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Faculty of Tropical Medicine, Mahidol University

Asia's Leader in Tropical Medicine

Annual Review 2008



Long Live the King! Celebrating the 80th Birthday of His Majesty King Bhumibol Hdulyadej 5 December 2007



The Year 2007 brought elation to the whole country, as we enthusiastically celebrated the 80th Birthday of His Majesty King Bhumibol Adulyadej, on 5 December 2007.

The following Annual Review 2008 represents an overview of the activities of the Faculty of Tropical Medicine for the calendar year 2007. The Faculty of Tropical Medicine is designated as the SEAMEO TROPMED Regional Center for Tropical Medicine (TROPMED/Thailand), the WHO Collaborating Centre for Environmental Management for Disease Vector Control, and the WHO Collaborating Centre for Clinical Management of Malaria. This Review reflects the integration of these roles into the Faculty's overall activities.

The Faculty continued to perform and advance strongly in all of its core operational areas--basic & clinical research, education & training, and the provision of healthcare & medical services--to consolidate our status as "Asia's Leader in Tropical Medicine". We also saw several substantial infrastructure improvements during the year, with staff occupying the new 8-floor administration building, and renovations at the Bangkok School of Tropical Medicine and the Hospital for Tropical Diseases. The Thai Government also approved a new Hospital building and accommodation for the Faculty's several Centers of Excellence, with construction due to start in early 2008.

Mahidol University was transformed into an autonomous institution on 16 October 2007. We hope this systemic change will strengthen and facilitate the Faculty's efficiency, flexibility, and ability to achieve our goals for the benefit of the Nation.

I wish to thank the staff of the Faculty, and all of our collaborators and supporters, for their admirable dedication to Tropical Medicine throughout 2007, and am pleased to commend this Annual Review 2008 to you.

Assoc. Prof. Pratap Singhasivanon Dean Faculty of Tropical Medicine Mahidol University





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Research

The Faculty of Tropical Medicine, Mahidol University, is strongly committed to high-quality research into the tropical diseases. Research activities range from molecular studies to community level, covering more than 20 diseases including vector-borne diseases, such as malaria and dengue, melioidosis, soil- and water-borne diseases, and other communicable and non-communicable diseases endemic to the Tropics. The current research programs include clinical trials, laboratory research, field and epidemiological studies, vaccine trials, assessment of future drugs for avian influenza, and assessment of the health effects of unpredicted natural hazards and disasters. The Faculty is a pioneer research hub in conducting HIV/AIDS vaccine trials, assessment of the efficacy of antiretroviral therapies, assessment of treatments for opportunistic infections, epidemiological surveys, and analyzing social risk factors. The Faculty is well placed to conduct high quality research, having a well-established Ethics Committee, Clinical Pharmacology Unit, a state-of-theart Data Management Unit, a board of research consultants, and the dedicated support of the Research and Academic Services Unit.

The Faculty encourages collaborative studies through both local and international linkages, and is currently affiliated with more than 20 leading research institutes on all continents of the globe. As part of our support for research into the tropical diseases all around the world, we provide international postgraduate education and training in tropical medicine for scientists, physicians, and others from all nations.

In the Year 2007, the research achievements of the Faculty included:

Malaria The Faculty of Tropical Medicine has provided a significant proportion of the biological, economic and clinical basis for the change in global antimalarial treatment recommendations to artemisinin combination treatments. We have been responsible for publishing 9.4% of all antimalarial trials since 1966, and enrolled 22% of all patients in such studies worldwide. Following a series of PK-PD studies in severe malaria, and a pilot comparison, we started the largest ever trial in severe malaria, in a multi-country prospective study to compare mortality in patients treated with intravenous artesunate or intravenous quinine. We have developed a mathematical-economic model of drug resistance which has been influential in guiding international recommendations.

Melioidosis The Faculty of Tropical Medicine has improved the accuracy and rapidity of the diagnosis of *B. pseudomallei* infection. Our studies of treatment for melioidosis have provided the currently recommended acute and eradication treatments for this important cause of lethal community-acquired infection in this region. Significant progress has been made in understanding the biology of *B. pseudomallei* since full sequencing of the genome. We demonstrated that horizontal gene acquisition was an important feature of its recent evolution and we have validated typing schemes based on MLST that will be essential for our understanding of disease epidemiology and pathogenesis



Leptospirosis In a large prospective study, we identified scrub typhus (caused by *Orientia tsutsugamushi*) and leptospirosis as major causes of febrile illness leading to hospital admission in rural areas of Thailand and Laos, and also an important and unrecognized cause of encephalopathy. We have conducted the largest ever randomized trial of antibiotic treatment in severe leptospirosis. We have conducted the first *in vivo* pharmacodynamic comparative assessment of the antifungal drug treatment of cryptococcal meningitis

Influenza Clinical Research The Faculty of Tropical Medicine is a partner in the research project "Pharmacologic study of Oseltamivir in Healthy Volunteers".



Discovery of new species of helminth, provisionally named "*Paragonimus pseudoheterotremus*". Assoc. Prof. Jitra Waikagul reported a new species of *Paragonimus* (Trematoda: Troglotrematidae) from a cat infected with metacercariae from mountain crabs *Larnaudia larnaudii* (*J Parasitol* 2007;93(6):1496-500).



New PCR method for detection and differential diagnosis of three *Entamoeba* species. The Department of Protozoology developed a single-round PCR and real-time PCR assay for the detection and differential diagnosis of 3 morphologically identical *Entamoeba* species found in humans--*E. histolytica*, *E. dispar* and *E. moshkovskii*. This PCR assay will serve as an accurate, rapid, and effective diagnostic method for the detection and discrimination of these *Entamoeba* species, in both routine diagnosis of amoebiasis and epidemiological surveys.

Development of new lymphedema treatment Dr. Wichai Ekataksin of the Liver Research Unit developed a new treatment for chronic lymphedema, which was granted a patent. The technique involves use of a twisting tourniquet to reduce fluid retention and swelling.

Dengue Diagnostic Center Several dengue research projects were conducted by Faculty staff. The "Epidemiological Study of Dengue Infection in Children in Ratchaburi Province", a cooperative project between the Faculty of Tropical Medicine and the Ministry of Public Health; is being supported by the Pediatric Dengue Vaccine Initiative (PDVI); the study will prepare for future dengue vaccine field trials, which will recruit in large population sizes in 2007-2011. The establishment of the TROPMED Dengue Diagnostic Center (TDC) is necessary for strengthening the Faculty's dengue research, applied research, and services.



Patent for new insect repellent The Department of Medical Entomology was granted a patent for a new formulation of insect-repellent cream/balm derived from various medicinal plants, which has a protective time against mosquito vectors of > 3 hours.

The Hematology Unit of the Hospital for Tropical Diseases was certified to be outstanding after an External Quality Assessment conducted by the Bureau of Laboratory Quality Standards, Department of Medical Science, Ministry of Public Health.

Centers of Excellence In addition to the existing Centers of Excellence within the Faculty—the Vaccine Trial Center, Pharmacokinetic Center of Anti-influenza Drugs (International Reference Laboratory), Bioequivalence Center, Influenza Clinical Research Center, Data Management Unit, and the Dengue Diagnostic Center—in 2007, the Faculty began setting up several new Centers of Excellence, and preparing the best possible facilities for them in new purposedesigned premises. The new Centers will include: the SEA Pharmacy Center of Anti-influenza Drugs, DNA Barcoding of Insect Vectors, Melioidosis Reference Center, Molecular Epidemiology Center of Antimalarial Drug Resistance, Biomedical and Public Health Informatics Center (BIOPHIC), and the Thailand Lymphedema Center.

