

Abstracts

Session 3 Parasitology

Plenary paper

S3.1 Studies on impact of mass drug administration and elimination of lymphatic filariasis in India

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Lymphatic filariasis (LF) is a major public health problem in India and many other developing countries. A global programme to eliminate LF was launched in the year 2000. Annual single dose mass drug administration (MDA) of anti-filarial drugs is the key strategy of the global programme. The potential of repeated mass administration of diethylcarbamazine (DEC) and ivermectin and DEC+Albendazole to eliminate LF is being examined in south India. During 9-10 rounds of MDA using DEC or ivermectin alone, 49% to 84% of the eligible population received treatment in different villages. Parasitological, entomological and immunological evidence from villages administered with ten rounds of DEC suggest that transmission interruption and elimination of LF appears to be relatively less difficult in the villages with low endemicity than in the villages with high endemicity. The latter may require more intensive or frequent MDA or alternative treatment regimens or additional measures such as vector control. Post 10th MDA, the study villages are being monitored to examine the disappearance/recrudescence of residual infection. The results with nine rounds of administration of ivermectin alone were slightly less impressive. Four rounds of administration of DEC+Albendazole lead to highly appreciable reduction in microfilaria prevalence and vector infectivity rates. These studies also suggest that MDA yields significant public health benefits in terms of significant reduction in chronic LF prevalence and other infections such as soil-transmitted helminths. The outcome of various research studies in India and their implications for LF elimination and costs and benefits of MDA will be discussed.



S3.2 Anti-malarial activity from *Erythrina variegata* (Leguminosae)

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Erythrina variegata (Leguminosae) is traditionally considered to have anti-malarial properties. In the course of our search for novel anti-malarial compounds from Indonesian plants, the methanol extract of *E. variegata* showed significant anti-malarial activity against *Plasmodium falciparum* in vitro. By using the active compounds to follow the separation, the methanol extract was separated by combination of column chromatography to yield five active compounds (1-5). The chemical structure of active compounds were determined on the basis of spectroscopic evidence and comparison with the previously reported and identified as flavonoid (1-2), triterpenoid (3-4), and alkaloid (5). The compound (1 and 3) showed anti-malarial activity against strain of parasite used with IC₅₀ of 0.013 and 0.243 µg/ml against FCR-3/A, respectively. The compound (2,4 and 5) showed anti-malarial activity against both strains of parasite used with IC₅₀ of 3.7, 4.3, and 3.2 µg/ml against K1 and 4.8, 24.9, and 25.5 µg/ml against 3D7, respectively.

S3.3 Preliminary clinical, biochemical and haematological findings in human *Plasmodium knowlesi* infections

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Following identification of human *P. knowlesi* malaria in Malaysian Borneo, we started a longitudinal observational study to characterize clinical and laboratory findings associated with this novel infection. Adults (aged ≥ 15 years) admitted to Kapit Hospital, Sarawak, Malaysia with PCR-confirmed single *P. knowlesi* infection were recruited. Those who had received previous antimalarial treatment or who were pregnant were excluded. All available admission data from the first 53 patients were analysed. Mean age was 44 (range 16-76) years and 60% were males. Median time from onset of symptoms to admission was 4 (range 1-23) days, with all patients experiencing fevers, chills and rigors. Other symptoms included headache (89%), myalgia (89%), anorexia (83%), arthralgia (62%), cough (59%), abdominal pain (40%) and diarrhoea (25%). On admission, 86% of patients were tachypnoeic, 49% tachycardic and 49% pyrexial (axillary temperature $>38^{\circ}\text{C}$), and 6% and 11% had palpable splenomegaly and hepatomegaly, respectively. Admission geometric mean parasitaemia was 1,210 (range 20-259,875) / μL whole blood. All patients were thrombocytopenic and 6% had a platelet count $<30,000/\mu\text{L}$. Hyponatraemia (34%) and mild changes in liver function (75%, including mild mixed hyperbilirubinaemia in 60% of these) were also observed. One patient (a hyperparasitaemic 68 year old woman) died of multi-organ failure. The fact that most infections occur in adults suggests that human knowlesi malaria has low endemicity in Malaysian Borneo. Despite relatively low parasitaemias in most cases, patients commonly present with a range of symptoms and laboratory abnormalities. Clinical severity can range from a mild to potentially fatal illness.



