decrease of parasitemia started from Day 1 through Day 7, then, proceded to Day 14, 21, dan 28 of regimen. The antimalarial efficacy of single sambiloto of 250 mg, 500 mg, combination of 250 mg, each with chloroquine and artesunate are 90.9%, 90.5%, 90.2% and 95.2% ($p > 0.3$) respectively. There is no side-effects or changes in laboratory blood parameter during the treatment. The extract of single sambiloto or its combination with either chloroquine or artesunate can be used as anti falciparum malaria.

O7.8 - Application of microwave heat treatment in the DNA extraction for the detection of positive blood sample with *Plasmodium falciparum*

Narong Jaturas[1,2], Naruemon Onnoi[2], Tan Tian Chye[1], Aunchalee Thanwisai[2], Shasheela Sri La Sri Ponnampalavanar[3], Thulasi Kumar[1], Subashini Onichandran[1], Lau Yee Ling[1] and Veeranoot Nissapatorn[1]

[1]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[2]Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, 65000 Phitsanulok, Thailand
[3]Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Malaria remains one of the most important communicable diseases in the world. A rapid, simple and accurate diagnosis is crucial component of malaria control strategies. In molecular analysis, DNA extraction would be the first step to be used and successful DNA amplification is vital for the detection of specific DNA target. Real-time PCR (RT-PCR) assay has been developed and it has become a promising tool for malaria detection of low parasitemia due to its high sensitivity. This study, we evaluated various DNA extraction methods in blood samples by comparing the DNA purity and DNA yield using RT-PCR and targeting *Plasmodium falciparum* lactate dehydrogenase (*pfldh*) gene after microwave heat treatment with seven different DNA extraction methods including Chelex with and without Proteinase K, FTA classic and elute card, phenol chloroform, QIAamp DNA minikit and rapid boiling technique. Real-time PCR results were positive after all DNA extraction methods except for FTA classic card. Of 7 DNA extraction methods, rapid boiling technique provided the highest DNA yield followed by microwave heat treatment and QIAamp DNA minikit respectively. However, among the three DNA extraction methods, the microwave heat treatment was the least time-consuming method as well as does not require the expensive equipment. This is the first study using microwave heat treatment, which is simple, rapid and a promising alternative method in detecting small amounts of *Plasmodium falciparum*. In addition, irradiation of blood samples with microwaves allows incorporation of PCR into a practical tool for routine diagnostic assessment of patients with malaria infection.

O7.9 - High diagnostic performance of fluorescence-based rapid kit diagnosis of malaria

SJ Yeo[1], HO Song[1], HJ Shin[2], JY Kim[3], WJ Lee[3], CK Chong[4], ET Han[5], and H Park[1]

[1]Zoonosis Research Center, Department of Infection Biology, Wongkwang University,
[2]Department of Microbiology, Ajou University School of Medicine,
[3]Division of Malaria and Parasitic Diseases, National Institute of Health, Korea CDC,
[4]GenBody, Republic of Korea.
[5]Department of Parasitology, Kangwon National University College of Medicine, Republic of Korea

ELISA, PCR, and RDT have been developed to diagnose malaria but due to time-consuming for diagnosis and inefficiency of mass screening, those approaches have practical limitations. In this study, the novel fluorescence immune diagnostic assay was developed with the monoclonal antibody against *Plasmodium* Lactate Dehydrogenase (LDH) and fluorescence dye optimized at Light Emitting Diode (LED). The monoclonal antibody against *Plasmodium* LDH was able to diagnose human *P. vivax* and *P. falciparum* as well as rodent *P. berghei*. Fluorescence-linked immunosorbent assay and fluorescent immunochromatographic test using fluorophore and monoclonal antibody determined parasitemia up to 0.2% and at least 0.1 ng of LDH antigen. FLISA and FICT showed 100% of the sensitivity and cross-reactivity was not found in other patient's specimens (i.e., HCV and HIV). Moreover, the intensity of fluorescence were found to be increased upon infection rate (R^2=0.9879). *P. berghei* infected mouse showed the same diagnostic performance of quantitative analysis (R^2=0.9922).Therefore, new fluorescent immunochromatographic assay are applicable for both qualitative and quantitative diagnosis of malaria.

O7.10 - Delayed parasite clearance after treatment with dihydroartemisin-piperaquine in uncomplicated *Plasmodium falciparum* patients in some sentinel sites in Vietnam

Nguyen Van Hong[1],Ta Thi Tinh[1],
Tran Cong Dai[2], Bui Quang Phuc[1]*

1 National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam
2 World Health Organization respresentative office, Vietnam

In Vietnam, artemisinin has been produced and used since the early 1990s, contributed greatly to reducing the morbidity and mortality from malaria in this country. However, the evidence of resistance has now been detected in several malaria areas in Vietnam. This study showed the results of regular monitoring of therapeutic efficacy of first line antimalarial drugs (dihydroartesunate-piperaquine) in four sentinel sites (Binh Phuoc, Dak Nong, Gia Lai, Quang Tri provices) in Vietnam.The study was designed as a 42-day follow-up study to assess the efficacy of DHA-PQ in treating clinical episodes with uncomplicated *P. falciparum* from August 2012 to March 2013. Patient were treated according to national treatment guidelines with an age depended dosing scheme of DHA-PQ. A total of 166 patients were enrolled. 100% adequate clinical and parasitological response to the DHA- PPQ in Binh Phuoc, Dak Nong and Gia Lai. A total of 35 (21.0%) patients showed parasites on day 3 with the rate of 30.6% ; 29.2% ; 22.8% in Binh Phuoc, Dak Nong , Gia Lai respectively. There were no delay parasite clearance detect in Quang tri province. No early treatment failure occurred but 1 late parasitological treatment failures was classified in Quang tri province, PCR analyses were confirm true late treatment failures. Compare with previous studies, the proportion of patients with positive parasites at D3 increases with each passing year in Vietnam. The results are suggestive of artemisinin resistance, urgent actions essential to contain artemisinin resistance in these areas.

O7.11 - High prevalence of dihydrofolate reductase (*dhfr*) an dihydropteroase synthase (*dhps*) mutations in *Plasmodium falciparum* isolates in Sabah

Nor Afizah Nuin[1], Timothy William[2], Prababakaran Dhanaraj[3], Lau Tiek Ying[1]*

[1]Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.
[2]Infectious Diseases Department, Queen Elizabeth Hospital, Karung Berkunci No. 2029, Jalan Penampang, Kota Kinabalu, 88560, Sabah, Malaysia.
[3]Kudat District Hospital, Peti Surat No. 22, 89057, Kudat, Sabah, Malaysia.

The emergence and spread of resistance caused by point mutations in dihydrofolate reductase (*dhfr*) and dihydropteroase synthase (*dhps*) genes in *Plasmodium falciparum* parasites towards sulfadoxine-pyrimethamine (SP) drug poses a serious public health problem in many endemic regions. This study aimed to study the prevalence of mutations in *dhfr* and *dhps* genes of *P. falciparum* collected from Kota Kinabalu, Keningau, and Kudat, Sabah. Nested-PCR was performed to amplify *pfdhfr* and *pfdhps* genes on the confirmed single *P. falciparum* infection. A total of 35 *pfdhfr* and 33 *pfdhps* partial sequences were amplified and mutations associated with resistance to SP drug were analyzed. High prevalence of mutations conferring resistance, particularly the double mutation in *pfdhfr*, C59R/S108N was detected in all samples while point mutation N51I was found only in one sample. It has been proposed that these mutations are associated with resistance in pyrimethamine. With respect to *pfdhps*, three point mutations were observed including A437G in all samples, K540T in 2 samples and A581G in 11 samples. Two of these mutations, A437G and A581G have been reported elsewhere to be associated with sulfadoxine resistance. The presence of *pfdhfr* C59R/S108N double mutation in combination with *pfdhps* A437G/A581G double mutation has resulted in *pfdhfr/pfdhps* quadruple mutation C59R/S108N/A437G/A581G in more than 30% of cases. This finding shows a remarkably high prevalence of mutations conferring resistance in *P. falciparum* from Sabah isolates. The level of resistance indicates the need for implementation of entire population access to the new line treatment, together with government monitoring in preventing the emergence of resistance to this resistance.

O7.12 - Modulation Of Th17-T regulatory cells by dendritic in malaria: Role of pro and anti-inflammatory cytokines

[1]*Tarun Keswani and* [1]**Arindam Bhattacharyya*

[1]*Immunology Laboratory, Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700019, West Bengal, India*

The spleen is a complex organ with several functions, with reports also demonstrating an immunopathologic involvement of this organ in human, rodent models and in experimental malaria models. The ability of proinflammatory cytokines such as IL-6 and regulatory cytokines like TGF-β to either promote or protect from severe malaria are likely to depend on many factors, including hosts ability to DC maturation and the immune status of an infected individual. Several researchers reported that Treg cells suppress the host immune system and aggravate malaria both in human and mice and also been reported that Treg cells protect hosts through suppression of severe inflammation. Other subsets such as Th17 cells have however not been described during *Plasmodium berghei* ANKA *(PBA)* infection. Given the delicate balancing act to be achieved between control of overwhelming infection and prevention of immune-pathology, it is very tempting to invoke a role for DC in Treg/ Th17 cells differentiation in malaria. IL-2 is crucial for the maintenance and expansion of natural Foxp3+ Treg cell populations and inhibits Th17 development, further supporting our findings. On the other hand, Foxp3 regulatory T cells control inflammation through multiple mechanisms including production of the cytokine IL-10 and TGF-β. In contrast, TGF-β and IL-6 trigger the coordinated activation of Smad3 and STAT3 to induce the transcription factor RORγt necessary for Th17 differentiation. We hypothesize that DC might play an important role in modulating the balance between the differentiation of Treg and Th17 cells in during the *PBA* infection by shaping Treg/Th17 response.

Forensic Science, Mosquitoes and Vector Borne Diseases

O8.1 - Total body score: A new scoring approach in assessment of post mortem changes

Teo Chee Hau, Sri Pawita Albakri Amir Hamzah, Khairul Osman, Noor Hazfalinda Hamzah.

Forensic Science Programme, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur.

Total Body Score (TBS) system is a system that allows quantitative assessment on decomposition process based on separate body parts. Body parts are scored independently and the total score is known as TBS score, which reflects the current overall decomposition process of the corpse. Twenty seven rabbits (*Oryctolagus cuniculus*) carcasses were used to study the effect of burial and type of clothing on rate of decomposition through TBS system assessment. Subjects were separated into 3 groups: No clothing, heavy clothing and plastic wrapping. The head, neck and limb regions were found to decay faster than the body trunk region. No clothing group showed the increase of TBS score at the fastest rate, closely followed by thick clothing group while plastic wrapping group showed the slowest development of TBS score. The delayed effect of plastic wrapping in decomposition became more obvious after three weeks post burial onwards. Meanwhile, variances of post mortem changes had been observed under the influence of clothing. TBS system was found to be suitable in assessment of post mortem changes for decomposed remains found on ground surface and in buried circumstances. However, the system required minor modification so that it is suitable for the faster rate of decomposition in Malaysia and bodies with adipocere.

O8.2 - Potential of pteridine fluorescence in age-determination of forensically important fly species

[1*]Roziah Ali, [1]Nazni Wasi Ahmad, [1]Hazmizam Hamzah, [1]Norazizah Ali, [1]Lee Han Lim

[1]Unit of Medical Entomology, WHO Collaborating Centre for Vectors, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia

In forensic entomology, minimum post-mortem interval (mPMI) is determined by using insects collected from the cadavers. By determining the age of the oldest immature stage, the minimum time elapsed since death can be estimated. Traditionally, the age of the larvae is estimated based on the morphological characteristics of the immature. However, this method needs expertise and is also laborious. Therefore, other age determination technique such as pteridine fluorescence analysis has been explored as an alternative age indicator. In this report, pteridine compounds were extracted daily from larvae until pupation from 3 different species, each from Calliphoridae, Sarcophagidae and Muscidae. The extracted pteridine was analysed using a fluorescence spectrophotometer (LS-55) with excitation at 330nm and emission 350nm-600nm. The concentration of pteridine ranged from 0.3 μgL^{-1} (day 1) to 60 μgL^{-1} (day 6-7).These results showed significant linear relationship between pteridine fluorescence and the age of the larvae, suggesting that pteridine concentration was strongly correlated with age. This technique is potentially a novel tool for determination of maggot age and hence establishing accurate mPMI.

O8.3 - Elevating of Cd4 and Cd8 T cells expression on placental tissue of pregnant *Plasmodium berghei* infected Balb/C mice are correlated with fetal low birth weight

Loeki Enggar Fitri,[1] Adilah Ulfiati,[2] Bougenvil Ungu.[2]
Maimun Zulhaidah Arthami, [3] Eviana Norahmawati [4]

[1]Department of Parasitology, Faculty of Medicine,
University of Brawijaya, Malang, Indonesia
[2]Medical Study Program, Faculty of Medicine, University of Brawijaya, Malang, Indonesia
[3]Department of Clinical Pathology, Faculty of Medicine,
University of Brawijaya, Malang, Indonesia
[4]Department of Pathology Anatomy, Faculty of Medicine,
University of Brawijaya, Malang, Indonesia

Malarial infection during pregnancy can cause maternal mortality and low birth weight of the fetal. Those can be influenced by elevating of mediator inflammatory and increasing of immune cells activities. This study was aimed to know the role of CD4 and CD8 T cells in pathological process of fetal development in pregnant *Plasmodium berghei* infected BALB/c mice. Female *Mus musculus* BALB/c (primigravida) were divided into positive control group (9 mice infected by *P. berghei* on the 9th day of pregnancy) and negative control group (8 normal mice). Surgery performed on the 18th day of pregnancy to isolate the fetus and the placenta. Expression of CD4 and CD8 T cells on placenta was counted by immunohistochemical staining and fetal weights were measured by analytical scale (Mettler AE 50). Independent T test showed that expression of CD4 T cells (p=0.009), expression of CD8 T cells (p=0.001), and fetal weight (p=0.003) increase significantly in positive control group. Moreover, Pearson correlation test showed that there was a negative correlation between expression of CD4 T cells (r=-0.665, p=0.004) and CD8 T cells (r=-0.633, p=0.006) and fetal birth weight in positive control group. Interestingly, expression of CD4 T cells had a moderate correlation with expression of CD8 T cells (Pearson correlation test r=0.661, p=0.004). The conclusion of this study is that elevating of CD4 and CD8 T cells have an important role in the pathogenesis of low birth weight during malaria infection.

O8.4 - Lipid formulation of Amphotericin B: An effective treatment option for the elimination of visceral leishmaniasis

Krishna Pandey[1], Pradeep Das[1], Shyam Sundar[2], Tara Kant Jha[3], Chandreshwar Prasad Thakur [4]*

[1]Rajendra Memorial Research Institute of Medical Sciences (Indian Council of Medical Research), Agamkuan, Patna-800007, Bihar, India.
[2]Kalaazar Medical Research Center, Rambag road, Muzaffarpur, Bihar-842001, India.
[3]Kala-azar Research Center, Brahmpura, Muzaffarpur, Bihar-842 003, India. 4Balaji Utthan Sansthan, Kalaazar Research Centre, Fraser Road, Patna -800 001, Bihar, India.

India accounts for 60% of total global visceral leishmaniasis (VL) cases. Oral (e.g. miltefosine) and parenteral drugs, considered the mainstay for treatment of VL, are now faced with resistance, decreased efficacy, low compliance and safety issues. Efficacy and safety of an alternative treatment option, single infusion of amphotericin B lipid emulsion (ABLE) vs liposomal amphotericin B (LAmB) was evaluated in VL patients. In this multicentric, open-label study; male/female patients (N=500) aged ≥5 to ≤65 years diagnosed with VL were randomized (3:1) to 15 mg/kg infusion of ABLE (N=376) or LAmB (N=124). Efficacy (initial cure [Day- 30/45], clinical improvement [Day-30], definite cure [Day-180]), and safety of treatments were evaluated. A total of 448(89.6%) patients completed the study (ABLE: 326[86.7%]; LAmB: 122[98.4%]). The initial cure rate was 95.9% vs 100% (ABLE vs LAmB) in the modified-intent- to-treat (MITT) population. Overall clinical improvement was comparable (98.9% vs 98.4%). Definitive cure was 85.9% vs 98.4% in ABLE and LAmB groups, respectively. Infusion-related pyrexia (37.2% vs 32.3%) and chills (18.4% vs 18.5%) were comparable between ABLE and LAmB. Treatment-related SAEs were 0.3% in ABLE and 1.6% in LAmB. Two deaths (one due to diarrhoea; second was sudden death) occurred in ABLE. No nephrotoxicity and hepatotoxicity were reported with either drug. ABLE was efficacious and well-tolerated. Given the high cost and limited supply of LAmB, ABLE is a valuable treatment option and can play a potential role in eradicating VL in endemic countries.

O8.5 - Infection of *Plasmodium berghei* in pregnant mice induces intrauterine growth restriction (IUGR) and down regulates placental glucose transporter 1 (GLUT-1) without correlation between them

Nur Fahma Pradiptasari[1], Teguh Wahju Sardjono[2], Loeki Enggar Fitri[2] and Eviana Norahmawati[3]

[1]Medical Study Program, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.
[2]Department of Parasitology, Faculty of Medicine, University of Brawijaya, Malang, Indonesia
[3]Department of Anatomical Pathology, Faculty of Medicine, University of Brawijaya, Malang, Indonesia

Glucose is the primary substrate for fetal and placental development. It is transferred across the placenta by sodium-independent facilitated diffusion along concentration gradient. Glucose transporter 1 (GLUT-1) is known as the main isoform of glucose transporter through placenta. In placental malaria, tissue hypoxia impairs transport of many nutrients, such as glucose. It is assumed that expression of placental GLUT-1 is down regulated during placental malaria. This experimental study used 17 pregnant Balb/c strain mice consisted of 9 mice which were infected by *Plasmodium berghei* on the 9th day post mating (treatment group) and 8 mice were not infected (control group). On the day of 18th post mating the mice were sacrificed, then their fetus and placenta were isolated. The weight of fetus was measured by analytical scale and the placenta was examined microscopically after staining by immunohistochemistry method. The mean of fetal weights of control group (0.94±0.19 g) and treatment group (0.63±0.12 g) were significantly different (p=0.002). GLUT-1 in treatment group showed lower expression than in control group significantly (p=0.000). There was no significant correlation between the expression of GLUT-1 and the fetal weight (r=0.284, p=0.269). It can be concluded that infection of *P. berghei* in pregnant mice induced fetal low birth weight (LBW) and downregulated expression of placental GLUT-1, but the fetal LBW was not caused directly by the decrease of GLUT-1 expression. It more indicates that Intrauterine Growth Restriction during malaria infection has multi factorial causes.

O8.6 - Endoglin expression and the level of TGF-β are increase in the placental tissue but only TGF-β has correlation with low fetal body weight in malaria pregnant mice

[1*]*Sujarot Dwi Sasmito,* [2]*Loeki Enggar Fitri,*
[3]*Teguh Wahju Sardjono,* [4]*Eviana Norahmawati*

[1]*Master's Program in Biomedical Science, Faculty of Medicine, University of Brawijaya, 65145,Malang, Indonesia*
[2]*Department of Parasitology, Faculty of Medicine, University of Brawijaya, 65145Malang, Indonesia*
[3]*Department of Parasitology, Faculty of Medicine, University of Brawijaya, 65145Malang, Indonesia*
[4] *Departement of Pathology Anatomy, Faculty of Medicine, University of Brawijaya, 65145Malang Indonesia*

Malaria infection during pregnancy cause accumulation of infected-erytrocyte in intervillous space of placenta and decrease blood flow from maternal to fetal. This condition triggers endoglin expression, a cofactor of TGF-β receptor and induces placental tissue hypoxia. This study was conducted on 17 Balb/c mice that consist of experimental group (9 pregnant mice infected with *Plasmodium berghei* on the day 9th of pregnancy) and control group (8 normal pregnant mice) to reveal the role of placental endoglin and placental TGF-β in pathophysiology of Intra Uterine Growth Restriction (IUGR). All samples were sacrificed at the 18th day of pregnancy. ELISA (R&D) was done to determine the level of tissue endoglin and TGF-β while immunohistochemical was performed to examine endoglin expression in placental tissue. Fetal body weight was measured by analytical scale (Mettler AE 50). The level of tissue endoglin was higher in experimental group than control group although independent t-test showed no significant difference (p=0,055). However the increase of endoglin expression by immunohistochemical staining showed a significant difference between control and experimental group (p=0,003). The level of tissue TGF-β in experimental group was higher than control group but no significant difference between them (p=0,064). The fetal body weight revealed significant difference between experimental group and control group (p=0,001). Pearson correlation test showed that only tissue TGF- β and fetal body weight had significant negative correlation (p=0,017, r=-0,571). The endoglin expression increased in placental tissue and tissue TGF- β has role in low fetal body weight during malaria infection in pregnant mice.

O8.7 - Bangle (*Zingiber cassumunar* Roxb.) extract as potential candidate for adjunctive therapy of malaria by increasing the phagocytic activity of macrophages

[1]*Yunita Armiyanti,* [2]*Devita Prima Nurmasari,* [3]*Lidya Ameliana ,* [1]**Wiwien Sugih Utami*

[1] *Department of Parasitology, Medical Faculty, Jember University, Jember, Indonesia*
[2] *Medical Undergraduate Programme, Jember University, Jember, Indonesia*
[3]*Department of Pharmaceutica, Pharmacy Faculty, Jember University, Jember, Indonesia*

Severe malaria that caused of high mortality rate involves immunological processes, such as the production of free radicals and inflammatory cytokines by immune cells. Therefore, it is necessary to use medications containing immunomodulator compounds as adjunctive therapy in severe malaria cases, such as Bangle (*Zingiber cassumunar Roxb*.). The aim of this study was to measure the effects of Bangle extract administration on the degree of parasitemia and phagocytic activity of peritoneal macrophages in mice infected with *Plasmodium berghei* and treated with artemisinin. The research was done using post-test-control-only design and 24 mice were randomly distributed into four groups. Two groups (K1 and K2) were orally administrated with a dose of 22.6 mg Bangle extract per mice for two weeks and one group (K4) was as control group. Furthermore, all group infected with *P.berghei*. The degree of parasitemia was measured everyday for four days after the parasite was positive in the blood smear. Artemisinin therapy was orally administrated to K2 and K3 for four days at a dose of 0.728 mg per mice. On the fifth day after the administration of Artemisinin, all of mice were terminated and assayed for phagocytic activity of peritoneal macrophages. All of mice that stimulated with Bangle extract had significantly increased phagocytic activity of peritoneal macrophages ($p < 0.05$) and the degree of parasitemia was lower than control group. This result showed that Bangle extract had immunomodulatory effect by increasing the phagocytic activity of macrophages, thus also increased the ability of parasite elimination.

O8.8 - Seasonal abundance of *Aedes (stegomyia) albopictus* and *Aedes (Stegomyia) aegypti* in Guwahati Metropolis and suburban settlements, Northeast India

V. Dev[1] and K. Khound[2]

National Institute of Malaria Research (Field Station),
Guwahati – 781 022, Assam (India),
National Vector Borne Disease Control Programme, Government of Assam,
Guwahati – 781 005, Assam (India)

Dengue is endemic in India with reported disease outbreaks in big metropolis cities, and spreading to areas hitherto free from disease onslaught. In Assam (Northeast India), ever since first report of dengue in 2010, there is progressive increase in case incidences, and vast majority (>70%) are being reported from Guwahati metropolis alone. Monthly surveys for larval breeding resources revealed that both *Aedes (Stegomyia) aegypti* and *Ae. (Stegomyia) albopictus* were prevalent during months of rainfall (April –October), and recorded breeding in variety of resources. *Ae. albopictus* were observed breeding in tyres, cut bamboo stumps, tin/plastic containers, flower vases and leaf axils both in urban and semi-urban areas. However, it was the predominant mosquito species in semi-urban areas breeding preferentially in flower vases, cut bamboo stumps and leaf axils. *Aedes aegypti* instead was the most common species in urban areas breeding predominantly in discarded tyres; other breeding sources included tin/plastic containers. Among *Ae. albopictus* subgroup of species prevalent in India, all mosquitoes dissected for male genitalia were morphologically identified to be true breeding *Aedes (Stegomyia) albopictus* (Skuse). For control of adult mosquito vector populations, it was observed that both *Ae. aegypti* and *Ae. albopictus* were resistant to DDT (4%), but fully susceptible to malathion (5%) at given diagnostic concentrations. However, both these species exhibited varied response to pyrethroids (deltamethrin and permethrin). *Aedes aegypti* was observed to be resistant to deltamethrin (0.05%) as well as permethrin (0.75%), but *Ae. albopictus* was susceptible to deltamethrin, and for permethrin the status was border line susceptibility (verification required).

O8.9 - *In vivo* dengue virus insecticide interaction to determine impact of fogging on dengue transmission

Nazni, W.A.[1] , Farah H[1], Ravindran T[2] , Zainah S.[1] , Lee H.L[1]., Sofian A[3].

[1] Medical Entomology Unit, Institute For Medical Research, Jalan Pahang,50588 Kuala Lumpur.
[2] Virology Unit, Institute For Medical Research, Jalan Pahang, 50588 Kuala Lumpur.
[3] Faculty Science, University Malaya.

Dengue virus serotypes 1-4 are the aetiologic agents of dengue are transmitted by *Aedes* mosquitoes. Controlling the mosquito vectors is the only mean of interrupting dengue transmission, in the absence of an effective vaccine and specific treatment. Insecticides such as permethrin and malathion are widely used for the control of dengue vectors. However, despite extensive use, there has been no report on the possible impact of insecticide on dengue virus replication. In this study, we investigated the *in vitro* effect of malathion and permethrin on dengue virus replication in *Aedes albopictus* C6/36 cell line. C6/36 cells were exposed to permethrin and malathion each at 0.2%, 0.4%, 0.6% and 0.8% for 24 hours. The effects of these insecticides on the replication of dengue virus were examined using tissue culture infective dosage (TCID50). Test concentrations of both insecticides did not exert any effects on uninfected C6/36 cell. However, introduction of dengue virus serotype 1-4 into insecticide treated cell-line showed presence of cytophatic effect, indicating replication of dengue viruses. Permethrin and malathion enhanced Den 1 and Den 3 replication with enhancement index of 1.6 times and 0.6 times respectively, while Den 2 and Den 4 replication was enhanced by malathion and permethrin by 1.2 and 0.8 times , respectively. Insecticide seems to exert certain effects on dengue virus replication and warrants further study to investigate the impact of chemical insecticide on dengue control.

O8.10 - First full genome sequence of chikungunya virus isolates from Malaysian non-human primates

Suhana O[1], Nazni, W.A.[2,] Apandi Y .[3] Azahari A.H[2], Farah H[2]. Lee H.L[2]. Zainah S.[3] Sofian A[1].

[1] Faculty of Science, University of Malaya. 50603 Kuala Lumpur
[2] Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur.
[3] Virology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur.

Four virus isolates belonging to genus *Alphavirus* in the family Togaviridae identified as Chikungunya virus (CHIKV) were obtained during a 2006 survey of arboviruses in the monkey (*Macaca fascularis*) in Pahang state, Peninsular Malaysia . The full genome sequence of the four CHIKV isolates, labelled as strain M125, M127, M128, M129 were determined. The full length sequence of the isolate was 11801bp with more than 95% of intraspecies sequence identity and more than 83% intraspecies amino acid similarity when compared with other genotypes. A unique amino acid, Glycine (G) was found in these monkey isolates in the NSP1 region, whereas Arginine (R) was found in the same region in human isolates. . An opal stop codon has been identified in each isolate in nsP3 region. Phylogenetic analysis was performed to determine the relationships of the monkey isolates with previously reported human isolates and showed that the 4 isolates from monkey belonged to the 2006 Bagan Panchor and 1999 Klang clades in an Asian genotype. Full genome sequence suggested that an Asian strain is endemic in Malaysia and monkey is one of the potential reservoirs of CHIKV in Malaysia. These findings warrant further investigation to characterise CHIKV from different geographical regions of the country to better understand the molecular epidemiology of CHIKV outbreaks in Malaysia. This is the first report of whole genome sequencing of CHIKV isolated from monkey in South East Asia.

O8.11 - Field evaluation of *Aedes* trapping efficiency of BG-sentinel™ trap and mosquito trap baited with synthetic mosquito lures

[1,2*]*Teoh Guat Ney,* [1]*Nazni Wasi Ahmad,* [3]*Renaud Lacroix,*
[1]*Lee Han Lim, and* [2]*Mohd. Sofian Azirun*

[1]*Unit of Medical Entomology, Institute for Medical Research, 50588 Kuala Lumpur,Malaysia.*
[2]*Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.*
[3]*Oxitec Ltd., 71 Milton Park, Oxford, OX14 4RX, United Kingdom.*

Mosquito surveillance is important in providing information and monitoring of entomologic risk factors in predicting the occurrence of vector-borne disease. Historically, most *Aedes* monitoring concentrated on assessments of juvenile stages, either egg, larval or pupal surveys. To date, *Aedes* adult mosquitoes are not strongly attracted to most standard mosquito adult traps; such as the Biogents Sentinel™ (BG-Sentinel™) trap which seems to capture more *Culex spp.* than *Aedes spp*. In this study, we developed and evaluated new tools to monitor the presence and abundance of *Aedes* mosquitoes in the field. Preliminary larval surveys and ovitrap surveillance were conducted in Tanah Merah Jaya, Melaka Tengah District, Taman Batu Muda and Kampung Batu Muda in Kuala Lumpur to monitor *Aedes* population since January 2012. High ovitrap index for *Ae.aegypti* in Tanah Merah Jaya (43%), Taman Batu Muda and Kampung Batu Muda (20%) indicated that these sites were suitable for investigating the efficiency of new traps and lures. The efficacy of the new adult trap; Mosquitito™ traps were compared with BG-Sentinel™ traps in field trials. Both trap types baited with BG-lure™ captured significantly higher (P≤0.05) *Culex spp.* adults compared to *Ae. Aegypti and Ae. Albopictus* adults. Therefore, further testing on the efficacy of new synthetic blend was applied to both trap types in order to re-assess the appropriateness in terms of mosquito recaptured, practicality of use and cost-effectiveness. An improved adult mosquito trapping system will provide better experimental sampling data and thus better understanding of the biology and behaviour of *Aedes* mosquitoes.

O8.12 - Chronic diarrhoea in protein energy deficiency

[1]R.Heru Prasetyo, [2] Joni Susanto, [3] Hani Plumerastuti

[1] Departement of Parasitology, Faculty of Medicine, Airlangga University, Indonesia
[2] Departement of Anatomy and Histology, Faculty of Medicine, Airlangga University, Indonesia
[3] Departement of Patology, Faculty of Veterenary Medicine, Airlangga University, Indonesia

Protein-energy deficiency is the most cause of mortality among under five malnourish children associated with intestinal disease and chronic diarrhoea. Objective of this research was to investigate the correlation of chronic diarrhoea and intestinal parasite infection in protein-energy deficiency. This research used five days fasted experimental mice *Balb/c* as model of protein-energy deficiency. The sample used in this research was small intestine that observed variables were histological change and immunohistochemistry of Hsp70, PGE_2, and sIgA in intestinal mucous. The result showed villus atrophy and epithelial mucous damage, increased of Hsp70 expression, decreased of PGE_2 and sIgA expression. These conditions cause absorption and secretion disturbance that cause of diarrhoea, decrease of intestinal motility and adaptive immunity. If intestinal parasite infection occur, the parasite will more easy to adhere on epithelial mucous, and it will more easy to proliferation and colonization, that all of it can increase intestinal mucous damage, and cause of severe and chronic diarrhoea. The conclusion that can be made from this research was intestinal parasite infection in protein energy deficiency can cause of severe and chronic diarrhoea and than it will increase mortality.

POSTER SESSION

P.1 - Fields' Stain: Ideal to differentiate *Dientamoeba fragilis* and *Blastocystis* sp.

Anitamalar Devi Ragavan, Suresh Kumar Govind

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

Dientamoeba fragilis, a trichomonad parasite is usually found in the gastrointestinal tract of human and it is known to be the cause for gastrointestinal symptoms. The parasite is globally distributed and mostly found in rural and urban areas. The parasite is not only found in humans but also in non-human primates such as the macaques, baboons and gorillas. Often the parasite is confused with another largely found organism in stools called *Blastocystis*. Although *D. Fragilis* is identified with two nuclei; *Blastocystis* is sometimes also seen with two nuclei. The parasite sizes are sometimes similar and often confused especially when isolated in cultures where they are grown together. The present study is the first to report of Modified Fields' stain being used to differentiate *D. fragilis* and *Blastocystis sp*. from cultures. The staining showed that *D. fragilis* can be differentiated from *Blastocystis* by the presence of a thinner outer membrane as well the presence of clearly demarcated red prominent nuclei which against the greenish background tends to have a better contrast than Giemsa stained smears. *Blastocystis* showed a darker and thicker stained outer membrane as compared to *D. fragilis*. The Fields stain' is a faster and more suitable stain than Giemsa when it comes to differentiating the two organisms and thereby providing a better and more reliable diagnostic method to differentiate one from the other.

P.2 - Comparison of two culture methods for detection of *Blastocystis* sp. in fresh stool specimens

Moosavi Ali[1], Haghighi Ali[1], Rafiei Sefid Dashti Raheleh[2], Nazemalhosseini Mojarad Ehsan[3], Arabmazar Zahra[1], Mohammadiha Anita[1], Naderi Farideh[1]

[1]Dept. of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Dept. of Medical Parasitology and Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3Reserch center for Gastroenterology and liver diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Blastocystis sp. is an enteric unicellular parasite and also it is a cause of gastrointestinal disorders when there are no other pathogenic agents present that could cause gastrointestinal symptoms. Although stool microscopy is a simple and economical procedure that can be done in any laboratory, short-term in vitro cultivation increases the sensitivity of detection compared to that of direct microscopy of fecal smears. A total of 420 stool samples obtained from 235 male (56%) and 185 Female (44%) were studied. Liver Infusion Tryptose (LIT) containing 10% fetal calf serum and Dulbecco's Modified Eagle Medium (DMEM) containing 20% calf serum in screw-capped tubes were used for the cultivation of stool specimens. They were incubated at 37°c for 72 hours. One hundred *Blastocystis*-positive cultures were isolated from LIT and a similar number was obtained from DMEM. This gives an isolation rate of 23.8%. While only 64 samples (15.2%) were positive with direct smear examination. Liver Infusion Tryptose (LIT) has been used for cultivation of aerobic parasites such as *Trypanozoma* and *Leishmania*. It has been found in this study that LIT can also be used for cultivation and diagnosis of *Blastocystis* sp. as an anaerobic parasite. Also Cultivation is a good method for diagnosis of *Blastocystis* sp. in stool specimens and no difference was found between the two culture media. However, DMEM is easier than LIT to buy and use.

P.3 - Proteolytic variation and hemoglobinolytic activity of *Blastocystis* spp.