In 2007, the Faculty published 126 international scientific papers, of which 70.6% (89 papers) are listed in the ISI Web of Science databases. There were 122 research projects, 33 of which commenced during 2007. Forty-seven (47) papers were presented at international conferences and 10 papers at national conferences.



The Faculty remains the leader in Asia in publishing research studies on malaria and other tropical diseases. We continue to encourage, support, and facilitate research in tropical diseases and other related global health problems. The research activities of the Faculty of Tropical Medicine will continue to strive to improve the education and health of the peoples in this region.

Education

The Faculty, through the Bangkok School of Tropical Medicine, offers 6 regular international postgraduate programs-the Diploma in Tropical Medicine and Hygiene, Master of Clinical Tropical Medicine, Master of Clinical Tropical Pediatrics, Master



of Science in Tropical Medicine, Doctor of Philosophy in Tropical Medicine, and Doctor of Philosophy in Clinical Tropical Medicine. In the year 2007, we enrolled 23 new Diploma in Tropical Medicine and Hygiene participants from 11 different countries, 9 Master of Clinical Tropical Medicine participants from 7 countries, 10 Master of Science in Tropical Medicine students from 4 countries, and 13 Doctor of Philosophy in Tropical Medicine students from 2 countries. Therefore, in total, 55 new international students were enrolled in the Faculty's international postgraduate programs. To date, participants from 56 different countries have attended these 6 programs.



Collaborations

The Faculty maintains 13 formal Memoranda of Understanding/Agreements with international institutes and a host of well-established cooperative and collaborative relationships with individuals, institutes, and other privateand public-sector organizations all around the world.

Joint International Tropical Medicine Meeting

The Faculty of Tropical Medicine holds the Joint International Tropical Medicine Meeting annually. In 2007, the Meeting was held 29-30 November at the Imperial Queen's Park Hotel in Bangkok. The main theme of the Meeting was "Health Security in the Tropics". This two-day event incorporated a unique scientific program, focusing on a broad range of tropical medicine topics. In 2007, the



Meeting attracted 1,070 registered participants from 24 countries. For more information about the Joint International Tropical Medicine Meeting series, please visit the JITMM website at <u>http://www.jitmm.com</u>

Finance

Total income amounted to 312.19 million Baht, of which 166.53 million Baht were supported by government budget, 177.36 million Baht from research funding and 145.66 million Baht from Faculty revenues.



Personnel

In the year 2007, the Faculty had 738 staff, including 96 academic staff, 196 technical staff, and 446 support staff. Seventy-two (72) staff (9.8%) had Ph.D. degrees, while 77 (10.4%) had Master Degrees or comparable qualifications. The ratio of Prof. : Assoc. Prof. : Assist. Prof. : Lecturer = 4 : 32 : 24 : 33. Nine (9) staff retired during the year.



Staff development

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Six (6) staff continued their education overseas, and 9 studied within Thailand. Two hundred and seventy (270) staff were trained within Thailand and 10 overseas. Forty (40) staff participated in international conferences and 529 in national conferences.

Infrastructure improvements

Construction of the "60th Anniversary of the King's Accession to the Throne" Building was completed in 2007. The 8 floors are designed to accommodate the Faculty's Executive and Administration office spaces, the Office of the Dean, SEAMEO TROPMED Network, the Mahidol University-Oxford Research Unit (MORU), the WHO Collaborating Centre for Clinical Management of Malaria, the WHO Collaborating Centre for Environmental Management for Disease Vector Control in Sustainable Development, and the Office of the Mekong Malaria Programme. Renovations of existing facilities included the Hospital for Tropical Diseases and the Bangkok School of Tropical Medicine. A new building, "Asia's Center of Excellence for Tropical Diseases", was approved by the Royal Thai Government, for construction starting in 2008. The building will serve as a Center of Excellence for clinical trials, research, and the treatment of tropical diseases.



Organization Chart



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Assoc. Prof. Pratap Singhasivanon Dean and Director of SEAMEO TROPMED/ Thailand



Assoc.Prof. Jitra Waikagul Deputy Dean for Academic Affairs and Special Projects



Assist. Prof. Varaporn Suphadtananphongs Deputy Dean for Policy and Human Resources



Mr. Chanathep Pojjaroen-anant Deputy Dean for Environment and Land Resource



Prof. Sasithon Pukrittayakamee Deputy Dean for Research



Assist. Prof. Phanorsri Attanath Deputy Dean for International Relations



Assoc. Prof. Porntip Petmir Assistant Dean for Research (To 30 September 2007); Deputy Dean for Administration and Finance (From 1 October 2007)



Assist. Prof. Usanee Suthisarnsuntorn Deputy Dean for Educational Affairs (To 30 September 2007)



Assoc. Prof. Wattana Leowattana Deputy Dean for Service and Hospital Director



Dr. Wichai Ekataksin Assistant Dean for Education and Information Technology



Assist. Prof. Kasinee Buchachart Deputy Dean for Administration and Finance (To 1 August 2007)



Assist. Prof. Chotechuang Panasoponkul Deputy Dean for Student Affairs and Special Activities; Acting Deputy Dean for Educational Affairs (From 1 October 2007)



Assoc. Prof. Supatra Thongrungkiat Assistant Dean for Research (From 1 November 2007)



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Heads of Department/Center/Unit



Prof. Punnee Pitisuttithum Department of Clinical Tropical Medicine & PI VTC



Assoc. Prof. Chalit Komalamisra Department of Helminthology



Assoc. Prof. Chamnarn Apiwathnasorn Department of Medical Entomology



Assoc. Prof. Manas Chongsa-nguan Department of Microbiology and Immunology



Assoc. Prof. Yaowalark Sukthana Department of Protozoology



Assoc. Prof. Emsri Pongponratn Department of Tropical Pathology



Prof. Nicholas J. White Chairman, SEA, Wellcome Trust



Assoc. Prof. Wijitr Fungladda Department of Social and Environmental Medicine Department of Tropical Hygiene and WHO CC for Environmental Management for Disease Vector Control



Assoc. Prof. Krisana Pengsaa Department of Tropical Pediatrics



Prof. Nicholas Day Director, MORU



Assist. Prof. Jaranit Kaewkungwal and DMU



Assist.Prof. Channarong Sanghirun Department of Tropical Radioisotopes



Assoc. Prof. Songsak Petmitr Department of Tropical Nutrition & Food Science



Prof. Polrat Wilairatana WHO CC for Clinical Management of Malaria



Mr. Somkid Nima Secretary of the Faculty (From 1 October 2007)

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Mrs. Vorapan Singhsilarak Secretary of the Faculty (To 30 September 2007)

Faculty Board



Faculty Senate

Assoc. Prof. Varee Wongchotigul	Chair
Assist. Prof. Pongrama Ramasoota	Vice-Chair
Assoc. Prof. Wijitr Fungladda	Lecturer Representative
Assoc. Prof. Watcharee Chokejindachai	Lecturer Representative
Dr. Jiraporn Ruangsittichai	Representative, Department of Medical Entomology
Assist. Prof. Kriengsak Limkittikul	Representative, Department of Tropical Pediatrics
Dr. Pornsawan Luengwutiwong	Representative, Department of Microbiology and Immunology
Dr. Teera Kusolsuk	Representative, Department of Helminthology
Ms. Rachatawan Chiabchalard	Representative, Department of Protozoology
Assist. Prof. Channarong Sanghirun	Representative, Department of Tropical Radioisotopes
Dr. Prapin Thanpoophasiam	Representative, Department of Social and Environmental Medicine
Dr. Natthanej Luplerdlop	Representative, Department of Clinical Tropical Medicine
Assist. Prof. Kesinee Chotivanich	Representative, Department of Tropical Hygiene
Dr. Sarunya Kaewprasert	Representative, Department of Tropical Nutrition and Food Science/Secretary
Assist. Prof. Urai Chaisri	Representative, Department of Tropical Pathology /Assistant Secretary

