South-East Asia Regional Office/Research and Training in Tropical Diseases – Small Grants Programme 2004–2010
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Foreword

The South-East Asia Region of WHO continues to face emerging tropical diseases e.g. malaria, dengue, lymphatic filariasis, kala-azar (visceral leishmaniasis), chikungunya, leprosy and tuberculosis. These diseases are increasingly causing more threat in the Region. To control or prevent or eliminate these diseases from the Region, we require innovative ideas in terms of case management, drug development, vector control and IEC materials for community mobilization.

In 2004, The South-East Asia Regional Office (SEARO) of the World Health Organization (WHO) in collaboration with the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) initiated the SEARO/TDR Joint Small Grants Programme (SGP) to develop capacity among the young scientists in the Member States. The aim of the SGP was to facilitate and strengthen control-oriented operational research in tropical and communicable diseases in Member States of the Region. The SGP supported those research projects through funding, on a competitive basis, that contributed to the prevention, control and treatment of communicable diseases, including improving the use of existing methods. The programme supported research activities that met its objectives and that focused on the following diseases: malaria, dengue, lymphatic filariasis, kala-azar, chikungunya, leprosy and tuberculosis. The duration for support is a maximum of 12 months with fund support not exceeding US$ 7500.

From 2004 to 2010, SEARO received 220 research proposals, out of which 118 proposals were recommended by technical units to the Research Review Committee (RRC). The RRC approved 62 proposals for support.

This publication documents the key findings of 38 proposals. It is hoped that the publication proves useful, interesting and informative for the Member States who may use this information for their control programmes.

Dr Samlee Plianbangchang
Regional Director
1. **Background**

The South-East Asia Regional Office (SEARO) of the World Health Organization (WHO) in collaboration with the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) initiated SEARO/TDR Joint Small Grants Programme (SGP) in 2004 to facilitate and strengthen control-oriented operational research in tropical and communicable diseases and to develop capacity among the young scientists in the Member States of the SEA Region. After discontinuation of support from TDR, SEARO continued supporting SGPs till 2010 and as many as 62 SGPs were supported during 2004 to 2010.

2. **Summaries of the SGPs**

2.1 **Title of the Study:** Treatment seeking behaviour for sputum smear-positive pulmonary tuberculosis patients in Bhutan (Project ID:SN1134), 2004

*Principal Investigator: Ms Manikala Laygoi, Academic Head, Royal Institute Health Sciences, Bhutan*

**Background:** Tuberculosis (TB) is one of the most challenging diseases prevalent in Bhutan, which has existed from the earliest time in the country’s history. The Ministry of Health established National Tuberculosis Control Programme in 1994 with the objective to achieve 80% case detection and 100% BCG coverage. Nationwide short-course chemotherapy was also implemented in 1994. The objective of the National TB Control Programme in Bhutan is to achieve 100% coverage of Directly Observed Short Course Therapy coverage, reduce prevalence to 1 per 1000 population and create awareness among people about TB, enabling early diagnosis and treatment. In 2005, 1018 cases were reported (310 new cases and 708 retreated cases) and TB mortality rate was 4.12%. This study was conducted to identify the factors leading to delay in seeking health care for tuberculosis among TB patients and TB suspects and barriers to seeking health care for respiratory symptoms in the general population.
Methods: Quantitative study was done in hospitals only with a sample of 200 confirmed TB patients and 200 lung TB symptomatic persons. Qualitative study was conducted in hospital as well as in the community. Focus group interviews were done with groups of ten samples from ten hospitals and ten districts. Personal interviews were held with five TB patients and five suspects who did not visit hospital. For focus group interview, participants were health workers and community people.

Result: The delay in seeking treatment ranged from one week to two months. The most common stigmas were “people covered their mouth while talking”, “people avoided TB patients”. Stigmas are more common in southern Bhutan. The reasons for personal delay were: “people did not think symptoms were due to TB”, “health system delay was due to non-availability of qualified persons to refer patients” and “lack of transport facility to refer”. The other factors included ignorance, illiteracy, difficult terrain and religious beliefs.

Conclusion: The study revealed that there is delay by the patients as well as health system delay, and that stigmas are still prevalent in Bhutan. The programme needs to take lead in improving these areas in order to be able to reduce prevalence to 1 per 1000 population and create awareness among people about TB, enabling early diagnosis and treatment.

2.2 Title of the Study: The prevalence of malaria parasite in placenta among pregnant women at Hospital Nacional Guido Valadares, Dili, Timor-Leste (Project ID: SN 1135), 2004

Principal Investigator: Dr Joao Soares Martins
First Vice Rector of Universidade da Paz (University of Peace), Manleuana, Dili, Timor-Leste

Background: The study to determine the prevalence of malaria parasite in placenta among pregnant women at Hospital Nacional Guido Valadares, Dili, Timor-Leste was designed to measure the prevalence of parasite among pregnant women in labour who came for delivery at its Maternity Ward, and to study the socio-demographic factors that were associated with malaria in pregnancy.
Methods: The study design was a cross-sectional study, the duration of which was three months from 11 January to 11 April 2006. Mothers were interviewed using questionnaires designed by the researcher; the questions focused on: socio-demographic factors, gravidity, etc. In addition, blood samples were taken from three sites, namely venous blood, placental blood and cord blood to examine for malaria parasite and haemoglobin level. Newborns were also assessed in respect of birth order, viability, APGAR Score, birth weight, and gestational age.

Result: Overall, 895 mothers participated in this study, mostly residents of Dili. A few came from districts. The age range of mothers was between 15 and 47 years. The prevalence of malaria found in this study was only 0.4%. The study only found four malaria cases in pregnant mothers in labour who came for delivery at the Hospital Nacional Guido Valadares (HNGV) – three cases of *Plasmodium falciparum* and one case of *Plasmodium vivax* during the three-month study period. The study documented good understanding of malaria terminology, malaria symptoms, risk recognition, and prevention practice and high utilization of bed nets. However, the use of chemoprophylaxis was still found to be very low (8.2%). Mothers’ education level appeared to be a significant factor in malaria risk recognition and bed net use. Income turned out to be a significant factor for symptoms recognition and prevention practice during pregnancy. Low birthweight (LBW) was found to be significantly affected by mothers’ age, gravidity and hemoglobin levels. The study recorded 19 (2.1%) stillbirths, 6 (0.6%) neonatal deaths, and 133 (14.9%) LBW.

Conclusion: It is recommended that malaria-focused health promotion effects would be increased and the ITN coverage for pregnant women is increased in order to continue to protect them from malarial infections. Further studies to explore and determine the cause of stillbirths and neonatal deaths are needed regarding malaria.
2.3 Title of the Study: Barriers in providing prompt and effective treatment for malaria patients: A health facility-based study (Project ID: SN 1138), 2005

Principal Investigator: Dr Karma Lhazeen  
Programme Manager, Vector-borne Disease Control Programme, Gelephu, Bhutan

Background: Malaria affects about 50% of the population and is one of the major public health concerns affecting mostly poor countries. Considering its burden on the public health, one of the Millennium Development Goals is to “have halted by 2015 and to begin to reverse the incidence of malaria”. Concerned with the health of the people, Royal Government of Bhutan has been vigorously combating malaria. The fruit of such commitment has resulted in drastic reduction in reported morbidity and mortality over the past years. Early diagnosis and prompt therapy are one of the major strategies utilized by malaria control agencies. Therefore, the vector-borne disease control programme of Bhutan has set a target to treat all fever patients within 24 hours of the onset of fever and within one hour of their arrival at the health facility. Despite such targets and resources pouring in, malaria is still a major public health concern causing morbidity and mortality. Therefore, this study was conducted with the objective to determine the factors causing delay in getting early diagnosis and prompt treatment by the patients with fever, and to determine the time spent in the health facility by patients.

Methods: This cross-sectional study was conducted by using structured questionnaires and focused group discussions.

Result: Our study showed that about 77% fever patients reported at the health facility only after 24 hours. Further focused group discussions revealed that lots of fever patients had sought alternative remedies. The main factors causing delay in reporting to the health facility were long distance to the health facility, sociocultural and religious practices, lack of finances, manpower, and knowledge, cultural norms, and quality of services at health facilities, etc.

Conclusion: Therefore, it can be concluded that there still is delay in treatment-seeking by a fever patient as defined by the programme. In order
to achieve this, further studies are needed with the objective to come up with model interventions to promote early diagnosis and prompt therapy so that the programme goals and objectives can be achieved.

2.4 **Title of the Study: Improving disease management of malaria by strengthening the participation of general practitioners**

(quote: Project ID: SN 1139), 2005

*Principal Investigator: Dr Thein Tun*

*Director-General/Head, Department of Medical Research (Upper Myanmar), Myanmar*

**Background:** The new antimalarial drug policy had been introduced in Myanmar with artemisinin-based combination therapy to be given to all *falciparum* malaria positives. Both public and private sectors were giving treatment to malaria patients. The objective of the study was to explore the participation of private general practitioners in disease management through educational intervention.

**Methods:** A total of 32 private general practitioners in Singu, Madayar and Kyaukse townships were given a one-day training covering relevant subjects. Monthly data collection was done by the research team. A focus group discussion and in-depth interview were carried out to collect additional information from private general practitioners during June 2006 to March 2007. Simultaneously, Population Service International introduced its franchising scheme at the private clinics by providing artemisinin-based combination therapy and rapid diagnostic tests at a highly subsidized rate. Microscopic facilities were made available for private general practitioners.

**Result:** Knowledge on disease management was significantly improved immediately after training and salient knowledge was retained up to the end of the study period. The majority of private practitioners were making only clinical diagnosis before this study. Private general practitioners used rapid diagnostic test in 92.42%, microscopy in 3.07% and clinical diagnosis in 4.51% of 4458 patients. Out of 4362, 47.73% (2082) were *falciparum* positives, diagnosed mostly by rapid diagnostic test. The number of *falciparum* positives, treated with artesunate and mefloquine combination accounted for 94.52% (1968/2082) of total *falciparum* cases. It was found
that 60.08% (1249/2079) of *falciparum* negatives were treated as vivax malaria. The treatment completion rate among patients treated with artemisinin-based combination therapy was 98.25% (1793/1825). Incomplete treatment rate among patients treated with the 14-day primaquine course was 15.93% (212/1331). Only 56.25% (18/32) of general practitioners participated in this study. The number of malaria patients who took treatment from private general practitioners (i.e. 4463) was 2.2 times higher than the number who took treatment from public sector (i.e. 2039).

**Conclusion:** This study revealed that by making artemisinin-based combination therapy and rapid diagnostic test available at a reasonable price, and through educational intervention, private general practitioners can actively participate in malaria disease management.

2.5 **Title of the Study:** Patient’s delay in tuberculosis-centre treatment among migrant population, south and east districts of Yangon division (Project ID: SN 1140), 2005

**Principal Investigator:** Dr Moe Thaw  
**Health Officer, Special Disease Control Unit Leader (Acting), Divisional Health Department, Yangon, Myanmar**

**Background:** A cross-sectional study on patient’s delay in tuberculosis (TB) centre treatment was conducted among migrants from Yangon south and east districts during June to October 2006. The objectives were to identify the period of migrant TB patient’s delay to seek treatment, to describe the predisposing, enabling and need factors to TB centre treatment and also to examine their relationship.

**Methods:** Purposively selected 218 pulmonary new smear-positive TB migrants were interviewed with a pre-tested questionnaire. Chi-square test and SPSS 11.5 software were used for analysis.

**Result:** A total of 57.8 % migrant TB patients were delayed to seek proper treatment, in which 31.2% was short-delayed (>30 days) and 26.6% was long-delayed (>60 days). The fair knowledge groups of 72.3% (p=0.005) patients were mostly delayed. The fifty three (p=0.031) percent of delayed group did not receive information from media. Travelling time
inconvenience in delayed group with \( p = 0.047 \) was noted. Patients did not require permission from employers and also did not receive advice from private doctors.