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

Department of Parasitology, Faculty of Medicine,
University of Malaya

Blastocystis spp. has been frequently incriminated for being pathogenic and associated with various gastrointestinal symptoms such as diarrhea, abdominal pains, flatulence and nausea. Although various studies have demonstrated protease activity as a possible virulent factor as shown in other intestinal parasites, however, there has been no studies carried out to compare the protease activity from different subtype (ST). The recent studies incriminating *Blastocytsis* spp. with iron deficiency anaemia and deficiency in hemoglobin in individuals infected led us to investigate the role of proteases from Blastocystis spp. in hemoglobinolytic activity. Symptomatic and asymptomatic isolates from different genotypes were tested for protease activity and the proteases were tested against human haemoglobin to determine hemoglobinolytic activity. The results showed that ST 3 possessed highest protease activity followed by ST1, ST5 and ST6 with intense bands which corresponds to azocasein unit. Based on the degradation of hemoglobin (Hg), ST3 showed the most intense activity compared to other ST's. The study substantiates another pathogenic property of ST3 and provides evidence that *Blastocystis* spp. does cause degradation of haemoglobin supporting earlier studies that revealed lesser Hg in *Blastocystis* spp. infected patients.

P.4 - Higher caspase-like activity in symptomatic isolates of *Blastocystis* spp.

D.B. Dhurga, K.G. Suresh

Department of Parasitology, Faculty of Medicine,
University of Malaya, 50603 Kuala Lumpur

Biochemical evidence of a caspase-like execution pathway has been demonstrated in a variety of protozoan parasites, including *Blastocystis* spp. Apoptosis was induced in *Blastocystis* spp. by treating cultures of symptomatic and asymptomatic isolates of 3 sub-types namely 1, 3 and 5 with two different concentrations, 0.1 and 0.0001 mg/ml of metronidazole (with and without pre-treatment with a pan-caspase inhibitor, zVAD.fmk). The experiment was repeated to assess the number of apoptotic cells in all the isolates of both conditions.Symptomatic isolates of subtype 3 (without pre-treatment with a pan-caspase inhibitor, zVAD.fmk) showed high fluorescence intensity for active caspase-like proteases [0.0001mg/ml, 88% ($p<0.001$) at 0.1mg/ml, 70% ($p<0.001$)] at the 72nd hour *in vitro* culture in comparison with asymptomatic isolates [0.0001mg/ml, 65%, at 0.1mg/ml, 55%]. The number of apoptotic cells was higher [0.0001mg/ml, 89% ($p<0.001$) and at 0.1mg/ml, 70% ($p<0.001$)] at the 72nd hour of *in vitro* culture in comparison with asymptomatic isolates [0.0001mg/ml, 66% ($p<0.001$) and at 0.1mg/ml, 45% ($p<0.01$)].The high number of symptomatic cells expressing active caspase-like proteases and becoming apoptotic compared to asymptomatic cells clearly demonstrates that the response to metronidazole treatment is isolate dependent.

P.5 - Effect of antibiotic on thermal stressed *Blastocystis* Sp.

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

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

One of the most important aspects of parasite survival is its ability to respond in stressful environments. *Blastocystis* sp. is known to be a common singled-celled intestinal parasite of human. In the tropics, the increasing incidences of vector borne diseases such as dengue and malaria can increase body temperature up to even 41^0C. Such patients would resort to treatment and often doctors prescribe antibiotics initially especially if they are not aware of the cause of the fever. It is highly probable that high temperatures can influence *Blastocystis* if such patients are also infected with the parasite. The question as to whether antibiotics have an influence on thermally stressed *Blastocystis* has never been investigated. The present study is aimed in determining the effects of antibiotic stress (Penicillin, Streptomycin, and Neomycin (PSN)) on thermal stressed *Blastocystis* sp. The *in vitro* cultures of *Blastocystis* were subjected to 41^0C and were further stressed with three different concentrations of PSN. The parasite growth of the thermal and antibiotic stressed *Blastocystis* sp. was doubled compared to the thermal stressed and the non-thermal stressed cultures. A reduction in the size was also observed in the thermal and antibiotic stressed parasite. These observations concur with our earlier postulation that thermal stress causes formation of granular forms of *Blastocystis* sp. and these forms reproduce viable vacuolar forms when treated with antibiotics. This will account for the higher parasite count of the stressed parasite compared to the non-stressed ones.

P.6 - Infectivity of *Blastocystis* isolates from different animal hosts in Sprague dawley rats

Hemalatha C[1]., Suresh Kumar G[1]., Chandrawathani P[2]., Premaalatha B[2]., Farah Haziqah M.T[1].,

[1] Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur.
[2] Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak.

A study was conducted to determine the infectivity of the enteric parasite *Blastocystis* sp. isolated from different animal hosts in 2 weeks old Sprague dawley male rats. *Blastocystis* sp. was obtained from peacock, orang utan, ostriches, pig, cow, goat and sheep reared at various government and private establishments. Only eight out of 14 isolates were able to infect the rats through oral inoculation which includes the ostrich, pig, goat and peacock. *Blastocystis* sp. isolated from all the non-human hosts were confirmed not to be any of the common human strain ranging from subtype1-7 except for the ostrich isolate which was identified to be subtype 6 when amplified with PCR using sequence-tagged site (STS). Meanwhile, only rats infected with the *Blastocystis* sp. of ostrich isolate were analyzed to be subtype 6 which was similar to the subtype of the inoculum. None of the isolates from experimental rats were subtypes 4 or 7 which usually predominates the rodent population. All the non-human isolates from the fresh faecal and in-vitro culture were subjected to transmission electron microscopy study in order to observe the internal cellular structures of the cystic and the vacoular form of *Blastocystis* sp. In conclusion, the data suggests that *Blastocystis* exhibits low host specificity and therefore the possibility of human-animal cross-infectivity cannot be ruled out.

P.7 - Phenotypic characterization of *Blastocystis* Sp. due to Metronidazole treatment

Kalyani, R., Tan, T.C., Suresh, K.

Department of Parasitology, Faculty of Medicine,
University of Malaya, 50603 Kuala Lumpur

Blastocystis infection causes symptoms such as diarrhea, abdominal pain, nausea, bloating and flatulence. The first line of drug treatment in eradicating *Blastocystis* infection is metronidazole. Success of eradicating *Blastocystis* infection varies from study to study. The conflicting results from 0% to 100% implicates that there are isolates which are resistant to treatment. In order to further characterize the differences observed between the isolates due to drug treatment, biochemical changes and ultrastructural differences were analysed. Biochemical changes were observed by acridine orange, fluorescein isothiocyanate (FITC) and 4',6-diamidino-2-phenylindole (DAPI) staining. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) shows vast difference between the control and drug treated isolates for different subtypes. Drug treated isolates of ST3 observed under TEM shows higher number of mitochondrion-like-organelle (MLO) present. ST1 and ST2 isolates did not show any MLO. Results implicate that resistance in *Blastocystis* could be due to subtype-dependent. Further genotypic analysis must be carried out to confirm this postulation

P.8 - Surface differences in *Blastocystis* isolated from asymptomatic individuals, symptomatic and irritable bowel syndrome patients

Nanthiney Devi Ragavan[1], Suresh Kumar Govind[1], Tan Tian Chye[1], Sanjiv Mahadeva[2]

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

Blastocystis, the most common human intestinal protozoan, has the faecal-oral route as the usual mode of transmission with its infective stage being the cyst form. Gut conditions which obviously varies in asymptomatic, symptomatic and irritable bowel syndrome (IBS) patients in terms of gut flora, pH, osmotic pressure and water potentials could play an important role in changing the surface of the parasite. The present study aims to elucidate if the gut environment from these three conditions can influence surface and ultrastructure of *Blastocystis*. A total of 8 *Blastocystis* isolates were obtained from four IBS patients (IBS1-4) and four symptomatic patients (S1-4) at a local gastroenterology clinic. Asymptomatic isolates (A1-4) were obtained from a field survey at a local village. All isolates were characterized as subtype 3. *Blastocystis* forms in three different groups, asymptomatic, symptomatic and IBS isolates appeared to have different surface respectively. Scanning electron microscopy showed that *Blastocystis* isolated from asymptomatic isolates possess a very smooth surface meanwhile the *Blastocystis* isolated from symptomatic isolates showed slightly rough surface with tiny pores. In IBS isolates, the surface of *Blastocystis* showed a very coarse and intensely folded surface. Therefore, our results showed that phenotypic differences at the surface level indicate that there were differences in the *Blastocystis* isolated from asymptomatic individuals, symptomatic and irritable bowel syndrome (IBS) patients.

P.9 - 70% alcohol – A more effective stool preservative for *Blastocystis hominis*

Nithyamathi K. and Suresh K.

Department of Parasitology, Faculty of Medicine, University Malaya

Preservatives used in human stool surveys protect parasites from disintegrating. However prevalence studies carried out globally tend to use different preservatives and this can influence interpretation when it comes to comparing the results. In the present study we compared the frequently used 10% formalin with polyvinyl alcohol and 70% alcohol for *Blastocystis hominis*, an intestinal organism known to cause gastrointestinal symptoms. Comparisons were made on the ability to interfere and reduce the quality of DNA, staining of specimens, storage days and storage temperature. Stools were spiked with *Blastocystis* from *in vitro* cultures and preserved respectively with the three preservatives. For each preservative faecal smear were made in triplicates and stained respectively with Fields', Giemsa and Trichome stains.The results showed that staining contrasts and clarity from stools preserved in 70% alcohol were comparable with that of smears made from 10% formalin. Storage at 4^0C was shown not to interfere with staining contrasts from both 10% formalin and 70% alcohol preservative. The study showed that storing samples in 70% alcohol and potassium dichromate up to 120 days at -20^0C did not interfere with DNA extraction. Since the staining contrasts were comparable to that preserved in 10% formalin it is suggested that stools be collected in 70% alcohol as the preservative confers the advantage of exploiting the samples for future molecular studies.

P.10 - *Entamoeba, Giardia* and *Blastocystis* infections among the Hausa-Fulanis of Northern Nigeria

Salwa Dawaki, Init Ithio, Jamaiyah Ibrahim and Hesham El-Mekhlafi

[1]Department of Parasitology, Faculty of Medicine, University Malaya, Kuala Lumpur, 50603, Jln Pantai, Kuala Lumpur, Malaysia

Intestinal protozoan infection has been incriminated to cause diarrhoea of different severity, from mild to persistent and dysentery that gives rises to enormous and grave pathological effects worldwide, especially in children. This study reports the incidence of infection with *Entameaba, Giardia* and *Blastocystis* species among the Hausa-fulanis in Northern Nigeria. The investigation was carried out in five communities (Kura, Bebeji, Gwarzo, Shanono and Minjibir) in Kano, Nigeria involving 550 volunteers. Incidence was found to be highest with protozoa species of *Blastocystis* (29.3%) followed by *Entamoeba* (17.3%) and *Giardia* (11.3%). In all the five communities, Minjibir has the maximum infection with all the protozoa (20.7%, 14.0% and 33.1%), followed by Gwarzo (38.1%, 17.5% and 10.9% respectively), Kura (26.2%, 10.3% and 11.1% respectively), Bebeji (24.4%, 14.3% and 14.3% respectively) and Shanono (22.2%, 23.2% and 9.1% respectively). The infection rate of these protozoa in the different settlement areas varies but is statistically insignificant ($p<0.05$). Socio-economic factors believed to be the main risk factors for these protozoa infection among the Hausa-fulanis of Northern Nigeria.

P.11 - *Blastocystis* exerts protective effect against 5-fluorouracil (5-Fu) in colon cancer cells, Hct116.

Vinoth Kumarasamy[a*], Umah Rani Kuppusamy[b*], Suresh Kumar Govind[a§]

[a]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b]Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Blastocystis is a unicellular organism found commonly in the intestinal tract of humans with an uncertain role in human disease. Blastocystis is considered opportunistic evidenced by its high prevalence in immunosuppressed individuals including CRC patients undergoing chemotherapy. However, the effect of Blastocystis towards the cancer cell inhibition in vitro in the presence of chemotherapy drugs has not been investigated. In this study, we investigated the possible synergistic effect between Blastocystis antigen and 5-Fluorouracil (5-FU) in HCT116 human colorectal cancer cell line and CCD 18-co, normal human colon fibroblast cells. 5-FU is a chemotherapeutic agent, commonly used to treat colorectal carcinoma by causing interference in the growth of cancer cells. Proliferation of HCT116 was inhibited in the presence of 5-FU at increasing concentration. MTT results showed that 5-FU alone had no effect on the inhibition (Abs: 0.169). However the inhibitory effect of 5-FU at 8µM was shown to be reduced in the presence of Blastocystis antigen (Abs: 0.309, $p<0.001$). Gene expression studies revealed the up-regulation of transforming growth factor (TGF-β) gene (5.22 fold change, $p<0.001$), nuclear factor E2-related factor 2 (Nrf2) gene (4.13 fold change, $p<0.001$), and cyclooxygenase-2 (COX-2) (3.33 fold change, $p<0.01$). This data indicates the presence of molecular pathogenesis and progression of cancer in HCT116 when co-incubated with Blastocystis antigen. Therefore, it is important to screen CRC patients who undergo chemotherapy as Blastocystis has the ability to interfere with activity of chemotherapeutic drugs.

P.12 - Seroepidemiology and molecular of visceral leishmaniasis in blood donors in Jahrom

Mohammad Hassan Davami

Department of Parasitology, School of Medicine,
Jahrom University of Medical Sciences, 74148-46199,Jahrom,Iran

Visceral leishmaniasis (kala-azar) is a zoonotic disease which is endemic in many parts of the world and the Middle East. The causing agent of this disease is an intracellular protozoon, *Leishmania spp*. The parasitic agent in Iran is *Leishmania infantum* that is endemic in some parts of the country especially in Fars province and Jahrom .The disease is mainly transmitted by infected sandflies. Because the parasite may also be transmitted through blood transfusion and due to lack of data in Jahrom, this project is conducted to find seropositivity and molecular study of visceral leishmaniasis in blood donors in Jahrom County in 2010. Blood samples were collected from 396 blood donors in Jahrom Blood Transfusion Centre. Sera were used to detect anti-*Leishmania* antibodies with DAT test. PCR was used to detect the parasite DNA in Buffy-coat of those with positive DAT. Data were analyzed by SPSS software. Two of 396 samples (0.5%) were positive by DAT test. According to data significant relation was found between the prevalence of VL and educational status and also blood Rh. No parasite DNA was detected from samples. In conclusion, existence of anti-*Leishmania* antibodies in the sera of 0.5 % of blood donors in Jahrom can be an important issue for blood transfusion center managers at the time of blood transfusion to pay more attention to unknown clinical signs of the disease.

P.13 - Alkyl galactofuranosides: a new class of anti-leishmanial drugs?

Muhammad Suleman [1], Jean-Pierre Gangneux [1,2], Laurent Legentil [3], Yari Cabezas[3], Rémy Dureau[3], Odile Sergent [1], Agnès Burel [4], Franck Daligault [5], Vincent Ferrières [3,], Florence Robert-Gangneux [1,21]

UMR INSERM U1085 – IRSET (Institut de Recherche en Santé Environnement et Travail), Université Rennes 1, 2 avenue Prof. Léon Bernard, 35033 Rennes, France, [2] Centre Hospitalier Universitaire de Rennes, Service de Parasitologie-Mycologie, 2 rue Henri Le Guilloux, 35043 Rennes, France, [3] Ecole Nationale Supérieure de Chimie, CNRS UMR 6226, avenue du Général Leclerc CS 50837, 35708 Rennes cedex 7, France, [4] mRIC (microscopy-Rennes Imaging Center), Université Rennes 1, 2 avenue Prof. Léon Bernard, 35033 Rennes, France, [5]Université de Nantes, FRE CNRS 3478, 2 Rue de la Houssinière, 44322 Nantes Cedex 3, France.

Visceral leishmaniasis (VL) is a neglected tropical disease, which has been set up as a priority by WHO. Conventional drugs used for the treatment of VL (antimonials, amphotericin B) require prolonged administration, have toxicity risks and are currently facing challenges of resistance in certain endemic regions, whereas new efficient therapeutic agents (liposomal amphotericin B and miltefosine), have a limited spread of use due to adverse events, cost, intravenous administration, or teratogenicity, respectively. The surface of the *Leishmania* parasite presents attractive targets for generating the future anti-leishmanial drugs. Particularly the lipophosphoglycan (LPG) covering the parasite surface and its components like galactofuranose (Gal*f*) and Gal*f* containing glycans are known to play a vital role for its survival and virulence. We evaluated new chemically synthesized octyl-β-D-galactofuranose (Gal*f*)-derivatives for their anti-leishmanial activity and explored their possible mechanism of action on *L. donovani* promastigotes. The effects of four Gal*f*-derivatives, i.e. n-octyl-□-D-galactofuranoside (**1**), 6-amino-galactofuranoside (**2**), 6-*N*-acetamido-□-D-galactofuranoside (**3**), and 6-azido-galactofuranoside (**4**) were evaluated on *L. donovani* promastigotes *in vitro*. Their interaction with parasite membrane was explored by using electron paramagnetic resonance spectroscopy (EPR) and saturation transfer difference nuclear magnetic resonance (STD-NMR), and ultrastructural alterations were investigated by transmission electron microscopy (TEM). Our results showed variable anti-leishmanial effects, with **1**>**2**>**3**>**4**. Compound **1** showed the most promising effects by inhibiting promastigote growth with an IC_{50} of 8.96 ± 2.5 µg/mL, and treated promastigotes showed a significant dose-dependent reduction of human macrophage infection *in vitro*. None of these derivatives were toxic towards human macrophages. EPR showed that compound **1** significantly reduced membrane fluidity, compared to control promastigotes and to compound **3**. The furanose ring was shown to support this effect since the isomer galactopyranose had no effect on parasite membrane fluidity or growth. STD-NMR showed a direct interaction of all compounds (**1**>**2**>**3**>**4**) with the promastigote membrane, as well as with octyl-galactopyranose and octanol, providing evidence that the *n*-octyl chain was primarily involved in the anchoring with the parasite membrane, followed by the putative crucial role of furanose ring in the anti-leishmanial activity. A morphologic analysis of compound **1**-treated promastigotes by TEM revealed profound alterations of parasite membrane and organelles, but not with compound **3**. Finally, compounds **1** significantly reduced amastigotes growth in macrophage cultures previously infected with *L. donovani*. In conclusion, our results point to an interesting anti-leishmanial activity and a high selectivity index of the n-octyl-galactofuranoside on *L. donovani* promastigotes, associated with strong surface interaction and membrane damage, thus making a potential head of a new class of anti-leishmanial drugs. Further experimentation shall be done to synthesize and screen other octyl-galactofuranoside derivatives, and explore their therapeutic potential *in-vivo*.

P.14 - Identification and characterization of an RNA binding protein, *Trypanosoma congolense* uridine binding protein-1

Keisuke Suganuma and Noboru Inoue

National Research Center for Protozoan Diseases,
Obihiro University of Agriculture and Veterinary Medicine,
Obihiro, Hokkaido 080-8555, Japan.

Our previous study revealed that gene expression of *congolense* epimastigote specific protein (*cesp*) was regulated by *cis*-elements located in the 3'UTR. In this study we examined specific interaction between the *cesp* 3'UTR and a RNA binding protein (RBP). *Trypanosoma congolense* uridine binding protein 1 (TcUBP1) was characterized *in silico* analysis. From the sequence analysis, the RNA recognition motifs (RRM) and two ribonucleo protein (RNP) domains were well conserved in TcUBP1 as other UBP1 orthologues. Three anti-TcUBP1 monoclonal antibody (mAb) clones were established and were purified. Among those mAbs, mAb #2 showed the highest affinity to TcUBP1. Northern blot analysis, Western blot analysis and immunofluorescent microscopy clearly showed that TcUBP1 was expressed as scattered cytosolic localization in each stage of trypanosome. RNA electro mobility shift assay (REMSA) clearly showed that specific interaction between rTcUBP1 and ARE-M-RNA, a required regulatory *cis*-element found in *cesp* 3'UTR. To analyze the protein-protein interaction of TcUBP1 and unknown RBPs, co-immunopresipitation assay was performed. The results suggested that some common and developmental stage-specific proteins interacted with TcUBP1. In conclusion, the stage-specific RBPs, which consisted of TcUBP1 and other unknown RBPs, might regulate EMF stage-specific *cesp* mRNA stabilization and destabilization via interaction with ARE-M-RNA.

P.15 - Origin of H7N9 virus transmission in China equation method using amino acid sequence motifs

M Yusuf Alamudi[1], Kholik[1] Setyarina Indrasari[1], Elsa Bahar Putri[1], Rahmalia[1],Emma Qurnianingsih[1,3], Kadek Rachmawati[1,2] Revianny V.Nidom[1,],C.A.Nidom[1,,2]

[1]Avian Influenza Research Center-Airlangga University
[2]Medical Veterinary Faculty-Airlangga University
[3]Medical Faculty-Airlangga University

The main objective of this study was to analyze or trace the origin of the H7N9 virus in China by using the amino acid sequence motifs equation. Forty seven amino acid sequence of the gene haemagglutinin H7N9 viruses circulating in the world from the year 1988 to 2013 was downloaded from GenBank and analyzed using the Mega software version 5.2.1. Based on the results of the analysis using the amino acid sequence motifs equation Mega Version 5.2.1 of the H7N9 virus in China, the origin H7N9 viruses can come from the environment, birds (ducks and chickens) which are derived from the Chinese territory. H7N9 viruses that infect humans come from the environment or from poultry (ducks and chickens) in the territory of China.

P.16 - Multi-drug Resistant *Pseudomonas putida* in Diabetic Foot Gangrene Patient in Karawaci District, Indonesia

Nata Pratama Hardjo Lugito, Cucunawangsih,
Andree Kurniawan, Theo Audi Yanto

Faculty of Medicine, Pelita Harapan University

Pseudomonas putida is a rod-shaped, non fermenting Gram-negative organism frequently found in the environment that utilizes aerobic metabolism, previously thought to be of low pathogenicity. It had been reported as cause of skin and soft tissue infection, especially in immunocompromised patients. A female green grocer, 51 year-old came to internal medicine out-patient clinic with gangrene and osteomyelitis on her 1st, 2nd and 3rd digit and wound on the sole of the right foot since 1 month prior. The patient had history of uncontrolled diabetes since a year ago. She was given ceftriaxone 2 grams BD, metronidazole 500 mg TD and amikacin 250 mg BD, and then undergone amputation of the digits and wound debridement of the foot. The microorganism's culture from pus revealed multi drug resistant *Pseudomonas putida.* She recovered well after antibiotics and surgery.

P.17 - Self reported haematuria and dip stick as diagnostic evidence of urinary schistosomiasis in poor resource setting

Umar S. Malina O. Syafinaz A.*

Department of Microbiology and Parasitology,
Faculty of Medicine and Health Sciences
University Putra Malaysia, 43400 Selangor, Malaysia

Schistosomiasis is water borne disease afflicting over 250 million people worldwide. Study in north western Nigeria reveals linear relationship between simple questionnaire and urine reagent strip (dip stick) testing for rapid diagnosis of *Schistosoma haematobium*. The objective of the study is to determine a simple criterion for diagnosis of *Schistosoma haematobium* in a disease poor resource endemic area. Participants provided 10 to 50 ml of urine sample and were examine under a microscope using concentration technique. Microscope objective lens 10x and 40x were use to confirm the presence of egg in urine. The remaining portion of urine was used for reagent strip testing. Simple questionnaire was administered to all participants for self report of blood in urine coupled with play and bathing in river. The Results of Microscopy of the urine samples obtained from 325 children both male and female aged 7 to 14 years revealed *S. haematobium* prevalence of 32.9%. The presence of any blood on a urine reagent strip was 62.1% sensitive, and 89.6% specific for *S. haematobium* diagnosis. Questionnaires were completed by 325 school children. Self-reported haematuria showed a sensitivity of 64.2% and a specificity of 94.3%. A dichotomous two-question panel was helpful in *S. haematobium* diagnosis, with bathing and playing in the river significantly associated with *S. haematobium* infection ($P < 0.001$).In Conclusion The use of dip stick (urine reagent strips) testing, together with questions regarding to passing blood in urine river water contact activities, could be a suggestive criteria considerable as point-of-contact diagnosis of *S. haematobium* in endemic zone and where microscopy facilities are not available.

P.18 - Gentamicin sensitive *Burkholderia pseudomallei*: Clinical features of culture positive cases of melioidosis in Bintulu.

Hon Siong Leng[1], Wong Jin Shyan[1,2], Niny Hwong Perng Leng[1], Chien Su Lin[3], Tan Lee See[3,4], Tee Teong Jin[1], Yuwana Podin[5, 6], Bart J Currie[6].

[1] Department of Medicine, Bintulu Hospital, Sarawak, Malaysia
[2] Department of Internal Medicine, Borneo Medical Centre, Kuching, Sarawak, Malaysia
[3] Department of Pathology, Bintulu Hospital, Sarawak, Malaysia
[4] Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia, Kampus Cawangan Bintulu, Bintulu, Sarawak, Malaysia
[5] Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Sarawak, Malaysia
[6] Global and Tropical Health Division, Menzies School of Health Research, Darwin, Northern Territory, Australia

Melioidosis is an important emerging infectious disease in Malaysia. Intrinsic resistance to gentamicin is considered an important component of laboratory identification of *Burkholderia pseudomallei*. Recently a novel strain of *B. pseudomallei* which is susceptible to gentamicin has been found to be common in Malaysian Borneo. A mutation within the amrB gene is responsible for the gentamicin susceptibility in these Malaysian strains. We reviewed 84 cases of culture confirmed melioidosis who were admitted to the Bintulu Hospital over a 6 year period. *Burkholderia pseudomallei* isolates from 52 cases were tested for susceptibility to gentamicin and 75% (39 cases) were found to be gentamicin susceptible. Sixty seven percent of patients were male, median age of 43 years (range 2-73; IQR 28,51). Twenty eight percent had diabetes mellitus. Median duration of fever was 7 days (IQR 5,14) and 29 (74%) presented with pneumonia of whom 15 needed ICU care. Abdominal ultrasound examination was performed for 21 cases and 60% had intra-abdominal abscesses. The overall mortality rate for the 52 cases was 36%, and the ICU mortality was 67%. The mortality rate in gentamicin sensitive and gentamicin resistant groups of patients was 41% and 23% respectively (not statistically significant). Further studies are required to understand the epidemiological and clinical implications of this novel strain of *B. pseudomallei*.

P.19 - Proteasome polyclonal antibodies inhibit the growth and affect the morphology of *Plasmodium falciparum in vitro* through polyubiquitinprotein accumulation.

[1*]*Alfian Wika Cahyono,* [1]*Wiji Astuti,* [1]*Dyah Ayu Laksmi,*
[1]*Stefan Bram Kanigia,* [1]*Lazuardiah Anandi and* [2]*Loeki Enggar Fitri*

[1]*Medical Study Program, Faculty of Medicine,*
University of Brawijaya, 65145 Malang, Indonesia
[2]*Department of Parasitology, Faculty of Medicine,*
University of Brawijaya, 65145 Malang, Indonesia

Ubiquitin Proteasome System (UPS) is a system that control protein quality in *Plasmodium falciparum* life cycle. It is responsible for the *Plasmodium*'s cell cycle regulation by conjugating all stage of the protein to form the poly ubiquitin-protein complex which finally will be degraded by the 20s proteasome. This study was an experimental study to explore the capability of proteasome polyclonal antibody produced from rabbit in inhibiting the growth of *P. falciparum* in vitro. *Plasmodium falciparum* cultures were divided into 1 positive control group (*P. falciparum* culture without any exposure), 3 treatment groups (*P.falciparum* culture exposed by proteasome polyclonal antibodies with dose of 100µg/mL, 200µg/mL, and 300µg/mL respectively) and 1 placebo group (*P. alciparum* culture exposed by 200µg/mL Tris-HCL and adjuvant). The degrees of parasitemia and the morphologies of the parasite were observed from the first time of exposure until the last exposure every 12 hours of 48 hours observation. The degrees of parasitemia observed every 12 hours using *ANOVA* analysis showed significant differences (p=0,000) among groups. The morphologies of *Plasmodium* were changed. There were many *crisis form* after the antibodies exposure, especially on the group of 300µg/mL. The *poly-ubiquitin* protein accumulation was measured using Western blot method. Western blot result showed higher density of poly ubiquitin-protein complex in exposure groups than control group. It can be concluded that proteasome polyclonal antibody is potential for a new vaccine candidate and malarial medication by inhibiting the UPS and cause the *Plasmodium's* death.

P.20 - Colonization of *Anopheles cracens*: A malaria vector of emerging importance

Amirah Amir, Jia Siang Sum, Yee Ling Lau,
Indra Vythilingam and Mun Yik Fong

Department of Parasitology, Faculty of Medicine,
University Malaya, 50603 Kuala Lumpur, Malaysia.

Anopheles cracens has been incriminated as a vector for the simian malaria parasite, *Plasmodium knowlesi,* that is the fifth *Plasmodium* species infecting humans. Little experimental data exists on this mosquito species due to the lack of its availability in laboratories. The population of *An. cracens*, collected from Kuala Lipis, Pahang was maintained at the insectary of the Department of Parasitology, Faculty of Medicine, University Malaya at 24-26°C and 60-80% relative humidity. The mosquitoes were maintained with artificial mating and blood-fed on humans and hamsters. The colony has been established since November 2011 and to date has reached its sixth generation. This is the first description of maintaining the Malaysian strain *An. cracens* colony by artificial mating. Colonization of *An. cracens* will provide fundamental information for genetic studies and will be useful in assessing comparative susceptibility to *Plasmodium* parasites.

P.21 - *In vitro* drug susceptibility of *Plasmodium falciparum* using a sybr green i assay

Kesinee Chotivanich[1,2], Sutida Roungboon[1], Juntima Sritabal[1],
Nattiya Chanthawongsa[1], Prakaykaew Charunwatthana[1,2],
Podjanee Jittmala[1,2], Nicholas P J Day[1,3], Arjen Dondorp[1,3],
Sasithon Pukrittayakamee[1,2], Nicholas J White[1,3]

[1]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [2]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, [3]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford, UK.

Antimalarial drugs resistance of human malaria parasites are being monitored by an **in** *vivo* therapeutic response and ex vivo susceptibility assay. Assessment of *in vitro* antimalarial drugs susceptibility using SYBR Green I is one of the rapid, simple, reproducible and reliable assay. Samples could be stored in the drug plate at minus 30° C up to 2 weeks before performing the assay. This becomes feasible to set up the in vitro susceptibility assay in the field sites particularly in the limited resources area. Minimal parasitaemia for reliable assessment (expressed as 50% inhibition concentration; IC50) is as low as 0.07%. Using this method, mean (SD) IC50 for artesunate after 72 hours incubation in 19 *P. falciparum* isolates from Srisaket on the Thai-Cambodian border was 1.5 (1.2) ng/mL. No significant difference in IC50 when compared with IC50 derived from a conventional schizont maturation inhibition assay (mean (SD) IC50 for artesunate 1.2 (0.4) ng/mL, P= 0.08). However, there was no correlation between in vitro IC50 and in vivo parasite clearance half- life. Evaluation of this method for susceptibility testing in other human malaria species will be further investigated.

P.22 - Which factors increase the risk for acquiring *Plasmodium knowlesi* malaria in Sabah, Malaysia: A case control study protocol

MJ Grigg[1,2], T William[2,3], C Drakeley[4], L von Seidlein[1], J Jelip[3], BE Barber[1], K Fornace[4] NM Anstey[1], TW Yeo[1], J Cox[4].

[1]Menzies School of Health Research, Darwin, Northern Territory, Australia
[2]Infectious Diseases Unit, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia
[3]Sabah Department of Health, Kota Kinabalu, Sabah, Malaysia
[4]London School of Hygiene and Tropical Medicine, London, UK

Human malaria due to zoonotic *P. knowlesi* has been increasingly reported throughout South-East Asia. In Sabah, Malaysia it is now the most common cause of malaria in both adults and children, and has the highest risk of severe and fatal disease of all *Plasmodium* species. Environmental and population changes may have led to increased human interaction with evolving mosquito vectors and primate hosts of *P. knowlesi*, with changes in transmission dynamics of human infection. Risk factors for acquiring *P. knowlesi* infection have not been explored previously and would assist in guiding future public health interventions. This will be a population based case-control study conducted over a 2-year period at adjacent district sites in north-west Sabah, Malaysia. Confirmed malaria cases presenting to the hospital sites will be enrolled if meeting relevant criteria. Three healthy community controls matched for the same village as the case will be randomly selected. Study procedures will include a questionnaire, household survey, blood sampling and GPS mapping to evaluate potential exposure risks between cases and controls. Analysis will be per protocol, with adjusted odds ratios for exposure variables between cases and controls calculated using conditional logistic regression models.

P.23 - Prevalence and identification of *Plasmodium knowlesi* in humans, macaques and mosquitoes in Sabah, Malaysia

[1]Mohammad Faruq Abd Rachman Isnadi, [1*]Rajasegar Anamalley,
[1]Rosida Abdullah,[1]Salleh Ismail, [2]Symphorosa Sipangkui.

[1]Faculty Of Health And Life Sciences,
Management And Science University, 40100 Shah Alam, Malaysia
[2]Lok-Kawi Wildlife Park, Sabah Wildlife Department, 88100 Kota Kinabalu, Malaysia

Previous studies managed to demonstrate large number of malaria cases caused by *Plasmodium knowlesi*, the simian malaria parasite in Malaysian Borneo. In response to unravel the nature of the parasite, several data and samples were collected from Sabah. Prevalence data have demonstrated the significant increase of *Plasmodium knowlesi* cases in rural area, contributed 53.8% from total malaria cases reported in 2010, 91.95% in 2011, and 92.16% in 2012 compared in urban area, denoted only 4.90% in 2012 ($P<0.05$). Meanwhile, based on the clinical features (n=10), the mean (□) age is 37.8 years old, followed by systolic blood pressure (124.5 mmHg), diastolic blood pressure (70.7 mmHg), pain score (2.7/10), and pulse rate (56.4 BPM). *Plasmodium knowlesi* was detected in all human blood samples (n=10; 100%) followed by 5 macaque blood samples (n=10; 50%) and 1 *Anopheles species* salivary gland (n=5; 20%) via conventional Polymerase Chain Reaction (PCR) using circumsporozoite protein (csp) gene. Single Pass DNA Sequencing results correlated with *Plasmodium knowlesi* isolates KH195h1 strain. It is concluded that there is an increasing prevalence of *Plasmodium knowlesi* especially in rural area of Sabah and cause moderately severe malaria in hospitalized patients. PCR results for macaque blood samples have proven the role of macaques as intermediate host and possibly to be widely distributed in urban area as well. Thus, it is crucial for the related organizations or boards on reviewing and conducting necessary steps to combat hence provide further awareness regarding this scenario.

P.24 - Effectiveness of gamma ray in inhibiting *Plasmodium falciparum* multiplication determined with the *in vitro* incorporation of tritium labeled hypoxanthine

Mukh Syaifudin, Darlina, Siti Nurhayati, Harry Nugroho Eko S. and Devita Tetriana

Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency of Indonesia, Jl. Lebakbulus Raya No. 49 Jakarta, INDONESIA 12440

Malaria remains a major public health threat in Indonesia. Therefore an attempt to create malaria vaccine for supporting the control of disease was taken by attenuating parasites with gamma rays and was proven effective based on microscopic observation. To obtain a more comprehensive insight, an isotopic based method was developed. A laboratory strain of *P. falciparum* (3D7) was *in vitro* cultured with standard procedure and was irradiated with gamma rays at doses of 150-250 Gy. Unirradiated (0 Gy) parasites served as control. Twenty four hours after 1-2 µCi of ^{3}H-hypoxanthine was added into culture, 100 µl of medium was taken at various times, and then hypoxanthine incorporation was measured. Microscopic observation of parasitemia in culture was also done. Results showed that there was a fluctuation in multiplication of parasites post irradiation mainly at higher dose. Irradiated parasites were more active in incorporate purine precursor up to 48 hours. Parasites returned to their highest activity at 116 hours after hypoxanthine addition. No significant difference was found among doses of irradiation ($p=0.05$). This was quite different with the finding from microscopic observation where 175 Gy was the most effective dose in inhibiting the parasite multiplication. Factors affecting these facts are discussed.