S3.4 Malaria situation in a forest district malarious province (Cambodia)

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In Cambodia, the majority of malaria cases and deaths occur in the mountainous and forested areas. Two Cross Sectional Surveys were conducted in 12 malaria endemic villages in 3 provinces (Pailin, Rattanakiri and Pursat) to evaluate the malaria transmission pattern in different forested settings and to estimate the risk factors of malaria infections. This preliminary analysis is only for 3 villages of Pursat province. Randomly selected individuals from the 3 villages participated in the surveys. About 92% were of Khmer origin and worked as farmers, loggers or hunters in the forest. Mosquito collections were also performed outside the houses in 3 locations for each study site: village, forest way and forest plots. No anophelines were obtained in forest ways. Dey Kraharm village, located in the evergreen forest, had highest density of *An. dirus*, *An. minimus* and *An. maculatus* s.l in August. Overall density of *Anopheles*, in villages and forest plots, was higher in November than in August. The overall parasite rate was 8.5% and 11.3 for surveys 1 and 2 respectively. *Plasmodium vivax* represented more than 84% of the species seen. People living in an evergreen forest (Dey Kraharm) or close to it (Don Neak) had significantly higher risk for malaria infections compared to the more distant village (Tang Yor) located in deciduous forest. Age group 0-19 years was higher at risk than age group 20-39 years. The anti-relapse (Primaquine) treatment for *P. vivax* should be taken into consideration along with the use of long lasting insecticidal nets.

S3.5 Oxidant state in *Plasmodium falciparum* culture incubated with artemisinin

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Malaria, in particular falciparum malaria, causes high morbidity and mortality especially in tropical country. Beside it's virulence, *Plasmodium falciparum* resistance against antimalarial drug has become a problem especially in South East Asia including Indonesia and South America. Artemisinin as a cyclic endoperoxide compound (sesquiterpene lactone), which is an antimalarial drug with high efficacy including against the resistant one, has several mechanisms of action producing free radical which is also toxic against the host. Because of that, we need to study the oxidant state of *P. falciparum* culture incubated with artemisinin. Supernatant of *P. falciparum* culture incubated with several artemisinin concentration for 24 hours were tested for MDA concentration. The result was analyzed using ANOVA which was continued using Duncan analysis. The MDA concentration, which reflected oxidant state, showed that there was a highly significant difference between artemisinin concentration $\leq 10^{-8}$ M and $\geq 10^{-7}$ M. There was no MDA concentration difference in artemisinin concentration 10^{-8} M, 10^{-9} M, 10^{-10} M, and 0 M also between 10^{-6} M and 10^{-7} M.



S3.6 The molecular identification of forensically important flies in decomposed corpses in Northern Peninsular Malaysia

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This study investigates the important aspects in forensic entomology during the identification of fly species i.e the utilization of the mitochondrial COI I (mtDNA) gene for precise forensic fly's identification in Northern Peninsular Malaysia. In second part of this study, the utility of mitochondrial cytochrome oxidase subunit 1 (COI 1) gene in the identification of forensically important flies occurring in Northern. Malaysia was investigated. Live larvae samples were collected from human corpses. DNA from the larvae obtained was extracted, a 300 bp region of cytochrome oxidase subunit I (COI I) gene was amplified and sequenced. Results analyzed using the PAUP software successfully differentiated between the two species of common forensic flies (*Ch. megacephala*, *Ch. rufifacies*) found on the different hosts.