Consultants to the Faculty of Tropical Medicine

Prof. Emeritus Chamlong Harinasuta Prof. Emeritus Danai Bunnag Prof. Emeritus Tan Chongsuphajaisiddhi Prof. Emeritus Santasiri Sornmani Prof. Emeritus Mukda Trishnananda Prof. Emeritus Prayong Radomyos Prof. Emeritus Chaisin Viravan Assoc. Prof. Mario Riganti Prof. Emeritus Sirivan Vanijjanonta Prof. Emeritus Wanpen Chaicumpa Assoc. Prof. Supranee Changbumrung Assoc. Prof. Suvanee Supavej Assoc. Prof. Yupa Rongsriyam Dr. E.B. Doberstyn Assoc. Prof. Chris Beyrer Dr. Gareth D.H. Turner Prof. David Ferguson Dr. Donald Pinkston Francis Dr. Jose Esparza Dr. Arjen M. Dondorp Dr. Hans L. Bock Dr. Ralf Clemens Dr. Francessco Castelli Assoc. Prof. Vangvarothai Singhasivanon Dr. Akravuj Viriyavejakul Prof. Srisakdi Charmonman Mr. Paisan Loaharanu Dr. Bounlay Phommasack Dr. Vivian Davis Tsu

Visiting Professors

Prof. Andrew Thompson, Murdoch University, Australia Dr. Hannes Wickert, University of Heidelberg, Germany

Office of The Dean

Telephone: 66 (0) 2354 9100-19 ext. 1326, 1328, Fax: 0 2354 9139



Mrs. Vorapan Singhsilarak Secretary of the Faculty (To 30 September 2007)



Mr. Somkid Nima Secretary of the Faculty (From 1 October 2007)

The Office of the Dean is a support unit facilitating the major tasks of the Faculty, such as teaching, research, academic services, and hospital services, to meet the goals of the Faculty. The Office of the Dean functions under the authority and supervision of the Secretary of the Faculty and Deputy Deans with related duties. It consists of 10 major units, i.e., Administration and General Affairs Unit, Personnel Unit, Policy and Planning Unit, Financial and Procurement Unit, Educational Affairs Unit, Information Technology Unit, Educational Technology Unit, International Relations Unit, Area and Maintenance Unit, and Research and Academic Affairs Unit.



SEAMEO TROPMED Regional Centre for Tropical Medicine (TROPMED/Thailand)



Assoc. Prof. Pratap Singhasivanon Director E-mail: tmpsh@mahidol.ac.th

Scooperative network established in 1967 for education, training and research in tropical medicine and public health under the *Southeast Asian Ministers of Education Organization (SEAMEO)*. The mission of SEAMEO TROPMED is to promote health and to prevent or control diseases, thus improving the living conditions of people through relevant programmes and services. The Network aims to develop the capacity of individuals and institutions in delivering quality healthcare. The Network operates through Regional Centres in Indonesia, Malaysia, the Philippines, and the SEAMEO TROPMED Regional Centre for Tropical Medicine in Thailand. The Regional Centres are all affiliated with academic and research institutions, which provide physical facilities, faculty, and technical support staff.



TROPMED/Thailand

The Faculty of Tropical Medicine, Mahidol University, was appointed the TROPMED National Centre for Tropical Medicine in 1967, and was later designated the SEAMEO TROPMED Regional Centre for Tropical Medicine, in 1994. It is now known as "TROPMED/Thailand". Its objectives are promoted through 3 major activities: (1) teaching and training, (2) research, and (3) services. The activities of the Faculty of Tropical Medicine and TROPMED/Thailand are therefore synonymous.

Tel: 66 (0) 2354 9100 to 19, ext 1680, 1682, 1683

TROPMED/Thailand Website: http://www.tm.mahidol.ac.th

Fax: 66 (0) 2644 7483, 2354 9198

SEAMEO Website: http://www.seameo.org



WHO Collaborating Centre for Clinical Management of Malaria



Prof. Polrat Wilairatana Director E-mail: tmpwl@mahidol.ac.th

The Faculty of Tropical Medicine, Mahidol University, has been nominated by the World Health Organization as the WHO Collaborating Centre for Clinical Management of Malaria. This report thus outlines the activities of the CC/Faculty.

Activities

- One doctor participated in the informal consultation "Containment of malaria multi-drug resistance on the Cambodia-Thailand Border", Phnom Penh, 29-30 January 2007.
- One doctor spoke on the topic "Cerebral malaria" at the ASEAN Neurological Association (ASNA) International Neurological Conference, Cha-am, Prachuab Khirikan, Thailand, 6-9 March 2007.
- One doctor and 1 nurse conducted training in malaria for nurses from SEARO, at the request of WHO Nursing and Midwifery/WHO SEARO and WHO/Bangladesh, Chittagong, Bangladesh, 11-18 May 2007.
- The Centre conducted the 5th International Training course for the Management of Malaria at the Faculty of Tropical Medicine, Mahidol University, Bangkok, 24-28 September, 2007, which was attended by 35 participants from the USA, Thailand, Myanmar, Vietnam, Hong Kong, Japan, Bhutan, Sri Lanka, Indonesia, and Poland. One malaria adviser came from India (Dr. Sanjib Mohanthy).
- One doctor was sent to the centre in Bangladesh at the request of WHO/Bangladesh for consultations and recommendations for establishing the Bangladesh Institute of Tropical Medicine (BITID), Chittagong, Bangladesh, 22 November-15 December 2007.

The activities of the CC (i.e. the Faculty of Tropical Medicine, Mahidol University) were implemented through collaboration with WHO SEARO, WR Bangladesh, WHO Mekong Malaria Programme, and SEAMEO TROPMED Network.

Collaboration between the CC and WHO

- Visits by WHO staff (HQ and SEARO office) to the Centre
- Utilization of Centre staff by WHO in international training on management of malaria outside Thailand; consultative meeting in Cambodia and Bangladesh; training in Bangladesh and Thailand.
- Staff from the Centre conducted training courses on the management of malaria in Thailand and Bangladesh.
- WHO SEARO supported fellows from SEAR countries and 1 malaria adviser from ISPAT General Hospital, Orissa, India, to participate in the training course on Management of Malaria in Thailand.

- WHO Mekong Malaria Programme and SEAMEO TROPMED Network supported 1 doctor from the Centre to participate in the consultative meeting in Cambodia.
- WR Bangladesh authorized APW to send 2 staff of the Centre to conduct a malaria training course for SEARO nurses in Bangladesh.





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Other collaborative activities

Collaboration with the Ministry of Public Health, Thailand, to revise doctors' guidelines for the management of malaria in Thailand--3-day regimen of artesunate-mefloquine for uncomplicated falciparum malaria.

Collaboration with other WHO Collaborating Centers

WHO Mekong Malaria Programme

WHO Collaborating Centre for Environmental Management for Disease Vector Control in Sustainable Development

Telephone: 66 (0) 2354 9100-19 ext. 1327, 1318 Fax: 66 (0) 2354 9141



Assoc. Prof. Pratap Singhasivanon Director (1 January-7 March 2007) E-mail: tmpsh@mahidol.ac.th



Assoc. Prof. Wijitr Fungladda Director (from 8 March 2007) E-mail: tmvfd@mahidol.ac.th

The Faculty of Tropical Medicine, Mahidol University, has been designated by the World Health Organization as the WHO Collaborating Center for Environmental Management for Disease Vector Control in Sustainable Development. This report thus outlines the activities of the CC/Faculty

Activities 2007

Technical Collaboration

The Centre collaborated with national and international institutes on research and training activities, namely:

- Ministry of Foreign Affairs, Thailand
- Ministry of Public Health, Thailand
- SEAMEO TROPMED Network
- Universita degli Studi di Brescia, Italy
- Japan International Cooperation Agency (JICA), Japan
- Japan International Corporation of Welfare and Services (JICWELS), Japan
- Rockefeller Foundation
- Global Fund to Fight AIDS, Tuberculosis, and Malaria
- WHO

Academic Services/Information Exchange

In 2007, the Centre provided scientific information and technical support in relation to disease vector control to various institutes, both national and international.