**Conclusion:** Under the National Tuberculosis Programme arrangements, a local midwife should be enough to check suspect TB cases among migrants by using the “countersign” system with local authorities, checking the sputum and then issuing special treatment relay cards. Migrant peer health education and the existing PPM-DOTS Project need to be strengthened by providing good migrant accessibility to treatment. Existing TB news and short spots with film stars should be continued and need to be strengthened. Similar studies with regard to different locations, system delays, and reasons for compliance and default among migrants are recommended.

### 2.6 Title of the Study: Effect of mass chemotherapy for filariasis control on soil-transmitted helminth infections in the Western Province of Sri Lanka (Project ID: SN1141), 2005

**Principal Investigator:** Professor Nilanthi De Silva  
**Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka**

**Background:** In July 2006, Sri Lanka completed five rounds of annual mass drug administration (MDA) with diethylcarbamazine citrate and albendazole as part of its national programme for elimination of lymphatic filariasis. Albendazole is also highly effective against soil-transmitted helminths (STH). This study was carried out to assess the impact of repeated annual MDA on STH infections in the western province of Sri Lanka, an area that is co-endemic for lymphatic filariasis and STH.

**Methods:** Seventeen schools in the western province were selected because they were included in a national survey of the health of school-children in Grade 5 in 2003, when one round of MDA had been completed. Faecal samples were obtained again in 2006 (after five rounds of MDA), from one randomly selected class of Grade 5 students in the same seventeen schools. In both surveys, faecal samples were examined using the modified Kato-Katz technique. The prevalence and intensity of roundworm,
whipworm and hookworm infestations in 2003 and 2006 were compared using chi-square or Z test for difference between two percentages.

Result: Faecal samples from 255 children were examined in 2003; 448 were examined in 2006. Roundworm prevalence was marginally lower in 2006 (4.0%) than in 2003 (4.7%), as was hookworm (0.2% vs 0.4%), whereas whipworm prevalence was higher (13.8% vs 9.4%). The mean egg counts for all three infections were marginally higher in 2006. However, none of these differences was statistically significant. The compliance rate with MDA in 2006, as reported by schoolchildren examined, was only 59%.

Conclusion: These results indicate that four annual rounds of MDA with diethylcarbamazine citrate and albendazole had virtually no impact on STH infections in the study area. It is likely that inclusion of albendazole in MDA for lymphatic filariasis does not have much impact on STH infections in areas of low endemicity, unless very high coverage rates are achieved.

2.7 Title of the Study: A study on delay in treatment of kala-azar patients related to elimination programme with Miltefosine in Bangladesh (Project ID: SN1142), 2005

Principal Investigator: Dr Syed Mohammad Arif
Associate Professor, Department of Medicine, Dhaka Medical College Hospital, Dhaka- 1000, Bangladesh

Background: Kala-azar is a major public health problem in Bangladesh. One district, Mymensingh, is producing about 60% of total cases. There were two types of delay namely “patient delay” (time interval from symptom recognition to initial medical consultation) and “system delay” (time interval from first medical consultation to the initiation of definitive treatment). The objective of this study was to find out the underlying factors of these two types of delay.

Methods: A cross-sectional study was carried out in two upazilas (subdistricts) of Mymensingh district from January 2006 to July 2007. Sixty five There are 65 patients from Bhaluka and 60 patients from Gafargaon upazila, who resided in these two upazilas and attended an Upazilla Health Complex (UZHC), and who had no previous history of kala-azar
were selected for this study. The suspected kala-azar patients who attended the Out Patient Department (OPD) were subjected to a dipstick test (rK39) for kala-azar. The positive cases were sent to Research Assistant (RA) for processing. Case-recording forms were filled up for each patient and the forms were reviewed periodically during the supervisory visits to study sites.

**Result:** Of the 125 patients, 80 (64%) were males and 45 (36%) were females. Their ages were between 4 and 60 years and most (80.8%) of them were from rural areas. Most patients (80%) consulted the non-qualified private practitioners, while only 15.2% consulted MBBS doctors. About 50% patients were referred to the UZHC by the family members or relatives. Eighty seven per cent and 50% patients required three and two health-care providers respectively for treatment. Patient delay ranged from 2 to 30 days, with a mean of 5.61 ± 4.179 days. The system delay ranged from 0 days to 225 days, with a mean duration of delay 50.34 ± 28.88 days. Patients of rural facilities experienced a longer system delay than those of urban facilities. Referred patients without any previous treatment had a shorter duration of delay. Health-care providers played a major role in system delay. Only the number of treatment providers (p value <.001) and residential status (p value <.05) had an impact on patient delay and system delay. Educational status also had impact on system delay (p<.05). Age, sex, BMI, marital status, economic status were not the contributor factors for delay.

**Conclusion:** Patient delay and system delay are important weaknesses of the kala-azar control programme in our country. Residence in rural areas, low educational background and treatment providers are associated with these delays. The educational programme may reduce both patient delay and system delay.
2.8 Title of the Study: Early identification of asymptomatic cases of kala-azar in endemic foci of Bihar, India: an epidemiological and sociobehavioural study
(Project ID: SN 1143), 2005

Principal Investigator: Dr Vidyanand Rabidas
Rajendra Memorial Research Institute of Medical Science (ICMR), Agamkuan, Patna, India

Background: In spite of the efforts by the Government of India to control kala-azar, spreading of the disease to newer areas i.e. asymptomatic cases pose a threat to the community in the light of continued transmission of the disease. There is scanty information on diagnosing latent infections and predicting the disease in asymptomatic persons. Very few socioeconomic studies have been conducted. In view of the above, the present study was undertaken.

Methods: A household-based study was conducted in two villages with around 1000 people restricted to 200 households due to limited availability of resources. The selection of sampling unit was done on the basis of kala-azar incidence. Every individual was subjected to serological examination and follow-up for positive cases in the first week of every month.

Result: A population of 1823 was enumerated. Out of 1823, 1048 were screened by rK-39 and 997 by PCR. About 9.2% and 9.5% were found to be positive respectively. The serological examination revealed that about 57.3%, 69.5% and 53.8% were positive by rK-39, PCR and both by rK-39 and PCR without any past/current history of kala-azar. The monthly follow-up data revealed that 25 sero-positives converted into full-blown cases. A regular follow-up of the sero-positive population revealed that 25 individuals had developed into full-blown cases. Therefore, asymptomatic cases may be of vital importance. Quantitative data on the socio-behavioural aspects need to be improved to provide community awareness.

Conclusion: The outcome obtained through this study may further be explored with a larger population size to authenticate the outcome, as well as to assess the actual proportion of asymptomatic cases prevailing in the population so as to foresee the disease burden.
2.9 Title of the Study: Study on transmission of dengue in Gampaha and Kurunegala districts in relation to the possible risk factors (Project ID: SN 1144), 2005

Principal Investigator: Dr Wimaladharma Abeyewickreme
Department of Parasitology, Faculty of Medicine, University of Kelaniya, Sri Lanka

Background: Dengue fever is a major infectious disease in Sri Lanka. Silent transmission of dengue virus has been suggested as a possible risk factor for the increasing incidence of dengue. The present study was carried out in the district of Gampaha using the cluster investigation method for understanding the silent transmission mode of dengue.

Methods: The cluster consisted of a minimum of 20 volunteers (family members and close neighbours) of a hospitalized, serologically/molecular biologically-confirmed dengue patient. Serum samples were collected from 148 volunteers in 7 clusters. Samples were tested for anti-dengue antibodies using Dengue Duo IgM and IgG Rapid Strip test. Of these, the positives were further tested for anti-dengue IgG antibody by haemaggutination Inhibition (HAI) assay, the gold standard test for serological diagnosis of virus infection.

Result: For the purpose of the study, 148 patients were identified. Forty one out of 148 participants presented with an evidence of exposure to dengue virus, which was diagnosed by Dengue Duo IgM and IgG Rapid Strip test. These 41 patients were positive for IgM: 28 (68.4%), IgM and IgG 7 (17%) and IgG: 6 (14.6%). Of the 41 patients, paired sera were collected from 36 patients and tested by HAI assay that confirmed dengue virus infection in 4 (11%) with a confirmed secondary infection 4 (100%). Additional 32 (88.9%) patients were diagnosed as recent dengue infections (probable secondary infection –17 (53.1%), probable dengue 15 (46.9%). Out of 36 patients, 12 (33.3%) were asymptomatic (confirmed secondary infection –1 (8.3%), probable secondary infection –10 (83.4%), probable dengue -1 (8.3%). The remains 24 (66.7%) patients were asymptomatic (confirmed secondary infection –3 (12.5%), probable secondary-7 (29.2%), probable dengue-14 (58.3%). Dengue vectors, Ae aegypti and Ae. albopictus were found in all seven study clusters.
**Conclusion:** The study serologically confirmed the persistence of silent transmission of dengue virus with a trend towards clustering around cases. The precedence of vector species in the area further supports this phenomenon.

2.10 **Title of the Study:** Monitoring of spatial-time pattern of DF/DHF incidence against space spraying
(Project ID: SN 1145), 2005

**Principal Investigator:** Dr Suwich Thammapalo  
**Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkal, Thailand**

**Background:** In Thailand, dengue is still one of the major public health concerns. This study aimed to apply the Geographical Information System (GIS) to evaluate the effect of space spraying in dengue control by longitudinal monitoring of DF/DHF cases in Songkhla Municipality, as well as response of spraying from May 2006 to April 2007. There were 244 DF/DHF cases residing in the Songkhla municipality, the mean age being 12.3 + 7.4 years.

**Methods:** Patient data collected from hospital were analysed during the study period. The GIS was used to evaluate the effect of space spraying.

**Result:** The occurrence of DF/DHF cases the peaked in January. There were 84 episodes of outbreak with an average of 3 cases in each episode (min=1, max=13). Most DF/DHF cases lived in “shop” houses (65.2%), concrete, constructed houses (54.9%), and houses with no window screens (85.7%). The DF/DHF cases occurred in the central and south western areas with dense populations. The control team could respond to an outbreak control within 24 hours of receiving notification, 75.4% of the time. The average area of spraying was 1702.7 + 2480.4 square metres (min=92.2, max=25,550, median = 1190.5) or around 10-15 households. The average operation time was 0.50+ 0.27 hours (min = 0.05, max =1.30, median = 0.4).

**Conclusion:** Space spraying was carried out inefficiently. Secondary cases occurred in sprayed houses in many areas. The influencing factors as well as extra spraying should be explored further. However, real-time monitoring
in the fields through GIS should be applied in areas where a computerized map is available. The coverage and quality of spray activities should be emphasized.

### 2.11 Title of the Study: Study on vector biology and human risk behaviours in an highly malaria-endemic area of Riau Province, Indonesia (Project ID: SN1150), 2005

**Principal Investigator:** Prof Dr Yoes Prijatna Dachian  
**Director, Tropical disease Center (TDC), Airlangga University, Kampus C, UNAIR JL. Mulyorejo, Surabaya, Indonesia**

**Background:** Malaria has been one of the most important diseases in Riau province, particularly in Indragiri Hilir district since the outbreaks of malaria in Belaras village, Tembilahan sub-district from 2003 to 2005, and since the inter subdistrict road construction project was taken up. The study was aimed at identifying the vectors in three selected subvillages of Belaras, and studied the vector bionomics and human behaviours in relation to malaria transmission and prevention, in order to help design an appropriate malaria control programme.

**Methods:** A cross-sectional study was done including an entomological study. Human behavioural survey was carried out at Batangsari, Priap Pancur, and Telaga subvillages, from July 2006 to April 2007.

**Result:** *Anopheles sundaicus*, *An. baezai*, *An. lesteri*, and *An. tesselatus* were observed by indoor and outdoor landing collection. *An. sundaicus* might act as the suspected major vector in these study sites, which is consistent with the presence of abundant *An. sundaicus* larvae in the permanently stagnant canal. It is presumed that malaria transmission could occur in these areas. The bad experiences of the local community during the outbreaks of malaria led the people to understand more about the risk of malaria (93.2%), prevention (98.1%) and health-care seeking for treatment by a nurse (90.4%) or a medical doctor (6.5%).