S3.7 Toxoplasmosis in patients with hematological malignancies : a Malaysian experience

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One hundred and thirty-one patients with hematological malignancies were recruited in this prospective and case-control study. The age range was from 16 to 81 years with a mean of 44.5 ± SD 17.6 years. The Chinese (78; 59.5%) composed the highest percentage when compared to Malays (32; 24.4%) and Indians (21; 16%). In all age groups, the majority of patients were unemployed males (51; 63.8%) who had a secondary level of education (67; 51.2%) (p=0.05). Interestingly, a significant majority of these subjects had leukemia (77; 58.8%) compared to lymphoma types (45; 34.4%) and other (9; 6.9%) hematological malignancies (p=0.03). Overall, latent *Toxoplasma* seroprevalence (IgG antibody) was found in 46 (35.1%) patients and more commonly in the age groups of ≥31 years (39; 30%, p=0.041), as well as in patients with leukemia (7/16) compared to other types of malignancies (p=0.45). Further analysis demonstrated that being female (15/16; p=0.045), Malay (12/13; p=0.003), a non-laborer (12/12; p=0.023), having a tertiary level of education (10/12; p=0.013), leukemia (25/29; p= 0.048), and a hometown outside Kuala Lumpur (33/39; P=0.05) were statistically significant contributors to the seroprevalence of toxoplasmosis, particularly in the age group of ≥31 years. Seropositive patients had significant contact with cats, particularly among Malays (p=0.006). Further analysis showed that home cooked (44; 95.6%) or outside foods (34; 74%), surprisingly, did not play any significant role in the acquisition of *Toxoplasma* infection (p>0.05). There was no clinical case of secondary reactivation of latent toxoplasmosis during the time of this study.



S3.8 Reduction of *Cryptosporidium* and *Giardia* by sewage treatment processes

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Cryptosporidium and *Giardia* are two important pathogenic parasites that have caused many waterborne outbreaks which affected hundreds of thousands of people. Contamination from effluent discharge by sewage treatment plants have been implicated in previous waterborne outbreaks of *Cryptosporidium* and *Giardia*. This study evaluated the reduction of *Cryptosporidium* and *Giardia* (oo)cysts in two sewage treatment plants (STPA and STPB) in Malaysia which employed different treatment processes for a period of a year. Raw sewage influents and treated sewage effluents were concentrated by repeated centrifugation, subjected to sucrose density flotation and concentrated to a minimal volume depending upon the levels of contaminating debris. *Cryptosporidium* oocysts and *Giardia* cysts were enumerated using epifluorescence microscopy. Results indicated that sewage treatment process which employed extended aeration could reduce *Cryptosporidium* and *Giardia* (oo)cysts significantly but treatment process which encompasses aerated lagoon could only reduce *Giardia* cysts but not *Cryptosporidium* oocysts significantly. This phenomenon is of great concern in areas whereby effluent of sewage treatment plants is discharged into the upstream of rivers that are eventually used for abstraction of drinking water. Further epidemiological studies on the occurrence and removal of pathogenic organisms from excreta and sewage are required, in order that the public health risks can be defined and the most cost effective sewage treatment options developed.

S3.9 Genotyping of *Blastocystis hominis* in Malaysian animal handlers

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Blastocystis hominis is one of the most common parasites found in man. Recent studies have provided evidence for the zoonotic transmission of *Blastocystis*. This is the first study to genotype *Blastocystis* in Malaysian animal handlers. Stool samples were collected from 90 animal handlers from the National Zoo (40), animal house of the University of Malaya (15) and a local abattoir (35). 13.3 % (12/90) of the animal handlers were found positive for *B. hominis* by *in vitro* culture using Jones' medium. Sequence-tagged site (STS) primer PCR detected subtype 3 (10/12), subtype 1 (1/12) and subtype 5 (1/12) of *Blastocystis*. Subtype 1 and subtype 5 were previously shown to be present in animals. Subtype 3 although, primarily a human genotype but was also shown to exist in cattle and pig. The present study provides evidence of the existence of these three subtypes in animal handlers and implies that these genotypes could have been acquired from animal sources through zoonotic transmission.