- Identification of mosquitoes, flies, Phebotomine sandflies and other medially important insects
- Determination of insecticide efficacy, persistence and resistance for private and government sectors based on WHO standard protocols
- Providing scientific information, technical support and advice on vector-borne diseases, vector biology, field surveys and vector control techniques to the Ministry of Public Health, Thailand
- Investigation of snails of medical importance in water resources development projects
- Development of IEC materials in regards to disease vector control

Training

Short training courses were conducted for local and international health personnel. These courses were offered in partnership with national, regional, and international organizations and academic institutions. In 2007, the following short training courses were organized:

- Spatial Epidemiology and GIS for Development of Environmental Surveillance System
- Regional Training Course on Spatial Statistics for GIS
- Basic GIS
- Spatial Statistics for GIS
- Tropical Medicine



Memoranda of Understanding and Agreements, 2007

- Canadian Food Inspection Agency, Government of Canada, Saskatoon, Canada
- Chiang Rai Regional Hospital, Ministry of Public Health, Thailand
- Department of Medical Sciences, Ministry of Public Health, Thailand
- Free University, Berlin, Germany
- Nagasaki University, Japan
- Southeast Asian Ministers of Education Organization (SEAMEO) and the Government of Thailand
- Swiss Tropical Institute, Basel, Switzerland
- Ubon Ratchathani University, Thailand
- University of Innsbruck, Austria
- University of Leicester, UK
- University of Shizuoka School of Pharmaceutical Science, Japan
- University of Texas Health Science Center at Houston, Texas, USA
- Wellcome Trust-Mahidol University-Oxford Tropical Medicine Research Programme

International Linkages

- SEAMEO-Australia: Tropical Health Programme
- SEAMEO-Canada: Institutional Partnership between Trop. Med. Centre and Canadian Lead Institutions
- SEAMEO-France: Applied Epidemiology, Geographic Information System, Diagnostic Laboratory, Research
- SEAMEO-GTZ: Community Nutrition, HIV/AIDS, M.Sc. Epidemiology, Health Sector Development
- SEAMEO-Rockefeller Foundation Health Equity Project
- SEAMEO-ADB-UNESCO Partnership Project on ICT & HIV/AIDS Preventive Education
- WHO: Roll Back Malaria, Tropical Disease Research, Research Capacity Strengthening
- UNESCO PROAP: Preventive AIDS Education
- UNAIDS: through CHASPPAR
- European Commission: Malaria Control
- Asian Forum for Health Research: Equity in Health Development
- JICA: Japan International Cooperation Agency
- Liverpool Lymphatic Filariasis Support Centre
- ACT Malaria



International Visitors

The Faculty was delighted to welcome overseas visitors from Austria (1), the Congo (3), the Dominican Republic (1), Egypt (2), Japan (14), Mexico (1), SEAMEO SEARCA (the Philippines) (2), the USA (Columbia University) (17), and the Yemen (1), during 2007. These visitors consisted of lecturers, academic staff, medical doctors, medical students, public health personnel, scientists and researchers.







• The Surveyers from the Office of National Education Standards and Quality Assessment visited the Faculty of Tropical Medicine on 17 January 2007



• Prof. Vicharn Panich, Chairman of Mahidol University Council, and his team visited the Faculty of Tropical Medicine on 27 March 2007

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• TROPMED congratulated Prof. John H. Cross for being awarded an Honorary Doctorate in Tropical Medicine





• Graduation Day, 4 July 2007



• Celebrating Thai New Year, Songkran Festival, 10 April 2007



The Minister for Public Health visited the Hospital for Tropical Diseases, Faculty of Tropical Medicine, 14 March 2007



• Student Academic Forum, 2007

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• Joint International Tropical Medicine Meeting 2007; the main theme of the Meeting was "Health Security in the Tropics", 29-30 November 2007



• Regional Training Course on Tropical Diseases, 12-16 November 2007



• Awards and Closing Ceremony, D.T.M.&H. 2007, 27 September 2007



• Opening Ceremony: Training Course on Global Infectious Disease Control, 10-21 September 2007

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• Bangkok School of Tropical Medicine Open House, 16 November 2007

Department of Clinical Tropical Medicine

Telephone: 66 (0) 2354 9100-19, ext. 1423, 1428 Fax: 66 (0) 2354 9168



Prof. Punnee Pitisuttithum Head E-mail: tmppt@mahidol.ac.th ; headtmmd@mahidol.ac.th

The Department of Clinical Tropical Medicine was established in 1960, to pursue research and training in research methods work that contribute to the advancement of knowledge and clinical care in tropical medicine. The Department of Clinical Tropical Medicine is composed of 13 units; each unit has one chief and unit staff. The units are: Biochemistry Unit, Critical Care Research Unit, Clinical Infectious Disease Research Unit, Clinical Pathophysiology Research Unit, Clinical Pharmacology Unit, Dermatology Unit, Gastroenterology Unit, Liver Research Unit, Nephrology Unit, Tropical Infectious Research Unit, Traditional Chinese Medicine Unit, Tropical Medicine Laboratory Unit, and Travel Medicine Research Unit. The Department has a total of 37 academic staff, with one Head of Department, one Deputy Head, 3 professors, 6 associate professors, 7 assistant professors, 14 lecturers, 4 scientists, 1 medical science associate and 2 general officers. The Department of Clinical Tropical Medicine has a long and proud history of contributions to clinical care and research in tropical medicine.

Education

The Department is actively involved in teaching and training in the Faculty's postgraduate programs:

- 1. Graduate Diploma in Tropical Medicine & Hygiene
- 2. Master of Clinical Tropical Medicine
- 3. Master of Clinical Tropical Pediatrics
- 4. Master of Science in Tropical Medicine
- 5. Doctor of Philosophy in Tropical Medicine
- 6. Doctor of Philosophy in Clinical Tropical Medicine

Departmental research students by Program

Master of Clinical Tropical Medicine

Andreas Ludwig Christian Neumayr Daw Aye Thida Elsideg Ahmed Mohammed Abdelrhaman Ho Thi Xuan Ha Sakhr Badawi Omar Elshiekn Shinsuke Miyano Tibor Pfleger Yin Nwe Tun

Master of Science in Tropical Medicine

Awab Ghulam Rahim Chatnapa Duangdee Jayathunge Parana Hewage Mangalasiri Juntima Sritabal Permjit Amonchai Sasikit Thepaboot Suparat Wanasilp

Doctor of Philosophy in Tropical Medicine

Ajin Songtub Natsuda Jamornthayawat Parkpoom Piyaman Piengchan Sonthayanon Preeyaporn Monatrakool Sriwipa Chuagchaiya Supinya Tanpongpichat Tassanee Panichchakul Tippawan Sungkapong