**Conclusion:** This study suggested that people in sub-villages need to be aware of mosquito breeding places, and that malaria surveillance should be done regularly in order to decrease malaria transmission and avoid an outbreak of malaria.
2.12 **Title of the Study: Reassessment of disease burden due to lymphatic filariasis in India using DALY as a summary measure** (SN 1151), 2006

*Principal Investigator: Dr. Kaliannagounder Krishnamoorthy*

*Director, Vector Control Research Centre, Indiranagar, Pondicherry 605006, India*

**Background:** A large-scale implementation of lymphatic filariasis (LF) elimination programme has been launched in India to cover all the 243 known endemic districts to achieve the national health policy of elimination of LF as a public health problem by 2015. Estimates of disease burden are necessary to assess the impact of the elimination programme and earlier estimates of Disability Adjusted Life Years (DALY) were based on historical data. In order to estimate the current burden, data from line-listing of LF cases, which is one of the activities under mass drug administration (MDA) to enlist diseased individuals for morbidity management, were used to reassess DALY.

**Methods:** A database was created from the district-level reports of line-listing of filarial cases during 2005–2006. Using descriptive epidemiology of the dynamics of filarial disease, the number of cases was interpolated to the recommended age classes of each gender. Disability weight derived by the Global Burden of Diseases (GBD) Study Group was used to estimate district-wise DALY from which the national burden was derived.

**Result:** A total of 597,835 lymphoedema and 340,210 hydrocele cases were line-listed in 222 endemic districts in India under the LF elimination programme. The number of cases ranged from 90 (Daman and Diu) to 342,200 (Bihar), with an average national disease prevalence of 0.17%. The nation burden due to LF was re-estimated to be 94,941 DALYs for the year 2006. The current burden is only 4.3% of the earlier estimates based on historical data. Spatial distribution of DALY showed that the burden ranged between 9 (Daman and Diu) and 33,761 (Bihar). Lymphoedema contributes to about 73% of the total burden in India. DALYs lost due to lymphoedema among females were about three times the number for males. The comparison of DALYs between age classes showed that the productive age class (15-44 years) contributes the maximum. Bancroftian filariasis contributes 98.3% of the DALYs while the DALYs lost due to
brugian filariasis is only 1572 (1.7%). The burden due to subperiodic *Wuchereria bancrofti* is only 151 DALYs. Clinical examination of all the available individuals in selected villages/wards from three endemic districts indicated underestimates of cases from line-listing. These estimates were 1.5 to 5.5 times of the reported cases. It is also evident that only 10 – 15% of the hydrocele cases were reported from line-listing. The level of underreporting was not related to disease rate.

**Conclusion:** The surveillance system needs to be strengthened to speed up the elimination process of lymphatic filariasis in the country.

### 2.13 Title of the Study: Impact of mass chemotherapy on low-count transmission of *Wucheria bancrofti* in Sri Lanka (Project ID: SN 1152), 2006

*Principal Investigator: Dr. Y.I. Nilminis Silva Gunawardene*

*Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya Thalagolla Road, Ragama, Sri Lanka*

**Background:** The mass drug administration (MDA)-based global lymphatic filariasis (LF) eradication programmes are aimed at stopping the transmission of *Wuchereria bancrofti* by its mosquito vector. The study was designed to compare the one-year post-treatment (mass distribution of Diethylcarbamazine-Albendazole) infection rates of *Wuchereria bancrofti* in *Culex quenquifaciatus*, the main vector of LF in Sri Lanka using the conventional dissection techniques and a polymerase chain reaction (PCR) assay based on parasite specific Ssp1 repeat that amplifies the fragment of 188 bp.

**Methods:** A field study was conducted in 45 sites in all areas in the Gampaha district, Sri Lanka. The sites were identified by the Anti Filariasis Campaign (AFC) as “high risk” for bancroftian filariasis transmission. Indoor-resting mosquitoes were collected by aspiration from 20 houses per each site. Some parts of mosquitoes were used for dissection while the rest were used for polymerase chain reaction (PCR) to detect the filarial parasites in mosquitoes.

**Result:** Mosquito dissection data revealed that 42.22% (19/45) sites were infested with *Wuchereria bancrofti*, indicating transmission-active 8 Ministry
of Health areas (53.33%; 8/15). An infection rate of 5.26% was observed among the mosquitoes caught from households and the larval density was 8.7 per positive mosquito. The PCR investigation revealed that 35.56% (16/45) sites were positive for *W. bancrofti* DNA, indicating transmission-active 9 areas (60%; 9/15).

**Conclusion:** The PCR was found to be more sensitive compared to microscopy in detecting the filarial parasite in field-collected mosquito samples with respect to the Ministry of Health areas. The PCR technique employed offers scope for detection of filarial parasites with higher sensitivity and specificity, and it is efficient and rapid. This technique was applied for the first time in Sri Lanka, and can be adopted as a diagnostic tool for the detection of filarial parasites in the vector population in surveillance to enable effective control of filariasis in the country.

### 2.14 Title of the Study: Artesunate plus amodiaquine versus chloroquine for the treatment of *vivax* malaria in Maumere, East Nusa Tenggara (Project ID: SN1153), 2006

**Principal Investigator:** Dr Armedy Ronny Hasugian  
**Center for Research and Development for Biomedic and Pharmacy, National Institute of Health and Research Development, Ministry of Health, Indonesia**

**Background:** Drug resistance is still a big problem for the malaria control programme in Indonesia, especially in its eastern part. Nusa Tenggara Timur (NTT) province, one of the provinces in the eastern part, is a meso-endemic malaria area. Seventy six per cent of total malaria cases in NTT were reported from Sikka district in Flores Island, one of its 16 districts. Of the four human plasmodia in Sikka, *P. falciparum* and *P. vivax* are the most common species. Both species are clinically resistant to chloroquine. Alternative antimalarial drugs are needed to replace the existing drugs. The objective of this study is to compare the efficacy, safety and tolerability of As+Aq versus chloroquine for the treatment of *P.vivax* malaria.

**Method:** To obtain the alternative antimalarial drug for treatment of *P. vivax* resistant to chloroquine and to have a practical regimen use in the field, we evaluated the efficacy of As+Aq. This is a prospective comparative clinical trial of the efficacy, safety and tolerability of As+Aq versus chloroquine for
treatment of uncomplicated vivax malaria with a 28-day follow-up. The study was carried out from April 2007 to August 2007 at Puskesmas Kota, Maumere, Sikka district, East Nusa Tenggara.

**Result:** We enrolled 105 *P.vivax* malaria patients for this study; 52 (49.5%) were treated with artesunate plus amodiaquine and 53 (50.5%) patients were treated with chloroquine. Sixty three (60%) patients were reported with adequate clinical and parasitological responses (ACPR), 22 (20.9%) patients with treatment failure (TF), 2 (1.9%) patients with recurrent vomiting, 11 (10.5%) patients were lost to follow-up, 5 (4.8%) patients with protocol violation, and 2 (1.9%) patients took another antimalarial drug. Of the 105 enrolled patients, cases of 100 patients (excluding protocol violation cases) were analysed by Intention to Treat (ITT) with Kaplan Maier (KM) survival analysis. We also analysed Per Protocol (PP) of all cases who completed therapy. The efficacies of As+Aq and chloroquine were 88% (44/50) and 40.8% (19/50) on day 28 by ITT, and 93.2% (41/44) and 47.2% (17/36) by PP, respectively. The mean survival times of As+Aq and Chloroquine were 26.8 days (95%CI: 25.5-28.1) and 22.4 days (95% CI: 20.3 – 24.6) by ITT analysis, and 27.7 (95%CI: 27-28.4) and 24 (95% CI: 22.1 – 25.9) by PP analysis. Regarding the clinical features of both study drugs, patients did not show significantly different readings during the follow-up days. Only dizziness and nausea (on day 2), and cough (on day 3) were documented as being significantly different between the AqAs and chloroquine groups.

**Conclusion:** Overall this study showed that the efficacy of AsAq is better than chloroquine. However, efficacy of AsAq was < 95%, below the WHO standard of 95%.

**2.15 Title of the Study: Utilization of insecticide-treated bednets in the hilly region of Myanmar** (Project ID SN 1154), 2006

*Principal Investigator: Dr Maung Maung*

*Department of Medicine Research (upper Myanmar), Sitha Village, Mandalay, Lashio Road, Myanmar*

**Background:** Malaria is still a major public health problem in Myanmar, because 71% of the total population of 51.12 million (2001) are residing in malaria-risk areas. Out of the 324 townships in the whole of Myanmar, 284
townships have local transmission and are become endemic areas. Because of this, the National Malaria Control Programme launched the Insecticide-treated bed Nets (ITN) programme in 35 townships, distributing 5,833,371 ITN to 213,683 households in 2003. The programme covers 36.6% of households in townships. The aim of this study was to find out the utilization of impregnated bednets by the local populace in hilly areas of Myanmar in order to determine the proportion of consistent users, intermittent users and non-users of impregnated bednets in three states and divisions, to study the reasons for use, and non-use of impregnated bednets, among family members, to ascertain the presence of parasiteaemia among consistent, intermittent and non-users of impregnated bednets and to correlate the utilization of impregnated bed nets and parasiteaemia among the local population.

**Methods:** A community-based cross-sectional evaluation study was carried out in order to determine the utilization rate of impregnated bednets by the local population.

**Result:** A total of 77.7% household members (villagers) used ITN consistently, 21.2% utilized them occasionally and 1.1% never used ITN. The majority of consistent users were women and children. The commonest reason for not using ITN was due to sleeping in the farms. Another reason for not using ITN was travelling to other malaria areas without ITN. Usually, people went into the forest to fetch wood. The attack rate of malaria in the study population was found to be 6.5%. However, it came down to 0.9% among consistent users. It shot up to 22.1% among intermittent users. ITNs offered a significant protective effect on malaria occurrence (OR+0.0#). In other words, ITN users are 39.3 times less likely to get malaria infection compared to non-users.

**Conclusion:** To increase the compliance rate, IEC activities should be strengthened. Otherwise, the expected result may not be achieved.
2.16 Title of the study: Vivax malaria patients and their drug adherence in northern Thailand (2008)

Principal Investigator: Nardlada Khantikul
Department of Disease Control, Office of Disease Prevention and Control, Ministry of Public Health, No 10 Chiang Mai, Thailand

Background: The main objective of this study was to increase the adherence of patients to anti-vivax malarial medications in the Mae Hong Son Province in northern Thailand.

Methods: The study conducted a retrospective base-line data collection in phase I and continued to a quasi-experimental design for Information, Education, and communication (IEC) implementation in phase II. For this study, nine malaria clinics in the Muang district and three clinics in the Mae Sa Riang district were selected. Two hundred and six patient histories concerning drug-taking behaviours were examined. In phase II, the IEC implementation was launched in the Muang malaria clinics (intervention site) and in clinics in Mae Sa Riang (control site). The implementation phase comprised programmes aimed at enhancing the capability of malaria officers concerning problem-solving and enhancing self-esteem and efficacy. Malaria officers designed and developed friendly, tailor-made IEC materials and an instruction package for vivax malaria patients who visited and received treatment at the study clinics. In contrast, the control areas received an unchanged routine treatment for anti-vivax medications as usual.

Results: The results from phase I revealed that 49 out of 206 patients (23.8%) completely adhered to the medications, while 157 out of 206 patients (76.2%) did not adhere to the medication prescription. Most in the non-adherent group stated that they were confused about the prescription from the clinics. Some neglected taking primaquine for 14 days, and some were troubled with side-effects of chloroquine. The determinants that were associated with the patient’s adherence were the knowledge concerning malaria (adjusted OR=2.22, 95%CI=1.10-4.45), and the ability to access information related to antimalarial medication (aOR=5.58, 95%CI=2.06-15.14). The results from phase II revealed that 100 out of 142 patients (71.1%) in the intervention group adhered to their prescriptions. However, only 35 out of 117 (29.9%) patients in the control group adhered to
treatment. The mean scores for the intervention group regarding knowledge, perception of malaria treatment, efficiency to perform drug adherence, access to information on antimalarial medication, and satisfaction with health services from malaria clinics were significantly higher after implementation than before implementation (p-value <0.05). The mean scores were also significantly higher among the intervention group than that of the control group (p-value<0.05).