P.25 -Malaria elimination in Sarawak: Decreasing *incidence of Plasmodium falciparum* and *Plasmodium vivax*, 2010-2012

Ooi Choo Huck[1], Mitchell Edward Jahai[1], Mohd Faizal Ali[1], Jitos Gubes[1], Jurizam Saili[1], Jamilah Hashim[2], Andrew Kiyu[3]

[1]Vector Borne Diseases Section, Sarawak Health Department, 93050 Kuching, Sarawak, Malaysia.
[2]Director's Office, Sarawak Health Department, 93050 Kuching, Sarawak, Malaysia.
[3]Public Health Research Section, Sarawak Health Department, 93050 Kuching, Sarawak, Malaysia.

Sarawak embarked on malaria elimination activities in 2011 and has set 2020 as its deadline for elimination of malaria transmission in Sarawak. The objective of this study was to investigate the progress of malaria elimination in Sarawak. A retrospective descriptive analysis was conducted on malaria epidemiological surveillance data in Sarawak State Health Department between January 2010 and December 2012. There were reduction of locally transmitted *Plasmodium falciparum* and *Plasmodium vivax* cases in Sarawak by 82.6% and 85.5% respectively from 2010 to 2012. The number of foci for *P. falciparum* and *P. vivax* cases reduced by 87.0% and 67.2% respectively from 2010 to 2012. The number of *P. falciparum* and *P. vivax* outbreaks reduced by 50.0% and 68.6% respectively from 2010 to 2012. There were also reduction of outbreak foci for *P. falciparum* and *P. vivax* by 50.0% and 72.0% respectively from 2010 to 2012. The total number of *P. falciparum* and *P. vivax* cases involved in outbreaks reduced by 20.0% and 91.4% respectively from 2010 to 2012. There were increase in the number of districts without *P. falciparum* and *P. vivax* cases in Sarawak by 38.9% and 55.5% respectively from 2010 to 2012. There were 13 districts without *P. falciparum* cases and 4 districts without *P. vivax* cases for three years consecutively (2010, 2001 & 2012). In conclusion, rapid reduction of locally transmitted *P. falciparum* and *P. vivax* malaria cases and outbreaks were observed in Sarawak from 2010 to 2012.

P.26 - *Plasmodium knowlesi* malaria in Malaysia

Ruhani Yusof[1], Yee Ling Lau[1], Rohela Mahmud[1], Mung Yik Fong[1], Jenarun Jelip[2], Ung Ngian Hie[3], Sahlawati Mustakim[4], Hani Mat Hussin[5], Noradilah Marzuki[6], and Marlindawati Mohd Ali[7] and Bee Bee Lim[8]

[1]Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Sabah State Health Department,Tingkat 1, 3 & 6, Rumah Persekutuan,Jalan Mat Salleh, 88590 Kota Kinabalu, Sabah.
[3]Hospital Kapit Jalan Mamora, 96800 Kapit, Sarawak.
[4]Department of Pathology, Hospital Tengku Ampuan Rahimah Jalan Langat 41200 Klang, Selangor.
[5]Kelantan State Health Department, Aras 5, Wisma Persekutuan, Jalan Bayam,15590 Kota Bharu Kelantan.
[6]Department of Pathology, Hospital Kuala Lipis, 27200 Kuala Lipis, Pahang.
[7]Department of Pathology, Hospital Tuanku Ja'afar Jalan Rasah70300 SerembanNegeri Sembilan.
[8]Department of Pathology, Pakar Sultanah FatimahJln Salleh, 84000 MuarJohor Darul Takzim.

Plasmodium knowlesi, a simian malaria parasite had been recognized as the fifth human malaria parasite due to its wide distribution of naturally acquired infection among the human populations in Southeast Asia. The aim of this epidemiological study was to determine the incidence and actual distribution of malaria parasites with special attention to *P. knowlesi* in Malaysia. Nested PCR assay was used to identify *Plasmodium* species in the DNA extracted from 455 microscopically malaria positive blood samples collected between September 2012 to December 2013 from 20 state and main district hospitals all over Malaysia. A total of 431 (94.7%) samples were positive for *Plasmodium sp* based on nested-PCR detection. Of all *Plasmodium* species identified, *P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi* contributed 11.6%, 32.0%, 0.2%, 0.45%, 52.9% respectively. Twelve mixed-infections were detected (1 *P. knowlesi*/*P. falciparum,* 10 *P. knowlesi*/*P. vivax* and 1 *P. falciparum*/*P. vivax*). *P. knowlesi* showed the highest prevalence among all malaria in Sarawak (72 cases, prevalence 63.2%), Sabah (71, 67.0%), Kelantan (35, 68.6%), Pahang (23, 95.8%), Terengganu (4, 80%) and Johor (5, 62.5%). *P. knowlesi* infection in Selangor and Negeri Sembilan was found to be 11.8% (n=14) and 40% (n=4) respectively. *P. knowlesi* was not found in Kuala Lumpur, Melaka, Perak, Kedah, Pulau Pinang and Perlis within the study period. This survey highlights the widespread distribution of *P. knowlesi* in Kelantan, Pahang, Selangor and Negeri Sembilan (Penisular Malaysia) on top of Sabah and Sarawak, which are well known for *P. knowlesi* infection.

P.27 - Laboratory diagnosis of malaria: Comparison of manual and automated diagnostic tests

Samina Naz Mukry, Madiha Saud, Gul Sufaida,
Kashif Shaikh, Arshi Naz and Tahir Sultan Shamsi

National Institute of Blood Diseases & Bone Marrow Transplantation (NIBD),
Karachi, Pakistan.

Malaria is the second most prevalent and devastating disease in Pakistan resulting in 30,000 annual deaths. Precise and timely diagnosis of malaria is imperative to overcome the associated risks. Comparison of various manual and automated methods of malaria diagnosis to propose better options. A descriptive cross-sectional study was conducted during the period of October (2012) to August (2013) at National Institute of Blood Diseases (NIBD), Karachi. 126 blood samples of malaria suspected patients and 50 blood samples from healthy individuals without clinical symptoms of malaria or any other infection were collected. Malarial parasite (MP) was screened by specie specific PCR, microscopy of blood smears, hematological analysis by Sysmex-XE2100 and rapid test devices (First response malaria and ICT malaria combo). Out of 176 samples, light microscopy detected MP in 125 samples (parasite load/µl [PL] 544-53712/µl); 70.19% were infected with *Plasmodium vivax*, 15.3% with *P. falciparum* while 14.5% had mixed *P. vivax* and *P. falciparum* infection. The mean PL for *P. vivax* and *P. falciparum* was 14496/µl and 24410/µl, respectively. The abnormal scatter grams of Diff, WBC/ Baso, IMI-channel and RET-EXT on Sysmex-XE2100 supported 99.2% MP detection whereas only 93% of confirmed malaria cases were detected by both rapid tests. Among the two tested devices; first response malaria was more specific (specificity: 88.33%) than ICT malaria combo (specificity: 86.21%). None of the control sample exhibited any abnormal signal on those scatter grams of Sysmex-XE2100. Sysmex XE-2100 analyzer can pick up the presence of MP with high sensitivity. Observer's training is required for identification of abnormal signals in scatter gram. Microscopy of thick and thin film remains the gold standard. Rapid diagnostic tests have acceptable sensitivity and specificity.

P.28 - Molecular identification and phylogeny and of *Anopheles* mosquitoes in peninsular Malaysia

Sum Jia Siang, Lee Wenn Chyau, Amir Amirah, Braima Kamil Ali, Lau Yee Ling, Fong Mun Yik and Abdul Aziz Noraishah Mydin

Department of Parasitology, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Different *Anopheles* species exhibit different susceptibility to the malaria parasite. To accurately identify these *Anopheles* species requires examination of adults together with its larval and pupal stages. It is common to misidentify *Anopheles* especially in closely related species complexes which shares morphological similarities. Injured or damaged specimen poses further complication to the identification process. This study utilized polymerase chain reaction to detect fixed differences between species in DNA sequence. The primers were designed to amplify fragments of internal transcribed spacer 2 of different *Anopheles* species. Various locations within Peninsular Malaysia were selected for the study. Mosquitoes were collected using human landing collection and human net bait methods. Six different species of *Anopheles* mosquitoes were successfully collected and identified. Phylogeny tree was constructed and shown the amplified DNA was able to distinctly differentiate each of the different species.

P.29 - Imported cerebral malaria among non-immune travelers

Vladimir Krcmery[1], Beata Saniova[2], Dusan Krkoska[3], Andrea Kalavska[1], Andrea Shahum[1], Martin Duris[1], Jaroslava Brnova[1,2], Nada Kulkova[1,2], Ivica Gulasova[1], Marta Taziarova[1]

[1]Slovak Topical Institute, St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia
[2]Department of Anaesthesiology and Intensive Medicine, Comenius University and University Hospital, Martin, Slovakia
[3]Institute of Public Health, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia
[4]Department of Laboratory Medicine, School of Health Science and Social Work, Trnava University, Trnava, Slovakia

Severe malaria represents less than 10% of all malaria cases, however it is associated with high mortality of 10 - 30%, which is highest in small children and non-immune patients, e.g. travellers to hyperendemic areas. The aim of this case series was to reported severe malaria in travellers within last 10 years in Slovakia. All cases of severe malaria in travellers reported within last 10 years from inpatient department in Slovakia to Slovak Tropical Institute (STI) are reported. Only those travelling as tourist to Sub-Saharan Africa were included. During the last 10 years, twelve (n=12) cases of cerebral malaria were reported. Concerning clinical picture, seven patients had deep coma (58,3%), 4 (33,3%) required ventilator support, 4 (33,3%) required dialysis, 5 (41,7%) had liver failure and 6 (50,0%) had severe acidosis. Three (25%) patients died and others (n=9) survived without sequelae and significant toxicity within 8 - 22 days of therapy. The one patient from non-survival cases (8,3%) was treated with quinine alone, the rest of the patients were treated with artemeter, artesunate (i.m. or i.v.), with artemeter/lumefantrin or quinine with clindamycin. Severe malarial cases were rare imported diseases in Slovakia within last 10 years. In survivors were mostly leaves sequelae, e.g. deafness, epilepsy, blindness, paresis, psychomotoric sequelae.

P.30 - Evidence to support natural hybridization between *Anopheles sinensis* and *An. kleini* (Diptera: Culicidae): Possibly a significant mechanism for gene introgression in sympatric populations

Wej Choochote[1], Gi-Sik Min[2], Pewpan M. Intapan[3,4] ,*
Chairat Tantrawatpan[3,5]Atiporn Saeung[1], Viraphong Lulitanond[3,6]

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
[2]Department of Biological Sciences, Inha University, Incheon 402-751, South Korea
[3]Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University, Khon Kaen 40002, Thailand
[4]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[5]Division of Cell Biology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Rangsit Campus, Pathum Thani 12121, Thailand
[6]Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand

The discovery of natural hybrids between *Anopheles sinensis* and *An. kleini* in Paju City, Republic of Korea, has led to systematic investigations of various aspects in order to clarify this event. Intensive hybridization experiments used iso-female line colonies of these anophelines together with DNA analysis of ribosomal DNA [second internal transcribed spacer (ITS2)] and mitochondrial DNA [cytochrome *c* oxidase subunit I (COI)] of the parental colonies, F_1-hybrids and repeated backcross progenies by using PCR-based assay and pyrosequencing technology. The results revealed that introgression of the COI gene between *An. sinensis* and *An. kleini* was involved in this phenomenon. The pure *An. sinensis* obtained from hybrids of repeated backcross progenies in both directions, i.e., $F_{2\text{-}11}$ progeny [(*An. sinensis* x *An. kleini*) x *An. sinensis*] and $F_{3\text{-}5}$ progeny [(*An. kleini* x *An. sinensis*) x *An. kleini*] were good supportive evidence. The results elucidated on a promising way to replace the population of a high potential vector (*An. kleini*) by that of a low potential vector (*An. sinensis*) through the mechanism of gene introgression.

P.31 - High parasitemia during *Plasmodium berghei* infection in pregnant mice induces high level of Il-17 and Il-10 In placenta but not in plasma

Zainabur Rahmah[1], Teguh Wahju Sardjono[2], Loeki Enggar Fitri[2], Budi Siswanto[3], Tatit Nurseta[3], Sasmito Djati[4]

[1]Doctoral Program in Medical Science Faculty of Medicine, University of Brawijaya, Malang Indonesia
[2]Department of Parasitology Faculty of Medicine University of Brawijaya, Malang Indonesia
[3]Department of Obstetrics and Gynecology RSSA/ Faculty of Medicine University of Brawijaya Malang Indonesia
[4]Department Biology Faculty of Science University of Brawijaya Malang Indonesia

Malaria infection in pregnancy is associated with accumulation of red blood cells in the infected placental intervillous space. The condition is referred to as placental malaria (PM). During pregnancy, interleukin (IL) -17 plays an important role in the induction of inflammation, which is necessary for the implantation process in the early stage of pregnancy. As soon as the implantation process is complete, the immune status dominance will shift the balance from Th1/Th17 response to a Th2 response that will produce IL-4, IL-9 and IL-10. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that acts to block production of inflammation mediators produced by macrophages such as TNF-α, IL-6, and IL-I. This study was conducted to prove the effect of parasitemia on levels of IL-17 and IL-10 levels in plasma and placenta. Seventeen pregnant mice Balb/c strain were divided into 2 groups, those were control group (8 normal pregnant mice) and treatment group (9 pregnant mice infected by *Plasmodium berghei*). Levels of IL-17 in the placenta were measured using ELISA R & D systems (catalog # M1700) and placental levels of IL-10 were measured using Elisa kit from Abcam (catalog # ab108870). Statistical analysis using t test showed that there were differences of the levels of IL-17 and IL-10 plasma between the treated mice and the control mice ($p = 0.04$; $p = 0,00$) and also the levels of IL-17 and IL 10 in placental tissue ($p = 0.02$; $p = 0.03$ respectively). Pearson correlation analysis showed the degree of parasitemia associated with placental levels of IL-17 ($r = 0.752$;$p = 0.02$;) and placental levels of IL-10 ($r = 0.680$; $p = 0.04$) but did not lead to high plasma levels of IL-17 ($r = 0.632$; $p = 0.06$) and IL-10 plasma levels ($r = 0.331$; $p = 0.38$). It can be concluded that malaria infection causes high levels of IL-17 and IL-10 in the placenta but not in plasma.

P.32 - Urbanization of taeniasis and cysticercosis? A serosurvey in Jayapura City, Apua Province-Indonesia

Lidwina Salim ,Samuel Sandy, Antonius Oktavian, Eva Fitriana

Institute of Research and Development for Biomedicine, Papua.Jl. Kesehatan no 10 Dok II Jayapura 99112

Papua Province has high prevalence in taeniasis and cysticercosis. Several studies have found 23.5 – 56.9% of cysticercosis in the central highlands of Papua. A traditional process to cook the pork called 'barapen' (grill the meat and vegetables with hot stone) is known as the major cause of the transmission. In recent years , the increase of urbanization from central highlands of Papua to coastal area such as Jayapura city, make the infection widespread. The above feature motivated the authors to carry out a serosurvey to measure the anti-taeniasis and anti-cysticercosis in the area of Jayapura city which have high density of people from highlands area. The study was carried out from August until December 2009. It consist of interview with questioner and serosurvey . For serosurvey we used an enzyme-linked immunoelectrotransfer blot (EITB) strip containing two recombinant antigens specific and sensitive for cystisercosis (rT24H) and taeniasis(rES33) produced by CDC Atlanta, in Parasitology Laboratory of National Institute of Research and Development for Biomedicine –Papua. The study found that prevalence of cysticercosis was 0.8% and taeniasis 0.9% from 632 sample in Jayapura City . On the other side, the prevalence of cysticercosis was 6.1% and 4.2% of taeniasis from 262 sample in District Keerom (border region with Jayapura City). We suggest that taeniasis and cysticercosis were spreading from central highlands of Papua to coastal area with urbanization. A more systematic and suistainable education program is needed to raise public health awareness, such as washing the hands and cooking the meat well.

P.33 - Knowledge, attitudes and practices (KAP) on lymphatic filariasis among population of Terengganu State, Malaysia

Nazeh Al-abd[1], Zurainee M. Nor[1], Mustafa Kassim[2]*

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

Lymphatic filariasis is a major cause of permanent disability in many tropical and sub-tropical countries. Malaysia being one of the endemic countries for this disease, have conducted five rounds of mass drug administration (MDA) in the effort to eradicate the disease as part of the Global Program for Elimination of Lymphatic Filariasis (GPELF) to be achieved by 2020. This study aimed to investigate the level of awareness about the disease and MDA program conducted. A descriptive cross-sectional survey was conducted involving 230 respondents aged ≥ 15 years in Terengganu state in Peninsular Malaysia. Demographic, socioeconomic and Knowledge, Attitudes and Practice (KAP) data of the respondents were obtained by using pre-tested questionnaires and analyzed using SPSS software version 13.0. Our study demonstrated that > 80% of the respondents were aware of the disease. 77% also aware that filariasis is transmitted by mosquitoes. Two-third of the respondents preferred hospital treatment whenever they are sick. Around 12% of the respondents only indicated having experience of filarial treatment during MDA. None of the respondents seemed to know the drug used for filariasis. In relation to MDA program, about 35% of the respondents were aware of it being conducted. Our statistical findings revealed that no significant association were observed between the knowledge of lymphatic filariasis with gender, age group, educational status, occupation and socioeconomic status of the respondents.

P.34 - Isolation of *Acanthamoeba* species in surface waters of Yasuj District, south of Iran

*Abdolali Moshfe[1,] *, Mohamad ali Kohansal[1], Sajjad Khorramrooz[1], Najmeh Parhizgari[1] Mojtaba Abbasi[1]*

[1]Cellular and Molecular Research Center,
Yasuj University Of Medical Sciences,Yasuj, Iran

Acanthamoeba spp. are free-living amoebae commonly found in the artificial and environmental sources such as water, soil, and air. This ubiquitous amoeba is the causative agent of Amoebic Keratitis(AK) and Granulomatous Amoebic Encephalitis(GAE). The objective of the present study was to investigate the presence of *Acanthamoeba* spp. in Yasuj district, south of Iran in 2013. A total of 30 surface water samples were collected from environmental sources, including natural rivers, springs and waterfall. The samples were filtrated and transferred to bacto agar medium plates seeded with *Escherichia coli* and were incubated for 3 to 14 days at room temperature. The plates were examined by microscope to morphologically identify *Acanthamoeba* species. Following DNA extraction, PCR approach was used to confirm the microscopically identification. A total of 11 out of 30 samples (36.6%) were positive for *Acanthamoeba* species based on the morphological criteria. Five out of 11 positive samples (45.46%) were confirmed by PCR method. In total, 5 (16.6%) samples out of 30 samples were positive for *Acanthamoeba* species based on PCR method. High frequency of *Acanthamoeba* spp. in different surface water sources specially Promenades in that region is an alert for the public health and highlights the needs for more awareness of health professionals and for the related risks.

P.35 - *In vitro* effectiveness of chlorhexidine (Cxd) and polyhexamethylene biguanide (Phmb) against *Acanthamoeba culbertsoni* isolated from keratitis patient

[1]Anisah Nordin, [2]Ng Sook Luan, [3]Mohamed Kamel Abdul Ghani, [1]Yusof Suboh, [1]Noraina Ab Rahim and [1]Norhayati Moktar*

[1]Department of Parasitology & Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jln Raja Muda Abdul Aziz, 50300 Kuala Lumpur
[2]Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jln Raja Muda Abdul Aziz, 50300 Kuala Lumpur
[3]Biomedical Science Programme, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jln Raja Muda Abdul Aziz, 50300 Kuala Lumpur

This study was conducted to determine the effectiveness of the drugs in therapeutic dose and the minimum cysticidal concentrations (MCCs) of each drug. Serial doubling dilutions of chlorhexidine digluconate and PHMB from 200µg/ml to 0.0977µg/ml were performed in a microtiter plate and tested against an *Acanthamoeba* isolate which was isolated from a local case of *Acanthamoeba keratitis* (AK) identified as *Acanthamoeba culbertsoni*. After exposure of the cysts to the drugs for 4 hours, it was washed free of drugs by rinsing and centrifugation. Washed cysts were cultured onto nonnutrient agar plates overlaid with heat-killed *Escherichia coli*. Replication and growth of the trophozoites from cysts exposed to each dilution were observed and recorded microscopically for 14 days to determine the MCC of each drug. The effectiveness of the drugs in therapeutic dose against the cysts was tested directly without any doubling dilutions. Both CXD and PHMB exhibited cysticidal activities in therapeutic dose (0.02%) and the MCC for both drugs was determined at 50µg/ml and 6.25µg/ml respectively. The conclusion that can be made from this study is that the MCC for both drugs was significantly lower than the actual therapeutic dose recommended for treatment. As such, in vitro sensitivity test can be used to explore the efficacy of drugs and recommend suitable therapeutic dose based on obtained MCCs.

P.36 - Current status of parasitic infections among Pangkor Island community in peninsular Malaysia

Arine Fadzlun Ahmad, Romano Ngui, Muhammad Aidil Roslan, Yvonne AL Lim, Rohela Mahmud

Limited data is available on the prevalence of parasitic infections among the island communities in Malaysia with most studies performed between 1960s-1970s. Since then, no subsequent studies were undertaken to determine the recent prevalence of parasitic infections in these communities. This study was conducted to determine the current prevalence of parasitic infections among Pangkor Island community in Peninsular Malaysia. A total of 131 stool and 298 serum samples were collected and subjected to microscopic examination for intestinal protozoa and helminths and detection of *Toxoplasma gondii* antibodies using commercial ELISA kits respectively. In addition, thin and thick peripheral blood films were prepared and microscopically screened for the presence of *Plasmodium* sp. and filarial nematodes respectively. The overall prevalence of intestinal parasitic infection among the Pangkor community was 9.9 % (13/131) with *T. trichiura* (5.3 %) being the most common intestinal parasite detected. For the first time, toxoplasmosis was reported in almost 60% of the community with significantly (p=0.038) higher prevalence rate among females (112/173; 64.7 %) compared to males (66/125; 52.8 %). None of the island community was infected with intestinal sarcocystosis, malaria and filariasis. The findings in this study revealed that the prevalence of intestinal parasitic infections among the Pangkor Island community has greatly reduced compared to those reported 35 years ago. Massive improvements in the socioeconomic status, personal hygiene, water facilities and sanitation may have contributed to the low prevalence of parasitic infections among the Pangkor community. Nevertheless, further studies need to be performed to determine the possible risk factors for the high prevalence of toxoplasmosis in the island community.

P.37 - The influence of immunocompromised condition to *Strongyloides stercoralis* infection on fecal samples at parasitology laboratory in Faculty Medicine of University Indonesia

Dewi Masyithah Darlan[1,2], Agnes Kurniawan[2], Heri Wibowo[2]

[1]Parasitology Departement, Faculty Medicine of University Sumatera Utara,
[2]Parasitology Department Faculty Medicine of University Indonesia

Strongyloides stercoralis infection is due to an intestinal nematode in the tropics or subtropics. Frequently, this infection can cause chronic, asymptomatic infection, but an alteration in immune status can increase parasite number, can result in hyper infection, dissemination, and unrecognized cause of death. This study was an observational analytical, case control study. It was conducted in Parasitology laboratory Faculty Medicine of UI, from March – August 2013. All samples which were sent to parasitology Laboratory were screened. Direct fresh stool from patients were diagnosed using harada mori culture and identified by binocular microscope 2x/3x. From 170 samples, 10.6% were positive for *S. stercoralis* and among them 33.3% were immunocompromised patients.

P.38 - Intestinal parasitic infection trends among children in Baghdad – Iraq

Waqar AL- Kubaisy[1], Hassanain AL-Talib[2], Alyaa Al-khateeb[3]
Mohammad Mazin Shanshal[4]

[1]Population Health and Preventive Medicine (PHPM) Department, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Selangor, Malaysia.
[2]Laboratory Medical Science Cluster, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Malaysia.
[3]Medical Sciences Cluster, Biochemistry & Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA(UiTM), Sungai Buloh, Malaysia.
[4]AL-Nahrain Faculty of Medicine,Baghdad Iraq.

Parasitic diarrhoea among children is a significant health problem worldwide. This cross sectional study described the burden of parasitic diarrhea among children, evaluated the impact of risk factors on the parasitic diarrhoea, and determined the parasitic profile among children in Baghdad-Iraq, during the period extending from September 2003 to June 2004. A total number of 2033 cases were included in the study. The estimated prevalence rate of parasitic diarrhoea was 22%. We identified the following major diarrhoea determinants: large households size, residential location, water source, low socioeconomic status, and low parent education. *Giardia lamblia* was found to be the most prevalent parasite with an infection rate of 45.54%. followed by *Entamoeba histolytica* 23.44%, *Enterobius vermicularis* 12.7%, *Hymenolepis nana* 9.82%, *Trichuris trichiura* 5.4%, and *Ascaris lumbricoides* 2.2%. In conclusions this study demonstrates that poor sanitation, inadequate environmental conditions, and low socioeconomic status are the main determining factors that predispose to parasitic diarrhea among children. Mass deworming programs are recommended for school children, as this population is easily accessible.

P.39 - Prevalence of worm infestation in a rural community in Sarawak, East Malaysia

[1]Sonia Sharma, [1]Sukhanthan Sivakumar, [2]Pangie Bakit,
[3]Hon Siong Leng, [3,4*]Wong Jin Shyan

[1] School of Medicine, King's College London University, London, United Kingdom
[2] Klinik Kesihatan Tatau, Bintulu, Sarawakm Malaysia
[3] Department of Medicine, Bintulu Hospital, Bintulu, Sarawak, Malaysia
[4] Internal Medicine Department, Borneo Medical Centre, Kuching, Sarawak, Malaysia

Soil-transmitted helminth infections are prevalent amongst children, especially those living in the rural areas of Malaysia. They are thought to be an important cause of malnutrition in children and can contribute to a lifetime of ill health. This is a cross-sectional point prevalence study to determine the prevalence of worm infestation in children living in rural longhouses in the Tatau sub-district in Bintulu, Sarawak. A health promotion activity was carried out in 4 long houses in August 2012. Parents were given talks on hygiene and sample pot were distributed to the families. Fresh stool sample were collected and examined using direct microscopy with saline mount and iodine staining. We received 60 stool samples out of 84 sample pots distributed. The median age was 8 years old, with the range from 1 to 15 years. Fifty two percent were males. The prevalence of worm infestation was 8.3% (5 out of 60 children). The rate of worm infestation differed between the 4 longhouses, ranging from none to 23%. Two of the children with worms had received de-worming medications within the past 1 year. Worm infestation is still a problem for rural communities living in longhouses. A regular education and health promotion activity about the risk of worm infestation is needed.

P.40 - Parasite contamination of water sources at Lingkungan V Kelurahan Pangkalan Masyhur, Kecamatan Medan Johor ,Medan, North Sumatera, Indonesia, 2012

Hutagalung Sunna

University Of North Sumatera

The World Health Organization (WHO) estimates that about 1,1 billion people globally drink unsafe water, hence the vast majority of diarrheal disease in the world is attributable to unsafe water, and the lack of sanitation and hygiene. The use of water by humans may produce biological pollutants, known to contaminate water, originating from human waste products which contain bacteria, protozoa, and other parasites. The purpose of this study is to determine the contamination of water by parasites at Lingkungan V Kelurahan Pangkalan Masyhur, Kecamatan Medan Johor, Medan, North Sumatera in 2012. This was a descriptive study with cross sectional design. Samples were randomly taken from each houses at Lingkungan V Kelurahan Pangkalan Masyhur, Kecamatan Medan Johor, Medan, North Sumatera in 2012. The sources of water were the home-wells, the nearby-river in which the people collected water for daily purposes from, and tap water from Perusahaan Air Minum Negara (PAM, the government-owned company). Samples were collected and put in clean and parasite free containers, and the Caldwell technique was applied for each of them. Twenty ml of each sample was centrifuged at the 2500 rpm speed for 5 minutes. The pellets were collected, and observed under the microscope with the addition of lugol solution. Out of the 103 water samples collected, 9 samples (8.7 %) were positive for *Paramecium caudatum*, the origin of these samples were from the home-wells & nearby river. One sample (1,3 %) was found positive for Hookworm larva, and the sample was obtained from tap water. No *Entamoeba histolytica*, *Cryptosporidium parvum*, and *Giardia lamblia* found in any of the samples. The presence of *Paramecium caudatum* in the samples collected from home-wells and nearby-river indicated that those water sources were possibly contaminated by bacteria and decaying organic materials surrounding them. The presumably clean uncontaminated tap water, however, by the presence of hookworm larva indicated chance that the tap water-pipe might be leaked or broken and in contact with contaminated soil nearby. Hence it is imperative to suggest proper water treatment ,such as boiling the water before drinks and continual maintenance of tap water-pipes, to the villagers.

P.41 - Study of *Giardia lamblia* isolates by using glutamate dehydrogenas gene in Tehran and Ahwaz Cities

Rouholla Valipour Nouroozi[1] *Javid Sadraei*[1], *Mehdi Forouzandeh*[2]

[1]*Parasitology Dept., Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
[2]*Biotechnology Dept., Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

Giardia lamblia is the main intestinal protozoa transmitted by water worldwide, as a result of inappropriate water treatment or cross-contamination of water with sludge. Transmission of *Giardia* species is by the fecal-oral route, and numerous waterborne outbreaks have been documented. Among the species identified in the *Giardia* genus, only *G. lamblia* infects humans and numerous other mammals as well. This study was conducted to molecular characterize *G. lamblia* isolates using glutamate dehydrogenase gene in Tehran and Ahwaz cities. Total of 1115 human stool specimen from Tehran and Ahwaz cities were examined by using microscopy. DNA extractions of samples were performed by phenol/ chloroform method. PCR assay, targeting the GDH gene, was used to differentiate between the isolates. Amplification of the GDH gene was performed as a single PCR. Thirty seven specimens) 22 from Ahwaz and 15 from Tehran) were positive for *G. lamblia*. GDH gene was amplified successfully for positive samples. The nucleotide sequences of 458bp of GDH of each of the 10 *G. lamblia* isolates (5 from each city) were determined. According to this study, genotype of G. *lamblia* isolates could be classified in to the 2 different assemblages. *Giardia lamblia* isolates were compared based on the nucleotide sequence of the GDH, and the results showed that the (Tehran=T) T8, T11, T14, (Ahwaz=A) A8, A13 isolates belong to assemblage A, and T2, T4, A1, A6, A14 isolate belonged to assemblage B and comparing samples from the two cities by this gene do not follow a specific pattern.

P.42 - Intestinal parasitic infection among school children in urban communities of Khon Kaen, Northeast region of Thailand

Kanchana Tomanakan[1] , Nuttiya Srisurat[2] ,
Suphakdee Sanseeha[3] , Kriangkrai Kongsuk[3]

[1] Parasitology Division , Khon Kaen Hospital, 40000 Thailand.
[2] Microbiology Division , Khon Kaen Hospital 40000 Thailand
[3]Central Laboratory , Khon Kaen Hospital 40000 Thailand

Epidemiological information on prevalence of intestinal parasitic infections also differed from region to region because of several environmental, social and geographical factors. The aim of this study was to determine the prevalence of intestinal parasitic infections in school children aged 5-13 years in 5 schools of Amphoe Muang , Khon Kaen province. In this cross-sectional study , 914 stool samples were collected by random sampling after we explained and asked the teachers and students for verbal inform consent. Then the specimens were examined for the intestinal parasites by wet direct smear and KATO-KATZ method. The presence of intestinal parasites was determined microscopically by two independent medical technologists. The prevalence of intestinal helminthic infections was 11.8%. The infected parasites were *Taenia* sp.(5.25%) , *Opisthorchis viverri* (2.63%), *Strongyloides stercoralis* (2.29%) , Hook worm (1.09%) and *Ascaris lumbricoides* (0.54%). Our findings suggested that there were intestinal parasites infecting the schoolchildren. However, in the urban communities of Khon Kaen. Health promotion by means of health education should be more emphasized. The school health educational program should focus on parasitic treatment. Prevention and control measures should be implemented at the school level.

P.43 - Urinary schistosomiasis among inhabitants of Aluu, Rivers State, Nigeria

Lucy Anyaegbunam[1], Zita Obi[2], Mervellous Ayanugwo[1]

[1]Department of Biological Sciences, Anambra State University,Uli Nigeria
[2]Department of Science Education, Anambra State University, Uli Nigeria

The investigation of Urinary Schistosomiasis was carried out in Aluu community of Rivers State, Nigeria. This was because of the life style of the inhabitants who are mostly farmers and fishermen. A total of 1,500 individuals were sampled from the seven quarters of the community made up of 755(50.3%) males and 745(49.7%) females. Urine samples were collected by the consented individuals in pre -numbered, screw capped bottles, between 10.00am to 1.30pm. Ten ml of each sample was centrifuged for parasitological examination. The overall infection recorded from the investigation was 2.0%. In the community related infection, the following were recorded, Mbodo (1.3%) and Omahunwo (0.7%) while other communities had no infection. The infection was found to be sex dependent that is all the positive results recorded were males, 30 (2.0%). According to age specific infection, the results were 40 – 49 (1.7%) and 50 – 59 (0.3%) respectively. The occupational assessment of the infection stood as, fishermen (1.47%) and farmers (0.53%). Aluu community, though recorded low infection rate, requires prompt treatment of the infected few to avoid subsequent spread to healthy areas.

P.44 - Fascioliasis in Iran during 2012

Hossein Masoumi Asl[1] , Moharram Mafi[1]

1-Center for Communicable Diseases Control,
Ministry of Health and Medical Education, Tehran, Iran

Iran is one of the endemic region of fascioliasis in the world and during the past three decades large outbreaks have been reported. In spite of previous large epidemics, disease occurs today sporadically. Fascioliasis is a zoonotic disease of the liver that can be transmitted to humans**.** *Lymnaea trunculata* and *Lymnaea gederosiana* are snail host for *Fasciola hepatica* and *Fasciola gigantica* parasites**.** The aim of this report is clarifying the last situation of fascioliasis in 2012 in Iran. A retrospective cross-sectional study was conducted by using fascioliasis national surveillance data during 2012. Epidemiologic investigation was conducted around all cases. A total 96 cases were reported to Center for Communicable Disease Control. Gilan province which is located in northern country reported 70 (73%) of cases. Tehran 12 (12.5%), Kermanshah 2 (2%), Khorasan Razavi 2 (2%) and 10 provinces have reported 10 (10%) cases . The age group of 40-49 years old was more affected. Eighty four % were in urban and 16% cases occurred in rural area. Thirty eight % of patients were males and reminder were female. The clinical findings include abdominal pain 62%, urticaria 21%, headache 34%, shoulder pain 11.3% and itching 28% . Eosinophilia more than 30% was seen in 11% of cases. The consumption of local vegetable such as Chouchagh and Khalvash were responsible of disease transmission. All of cases have been treated with Triclabendazole without remarkable complications. The socio-economic conditions improvement and implementation of different preventive and control measures resulted in decreasing of cases dramatically. Outbreaks occurred in most of the provinces, therefore, inter sectoral coordination including veterinary organization is necessary to prevent re emerging of disease.