Training

- Clinical Research Methodology; 1 Aug. 2007; 147 participants. Protocol design/research planning Select appropriate study design Statistical analysis for non-statisticians Basic statistical concepts (summarizing data/probability and statistics/ estimation/hypothesis testing) Sample size/power Statistical analysis plan How study data are examined statistically
- Advanced Good Clinical Practice (GCP) in Clinical Research Part 1; 2 Aug. 2007; 117 participants Development of ICH GCP and overview of clinical research in advance aspects Responsibilities of the Ethics Committee in Human Research and processing Responsibilities of the sponsor Responsibilities of the investigator Informed consent (advanced issues) Essential documents in a regulatory framework Group discussion: essential documents
- Advanced Good Clinical Practice (GCP) in Clinical Research Part 2; 3 Aug. 2007; 120 participants Safety reporting in special situations Round table exercise and discussion: safety reporting Preparation for GCP/FDA audit Fraud and misconduct in clinical research Data management systems (DMS) DMS design and technology Electronic records/signatures (21 CFR Part 11) and data quality Electronic data capture (EDC) and data security New approaches in EDC

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2.	Suppressive effects of the anti-oxidant N-acetylcysteine on the anti-malarial activity of artesunate	74
3.	Serological and blood culture investigations of Nepalese fever patients	74
4.	Immune dysfunction in HIV-seronegative, Cryptococcus gattii meningitis	75
5.	Oral versus intravenous flucytosine in patients with Human Immunodeficiency Virus-associated cryptococcal meningitis	75
6.	Biological relevance of colony morphology and phenotypic switching by Burkholderia pseudomallei	76
7.	Accuracy of enzyme-linked immunosorbent assay using crude and purified antigens for serodiagnosis of melioidosis	76
8.	A randomized controlled trial of granulocyte colony-stimulating factor for the treatment of severe sepsis due to melioidosis	
	in Thailand	77
9.	Release of granzymes and chemokines in Thai patients with leptospirosis	77
10.	Reduction of parasite levels in patients with uncomplicated malaria by treatment with HE2000	77
11.	Src-family kinase-dependent disruption of endothelial barrier function by Plasmodium falciparum merozoite proteins	78
12.	Significant association between TNF-a (TNF) promoter allele (21031C, 2863C, and 2857C) and cerebral malaria in	
	Thailand	78
13.	Contrasting genetic structure in Plasmodium vivax populations from Asia and South America	79
14.	Relapses of <i>Plasmodium vivax</i> infection usually result from activation of heterologous hypnozoites	79
15.	Efficacy of atovaquone-proguanil for treatment of acute multidrug-resistant <i>Plasmodium falciparum</i> malaria in Thailand.	80
16.	Clinical efficacy of chloroquine versus artemether-lumefantrine for <i>Plasmodium vivax</i> treatment in Thailand	80
17.	In vitro-clinical correlations for amphotericin B susceptibility in AIDS-associated cryptococcal meningitis	81
18.	Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of oseltamivir	81
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19.	Patient and sample-related factors that effect the success of in vitro isolation of Orientia tsutsugamushi	
20.	Skin reaction after desensitization does not predict the outcome of short-course sulfa desensitization protocol among AIDS	82
	patients	
21.	Combined molecular and clinical assessment of Plasmodium falciparum antimalarial drug resistance in the Lao People's	
	Democratic Republic (Laos)	83
22.	Independent evolution of pyrimethamine resistance in <i>Plasmodium falciparum</i> isolates in Melanesia	83

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23.	The genotypes of GYPA and GYPB carrying the MNSs antigens are not associated with cerebral malaria	84
24.	Analyses of cytochrome b mutations in Plasmodium falciparum isolates in Thai-Myanmar border	84
25.	In vitro antimalarial activity of azithromycin, artesunate, and quinine in combination and correlation with clinical	
	outcome	85
26.	Effects of malaria heme products on red blood cell deformability	85
27.	Production of erythropoietic cells in vitro for continuous culture of Plasmodium vivax	86
28.	Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria	86
29.	Doxycycline versus azithromycin for treatment of leptospirosis and scrub typhus	87
30.	IgG antibody profile to c-terminal region of <i>Plasmodium vivax</i> merozoite surface protein-1 in Thai individuals exposed to	07
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3Z.	Concurrent Salmonella bacteremia in P. vivax infectiona report of 2 cases at the Hospital for Tropical Diseases, Thailand	00
33.	Bayesian approach	88
34.	Predictive score of uncomplicated falciparum malaria patients turning to severe malaria	89
35.	IgG subclasses and IgE antibody regulation in malaria. Differential regulation of IgG subclasses and IgE antimalarial antibody	
	responses in complicated and uncomplicated Plasmodium falciparum malaria	89
36.	Relative levels of IL4 and IFN-g in complicated malaria: association with IL4 polymorphism and peripheral parasitemia	90
37.	Genetic analysis of the dihydrofolate reductase-thymidylate synthase gene from geographically diverse isolates of <i>Plasmodium</i> malariae	90
38	Outcomes in HIV-infected patients on antiretroviral therapy with tuberculosis	91
39	Predisposing factors for nevirapine toxicity among AIDS patients with low baseline CD4 count	91
40	A dominant clone of <i>Lentosnira interrogans</i> associated with an outbreak of human lentosnirosis in Thailand	92
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42	Short-term in vitro culture of field isolates of Plasmodium vivax using umbilical cord blood	93
43	Toll-like receptor 2 impairs host defense in gram-negative sensis caused by Burkholderia nseudomallei (melioidosis)	93
44	Ontimization of culture of Lentosnira from humans with lentosnirosis	94
45	Short Report: quantitation of <i>B</i> neudomallei in clinical samples	94
46	Clinical diagnosis and geographic distribution of lentospirosis. Thailand	94
47	Differential roles of CD36 ICAM-1 and P-selectin in Plasmodium falcinarum cytoadherence in vivo	95
48	Cloning and characterization of Plasmodium vivas cerine hydroxymethyltransferase	95
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2.	Women's participation in the phase III efficacy trial of ALVAC HIV-1 vaccine priming. AIDSVAX vaccine boosting in	
	Thailand	96
3.	Thai prime-boost HIV vaccine phase III trial: update 2007	97
4.	Social impact of participation in a phase III HIV vaccine trial in Thailand	98
 5	Safety and immunogenicity of the MRKAd5 gag HIV-1 vaccine in a worldwide phase I study of healthy adults (Merck	20
۶.	V520-018/HVTN 050).	98
6.	HLA Class II alleles and neutralizing antibody responses to HIV-1 protein subunit boosting in a Thai population	99
7.	Outcome of tuberculosis in advanced HIV-infected patients on antiretroviral therapy	100
8.	Thailand's experiences in conducting HPV vaccine trials and HPV vaccine update	100

Department of Helminthology

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Assoc. Prof. Chalit Komalamisra Head E-mail: tmckm@mahidol.ac.th

Education

The Department teaches medical helminthology in the Faculty's regular programs: the graduate Diploma in Tropical Medicine and Hygiene (DTM&H), Master of Science in Tropical Medicine (MSc TropMed) and Doctor of Philosophy in Tropical Medicine (PhD TropMed).

The Department teaches in the following subjects in the DTM&H: Fundamentals of Tropical Medicine and Hygiene, Food-and Water-borne Diseases (I) and Soil-transmitted Helminthiases, Food- and Water-borne Diseases (II) (Tissue Parasites), Mosquito-borne Diseases (I), Field Study, and Medical Parasitology and Entomology.

In the MSc and PhD programs: Tropical Medicine I, Medical Helminthology, Practical Parasitology, Essential Systematic Helminthology, Advanced Parasitology, Experimental Techniques in Parasitology, Molecular Biology of Parasites and Vectors, Special Topics in Helminthology, Special Topics in Parasitology and Medical Entomology.