Conclusion: The study may be beneficial to malaria officers as it could help them be cognizant of sociocultural factors in developing an innovative intervention programme for vivax malaria patients concerning adherence to treatment prescriptions.

2.17 Title of the Study: Tuberculosis contact-tracing through PPM in Kyaukse, Myanmar (SN 1156), 2006

Principal Investigator: Dr Maung Maung
Department of Medicine Research (upper Myanmar), Sitta Village, Mandalay, Lashio Road, Myanmar

Background: Contact-tracing is an essential component of tuberculosis (TB) control. The aims of contact-tracing are: to identify those infected by the index case, and the source of infection, counsel these persons and offer appropriate therapy. We conducted a feasibility study of contact-tracing through general practitioners (GPs) in Kyaukse Township, Mandalay Division, Myanmar with the aim to find out the sputum smear positive (Category I) cases and X-ray positive cases among the family contacts in the study area, to determine the secondary attack rates among the contacts of smear-positive cases, and to compare the number of cases scanned and diagnosed by the National Tuberculosis Programme (NTP) and GPs.

Methods: The longitudinal observational study design was used. The contacts of index cases treated by GPs and NTP were scanned for tuberculosis using sputum examination and chest X-ray.

Result: A total of 250 close contacts of 71 index cases were scanned; 19 contacts failed to continue scanning. The compliance rate was 92.4%. Of scanned contacts, 6% and 3.4% had primary complex and adult tuberculosis respectively. Secondary attack rate was found to be 9.5%. GPs contributed
76.4% of contact tracing activity whereas NTP contributed only 23.6%. Attrition among contacts scanned by GPs was low (3.7% Vs 20.3%). It is concluded that contact tracing was feasible for GPs.

**Conclusion:** Health planners, NTP managers and TB officers may note that there was one new undiagnosed case among the contacts of every 10 smear-positive cases that were registered for treatment.

### 2.18 Title of the Study: Study of entomological, ecological, environmental and human risk factors in malaria transmission in study areas in Bhutan

(Project ID: SN 1159), 2006

**Principal Investigator:** Mr Rinzin Namgay
**Vector-Borne Disease Control Programme, Post Box. 191, Gelephu, Bhutan**

**Background:** The Vector-borne Disease Control Programme’s entomological surveys were carried out from April to October 2007 to study probable malaria vector prevalence, bionomics in relation to ecology, and environment and human behaviour factors. The studies were planned to coincide with Anopheline mosquito density build-up and appearance of malaria cases in Jomotshangkha, Lhamoizingkha and Sipsu subdistricts, which contributed maximum malaria cases during the past five years in the country.

**Methods:** Anopheline surveys were made monthly at fixed-time intervals and randomly selected fixed sites.

**Result:** A total of 2270 adult female Anopheline mosquitoes belonging to 11 species and 1078 larvae belonging to 9 species were collected. The breeding sites consisted of seasonal or temporary rain-fed ponds, irrigated paddy fields, streams, marshy areas, fishery ponds and river beds. However, permanent streams, fishery ponds and open drainages that act as breeding foci in the dry winter season were also included. Heavy rainfall had always had a negative impact on larval densities in these areas. An. pseudowillmori were found to be the prominent species that bit humans as well as cattle when compared with other species. These species were also found to be the most fully fed through human biting and gravid females were sampled
through 23.00-25.00 hour resting surveys. This indicates that it could be the probable malaria vector in the country.

**Conclusion:** Early biting habit (19-21 hours) of *An. pseudowillmori* and late sleeping habit of humans, including the habit of covering their bodies only partially, were the main factors for persistent malaria transmission in the areas covered, despite adequate control measures with two rounds of residual sprays and high coverage with longlasting insecticide-treated bed-nets.

### 2.19 Title of the Study: Utilization of basic health staff by rural community in disease management of malaria
(Project ID: SN 1163), 2007

**Principal Investigator:** Dr Hla Soe Tint  
**Medical Statistic Division, Department of Medical Research**  
*(Upper Myanmar), Sithar Village Tract, Near Anisakan Airstrip, Pyin Oo Lwin Township, Mandalay Division, Myanmar*

**Background:** In Myanmar, malaria is the first leading cause of mortality and third the leading cause of morbidity. The national malaria control programmes aims to make early diagnosis as well as prompt and adequate treatment in the country accessible. The basic health staff (BHS) are frontline workers implementing public health services in the community including disease management of malaria. The objectives of the study are to assess the utilization of BHS by rural people in disease management of malaria and to explore the factors influencing utilization.

**Methods:** This was a community-based cross-sectional analytical study that was carried out during the period of September 2007 to September 2008. A total of 1500 households (HH) were randomly selected and all BHS (total 153) were assigned to public health facilities of selected rural areas included in the study. Quantitative data were collected through a questionnaire survey at HHs level and focus group discussions were carried out to collect qualitative data.

**Result:** The study was carried out among 7974 rural people; the majority of respondents (75.3) were labourers. Out of the total population, 153 suffered from malaria. Among the malaria patients, 61.4% were confirmed
by RDT and 7.2% by microscopy, and were treated. The remaining 31.4% patients were treated based on a clinical suspicion of malaria. The result showed that 64.5% families resided within 1.5 kilometres of a rural health centre. Among malaria patients (153) in a year, only 33.3% were treated by BHS. However, the majority (82.7%) of respondents knew the names of BHS working in their community while only 18.3% respondents knew the proper anti-malarial treatment. Only 18% of rural people accepted that BHS could treat malaria patients. While the majority (68.6%) thought that BHS could not provide health care for malaria.

**Conclusion:** The utilization of BHS by rural communities should be promoted through information, education and communication. Information on availability of diagnostics and treatment facilities at the BHS level should be widely disseminated to the target population.

### 2.20 Title of the Study: Miltefosine in post-kala-azar dermal leishmaniasis (Project ID: SN 1164), 2007

**Principal Investigator:** Dr V Ramesh  
**Department of Dermatology and Regional STD Centre**  
**Safdarjung Hospital, New Delhi 110029, India**

**Background:** Drugs like the pentavalent antimonials and amphotericin B, which are in current use for the treatment of post-kala-azar dermal leishmaniasis (PKDL) have to be given parenterally over a prolonged period, often necessitating hospitalization of the patient. Only a few patients are able to complete this prolonged, recommended duration of treatment. Miltefosine, an orally administered drug found to be effective in kala-azar, has also been found to cure PKDL. The objective of the current study was to study the efficacy of this drug in PKDL including the evaluation of optimal dose, duration and side-effects.

**Methods:** Thirty patients of PKDL were inducted into the study. Patients who were pregnant or lactating were excluded. Complete history was taken with special emphasis on previous episode of kala-azar and its treatment. Skin eruptions were fully evaluated on the basis of patient history and thorough examination. The diagnosis was based on clinical features, slit-skin smears for Leishman-Donovan bodies (LDB), histopathology of the skin lesions, rk 39 antigen in the blood, and PCR studies on the skin tissue. After
baseline investigations (haemogram, liver and renal function tests, urine examination and chest X-ray) miltefosine was given in the dose of 50 mg thrice per day to adults and 2.5mg/kg to children. The patients were followed up every fortnight when the liver and renal functions were repeated. Skin biopsies were performed at the beginning of therapy, after one month and at the end of therapy when the patient was declared cured. All patients who completed the therapy were followed up at the end of 2, 6 and 12 months after being declared cured. Though initially it was intended to have two groups comprising 15 patients in each group taking miltefosine 50 mg thrice daily for 60 days and miltefosine 50 mg twice daily for 90 days respectively, it was decided to uniformly adopt the former for all patients based on published material, according to which the duration could be considerably reduced when the drug was given thrice daily in place of twice daily in adults. The patient was considered cured when all papules and plaques had subsided and histopathology revealed that the infiltrate was either absent or only mildly inflammatory. In the macular form of PKDL, the treatment was simply given for 60 days or 90 days till 180 capsules had been consumed by the patient.

**Results:** A total of 30 patients including 3 children were evaluated. There were 23 males and 6 females aged 9 – 60 years. All patients hailed from the state of Bihar; past history of kala-azar could be elicited in 25 of them. Twenty-two had received sodium stibogluconate (SSG) for kala-azar, one had received amphotericin-B, and 2 of them had been enrolled for miltefosine trials on kala-azar in Bihar. The interval between kala-azar and development of PKDL varied from 3 months to as long as 33 years. The two main clinical types of PKDL were those with papules, plaques and hypopigmented macules (n=18), while the second clinical type had predominantly papules and plaques only (n=10). A small third group of only 2 patients comprised those having predominantly hypopigmented macules. Slit-skin smears for LDB were positive in 14 patients. In the majority of patients the histopathology revealed a diffuse infiltrate of lymphocytes, histiocytes and plasma cells. Occasionally epithelioid cell granulomas were seen. The PCR and rk39 were positive in all patients.

**Therapeutic response and adverse effects:** The main adverse effects noted were anorexia, vomiting, abdominal pain and vertigo. One young man aged 23 took the drug thrice daily for 11 days but could not continue because of severe abdominal pain, which recurred even with a dose of
1 capsule twice a day and later once a day. Another 30-year-old woman who was taking the capsules twice daily and had completed 35 days with improvement did not complete the treatment. They had opted out of the study leaving a total of 28 patients on whom the therapeutic response could be studied. Sixteen, including 3 children, out of the total of 28 patients were able to take the drug with minimal side-effects like anorexia and vomiting. Of the 13 adults who took the drug thrice daily, 7 achieved cure after taking a total of 180 capsules over two months (60 days); in 4 patients the drug had to be prolonged beyond two months for periods varying from one week to a month; and in one patient with extensive lesions complete cure could not be achieved even after extending the treatment for 45 days after completion of two months. He is currently under follow-up without any therapy. Of the three children, one boy and a girl were cured after they had taken 90 capsules over three months. The third child, a 12-year-old boy completed 90 capsules over 45 days by taking them twice daily, and responded well with complete cure. The other group of 12 patients experienced vomiting, diarrhoea and abdominal pain more often. However five adults from this group managed to tolerate the symptoms to complete the therapy till they were declared cured, though two of them had to discontinue the drug for short periods in between the therapy for three days and for seven days respectively, owing to increased bouts of vomiting and vertigo. The remaining seven could not tolerate a dosage of thrice daily and could only take the drug twice a day after meals and were declared cured after they had taken a total of 180 capsules.

**Conclusion:** Miltefosine is an effective drug in the treatment of PKDL. In the present study 27 patients out of 28 who completed the period of therapy were cured of their disease. However, the dose had to be reduced in some and consequently the duration of treatment had to be prolonged. Most adult patients appear to require a total of 180 capsules to achieve cure. The duration depends on whether the patients are able to tolerate the drug thrice daily or twice daily. Ten patients completed a follow-up of one year after achieving cure. They have not shown signs of relapse.
2.21 Title of the Study: Serological and molecular study of Japanese encephalitis and dengue viruses in Nepal (Project ID: SN 1165), 2007

Principal Investigator: Dr Basu Dev Pandey
Vector-Borne Disease Research and Training Centre, Bhutani Devi, Hetauda, Makwanpur, GPO Box 9045, Kathmandu
Nepal Ministry of Health, Nepal

Background: Since 2006, Nepal is reporting dengue cases. The peak season for JE and dengue is usually August to November. The objectives of the present study are to know the epidemiology, serology of Japanese encephalitis and dengue viruses in the Taria region of Nepal. The study further intended to characterize the isolated viruses from Nepal at molecular level, to determine the serotype of dengue viruses in Nepal and perform phylogenetic analysis from the isolated viruses.