P.45 - Parasitic helminths among Hausa communities in Kano, Nigeria

Salwa Dawaki, Init Ithio, Jamaiyah Ibrahim and Hesham El-Mekhlafi

Department of Parasitology, Faculty of Medicine, University Malaya, Kuala Lumpur,50603, Jln Pantai, Kuala Lumpur, Malaysia

A survey was carried out to investigate the prevalence of parasitic helminths infections in 550 stool samples obtained from the Hausa-fulanis (consist of 5 five communities namely, Kura, Bebeji, Gwarzo, Shanono and Minjibir) in Northern Nigeria. The prevalence of helminthes was found high with *Schistosoma mansoni* (9.27%) followed by *Schistosoma haematobium* (8.73%), *Ascaris lumbricoides* (8.18%), hookworms (7.45%), *Hymenolepis nana* (3.82%), *Fasciola hepatica* (3.09%), *Strongyloides stercoralis* (2.91) and *Enterobius vermicularis* (2.00%). Among the communities, the infection of helminthes was highest in Gwarzo (59.8%) followed by Shanono (51.5%), Manjibir (48.8%), Bebeji (48.7%) and Kura (42.9%). Among these helminthes, only *Schistosoma haematobium* showed significantly high in Gwarzo community (X^2=, $p > 0.05$). Prevalence of helminthes in the Hausa-fulanis population may be due to social-economic factors and the highest prevalence of *S. haematobium* infection in Gwarzo community was highly due to the volunteers who were mainly secondary school children (age 11-17years old) who lives nearby infested ponds and rivers. Further more this community has no campaign programme on schistosomiasis treatments.

P.46 - Distribution of *Giardia duodenalis* assemblages A and B among Orang Asli in Malaysia: Risk factors and correlation with clinical symptoms

Tengku Shahrul Anuar[1], Norhayati Moktar[2] and Fatmah Md Salleh[2]

[1]Department of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Selangor, Malaysia.
[2]Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

Giardia duodenalis is a flagellate parasite which has been considered the most common protozoa infecting human worldwide. Molecular characterization of *G. duodenalis* isolates have revealed the existence of seven groups (assemblage A to G) which differ in their host distribution. Therefore, this cross-sectional study was conducted to identify assemblage's related risk factors of *G. duodenalis* among Orang Asli in Malaysia. Stool samples were collected from 611 individuals aged between 2 and 74 years old of whom 266 were males and 345 were females. Socioeconomic data were collected through a pre-tested questionnaire. Meanwhile, stool samples were processed with formalin-ether sedimentation and Wheatley's trichrome staining techniques for the primary identification of *G. duodenalis*. Molecular identification was carried out by the amplification of a triose phosphate isomerase gene using nested-PCR assay. Sixty-two samples were assemblage A and 36 were assemblage B. Risk analysis based on the detected assemblages using univariate and logistic regression analyses identified three significant risk factors of giardiasis caused by assemblage B. These factors were (i) children ≤15 years old, (ii) consuming raw vegetables and (iii) presence of other family members infected with giardiasis. On the other hand, subjects who have close contact with household pets were found to be significant predictors for assemblage A. Individuals infected with assemblage A were also at higher risk of manifesting diarrhoea (P = 0.016) and other gastroenteritis symptoms (P = 0.024). It has been concluded that *G. duodenalis* infection is still a public health problem among Orang Asli and was caused by both assemblages. The dynamic of transmission is most probably through zoonotic and anthroponotic, which is human-to-human either directly or indirectly through contaminated food. These routes of transmission should be considered in the control strategy of the disease.

P.47 - *Cyclospora cayetanensis* infections among diarrheal patients in Riyadh, Saudi Arabia

WAFA A. I. AL-MEGRIN

Department of Biology, Princess Nora Bint Abdul Rahman University, P.O.Box 25701, Riyadh 11476, Saudi Arabia.

This study was conducted to determine the prevalence of *Cyclospora cayetanensis* as a cause of diarrhea among patients in Riyadh hospitals, Saudi Arabia. Three hundred and eighty male and female patients of different age groups (3 month to 63 year old) presenting with diarrhea were investigated over a 12 month period. Modified Ziel-Neelsen staining of formalin-ether stool concentrates was used for identification of the coccidium. Forty patients were positive for *C. cayetanensis*, giving an overall prevalence of 10.53%. The lowest infection rate (5.97%) was recorded in infants (3 month – 2 year) and the highest (13.54%) in older children (3-12 year). However, differences between different age groups were statistically non-significant. Similarly, no significant difference in the infection rate was found between male and female patients, whereas a highly significant difference ($P<0.001$) was recorded between Saudi (6.5%) and non-Saudi (17.9%) patients. The results also indicated a highly significant seasonal variation ($P<0.001$), with highest prevalence in summer (20.53%), followed in descending order by autumn (9.68%), winter (6.94%) and spring (2.9%).

P.48 - Nephro - and tsticulopathies in chronic experimental *Trypanosoma evansi* infection in rabbits

Yasameen, S. G., Sharma, R. S. K., Hemn, H. O.,
Zuki, A. B. Z. and Noordin, M. M.*

Faculty of Veterinary Medicine Universiti Putra Malaysia,
43400 UPM, Serdang, Malaysia

The pathogenesis of surra caused by *Trypanosoma evansi* is still obscured especially with regards to nepho-and testiculopathies and this forms the basis of the present investigation. Thirty five male rabbits were divided into seven groups randomly, each consist of five rabbits. Animals from trypanosome-infected groups were inoculated intravenously with 10^5 trypanosomes while the control group received normal saline via the same route. Five rabbits from each group were sacrificed and examined at 1, 2, 3, 4, 5 and 6 months post-infection (pi). At necropsy, nephropathy, hepatomegaly, testicular enlargement were observed. Histopathological changes in the kidney include moderate to severe tubular degeneration and necrosis, atrophied glomeruli with proliferative glomerulonephritis. Amylidiosis were demonstrated 5 and 6 months pi. Testicular changes, especially aspermia were observed since first month pi. Chronic inflammatory reaction demonstrated by chronic mononuclear inflammatory cell infiltration accompanied with the cellular degeneration in liver. Our findings indicated that the pathogenesis of Malaysian isolate of *T. evansi* in chronically infected laboratory model is associated with obvious pathological changes in the kidneys and testicles.

P.49 - Seroprevalence and molecular study of *Toxoplasma gondii* infection among healthy blood donors in Southern Iran

*1*Bahador Shahriari Rad, 2Reza Shafiei,2*
Mani Zare, 2Sattar Sohrabpour, 3Leila Kasraian

1Basic Sciences in Infectious Diseases Research Center,
Shiraz University of Medical Sciences, Shiraz, Iran
2Department of Parasitology and Mycology, School of Medicine,
Shiraz University of Medical Sciences, Shiraz, Iran
3Education Unit, Shiraz Blood Transfusion Organization, Shiraz, Iran

Toxoplasma gondii is a protozoan parasite which can be transmitted to human through a variety of ways including blood transfusion. The current cross sectional study aimed to evaluate the seroprevalence of Toxoplasma infection and its related epidemiological features among healthy blood donors in Fars province, southern Iran. A total of 1480 healthy blood donors from five blood service centers in Fars province were evaluated for Toxoplasma infection. The blood samples were tested for anti-T. gondii IgG and IgM antibodies by enzyme immunoassay. IgM-positive samples were tested for presence of Toxoplasma DNA by PCR. Demographic characteristics of participants were also recorded while collecting samples. Anti T. gondii antibodies was detected in sera of 286 of 1480 blood donors corresponding to overall seroprevalence of 19.3% in this population. From these, 182 (12.3%) were seropositive for only IgG, 81 (5.47%) were seropositive for only IgM and 23 (1.6%) were positive for both IgG and IgM. PCR detected active parasitemia in 2 (1.9%) of IgM-positive subjects. Association between age, place of residence and educational level with seropositivity to Toxoplasma were statistically significant ($P<0.05$). Findings of this study provide appropriate information about the seroepidemiology of T. gondii among healthy blood donors in south of Iran. The findings highlighted that asymptomatic blood donors, especially those with active parasitemia, may constitutes a significant risk of transmitting of toxoplasmosis to susceptible recipients.

P.50 - Antitrypanosomal compound isolated from an indigenous *Streptomyces* Sp.

Lili Sahira H., Getha K., Mohd Ilham A., Muhd Syamil A.,
Hema Thopla G., Norhayati I., Muhd Haffiz J.,
Nur Fairuz M.Y., Siti Syarifah M.M.

Natural Product Division, Drug Discovery Centre, Forest Research Institute Malaysia, 52109, Kepong, Selangor.

The unique ecosystems in Malaysia can be potential sources for isolating actinobacteria strains that produce interesting bioactive secondary metabolites. In the present study, growth profile of *Streptomyces* sp. strain FACC-A032 which exhibited strong antitrypanosomal activity (IC_{50} 0.23 ug/ml) in culture broth extract was studied to determine the incubation period for antitrypanosomal metabolites production. Data onbroth pH, wet weight of biomass and antitrypanosomal activity of culture broth extracts were recorded during shake-flask fermentation. Further studies were carried out to isolate the active fraction/s from the culture extracts of strain FACC-A032. Using bioassay-guided isolation techniques, active fraction 2SnF8 was fractionated from the crude extracts of culture filtrate. HPLC chromatogram of the active fraction showed the presence of a major peak at retention time of 7.2 min. with UV absorption max at 292 nm. This active peak was further purified using Preparative HPLC and identified by NMR spectroscopy. The structure of the compound closely resembled that of staurosporine when compared with the spectral data from literature. Staurosporine is known as a class of protein kinase inhibitor, originally discovered from a *Streptomyces* sp.

P.51 - Interaction of *Toxoplasma gondii* Sag1 and Sag2 with human cDNA library

Meng Yee Lai[1], Yee Ling Lau[1], Mun Yik Fong[1] & Nazia Abdul Majid[2]

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

Toxoplasma gondii (T. gondii) is an obligate intracellular protozoan parasite that invades any nucleated cells in human and other warm-blooded animals. Approximately 25% to 30% of the world's human population is infected by *T. gondii*. Many proteins involved in *T. gondii* invasion have been characterized and contribution for parasite entry has been proposed. However, their molecular interactions remain unclear. Surface antigens (SAGs) of *T. gondii* play a major role in host cell invasion. In this study, Yeast two-hybrid system was used to analyze the interaction of SAG1 and SAG2 of *T. gondii* with the host cell using human cDNA library. The SAG1 and 2 proteins were expressed by pGBKT7 plasmid using Y2HGold yeast. At the same time, host cell proteins were expressed by pGADT7-RecA plasmid using Y187 yeast. Following a selection procedure, a total of 89 and 20 colonies of SAG1 and SAG2 were found on the QDO/X/A (quadruple dropout medium supplemented with X-α-Gal and aureobasidin A) plates. Among these colonies, 69 and 15 colonies from SAG1 and SAG2 respectively were positive for colony PCR. Beta-galactosidase assay was performed and colonies with high beta-galactosidase activity were chosen for further analysis.

P.52 - The influence of *Toxoplasma gondii* on rat behavior

Minoo Shaddel

AJA University Of Medical Sciences

Toxoplasma gondii, an obligate intracellular protozoan, is able to cross the blood brain barrier (BBB), and migrates to the central nervous system which may form cyst and causes chronic infection without any symptom. It seems, this infection may causes different mental disorders, and behavioral changes in it's different vertebrate hosts. The aim of this study was to assess the effect of infection by *Toxoplasma gondii (RH strain) on Wistar* male rat behavior. Rats were assigned into three groups. Experimental chronic Toxoplasmosis was initiated by injection of up to 10^7 alive *Toxoplasma gondii* tachyzoite (ip) in infected group (n=14). Sham control (n=10) and control (n=14) groups received physiological serum (ip) and nothing respectively. Animals were kept in standard condition. After 3 weeks, all rats were evaluated for behavioral tests included sterotypic movement and social interaction test. The data were analyzed by SPSS software version 20. According to the grooming test, infected group was significantly more than sham control and control groups (p<o.o5). About the time of being together in the social interaction, infected group was significantly less than sham control group (p<0.05). There were not significant differences among group and other tests. The results of stereotypy and social interaction tests are similar to the animal model of Schizopherenia in the rat described by increased grooming and decreased time to be together.

P.53 - *Nigella sativa*: Potential herbal treatment for lethal infection with *Toxoplasma gondii*

Wan Omar, A[1]., Kumareswaran[1], D., Ngah Zasmy, U[1]., Rukman A.H[1]. and Rusliza, B[2].

[1] Department of Medical Microbiology and Parasitology; Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor Darul Ehsan;
[2] Department of Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor Darul Ehsan

Toxoplasmosis drugs have the longest history and are still the first choice for most conditions. Alternative drugs such as Co-trimoxazole and Tetracycline have been tried and acclaimed successful. The lack of general acceptance, however, is an indication that the results are not very convincing. A wide range of antibiotics is urgently needed for patients with drug reaction or resistance problems. *Nigella sativa* (black seed) is commonly known as “habatu saudah” in Arabic and Moslems believe it as a panacea to all diseases. The antitoxoplasmic activity of water and ethanol extracts *N. sativa* was evaluated in murine models of intraperitoneal infection using the RH strain of *Toxoplasma gondii*. Black seed oil was purchased directly from Arab Grocery Shop at Masjid India, Kuala Lumpur. Two separate studies were performed, viz prophylactic and therapeutic studies. Mice were infected with 2×10 tachyzoites/ml, and then treated orally with the oil at 100 and 200mg/kg/day for ten days. Mice were observed for mortality incidence for ten days. Both prophylactic and therapeutic groups survived significantly better (P=0.01) than infected untreated group (69.96%). The therapeutic group showed 87.9 % cumulative survival rate. *N. sativa* prolonged the time of death of the treated group compared to the control group (mean time of death was 21.36 ± 2.4 and 18.6 ± 5.4 compared to the control group, (P = 0.4) and (P= 0.6), respectively. *Nigella sativa* showed promising prophylactic and infection with *T. gondii* in the mice model. Studies are undergoing in our laboratory in both *in vitro* and *in vivo* testing of bioactive compounds of *N. sativa* against *T. gondii*.

P.54 - Evaluation of the *in vitro* properties of selected Malaysian medicinal plants against *Toxoplasma gondii* in mice

Wan Omar, A[1]., Kumareswaran, D[1]., Ngah Zasmy, U[1]., Init, I[2]., and Rusliza, B[3].

[1] Department of Medical Microbiology and Parasitology; Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor Darul Ehsan;
[2] Department of Parasitology, Faculty of Medicine, Universiti Malaya, Kuala Lumpur 50605, Malaysia;
[3] Department of Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor Darul Ehsan

Toxoplasma gondii infection causes toxoplasmosis, an infectious disease with worldwide prevalence. The limited efficiency of drugs against this infection, their side effects and the potential appearance of resistant strains make the search of novel drugs an essential need. We examined *Nigella sativa, Tinospora crispa*, *Andrographis paniculata* and *Ginkgo biloba* extract as potential sources of new compounds with high activity and low toxicity. The main goal of this study was to investigate the anti-*T gondii* activity of these plant crude extract, with clindamycin as the positive control. *In vitro* toxoplasmacidal evaluation was performed using Vero cells as host for *T. gondii*. Light microscopy technique was used to study in situ antiparasitic activity. Significant anti-*T.gondii* activity was observed with clindamycin (IC_{50}=16.57 ±_0.36 µg/ml), followed by *N. sativa* (IC_{50}=18.03 ± 0.69 µg/ml), *T. crispa* (IC_{50}=24.45 ± 0.82µg/ml), *A. paniculata*, (IC_{50}=31.24 ± 1.44µg/ml), and *G. biloba* (IC_{50}=34.68 ± 1.32 µg/ml). Our study demonstrated that *N. sativa* extract showed the highest in vitro antiparasite activity compare to *T. crispa*, *A. paniculata* and *G. biloba*. All these plants extract potentially can be new sources of anti *T. gondii* bioactive compounds.

P.55 - Alpha-Feto protein IGM antibody cross reaction with *Toxoplasma* antibodies among women in Kirkuk-Iraq.

Yahya Jirjees Salman

College of Medicine-Kirkuk University

Toxoplasmosis is one causative agents of women abortion and congenital outcome.While alpha-feto protein(AFP) is measured in pregnant women through the analysis of maternal blood or amniotic fluid, as a screening test for a subset of developmental abnormalities.This study aimed to determine *Toxoplasma* incidence among women with abortions and to assess cross reaction between *Toxoplasma* antibodies and AFP IgM antibodies. 610 sera were extracted fromvenous blood of women with abortions,all sera were tested for *Toxoplasma* IgM,IgG and AFP IgM using ELISA technique. The rate of *Toxoplasma* infection was 20.76 % in 152 samples. High rates of toxoplasmosis 47.71 % and 25.29 % were recorded in summar andAutunm compare dto low rates in winter and spring , $P<0.05$. From a total of 135 sera positive for toxoplasmosis only 11 sera with the rate 8.15 % were positive for AFP-IgM compared to 0.74% in control group, $P<0.05$. *Toxoplasma* antibodies classification revealed 51.56 % of *Toxoplasma* IgG followed by 20.52 % *Toxoplasma* IgM.While *Toxoplasma* IgM+IgG rate was 27.92 %<P<0.05. High rate of antibody crossing of AFP was seen with *Toxoplasma* -IgG 8.97 % compared to 2.64 % AFP-IgM cross with *Toxoplasma* IgM, while no AFP antibody crossing was detected with seropositive *Toxoplasma* IgM+IgG, $P<0.05$. Toxoplasmosis was high among women in Kirkuk city. High rates of *Toxoplasma* antibodies were seen in summar and autunm seasons , $P<0.05$. High rate of AFP antibodies cross was seen with *Toxoplasma* IgG than crosses with *Toxoplasma*-IgM. AFP-IgM ELISA test should be applied with other tests to detect the causative agents of women abortion.

P.56 - Larvicidal activities of *Alpinia galanga* and *Boesenbergia rotunda* extracts against *Aedes aegypti, Aedes albopictus* and *Culex quinquefasciatus*

Adibah, N.M.Y[1]., Ibrahim, H[1]., Yusoff, M.M[2]., Nurulhusna, A.H[3].

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.
[2]Faculty of Industrial Sciences & Technology, University of Malaysia Pahang, Malaysia.
[3]Entomology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia.

The search for environmentally friendly and biodegradable natural insecticides of plant origin has received renewed interest from scientists. This research aimed to evaluate the effectiveness of hexane and dichloromethane extracts of *Alpinia galanga* and *Boesenbergia rotunda* against larvae of *Aedes aegypti, Aedes albopictus* and *Culex quinquefasciatus.* The rhizomes of *A. galanga* and *B. rotunda* were extracted with hexane and dichloromethane and tested on mosquito larvae at various concentrations (25mg/L, 50mg/L, 70mg/L, 100mg/L and 200mg/L) following WHO standard bioassay method. Results of the study showed that both plants exhibited larvicidal activities against tested larvae. *A. galanga* hexane extract was more effective at 70mg/L in killing larvae of *Ae. aegypti* (88.00%) and *Ae. albopictus* (81.33%). While *B. rotunda* hexane extract was effective in killing the *Cx. quinquefasciatus* larvae (54.67%) at 50mg/L. The larvae of *Ae. aegypti* and *Ae. albopictus* showed 66.67% and 88.00% mortality respectively, when exposed to dichloromethane extract of *A. galanga* at concentration of 70 mg/L. However, the dichloromethane extract of *B. rotunda* was effective in killing larvae of *Cx. quinquefasciatus* (62.37%) at 70 mg/L. This preliminary study suggests that *A. galanga* rhizome extract is effective in killing larvae of *Ae. aegypti* and *Ae. albopictus*, while *B. rotunda* rhizome extract is effective in killing larvae of *Cx. quinquefasciatus*. Further detailed studies will be conducted to investigate the insecticidal properties of both plants.

P.57 - Scanning electron microscopy of *Anopheles hyrcanus* Group (Diptera: Culicidae) eggs in Thailand and an ultrastructural key for species identification

Atiporn Saeung[1], Chayanit Hempolchom[1], Thippawan Yasanga[2], Yasushi Otsuka[3], Sorawat Thongsahuan[4], Wichai Srisuka[5], Udom Chaithong[1], Kritsana Taai[1], Pradya Somboon[1], Wej Choochote[1]

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, [2]Medical Science Research Equipment Center, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
[3]Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, 879-5593, Japan
[4]Faculty of Veterinary Science (Establishment Project), Prince of Songkla University, Songkhla 90110, Thailand
[5]Entomology Section, Queen Sirikit Botanic Garden, P.O. Box 7, Chiang Mai, 50180, Thailand

The eggs of *Anopheles argyropus*, *An. crawfordi*, *An. nigerrimus*, *An. nitidus*, *An. paraliae*, *An. peditaeniatus*, *An. pursati* and *An. sinensis* are described with the aid of scanning electron micrographs. Comparisons of egg structure among the eight species showed that the eggs differed with respect to the following characteristics. The deck: complete (*An. argyropus*, *An. nigerrimus*, *An. paraliae*, *An. peditaeniatus* and *An. sinensis*), variable (complete or incomplete/*An. crawfordi*) and division into an area at each end (*An. nitidus* and *An. pursati*). The ratio of the entire length per maximal deck width within the area covered by floats was 3.12-4.50 (*An. sinensis*), 8.28-11.50 (*An. peditaeniatus*), 13.67-17.80 (*An. nigerrimus*), 26.33-44.25 (*An. paraliae*), 26.99-75.94 (*An. argyropus*), 29.33-88.93 (*An. crawfordi*); and the total numbers of anterior and posterior tubercles were 4-7 (*An. crawfordi*), 6-8 (*An. paraliae*) and 9-11 (*An. argyropus*). The numbers of float ribs were 21-27 (*An. peditaeniatus*) and 28-34 (*An. nigerrimus*), and types of exochorionic sculpturing were reticulum type (*An. argyropus*, *An. crawfordi*, *An. nigerrimus*, *An. nitidus*, *An. paraliae*, *An. peditaeniatus* and *An. sinensis*) and pure tubercle type (*An. pursati*). Attempts are proposed to construct a robust key for species identification, based on the morphometrics and ultrastructures of eggs under scanning electron microscopy.

P.58 - The effect of essential oils of two common medicinal and culinary gingers (Family: Zingiberaceae) as repellent against house dust mites, *Dermatophagoides pteronyssinus* (Acari: Astigmata)

[1]*Azima Laili Hanifah,* [1]*Mariana Ahamad,* [2] *Nurulhusna Ab Hamid,*
[1]*Vishalani Vishnu Narainasamy and* [3]*Halijah Ibrahim*

[1]*Acarology Unit, Institute for Medical Research,*
Jalan Pahang,50588 Kuala Lumpur, Malaysia.
[2]*Entomology Unit, Institute for Medical Research,*
Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
[3]*Institute of Biological Sciences, Faculty of Science,*
University of Malaya, 50603Kuala Lumpur

Ginger is an herbaceous plant that is often used in a variety of dishes. It is easily found and grown. Apart from its use in cooking, aroma of ginger is also able to repel the presence of insects; including house dust mites. Dust mites have a cosmopolitan distribution. They will produce approximately 2000 fecal particles and an even larger number of partially digested enzyme-covered dust particles in its whole life span. Both decomposing animal parts and the protein that surrounds mite fecal pellets cause mite allergy. Therefore it is more effective to repel than to kill them. This study examined the repellency effect of essential oils of 'halia bara' (*Zingiber officinale var rubrum*) and 'temu kunci'/ fingerroot (*Boesenbergia rotunda*) at different concentration; 20%, 10%, 5%,2.5%,1.25% for 3 hours exposure time against house dust mites, *Dermatophagoides pteronyssinus* . Thirty adult mites were placed on square white cotton fabric (Ø45mm) that had been divided into two sides; untreated and treated with essential oil solution. Mites initially are placed on the untreated area and after 3 hours whoever mites found on the treated area were considered not repel to the particular essential oils. As a result, both essential oils have been shown to be effective repellents against *D.* pteronyssinus and their repellencies were found similar ($p = 0.249$). On the other hand, there was a significant difference ($p<0.05$) between concentrations of each essential oil on repelling the dust mites. The repellencies increase with increasing concentrations of essential oil. At 1.25% concentration both gave more than 50% repellencies and at higher concentration both essential oils gave more than 90% repellencies. These essential oils have the potential to be commercialized as repellants against house dust mite.

P.59 - Effectiveness of ethanol extract of papaya leaf (*Carica papaya* L) against larvae of *Culex* Sp

Emma Mardliyah Hidayat

Faculty of Medicine, Jenderal Achmad Yani University, Indonesia

Culex sp. is a type of mosquito that is important in Indonesia because of its role as vectors of disease. In Cimahi, *Culex sp*. is a potential vector of filariasis. Several measures have been taken to control the mosquito presence, among others, the use of larvicides. Papaya (*Carica papaya L*) is a fruit that contains papain, in particular on the leaves. The papain active substance has the power to kill the mosquito larvae that can be used as an alternative natural larvicides for mosquito. This study is experimental and aimed to determine the effect of ethanol extract of papaya leaves as mosquito larvicides of *Culex sp*. In this study, the 20 mosquito of larvae *Culex sp* were included in the solution of ethanol extract of papaya leaves with a concentration of 0.0006 g/ml, 0.0007 g/ml, 0.0008 g/ml, 0.0009 g/ml , 0.0010 g/ml, 0.0011 g/ml, 0.0012 g/ml, 0.0013 g/ml, 0.0014 g/ml and 0.0015 g/ml with four replicates. Observations were made by counting the number of dead larvae after 24 hours. The results showed that the extract of papaya leaves (*Carica papaya L*) is effective against larvae of *Culex sp*. The use of papaya leaves which are very easy to get in Cimahi as a complementary food (vegetables) turns out to be that it can also be an alternative natural larvicide which is environmentally friendly.

P.60 - Effectiveness of using artificial versus natural membrane for artificial blood feeding of *Aedes albopictus*

Izzah Maziyyah Mohd Yusof[1], Noor Idzatul Aqma Misran[1], Ummu Hanie Baharum[1], Selvi Subramaniam[2], Nazni Wasi Ahmad[2], [2]Lee Han Lim[2] and Edah Md Aris[1]

[1]Institute of Biological Science, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Entomology Division, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

Female mosquito needs blood meal for egg maturation and reproduction. Often, in small scale laboratory work, blood is sourced from live animals for mosquito feeding. However, nowadays using animal for feeding incurs ethical issue and cost expensive. Therefore it is essential to find an alternative source of feeding mosquito that is easily available and cost effective. In our present study we have designed an artificial membrane device called Glytube with some modifications that was adapted from previous study. The Glytube was used to test on natural membrane i.e cattle membrane, thinned chicken skin and cattle diaphragm against artificial membrane namely parafilm, clean film, gauze sealed with wound dressing to feed lab-bred *Aedes Albopictus*. Parameters that were assessed from this membrane feeding experiment i.e feeding rate, eggs production per female, total egg production and hatch rate will be discussed further.

P.61 - Human practices: A case in breeding of mosquito vectors in Ekwulobia, Anambra State Nigeria

Zita Obi[1], Lucy Anyaegbunam[2], Oscar Emmy-Egbe[2], Martha Nwadiugwu[2]

[1]Department of Science Education, Anambra State University, Uli Nigeria
[2]Department of BiologicaSciences, Anambra State University, Uli Nigeria

Mosquitoes are associated with different breeding sites which are both natural and man-made. With the level of development in the Tropics and literacy among the inhabitants, different breeding grounds and conflicting diseases transmitted by mosquitoes abound. The survey of mosquito vectors and human practices that encourage their breeding was carried out in Ekwulobia, Anambra State, Nigeria. A total of one thousand, one hundred and eighty-seven (1,187) mosquitoes were recovered from the sampled households of four quarters in Ekwulobia. Using different collection methods, the following were involved, human bait (85), pyrethrum (102), sweep net (677), and suction tube for those in resting position (323). The mosquitoes sampled were identified by a specialist in the area at National Arbovirus and Vector Research Division, Federal Ministry of Health Enugu, Nigeria. The mosquitoes *Culex quinquenfasciatus*(69.97%), *Aedes aegypti* (18.0%), *Aedes albopitus* (6.4%), *Anopheles gambiae* (4.1%) and *Mansonia africana* (1.2%). The abundance of mosquitoes in relation to sex were, females (81.9%) and males (18.1%). Considering the period of survey which was between June – October 2011, the relative abundance of mosquitoes were as follows, August – October, *Culex* was predominant (724) followed by *Aedes* (229) while *Anopheles* (42) was relatively high between June and July. In the sampling methods, sweep net was best for *Culex* and *Aedes* while human bait and pyrethrum were good for *Anopheles* and *Mansonia* which were mostly indoors. Different human practices favoured the distribution and abundance of mosquito vectors in the community which were fermented cassava (489), farms (102), sucker away pits (154), and earthen wares (77). The abundance of mosquito vectors in the four sampled quarters were, Ula (581), Agba (390), Eziagulu (163) and Abogwume (53). Owing to the abundance of mosquitoes in the study area, several preventive/control methods were used to avoid mosquito bites. Use of insecticide spray, burning of mosquito coils, door and window screens, sleeping under insecticide treated nets, as well as use of herbs, which include, Lemon grass (*Cymbopogon citraus*), Rosemary (*Romarinus officinalis*), Mosquito plant (Citronella) and Neem (*Azadirachta*). Further study on mosquito surveillance is expected in the area for its grass root control and related diseases.

P.62 - *Aedes aegypti* subdistrict of Cigugur Tengah, Cimahi City, Indonesia

[1]*Lutfhi Nurlaela,* [1]*Emma Mardliyah Hidayat*

[1]*Faculty of Medicine, Jenderal Achmad Yani University, Cimahi-West Java, Indonesia*

The mortality and mordibity rate due to Dengue Fever in Indonesia has been high up to the present time with Case Fatality Rate of 0,86% in year 2012. To reduce the CFR rate, there is a need of a simple treatment but is easy to do by everyone, one of which is the surveillance of the larvae of dengue fever vector mosquito, which is Aedes aegypti, ran by the community. The measurement that can represent the vector density in one area is conducted by counting the number of larvae-free. The higher the number of larvae-free in one area, the less the population of the vector, and it is expected that the CFR will also decrease. The research conducted was a descriptive study, data collection was done by consecutive sampling, by seeing the presence of the Aedes aegypti larvae in the water reservoirs both inside and outside of the houses of the settlers in the area of subdistrict of Cigugur Tengah Subdistrict of Cimahi Tengah, Cimahi. Of the 497 homes sucessfully visited and examined, 150 homes were tested positive for Aedes aegypti larvae, while 347 homes were tested negative for Aedes aegypti larvae meaning there was no indication that Aedes aegypti was present. Therefore the number of larvae free was 69.8%. Although it has been more than 50%, this figure is still far from the real target as the expected number larvae free is >90%. This was probably related to frequency of the application of temefos which was still lacking in the community (38.25%), or due to less involvement of the the community in running the surveillance of the mosquito larvae periodically at their own homes. Therefore, the presence of “jumantik” (larvae monitoring officer), extension, education and cadre trainings and home visit to the community should be made regularly to make the settler the “jumantik” of their own home.

P.63 - Effect of *Citrus grandis* (L.) Osbeck fruit peels extracts against *Aedes aegypti* (Linn.) larvae

Manorenjitha Malar[1*]*. S, Zairi Jaal*[2]*, Ling Sui Kiong*[3]*, Mailina Jamil*[3] *and Nuziah Hashim*[3]

[1]*Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia, Malaysia*
[2]*Vector Control Unit, Universiti Sains Malaysia, Malaysia*
[3]*Biotechnlogy Division, Forest Research Institute Malaysia*

Botanical derivates of *Citrus grandis* fruit peels are known to contain alkaloids and tannins which are known to exhibit larvicidal activities. Various extracts of *C. grandis* (hexane, ethyl acetate, methanol, aqueous and water extracts) and *C. grandis*’s essential oil were tested against early fourth instar *Ae. aegypti* larvae. Bioassay was conducted under laboratory conditions at different concentrations to determine the LC_{50} and LC_{90} values at 24 and 48 hours post treatment. Of the various extracts tested, hexane and essential oil were observed to be more efficient. The finding indicates that extract of *C. grandis peel* have the potential to be used as alternative larvicides against *Ae. Aegypti* larvae.

P.64 - A campus community's knowledge, beliefs and practices regarding prevention of tick bite and tick-borne diseases

Masoumeh Ghane Kisomi[1], Li Ping Wong[1], and Sun Tee Tay[2],

[1]Department of Social and Preventive Medicine, University of Malaya, Kuala Lumpur, Malaysia
[2]Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Malaysia

Ticks are second important vector for human infection diseases after mosquitoes, and the key method for controlling tick borne diseases is prevention. This survey examined students' perspectives of knowledge, beliefs, experiences and practices regarding prevention of tick bite and tick borne diseases. Four hundred and forty one Malaysian students in the University of Malaya aged 18 years old and above responded a cross-sectional web-based questionnaire survey during July to October 2013. Three hundred and forty eight (79%) out of 441 students stated they knew ticks and among them 140 (40.2%) students had experience of tick bite. Students with experience of tick bite were more likely to have higher score of knowledge compared to who did not experience tick bite (odds ratio [OR] = 1.91, 95% confidence interval [CI] =1.21-3.01). Students with high perception of severity were less likely to experience tick bite (OR=0.63, 95% CI =0.41- 0.97) versus those with low perception of severity. Students who ever had pets (42%) had high perception of susceptibility to get tick bite (OR=2.15, 95% CI =1.27-3.64) compared to those who did not own a pet. The findings suggest that the knowledge of ticks was low among the study's group and perceived severity and susceptibility of tick bite and tick borne diseases play a role to adopt preventive measures and decrease experience of tick bite.

P.65 - Types of mosquito larvae at Kelurahan Baru – Ladang Bambu, Kecamatan MedanTuntungan, Medan, North Sumatera, Indonesia

Merina Panggabean[1], Logeswaran Gunasegaran[1]

[1]Department of Parasitology, Faculty of Medicine, University of North Sumatera, Indonesia

Mosquitoes are the cause of some dangerous disease to humans. Larvae is an important stage in mosquitoe's life cycle and it is important to identify its presence and stop its development. This study aims to identify the type of mosquito larvae which are found in Kelurahan Baru – Ladang Bambu, Kecamatan Medan Tuntungan, Medan, North Sumatera, Indonesia. Samples of mosquito larvae were collected in Kelurahan Baru – Ladang Bambu, Kecamatan Medan Tuntungan and examined under microscope to identify the species with the help of taxonomic keys. Type of mosquito larvae is identified by the morphology of the larvae and the location where larvae were found. The results showed that type of mosquitoes found in the study site was the *Aedes sp*. Amount 83 samples were collected from 230 houses, all of which were the same type, that is 100% *Aedes* sp.