Postgraduate program participants

Master of Science in Tropical Medicine	4
Doctor of Philosophy in Tropical Medicine	4
Total	8

Research

On-going Departmental Research

Angiostrongyliasis

- Angiostrongyliasis: partially purified antigens of *Angiostrongylus* cantonensis adult worms for diagnosis using immunoblot
- Angiostrongyliasis: potential fractionated antigens of *Angiostrongylus cantonensis* adult worms for diagnosis using ELISA
- Angiostrongylus cantonensis: s-adenosyl methionine decarboxylase
- Analysis of a protein expression from Angiostrongylus cantonensis DNA cloning for serodiagnosis of angiostrongyliasis
- Detection of IgG-subclass for serodiagnosis of angiostrongyliasis
- Experimental infection of freshwater fish in Thailand with infective stage of *Angiostrongylus*

Cysticercosis & Taeniasis

- Comparison of biochemical extract preparations of *Cysticercus cellulosae* by SDS-polyacrylamide gel electrophoresis and immunoblot technique
- Differentiation of fractionated larval antigens (*Cysticercus cellulosae*) responsible for antibody of neurocysticercosis patients
- mtDNA sequences of Taenia spp.
- Seroepidemiology for cysticercosis

Echinococcosis

Analysis of fluid antigens of *Echinococcus* cyst for diagnosis





- Mitochondrial DNA sequence of protoscoleces of *Echinococcus* cysts from four patients
- Serodiagnosis of suspected echinococcosis cases using native, partially-purified, and DNA recombinant antigens







Filariasis

- Rare or unrecognized evidence of human dirofilariasis in Thailand: possible immunoblot diagnosis of one Thai patient
- Study on prevalence of *Wuchereria bancrofti* infection in Kanchanaburi and Ratchaburi provinces

Gnathostomiasis

- Effect of ivermectin on *Gnathostoma spinigerum* morphology
- · Immunoblot--diagnostic specificity for gnathostomiasis
- Prevalence of *Gnathostoma spinigerum* in cats and dogs in Buddhist Temples in Bangkok

Opisthorchiasis

- Research and development of an application to purify *Bithynia* snail antigen in serodiagnosis of opisthorchiasis
- Studies on the efficacy of Thai traditional herbal medicines in the treatment of opisthorchiasis in hamsters

Paragonimiasis

- 32-33 kDa-eluted *Paragonimus heterotremus* antigen for serodiagnosis
- Molecular systematic of *Paragonimus* lung flukes and intestinal trematodes
- Studies on *Paragonimus* populations: morphology, enzymology, molecular biology and epidemiology aspects

Soil-transmitted helminthiases

- Effect of mebendazole on *Trichuris trichiura* morphology
- Efficacy of high dose mebendazole against trichuriasis in adult patients
- Epidemiology and treatment of strongyloidiasis with ivermectin
- Herbal medicinal effects on soil-transmitted helminthiasis
- Impact of deworming on anemic status of children and adolescent girls
- Relationship between behavior and soil-transmitted helminthiasis in seamen
- Soil-transmitted helminthiases control through schoolbased intervention

Toxocariasis

• *Toxocara* canis larval antigens for serodiagnosis of human toxocariasis

Trichinellosis

- Monoclonal antibody-based competitive ELISA and indirect ELISA for immunodiagnosis of trichinellosis *Other parasites*
- Diagnosis of liver and intestinal flukes by PCR
- Differential serodiagnosis between liver and intestinal trematodes
- Fish-borne trematodes in Thailand
- Research and development of the integrated project on chemotherapy on control of malaria and parasitic infections

Services

- 1. Helminth immunodiagnosis
- 2. Fecal diagnosis by Katz's modified thick smear technique
- 3. Project to provide Quality Control Sample services
- 4. Fecal diagnosis of helminth infection
- 5. Project to provide educational helminthology materials

Center of Excellence

International Reference Centre for Food- and Waterborne Parasitic Zoonoses

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1.	A new species of Paragonimus (Trematoda: Troglotrematidae) from a cat infected with metacercariae from mountain crab	
	Larnaudia larnaudii	101
2.	Sympatric occurrence of Taenia solium, T. saginata, and T. asiatica, Thailand	101
3.	Responses to albendazole treatment for hookworm infection in ethnic Thai and immigrants in west-central Thailand	102
4.	Cysticercosis and taeniasis in Thailand	102
5.	Discrimination of Opisthorchis viverrini from Haplorchis taichui using COI sequence marker	103
6.	Fish-borne zoonotic intestinal trematodes, Vietnam	103
7.	Disease burden and epidemiology of soil-transmitted helminthiases and schistosomiasis in Asia: the Japanese perspective	104
8.	Heliconema longissimum (Ortlepp, 1923) (Nematoda: Physalopteridae) from Pisodonophis boro (Teleostei: Ophichthidae) in	
	Thailand, with remarks on the taxonomy of the Proleptinae Schulz, 1927	104
9.	Beyond deworming: the promotion of school-health-based interventions in Japan	105
10.	Anaphylaxis as an initial manifestation of cholangiocarcinoma	108
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1.	Evaluation of a helminth control programme in northern Thailand	105
2.	Paragonimiasis heterotremus in Saraburi Province, Thailand	106
3.	Cysticercosis and taeniasis in Thailand	106
4.	Molecular phylogenetic relationship of Paragonimus pseudoheterotremus	106
5.	Differential serodiagnosis of Brugian and Bancroftian filariases using antigenic products from Dirofilaria immitis adult	
	worms	107
6.	Capillariasis: a chronic watery diarrhea not only from microorganisms; what do you think?	107
7.	Effect of albendazole on lipid peroxidation in rats infected with Trichinella spiralis	108

Department of Medical Entomology

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Assoc. Prof. Chamnarn Apiwathnasorn Head E-mail: tmcaw@mahidol.ac.th

The Department of Medical Entomology was established in 1960. Currently, the Department has of 8 academic staff, 1 researcher, 3 scientists, 2 medical science associates, 10 medical science assistants, 1 administrative officer and 3 general assistants. The Department is actively involved in the teaching, research and services of the faculty of Tropical Medicine. In addition to the regular Medical Entomology subjects in the postgraduate Diploma in Tropical Medicine and Hygiene, Master of Science in Tropical Medicine and Doctor of Philosophy in Tropical Medicine programs, the Department provides training programs on the medically important insects / arthropods and vector control for individuals or groups, upon request. As a Reference Centre, the Department of Medical Entomology develops and maintains the Mosquito Museum and runs the projects on Application of Computer Technology to the Management of a Biological Museum, and Courseware Development for Computer-aided Instruction in Medical Entomology.

Education

The Department teaches in the postgraduate courses of the Faculty, including the Master of Science in Tropical Medicine, Doctor of Philosophy in Tropical Medicine, and the Graduate Diploma in Tropical Medicine & Hygiene.

MSc/PhD program participants

Master of Science in Tropical Medicine	1
Doctor of Philosophy in Tropical Medicine	13
Total	14

Training Course

Detection and monitoring technique for insecticide susceptibility of mosquito vectors, 1 October 2007-18 March 2008

Research

Highlight activities. Study of the insecticidal activities of several species of Thai herbal extracts for controlling mosquito vectors. Promising oil extracts showing mosquito repellency included qinghao (*Artemisia annua*), "may chang" (*Litsea cubeba*), clove (*Syzygium aromaticum*), and "makaen" (*Zanthoxylum limonella*), were formulated into cream or gel. "Thong pun chang" (Rhinacanthus nasutus) extract provided high larvicidal activity, and has been prepared in tablet form. A mosquito-repellent formulation of mixed volatile oils from Artemisia annua is in process of patent approval.

Mosquito repellents extracted from various medicinal plants were screened and their efficacies determined to develop commercial formulations for public use. Recently, the Department was granted a petty patent for a natural mosquito repellent cream formula, with a protective time against major mosquito vectors of > 3 hours.