Methods: Samples were collected from nine districts of Taria region from hospitals for two weeks. Informed consent was taken from all study subjects. A total of 538 serum samples were collected from nine sites of the Taria region. These samples were collected from febrile patients (both inpatients admitted in the hospital and from outpatients) with the probable diagnosis of Japanese encephalitis, dengue fever or other viral fever from the south-east to the west of the Taria region. Serum samples were assayed by IgM-Capture ELISA against anti-dengue IgM and anti-JE IgM. Particle agglutination (PA) assay (Pentax Ltd Tokyo, Japan) and IgM-capture ELISA (Dengue/JE IgM Combo ELISA kit, Panbio Ltd, Brisbane, Australia) were used. The RT-PCR was performed on these serum samples in acute-stage febrile patients. Sample preparation; RNA extraction was performed on the serum samples by using QIAGEN- RNA extraction kit. Primers used: Initially dengue-consensus primers were used, followed by serotype-specific primers to know the dengue serotype prevalent in Nepal. JE-specific primers were used for JE RT-PCR.

Result: A total of 538 serum samples were collected from febrile patients (both from outpatient and inpatient departments) of JE and dengue in the endemic area of Taria region. A diagnostic dilemma was faced due to the cross-reaction between dengue and JE prevalent in the area. About 28% febrile cases in the study areas showed positive for dengue infection and...
32% of the suspected JE patients were actually positive for anti-JE antibodies. *Aedes aegypti* has been identified in these areas and there is frequent movement in the border area between India and Nepal indicating a high risk of dengue virus infection in the other area too. However, we could not isolate dengue or JE viruses in this study and could not further construct phylogenetic analysis.

**Conclusion:** The results suggest that dengue infection is firmly established from east to west Tarai region of the JE-endemic areas. There is an urgent need for cell culture facilities at least in the centre to do molecular study of JE and dengue in Nepal. The results also suggest that dengue and JE diagnostic facilities should be strengthened. Also, potential risk factors for a future outbreak of dengue as well as for the spread of JE to new areas need to be carefully monitored.

### 2.22 Title of the Study: Rapid dengue stereotypic assay by single tube-multiplex real-time PCR (Project ID: SN 1168), 2007

**Principal Investigator:** Dr Natthanaj Luplerdlop  
*Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawitthi Road, Bangkok 10400, Thailand*

**Background:** Dengue is one of the most important public health concerns in the country. It has a high impact on morbidity and mortality. The dengue virus is a positive-strand RNA virus that belongs to the flavivirus genus in the Flaviviridae family. Dengue has four distinct serotypes (DEN-1, -2, -3, -4) that can be differentiated by molecular methods. Some assays require major manipulation of the sample as each of the four serotypes require individual assay for serotyping. The objectives of the study were to develop a single-tube multiplex RT-PCR-based rapid and cost-effective diagnostic test for detection, as well as specific-serotypic characterization of all four dengue viruses in the dengue samples, and to use the single-tube multiplex PCR for standard specific-serotypic diagnostic protocol in field and hospital applications.

**Methods:** In the present study, we used single-tube multiplex RT-PCR primers with a variability of NS5 for designing the multiplex primers; and optimized a multiplex RT-PCR for a simultaneous detection of viral RNA genomes of the dengue virus from virus-containing cell culture
supernatants, mosquito inoculation and different disease severity (dengue fever: DF; dengue haemorrhagic fever: DHF and dengue shock syndrome: DSS) patients’s sera. We examined the sensitive and specificities of these multiplex RT-PCR based on viral RNAs extracted from viruses of known titer, compared with other pathogens that induced the fever in their clinical presentation.

**Result:** The experiment showed the development of a rapid dengue (DEN) virus nucleic acid detection assay that can identify the serotype of the infecting DEN virus in a single reaction mixture. The single-tube multiplex PCR assays were optimized so that all four sets of “primer pairs” molecular methods for detecting DEN nucleic acid included nested RT-PCR and real-time RT-PCR, in which the serotype is identified in separate reaction mixtures. The single-tube multiplex PCR assays were found to be more sensitive and faster than the gold standard Lanciotti RT/nested PCR assay that is widely used in laboratories to identify DEN serotypes. In addition, amplification and detection of DEN nucleic acid is carried out in a closed system in the single-tube multiplex PCR assays, which is an advantage over the two-step, nested RT-PCRs, where contamination of the PCR product can occur. With the single-tube multiplex PCR assays, the DEN serotype can be identified in a single-reaction mixture, compared with the separate reactions necessary in other DEN serotype-specific nucleic-acid amplification assays. The assay was $10^{-2}$ pfu more sensitive for DEN-2 and DEN-3 viruses, with limits of detection from $10^{-1}$ pfu, than for DEN-1 and DEN-4 virus. DEN viruses are positive-strand RNA viruses and as such have a high potential for mutation, resulting in nucleotide differences between genotypes and also within a genotype. Dengue virus infections continue to be an important public health problem, and rapid, accurate diagnosis of DEN virus in acute infections is crucial for treatment of patients and for effective surveillance and control of DEN outbreaks. Because of cross-reactivity among flaviviruses in serological assays and anamnestic antibody response in secondary DEN virus infections, antibody detection assays are not adequate diagnostic tools. In acute DEN virus infections, virus isolation has been the “gold standard”; however, many laboratories do not have virus culture capabilities, or viremia may be very low so that infectious virus cannot be isolated. This single-reaction, fourplex real-time RT-PCR nucleic-acid detection assay can be used as a method for differential diagnosis of a specific DEN serotype in viremic dengue patients and as a tool for rapid identification and serotyping of DEN virus isolates.
**Conclusion:** The single-tube multiplex PCR or Developed Probe Hybridization Real-time PCR can be used for surveillance, disease control and early treatment to reduce the morbidity and mortality rate of dengue viral-infected cases.

**2.23 Title of the Study:** Predicted Factors and model development for reducing delay of treatment among new smear-positive pulmonary tuberculosis patients on the Thai-Cambodia border: case studies in Surin and Sisaket Province, Thailand.

I.D. SN 1177, 2008

**Principal Investigator:** Mrs Nongluck Tesana

181/37 Srijan Road, Muang District, Khon Kaen Province 40000, Thailand

**Background:** The study was conducted in Surin and Sisaket provinces which are two of the highest TB prevalence provinces in northeast Thailand, especially along border areas (eight districts). It aimed to identify the factors responsible for delays (total delay, patient delay, health system delay) of treatment among new smear-positive pulmonary tuberculosis patients on the Thai-Cambodia border, and to develop an appropriate model of intervention for reducing the delay in treatment in border areas. The objectives of the study were to find out the causes of delay of treatment and factors that would help predict the delay in treatment among new smear-positive pulmonary tuberculosis patients on the Thai-Cambodia border.

**Methods:** The eligible TB patients and health-care providers in eight districts were interviewed to collect the variables through structured questionnaires. Answers were recorded in the computer database and multiple regression analysis was conducted to identify the risk factors that could help in predicting the delay. The intervention models were developed on the basis of such risk factors (border check-point area in Phusing district).

**Result:** In the case of duration of patient delay, health system delay and total delay, we found that there were 74.04% patient delays (med=51, Q1=28, Q3=98); 25.51% health system delays (med=4, Q1=0, Q3=16);
and 53.72% of total delays (med=62, Q1=31, Q3=112). As determined from data associated with patients’ delays, the backward elimination regression analysis yielded an eight-variables equation for estimating patients, delays. The selected variables were: pay for travel; cough-no sputum; cough with sputum; hemoptysis; chest pain; weakness/anorexia; fever; weight loss > 10%. The coefficient of determinants expressed as a percentage indicated that 48% variance in patients, delay among the validation group was accounted for by the variance of the eight prediction variables ($R^2 = 0.48, P<.05$).

Conclusion: The national TB control programme should take care of factors relating to all types of delay in the treatment of patients. Hence, the programme should be strengthened.

2.24 Title of the Study: Correlation of alpha-tubulin II and osmiophilic body gene expression in *Plasmodium vivax* gametocyte and mosquito infection I D SN 1179, 2008

Principal Investigator: Dr Sureemas Buates
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Background: It is not feasible during malariometric surveys to measure human infectivity by feeding mosquitoes directly on people or their blood. Thus, for epidemiological study of malaria transmission, an index of infectiousness based on gametocyte rates from blood films has been used. In Thailand and South-East Asia, malaria transmission is low but stable in most endemic areas with low gametocyte density. Using only gametocyte density to determine the status of malaria transmission may result in unreliable information, and have an impact on evaluation of any control programme. We proposed to investigate potential molecular marker(s) for gametocyte maturation and infectivity to the vectors. The aim of this study was to investigate expression of α-tubulin II and osmiophilic body protein, Pf377 ortholog, genes in *Plasmodium vivax* and correlation of their expression levels to *Anopheles dirus* infection.

Methods: *Plasmodium vivax*-infected blood samples and mosquito feeding data were provided by the Entomology Department, Armed Forces Research Institute of Medical Sciences (AFRIMS). The *An. dirus* mosquitoes
were fed on these blood samples to determine the infectiousness of the gametocytes. Total RNA was prepared from each blood sample. The transcripts of α-tubulin II and Pf377 ortholog genes levels in *P. vivax*-infected blood samples were determined by quantitative real-time PCR using the SYBR green system. The correlation of expression levels of alpha-tubulin II, Pf377 ortholog genes in *P. vivax* and the parasite infectivity to *An. dirus* was analysed using the SPSS programme version 11.

**Result:** A total of 73 clinical blood samples positive for *P. vivax* gametocytes by microscopy were fed to *An. dirus* mosquitoes and the infectiousness of gametocytes was determined by examination of the number of oocyst in mosquito mid-guts. Fifty three of all blood cases were infective to mosquitoes. The number of oocysts in mosquito mid-guts ranged from 1-350.8(47.5864 ± 10.70214). There was no significant difference in the number of asexual stages, and in male and female gametocytes between the blood samples that were infective and non-infective to mosquitoes. Forty seven cases from the 53 infective cases were positive for α-tubulin II and Pf377 ortholog gene expression, and the copy number of the genes ranged from 1-15096.67 (1077.94 ± 407.83) and 1-54349.17 (3574 ± 1396.24) respectively. For 20 cases in the non-infective group, expressions of α-tubulin II and Pf377 ortholog genes were detected in 13 cases, and the copy number of the genes ranged from 2.96 – 594.42 (109.29 ± 47.82) and 1 – 2190.42 (283.85 ± 169.16), respectively. The expression levels of these two genes in blood samples infective to mosquitoes were significantly higher than those of non-infective ones (Mann-Whitney U test, P < 0.05). The results demonstrated that the expression levels of α-tubulin II and Pf377 ortholog genes were significantly correlated with the number of oocyst in mosquitoes (Peason, r = 0.716, P < 0.01 and r = 0.447, P < 0.01, respectively). Whereas there was no correlation between the parasite number (male and female gametocytes, total gametocytes, ratio of male/female gametocytes and parasitemia) determined by microscopy and the number of infected mosquitoes or mean number of oocysts in each batch of mosquitoes.

**Conclusion:** The parasites from blood samples that were infective to mosquitoes expressed significantly higher levels of α-tubulin II and Pf377 ortholog genes than those in the non-infective group. However, there was no direct correlation between the expression level of these genes with the
number of infected mosquitoes or the mean oocyst number in each batch of mosquitoes.

2.25 **Title of the Study: Plasmodium infection rates in Anopheles mosquitoes in epidemic-prone border areas of Bangladesh**

I.D. SN 1181, 2008

*Principal Investigator: Mohammad Shafiul Alam*

*International Centre for Diarrhoeal Disease Research (ICDDR,B)*

68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh

**Background:** The information related to malaria vectors is very limited in Bangladesh. Due to the changing environment, various *Anopheles* species may be incriminated and play a role in the transmission cycle. This study was designed with the intention to identify *anopheline* species and possible malaria vectors in the border areas, where malaria is endemic in Bangladesh.

**Methods:** *Anopheles* mosquitoes were collected from three border areas (Lenguara, Deorgachh and Matiranga) during the peak malaria transmission season (May to August). Three different methods were used: human landing catches; resting collecting by mouth aspirator; and CDC light traps. Enzyme-linked immunosorbent assay (ELISA) was done to detect *Plasmodium falciparum*, *Plasmodium vivax*-210 and *Plasmodium vivax*-247 circumsporozoite proteins (CSP) from the collected female species.