P.66 - Comparison on larvicidal activity of synthetic and natural insecticides against *Aedes albopictus*

Noor Idzatul Aqma Misran[1], Izzah Maziyyah Mohd Yusof[1], Ummu Hanie Baharum[1], Selvi Subramaniam[2],Nazni Wasi Ahmad[2],Lee Han Lim[2] and Edah Md Aris[1]

[1]Institute of Biological Science, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Entomology Division, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

The synthetic insecticides are toxic and adversely affect the environment. There is a need to find alternatives to these synthetic insecticides. Natural insecticides are promising in that they are effective, environment-friendly and inexpensive to kill mosquitoes. Mosquitoes in the larval stage are attractive targets for insecticides because mosquitoes breed in water and thus it is easy to deal with them in this habitat. Larvicidal activity of synthetic insecticides versus natural insecticides were tested against larvae of *Aedes albopictus*. Three different types of synthetic insecticides were used in this experiment (malathion, permethrin, temephos) and the results on the larvicidal activity was compared with three different types of natural insecticides (citronella oil, clove oil, cinnamon oil). The method of WHO larval bioassay was applied to determine the larvicidal activity of *Ae. albopictus*. The susceptibility and resistance of *larvae Ae. albopictus* towards synthetic and natural insecticides was measured by LC_{50} value. The larvae was immersed into the water containing particular insecticides for 24 hours and the rate of larvae mortality was counted and the LC_{50} value was determined. The results of the comparison on larvicidal activity of synthetic and natural insecticides against *Ae. albopictus* will be discuss further.

P.67 - New candidates for plant-based repellent against *Aedes aegypti*

Norashiqin Misni[1], Zurainee Mohamed Nor[1], Rohani Ahmad[2]

[1]Parasitology Department, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Entomology Unit, Institute of Medical Research, 50588 Kuala Lumpur, Malaysia

An ethnobotanical study was conducted on plants collected from Kota Tinggi, Johor, traditionally used against nuisance insects. Out of sixteen plants chosen, ten were reported to be used against mosquitoes (*Cymbopogon nardus*, 13.4%; *Pelargonium radula*,5.7%; *Citrus aurantifolia*, 5.4%; *Alpinia galanga*, 3.4%; *Lantana camara*, 3.4%; *Citrus grandis*, 3.1%; *Etlingera eliator*, 1.1%; *Pogostemon cablin*, 1.1%; *Sesbania grandifolia*, 0.9% and *Tagetes erecta*, 0.3%). Three plants were finally selected for this study. Leaves *of Citrus aurantifolia*, fruit peel of *Citrus grandis* and rhizome of *Alpinia galanga* were extracted using hydrodistillation method to produce essential oil. These essential oil-based products were then formulated and then tested for their repellent effect against *Ae. Aegypti*. This study revealed that at 5% concentration, these plant-based products provided a complete protection (100%) against mosquito landing and/or bites for 2 hour post application. At 15% and 20% of concentrations, they demonstrated high protection (< 90%) for 4 hour post application. These findings however was found having no significant difference of protection when compared to DEET-based repellent (p=0.632). When compared to commercial plant-based repellents (those using *Cymbopogon nardus* and lemon extract as the active ingredient), significant difference were detected (p=0.04). In conclusion, the three plant-based products tested proven to possess prolong protection effect against *Ae. Aegypti* landing and/or bites, thus could be considered as candidates to be commercialized as an alternative plant-based repellent in the market.

P.68 - Residual efficacy of vectobac ® WG against *Aedes albopictus* and *Culex quinquefasciatus* by spray application

Rosilawati, R. [1,] Nazni, W.A.[1], Teoh, G.N.[1], Seleena, B.[2]

Medical Entomology Unit, Institute for Medical Research,
Jalan Pahang, 50588 Kuala Lumpur
Valent BioScience Corporation

The only effective method to breakdown the transmission of dengue virus is still depending on vector control program since no vaccine available until today. Most of the program involved in the usage of chemical insecticide which will become a major threat to the effectiveness of the program when the vector develops resistance. However, a safe alternative bio-agent so called *Bacillus thurinngiensis israelensis* (*Bti*) show promising larvicidal activity against *Aedes aegyti* with no resistance had been reported so far, even in regions heavily treated infected area with *Bti*. The method of delivery of *Bti* and its residual activity are two main considerations to ensure effective use of *Bti.* In order to evaluate the effectiveness of this *Bti* by spray application, a small scale study was conducted by spraying 3 lots of VectoBac® WG and further evaluates the residual efficacy against *Aedes albopictus* and *Culex quinquefasciatus.* There were a slightly different pattern of residual toxicity in Lot B, Lot C and Lot D ($p<0.05$). Within 24 hours post exposure, Lot C provided the higher mortality against *Ae. albopictus* and *Cx. quinquefasciatus* from day 1 up to day 14, while, Lot B performed much better only after day 7 onwards, moreover, Lot D induced consistent residual efficacy at near complete mortality (>98% mortality) against *Cx. quinquefasciatus* than *Ae. albopictus* (90% to 97% mortality). This study indicated all tested *Bti* products were capable of killing both larvae species (>90% mortality) within 24 hours exposure and recorded residual toxicity up to 14 days under simulated field conditions

P.69 - Morphometric study of a female-specific flightless phenotype of RIDL *Aedes albopictus* Skuse

Selvi Subramaniam[1], Derric Nimmo[2], Lee Han Lim[3], Nazni Wasi Ahmad[3], Paul Eggleston[4], Edah Mohd Aris[1], Sofian-Azirun M[1]*

[1] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[2] Oxitec Limited, 71 Milton Park, Oxford OX14 4RX, United Kingdom.
[3] Institute for Medical Research, Medical Entomology Unit, Infectious Diseases Research Centre, Jalan Pahang, Kuala Lumpur, 50588, Malaysia.
[4] Institute for Science and Technology in Medicine, School of Life Sciences, Keele University, Huxley Building Keele, Staffordshire, ST5 5BG.

Genetic based mosquito control is a potential vector control method which has been developed based on SIT– like system known as RIDL (Release of insects Carrying a Dominant Lethal gene) for suppression of vector populations particularly *Aedes aegypti* and *Aedes albopictus*, the vectors of dengue, chikungunya and yellow fever. The success of RIDL technology application into natural population and thus eliminating the vector mosquitoes rely on fitness cost. In this regard, one of the essential components of fitness cost is analyzing the developmental rate of transformed mosquito. In this study we have carried out a morphometric examination on genetically modified female-specific flightless phenotype of RIDL *Aedes albopictus* in comparison to lab-bred *Ae. albopictus*. The parameters assayed on morphometric study were based on larval growth rate and number of days spent in each life stages between the RIDL *Ae. albopictus* and lab strain and this will be discussed further.

P.70 - Isolation and identification of midgut bacteria from *Anopheles minimus* in Thailand

Thikhumporn Sittivicharpinyo[1], Uraiwan Arunyawat[1],
[1]Anchanee Kubera[1], Theeraphap Chareonviriyaphap[2] and Wunrada Surat[1]*

[1]Department of Genetics, Faculty of Science, Kasetsart University,
[2]Department of Entomology, Faculty of Agriculture, Kasetsart University,
10900 Bangkok, Thailand

Anopheles minimus is one of the main malaria vectors in Thailand. Transmission of *Plasmodium* depends on the success of the parasite survival in the mosquito, including midgut microbiota. Here, we isolated bacteria from *An. minimus* mosquito midgut using culture-dependent method. Mosquito midgut was ground in sterile saline buffer and spread on Luria-Bertani agar (LA) plates. Nine different colonies based on colony morphology were found. Then, DNA from each colony was extracted and used for bacterial species identification by PCR method using 16S rRNA primers. One band sized approximately 1500 base pair was detected from each sample. After DNA sequencing and sequence analysis, the results showed that seven species in six genera were identified including *Candidatus Chryseobacterium*, *Cellulosimicrobium cellulans*, *Cedecea davisae*, *Paenibacillus uliginis*, *Acinetobacter sp*., *Enterobacter sp*. and *Bacterium sp*. Among them, *Cellulosimicrobium* was firstly detected in the midgut of *Anopheles* species while the others were identified in other *Anopheles* mosquitoes.

P.71 - The biochemical basis of insecticide resistance in *Culex quinquefasciatus*: A nationwide survey in Malaysia

Van Lun Low[1], Chee Dhang Chen[1], Han Lim Lee[2], Tiong Kai Tan[3], Chin Fong Chen[1], Cherng Shii Leong[1], Yvonne Ai Lian Lim[3], Phaik Eem Lim[1,4], Yusoff Norma-Rashid[1] and Mohd Sofian-Azirun[1]

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.
[2]Medical Entomology Unit, WHO Collaborating Centre for Vectors, Institute for Medical Research, Kuala Lumpur, Malaysia.
[3]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.
[4]Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia.

There has been no comprehensive study on biochemical characterization of insecticide resistance mechanisms in field populations of Malaysian *Culex quinquefasciatus*. To fill this void in the literature, a nationwide investigation was performed to quantify the enzyme activities, thereby attempting to characterize the potential resistance mechanisms in *Cx. quinquefasciatus* in residential areas in Malaysia. *Culex quinquefasciatus* from 14 residential areas across 13 states and one federal territory were subjected to esterases, mixed function oxidases, glutathione-S-transferase and insensitive acetylcholinesterase assays. Enzyme assays revealed that α-esterases and β-esterases were elevated in 13 populations and 12 populations, respectively. Nine populations demonstrated elevated levels of mixed function oxidases and glutathione-S-transferase. Acetylcholinesterase was insensitive to propoxur in all 14 populations. Activity of α-esterases associated with malathion resistance was found in the present study. In addition, an association between the activity of α-esterases and β-esterases was also demonstrated. The present study has characterized the potential biochemical mechanisms in contributing towards insecticide resistance in *Cx. quinquefasciatus* field populations in Malaysia. Identification of mechanisms underlying the insecticide resistance will be beneficial in developing effective mosquito control programs in Malaysia.

P.72 - Laboratory evaluation of imidacloprid gel bait against the German cockroach, *Blattella germanica* (L.) (Dictyoptera : Blattellidae)

Wan-Norafikah, O[1]., Lee, H.L[2]., Sofian-Azirun, M[3].,
Nura-Muna, A.H[3]., and Chen, C.D.[3]

[1]Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia.
[2]Medical Entomology Unit, Infectious Diseases Research Centre (IDRC), Institute for Medical Research (IMR), Jalan Pahang, 50588 Kuala Lumpur, Malaysia.
[3]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.

The toxicity of a 2.15% imidacloprid gel bait against laboratory strain German cockroach, *Blattella germanica* was determined in the laboratory. Two tests: primary poisoning and secondary poisoning were carried out. Total mortalities were demonstrated in adult male, adult female and nymph of *B. germanica* within 10 days of primary poisoning testing. *B. germanica* adult male was the most susceptible towards imidacloprid (LT_{50} = 1.58 h; LT_{95} = 20.93 h). On the other hand, only *B. germanica* adult male (LT_{50} = 54.66 h) obtained 50% mortality within 10 days of secondary poisoning testing. Complete mortalities were not observed in any stages of laboratory strain *B. germanica* within the secondary poisoning testing period. Hence, imidacloprid gel bait tested was able to cause complete mortalities of nymph, adult male and adult female of laboratory strain *B. germanica* within 10 days of primary poisoning testing but not in the 10-day secondary poisoning testing.

P.73 - Molecular docking of compounds against *Anopheles* carboxypeptidase B using for blocking malaria transmission

[1]Watcharakorn Mongkol, [1]Wunrada Surat, [1]Uraiwan Arunyawat,
[2]Waranyoo Phoolcharoen, [3]Somchai Pornbanlualap and [1*]Anchanee Kubera

[1] Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand.
[2] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.
[3] Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand.

Malaria is the major public health problem which is caused by *Plasmodium* and transmitted by *Anopheles* mosquitoes to human and other animals. To prevent the spread of malaria and limit the area of drug-resistant *Plasmodium*, Transmission blocking vaccine (TBV) is introduced. From previous study, carboxypeptidase B (CPB) in the *Anopheles* mosquito midgut was found necessary for *Plasmodium* development in mosquito and mosquito reproduction, so the CPB could be a potential target to generate TBV. This research aimed to obtain the compounds from NCI diversity dataset and FDA approved drug dataset in ZINC database that can reduce the *Anopheles* CPB activity. The cDNA fragment of *cpb* gene from *An. maculatus* was amplified and sequenced to predict the protein structure of CPB. The virtual screenings of the compounds from two datasets were performed against CPBAm. The 880 of 1,169 compounds from NCI diversity set and 1,894 from 3,180 compounds from FDA approved drug dataset could bind to the active site of CPBAm homology structure. The top-scoring molecules will be further characterize their inhibition activity against *Anopheles* CPB, *in vitro*. The results of this study will reveal the active compounds that can be used for blocking malaria transmission.

P.74 - Eperythrozoonosis (*Mycoplasma Sp.*) in Malaysian pangolin

Jamnah O.[1], Chandrawathani P.[1], Premaalatha B.[1], Erwanas A.I.[1],
Lily Rozita M.H.[1], Ramlan M.[1] & Faizal H.[2],

[1]*Veterinary Research Institute, 59 , Jalan Sultan Azlan Shah , 31400 Ipoh, Perak , Malaysia*
[2] *Faculty of Science and Biotechnology, University Selangor, Bestari Jaya, Selangor ,Malaysia*

The Malaysian Pangolin (*Manis javanica*) is an endangered species, but is widely hunted for its medicinal value in body parts. A total of sixteen pangolins were screened for blood protozoa and six pangolins were confirmed to be positive for eperythrozoonosis infection based on the morphology from blood smears stained with 8% Giemsa. The causative organism, *Eperythrozoon sp*., with a size of 0.3 µm were observed under a compound microscope at 100x magnification on the surface of red blood cells as blue coloured dots. This is the first report of Eperythrozoon infection from pangolins in Malaysia. Further identification using polymerase chain reaction (PCR) is necessary to confirm *E. ovis* or *wenyonii*, which is uncultivatable in artificial media.

P.75 - The identification of echinococcus species and genotypes in human tissue samples from Riga Pauls Stradins Clinical University Hospital, Latvia During Years 2003 – 2013

Angelika Krūmiņa[1], Māra Pakalnišķe[2], Inga Piģiņka-Vjačeslavova[3], Inese Gavarāne[2], Muza Kirjušina[2]

[1] Infectology Department, Riga Stradins University, Linezera str. 3, Riga, Latvia, LV-1006
[2] Institute of Systematic Biology, Dauagavpils University, Vienibas str. 13, Daugavpils, Latvia, LV-5401
[3] Institute of Food Safety, Animal Health and Environment – BIOR, Lejupes Street 3, Riga, LV-1076

Various species from genus *Echinococcus* are known to use humans as intermediate hosts. Two species are reported in Latvia – *Echinococcus granulosus* and *E. multilocularis* which respectively cause the diseases named cystic echinococcosis (CE) and alveolar echinococcosis (AE). Dogs and foxes are often among the definitive hosts of these zoonotic diseases. The most affected organs in the human body are liver and lungs. Animals involved in the life cycle of *E. granulosus* are closely living with the human – dogs as definitive hosts and sheep, horses, pigs, camels etc. as intermediate hosts, whereas animals involved in the *E. multilocularis* cycle are wild animals like fox as the definitive hosts and various rodents (e.g., voles) as intermediate hosts. However, both forms of echinococcosis are zoonoses of increasing concern. There is no data about *E. granulosus* in domestic animals of Latvia and regarding wild animals, it has been found only once in an adult male wolf. However, *E. multilocularis* has been detected in several wild animals – foxes, raccoon dogs and wolves. There are no studies conducted in Latvia regarding the intermediate hosts (rodents) of *E. multilocularis*. Unfortunately, there are not enough data about the *Echinococcus* exact status of the genotype in animals and humans in Latvia. *Echinococcus granulosus* is characterized by high intra-specific variability (genotypes G1-G10) and the molecular characterization of *E. granulosus* isolates is fundamental to understand the epidemiology of this complex. We aimed to characterize the genotypes of *E. granulosus* that could be responsible for the transmission cycle. The data of a total of 50 infected individuals (age 17 – 78 years) was used. The cases involved patients with the diagnosis of echinococcosis, as well as ones with a doubtful diagnosis where echinococcosis was stated as one of the possibilities. The data was collected from biopsies and operations of patients from Riga Pauls Stradins Clinical University Hospital during years 2003 – 2013.

P.76 - *In vitro* larvicidal activity of garlic in goats

Nurulaini R[1,2]., Nik Ahmad Irwan[1], N.H., Hamdan,A[1].,
Premaalatha B[2]., Erwanas A.I[2]., and Chandrawathani P[2].

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800,USM, Penang, Malaysia
[2]Veterinary Research Institute, Department of Veterinary Services, 59, Jalan Sultan Azlan Shah, 31400,Ipoh, Perak, Malaysia

Livestock industry under the agriculture sector is an important contributor in the government Economic Transformation Programme (ETP) to ensure national food security. Infectious diseases such as parasitic worm infection are affecting the small ruminant industry thus causing animal mortality and morbidity as well as increasing cost for treatment. Many studies have been conducted on medicinal plants usage on animals which is very costly and time consuming. Therefore, this study was conducted to investigate *in vitro* larvicidal effect of aqueous extract of garlic bulb against strongyle nematode larvae (L3) of goats. In this study, *in vitro* larvae migration inhibition assay was conducted where larvae were incubated with aqueous garlic extracts at different concentrations of 5, 10, 20,40, 50,100, 150 and 200 mg/ml. For the control, larvae were incubated using distilled water and levamisole. Results showed that aqueous extract of garlic were able to inhibit the migration of third stage larvae at high concentration. Further study will be conducted in goats to evaluate garlic efficacy in reducing Worm Egg Count (WEC).

P.77 - Strongyle nematode helminth larvae in goats in Perak - A preliminary report

Nurulaini R[1,2]., Nik Ahmad Irwan[1], N.H., Hamdan,A[1].,
Premaalatha B[2]., Erwanas A.I[2]., Chandrawathani P[2]. and Ramlan, M[2].

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800,USM, Penang, Malaysia
[2]Veterinary Research Institute, Department of Veterinary Services, 59, Jalan Sultan Azlan Shah, 31400, Ipoh, Perak, Malaysia

Livestock industry is one of the important economic contributors not only in Malaysia but also worldwide and are a significant global asset with a value of at least $1.4 trillion. Livestock census from Department of Veterinary Services reported the total numbers of sheep and goats have increased in the country from 650, 518 heads in 2009 to 680,090 heads in 2010. However, one of the key constraints affecting animal productivity is diseases such as pneumonia and gastrointestinal worm infection which causes mortality and morbidity. Strongyle nematode infection mainly due to *Haemonchus contortus* was highly significant, especially in weaners. Therefore, this study was conducted to investigate strongyle nematode larvae species in local sheep and goats in Perak. Faecal samples were collected from six farms and larvae culture and species identification was conducted. Larvae were identified as *H. contortus* (38%), *Trichostrongylus* spp. (31.6%), *Cooperia* spp. (19.9%), and *Oesophagustomum* spp (12.4%). Results showed that *H. contortus* is still the predominant species affecting goats followed by *Trichostrongylus* spp. Haemonchosis can cause severe problems such as anemia, morbidity and even mortality. Further studies will be conducted to determine the strongyle nematode prevalence in Malaysia for more effective policies in worm control.

P.78 - Characterization of *Trichuris* Sp. isolated from rats in Sagamihara, Japan

Sayaka Kakuda[1] Kensuke Taira[1],Koichi Koyama[2],
Viliam Snabel[3], Hong-Kean Ooi[1],

[1]Department of Veterinary Medicine, Azabu University, Sagamihara-shi, Japan
[2]Department of Parasitology, Kitasato University School of Medicine, Japan
[3]Institute of Parasitology, Slovak Academy of Science, Kosice, Slovakia

In a routine parasitic fauna survey of rats in Sagamihara city, Japan, whipworms found in the rats were observed to be taxonomically distinct from those reported in the literature. Of the 35 *Rattus norvegicus* and 12 *R. rattus*, examined, whipworms were found in 6 (17.1%) of the former and 1 (8.3%) in the latter species of the rat. All whipworms were found parasitizing only in the caecum. Morphometry, phylogenetic analysis of the rDNA ITS1-5.8S-ITS2 region and experimental infection studies were conducted to identify this whipworm isolate. A total of 46 adult worms and 50 eggs of the whipworm were morphometrically measured. The whipworm was tentatively designated as *Trichuris* sp. (Sagami isolate). Oral inoculation of embryonated eggs of *Trichuris* sp. (Sagami isolate) into prednisolone-treated ddY mice, nude rats, SCID mice and wild *R. norvegicus* was not successful. *Trichuris* sp. (Sagami isolate) differed from the classical *T. muris* (E/J isolate, originally from UK but maintained in Japan) in the length of their spicules, distance between the posterior tip of testis and the worm posterior end, distance between the posterior tip of seminal receptacle and the worm posterior end as well as length and width of their eggs. The rDNA sequence of ITS1-5.8s-ITS2 region of *Trichuris* sp. (Sagami isolate) of *R. norvegicus* and *R.rattus* were completely identical but formed a different clade from that of *T. muris* and *T. arvicolae*, differing in 28.8-31.3% of nucleotides from these species, in a constructed phylogenetic tree. Thus, it is concluded that *Trichuris* sp. (Sagami isolate) is different from the classical *T. muris* and may probably belong to a new species.

P.79 - Ticks of dogs residing in beaches and fields fishing of central zone of Sinaloa, México

Rubio Robles Mario César, Gaxiola Camacho Soila Maribel, Castro del Campo Nohemí, Enríquez Verdugo Idalia, Borbolla Ibarra Jaime Eleazar, Cota Guajardo Silvia del Carmen, Gaxiola Montoya Joel, Pérez Corrales Ascención, , Quintero Osuna Isabel, Barraza Tizoc Claudia, Romo Valdez Manuel, Castro Angulo Antemio

Facultad de Medicina Veterinaria y Zootecnia,
Universidad Autónoma de Sinaloa, Culiacán, México

The ticks are bloodsucking ectoparasites, feeding on vertebrates, but particularly on skin of the dogs. They can be found infesting pets, residences, and gardens, and can act as reservoir or vector of zoonotic parasites, and potentially can infect the host causing pain and can transmit diseases such as Lyme disease, Rocky Mountain Spotted Fever, Typhus, Rickettsial, Tularemia, Babesia and Anaplasma. Generally, these different diseases are unique to different ticks which carry causal organisms of these diseases and can be confined to certain regional areas. The objective was to determine the prevalence of ticks in dogs of the central zone of Sinaloa, Mexico. The samples were determined for a representative sample with both sexes and cradle described by the technique of Thrusfield (2005) was used: n=[t*SD/L]2. Where n = sample size, t = value of the normal distribution (Student t) for a 95% confidence level (t = 1.96), L = accepted error or precision (5%), and SD = weighted disease prevalence (%). With this technique, the number of animals determined for random sampling was 320. Ticks were collected into identifed plastic bags and identified using a microscope. 267 dogs (83.44 %) were positive to ticks, and 100 % were *Rhipicephalus sanguineus*. Thus a large number of positive dogs can be a zoonotic potential risk to health of the pets and public of these recreation places. Because the area presents optimal characteristics for this ticks, it is necessary implement control strategies and education for the prevention of the infestations.

P.81 - Molecular characterization of zoonotic isolates of *Enterocytozoon bieneusi* in Iran

Sadraei J.[1], Pirestani M.[1], Forouzandeh M.[2]

1- Parasitology Dept., Faculty of Medical Sciences,
2- Tarbiat Modares University, Tehran, Iran.
3- Biotechnology Dept., Faculty of Medical Sciences,
4- Tarbiat Modares University, Tehran, Iran.

Microsporidia infections occur in all invertebrate and vertebrate hosts. The most common microsporidia infecting humans and animals are *Enterocytozoon bieneusi*. This study aimed to characterize the zoonotic isolates of *E. bieneusi* using a PCR among the slaughtered cattle in Tehran. In this descriptive study, 126 fecal samples from slaughtered cattle in Tehran were analyzed for *E. bieneusi*. A transcribed spacer region (500 bp) for rRNA gene of *E. bieneusi* was amplified using a nested PCR technique. For genotyping, positive samples were sequenced and the phylogenetic tree was reconstructed to determine the relationship between the isolates from human, animal and zoonotic isolates. Nineteen out of 126 *E. bieneusi* PCR-positive samples were sequenced. A high degree of genetic polymorphism, represented by four genotypes (IREb4, IREb5, D, M), was found among the *E. bieneusi* isolated from cattle. In this study, the most common genotypes were D (38.6%), M and IREb4 (26.3%), respectively followed by IREb5 (10.5%) in the next stage. In phylogenetic analysis, 89.5 percent of the isolates (D _IREb4 _ IREB5) formed a distinct cluster consisting of genotypes from humans and other domestic animals, but one genotype clustered as *E. bieneusi* genotypes taken from cattle and pig. Only some *E. bieneusi* isolates taken from cattle may be of public health importance. However, further studies should be conducted on cattle and other hosts to determine the role of animals in the transmission of infection to human.

P.81 - Anthelmintic effect of green synthesized silver nanoparticles on some biochemical parameters of *Haemonchus contortus*

Shabad Preet and Rahul Singh Tomar

Department of Zoology, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra-282005, India.

The present work was aimed to evaluate the efficacy of silver nanoparticles (AgNPs) synthesized by using the aqueous extract of Ziziphus jujuba leaves against a gastrointestinal nematode of small ruminants, Haemonchus contortus. In vitro assay was based on egg hatch assay (EHA) and adulticidal study. The highest concentration induced 91 ± 1.76 percent egg hatch inhibition. IC_{50} and IC_{90} values for EHA was 0.007 ppm and 7.71 ppm in case of AgNPs treatment and 301.83 ppm and 849.43 ppm in Z. jujuba leaf extract (aqueous). LC_{50} and LC_{90} value for in vitro effect on adult H. contortus was 15.29 ppm and 33.87 ppm in case of AgNPs treatment and 942.68 ppm and 2047.50 ppm in Z. jujuba leaf extract (aqueous). Remarkable changes were induced by AgNPs on the eggs hatching which is evident from shrinkage and disintegration of embryo when compared to control. Relatively less damage was observed in aqueous Z. jujuba extract treated eggs. Coiling was observed in adults following treatments with AgNPs and Z. jujuba leaf extract. In biochemical analysis, the glycogen, lipid and protein content decreased very significantly ($P<0.05$) with increasing concentration of both green synthesized AgNPs and Z. jujuba leaf extract. The overall findings of the present study have shown that our experimental plant extracts contains reducing properties for the synthesis of AgNPs which in turn proved to decrease the glycogen, lipid and protein content very significantly with increasing concentrations of AgNPs and posses potent anthelmintic properties.

P.82 - Epidemiological investigation on botulism of cattle In Northern Gyeonggi- do between 2011 and 2012

Wooseog Jeong, Oun-Kyong Moon, Hachung Yoon,*
Woo Seok Chang, Do-Soon Kim

Animal and Plant Quarantine Agency, Anyang,
Gyeonggi-do, 430-82, South Korea

Botulism (types A to G) is neuroparalytic disease caused by a toxin produced by anaerobic bacteria *Clostridium botulinum*. Most incidents of botulism in cattle are associated with contaminated feeds, carcasses of small animals present in the environment and broiler litter. The aim of this study is to provide knowledge on the recent outbreak of Botulism in Pocheon and Yeoncheon, Gyeonggi-do between August 2011 and July 2012 which specifically occurred in cattle farms (24 farms, 431 cattle). It is necessary to find out the causes and potential risk factors of the outbreak and apply it to bio-security policies. The initial case occurred in summer on the 29th August 2011 that started preventive vaccination against mosquito borne diseases. Later they confirmed *C. botulinum* and started vaccinating against botulinum toxin type B, C and D in February 2012 on affected farms. The geographical range of vaccination was widened and the outbreak was stabilized. Most incidents of botulism in cattle are believed to be associated with the ingestion of preformed toxin (foodborne botulism), due either to the primary growth of *C. botulinum* in spoiled feeds (forage botulism) or to the contamination of feed with toxin-containing carrion (carrion-associated botulism). Disease in cattle is produced primarily by types B, C and D. Most non-carrion-associated botulism is due to type B toxin, whereas type C and D toxins are usually associated with the putrefied carcasses of birds or small animals that have contaminated water, feed or the environment. The study concludes that the outbreak was caused by *C. botulinum* due to flooding as confirmed by botulinum type B, C, D in environmental specimens including fowl droppings.

P.83 - Diagnostic-kit candidate to detect dengue antibody, using coaglutination method, utilizing protein a positive *Staphylococcus aureus* as a carrier

Eka Noneng Nawangsih

Department of Microbiology, Faculty of Medicine,
Jenderal Achmad Yani University, Indonesia.

The principle employed in this diagnostic-kit is the utilization of the ability of protein A, which could be found in the walls of *Staphylococcus aureus,* to bind Fc imunoglobulin fractions; without altering the ability of that immunoglobulin to bind antigen. To enhance the diagnostic-kit's sensitivity and specificity, chicken IgY is used as one of it's component. Since protein A cannot be bond with chicken IgY, indirect methods is then used. Rabbit immunoglobulin chicken anti-IgY is used as an intermediary connection between protein A positive *S. aureus* with chicken IgY. Those components are then prepared as A solution and B solution. The A solution contains *S. aureus* Cowan I and rabbit immunoglobulin chicken anti-IgY serum. While the B solution contains anti-dengue chicken serum and dengue antigen. The laboratory experiments produced a desirable AB composition, with the formula of: A : B = 1~3 : 1~3 (v/ v). This composition had been, then, tested to the samples of human serum and then compared with the results of previously done ELISA IgM and IgG tests, as the gold standard. The test to the 65 samples resulted in: 47. 69 % (31/ 65) true positive, 4.61 % (3/ 65) false positive, 41. 54 % (27/ 65) true negative, and 6. 15% (4/ 65) false negative. The sensitivity and specificity compared to the ELISA results showed 89% and 90% respectively. The chi-square analysis to the sensitivity and specificity proved that there were no significant differences between the sensitivity and specificity of the diagnostic-kit candidate compared with the ELISA results ($p > 0.05$).

P.84 - Treatment of oropharyngeal candidiasis in patients infected with HIV by herbal medicine

Samira Salari *[1, 2], *Ali Reza Khosarvi* [3], *Seyyed Amin Ayatollahi Mousavi* [1, 2], *Gholam Reza Nikbakht Brojeni*[4]

[1]*Department of Medical Mycology and Parasitology, School of Medicine, Medical University of Kerman, Kerman, Iran*
[2]*Research Center for Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran*
[3]*Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[4]*Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran,*

Oropharyngeal Candidiasis (OPC) continues to be considered the most common opportunistic fungal disease in HIV/AIDS patients globally. The present study was undertaken to determine the antifungal susceptibility of *Candida* species isolates were obtained from Iranian PLWH (people living with HIV) and cultured on CHROMagar and Sabouraud's dextrose agar. All isolates were identified according to assimilation profile, germ tube, colony color and other conventional methods. Disk diffusion testing and Broth Micro dilution of Fluconazole according to the methods described in CLSI was performed. In addition, Cuminum cyminum essential oil was used to evaluation in vitro activity it's against against fluconazole resistant and susceptible *Candida albicans*. In our study, *C. albicans* (50.2%) and *C. glabrata* (22%) were the most frequent isolated, from these isolates, 25.7% were resistant to fluconazole (MIC 64 µg/ml. Complement data showed mean MIC, 0.575% ± 0.6810% (range: 0.25%-2%) for *Cuminum cyminum* essential oil to Fluconazole-resistant *C. albicans* isolates and mean MIC, 0.306% ± 0.2640% (Range, 0.125%-0.5%) for susceptible *C. albicans* isolates with significantly difference ($p<0.001$). Based on our result we conclude that screening of resistance candida isolates in clinical laboratory is idealistic for surveillance of antifungal resistance to patient's managements, And *Cuminum cyminum* essential oil having antifungal properties, can be helpful to treat of candidiasis.

P.85 - Differences of MRI features between tuberculosis and bacterial spondylitis: A report from the endemic area

Thammaroj, J.[1], Kitkuandee, A.[2], Sawanyawisuth, K.[3,4]

Department of [1]Radiology, [2]Surgery, [3]Medicine, Faculty of Medicine, Srinagarind Hospital, Khon Kaen, Thailand, 40002
[4]The Research and Training Center for Enhancing Quality of Life of Working-Age People, Khon Kaen University, Khon Kaen, Thailand

Pyogenic and tuberculosis spondylitis are the common causes of infectious spondylitis. The purpose of this study is to determine the specific MRI findings that can differentiate these two types of infectious spondylitis. This study retrospectively analyzed MRIs in patients diagnosed as infectious spondylitis from January 1, 2005 to December 31, 2009. Tuberculosis spondylitis was diagnosed by histopathological findings of caseous granuloma, while pyogenic spondylitis was diagnosed by positive cultures of tissue, fluid, or blood. The MR findings were recorded in terms of location and extension of the lesions, and the presence or absence of findings of specific imaging criteria. Statistical analysis was performed with the Fisher's Exact Test. A significant difference was considered at p value < 0.05. During the study period, there were 33 patients who met the criteria. Of those, 24 patients were tuberculosis spondylitis and 9 patients were pyogenic spondylitis. Two suggestive findings for tuberculosis spondylitis were thin and smooth abscess walls (75% in tuberculosis spondylitis vs 0% in pyogenic spondylitis, p < 0.001) and well defined paraspinal soft tissue (66.7 % in tuberculosis vs 11.1 % in pyogenic, p 0.007). Thin and smooth abscess walls, and well defined paraspinal soft tissue were two MRI findings that may be helpful to differentiate between tuberculosis and pyogenic spondylitis.

P.86 - Potency of soy milk probiotics as biotherapy in gastrointestinal infections

Sayu Putu Yuni Paryati, Eka Noneng Nawangsih, Evi Silvia, Danirmala Lalita

Medical Faculty, University of Jenderal Achmad Yani, Indonesia

Probiotic bacteria may counteract the inflammatory process by stabilizing the gut microbial environment and the intestine's permeability barrier, and by enhancing the degradation of enteral antigens and altering their immunogenicity. Most commercial products of probiotics have been derived from food sources, especially cultured milk products. Unfortunately, not everyone can enjoy the probiotic benefits of dairy yogurt because they're lactose intolerant or allergic to milk. One alternative for this problem is probiotics from Indonesian soy milk. This research aims to determine the growth of *Lactobacillus acidophilus* colonies in soy milk medium with sucrose added (0%, 5%, 10% and 15%) and to know the influence of soy milk added with 10% glucose to the growth of *L. acidophilus* in the digestive tract of neonatal mice. Growth of bacteria were counted by Total Plate Count (TPC) at 12 hour, 24 hour, and 48 hour after the tretment. Mice divided into three groups. There are negative controls, *L. acidophilus* and *L. Acidophilus*+soy milk+10% glucose. The results indicate that *L. acidophilus* can grow well in soy milk medium, and the best growth of *L. acidophilus* is in the soy milk with 15% sucrose, followed by 10%, 5% and 0% of sucrose. In soymilk medium with 10% glucose, *L. acidophilus* grow better ($p<0,05$) in passing through the digestive tract. There was an increase the growth of *L. acidophilus* in the GI tract at 12 hour after the treatment, and the maximum growth of *L. acidophilus* occurs at 24 hour after the treatment.