Current Research Activities. Research involved the application of both basic and applied science for controlling insect and arthropod vectors of tropical diseases, with an emphasis on mosquito-borne-diseases. The biology of vectors and their relationships with pathogens at the molecular level are of research interest. Studies of the population dynamics and vector competence of dengue vectors in urban and

suburban areas continued, as well as the population genetics of vectors of malaria, dengue fever, and lymphatic filariasis, such as *Anopheles dirus* and *An. minimus* species complexes, *An. barbirostris* group, *An. sundaicus*, *Aedes niveus* subgroup and *Ae. albopictus*.

Another important goal was to resolve entomological problems through research and to make the results available to other scientists, educators, and the general public. As a result of a previous study on the impact of the Asian Tsunami on mosquito vectors in Phang Nga Province, a small-scale project for controlling mosquito larvae inhabiting swamps and ponds contaminated by seawater was successfully conducted using Bacillus thuringiensis israelensis (VectoBac® WDG); its use has been extended to the worst-affected area, Ban Nam Khem Village. The Department, with the Department of Disease Control, Ministry of Health, is conducting a project in collaboration with the European Research Community entitled "Toward successful dengue prevention and control" to determine the field efficacy of commercial impregnated, long-lasting curtains and IGR in control populations of dengue vectors in Laem Chabang Commune municipal area, Chonburi Province.

The Department works with local public-health officials to help investigate emerging and re-emerging diseases, e.g., investigations of autochthonous leishmaniasis cases in Nan and Phang Nga provinces, infestations of mites in the external ear canals of shallot farmers in Si Saket Province, and an outbreak of contact dermatitis due to infestation of rove beetles in apartments in Phra Nakhon Si Ayutthaya Province, as well as identification of medically important insects, such





as maggots causing myiasis, and ticks and mites for general hospitals.

Laboratory colonies of different strains of mosquito vector species of *Anopheles, Aedes, Culex,* and *Mansonia* as well as other medically important insects, for example, cockroaches and flies, are continuously maintained in the insectarium for further use.

The Department of Medical Entomology acts as a reference center on mosquito vectors in Thailand through establishment of the Mosquito Museum Annex. Academic consultations, especially on mosquito-borne diseases and their control measures, and services for detecting filarial parasites, identification of mosquitoes and other medically important insects and arthropods, are regularly provided.

Current Research

- Dynamic and temporal structure of the troglobitic fauna of medically important insects and arthropods in caves of Kanchanaburi Province
- Molecular identification of *Anopheles sundaicus* in natural breeding sources, the coastal area of Andaman and the Gulf of Thailand
- Survey and study on geographical distribution of *Aedes albopictus* in Bangkok Metropolitan area
- Field application of *Bacillus thuringiensis israelensis* (VectorBac ,WDG) in the control of mosquito larvae in salt-marsh habitats in tsunami-affected areas, Phang-Nga Province, Thailand: monitoring by reduction in adult density
- Novel methods for dengue prevention by vector control (DENCO)
- Implementation of research on new intervention tools (DENCO)
- Research on efficacy of insecticide-impregnated products against the dengue vector, *Aedes aegypti*, in endemic houses,

Thailand

- Research on emerging and reemerging viral infectious diseases, especially prevention, diagnosis, treatment and prognosis
- Mosquito repellents from medicinal plants
- Effect of heavy metal (Pb²⁺, Cd²⁺) on enzymes of *Culex quinquefasciatus*.
- Control of mosquito vectors of tropical infectious diseases in Thailand by the use of mosquito coils containing several pyrethroids and synergist, in the laboratory and in the field
- Dengue virus detection in dark-and pale-form *Aedes aegypti* collected as immature stages from breeding sites
- Technology transfer of research and development for essential oils of *Lisea cubeba*, qinghao (*Artemisia annua*) and kaffir lime (*Citrus hystrix*) as mosquito repellents for control of mosquito-borne diseases to local communities in northern, southern and northeastern Thailand
- Population dynamics of dengue vectors and dengue-virus infection in *Aedes aegypti* and *Aedes albopictus*, in urban and suburban areas

International Collaborations

- Oita University, Japan
- Liverpool School of Tropical Medicine, UK
- Institute of Tropical Medicine Nationalestraat, Antwerpen, Belgium
- Dainihon Jochugiku Co. Ltd. ,Japan

National Collaboration

• Department of Disease Control, Ministry of Public Health, Thailand

Academic Services

- Identification of arthropods and insects from biological specimens
- Determination of filarial worms by Knott's concentration technique

Centers of Excellence

- 1. Center of Pesticide Research and Services
- 2. Center of Excellence for DNA Barcode of Medically Important Insects and Arthropods of Thailand

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List of publications

1.	Effects of food, embryo density and conspecific immatures on hatchability in the dengue vector Aedes albopictus	109
2.	An empowerment program to enhance women's ability to prevent and control malaria in the community, Chiang Mai	
	Province, Thailand	109
3.	A new densovirus isolated from the mosquito Toxorhynchites splendens (Wiedemann) (Diptera: Culicidae)	110
4.	Influence of larval density or food variation on the geometry of the wing of Aedes (Stegomyia) aegypti	110
5.	Rapid determination of viral RNA sequences in mosquitoes collected in the field	111
6.	The geometry of the wing of Aedes (Stegomyia) aegypti in isofemale lines through successive generations	111
E	ist of presentations	
1.	Effectiveness of Bacillus thuringiensis H-24 against temephos-resistant Aedes aegypti	112
2.	Molecular identification of coastal malaria vector, Anopheles sundaicus, in natural breeding sources, the coastal area of	
	Andaman and the Gulf of Thailand	112
3.	Possibility of domestic sand flies as a vector of leishmaniasis in Thailand	112
4.	Geometry of Aedes (Stegomyia) aegypti wings under the influence of larval density and food supply	113

Department of Microbiology and Immunology

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Assoc. Prof. Manas Chongsa-nguan Head E-mail: tmmcs@mahidol.ac.th

The Department of Microbiology and Immunology was founded in 1960. The Department is actively involved in education by teaching in the Faculty's postgraduate programs, the postgraduate Diploma in Tropical Medicine and Hygiene, Master of Science in Tropical Medicine and Doctor of Philosophy in Tropical Medicine. Many research articles prepared by Departmental staff have been accepted for publication in renowned journals, and some have been recognized for outstanding achievements in related research fields, especially in the tropical diseases. The Department's aim to continue this research work on various tropical diseases, especially those affecting human health, so that the results can be applied to improving health in Thailand and other regions. The Department's medico-scientific services consist of an array of laboratory diagnostic tests.

Research

The current research activities of the Department include immunology, biology, and molecular biology of infectious agents/diseases, particularly those causing problems in tropical areas, with the ultimate aims being 1) development of simple, rapid, specific, sensitive, costeffective and practical diagnostic methods for use in remote areas and for the self-reliance of the country; 2) identification of potential protective antigens for vaccine development; 3) understanding of host responses and immunity; 4) understanding pathogenic mechanisms and virulence factors of pathogens and pathophysiology in hosts; and 5) acquisition of, and acquaintance with, modern technologies, e.g. genetic analysis of bacterial pathogens for epidemiological study, immunogenetic characteristics of severe malaria, gene polymorphism of malaria parasites.

Education

The Department supports the educational objectives of the Faculty and the Nation through teaching in the Faculty's postgraduate programs, from graduate diploma to doctoral levels. Short training courses are arranged according to demand.