**Result:** A total of 634 female *Anopheles* mosquitoes belonging to 17 species were collected. *Anopheles vagus* was the dominant species (18.6%) followed by *Anopheles nigerrimus* (14.5%) and *Anopheles philippinensis* (11.0%). The infection rate was found to be 2.6% in 622 mosquitoes tested with CSP-ELISA. Eight (1.3%) mosquitoes belonging to five species were positive for *P. falciparum*, seven (1.1%) mosquitoes belonging to five species were positive for *P. vivax*-210 and a single mosquito (0.2%) identified as *Anopheles maculatus* was positive for *P. vivax*-247. No case of mixed infection was found. The highest infection rate was found in *Anopheles karwari* (22.2%) followed by *An. Maculates* (14.3%) and *Anopheles barbirostris* (9.5%). The other positive species were *An. nigerrimus* (4.4%), *An. vagus* (4.3%), *Anopheles subpictus* (1.5%) and *An. philippinensis* (1.4%).
Anopheles vagus and An. philippinensis were previously incriminated as malaria vectors in Bangladesh. In contrast, An. karwari, An. maculatus, An. barbirostris, An. Nigerrimus and An. subpictus had never previously been incriminated in Bangladesh.

**Conclusion:** The findings of this study suggested that in the absence of major malaria vectors, there was a possibility that other Anopheles species may have been playing a role in malaria transmission in Bangladesh. Therefore, further studies were required with regard to the positive mosquito species found in this study to investigate their possible role in malaria transmission in Bangladesh.

### 2.26 Title of the Study: Serological and molecular epidemiological study of dengue viruses in Nepal

**I.D. SN 1183, 2008**

**Principal Investigator:** Dr Basu Dev Pandey  
**Vector Borne Disease Research and Training Center, Ministry of Health, Ministry of Health, Bhutan Devi, Hetauda Makwanpur, GPO Box 9045 Kathmandu, Nepal**

**Background:** The first dengue cases were reported from Nepal in 2006 when it was proved that there was local transmission. The objectives of the present study were to know the epidemiology and serology of dengue viruses in the epidemic-prone Teria region of Nepal. The study is a continuation of previous serological study conducted in 2007–2008. It further intended to characterize the isolated viruses from Nepal at molecular level, to determine the serotype of dengue viruses in Nepal and perform phylogenetic analysis from the isolated viruses.

**Methods:** Epidemiological data were collected through questionnaires after consent was obtained from study subjects (in case of children, from their father/mother). The study team collected blood sample of patients from hospitals. A total of 485 serum samples were collected from febrile patients (both outpatients and inpatients) and Serum samples were assayed by IgM-Capture ELISA against anti-dengue and IgM capture ELISA (Dengue/JE IgM Combo ELISA kit, Panbio Ltd, Brisbane, Australia) was used. Serum samples from early febrile cases were inoculated in C6/36 cells for virus isolation.
Results: A total of 485 serum samples were collected and analysed. The clinical, epidemiological and serological results show that dengue infection is firmly established from east to west of the Taria region. About 10% febrile cases in the study areas showed up as “positive” for dengue infection. However, we could not isolate dengue viruses in this study. Since Aedes aegypti has been identified in these areas and because of frequent movement in the border area, there is a high risk of dengue virus infection spreading to other areas too. Japanese encephalitis (JE) is another flavivirus that too is endemic in these areas.

Conclusions: At present, there is a diagnostic dilemma due to the cross-reaction between dengue and JE prevalent in the area. Sero-surveillance of dengue should be expanded throughout the endemic areas. The results also indicate that dengue diagnostic facilities should be strengthened, and that careful monitoring to prevent future outbreaks of dengue in Nepal is essential.

2.27 Title of the Study: Compliance with the 14-day course of primaquine in Plasmodium vivax malaria. I.D. SN 1184, 2008

Principal Investigator: Ms Manusika Rai
Department of Medical Services, Ministry of Health, Drugs, Vaccines and Equipment Division, Department of Medical Services, Ministry of Health, Post Box 985, Thimphu, Bhutan

Background: The Royal Government of Bhutan has provided top priority to health care of its population and provision of all health services including the provision of essential drugs for free supply. All 15 districts, with a population of 468,993 have climatic conditions that are conducive for malaria vector and are thus at a higher risk of malaria. These fifteen districts are divided into perennial transmission districts and seasonal transmission districts. The Annual Blood Smear Examination Rate (ABER) during the last two decades was more than the minimum prescribed level of 10%. During the same period, the Annual Parasite Incidence (API) rate was between 1.5 to 140.00. The Slide Positivity Rate (SPR) ranged from 2 – 30% and Plasmodium falciparum (Pf) % over the 15-year period ranged from 11 – 60 %. There was an increase in the percentage of Pf cases from 31.5%
to 59.3% from 1990 to 2005, which is of great concern for the programme as it is the more fatal form of malaria. The objectives of the study are to estimate the effects of patient adherence on therapeutic response; to determine the factors affecting compliance.

**Methods:** This was a cross-sectional study aimed to assess the rate of adherence to *P. vivax* malaria treatment regimen and to determine the factors contributing to patient adherence. Two health workers from each health centre i.e. one prescriber/doctor and one malaria technician was trained for data collection. Patients who met the inclusion criteria were approached by the health worker and invited to participate in the study. The written consent of patients was obtained.

**Results:** A total of 29 *P. vivax* positive patients consented to take part in the study over a five-month period. Zero cases were reported from one of the five sites that took part in the study. Twenty six patients (89.7%) completed the 14-day follow-up and made up the analytical sample for this study. Three patients could not be followed up on day 14 and hence were considered as dropouts. The mean age (± SD) was 26 years with a range of 4 – 61 years. Using the pill count, 92.3% (n=24) patients demonstrated full compliance with a mean adherence rate of 99%. Of the two who did not show full compliance, their adherence rate was 85.7% and 89.3% respectively, calculated as the percentage of prescribed dose taken during the 14-day course. Study results have demonstrated good concordance between the different adherence measures. The results generated from pill counts were comparable with the adherence rate determined by patient interviews. All patients, irrespective of their medication adherence, showed adequate clinical response and this were confirmed by assessing parasite clearance at the end of treatment. Furthermore, no patient missed more than two doses of primaquine. All findings validate the notion that there is a strong correlation among results using different adherence measures.

**Conclusions:** Good compliance with the 14-day course of primaquine in *P. vivax* malaria was shown in the study, as confirmed by different methods of measuring medication adherence. This was translated into adequate clinical response. Further studies with adequate sample size are needed to measure the true adherence rate to the therapy.
2.28 Title of the Study: Challenges in utilization of insecticide-treated bed-nets (ITNs) in migrant plantation workers in two sentinel sites (Mon state and Tanintharyi region) in Myanmar (WHO Reference 2011/167621-0, Purchase Order 200416043: Reg. File SEA-2011-T16-TSA-0008)

Principal Investigator: Dr Myat Htut Nyunt  
Research Officer, Parasitology Research Division  
Department of Medical Research (Lower Myanmar)

Background: High population coverage of insecticide-treated nets (ITN) can reduce mortality and morbidity of mosquito-borne diseases including malaria. To assess the challenges in utilization of ITN in migrant workers, a cross-sectional study was done in two sentinel sites in Myanmar. A multistage stratified cluster sampling was used to select the participants for face-to-face interview by using structured pre-coded questionnaire. Focus group participants were selected by stratified purposive sampling and focus group discussions were held to obtain in-depth information on effective utilization of bednets. The result provided useful information for universal coverage and utilization of ITNs in migrant workers. The study was conducted to assess the challenges in utilization of ITNs in migrant plantation workers in Mon state and Tanintharyi division, Myanmar.

Methods: A field-based, cross-sectional descriptive study was carried out in plantation sites in Mon state and Tanintharyi division from November to December 2011. A multistage stratified cluster sampling technique to select participants, and collection of data through face-to-face interviews during household survey (HHS) using pre-coded questionnaires and focus group discussions were used for the study. The questionnaire was designed to collect information on population characteristics, education status, family size, net ownership, knowledge about mosquito nets, and source of nets, bednet utilization, total number of family members sleeping under nets, presence of unused nets in households and problems faced while using the net. Interviews were conducted by trained interviewers in local languages using questionnaires adapted and modified from the WHO Malaria Indicator Survey (MIS) tools (WHO 2005). Net installation was observed in participants’ households by researchers.
Result: A total of 345 respondents from two sentinel sites were included in this study to explore the actual utilization pattern of ITNs and problems and experiences of end-users (170 migrant workers in palm oil plantation sites in Tanintharyi region and 175 in rubber plantation sites in Mon state). Although 13 respondents mentioned they had no bednets at all, 52 out of 345 (15%) said there were no ITNs in their families, while 94 (27%) had no non-ITN, in their families. Frequent washing (more than 12 times per year) was still not uncommon (12.75%). Although more than 80% of them were using bednets during all three seasons regularly, some of them refused to use the nets in the summer. Among the non-ITN users, irregular utilization of ITN was common due to the nature of work (108 (31.3%) out of 345 respondents). More than half the respondents (59%) reported that they had unused nets in their families and 33% of them had one or two unused nets.

Among the ITN users, 28.9% reported that they faced problems while using ITNs such as insecticide smell (56.88%), dizziness (20.18%), headache (12.84%), itchiness (9.17%) and difficulty in breathing (0.9%). More than 75% respondents received ITNs from local health authorities and NGOs free of charge. More than 70 % mentioned that they wanted to buy, but 64% said that they could not afford the nets. They also mentioned that they received no methods or materials for retreatment of bednets (87.9%). Observations carried out during installing of nets in participants’ homes, revealed that there were just five families without bednets; however, 80% showed one to three bednets in their families. The information was mainly obtained from health personnel followed by friends and families, and community leaders.

Although ITN utilization was noted in most families in this study, consistent utilization was noted only in 189 out of 145 respondents (53.1%). Consistent utilization of the ITNs was higher in palm oil plantation rather than rubber plantation workers (X²= 94.251, p= 0.0001). This was mainly due to the nature of the work at night among rubber plantation workers. The perceived malaria risk was also significantly higher in consistent users of ITNs than non-users (X²= 12.517, p=0.0004). Consistent users of ITNs showed better attitude to buy ITNs than non-users (X²=12.237, p=0.0005), but consistent users found ITNs to be less affordable than non-users (X² = 26.374, p=0.0001). Misuse of ITNs such as using nets to catch fish, and using the nets for animals, were also noted and free redistribution of ITNs after taking back the old ones should be
considered. Some respondents commented that the nets distributed should be more durable, and that the netting should be smooth with small holes in lace. Respondents also reported that the effectiveness of nets was reduced after six months and after two-three washes.

**Conclusion:** The need for behaviour change communication and health promotion to scale up the 100 per cent effective utilization of ITNs in migrant populations should be emphasized.

### 2.29 Title of the Study: Detection of cryptic Leishmania infection among blood donors in the kala-azar endemic area of Bangladesh (Project ID: SECDS 1003954), 2009

**Principal Investigator:** M. Mamun Huda,  
**Research Investigator, Parasitology Research Group,**  
**Centre for Communicable Diseases (CCD), ICDDR, Mohakhali,**  
**Dhaka-1212, Bangladesh**

**Background:** Human visceral leishmaniasis (VL)/kala-azar (KA) affects approximately half a million new patients each year worldwide; 60% of them are from the Indian subcontinent. Mymensingh is the most endemic district out of the 45 affected districts in Bangladesh. The disease is transmitted by sandfly bite, but it could also be transmitted through blood transfusion. No information is available about the prevalence of leishmania infection among blood donors in Bangladesh. Our study aimed to determine the prevalence of leishmania *donovani* among blood donors in a kala-azar endemic district of Bangladesh.