S3.10 Risk factors for endemic giardiasis-highlighting the association with contaminated water and food

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This study was carried out to assess the risk factors of giardiasis in Orang Asli population living in Pahang, Malaysia. Stool samples were collected from 321 individuals aged between 2-76 years old, of whom 160 were males and 161 were females. Faecal samples were collected in screw-capped containers, fixed in PVA and examined using trichrome staining technique. Biodata, personal hygiene, sanitation and socioeconomic variables were also collected via pre-tested standard questionnaires. The overall prevalence of *Giardia intestinalis* infection was 22.8%. Results showed a significant association between age groups and giardiasis ($p < 0.005$). Children ≤ 12 years old had the highest infection rate (OR = 6.2, 95%CI = 1.5-27.0, $p < 0.005$). The risk of getting giardiasis in the study population appears to be associated with drinking pipe water (OR = 5.1, 95% CI = 0.06-0.7, $p < 0.005$), eating raw vegetables (OR = 2.4, 95% CI = 0.2 -0.6, $p < 0.005$) and eating fresh fruits (OR = 2.7, 95% CI = 1.03-7.3, $p = 0.036$). Risk of giardiasis was also observed among individuals who do not use toilet (OR = 1.8, 95% CI = 1.1-3.0, $p = 0.022$). Logistic regression analysis confirmed that age group ≤ 12 years old, drinking pipe water and eating raw vegetables are risk factors for giardiasis. A significant association between giardiasis and diarrhea and other gastroenteritis symptoms has been noted in this study ($p < 0.05$). It has been concluded that giardiasis is still a public health problem in Malaysia.

S3.11 An unceasing problem: soil-transmitted helminthiases in rural Malaysian communities

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Despite great development in socioeconomic status throughout 50 years of independence, Malaysia is still plagued with soil-transmitted helminthiases (STH). STH continue to have significant impact on public health particularly in rural communities. In order to determine the prevalence of STH among rural Orang Asli children and to investigate the possible risk factors affecting the pattern of this prevalence, fecal samples were collected from 292 Orang Asli primary schoolchildren (145 males and 147 females) aged 7-12 years, from Pos Betau, Kuala Lipis, Pahang. The samples were examined by Kato-Katz and Harada Mori techniques. Socioeconomic data were collected using pre-tested questionnaires. The overall prevalence of ascariasis, trichuriasis and hookworm infections were 67.8%, 95.5% and 13.4% respectively. 29.8% of the children had heavy trichuriasis while 22.3% had heavy ascariasis. 67.7% of them had mixed infections. STH eggs were also detected in dogs and rats faeces with moderate and heavy egg loads. Moreover, eggs were reported in 70% of the soil samples and 20% of river water samples. Age ≤ 10 years ($p=0.016$), no toilet in house ($p=0.012$), working mother ($p=0.040$), low household income ($p=0.033$) and large family size ($p=0.028$) were identified as risk factors of ascariasis. Logistic regression confirmed low income, no toilet in house and working mother as significant risk factors of ascariasis. STH may also contribute to other health problems such as micronutrient deficiencies, protein-energy malnutrition and poor educational achievement. Public health personnel need identify innovative and integrated ways in order to reduce STH in the rural communities.



S3.12 Kinetics of antigen specific IgG subclass antibody responses in visceral leishmaniasis patients of Bangladesh

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Visceral leishmaniasis (VL) or Kala-azar is endemic in many countries of the world including Bangladesh. *Leishmania donovani*, the causative agent, induces antibody response in infected person which is currently the most useful diagnostic marker. Leishmanial antigens may preferentially stimulate a particular IgG subclass antibody in active infection. The present study was carried out to investigate antigen-specific IgG subclass antibody response in different phases of infection in VL patients of Bangladesh. A total of 131 VL patients from 449 households of 10 villages were included in the study. Active cases were diagnosed by demonstration of parasite in bone marrow aspirate. A validated ELISA test, using K-39 antigen, was employed to determine anti-leishmania total IgG and its subclass (IgG1-4) in pre and post treated cases. There was significant preferential IgG1 anti-leishmanial antibody response in active stage of the diseases. IgG2, 3 and 4 were not significantly raised following infection. Sensitivity and specificity of detecting active kala azar cases by total IgG and IgG1 were 97% and 100% respectively. Post treatment follow up of 78 cases revealed consistent gradual decline of total IgG and IgG1 antibodies over 9-month period and 94% and 80% became negative ($<$ cut off OD) respectively by 9 months. The findings indicate that K-39 specific total IgG and IgG1 is an indicative marker for active infection and *L. donovani* selectively modulates subset of T helper lymphocyte.