P.87 - Discriminative value of elevated transaminases for dengue fever (DF) and dengue haemorrhagic fever (DHF)

Dr.Somia Iqtadar[1], Nabeel Akbar[2],Naeem Majeed[3],Numan Majeed[3]

[1] Department of Medicine, King Edward Medical University Lahore, Pakistan
[2] Department of Medicine, Sir Ganga Ram Hospital, Lahore ,Pakistan
[3] Services for Public Health and Research (Sphere),Lahore, Pakistan

To assess the relationship of liver derangement with Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) using elevated transaminases level, 220 seropositive cases of DF and DHF who were admitted to Mayo Hospital Lahore in 2013 epidemic were retrospectively studied. There were more (60%) DHF patients with hepatomegaly compared to DF (40). Analysis of the liver profile showed that liver dysfunction was more common in DF compared to DHF (38.15 vs 18.6%), indicating that the degree of liver impairment is not related to the severity of Dengue infection. The mean (range) serum AST levels were 98.92(50-280) U/l in DHF and 68.2(20-287) U/l in DF. Although patients with elevated transaminases were more commonly male but the mean value of AST and ALT were higher in females (87.3 vs 73.0 and 84.1 vs 71.7) respectively. Both ALT as well as AST were most elevated in middle age group 31-40. (ALT 104.5, AST 94.3) while least elevated in patients above the age of 50 (60.2 and 53.6 respectively).None of the patients developed fulminant hepatitis. This study showed that there was a significant overlap in ALT and AST values among patients of DF and DHF showing a poor discriminatory value of these enzymes in DF and DHF

P.88 - Ten years follow up of HAART in Cambodian children – Cohort study in 121 children

Irad Beldjebel[1] , [2]Andrea Sahum[1], MCH Vichet[1] Gorge Benca[1], Veronika Sladeckova[1], Silvia Foretkova[1], Jaroslava Brnova[2,3], Vladimir Krcmery[2]

[1]St. Maximilian Kolbe Clinic, Tropical programme St. Elizabeth University of Health and Social Sciences, Phnom Penh, Cambodia
[2] Slovak Tropical Institute, St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia
[3]Department of Laboratory Medicine, School of Health Science and Social Work, Trnava University, Trnava, Slovakia

Failure of first line and second line HAART is one of major concerns in assessment of successfully treatment of HIV in children. The purpose of this study was to define the failure risk of first line antiretroviral therapy (ART) within 10 years of follow up in HIV positive and ART naïve children in Phnom Phen Cambodia. One hundred twenty-one (121) children were assessed, of them 118 were followed up 9-10 years. CD4 cells counts and viral load (VL) was measured every 6 months or when CD4 drop has been observed, consequently minimally twice. Laboratory and genotypic resistance tests were performed in case of clinical or immunological failure by National Paediatric Hospital laboratory affiliated to Institute Pasteur Cambodia, Phnom Penh. First line therapy was consisted with Zidovudine + Lamivudine + Nevirapine or Efavirenz in those with concomitant tuberculosis infection. Within 8-10 years follow up only 20 of 121 children (17%) failed during 10 years of first line ART. Median time from the onset of therapy to the failure was 8,5 years (5-9 years). Major cases of failure were non-compliance, treatment outside of the project in complete family children. All our children were placed in 2 orphanages with good nutrition (five times a day) on inpatient basis, which contribute to low failure rate. Paradoxically orphan status, because of better compliance due to fulltime facility treatments was protective, comparing non-compliance, which were the major risk factors of failure of first line HAART.

P.89 - DNA barcoding: Complementing morphological taxonomy of mosquito species in Singapore

Abigail Chan[1], Chiang Lee Pei[2], Hapuarachchige Chanditha Hapuarachchi[1], Tan Cheong Huat[1], Pang Sook Cheng[1], Ruth Lee[1], Lee Kim Sung[1], Ng Lee Ching[1], Lam-Phua Sai Gek[1]

[1]Environmental Health Institute, National Environment Agenc,11 Biopolis Way, Helios Block, #06-05/08 Singapore 138667
[2]Nobicell Biosciences, 48 MacTaggart Road #03-02, MAE building, Singapore 368088

Standard identification of mosquito species is achieved with the aid of taxonomic keys that utilize morphological characteristics. However, morphological identification can be challenging when the expertise is lacking and characters are either missing or damaged. With the advancement in molecular techniques, we explored the use of mitochondrial cytochrome C oxidase subunit 1 (COI) gene as a DNA marker to identify mosquito species in Singapore. Here, comparing both morphological taxonomy and DNA barcoding, we seek to establish a more reliable identification system for the mosquito species found in Singapore. We analysed 129 adult mosquito specimens, belonging to 46 species of 11 genera. Phylogenetic analyses were performed for Aedes, Anopheles, Culex and other genera of mosquitoes and the distinctive clustering of different species was compared with their taxonomic identity. The COI barcode accurately identified 91% of the species analyzed here. We also report COI sequences of 18 mosquito species which were not available previously in the GenBank database. Our study presents the first evidence of utilizing DNA barcoding for the identification of mosquito species in Singapore. COI-based DNA barcoding is a useful tool to complement taxonomy-based identification of mosquito species.

P.90 - Microevolution of dengue virus 1 genotype III during the 2013 epidemic in Singapore

Carmen Koo, Hapuarachchi Hapuarachchige Chanditha, Kek Relus, Rajarethinam Jayanthi, Zhao Yasi Jacqueline, Lai Yee Ling, Pok Kwoon Yong, Lee Kim Sung, Ng Lee Ching

Environmental Health Institute, National Environment Agency, Singapore

Singapore has experienced the worst known dengue epidemic in its history with over 22000 cases reported by the end of 2013. This unprecedented increase in dengue incidence coincided with a switch from DENV-2 to DENV-1 in early 2013. Since then, DENV-1 has been the predominant serotype, contributing to 61% (n=4537) of dengue cases serotyped in 2013. In the present study, we analysed 466 envelope (E) genes and 6 whole genomes of DENV-1 strains obtained from different parts of the country since 2012 to determine their ancestry, genetic characteristics and evolution during the epidemic. Our phylogenetic analysis revealed that the epidemic strain belonged to genotype III of DENV-1 and it was first detected in Singapore in the last week of October 2012. Although the current strain shared a South and Southeast Asian ancestry, it was genetically distinct from those reported previously in these regions. The nucleotide analysis of E gene sequences revealed extensive heterogeneity within the epidemic strain of DENV-1 genotype III. We identified 12 variants of DENV-1 genotype III isolates, each of which was characterized by fixed nucleotide substitutions. The variants showed a spatial-temporal distribution pattern, indicating a consistent micro evolutionary process within the DENV-1 genotype III population during the epidemic period. The intense transmission is likely to have driven the in-situ evolution of DENV-1 genotype III strain to generate a highly heterogeneous virus population. Further investigations are required to determine whether those micro genetic changes were resulting from host adaptation of the virus during the epidemic.

P.91 - Micro-environment as a factor of recrudescence of cutaneous leishmaniasis in Foum Jamâa (Azilal, Morocco)

Arroub Hassan[1], Lemrani Meryem[2] and Habbari Khalid[1].

[1]Laboratory of Parasitology, Faculty of Sciences and Technics, Sultan Moulay Slimane University, M'GHILA Route de Fes, B.P. 523, Beni Mellal, Morocco.
[2] Laboratory of Leishmaniasis, Pasteur Institut of Marocco, Casablanca, Morocco.

Leishmaniasis is a parasitic diseases with a wide range of clinical symptoms and currently threaten 350 million persons in 88 countries. Cutaneous Leishmaniasis (CL) has been reported in many areas of Morocco including the province of Azilal. The region of Foum Jamâa (province of Azilal in the Atlas of Morocco) has become an endemic for CL. This study summarize the result of repartition of the 479 positive cases according to micro-environmental factors that may act as a factor of recrudescence of this parasitic disease. These epidemiological assessments were conducted from January 2006 to December 2009. Among CL positives cases, free distribution tests were used to analyze the effect of each factor. No association between gender and the rate of Leishmaniasis was observed, while the highest rate of positive lesions was found in the age group of 10 years or under. The distribution of positive cases was more significantly influenced by environmental factors common to each sector (Altitude, Sewerage, Garbage, etc.) than by individual specific lifestyle (Habitat type, source of light, breeding, dogs, etc.). The survey yielded many recommendations to be made in planning a more accurate program of Leishmaniasis Control and many preventive actions to be undertaken to tone down the drawbacks of urbanization.

P.92 - Evaluation of knowledge, attitude and performance of the population affected by cutaneous leishmaniasis in Morocco (Foum Jamâa, Azilal Case)

Hassan Arroub[1], Khalid Habbari[1]*, Hassan Latrache[2]**

[1]Laboratory of Management and Valorization of Naturals Resources,
[2]Laboratory of Valorization and Food Safety
FST, Sultan Moulay Slimane University, M'GHILA Route de Fes,
B.P. 523, Beni Mellal, Morocco.

Cutaneous leishmaniasis (CL) is one of the health problems of many tropical and subtropical regions and is endemic in many parts of Morocco. The region of Foum Jamâa (province of Azilal in the Atlas of Morocco) has become an endemic for the disease. Education about health for high risk population seems to have a critical role in prevention of leishmaniasis and therefore evaluation of knowledge, attitude and performance are of importance. For the success of prevention and control programs of any disease, the most important prerequisite is community participation. Program implementers need to understand the disease-related knowledge, attitude, and practices of the community, because these are the important determinants of community participation. There is no data from Foum Jamaà focusing on these aspects, and thus this study presents the information related to CL. In this study, 655 patients were studied. Among CL positives cases, free distribution tests were used to analyze the effect of each factor. According to our results, no association between gender and the rate of Leishmaniasis was observed, while the highest rate of positive lesions was found in the age group of 10 years or under. A large mass of the patients had inappropriate knowledge, attitude and performance score about leishmaniasis highlighting the fact that of the Foum Jamaà population needs practical education about combating with leishamaniasis. To close the gap between the knowledge and practice of the patients, face to face education and use of instructional aides are recommended.

P.93 - Epidemiology of imported malaria in Morocco

*R. Harbous[1], F-Z Morchid[1], KH. Habbari[1], N. EL Maimouni[2] and M. Bachaoui[1]**

[1] *Sultan Moulay Slimane University, Faculty of Science and Technology, BeniMellal Morocco*
[2]National Institute of Hygiene, Morocco

Malaria is a public health problem in endemic countries. In Morocco, this infection has not been recorded locally since 2004. However, the problem of imported malaria persists and poses a risk of reintroduction of the disease in Morocco. In order to assess the situation of the imported malaria in Morocco, we studied the main epidemiological characteristics of malaria infections diagnosed during last decade. The Study of 416 cases of confirmed imported malaria allowed to identify the characteristics of this parasites in Morocco. Most cases are Moroccan peoples (about 64%) older than 15 years and have resided in sub-saharan Africa. *Plasmodium falciparum* is the most commonly imported plasmodium (about 83%), especially during the summer holidays. We present at the 6th ACTMP the evolution and the spatial distribution of cases detected and precautions to be taken by the competent authorities to avoid the risk of introduction or re-emergence of malaria in Morocco.

P.94 - Retrospective study of the cutaneous leishmaniasis and first part of the molecular diagnosis of the parasite circulating in the area Souss-Massa-Daraa (Morocco)

Nezha Mejjati[1], Khadija Lahbab[1], Faiza Sebti[2], Khalid Habbari[1], Mostafa EL Louali[1]

[1]Laboratory of Parasitology, Faculty of Sciences and Technics, Sultan Moulay Slimane University, M'GHILA Route de Fès, B.P. 523, Beni Mellal, Morocco.
[2]National Institut of hygiene, Rabat, Morocco.

To cope with the problems which Leishmaniasis can generate, the Ministry for Health considered in 1997, to work out a national program of fight called "Program of fight against the Leishmaniasis" (PLCL), and to create a national reference laboratory in Leishmaniasis (LNRL) responsible to form and install laboratories of the disease within the endemic provinces of Morocco. Thanks to active tracking and the obligatory declaration of the disease all the cases are declared; what makes it possible to follow the evolution of the disease in the various regions of the country and to envisage the fight plan. Since the year 2000, the laboratory received all samples from the various laboratories of the kingdom for the quality control. From the results of control, a synthesis of the periodic data proved to be essential to know the state of the Leishmaniasis and its evolution in the course of time in the various provinces. Within this framework, our work was integrated and consisted in making: - A retrospective study of the evolution of the cutaneous leishmaniasis during the 10 last years in the area of the south "Souss-Massa-Draa" (SMD) (number of cases, sectoral distribution, distribution of the cases according to the age and the sex), while exploiting: * Data of the direction of epidemiology and fight against diseases. * Information sheets on the level of the National reference laboratory of Leishmaniasis (LNRL). A data analysis of control and confirmation of the diagnosis of leishmaniasis of area SMD. A first stage of the molecular diagnosis by PCR ITS1 of the parasite in the most affected sectors area while basing itself on the blades received on the level of the LNRL during the year 2010. Our results will be useful in a forthcoming study to make the enzymatic digestion of the obtained PCR ITS1 products, which will enable us to identify the species of leishmania circulating in the study area

P.95 - *Heligmosomoides polygyrus* larva 3rd extract inhibited Tnf-A expression in mice induced colitis

Agustina Tri Endharti[1], Aswin D. Baskoro[1], Eviana Norahmawati[2], Donabella F. Santosa[1] ,Mangkubumi P Wijaya[1]*

Department of Parasitology, Faculty of Medicine, University of Brawijaya[1], Department of Pathology, Anatomy Faculty of Medicine, University of Brawijaya[2]

Ulcerative colitis is an ulcero inflammatory disorder that affects the colon and chronically caused by an imbalance of the immune system. The aim of this study was to determine the effects of *Heligmosomoides polygyrus* extract on the pathogenesis of DSS-induced colitis. *Heligmosomoides polygyrus* extract can be used as an alternative therapy to help the balance of the immune system. Extracts of *H. polygyrus's* colon can enhance Th2 and suppress Th1 improvement. This study showed the expression of TNF-α in Balb/c mouse with and without treatment of *H. polygyrus* extract. There are 5 groups, there were the negative control group, positive control and three groups with *H. polygyrus* extract at various doses (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg, respectively. Mice were induced colitis by 1-week oral exposure to 3% DSS in drinking water, then treated with *H. polygyrus* extract for 12 weeks and followed by RNA isolation to detected TNF-α expression. *Intracellular* expression of TNF-α on each group also were assessed by flow cytometer. One way ANOVA showed both of TNF- α expression decreased, at a dose of 0.4 mg/kg body weight. It can be concluded that the extract of *H. polygyrus* at 0.4 mg/kg body weight dose could reduce the expression of TNF-α in mice induced colitis.

P.96 - Ursolic acid from *Nyctanthes arbortristis*: A potential compound for the treatment of bancroftian filariasis

*Prasanta Saini, Prajna Gayen, Niladri Mukherjee, Suprabhat Mukherjee, *Santi P. Sinha Babu*

Parasitology Laboratory, Department of Zoology (Centre for Advanced Studies), Visva-Bharati University, Santiniketan- 731 235, West Bengal, India

Lymphatic filariasis is a neglected tropical disease. The World Health Organization considers lymphatic filariasis to be a global health problem affecting 120 million people in over 81 tropical countries. One-sixth of the world population is at risk of infection. In this paper, we are reporting that ursolic acid (UA) showed significant micro- as well as macrofilaricidal activities and it was isolated from the leaves of *Nyctanthes arbortristis.* The antifilarial efficacy was tested against the oocyte, microfilaria and adult of *Setaria cervi,* a bovine filarial parasite by dye exclusion test and MTT reduction assay. Significant microfilaricidal activity of UA was further proved against mf of *W. bancrofti* by viability assay. UA causes apoptosis in different life cycle stages of filarial parasite, which was proved TUNEL, Hoechst staining, Annexin V-Cy3, flow cytometric analysis and DNA fragmentation assay. Differential expressions of pro- and anti-apoptotic genes were observed at the transcription and translational level in a dose-dependent manner. Depletion in the worm GSH level and elevation in the parasite GST, SOD and super oxide anion indicated the generation of ROS. In this investigation we are reporting for the first time that UA acts its antifilarial effect through induction of apoptosis and by downregulating and altering the level of some key antioxidants like GSH, GST and SOD of *S. cervi*. The findings thus provide a new lead for development of a suitable filaricide from natural products. UA may serve as a new promising agent in the treatment of bancroftian filaraiasis.

P.97 - Seasonal fluctuations of Phlebotomine sand fly populations (Diptera: Psychodidae) in a focus of cutaneous leishmaniasis in Foum Jamâa (Azilal), Atlas of Morocco

Arroub Hassan[1]., Lemrani Meryem[2],Kouider Nourreeddine[3], Habbari Khalid[1].

[1]Laboratory of Parasitology, Faculty of Sciences and Technics, Sultan Moulay Slimane University, M'GHILA Route de Fes, B.P. 523, Beni Mellal, Morocco.
[2] Laboratory of Leishmaniasis, Pasteur Institut of Morocco, Casablanca, Morocco.
[3]Laboratory of Industrial engineering, Faculty of Sciences and Technics, Sultan Moulay Slimane University, M'GHILA Route de Fes, B.P. 523, Beni Mellal, Morocco.

Leishmania infection is transmitted to human host as a result of a bite by an infected female sand fly of the genus Phlebotomus on exposed parts of the human body. we carried out an entomological survey of Phlebotomine sand flies (Diptera: Psychodidae) in Foum Jamaà during January–December 2010 . Morphological identification was performed on a total of 1152 sand flies (23% females and 77% males) collected by sticky paper traps. 80% of the total collected flies were identified as Phlebotomus (Paraphlebotomus) sergenti (Parrot) (57%) and Phlebotomus (Larroussius) longicuspis (Nitzulescu) (23%). In addition to these dominant species, four other species were found, Phlebotomus (Phlebotomus) papatasi (Scopoli), Sergentomyia (Sergentomyia) minuta (Rondani), Phlebotomus (Larroussius) perniciosus(Newstead) and Phlebotomus (Paraphlebotomus) chabaudi (Croset). Overall, the population dynamics show a yearly bimodal pattern related to rainfall and temperature, and with high density around human dwellings. The spatiotemporal distribution of sand fly species was helpful to discuss strategies that might be useful in controlling cutaneous leishmaniasis transmission in this endemic focus.

P.98 - Poc diagnostic tool for rapid detection of influenza A/H1N1 virus in human clinical specimens using labchip real-time PCR

[1]Chun-Ying Li, [1]Hyun-Ok Song, [1]Je-Hyoung Kim, [2]Ho-Sun Ryu, [2]Dong-Hoon Lee, [2]Sun-Jin Kim, [2]Deog-Joong Kim, [3]In Bum Suh, [4]Soo Ho Lee, [4]Seung Taek Yu, [2*]Sung-Woo Kim, [1*]Hyun Park and [4*]Du Young Choi

[1]Zoonosis Research Center, Department of Infection Biology, Wonkwang University School of Medicine, Iksan, Jeonbuk, Republic of Korea
[2]NanoBioSys Inc., 68-18 Gasan-dong, Geumcheon-gu, Seoul, Republic of Korea
[3]Department of Laboratory Medicine, Kangwon National University School of Medicine, Chuncheon, Kangwon-do, Republic of Korea
[4]Department of Pediatrics, Wonkwang University School of Medicine, Iksan, Jeonbuk, Republic of Korea

It is important to detect influenza A virus using a fast, portable, and accurate system that has high specificity and sensitivity. To achieve this goal, it is necessary to develop a highly specific primer set that recognizes only influenza A viral genes and a rapid real-time PCR system that can detect even a single copy of the viral gene. In this study, we developed and validated a novel fluidic chip-type real-time PCR (LabChip real-time PCR) system that is sensitive and specific for the detection of influenza A/H1N1. This LabChip real-time PCR system has several remarkable features: (1) It allows rapid quantitative analysis, requiring only 15 min to perform 30 cycles of real-time PCR. (2) It is portable, with a weight of only 5.5 kg. (3) The reaction cost is low, since it uses disposable plastic chips. (4) Its high efficiency is equivalent to that of commercially available tube-type real-time PCR systems. The developed disposable LabChip is an economic, heat-transferable, light-transparent, and easy-to-fabricate polymeric chip compared to conventional silicon- or glass-based labchip. The efficiency of LabChip real-time PCR system was confirmed using novel primer sets specifically targeted to the hemagglutinin gene of influenza A/H1N1 and clinical specimens. Eighty-five human clinical swab samples were tested using the LabChip real-time PCR. The results demonstrated 100% sensitivity and specificity. These results were identical to those from a tube-type real-time PCR system. This indicates that the novel LabChip real-time PCR could be a point-of-care diagnostic tool for influenza A/H1N1 with a high sensitivity and specificity.

P.99 - Efficacy of combination therapy (Metronidazole and/ or Artemether) in experimental giardiasis and its Impact on non-enzymatic oxidative stress biomarkers

Eman M. Aly, Hoda Y. Sabry, Zeinab H. Fahmy, Rabab S. Zalat

Department of Parasitology, Theodor Bilharz Research Institute, Imbaba, P.O. Box 30, Egypt

Giardia lamblia trophozoites colonize in the upper small intestine resulting in diarrhea and various clinical manifestations including abdominal pain, anorexia and signs of malabsorption. A decrease in the level of trace elements due to this absorption deficiency resulting from giardiasis might occur. Experimentally, the excretory secretory products of G. lamblia trophozoites increased the level of reactive oxygen species in mice enterocytes. This study was designed in order to reveal the changes in iron (Fe), manganese (Mn), copper (Cu) and chromium (Cr) serum levels pre and post treatment with the combination therapy of metronidazole (MTZ) and artemether (ART) in experimentally Giardia-infected hamsters. A secondary objective is to evaluate the impact of this therapy on serum levels of bilirubin, uric acid and albumin as non-enzymatic oxidative stress biomarkers. Hamsters were divided into 4 groups: control group I including 2 subgroups Ia (non-infected, non-treated) and Ib (infected non-treated); group II (infected and treated with MTZ); group III (infected and treated with ART); and group IV (infected and treated with combination therapy (ART+MTZ)using half the dose for each drug. Hamsters of all four groups were sacrificed 5 week post-infection, i.e. 2 weeks after treatment to evaluate drug efficacy. Stool samples and duodenal contents were examined to count the number of G. lamblia cysts and trophozoites, respectively. Blood samples were also collected to estimate trace elements (Fe, Mn, Cu and Cr) as well as non-enzymatic oxidative stress biomarkers (bilirubin, uric acid and albumin). There was a significant reduction of trophozoite count in intestinal tissue following treatment with ART alone (88%) as compared to infected control (Ib). Group IV given both drugs in reduced dose also yielded a very high percentage of reduction in both trophozoite (98.3%) and cyst counts (95.5%). Uric acid, on the other hand, increased in infected control (Ib) as compared to normal (Ia). Treatment with either ART or MTZ decreased uric acid levels lower than normal level. The combination of both drugs (group IV) normalized serum uric acid levels in hamsters. The trace elements in serum of infected hamsters displayed decrease in Fe and Mn, as compared to their levels in hamsters of group Ia, while Cu levels increased in the infected group and were still increased even after treatment in all groups.The effect of giardiasis on the changes in the level of trace elements and non-enzymatic oxidative stress biomarkers is relevant in this work. The combination therapy normalized these parameters with significant parasite eradication. Further studies are needed to evaluate these data in under-nourished and chronically infected hamsters.

P.100 - Impact of climate change on the dengue vector, *Aedes aegypti* in different agro-climatic zones of Tamil Nadu, India

Kalimuthu Kovendan[1], Kadarkarai Murugan[1], Palanisamy Mahesh Kumar[1], Savariar Vincent[2],*

Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore - 641 046, Tamil Nadu, India.

Centre for Environmental Research and Development, P.G. Research & Department of Advanced Zoology and Biotechnology, Loyola College, Nungambakkam, Chennai – 600 034, Tamil Nadu, India.

Aedes aegypti, is responsible for dengue fever in India where the number of dengue fever cases has increased significantly in recent years. Dengue is a major public health problem worldwide, especially in the tropical and subtropical regions. *Solanum elaeagnifolium* as a natural pesticide against *A. aegypti* a potent dengue vector. In the present study, the mosquito breeding sites have been made at different temperature regions of Tamil Nadu, India, viz., Ooty (25ºC) with the elevation of 2,623 m., Coimbatore (33.7°C), Madurai (37.5°C), Sivakasi (40.2ºC), Chennai (43.4ºC). Biopesticides such as plant extract have been used to control the vector mosquito of *A. aegypti*, laboratory lethal toxicity different larval stages of mosquitoes. To study the impact of different temperatures on the mosquito prevalence at different agro-climatic regions of Tamil Nadu, India. Colonization of *A. aegypti*, and mosquito culture, Collection of plant materials, Preparation of plant extracts, Larval and pupal toxicity test of plant extract, Different temperatures, Statistical analysis. The *S. eleagnifolium* methanol leaf extract in different temperatures against dengue vector, *Ae. aegypti*. The larval and pupal mortality was observed after 24 hrs of exposures. The values of LC_{50} = 484.09, 574.58, 700.85, 818.38 and 1222.26 ppm; LC_{90}=1019.65, 1199.54, 1399.68, 1586.81 and 2154.81 ppm, respectively. Results with $P< 0.05$ were considered to be statistically significant. This plant extract clearly indicates that the climate change does not affect the plant pesticide efficacy (degradation) whereas in the chemical or synthetic pesticides the degradation capacity was very low and the residues were left back in the environment which also plays a role in the climate change. Therefore, the use of plant-based insecticides could substantially inhibit the insecticide resistance considerably. This ideal eco and user-friendly vector control strategy could minimize the dengue burden and eventual elimination in the near future.

P.101 - A Study of parasites of one humped camel (*Camelus dromedaries*) In Tehran and Najaf Abad Abattoirs, Iran

*Morteza Majidi rad *[1], Seyed Hoseini Hoseini[1], Fatemeh Jalousian[1], Mohammad Rajabloo[1], Abbas Gerami Sadeghian[1]*

Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran Iran

Camels are important animals especially in arid and semi-arid areas and generally infected with numerous parasites. Despite the economic importance of camel in Iran, a few studies were perfumed on different infection including parasitic diseases. This study was conducted in Tehran and Najaf Abad abattoirs to determine the parasitic infection in *Camelus dormedarius* in Iran. A total of 286 camels from abattoirs of Tehran and Najaf Abad were examined during July 2011 until August 2012 from which 100 camels were evaluated for protozoa and external parasite infection, and 186 camels were evaluated for internal parasite. A total 9 species nematodes, 4 species cestodes and 7 species of external parasite were identified as follow: Nematodes: *Haemonchus longistipes* (36%), *Parabronema skrjabini* (2.1%), *Camelostrongylus mentulatus* (5.3%), *Trichostrongylus mentulatus* (0.5%), *Physocephalus sexalatus* (0.5%), *Nematodirella longissimespiculata* (0.5%), *Nematodirus oiratianus* (0.5%), *Nematodirella cameli* (1.6%), *Onchocerca fasciata (*15%). Cestodes: *Momiezia expansa* (5.9%), *Moniezia benedeni* (2.6%), *Stilesia globipunctata* (23.6%), Hydatid cyst (in lung 16.6%, in liver 5.9% and in spleen 1.6%)Arthropoda: *Hyalomma dromedary* (63.8%), *Hyalomma anatolicum.anatolicum* (4.6%) *Hyalomma schulzei* (0.4%), *Hyalomma detritum* (0.4%), *Rhipicephalus turanicus* (0.9%), *Cephalopina titilator* (48/3%), *Linguatula serrate* (64/7%). According the results of this study*Haemonchus longistipes* and *Hyalomma dromedary* were more prevalent in camel.The results of this study showed that strategic control, including external and eternal parasite of camel should be considered. According the results, *Haemonchus longistipes* and *Hyalomma dromedary* were more prevalent in camel. Strategic control, including external and eternal parasite of camel should be considered.

P.102 - Tissue mast cells density in mefloquine, artemether and praziquantel treated *Schistosoma mansoni* infected mice

Maysa M.K. Sobhy[2], Soaad I. Hassan[1], Ayman A. El-Badry[2], Shaimaa H. El-Sayed[1] Soheir S. Mahmoud[1], Olfat A. Hammam[3], Hoda Y.Sabry[1]

[1]Theodor Bilharz Research Institute,Parasitology[1]and Pathology[3] departments Imbaba, Giza, Egypt
[2] Medical Parasitology department, Kasr Al-Ainy Faculty of Medicine,Cairo University, Cairo, Egypt

Schistosomiasis remains an important health problem in endemic areas. The antimalarial drugs mefloquine and arthemether proved to have interesting antischistosomal properties. Mast cells infilteration is a common feature of *Schistosoma mansoni* infection and may contribute to tissue granuloma. The aim of this study was to describe tissue mast cells density in mefloquine, artermether treated *S. mansoni* infected mice compared with praziquantel. Age-matched male Swiss albino mice, weighing 20 ± 2 gm were grouped into 4 main groups, of which two groups were uninfected (gr.I) and(gr.II), the later was further subdivided into 3subgroups,namely(IIa,IIb and IIc).While(gr.I)remained normal,the 3 subgroups of (gr.II)received mefloquine, artemether and praziquantel as a single oral dose of 400, 400 or 500 mg/kg body weight, respectively.As regards the other two groups (gr. III) and(gr. IV)they were infected with the Egyptian strain of *Schistosoma mansoni* cercariae (90 ± 10 cercariae/ mouse) sub-cutaneously. At week 6 post-infection, the infected group (IV) was also subdivided into(gr.IVa),(gr.IVb)and(gr.IVc)and they were treated with mefloquine(MFQ), artemether(ART) and praziquantel(PZQ) as a single oral dose of 400, 400 or 500 mg/kg body weight, respectively. All infected mice were scarified at week 8post-infection, while the uninfected treated subgroups were sacrificed 2 weeks post treatment. The intestinal and liver tissues of all groups were examined for mast cells infiltration using toluidine blue stain. The parasitological data as well as in infected groups were collected, inspection of granuloma was also undertaken. The present study showed that the density of mast cells in tissues was significantly higher in *S. mansoni* infected mice compared to un-infected untreated control animals. Tissue mast cell numbers showed that mefloquine and artemether induced mastocytosis in both infected and un-infected treated control mice, on the other hand praziquantel reduced mast cells count(gr.IVc). There was no association between tissue mast cell density and clearance of infection in treated mice. Also, there was no association between the count of tissue mast cells and both the worm burden and tissue egg count in infected treated mice. The increased aggregation of mast cells is associated with infection,treatment with MFQ(gr.IVa) resulted in an insignificant increase in mast cells compared to infected control group.ART(gr.IVb)yielded a significant mastocytosis when compared to corresponding control ,although both drugs induced mastocytosis in uninfected mice. Mast cells may play a role in the dynamic of the granuloma of *Schistosoma mansoni* infected mice but may not have a key role in clearance of schistosomiasis *mansoni* infection. The exact role of mast cells within schistosoma granulomas during clearance of infection remains to be clarified.The impact of treatment on mastocytosis needs further investigations.

P.103 - Neem (*Azadirachta indica*) for the control of mites and helminths in mice

Jamnah O.[1], Siti Munirah A.H.[2], Noor Hidayah R.[2], Chandrawathani P.[1], Premaalatha B.[1], Erwanas A.I.[1], Zaini C.M.[1] & Lily Rozita M.H.[1]

[1]Veterinary Research Institute, 59 , Jalan Sultan Azlan Shah , 31400 Ipoh, Perak , Malaysia
[2]Faculty of Pharmacy, The National University of Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur

The aim of this project was to study the effectiveness of neem leaf powder and neem leaf decoction as an alternative treatment for controlling mites and helminths in mice. The two products were developed in the Veterinary Research Institute , Ipoh as an eco herbal product. This project was carried out using three BALB/C mice that were infected with mites and helminths. The two neem products showed variable degrees of efficacy in naturally infected mice. The type of mites and helminth were identified as *Aspicularis tetraptera* and *Myocoptes musculinus* based on their morphological features.

P.104 - Parasitic infections found in pet and stray dogs in Ipoh, Malaysia

Erwanas A.I.[1], Chandrawathani P.[1], Premaalatha B.[1], Zaini C.M.[1], Lily Rozita M.H.[1], Kumutha M.[2], Norashikin M.S.[1], Norazura A.H.[1], Niny Fariza J.[1], Rajandran K.[3] & Ramlan M.[1]

[1]Veterinary Research Institute, 59 , Jalan Sultan Azlan Shah , 31400 Ipoh, Perak , Malaysia
[2]The National University of Malaysia, 43600 Bangi, Selangor
[3]Department of Veterinary Services, Jalan Sultan Azlan Shah, 30740 Ipoh, Perak

A study carried out on the presence of parasites, both internal and external, in 29 stray and 38 pet dogs in Ipoh, Perak indicated that four species of ectoparasites and four species of endoparasites were identified. Samples collected were diagnosed at the Veterinary Research Institute (VRI) whereby it was found that a higher percentage of stray dogs were infected (76%) with parasites as compared to pet dogs (16%) that were presented at the government veterinary clinic. Parasitic infections in dogs are especially important as *Demodex* sp., *Giardia* sp., *Toxocara* sp. and *Ancylostoma* sp. are zoonotic and can cause skin infections, mange, diarrhoea and anaemia in humans. Regular screening of pets is important to stave off unwanted infections. As for stray dogs, strict enforcement to control stray dog population and public awareness campaigns on uncontrolled breeding of dogs needs to be emphasised.

P.105 - Testing neem leaf products on goats in Infoternak, Perak – A preliminary trial for neem capsules, neem juice, neem extract & neem decoction for worm control.

Premaalatha B.[1], Chandrawathani P.[1], Shanmugapriya P.[2], Renuga K.[2], Norhafiza N.H.[3], Nor Andilla I.[4], Zary Shariman Y.[3], Erwanas A.I.[1], Jamnah O.[1], Lily Rozita M.H.[1], Zaini C.M.[1] & Ramlan M.[1]

[1]Veterinary Research Institute, 59 , Jalan Sultan Azlan Shah , 31400 Ipoh, Perak , Malaysia
[2]University Industry Selangor , (UNISEL) , Shah Alam , Selangor
[3]School of Biological Sciences , University Sains Malaysia (USM), Minden , Penang
[4]Ladang Infoternak Sungai Siput, Perak Darul Ridzuan , Malaysia

Four types of products made from Neem namely, neem leaf decoction, neem capsule, neem fresh juice and neem extract were given to 4 groups of goat for a period of 10 weeks to evaluate the effectiveness of these products in controlling natural gastrointestinal helminth infection. During the course of the study , faecal egg counts, Packed Cell Volume and FAMACHA readings were monitored weekly. Results of feeding these products were variable when compared to untreated control animals, however, a 40-60% worm control was observed. Further testing is required to fine tune these products for use in the field especially where anthelmintic resistance deems drugs to be ineffective.

P.106 - Bovine brucellosis in Malaysia: Cases diagnosed in VRI from 2010 to 2013

Nurul Fatiha A.S., Mazlan L., Chin S.W., Zainab Z., Aminah Kadariah A.L., Surayani A.R., Azmaliza A.A., Norashikin M.S., Noor Akmi M.N, Ramlan M.

Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400, Ipoh, Perak

A total of 99,263 bovine serum samples from Peninsular Malaysia were tested using Complement Fixation Test (CFT) for *Brucella abortus* under the National Surveillance Programme from the year 2010 to 2013. The results of diagnosis in cattle, made up of several local and imported breeds, indicates a positive reactor rate of 4.07% (n = 4,036 sera) for this period of study, while 91.23% of the tested serum were negative, 1.23% were found to have doubtful titres (indicating borderline negative titres) and 3.48% showed anticomplementary readings (indicating damaged or contaminated samples). The annual mean reactor was 4.30% with the highest rate in 2013 at 6.90%, and the lowest in 2011 at 2.26%. This result shows an increment of the reactor rates in cattle compared to previous studies (2.5%) in 2008. This clearly indicates that The National Surveillance Programme for Brucellosis Control should be strengthened to continuously monitor the health status of the animal population. Moreover, strict enforcement by the Department of Veterinary Services especially in culling positive animals is crucial in controlling the disease in Malaysia.

P.107 - Fascioliasis in domestic large ruminants in Perak, Malaysia

Fazly Ann Z.A., Muhamad Syamsul Naim N.A., Wan Normaziah W.O.B., Geethamalar S., Erwanas Asmar I., Chandrawathani P. & Ramlan M.*

Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia

Fascioliasis or liver fluke infestation is considered as one of the most important parasitic diseases affecting domestic large ruminants. *Fasciola hepatica* and *Fasciola gigantica* were known as the common causative agents that require fresh water snails, *Lymnaea spp.* as an intermediate host. In the affected animals, the flukes may cause destruction of the tissues during their migration in the liver. In severe cases, the disease may affect the meat production by reduction in body weight gain and growth rate. The aim of this study is to determine the prevalence rate of fascioliasis in domestic large ruminants in Perak, Malaysia. A total of eighty three fresh liver samples from sixty nine Kedah-Kelantan crossbred cattle and fourteen Murrah buffalo were collected from 4 abattoirs in Perak (Ipoh, Taiping, Teluk Intan and Tapah) commencing from February 2013 to October 2013. The samples were examined macroscopically after slaughtered during the meat inspection process. Samples were then stored at 4° to 6°C to confirmed the presence of the flukes and proceed with species identification under the stereo microscope in parasitology laboratory. Out of 83 animals, 6 (7.23%) animals were diagnosed fascioliasis. 7.25% (5 of 69) and 7.14% (1 of 14) were detected positive from cattle and buffalo sample respectively. Based on the results, only *F. gigantica* were detected upon examination.

P.108 - Development of an RT-Lamp diagnostic kit for chikungunya virus

Nico Gabriel Carlos, Brian Schwem, Joy Ann Petronio, and Raul V. Destura

Institute of Molecular Biology and Biotechnology, National Institutes of Health, University of the Philippines Diliman

Chikungunya (CHIKV) is an arbovirus transmitted by *Aedes aegypti* and *Aedes albopictus,* and is found throughout the Philippines. Recently, outbreaks have been reported across the 3 island groups, including 3 outbreaks in 2012 and 12 outbreaks in 2013. The current diagnostic tests used for CHIKV have drawbacks; ELISA can take 10 or more days to detect the virus and the cost PCR is out of reach for most Filipinos. To address this problem, this project sought to develop an RT-LAMP assay for the detection of CHIKV in patients in the Philippines. LAMP is capable of detecting the virus during the 1st day of illness and is inexpensive, which addresses the problems presented by the previously mentioned tests. Twenty-two patient samples that were positive in RT-PCR were sequenced, which revealed them to be of the Asian genotype. LAMP primers were designed based on the Asian genotype. The serum samples were first extracted for RNA and tested with PCR. Results of the PCR were used to compare the accuracy of the new RT-LAMP assay. To date, a total of 7 positives and 5 negatives have been tested and showed complete agreement with PCR results. Strong positives (from PCR) yielded a bright green fluorescence when SYBR green was added, while weak positives yielded lesser fluorescence. Negative samples did not turn green when SYBR green was added.

P.109 - Species identification of *Aleuroglyphus* mites (Acari: Acaridae) using Its2 gene as a molecular marker

Ernieenor Faraliana Che Lah and Mariana Ahamad*

Acarology Unit, Infectious Diseases Research Center, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

Aleuroglyphus is a storage mite that has been proven to be allergenic. Species identification using morphological characteristics of these mites is difficult due to their minute size, polymorphism in adults, not many taxonomic images available and lack of expertise. Polymerase Chain Reaction (PCR) and available sequences databases in GenBank are often used for molecular systematics to overcome all the shortcomings. To develop a molecular technique using PCR for identification of *Aleuroglyphus* mites based on of their amplified second internal transcribed spacer region (ITS2) gene. PCR using the following primer set: ITS2 forward primer 5'-CGACTTTCGAACGCATATTGC-3' and ITS2 reverse primer 5'-GCTTAAATTCAGGGGGTAATCTCG-3' (which complementary ITS2 gene) was performed on DNA of *Aleuroglyphus* mites from a pure colony in the Acarology Unit, Institute for Medical Research. Their genomic DNA was extracted, amplified and the products were then visualized on gel electrophoresis before purified and sequenced. The sensitivity for PCR amplification of ITS2 gene was evaluated by gradient volume starting from 1 – 10 µl of DNA template. The ITS2 gene of *Aleuroglyphus* was successfully amplified and the length of the ITS2 sequence was > 400 bp. The sequences exhibited up to 97% similarities to the available sequences of *Aleuroglyphus ovatus* in the NCBI GenBank. With regards to sensitivity, the PCR products can be detected by the established PCR using as low as 4 µl of DNA template.

P.110 - Seroprevalence of cryptococcosis in Malaysia: A retrospective study

Ratna Mohd Tap and Norazah Ahmad*

Bacteriology Unit, Institute for Medical Research,
Jalan Pahang, 50588 Kuala Lumpur, Malaysia

This study describes the prevalence of Cryptococcosis in Malaysia from year 2010 to August 2013. Samples from suspected cases of cryptococcosis were received from 32 hospitals and private laboratories in Malaysia over the years 2010 to August 2013. The samples consisting serum and cerebrospinal fluid (CSF) were tested for cryptococcal antigen using Latex-Cryptococcus Antigen Test kit (IMMY). A total of 194 samples, 34% (n=65) was CSF and 66% (n=129) was serum. Thirty two samples (16%) were positive for Cryptococcal antigen, in which fifteen percent (15%) was CSF and 85% was serum samples. Hundred and sixty two (84%) samples were negative for Cryptococcal antigen. Out of 55 HIV positive cases, five samples (9%) were positive for cryptococcal antigen. Seventy nine percent (79%) of Cryptoccosis occurred in male, aged from 17 to 50 years old. We found a 15% prevalence of cryptococcosis in Malaysia. A national surveillance program of cryptoccosis needs to be established in generating the factual prevalence rate in Malaysia.

P.111 - Hla-A*11:01 And Hla-B*18:01 are associated with a lower risk of severe dengue in a Malay population

[1]Salsabil Sulaiman, [1]Chun Lai Too, [2]Ravindran Thayan, [1]Norfarhana Khairul Fahmy, [1]Geetha Gunasekaran, [3]Shahnaz Murad, [1]Jasbir Singh Dhaliwal.*

1. Allergy and Immunology Research Center, Institute for Medical Research, Jalan Pahang,
2.Virology Unit, Infectious Disease Research Center, Institute for Medical Research,
3. Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

Dengue fever is a significant public health problem in tropical and subtropical regions. It may be asymptomatic, symptomatic or severe, and most deaths occur in the latter group. There is a need to identify patients at risk, and to understand the factors/mechanisms involved in severe dengue. In this study, we investigated the HLA association with severe dengue in the Malay population. A total of 224 Malay patients diagnosed with dengue infection (symptomatic dengue, n=110 and severe dengue, n=114) were recruited between December 2010 and 2012. The dengue infection was confirmed by the presence of dengue IgM/IgG antibodies using ELISA and/or reverse transcriptase PCR. High resolution HLA molecular typing was carried out on all patients by PCR. Statistical analyses demonstrated that the frequencies of two HLA alleles were significantly reduced in severe dengue: HLA-A*11:01 (OR=0.51, 95% CI: 0.32 – 0.84, p<0.01) and HLA-B*18:01 (OR=0.32, 95% CI: 0.14 – 0.73). Additionally, there was an increased frequency of HLA-B*38:02 in patients with severe dengue compared to the symptomatic dengue patients (severe dengue versus symptomatic dengue, 8% (n=17) vs. 3% (n=7), OR=2.38, 95% CI: 0.97-5.87). In summary, our data suggest that the frequencies of HLA-A*11:01 and HLA-B*18:01 are significantly reduced in severe dengue compared to symptomatic dengue patients in the Malay population.

P.112 - Toxicological substances detection from empty puparial cases – sample 060/13

Rozie Sarip1,, Maizatul Hasyima Omar2 and Nazni Wasi Ahmad3*

1Bioassay Unit, Herbal Medicine Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur 2Phytochemistry Unit, Herbal Medicine Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur 3Entomology Unit, Infectious Diseases Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur

The research presents here describes a sensitive UHPLC-MS/MS (ORBITRAP) method for quantification of toxicological substances in empty puparial cases of calliphoridae. Samples were collected from a crime scene where human samples were unavailable. A class of amphetamines and sarin, the toxicological substances was predicted to be existed in the corpse. Hence, entomotoxicological detection is important in providing the evidence. An analysis of HPLC and GC- MS were done as initial insight on the substance determination but both techniques gave negative outcome due to low and/or none concentration of the substances in the samples and insensitive equipment. None of the six amphetamines classes of drugs were detected utilising both analytical techniques after three replicates and even after spiking with the respected standards. Therefore, those candidates were eliminated. The next best target of toxic substance was sarin. The use of ORBITRAP shows significant peaks at the region responsible for sarin (m/z 140.04024 ± ≤ 50 ppm). Sarin (O-isopropylmethylphosphonofluoridate) is a highly toxic nerve agent produced for chemical warfare. Death by sarin is due to anoxia resulting from airway obstruction, weakness of the muscles of respiration, convulsions and respiratory failure. The main clinical symptoms of acute toxicity of sarin are sizures, tremors and hypothermia. The confirmation of sarin detected was done by comparing with an online database for the observed and targeted m/z peak in the mass chromatogram with a reliable mass accuracy that leads to an unambiguous interpretation. The ORBITRAP system is the most sensitive technique and had been used to monitor the existence of sarin and it is ‘fit for purpose’. However, no relationship between the sarin concentration in the substrate and empty puparial cases could be established due to the lack of sarin standard available in Malaysia. Nonetheless, the preliminary results indicated that the empty puparial cases could be a useful alternative to toxicological specimen for the detection of toxicological substances.

P.113 - Laboratory evaluation of the bioefficacy of a long-lasting insecticide treated net (lifenet®) against *Aedes aegypti* (linnaeus) and *Culex quinquefasciatus* Say

[1]Tan Swee Beng, [1*]Rohani Ahmad, [2]Do Van Nguyen, [1]Mohd Ariffin Majid, [1]Rosman Salleh, and [1]Lee Han Lim

[1]Medical Entomology Unit/WHO Collaborating Centre for Vectors, Infectious Disease Research Centre, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia.

[2]School of Diploma in Applied Parasitology & Entomology, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia.

This is the first laboratory evaluation in Malaysia on deltamethrin treated LifeNet®, a long-lasting insecticide treated net against vector of dengue and filariasis. The objectives of this study were to determine the bioefficacy of LifeNet® against *Ae. aegypti* and *Cx. quinquefasciatus* using WHO contact bioassays and blood-feeding inhibition using WHO tunnel test. Lifenet® was very effective against *Ae. aegypti* and *Cx. quinquefasciatus* after 3 minutes of exposure in WHO contact bioassay. It took less than 1 minute and about 4 minutes to knock down 50% and 90% of both mosquito species respectively, with 100% delayed mortality 24 hours post-holding period. In the control tunnel without LifeNet®, more live and fully blood fed *Ae. aegypti* were found in outer cage, but more *Cx. quinquefasciatus* were found in mouse cage ($p<0.05$). In the tunnel with treated net, more dead but unfed females were found in outer cage for both species of mosquitoes ($p<0.05$), indicating the bioefficacy and effectiveness of blood feeding inhibition effect of Lifenet®. The ratio of inhibition of blood feeding in the tunnel with treated LifeNet® and untreated control net for *Ae. aegypti* and *Cx. quinquefasciatus* was1:25 and 1:30 respectively, showing that the number of live and blood fed *Cx. quinquefasciatus* was 1.2 times more than *Ae. aegypti.* The delayed mortality in tunnel test for *Ae. aegypti* and *Cx. quinquefasciatus* was 92.67±5.28% and 95.60±3.67% respectively. LifeNet® therefore was highly effective against vector mosquitoes.

P.114 - The presence of *Borrelia* antibodies among suspected human lyme borreliosis in Malaysia

[1*]*Jama'ayah Mohd Zahidi,* [1]*Tay Bee Yong,* [1]*Siti Sakdaton Shahit,*
[1]*Heu Jen Yuh,* [1]*Norazah Ahmad*

[1]*Department of Bacteriology, Institute for Medical Research,*
50588 Kuala Lumpur, Malaysia

Lyme borreliosis is a tick borne spirochetal infection caused by *Borrelia burgdorferi* sensu lato complex comprising of three different *Borrelia* species namely *Borrelia afzelii*, *Borrelia burgdorferi* and *Borrelia garinii*. The disease is underreported in Malaysia mainly because it is rarely clinically suspected, and laboratory diagnosis is not readily available in hospitals. The diagnosis of Lyme borreliosis is based on serological methods. A two-tiered system is recommended for serological testing (CDC, Atlanta). All sera tested positive using indirect immunofluorescent assay (IFA) or enzyme immunoassay (EIA) should be confirmed using the western blot assays (WBA). In the present study, a total of 61 serum samples from 61 patients suspected of Lyme borreliosis were tested. All samples were screened using IFA technique to detect IgM and IgG against Borrelia (Diagnostic Automation, USA). All positive sera on screening were then subjected to western blot assay using the Euroimmun Western Blot IgM and IgG test kit. The results of the western blot assay were evaluated and interpreted using EUROLineScan software. Out of 61 suspected Lyme borreliosis patients, 21 (34.4%) sera were tested positive with the IFA where 9 (14.7%) samples were positive for IgM antibody only, 5 (8.2%) were positive for IgG antibodies, and 7 (11.5%) were positive for both IgM and IgG antibodies. By WBA, 7 (33.3%) out of 21 the IFA positive sera were positive for IgM antibodies only. Four of the sera showed the presence of antibodies against of *B. afzelii* and *B. burgdorferi* while 2 samples were positive for *B. afzelii*, *B. burgdorferi* and *B. garinii* antibodies. Only one serum was positive for *B. garinii* antibody. This study showed the presence of Borrelia antibodies in 11.4% of the suspected Lyme borreliosis cases. Lyme borreliosis should be one of the differential diagnosis in cases of fever of unknown origin.

P.115 - Behavioural response of female *Aedes* species against two synthetic semiochemicals

[1]Nurulhusna AH, [1] Mohd-Iqbal MN, [2]Taraumae C,
[1]Khadri MS and [1]Lee HL

[1] Medical Entomology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur
[2] School of Diploma in Applied Parasitology and Entomology,
Institute for Medical Research, Jalan Pahang,50588 Kuala Lumpur.

Semiochemicals are chemical compounds found in insects that can elicit behavioural response in both sexes. *n*-Heptadecane and *n*-nonadecane are two such compounds isolated and identified from extract of *Aedes aegypti*. These two compounds are commercially available and were tested against female *Ae. aegypti* and *Ae. albopictus* in this study. The modified single tunnel cage test based on WHO (2005) was used to evaluate the female *Aedes* species behavioural response against two concentrations (10 mg/L and 100 mg/L) of *n*-heptadecane and *n*-nonadecane for 24 hours. Both *n*-heptadecane and *n*-nonadecane at 10 mg/L exhibited 74.88% and 62.32% repellency against *Ae. aegypti,* respectively. However, at 100 mg/L *n*-nonadecane was able to repel 81.12% of the females in the presence of the bait (mouse) in the bait chamber, while *n*-heptadecane repelled 67.60% *Ae. aegypti* female at the same concentration. Females of *Ae. albopictus* exhibited higher percentage of repellency against *n*-nonadecane at both concentration; 10 mg/L (64.64%) and 100 mg/L (67.56%) compared to *n*-heptadecane at 10 mg/L (57.44%) and 100 mg/L (56.24%). *n*-Heptadecane was effective as a repellent at lower dose, while *n*-nonadecane was effective at higher dose for both species tested. This preliminary study showed that these compounds are potential repellents against female *Aedes* mosquitoes.

P.116 - Phylogeny and PCR-based classification of *Wolbachia* strains isolated from *Aedes albopictus* using Wsp gene sequences

[1*]Noor Afizah Ahmad, [1]Roziah Ali, [1]Nazni Wasi Ahmad, [1]Lee Han Lim

1Unit of Medical Entomology, WHO Collaborating Centre for Vectors, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur.

Wolbachia-based vector control strategies have been proposed as a mean to augment the current existing measures for controlling dengue vector through population replacement. The successful application of *Wolbachia* in insect control is critically dependent on the ability of the agent to invade and maintain itself at a high frequency in the natural population with desired genotype. In this study, partial *wsp* gene sequences from 2 strains of *Wolbachia* (*w*AlbA and *w*AlbB) isolated from *Aedes albopictus* were successfully amplified using polymerase chain reaction (PCR). The sequencing results for *wsp* gene yielded a fragment of 341bp and 463bp for strain A and B respectively. BLAST results of the sequence from *wsp* strain A gene (GenBank accession number KC004024) corresponded to the *Wolbachia* endosymbiont of *Ae. albopictus* surface protein A-like gene, whereas *wsp* strain B gene (GenBank accession number KC004025) from the same sample showed 100% nucleotide sequence homology with outer surface protein *Wolbachia* strain B isolate in *Ae. albopictus.* A neighbour-joining (NJ) tree of all sequences was constructed based on the Kimura two-parameter model. Bootstrap analysis was done with 1000 replications and showed that *Wolbachia* isolate from the present study is closely related to *Wolbachia* isolated from *Ae. albopictus* collected from different locations and the sequences were grouping together according to the *wsp,* strain A and B respectively.

P.117 - Effect of sublethal dosage of insecticides on dengue virus replication in *Aedes aegypti* (L.)

Nazni, W.A.[1] , Azman, M[1], Ravindran,T[2] , Chandru,A [1], Zainah S.[1] , Lee H.L[1]

Dengue is an acute viral infection and a major public health problem in Malaysia since it first reported in 1901. Until today, there is no effective vaccine and specific treatment for dengue infection. The only option to control dengue is via vector control and chemical insecticides still play the pivotal role of controlling the *Aedes* adults. The insecticides of choice are mostly organophosphates or pyrethroids applied through thermal or ultra-low-volume (UL V) fogging, mostly during an outbreak. However, despite its long history of application, the effect of these chemicals on the vector susceptibility to dengue infection remains unknown. The objective of this study was to determine the effect of insecticides namely, malathion and permethrin in *in vivo* dengue virus replication in *Aedes aegypti*. We employed two protocols, firstly, exposure of *Aedes aegypti* to insecticide prior to feeding with blood infected with dengue serotype 1 virus (Protocol 1) and secondly feeding the *Aedes aegypti* with Den-1 prior to exposure to sub-lethal dosage of permethrin and malathion (Protocol 2). Control consisted of mosquitoes fed with Den-1 but were unexposed to the insecticides. Dengue viral load in the mosquitoes was quantified using real time Real Time-PCR. The 50% knock down rate (KT_{50}) of the sublethal dosage of permethrin and malathion was 45 sec and 5.61 min, respectively. The results indicated that Den-1 viral load in the control was 1.88×10^9 Relative Fluorescence Unit (rfu). Mean viral load of Den-1 using Protocol 1 was 2.65×10^9 rfu and 1.14×10^9 rfu and Protocol 2 was 1.95×10^9 rfu and 1.97×10^9 rfu respectively for permethrin and malathion. . Our results indicated that pre-exposure of *Ae aegypti* to insecticide followed by infection with Den-1 virus enhanced virus replication but overall there were no significant differences between protocols 1 and 2 compared to control ($p>0.05$). This warrants further investigation on the impact of fogging on dengue replication in the vector.

P.118 - Prevalence and genotyping of *Acanthamoeba* in natural aquatic and paws animal shelter

Mohamad Hafiz Abdul Basher[1], Init Ithoi[1], Rohela Mahmud[1],Nadiah Md Sarani[1], Awatif Mohamed Abdulsalam[1] and Wan Omar Abdullah[2]

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

[2]Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Acanthamoeba is a free living amoeba found in all habitats worldwide. Many pathogenic species of this amoeba are able to infect the corneas and CNS of both animals and humans. In this study, a total of 45 samples (surface water, debris and hard surface swab) from natural aquatic environments (ponds, rivers and lakes) and 55 swab samples (animal bedding, food and drinking containers) from paws animal shelter, were respectively cultured and subjected to PCR, sequencing and phylogenetic analysis. Result from cultivation showed that the prevalence of *Acanthamoeba* spp. was high in natural aquatic environments (84.4%) compared to paws animal shelter (70.9%). In natural aquatics, it prevalence was 100% in both of surface water and debris, and 53.3% in hard surface swab of stones and rocks. In paws animal shelter, the highest prevalence was at food containers (73.68%) followed by animals bedding (70%) and drinking containers (68.75%). Phylogenetic analysis showed the presence of genotype T3, T4 and T5, in which T4 was the most prevalence in both natural aquatic and paws animal shelter.

P.119 - Latex-agglutination based surveillance of equine toxoplasmosis in Punjab,Pakistan

Muhammad Saqib[1], Muhammad Hammad Hussain[1], Muhammad Sohail Sajid[2], Mansoor Khalid[3], Muhammad Nadeem Asi[1], Ali Zohaib[4], Ghulam Muhammad[1], Irfan-ullah[2]*

[1]Department of Veterinary Clinical Medicine and Surgery, [2]Department of Parasitology, [3]Institute of Microbiology, University of Agriculture, Faisalabad-38040, Pakistan

**Corresponding Author: Assistant Professor, Department of Veterinary Clinical Medicine and Surgery, University of Agriculture, Faisalabad-38040, Pakistan*

Toxoplasmosis is a serious threat for livestock having zoonotic significance. In this study, sero-diagnosis of equine toxoplasmosis was conducted in a selected population (three districts) of Punjab, Pakistan. To this end, 272 draught equines were screened using commercial latex agglutination assay kit. Association of probable risk factors of equine toxoplasmosis was also documented. A total of 91 (35.5%) equines were found sero-positive for *Toxoplams (T.) gondii* having Ab-titer ranging between 1:32 to 1:612. The highest rates of sero-positive cases were observed in donkeys followed by horses and mules. Age, sex and species of draught equines were not statistically found associated with the antibody titer. The results of our study provide a baseline status of the exposure of equine population in the area. Objective of this presentation is to provide probably first sero-epidemiological investigation of the equine toxoplasmosis in Pakistan. Extensive epidemiology of this nuisance in tropical countries like Pakistan is immensely needed to sort out the risk factors which could affect in minimizing the infection.

P.120 - The discovery of the malaria transmission and of its treatment

[1]*Andreas Diedenhofen*

[1]*Sigma-Tau Industrie Farmaceutiche Riunite S.P.A,*
Viale Shakespeare, 47 - 00144 Rome – Italy

The discovery of the malaria transmission and of its treatment goes a long way back. The journey started with the first clinical trial carried out in 1898 on a patient, Abele Sola, and led to fundamental researches carried out in China to help the North Vietnam army during the Vietnam war. These resulted in the isolation and purification of artemisinin from the plant Artemisia annua. Dihydroartemisinin/Piperaquine, or DHA/PQP (Eurartesim, Sigma Tau, Italy) in 2011 has been the only Artemisinin-based Combination Therapy (ACT) approved by the European Medicine Agency for the treatment of uncomplicated malaria. Nowadays, great attention is given to the increased tolerance to the ACTs in South East Asia. If it were to expand from Cambodia to Myanmar, Bangladesh and India, the worldwide spread would become a nightmare. An update on this topic will be presented, as well as the possible measures for the containment. DHA/PQP has been tested as a drug for the treatment of the blood stage of P. vivax malaria and, together with primaquine for the radical cure, it has been shown to be very effective in areas where chloroquine resistance is common. In the Symposium optimization of the DHA/PQP doses will also be presented with pharmacokinetic models that would increase the efficacy on very young children. The development and the registration of Eurartesim has been the outcome of the joint efforts of Sigma Tau (Rome, Italy) and Medicine for Malaria Venture (Geneva, Switzerland) to allow the availability of DHA/PQP, produced according the highest standard requested by Good Manufacturing Practice.

P.121 - Application of multiplex PCR for detection and differentiation of *Entamoeba histolytica*, E*ntamoeba dispar* and *Entamoeba moshcovskii*

Zebardast N [1], Haghighi A [1], Yeganeh F [2], Seyyed Tabaei SJ [1], Gharavi MJ [3], Fallahi SH [1], Lasjerdi Z [1], Salehi N [1], Taghipour N [1], Kohansal K [4], Naderi F [1].*

[1]Dept. of Parasitology and Mycology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [2] Dept. of Immunology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [3]Dept. of Parasitology, faculty of Allied Medicine, Iran University of Medical Sciences,Tehran, Iran, [4]Tamin ejtemaee hospital Ahvaz, Iran.

Entamoeba moshkovskii and *Entamoeba dispar* are impossible to differentiate microscopically from the pathogenic species *Entamoeba histolytica*.

Multiplex polymerase chain reaction (Multiplex PCR) is a widespread molecular biology technique for amplification of multiple targets in a single PCR experiment. For detection and differentiation of the three microscopy indistinguishable *Entamoeba* species in human, Multiplex PCR assay using different DNA extraction methods was developed. A conserved forward primer was derived from the middle of the small-subunit rRNA gene, and reverse primers were designed from signature sequences specific to each of these three *Entamoeba* species. A 166-bp PCR product with *E. histolytica* DNA, a 580-bp product with *E. moshkovskii* DNA and a 752-bp product with *E. dispar* DNA were generated in a single round PCR reaction. We recommend this PCR assay as an accurate, rapid, and effective diagnostic method for the detection and discrimination of these three *Entamoeba* species in both routine diagnosis of amoebiasis and epidemiological surveys.

P.122 - The first record of potentially zoonotic avian schistosome in lymnaeid snail (*Austropeplea viridis*) in Vietnam

[1,2*] *Bui Thi Dung,* [2] *Yannick Caron,* [2] *Claude Saegerman,* [2] *Bertrand Losson*

[1] *Department of Parasitology, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Vietnam.*
[2] *Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Bld de Colonster, 20 B-4000, Liège, Belgium.*

Cercarial dermatitis or swimmer's itch is a cutaneous inflammatory response usually associated with penetration of the skin by cercariae of avian schistosomes. The life cycle of these schistosomes requires freshwater snails and waterfowls. Humans engaged in professional or recreational water activities are at risk as repeated exposures to cercariae can lead to skin sensitization with the induction of pruritic skin lesions. Indeed, there is a lack of information on avian schistosome and cercarial dermatitis in Vietnam although numerous human cases with compatible cercarial dermatitis symptoms are known. In this study, we provide the first report of snails naturally infected with ocellate furcocercariae. Lymnaeid snail samplings were conducted in Tu Nhien, Chuong Duong, and Le Loi communes (Thuong Tin district, Ha Noi) in February 2011. Crushing and microscopical examination were used to detect cercariae in snail. The snail species *Austropeplea viridis* was collected in rice-fields and small channels where duck are feeding. Ocellate, brevifurcate and apharyngeate cercariae, potentially zoonotic were found in *A. viridis* with the following morphological characteristics: forked tail, two eye spots, body without dorsomedial finfold and ventral sucker well developed. The infection rate was 0.48% (9/1855). Infected snails were found in Le Loi commune where ducks were common in the field. This is the first record of ocellate furcocercariae (Schistosomatidae) in lymnaeid snail in Vietnam. Further studies are necessary on the biology, the ecology, the molecular identification and the epidemiology of avian schistosome in Vietnam to assess public health concern.

P.123 - Entomological survey and mapping of immature mosquito breeding sites in four urban villages in Puerto Princesa City, Palawan

Karis Lee Del Castillo, Aldwin Velasco, Majhalia Torno, Estrella Cruz, Kristian Marollano, Ferdinand Salazar, Fe Espino

Research Institute for Tropical Medicine, Alabang, Muntinlupa City

The increasing incidences in dengue transmission in Puerto Princesa City, Palawan can be considered a public threat not only to the local residents but also to the hundreds of tourists who visit the island every day. As an enhancement of dengue vector surveillance in the city, a survey of immature mosquito breeding sites had been conducted in 80 household structures and 20 non household structures in 4 purposively selected urban villages during the dry and rainy seasons. Specific types of containers were recorded and described using WHO standard larval/pupal survey form. Coordinates of positive breeding sites had been recorded and were layered over coordinates of serologically confirmed dengue cases in Puerto Princesa. Immature mosquitoes collected were reared and allowed to emerge as adults in order to speciate and count the female species in the collected immatures. House index, container index, and Breteau index had been computed as estimates of transmission risk to people residing in the inspected vicinity.Results showed that containers belonging to “other types” and bamboo are most common positive breeding sites of immature mosquitoes. Female species identified were 57.8% *Aedes aegypti,* 14.6% *Aedes albopictus,* and 27.6% *Culex quinquefasciatus*. Upon mapping, breeding sites were seen to be proximal to denguge patients’ addresses. Larval entomological indices computed were indicative of high transmission risk in all 4 villages.

P.124 - The effect of heligmosomoides polygyrus larva 3rd extract to the expression of TNF-α induced ulcerative colitis

[1*],Agustina Tri Endharti, [1]Aswin D. Baskoro, [1]Donabella F. Santosa ,[1]Mangkubumi.

Department of Parasitology, Faculty of Medicine, University of Brawijaya[1],

Ulcerative colitis is an ulcero inflammatory disorder that affects the colon and chronically caused by an imbalance of the immune system. The aim of this study was to determine the effects of *Heligmosomoides polygyrus* extract on the pathogenesis of DSS-induced colitis. *Heligmosomoides polygyrus* extract can be used as an alternative therapy to help the balance of the immune system. Extracts of intestinal nematode *Heligmosomoides polygyrus* can enhance Th2 and suppress Th1 improvement. In this study, there were differences in the expression of TNF-α in Balb/c mice with and without treatment with extract of *Heligmosomoides polygyrus*. There are 5 treatment groups, the negative control group, positive control, and three groups of mice were given treatment with *Heligmosomoides polygyrus* extract with various doses (0.1 mg/kg, 0.2 mg/kg, and 0.4 mg/kg, respectively. Mice were induced colitis by 1-week oral exposure to 3% DSS in drinking water, then treated with *Heligmosomoides polygyrus* extract for 12 weeks and followed by RNA isolation from colon of mice to the expression of TNF-α. One way ANOVA analysis of variance showed the expression of TNF-α were significantly decrease at a dose of 0.4 mg/kg body weight($p<0.05$) among control and treatment groups. Histological assessment of colon sections confirmed amelioration of the disease. It can be concluded that the extract of *Heligmosomoides polygyrus* at 0.4 mg/kg body weight dose could reduce the expression of TNF-α in mice induced colitis.

P.125 - *Blastocystis* sp. infections in wild animals in captivity from a zoological park

Farah Haziqah M.T.[1], Chandrawathani P.[2], Mohd Zain S.N.[1], Suresh Kumar G.[3], Sabapathy D.[4], Hemalatha C.[3], & Premaalatha B.[2]*

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur.
[2]Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur.
[3]Department of Parasitology and Hematology, Veterinary Research Institutes, Ipoh, Perak.
[4]Bukit Merah Laketown Resort Ecopark, Jalan Bukit Merah, Semanggol, Perak.

Blastocystis sp. is an enteric protozoan parasite commonly found in most animals. It is considered a zoonotic disease and animals constitute a large reservoir for human infection via the faecal-oral route. Animals under captivity are also susceptible to this infection due to their close proximity to other animals. This study is the first report on the presence of *Blastocystis* infection of wildlife in captivity in a mini zoological park. A total of 43 faecal samples were randomly selected from several species of birds (eagle, sparrow and macaw), chickens (village chicken, peacock and Malaysian serama), small animals (rabbit and porcupine), hoofed mammals (deer and goat) and primates (lar gibbon). Results showed that village chickens, peacocks and lar gibbon were positive for *Blastocystis* sp. infection with infections were mainly asymptomatic. Monitoring of the presence of *Blastocystis* sp. in animals in captivity is imperative in zoo management in the formulation and implementation of preventive and control measures against the spread of infectious zoonotic diseases among animals within the zoo or to humans.
Keywords: Blastocystis sp., Zoonotic, Wildlife, Morphology, *In vitro* cultur

P.126 - The use of insecticide treated curtiains (ITCS) as malaria vector control in two holoendemic communities of Adamawa, Nigeria

Nassar A.S and ALO E.B

Biomedical Science Department, Ladoke Akiintola University of Technology, Ogbomoso
Zoology Department, Modibo Adama University of Technology, Yola

This trial study was conducted between March 2008 and August 210 in two rural villages of Rugange and Geriyo fishing communities of Adamawa, North Eastern Nigeria to assess the impact of Insecticide Treated Curtains (ITCs) on malaria infection using Lamdacyhalothrin as an insecticide of choice. A total of 384 Subjects in 28 randomly selected households participated in the study after approval and informed consent obtained from local Government Authorities, Village Heads and Head of Households. The study was divided into three phases ;Pre-intervention where malaria prevalence of the study subjects determined by microscopic examination of Giemsa stained thick and thin blood films .Intervention then followed by treating the curtains provided to the subjects with insecticide and then monitored for parasitaemia at three months interval .Mean Mosquito Density was also determined as part of Post-Intervention activity .When mean Mosquito Density was assessed after insecticide Treated Curtain Usage, there was reduction in the values from 1073.3 ± 0.89 to520.6 ± 0.33 and the difference was statistically significant($P<0.05$).Relating ITCs with malaria prevalence there was slight difference but the value recorded for post ITCs(16.1%) was not significantly lowered than that of Pre-ITCs (10.5%).

P.127 - Puerto Princesa City – A global tourist hotspot

Shellemai Roa, Fe Espino, Aldwin Velasco, Karis Lee Del Castillo & Mary Vinessa Villaruel,

Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Philippines

Travel and tourism are the primary sources of foreign exchange earnings in many developing countries and among the fastest growing industries around the world. Urbanization is a good indicator of development, yet it has an inverse relation to environment. This study aims to know the observed tourism-related changes and existing socio-economic and environmental condition of Puerto Princesa. It focuses on the land use, tourist arrival, tourism-related revenue and establishment, employment, population and dengue cases. This was conducted in Puerto Princesa City, Philippines from June to September 2013. Key informant interviews and extensive review of secondary data were utilized in data gathering. As a renowned global tourist destination site, Puerto Princesa experienced rapid changes in land use especially in tourist industry. From four daily flights in 2006 to 21 commercial flights today. The revenues collected with its famous Underground River for half of year 2012 contributes for about 47.4% to the total gross income of the city. Due to increasing tourists' demand, many tourism-related establishments were built up that resulted to conversion of thick forested areas into concrete regions. *Ae. Aegypti* is the primary dengue vector and very adaptable to environmental changes – from forested habitat into artificial containers such as bamboo stumps and tires. During late 1900s, there were no recorded dengue cases in Puerto Princesa but today it becomes one of the major problems in the city even in whole province of Palawan.