**Methods:** The study was carried out in the Blood Transfusion Unit of Mymensingh Medical College Hospital. One thousand one hundred and ninety five adult healthy blood donors attending this unit were enrolled in the study from August 2010 to April 2011. After obtaining written consent, the socio-demographic data and information about illness in the past were collected. The medical officer in the unit performed a complete physical examination to exclude any acute or chronic diseases, which was followed by a sero-diagnosis for exposure to leishmania using the rK39 strip test using finger-pricked blood. Blood donors, positive for the rK39 strip test underwent a PCR test for detection of leishmania DNA in their peripheral blood buffy coat.
Results: Eighty two per cent of blood donors were males and 18 per cent were females. The mean age of blood donors was 27 (SD, 7.95). The majority donors were literate and had a better socioeconomic condition reflected by better household condition. Only 2.6% had a family member with VL in the past. Three blood donors tested positive for leishmania infection by the rK39 strip test (0.3 %, 95%CI, 0.05-0.73). None of these three had active leishmania infection as demonstrated by PCR. During the six months of follow-up, neither rK39 positive (N=3) or rK39 negative (N=1192) donors developed VL.

Conclusions: The risk of leishmania donovani transmission by blood transfusion is very low. Therefore, the chance for transmission of VL through blood transfusion is negligible. We believe the National VL Elimination Programme does not need to set up a routine screening for leishmania donovani infection at the blood transfusion centre, located in the VL-endemic areas of Bangladesh.

2.30 Title of the study: Assessing involvement of community volunteers in TB control activities initiated by INGOs in selected townships, Myanmar
(SEA-2011-T16-TSA0002, Award No. 53411)

Principal investigator: Dr Le Le Win
Department: Department of Medical Research (Lower Myanmar)

Background: The National Tuberculosis Programme (NTP) of Myanmar had planned to sustain its global achievement of case detection rate (CDR) and treatment success rate (TSR) (i.e., CDR (over 70%) since 2003 and TSR (85%) since 2005. To sustain this achievement is one of the STOP TB strategies. Accordingly, in Myanmar, NTP had performed the control activities through numerous means and ways. Collaboration with international nongovernmental organizations (INGO) is one of the methods. The respective INGOs trained the local people as community volunteers for control activities in some townships. The NTP collaborated with 10 INGOs of which, five INGOs (i.e. Pact Myanmar, World Vision International, Malteser, International Organization for Migration - IOM, Merlin) mainly participated in TB control activities through community volunteers. The main objective of the study was to assess the existing situation of
involvement of community volunteers in TB control activities initiated by INGOs and contribution of INGOs for NTP in terms of referral, case detection and treatment adherence in selected townships of Myanmar.

**Methods:** In this study, two INGOs – World Vision and IOM were involved. Cross-sectional study was conducted to assess the existing situation of involvement of community volunteers in TB control activities initiated by INGOs and contribution of INGOs to NTP. The study was conducted in Loikaw and Mudon townships where World Vision and IOM are working. Community volunteers, people affected with TB, and staff from NTP from the selected townships, were involved. Information was gathered through reviews, interviews with community volunteers, in-depth interview (IDI) with some volunteers and key informant interviews (KII) with people affected with TB, midwives, staff from State Health Department and the NTP. Patients were asked about activities performed by community volunteers, opinions, barriers, and suggestions about TB control being activities implemented by the respective organizations. The study involved 54 volunteers for face-to-face interviews and 58 respondents for IDI and KII. The interviews were conducted by trained interviewers after getting the consent from each respondent.

**Results:** Although the study did not aim to evaluate and compare the activities of organizations, in order to avoid the complexity, throughout the report, pseudo names will be used, such as Organization A and Organization B respectively. The findings will be expressed according to answers and responses of the study respondents.

The findings indicated that volunteers from both organizations were actively involved in TB control activities. They were well recognized and accepted by the community. It was also noted that the community including patients relied too much on volunteers. This could be due to getting benefits at no cost and easy accessibility of volunteers. It was interesting to find that most midwives from the study townships also depended on volunteers to some extent. The reasons for this situation were that since mostly midwives could not spare time to go to the city to accompany the patients, to send sputum and to get results of the patients because of being engaged with their routine duties and also because it was unaffordable for them to commute. Although there was coordination
between the organizations and public health staff at state level, collaboration between midwives and volunteers at the peripheral level, particularly for case-finding and health education activities, was found to be weak. Rather, the volunteers had more connections with persons from NTP for initial and follow-up sputum examination, and getting treatment.

Regarding the magnitude of new cases detected after the involvement of organizations, in both study areas, although on the same fact, a slightly different opinion on changes in case detection was explored. While the majority of respondents believed that the number of new cases had increased because of health message dissemination to improve community awareness and intensive case-finding activities, some felt that after the initial increase in new cases, the number had started declining over the years.

The findings point out that TB activities conducted by volunteers were fully supported by the respective organizations and the community. This resulted in their being able to reduce and phase out some villages and to have an opportunity to include new villages for TB activities. The situation of free services and support currently provided by the respective organizations could be considered as a questionable situation for the long run to sustain the activities, especially when the international support stops one day.

**Conclusions:** In conclusion, the findings reveal that the volunteers are contributing to NTP in raising community awareness, case detection, and treatment completion outcome to some extent. The information from this study will help NTP in developing policy on community volunteers’ involvement in TB control, and in strengthening the partnership with NTP. Based on the findings, we recommended strengthening of collaboration between midwives and volunteers from both sides, utilization of trained volunteers by NTP once the INGOs pull out some day, and formation of village-based support groups like village member working group (VMWG), self-help group (SHG) to work together with volunteers, basic health staff and NTP.
2.31 Title of the Study: Awareness regarding tuberculosis and treatment-seeking behaviour for chest symptoms among factory workers in Yangon Division, Myanmar (Project ID: SN 1157), 2006

Principal Investigator: Dr Aung Thu
Clinical Research Division, Department of Medical Research (Lower Myanmar), Dagon PO 1191, Yangon, Myanmar

Background: The awareness regarding tuberculosis and treatment-seeking behaviour for chest symptoms among factory workers in Yangon Division of Myanmar has been a concern. The objective of the study was to assess workers' knowledge about TB, their health-seeking behaviour, acceptability of TB screening and predictors for approval regarding dismissal of TB patients.

Methods: A cross-sectional survey, and structured interviews with 349 factory workers were followed by 27 in-depth interviews and two focus group discussions with employers.

Results: Among 349 workers, 95% perceived TB as being curable, 50% correctly reported air as the main mode of transmission and 68% were aware of free treatment. Although 88% perceived screening before employment as necessary, only 14% underwent screening; 96% were willing to undergo contact screening for TB, but only 55% could afford it; 33% agreed with the dismissal of workers with TB, which was associated with lower education, shorter time in employment, not having a history of TB contact and unwillingness to work with an index TB case due to fear and lack of knowledge.

Conclusions: More effective communication strategies towards factory workers are needed to increase workers' knowledge about transmission and reduce stigma. Employers should be sensitised to protect employees with TB and invest in preventive activities.
2.32 Title of the study: An intervention model of dengue using demographic and behaviour approach in Medan, North Sumatra, Indonesia (Project ID: SN 1194), 2010

Principal Investigator: Fotarisman Zaluchu
Epi Treat Unit, University of North Sumatra, Medan, Indonesia

Background: Every year, clinical manifestation of dengue outbreak occurs in Indonesia and especially in Medan. However, it is important to assess different profiles of dengue risk in order to provide specific intervention. The objectives of this study were to: (i) measure community demographics profile in Medan, Indonesia; (ii) measure and explore community knowledge, attitude and practice of dengue disease in Medan, Indonesia; (iii) determine the Breteau Index and Container Index; (iv) develop and implement limited-scale intervention model, based on demographic factors and human behaviour in Medan, Indonesia; and (v) recommend dengue infection prevention model and planned expansion of the model.

Methods: Two sub-districts were selected based on case reports on the 2009 data. One subdistrict had the highest incidence (Medan Baru) while one subdistrict (Medan Area) had the lowest incidence. Both areas had a similar latitude and population profile. A total of 840 respondents were included in the study. Calculations were based on WHO-recommendation rapid survey (2 x 210 from each subdistrict). Respondents were heads of households and had been living in the area for a minimum of two consecutive years. Questionnaires were distributed to assess the demographic profiles, knowledge, attitudes and practices. Environmental assessments were made to calculate the Container Index. Well-trained enumerators were involved. Ethical clearance was provided by the Ethical Committee, Medical Faculty, University of North Sumatra.

Results: Most respondents were >35 years old at both locations, were females, were engaged in permanent work. Most of them had primary school, and were owners of their houses. Based on fever history, only 1% respondents in Medan Baru had experienced dengue compared with 1.4% at Medan Area. However, more respondents in Medan Area had a fever experience (6.4%) compared with Medan Baru (2.4%). Behaviour assessments showed that only half of the respondents had “good” knowledge and attitude. Respondents figuring at the lowest level of the
dengue case report expressed their availability to participate in preventing (61%) and controlling dengue (57.6%) compare with those figuring at the highest level of the dengue case report (46.9% and 45.5% respectively).

**Conclusions:** It was concluded from the study that behaviour and environmental profile contributes to dengue cases. Different types of community participation was also found to be an important factor in producing different results for dengue cases in Medan City. Thus, the mode of intervention should be based on the community-specific profile.

2.33 **Title of the Study:** Drug-resistant patterns among Category 2 treatment-failure patients attending different TB centres, *Myanmar* Project ID: SN No. 1171 (2008)

**Principal Investigator:** Dr Wint Wint Nyunt, Microbiologist, *National TB Reference Laboratory, National Tuberculosis Control Programme, Myanmar*

**Background:** Myanmar is one of the 22 TB high-burden countries that account for 80% of all new TB cases arising each year, and one of the 27 countries that account for 85% of the global MDR-TB burden. Nationwide drug-resistant TB surveys that were carried out twice revealed that the proportion of MDR-TB among new cases was 4% and among previously treated cases was 15.5% in 2002–2003, and 4.2% and 10% in 2007–2008, respectively. The objective of this study was to determine the prevalence of drug-resistant TB among Category 2 treatment-failure patients in different TB centres in Myanmar, and to revise the treatment regimen for MDR-TB patients.

**Methods:** This was a cross-sectional descriptive and analytical study. It took into consideration 230 Category 2 treatment-failure patients attending 17 different TB centres in Myanmar. A total of 230 Category 2 treatment-failure patients, 155 males and 75 females, from state and regional TB centres were enrolled. Two sputum samples from each patient were collected and transported to the National TB Reference Laboratory (NTRL) for microscopic examination, culture and drug susceptibility testing of four first-line anti-TB drugs, namely streptomycin, isoniazid, rifampicin and
ethambutol by the proportion method. All culture-positive isolates were sent to the NTRL, Bangkok, which performed the second-line anti-TB drug susceptibility testing.

**Results:** Among 230 Category 2 treatment-failure patients, 218 patients (94.8%) were resistant to four first-line anti-TB drugs, 9 patients (3.9%) were resistant to other three firstline anti-TB drugs apart from ethambutol, and 3 patients (1.3%) were resistant to other three first-line anti-TB drugs apart from streptomycin. All culture-positive isolates were sent to NTRL, Bangkok and we received the second-line anti TB drug susceptibility testing results of 189 patients. Of these, 149 patients (78.8%) were sensitive to six second-line anti-TB drugs namely ofloxacin, kanamycin, cycloserine, ethionamide, P-aminosalicylic acid and capreomycin. Thirty four patients (18.0%) were resistant to one or two second-line anti-TB drugs not simultaneously resistant to ofloxacin and kanamycin, 3 patients (1.6%) were resistant to ofloxacin and kanamycin, 1 patient (0.5%) was resistant to ofloxacin, kanamycin, cycloserine and Capreomycin. The culture isolates of two patients (1.1%) were identified as nontuberculous mycobacteria (NTM). Out of 189 patients, 4 patients (2.1%) were extensively drug resistant TB (XDR-TB) i.e. resistant to at least one of fluro-quinolone group and one injectable form of second-line anti-TB drugs in addition to multi drug resistant TB.

**Conclusion:** This study revealed that almost all Category 2 treatment-failure patients are MDR-TB patients. Among them, 155 patients (67%) were males and 75 (33%) were females showing that MDR-TB burden in males is twice as much as in females. By knowing the resistant patterns of second-line anti-TB drugs, the NTP can revise the treatment regimen for patients with MDR-TB for the DOTS Plus Project.
Title of the study: Screening of Glucose-6-Phosphate Dehydrogenase enzyme deficiency in three ethnic groups living in malaria-endemic areas of Myanmar.
(I.D. SN 1178) (2008-9)

Principal investigator: Dr Nwe Nwe Oo, Deputy Director/Head, Biochemistry Research Division, Department of Medical Research (Lower Myanmar)

Background: In chemotherapy of Plasmodium vivax infection, the oxidant drug primaquine is required for elimination of the infection. There is a risk of relapse of Plasmodium vivax malaria infection without primaquine therapy. Among the side-effects of the antimalarial drug primaquine, the most salient effect is drug-induced haemolysis in G-6-PD enzyme-deficient persons. In our country 80% people reside in malaria-endemic areas. The incidence of Plasmodium vivax was 21.55% in malaria-confirmed cases over the five-year period: 2001–2005. As G-6-PD enzyme deficiency is a genetic condition its deficiency is different for different ethnic groups. In our country, there are eight main ethnic groups. The study to detect G-6-PD enzyme deficiency rates for five ethnic groups had already been conducted. For the remaining three ethnic groups, namely Chin, Kayah and Rakhine, we conducted the study to determine the G-6-PD enzyme deficiency rates.

To find out the G-6-PD enzyme deficiency rate in Chin, Kayah and Rakhine ethnic groups. The specific objectives were to: (i) Find out the genotype and phenotype of G-6-PD enzyme deficiency; and (ii) Differentiate heterozygote and homozygote in G-6-PD-deficient females.

Methods: All subjects were unselected, unrelated, healthy adult males and females of Chin, Kayah and Rakhine races. Moreover, they were born of parents belonging to the same race. The G-6-PD enzyme deficiency was screened by Methaemoglobin Reduction Test (Brewer's 1967), while the differentiation of G-6-PD homozygote and heterozygote females was done by the cytochemical staining method (Van-Nooder and Vogels (1987). Phenotypic and genotypic alleles were detected in males by agarose gel electrophoresis.(Myint-Oo 1995)
**Results:** In respect of the Chin race, 147 male and 261 female subjects were recruited, among them 4.08% males and 2.29% females & were mildly deficient and 0.68% males and 0.38% females were severely deficient. In the case of the Kayah race, 152 males and 252 females were recruited, among them 1.3% males and 0.4% females were mildly deficient and 1.98% females were severely deficient; there were no severely deficient males. In case of the Rakhine race, 151 males and 250 females were recruited, among them 3.98% males and 0.8% females were mildly deficient; no severely deficient person was found.

**Conclusion:** According to the above findings, the G-6-PD enzyme deficiency in Myanmar is not as high as expected and the rate of severely deficient persons is less than 2%.

### 2.35 Title of the Study: Clinical and bacteriological factors relating to the treatment outcome in multi-drug resistant tuberculosis (MDR-TB) patients attending the private sector (Project I.D. SN 1185, 2008)

**Principal Investigator:** Dr Han Win  
**Clinical Research Division, Department of Medical Research (Lower Myanmar), Yangon, Myanmar**

**Background:** Tuberculosis is one of the major health problems in Myanmar. The problem is further complicated by substantial increase in MDR-TB. The aim of this study is to determine the demographic, clinical and bacteriological factors and their relation to treatment outcome in MDR-TB patients attending the private clinics in Yangon.

**Methods:** Forty MDR-TB patients who attended the two chest physicians’ clinics in Yangon were included in the study. Face-to-face interviews were conducted with a set of structured questionnaires to obtain the sociodemographic and clinical data. Patients’ treatment records were also reviewed. They were followed up clinically, radiologically and bacteriologically by sputum smear and culture at regular intervals.

**Result:** Out of 40 MDR-TB cases, 24 (60%) were males and 16 (40%) were females. Their mean age was 34.95 ± 9.87 years (range 18 – 51 years). All of them had a history of previous treatment ranging from 6 to 36 months.
Cavities were visible in 29 (72.5%) chest x-rays, while 17 (42.5%) showed extensive involvement. Twelve patients were resistant to isoniazid (H) plus rifampicin (R), and 10 patients to HR plus streptomycin (S) or ethambutol (E). Eleven patients were resistant to HRSE, and 7 patients to HRSE plus pyrazinamide (Z). Patients received at least four “second-line drugs” during their treatment with a mean of 6.4 antitubercular drugs during their intensive phase. The common adverse effects that were reported were dizziness, insomnia, skin itching and arthralgia. Regarding treatment outcome, 26 patients were cured, 5 suffered treatment failure, 7 defaulted and 2 expired. Three predictors were identified for a successful outcome of treatment (cured). They included weight gain at six months, radiological improvement during treatment, and resistance to three or less antitubercular drugs.

**Conclusion:** Recording the weight, and radiological follow-up during MDR-TB treatment are integral parts of monitoring the response to predict the outcome of therapy.

### 2.36 Title of the Study: Effects of insecticide-treated nets on malaria in pregnancy in Myanmar

*Project ID: SN 1182, 2008*

**Principal Investigator:** Dr Kay Thwe Han  
**Parasitology Research Division, Department of Medical Research (Lower Myanmar), Yangon, Myanmar**

**Background:** Malaria in pregnancy is a major but preventable contributor to maternal morbidity and mortality. A study to determine the burden and determinants of malaria in pregnancy was conducted in Thaton, Mon State during 2004–2005. In late 2005, distribution of insecticide-treated nets was carried out by the National Malaria Control Programme in some villages in the state. To assess the effects of insecticide-treated nets (ITN) on malaria in pregnancy, malaria-related information was collected in Thaton district during 2008–2009.

**Methods:** A community-based comparative study was conducted in two rural health centres (RHC) where an ITN project was implemented (study area) and another two in RHCs with no ITN project (control area). A total of 101 deliveries and 113 antenatal care (AN) cases were recruited, divided
evenly between study and control areas. To assess the disease burden, rapid assessment tools for malaria in pregnancy (WHO/CDC 2005) was applied.

**Results:** Maternal malaria rates were lower in the study area than in the control area (7.7% vs 11.5% in AN cases, and 7.8% vs 12.0% in delivery cases). Reported use of ITNs was significantly higher in the study area. The placental malaria rate was lower in the control area (11.8% vs 14.0%). Higher use of ITNs was reported among women without placental malaria. The proportion of pregnant women with low-birth-weight babies (LBW) in the control area was double of the control area (3.9% vs 8.0%). All women with LBW babies, and all placental malaria-positive women were ITN non-users. In comparison with baseline data from 2004–2005 before ITN implementation, the proportion of LBW babies and anaemia prevalence were significantly reduced in the study area ($p=0.017$, and $p=0.0005$) although placenta malaria was slightly reduced (11.8% vs 15.0%).

**Conclusion:** These results highlight the benefits of using ITN in preventing malaria during pregnancy in terms of malaria prevalence rate, anaemia rate, and proportion of low-birth-weight babies.

2.37 **Title of the Study:** Association of visceral leishmaniasis and HIV co-infection: clinical management and the immune status in Bihar, India.

**Principal Investigator:** Dr P.K. Sinha, MD. Department of Clinical Medicine, RMRIMS (ICMR), Patna, India

**Background:** Co-infection of HIV and kala-azar infection has an impact on the epidemiology and natural history of visceral leishmaniasis and increases the risk of developing VL in endemic areas, reduces the likelihood of therapeutic response and greatly increases chances of relapse. Kala-azar (visceral leishmaniasis) is an AIDS-refining condition and a valid entry point for starting ARV treatment, irrespective of the CD4 count. The baseline CD4 count is lower in VL-HIV co-infected patients, as VL itself causes a reduction in CD4 cells.

HIV and VL infection reinforce each other. HIV patients are more likely to develop VL (due to re-activation of a dormant infection or clinical
manifestation of primary infection). Patients characteristically have high disseminated parasite loads. VL also negatively affects the response to ARV treatment and is difficult to cure in co-infected patients, especially those with CD4 counts of less than 200, who typically relapse.

**Methods:** All VL cases visiting district hospitals (Vaishali) and other centres offering VL treatment will be routinely screened for HIV infection and all subjects visiting HIV sentinel sites and VCTC will be clinically and serologically screened for VL. The co-infected cases will be treated with anti-leishmanial drugs and ART. Cases will be followed up to assess the output.

Kala-azar (VL) is being treated with 20mg/kg ambisome in District Hospital, Hajipur, Vaishali, Bihar, since 2007. It was because of the facility of treatment being offered by the Bihar government that we chose Vaishali as our centre for the study.

**Result:** To date, approximately 121 HIV-VL co-infected patients have been identified and treated with this regimen, of which 64 were primary KA patients. Among, 55 identified patients, 49% were relapse cases, and they demonstrated survival by one and two years after treatment with liposomal amphotericin B and antiretroviral therapy as per NACO guidelines. None had an initial treatment failure. A probability of relapse of 0% at six-monthly follow-up, 11.7 % at yearly follow-up was observed.

**Conclusion:** The present study confirms that the prevalence of HIV-VL co-infections is increasing.

2.38 **Title of the Study:** Adherence to the recommended regimen of artemether-lumefantrine for treatment of uncomplicated *falciparum* malaria in Myanmar. (Project ID: SN1193), 2010

**Principal Investigator:** Dr Zaw Win Tun  
**Research Officer, Department of Medical Research (Upper Myanmar), Myanmar**

**Background:** Malaria is one of the priority diseases in Myanmar. About 70% of the population are residing in malaria-endemic areas. Artemether-lumefantrine (AL) is the recommended artemisinin-based combination
therapy (ACT) for treatment of uncomplicated *falciparum* malaria in Myanmar. It is a six-dose regimen and each dose is best taken with fatty food. It is a very effective antimalarial. However, for long-term use of this ACT, adherence of patients has to be considered. Poor levels of compliance may decrease the cure rates and may precipitate the development of the drug resistance. In current practice, the *Plasmodium falciparum* positive patients are instructed to take the first dose of AL under observation of the practitioner at the clinic and they are supplied with the remainder of tablets, and explained how and when to take them at home. It is assumed that the drug is taken as prescribed. This study aimed to assess the adherence of patients to the recommended six-dose regimen of AL in uncomplicated *falciparum* malaria patients in the community.

**Methods:** A cross-sectional comparative study was conducted on 248 uncomplicated *falciparum* malaria patients in four townships of the Mandalay region. Three categories of adherence were analysed as proportions and compared among four age groups (less than 5 years; 5-<9 years; 9–14 years; 14 years and above) using the Chi-square test.

**Result:** Thirty-three percent patients (95% CI= 27.5-39.1) took the medicine with fatty food. Ten patients (4%, 95% CI=2.2-7.3) were classified as definitely non-adherent. Patients in the probably non-adherent category were 16 (6.5%, 95% CI=4-10.2). Reasons for non-adherence were 18 (69.2%, 95% CI=50-83.5) thought of being cured, 3 (11.5%, 95% CI=4-29) expected rapid cure by taking more than prescribed dose, 3 (11.5%, 95% CI=4-29) feared side-effects, and 2 (7.7%, 95% CI=2.1-24.1) shared the drug with another patient. Two hundred and twenty-two patients (89.5%, 95% CI=85.1-92.7) were probably adherents to the recommended regimen. The youngest age group had a significantly higher adherence than the eldest age group (p=0.02) but no significant differences were seen among other age groups. The background characteristics of patients or care-givers were not associated with a probable adherence to the recommended regimen of AL.

**Conclusion:** The high level of adherence to AL found in our study is likely a result of the effectiveness of the health care programmes, and of the awareness of malaria among community members. Misperception towards the regimen is still present, nevertheless.
Communicable diseases remain an important cause of morbidity and mortality, which require constant updating of knowledge and evidence through research. To fill in these knowledge gaps, the South-East Asia Regional Office/Research and Training in Tropical Diseases Joint Small Grants Programme (SGP) was initiated in 2004 by WHO Regional Office for South-East Asia (SEARO) in collaboration with the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). This programme aims at facilitating and strengthening control-oriented operational research in tropical and communicable diseases and developing capacity among the young scientists in Member States of the South-East Asia Region. The key findings of 38 such projects are documented in this publication. It is hoped that the publication proves useful, interesting and informative for the Member States.