P.128 - Geo-location of clinically-confirmed dengue cases in Puerto Princesa City, Philippines

[1]*Mary Vinessa Lacao Villaruel,* [1*]*Ava Krist Sy,*
[1]*Aldwin Velazco,* [1]*Fe Esperanza Espino*

[1]*Research Institute for Tropical Medicine, Filinvest, Alabang, Muntinlupa City, Philippines*

Dengue fever is a public threat to every tropical country in the world. In the Philippines, dengue is one of the top 10 leading causes of morbidity. A UNESCO Heritage Site – Puerto Princesa City has been reporting suspected cases of dengue fever even before the surveillance was enhanced in the area. To visualize its distribution in space and time, a technology was used to interpret its global position. A Geographical Information System was used to place all data in one output map. A total of 409 clinically-confirmed dengue fever cases were geo-tagged and was placed in a base map. The clinically confirmed dengue cases were obtained from the database of the reference lab. The base map was from the city planning office that uses Geographic Information System and shape file in classifying land use. The map showing dengue fever cases is then used as an initial tool to present data to the stakeholders. The cases in the city was easily visualize because of this kind of data rather than using graphs to show the number of cases. It also shows the trend of the virus where people in the community acquire dengue. Interpreting the location of dengue fever cases can be enhanced through the use of analytical tools that are found in Geographic Information System. This can be implemented as a routine in dengue surveillance with the aid of government and non-government organizations having such resources in creating maps.

P.129 - Adult-vector surveillance using moshouse (sticky ovitrap): pilot testing in Barangay San Pedro, Puerto Princesa City, Philippines

[1]*Mary Vinessa Villaruel,* [1*]*Kristian Marollano,* [1]*Karis Lee Del Castillo,*
[1]*Aldwin Velazco,* [1]*Mahjalia Torno,* [1]*Fe Espino*

[1]*Research Institute for Tropical Medicine, Filinvest,*
Alabang, Muntinlupa City, Philippines

Dengue fever is the most globally widespread arboviral disease, causing 50 – 100 million cases per year which is endemic throughout tropical and subtropical countries of the world. Puerto Princesa City reported 436 suspected cases from the record of Ospital ng Palawan (Provincial Hospital) and City Health Office in the year 2012. From the two-season entomological survey that was done in the city by the Research Institute for Tropical Medicine last October 2012 and April 2013, larval and pupal surveys captured *Aedes aegypti* and *Aedes albopictus*. Methods of collecting adult mosquitoes such as landing collections, resting collections, or traps were not used. A MosHouse is a low-cost model of sticky ovitrap used to trap gravid female *Aedes aegypti* mosquitoes, the primary vector for dengue fever. The study took nine weeks in Barangay San Pedro. A total of 180 mosquitoes were trapped in the MosHouse and 50 were identified as *Aedes aegypti*. The MosHouse was an effective tool in collecting adult vectors for dengue fever as well as other vectors of mosquito-borne diseases. It can be used widely by health services for dengue control programs and dengue surveillance.

P.130 - Comparison of scolicidal effectiveness of physical agents (temperature and osmolarity), chemical agents (albendazole) and aquatic extract of Harmel (*Peganum*H*hrmala* L.) an *in vitro* study

Ahmad Nematollahi, Hamed Ghazi

Department of Pathobiology, Faculty of Veterinary Medicine
University of Tabriz, Iran

The aim of present study was to comparison of scolicidal effectiveness of physical agents (temperature and smolarity), chemical agents (Albendazole) and aquatic extract of Harmel (Peganum harmala). For this purpose, protoscolices were collected aseptically from sheep livers and lungs containing hydatid cyst in slaughterhouse. In laboratory, viability of protoscolices was determined by 0.1% eosin staining. In this study, the effects of temperature (-20^{oC}, 40^{oC} and 60^{oC}) and smolarity (20%Nacl, 10% Nacl, 50% Dextrose) and chemical agent (2% Albendazol), and aquatic extract of Harmel (Peganum harmala) (25%, 50%, 75% and 100%) on protoscolices were investigated. After 5, 10, 15 and 60 min for each agent, the first 100 protoscolices were counted and viability of protoscolices was determined by 0.1% eosin staining. The results of present study showed that 20% Nacl after 60 min has significantly ($P<0.05$) than other agents but due to side effects of it, this agent cannot be seen as an ideal scolicidal agents.

P.131 - Evaluation of phytochemical and bioactivity of commonly used antihelmintic plants in Kenya

Jackson Mutembei[2], Francis Njonge[1*], Patrick Kareru[2],
Hellen Kutima[3], Joseph Karanja[2]

[1] Department of Animal sciences, Jomo Kenyatta University of Agriculture and Technology, K
[2] Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, Kenya
[3] Department of Zoology, Jomo Kenyatta University of Agriculture and Technology, Kenya

Plants used in traditional medicine are likely to yield pharmacologically active compounds. Most rural communities in developing nations rely on herbs to deworm their livestock due to inaccessibility of conventional drugs and veterinary services that are at times too expensive to afford. Most secondary metabolites produced by plants for protection against predation by microorganisms, insects, and herbivores possess medicinal properties.
Brine shrimp lethality test (BST) is a simple tool used in the screening and fractionation of physiologically active plant extracts. In this work, *Vernonia lasiopus*, *Rapanea rhododendroides*, *Entada leptostachya*, and *Albizia anthelmintica* were investigated for their cytotoxicity and phytochemicals, with the main aim of evaluating the nature of bioactive phytochemicals present in these medicinal plants, and whether there exist a correlation between bioactivity and the phytochemicals present.The plants are widely used to treat various types of worms in livestock by communities of Meru-South and Mbeere Districts in Kenya.
All the plants were found to contain saponins, alkaloids, terpenoids, and tannins, with saponins present abundantly in all of them. Reducing sugars were also present in all the plants except in *Albizia anthelntica*. Anthraquinones were found to be present in *Vernonia lasiopus* and *Rapanael rhododendroides*. No steroids or steroidal nucleus were present in all the plants. The anthelmintic properties of these plants can thus be associated with saponins and tannins.
Cytotoxicity tests results revealed a concentration dependent bioactivity, with activity increasing with increasing concentration. *Vernonia lasiopus*, *Rapanea rhododendroides*, *Entada leptostachya*, and *Albizia anthelmintica* were found to have LD_{50} of 25.57 mg/mL, 25.61mg/mL, 25.57mg/mL and 41.28mg/mL respectively. However, the tests showed that the medicinal property of these plants was due to active phytoconstituents present in the plants and not toxicity of the plant. Thus, there is need for further isolation and purification of different phytochemicals that are already known to be antihelmintic such as tannins and develop antihelmintics herbal products to deworm livestock.

P.132 - Comparison of the re and b1 gene for detection of *Toxoplasma gondii* infection in children with cancer

Sh. Fallahi[a,b] *, *B. Kazemi*[a,c], *S.J. Seyyed tabaei*[a], *N. Zebardast*[a], *Z. Arab Mazar*[a]

Department of Medical Parasitology and Mycology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Department of Medical Parasitology and Mycology, Lorestan University of Medical Sciences, Khorramabad, Iran
Department of Biotechnology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Early, accurate and effective diagnosis of toxoplasmosis can make an important contribution to the prevention and control of disease, especially in people who are at risk. In this study, two commonly used genomic repeats of *Toxoplasma gondii*, RE (AF146527) and B1, were compared to each other in nested-PCR assay. Five hundred and thirty-five blood samples from children with leukemia were tested for the presence of *T. gondii* antibodies using enzyme immunoassays. One hundred and ten DNA samples of these patients were analyzed by nested-PCR. The specificity of two nested PCR assays was determined using the DNA samples of other parasites and human chromosomal DNA. 82% and 68% of the IgM+, IgG+ samples were positive on duplicate RE and B1-nested PCR analyses, respectively. None of the 10 IgM-, IgG+ seropositive samples was detected positive after testing RE and B1-nested PCR assays in duplicate. One of the 50 seronegative samples was positive by RE-nested PCR but none of them were positive by duplicate B1-nested PCR. The detection limit of the RE-nested PCR assay was 640 fg of *T. gondii* DNA whereas this rate for B1-nested PCR was 5.12 pg of the DNA template. No cross-reactivity with the DNA of other parasites and human chromosomal DNA was found. The results indicate that an RE-based nested PCR assay is more sensitive than B1 genomic target, of those tested, for detection of *T. gondii*. It is noteworthy that in comparison with B1-nested PCR, RE-nested PCR could detect the *T. gondii* DNA in seronegative samples too.


BOOK OF ABSTRACTS
International Conference on Interdisciplinary Research and Development in ASEAN Universities
8-10 August 2013
Imperial Mae Ping Hotel
Chiang Mai, Thailand
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ORAL PRESENTATION (Afternoon Session)		
Venue: Grand Hall 2		
Date/Time	**Description**	
August 8, 2013	**SESSION: MEDICAL PARASITOLOGY AND ENTOMOLOGY**	
13.00-13.50	**Invited paper** Renal Injury and Animal Toxins *Prof. Dr.Visith Sitprija* **Invited speaker** Taking Research on Transmission of Vector-borne Parasites from the Lab to the Field *Prof. Dr.Paul A. Bates*	
13.50-17.30	**Oral presentation**	
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OM-3	• Midgut Ultrastructure of the Fourth Instar of Ochlerotatus togoi (Diptera : Culicidae) *Wetpisit Chanmol, Narissara Jariyapan, Sriwatapron Sor-Suwan, Kritsana Taai, Benjarat Phattanawiboon, Nuchpicha Intakhan, Atiporn Saeung and Wej Choochote*	22
OM-4	• Invasion of *Brugia malayi* Microfilariae within the Midgut of a Refractory Vector, *Aedes aegypti* (Sukhothai, Thailand Strain) *Nuchpicha Intakhan, Narissara Jariyapan, Wetpisit Chanmol, Sriwatapron Sor-Suwan, Benjarat Phattanawiboon, Kritsana Taai, Atiporn Saeung and Wej Choochote*	23

ORAL PRESENTATION (Afternoon Session)		
Venue: Grand Hall 2		
Date/Time	**Description**	
OM-5	• A Multiplex PCR Assay for Species Identification of Eight Species Members of the Thai *Anopheles hyrcanus* group *Chayanit Hempolchom, Visut Baimai, Sorawat Thongsahuan, Otsuka Yasushi, Atiporn Saeung, Kritsana Taai and Wej Choochote*	24
OM-6	• Development of an Allele-specific PCR Assay to Detect and Determine the Distribution of the Val1016Gly Mutation of the Voltage Gated Sodium Channel Gene of *Aedes aegypti* Populations from Thailand *Steven Andy Stenhouse, Suriya Plernsub, Nongkran Lumjuan, Jintana Yanola, Wej Choochote and Pradya Somboon*	25
OM-7	• Relationship between the Frequencies of the F1534C and V1016G Mutations in the Voltage-Gate Sodium Channel Gene and Deltamethrin Susceptibility in *Aedes aegypti* *Suriya Plernsub, Steven Andy Stenhouse, Nongkran Lumjuan, Jintana Yanola, Wej Choochote and Pradya Somboon*	26
OM-8	• Biology of the Blow Fly, *Hypopygiopsis Tumrasvini* Kurahashi (Diptera : Calliphoridae) : Adult Fly Emergence and Mating Behavior *Sangob Sanit, Chutharat Samerjai, Narin Sontigun, Tunwadee Klongklaew, Kwankamol Limsopatham, Piyawat Marswatt, Kom Sukontason and Kabkaew L. Sukontason*	27
OM-9	• The Blow Fly, *Achoetandrus rufifacies* (Macquart) : Spatiotemporal Distributions and Ovarian Development *Tunwadee Klongklaew, Ratchadawan Ngoen-Klan, Kittikhun Moophayak, Kom Sukontason, Kimberley N. Irvine, Hiromu Kurahashi and Kabkaew L. Sukontason*	28
Venue: Grand Hall		
18.30-22.00	Welcome dinner with Thai culture show	

OM-2

Genetic Compatibility Between *Anopheles lesteri* from Korea and *Anopheles paraliae* from Thailand

Kritsana Taai[1,*], Visut Baimai[2], Atiporn Saeung[1], Sorawat Thongsahuan[3], Gi-Sik Min[4], Yasushi Otsuka[5], Mi-Hyun Park[4], Masako Fukuda[6], Pradya Somboon[1] and Wej Choochote[1]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[2] Department of Biology and Centre for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Bangkok, Thailand
[3] The Establishment Project of Faculty of Veterinary Science, Prince of Songkla, Songkhla, Thailand
[4] Department of Biological Sciences, Inha University, Incheon, South Korea
[5] Department of Infectious Disease Control, Faculty of Medicine, Oita University, Hasama, Oita, Japan
[6] Department of Infectious Disease Control, Faculty of Medicine, and Promotion Project, Oita University, Hasama, Oita 879-5593, Japan
[*] Corresponding author, E-mail : kritsana_taai@hotmail.com

To assess differentiation and relationship between *Anopheles lesteri* and *Anopheles paraliae* three and five iso-female lines of *A. lesteri* from Korea and *A. paraliae* from Thailand were established. These isolines were used to investigate the genetic relationship between the two taxa by crossing experiments and by comparing DNA sequences of ribosomal DNA second internal transcribed spacer (ITS2) and mitochondrial DNA cytochrome c oxidase subunit I (COI) and subunit II (COII). Reciprocal and F1-hybrid crosses between *A. lesteri* and *A. paraliae* indicated that they were compatible genetically producing viable progenies and complete synaptic salivary gland polytene chromosomes without inversion loops in all chromosome arms. The pairwise genetic distances of ITS2, COI and COII between these morphological species were 0.040, 0.007-0.017 and 0.008-0.011, respectively. The specific species status of *A. paraliae* in Thailand and/or other parts of the continent are discussed.

Keywords: *Anopheles lesteri*, *Anopheles paraliae*, Crossing experiments, Ribosomal DNA, Mitochondrial DNA, Genetic compatibility

OM-3

Midgut Ultrastructure of the Fourth Instar of *Ochlerotatus togoi* (Diptera : Culicidae)

Wetpisit Chanmol[1], Narissara Jariyapan[1,*], Sriwatapron Sor-Suwan[1], Kritsana Taai[1], Benjarat Phattanawiboon[1], Nuchpicha Intakhan[1], Atiporn Saeung[1] and Wej Choochote[1]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand
[*] Corresponding author, E-mail : narsuwan@mail.med.cmu.ac.th

The midgut is the largest organ in the mosquito larval body sustaining ion transport, biomolecule absorption and an entry site for several pathogens. In this study, the ultrastructure of the midgut of *Ochlerotatus togoi* fourth instar was investigated by light scanning and transmission electron microscopy. In early 4th instars, the larval midgut was approximately 2 mm in length and formed by a monolayer of epithelial cells with the plasma membrane showing multiple folding where it adjoined the basement membrane. It consisted of at least 3 morphologically distinct cell types including columnar, endocrine, and regenerative cells. Regenerative cells were scattered throughout the basal portion of the epithelium, along with endocrine cells. Other midgut cells containing large, microvilli-lined apical cavities were identified in most specimens. No evidence of division or differentiation was obtained for any cell types. At least six layers of peritrophic matrix (PM) were observed in the gut lumen. The PM separated foods from the midgut epithelial cells. For late 4th instars before defecation, cytoplasmic protrusion in many areas of the luminal midgut surface and numerous phagocytosomes in the epithelial cells were observed suggesting that autophagy process occurred at the late stage of the 4th instars. This information provided an understanding of the normal larval midgut development for further studies on factors that control the growth and nutritional state of *O. togoi* larvae to reduce adult fecundity and physiological roles of the larval midgut in interaction with biological control organisms.

Keywords: *Ochlerotatus togoi*, Larva, Midgut, Ultrastructure

OM-5

Multiplex PCR Assay for Species Identification of Eight Species Members of the Thai *Anopheles hyrcanus* Group

Chayanit Hempolchom[1,*], Visut Baimai[2], Sorawat Thongsahuan[3], Otsuka Yasushi[4], Atiporn Saeung[1], Kritsana Taai[1] and Wej Choochote[1]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[2] Department of Biology and Centre for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Bangkok, Thailand
[3] Faculty of Veterinary Science (Establishment Project), Prince of Songkla University, Songkhla, Thailand
[4] Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan
[*] Corresponding author, E-mail : chayanit.hem@gmail.com

Anopheles hyrcanus group comprises important vectors of malaria, due to Plasmodium vivax and filariasis caused by Brugia malayi in many countries of South, Southeast and East Asian regions. In Thailand, 8 species members, including *Anopheles argyropus, A. crawfordi, A. nigerrimus, A. nitidus, A. paraliae, A. peditaeniatus, A. pursati, and A. sinensis of the A. hyrcanus* group have been recognized. Due to morphological overlap, adult females of the *A. hyrcanus* group in Thailand have been markedly misidentified among the 8 species members, particularly by the traumatic scales of wild-caught specimens from epidemiology and control approaches. Therefore, this study firstly developed a simple and robust multiplex PCR assay, based on second internal transcribed spacer (ITS2) sequences of ribosomal DNA, for differentiating the 8 species members of the Thai *A. hyrcanus* group.

Keywords: *Anopheles hyrcanus* group, Multiplex PCR assay, ITS2

OM-6

Development of an Allele-specific PCR Assay to Detect and Determine the Distribution of the Val1016Gly Mutation of the Voltage Gated Sodium Channel Gene of *Aedes aegypti* Populations from Thailand

Steven Andy Stenhouse[1,*], Suriya Plernsub[1], Nongkran Lumjuan[2], Jintana Yanola[3], Wej Choochote[1] and Pradya Somboon[1]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[2] Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand
[3] Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
[*] Corresponding author, E-mail : steven.stenhouse@gmail.com

Resistance to pyrethroid insecticides is widespread among populations of *Aedes aegypti*. Several different point mutations within the voltage gated sodium channel (VGSC) gene contributed to such resistance. This study developed and utilized an allele specific PCR (AS-PCR) assay that could be used to detect the Val1016Gly mutation in domain II, segment 6 of the VGSC in *Ae. aegypti*. The assay was validated against a number of sequenced DNA samples of known genotype and was found to be in complete agreement. Larvae and pupae were collected from various localities in Thailand. Samples were reared to adulthood and their resistance status was determined by deltamethrin susceptibility bioassays. Deltamethrin-resistant and susceptible insects were then tested by our AS-PCR assay. This testing indicated that the mutation is positively associated with deltamethrin resistance and is widely distributed at high frequencies throughout the country. The usefulness of this assay in determining the distribution of this mutation outside of Thailand had also been discussed.

Keywords: *Aedes aegypti*, AS-PCR, Deltamethrin resistance, Thailand

OM-7

Relationship between the Frequencies of the F1534C and V1016G Mutations in the Voltage-Gate Sodium Channel Gene and Deltamethrin Susceptibility in *Aedes aegypti*

Suriya Plernsub[1,*], Steven Andy Stenhouse[1], Nongkran Lumjuan[2], Jintana Yanola[3], Wej Choochote[1] and Pradya Somboon[1]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[2] Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand
[3] Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand
[*] Corresponding author, E-mail : immuno_six@hotmail.com

Pyrethroid resistance in *Aedes aegypti* poses a problem for vector control. Mutations within the voltage-gate sodium channel (vgsc) gene may contribute to insecticide resistance. Single nucleotide polymorphisms within the gene can lead to amino acid substitutions, particularly a valine to glycine substitution at codon 1016 (V1016G) in domain IIS6 and a phenylalanine to cysteine substitution at codon 1534 (F1534C) in domain IIIS6. These are the most important knockdown resistance (kdr) mutations in *A. aegypti* found in Thailand. The relationship between the two mutations in *A. aegypti* and deltamethrin resistance was investigated in a laboratory-selected strain originated from Chiang Mai, Thailand. Wild caught mosquito larvae from Chiang Mai city were reared into adults. Resistant mosquitoes were selected with 0.05% deltamethrin impregnated papers for 10 generations. Samples of dead and surviving mosquitoes were analyzed for the F1534C and V1016G mutations by using allele specific PCR techniques. Mortality rates and the allele frequencies were recorded for each generation. It was shown that the mortality rates of the F0-F10 generations decreased during selection (48.6%-1.01%), while the frequency of the V1016G mutation increased. By contrast, however, the frequency of the F1534C mutation decreased with selection. These results suggest that deltamethrin resistance in *A. aegypti* is positively correlated with the V1016G mutation in the voltage-gate sodium channel gene.

Keywords: *Aedes aegypti*, Deltamethrin susceptibility, Voltage-gate sodium channel gene


GREATER MEKONG SUBREGION
MYANMAR
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CHINA
Proceedings of
The 1st Asian Parasites
by Asian Parasitologists (APAP):
Helminthiases in Greater Mekong Subregion
12 May 2013
Faculty of Medicine, KKU
Khon Kaen, Thailand
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"แก่นคูน"
Khon Kaen University

P23 - Bile canalicular alteration and bile secretory defect in acute and chronic *Opisthorchis viverrini*-infected hamsters. (Lakhanawan Charoensuk, Porntip Pinlaor, Wunnee Chaijaroonkhanarak, Puangrat Yongvanit, Chawalit Pairojkul, Yukifumi Nawa and Somchai Pinlaor)

P24 - Encapsulated curcumin enhances periductal fibrolysis in *Opisthorchis viverrini*-infected hamsters after praziquantel treatment. (Porntip Pinlaor, Lakhanawan Charoensuk, Rucksak Rucksaken, Puangrat Yongvanit and Somchai Pinlaor)

P25 - Proteomic identification of expressed proteins associates with periductal fibrosis in chronic experimental opisthorchiasis. (Ornuma Haonon, Rucksak Rucksaken, Sudarat Onsurathum, Porntip Pinlaor, Thidarut Boonmars and Somchai Pinlaor)

P26 - The hydatid-killing effects of high intensity focused ultrasound enhanced with ultrasound contrast agent and superabsorbent polymer. (Hui Cai, Bin Ye, Lu-Lu Chen, Ai-Bo Liu, Jing Zhang, Yi-Feng Zhao and Xiao-Yi Zou)

P27 - *Trichinella spiralis* adult worm but not muscle larvae products ameliorate acute DSS-induced colitis (Xiaodi Yang, Yaping Yang, Yunyun Wang, Yuan Gu and Xinping Zhu, Xiaodi Yang and Yaping)

P28 - A prospective cohort study on the factors associated to the participation in and compliance to the mass drug administration for Schistosomiasis in Barangay Bethel and Barangay Canaan, Victoria, Oriental Mindoro. (Co, Stephanie N, Ong, Ronnah Marie M, Lenon, John Lemuel L, Ramos, Carmela Cecilia S, Mirano, Ma. Veronica D.T., Valdes and John Xavier R.)

P29 - *Gnathostoma spinigerum* infection involving the inner lip of a Korean woman: the first autochthonous case. (Jae Hee Kim, Hyemi Lim, Young-Sang Hwang, Tae Youn Kim, Eun Mee Han, Eun-Hee Shin and Jong-Yil Chai)

P30 - Current status of *Paragonimus* spp. and paragonimiasis in north and central Vietnam. (Pham Ngoc Doanh, Hoang Van Hien, Yoichiro Horii, Yukifumi Nawa)

P31 - High prevalence of haplorchiasis in Nan and Lampang province, Thailand, following antehelminthic treatments of suspected opisthorchiasis cases. (Adulsak Wijit, Nimit Morakote and Jaewwaew Klinchid)

P32 - Susceptibility of five species members of the Korean *Anopheles hyrcanus* group to *Brugia malayi*. (Atiporn Saeung, Gi-Sik Min, Sorawat Thongsahuan, Kritsana Taai and Wej Choochote)

P33 - Prevalence of intestinal parasitic infections among schoolchildren of Phitsanulok Province, northern Thailand. (Raxsina Polseela and Apichat Vitta)

P34 - Recovery of *Enterobius vermicularis* and *Taenia* eggs by Scotch tape technique. (Apichat Vitta Aunchalee Thanwisai, Wilawan Poomidonming, Seangchai Nateeworanart and Raxsina Polseela)

P35 - Intestinal Parasitic infection among school children in an urban communities of Khon Kaen, Northeast region of Thailand. (Kanchana Tomanakan, Nuttiya Srisurat, Suphakdee Sanseeha and Kriangkrai Kongsuk)

Susceptibility of five species members of the Korean *Anopheles hyrcanus* group to *Brugia malayi*

Atiporn Saeung[1], Gi-Sik Min[2], Sorawat Thongsahuan[3], Kritsana Taai[1] and Wej Choochote[1]

[1]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand; [2]Department of Biological Sciences, Inha University, Incheon 402-751, South Korea; [3]Faculty of Veterinary Science (Establishment Project), Prince of Songkla University, Songkhla 90110, Thailand

E-mail: atsaeung@mail.med.cmu.ac.th

ABSTRACT

Five species members of the Korean *Anopheles hyrcanus* group, i.e., *An. pullus*, *An. sinensis*, *An. kleini*, *An. belenrae* and *An. lesteri* were tested for susceptibility to *Brugia malayi*. They were allowed to feed artificially on blood containing *B. malayi* microfilariae, and dissected 14 days after feeding. The susceptibility rates were 60%, 65%, 90%, 100% and 100% in *An. pullus*, *An. sinensis*, *An. kleini*, *An. belenrae* and *An. lesteri*, respectively. As determined by levels of susceptibility, results indicated that *An. pullus* and *An. sinensis* were moderate potential vectors, while *An. kleini*, *An. belenrae* and *An. lesteri* were high potential vectors when compared with the 95% susceptibility rate of an efficient control vector, *Ochlerotatus togoi*. The satisfactory susceptible rates obtained in this study from *An. sinensis* and *An. lesteri* have confirmed previous reports that these 2 anopheline species played an important role as natural vectors of Brugian filariasis in East Asia. Even though this filarial parasite has been eradicated up to now in the Republic of Korea (ROK), beneficial results, reported herein, emphasize the potential role of these anopheline species in transmitting *B. malayi* in other countries, in which this filarial parasite is still endemic and/or re-emerging, similarly to the recent re-emergence of other mosquito-borne diseases, e.g., malaria due to *Plasmodium vivax* in ROK.

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POSTER PRESENTATION (Afternoon Session)		
Venue: Imperial 5		
Date/Time	**Description**	
August 8, 2013	**SESSION: INFORMATION TECHNOLOGY**	
13.00-17.00	**Poster presentation**	
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PI-5	• Research Trends in the Online Environment and Natural Resources Management Program of the University of the Philippines Open University *Maripres Sarinas*	236
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PM-2

Development of a Facile System for Mass-Production of *Brugia malayi* in a Small-Space Laboratory

A. Saeung[1] and Wej Choochote[1,*]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[*] Corresponding author, E-mail : wchoocho@mail.med.cmu.ac.th

Brugia malayi is one of the important lymphatic filarial nematodes that cause elephantiasis and disability in humans in the Asian region. Mass production of this nematode in both small laboratory animal hosts and mosquito vectors is still necessary in order to continue various research aspects. This study elucidated on the use of non-blood feeding or the autogenous *Ochlerotatus* (=Aedes) *togoi* (Thailand strain) and male Mongolian jird (*Meriones unguiculatus*) system. This was a low cost and highly effective procedure for the mass-production of blood containing microfilariae, infective (L3) larvae and adults of *B. malayi* under non animal-blood feeding insectary and small-space animal housing conditions. The highly infective rates and parasite loads of autogenous *O. togoi* to *B. malayi* and high adult worm recovery from *B. malayi*-infected male jird were good supportive evidence. In addition, all special techniques required for the success in the establishment of a facile system regarding these matters were discussed.

Keywords: *Brugia malayi,* Mass-production, *Ochlerotatus togoi*, Mongolian jird, Small-space laboratory

POSTER PRESENTATION (Afternoon Session)		
Venue: Imperial 5		
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PM-6	• Susceptibility of Five Species of Korean *Anopheles hyrcanus* to *Brugia malayi* and Hybridization between *B. malayi* -Susceptible and -Refractory *A. sinensis* Strains *Atiporn Saeung, Gi-Sik Min, Sorawat Thongsahuan, Kritsana Taai, Siripan Songsawatkiat and Wej Choochote*	243
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PM-8	• Morphological and Protein Analyses of the Female Salivary Glands of *Anopheles Barbirostris* Species A1 (Diptera : Culicidae) during Adult Development *Benjarat Phattanawiboon, Narissara Jariyapan, Sriwatapron Sor-Suwan, Atiporn Saeung, Yong Poovorawan, Paul A Bates and Wej Choochote*	245
PM-9	• Screening for Larvicidal Bioactivity of Thai Indigenous Plants Against the Dengue Vector, *Aedes aegypti* *Daruna Champakaew, Anuluck Junkum, Benjawan Tuetun, Wej Choochote, Udom Chaithong, Atchariya Jitpakdi, Doungrat Riyong, Jitrawadee Intirach, Rukpong Sanghong and Benjawan Pitasawat*	246
PM-10	• Repellency of *Conioselinum univitatum* Trucz. (Family : Umbelliferae) Rhizome Extracts for Personal Protection against Mosquito Bites *Rukpong Sanghong, Anuluck Junkum, Wej Choochote, Udom Chaithong, Atchariya Jitpakdi, Doungrat Riyong, Daruna Champakaew, Jitrawadee Intirach, Roongtawan Muangmoon, Arpaporn Chansang and Benjawan Pitasawat*	247
PM-11	• Ant Colony System-based Applications to Healthcare Distribution System Optimization *Boonyong Punantapong*	248
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18.30-22.00	Welcome dinner with Thai culture show	

PM-6

Susceptibility of Five Species of Korean *Anopheles hyrcanus* to *Brugia malayi* and Hybridization between *B. malayi* -Susceptible and -Refractory *A. sinensis* Strains

Atiporn Saeung[1,*], Gi-Sik Min[2], Sorawat Thongsahuan[3], Kritsana Taai[1], Siripan Songsawatkiat[1] and Wej Choochote[1]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[2] Department of Biological Sciences, Inha University, Incheon, South Korea
[3] Faculty of Veterinary Science (Establishment Project), Prince of Songkla University, Songkhla, Thailand
[*] Corresponding author, E-mail : atiporn44@yahoo.com

Five species of Korean *A. hyrcanus*, i.e., *A. pullus, A. sinensis, A. kleini, A. belenrae* and *A. lesteri* were tested for susceptibility to *Brugia malayi*. They were allowed to feed artificially on blood containing *B. malayi* microfilariae, and dissected 14 days after feeding. The susceptibility rates were 60%, 65%, 90%, 100% and 100% in *A. pullus, A. sinensis, A. kleini, A. belenrae* and *A. lesteri*, respectively. As determined by levels of susceptibility, it was indicated that *A. pullus* was a moderate potential vector, while *A. sinensis, A. kleini, A. belenrae* and *A. lesteri* were high potential vectors, when compared with the 90-95% susceptibility rates of an efficient control vector, *Ochlerotatus* (=*Aedes*) *togoi*. An introgressive study of *B. malayi*-susceptible/-refractory genes was performed intensively by hybridization experiments between a high (Korean strain) and a low (Thailand strain) potential *A. sinensis* vectors, and the susceptibility rates of F1-hybrids and backcross progenies were compared with parental stocks. It was revealed that the *B. malayi* -susceptible genes could be introgressed from a high to low potential *A. sinensis* vector by increasing the susceptibility rates from 0-5% in the parental stocks to 55% and 70% in F1-hybrids and backcross progenies, respectively. The increase in susceptibility rates related clearly to the increase of normal larval development in the thoracic muscles of F1-hybrids and backcross progenies.

Keywords: *Anopheles hyrcanus*, *Brugia malayi*, Susceptibility level, Hybridization, Susceptible/refractory genes

POSTER PRESENTATION (Afternoon Session)		
Venue: Imperial 5		
Date/Time	**Description**	
PM-6	• Susceptibility of Five Species of Korean *Anopheles hyrcanus* to *Brugia malayi* and Hybridization between *B. malayi* -Susceptible and -Refractory *A. sinensis* Strains *Atiporn Saeung, Gi-Sik Min, Sorawat Thongsahuan, Kritsana Taai, Siripan Songsawatkiat and Wej Choochote*	243
PM-7	• Cytogenetic, Hybridization and Molecular Evidence of Four Cytological Forms of *Anopheles Nigerrimus* (Hyrcanus Group) in Thailand and Cambodia *Siripan Songsawatkiat, Visut Baimai, Atiporn Saeung, Sorawat Thongsahuan, Yasushi Otsuka, Wichai Srisuka and Wej Choochote*	244
PM-8	• Morphological and Protein Analyses of the Female Salivary Glands of *Anopheles Barbirostris* Species A1 (Diptera : Culicidae) during Adult Development *Benjarat Phattanawiboon, Narissara Jariyapan, Sriwatapron Sor-Suwan, Atiporn Saeung, Yong Poovorawan, Paul A Bates and Wej Choochote*	245
PM-9	• Screening for Larvicidal Bioactivity of Thai Indigenous Plants Against the Dengue Vector, *Aedes aegypti* *Daruna Champakaew, Anuluck Junkum, Benjawan Tuetun, Wej Choochote, Udom Chaithong, Atchariya Jitpakdi, Doungrat Riyong, Jitrawadee Intirach, Rukpong Sanghong and Benjawan Pitasawat*	246
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PM-11	• Ant Colony System-based Applications to Healthcare Distribution System Optimization *Boonyong Punantapong*	248
WELCOME DINNER		
Venue: Grand Hall		
18.30-22.00	Welcome dinner with Thai culture show	

PM-7

Cytogenetic, Hybridization and Molecular Evidence of Four Cytological Forms of *Anopheles nigerrimus* (Hyrcanus Group) in Thailand and Cambodia

Siripan Songsawatkiat[1,*], Visut Baimai[2], Atiporn Saeung[1], Sorawat Thongsahuan[3], Yasushi Otsuka[4], Wichai Srisuka[5] and Wej Choochote[1]

[1] Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[2] Department of Biology and Centre for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Bangkok, Thailand
[3] Faculty of Veterinary Science (Establishment Project), Prince of Songkla University, Songkhla, Thailand
[4] Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan
[5] Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand
[*] Corresponding author, E-mail : siripan471@gmail.com

Thirteen isoline colonies of *Anopheles nigerrimus* were established from individual wild-caught females collected from cow-baited traps at 4 and 1 locations in Thailand and Cambodia, respectively. Three types of X (X1, X2, X3) and 4 types of Y (Y1, Y2, Y3, Y4) chromosomes were recovered, according to differing amounts of extra heterochromatin. Four karyotypic forms were designed depending upon apparently distinct figures of X and Y chromosomes, i.e., Form A (X1, X2, X3, Y1), B (X2, X3, Y2), C (X1, Y3) and D (X3, Y4). Forms C and D were new metaphase karyotype discovered in this study. Form A appeared to be common in both Thailand and Cambodia. Forms B and D were found to be rather specific to southern and northeastern Thailand, respectively, whereas Form C was confined somewhat to Cambodia. Hybridization experiments among the 8 isoline colonies, which were representative of 4 karyotypic forms of *A. nigerrimus*, demonstrated genetic compatibility in giving viable progenies and synaptic salivary gland polytene chromosomes through F2-generations. These results elucidated the conspecific relationship, comprising 4 cytological forms within this taxon. Supportive evidence was confirmed further by very low intraspecific sequence variations (average genetic distance=0.002-0.007) of the nucleotide sequences in ribosomal DNA [second internal transcribed spacer (ITS2)] and mitochondrial DNA [cytochrome c oxidase subunit I (COI) and subunit II (COII)].

Keywords: *Anopheles nigerrimus*, Metaphase karyotypes, Hybridization, Ribosomal DNA, Mitochondrial DNA