Services

- Aerobic bacterial culture & identification
- Culture for bacteria/fungi in herbal medicine
- Production of microbiological teaching materials
- · Indirect immunofluorescent assay (IFA) for scrub typhus
- · Scrub typhus IFA, production of instant test kit
- · Microscopic agglutination test for leptospirosis
- Flow cytometry for CD4 and CD8





E	ist of publications	Pay
1.	Relative levels of IL4 and IFN-g in complicated malaria: association with IL4 polymorphism and peripheral parasitemia	90
2.	Differential regulation of IgG subclasses and IgE antimalarial antibody responses in complicated and uncomplicated	
	Plasmodium falciparum malaria	89
3.	IgG antibody profile to C-terminal region of <i>Plasmodium vivax</i> merozoite surface protein-1 in Thai individuals exposed to malaria	8
4.	Multiple mutation in katG and inhA identified in Thai isoniazid resistant Mycobacterium tuberculosis	11
5.	Proteome and immunome of pathogenic <i>Leptospira</i> spp. revealed by 2DE and 2DE-immunoblotting with immune serum	11
6.	OmpL1 DNA vaccine cross-protects against heterologous <i>Leptospira</i> spp. challenge	11
7.	Monoclonal antibody that neutralizes pertussis toxin activities	11
8.	Virulence genes of clinical Vibrio cholerae O1 isolates in Thailand and their ribotypes	11
9.	Novel linear polymers bearing thiosialosides as pendant-type epitopes for influenza neuraminidase inhibitors	11
10.	Thiosialoside clusters using carbosilane dendrimer core scaffolds as a new class of influenza neuraminidase inhibitors	11
11.	Characterization of atypical lymphocytes and immunophenotypes of lymphocytes in patients with dengue virus infection	11
12.	An avian influenza H5N1 virus that binds to human-type receptor	11
13.	Long-lasting protective immune response to the 19-kilodalton carboxy-terminal fragment of <i>Plasmodium yoelii</i> merozoite surface protein 1 in mice	11
14.	Molecular typing of <i>Leptospira</i> spp. based on putative O-antigen polymerase gene (WZY), the benefit over 165 rRNA gene	11
15.	Significant association between TNF-a (TNF) promoter allele (-1031C, -863C, and -857C) and cerebral malaria in Thailand	78
16	The genotypes of GYPA and GYPB carrying the MNSs antigens are not associated with cerebral malaria	84
17	Rapid dissemination of <i>Plasmodium falcinarum</i> drug resistance despite strictly controlled antimalarial use	11
18.	FTO polymorphisms in oceanic populations	11
E	st of presentations	
1.	Regulation of immune response in complicated malaria: balance between Th1 and Th2 cytokines and cytokine gene	
	polymorphism	12
2.	Polymorphism of the gene encoding buffy binding protein of <i>Plasmodium vivax</i> from Thai patients	12
3.	Detection of dengue NS1 antigen for early diagnosis of dengue virus infections (Presented in two conferences)	12
4.	The enhancing antibodies of dengue virus infection in Thai patients' sera	12
5.	Dengue virus infection rates in Aedes albopictus related to environmental factors (Presented in two conferences)	12
6.	Avian influenza-related KABP among school students, parents and teachers in Thailand	12
7.	Dengue infection rates in Aedes aegypti, Petchabun Province	12
8.	A comparative evaluation of four techniques for the serological diagnosis of scrub typhus	12
9.	Serodiagnosis of scrub typhus and melioidosis by dual immunoperoxidase test	12
10.	Identification of genus-and/or species-specific antigens of Aeromonas by western blotting	12
11.	Production of monoclonal antibody against Aeromonas veronii	12
12.	Construction of single chain antibody library specific to H5N1 avian influenza virus	12
13.	Correlation of CD4+CD25+ regulatory T cells and active autoimmunity in C4KO mice	12
14.	Construction of antibody phage library specific to H5N1 avian influenza virus	12
15.	Recombinant tropomyosin of American cockroach, Periplaneta americana	12
16.	Development of novel cockroach allergy model	12
17.	Preparation of human monoclonal antibody that neutralizes tetanus toxin using phage display technology	12
18.	Development of novel multiplex reverse transcriptase-PCR assays for rapid detection of arboviruses	12
19.	Detection of specific antibodies to recombinant dengue viral protein using immunoblot assay.	12

Department of Protozoology

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The Department of Protozoology provides diagnostic techniques based on up-to-date research, from enzyme to community level, and is responsible for the Research-based Diagnostic Unit.

Training

1. Workshop on "Laboratory detection of opportunistic and pathogenic protozoa in fecal specimens"

- Group 1: 15-16 March 2007; 60 participants
- Group 2: 22-23 March 2007; 60 participants

2. Workshop on "Laboratory diagnosis of malaria", 23-24 August 2007: 80 participants

Research

- Ultrastructure of acute and chronic toxoplasmosis after pyrimethamine and artesunate administration in vivo study
- Viability of Giardia in water
- Effect of ultraviolet irradiation on the viability of *Cryptosporidium*
- Biological contamination-free Thai frozen food
- Molecular technique for diagnosis of toxoplasmosis and neosporosis in Thai dairy cows
- Molecular and immunohistochemistry studies on stage interconversion of toxoplasmosis in immunocompromised hosts
- · Protozoa contamination on Thai vegetables
- Studies on DNA replication and DNA repair in *Plasmodium falciparum*.
- Differential diagnosis of *Entamoeba* spp. found in humans by molecular biology method
- Development of molecular methods for drug resistance markers in *Plasmodium falciparum*

National linkages

- Inter-laboratory Network for Parasitic Immunology hosted by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health
- Project for making *Plasmodium* slides to distribute nationwide for laboratory QC at request of Department of Medical Sciences, Ministry of Public Health

International linkages

- Department of Parasitology, School of Veterinary Science, Murdoch University, Western Australia
- Nuffield Department of Pathology, University of Oxford, John Radcliffe Hospital, UK
- Department of Parasitology and Tropical Hygiene, Heidelberg University, Germany.
- Division of Enteric Infections, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

Laboratory services

- Diagnosis of *Toxoplasma gondii* by Sabin-Feldman Dye Test
- Diagnosis of intestinal protozoa by using special stain techniques

Center of Excellence

• Research-based Diagnostic Unit: workshops on diagnosis of intestinal protozoa and malaria

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1.	Canine parasitic zoonoses in Bangkok temples	130
2.	Immunohistochemical study of acute and chronic toxoplasmosis in experimentally infected mice	131
3.	Novel drug compounds against Neospora caninum and Toxoplasma gondii in vitro	131
4.	Partial purification and characterization of DNA polymerase β -like enzyme from Plasmodium falciparum	131
5.	IMS-free DNA extraction for the PCR-based detection and quantification of Cryptosporidium parvum and Giardia lamblia	
	surface and waste water	132
6.	Formation, physical stability and in vitro antimalarial activity of dihydroartemisinin nanosuspension obtained by co-grinding	
	method	134
L	ist of presentations	
1.	Biological contamination-free Thai frozen food	132
2.	Giardia and Cryptosporidium as biohazard in frozen food products	133
3.	Detection of food-borne pathogenic protozoa contaminated in Thai vegetables	133
4.	Detection of Entamoeba spp. from surface and waste water samples by PCR using genus-specific primers	134

Department of Social and Environmental Medicine



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Assoc. Prof. Wijitr Fungladda Head E-mail: tmvfd@mahidol.ac.th

The Department of Social and Environmental Medicine, formerly known as the 'Department of Tropical Medicine', was one of the 5 departments established at the foundation of the Faculty of Tropical Medicine, in 1960. Major responsibilities of the Department include teaching, research and academic services. The Department offers various postgraduate courses leading to the Master of Science in Tropical Medicine and Doctor of Philosophy in Tropical Medicine programs. Specialization focuses on three tracks, i.e., Social Medicine, Environmental Health and Environmental Toxicology. Research involves both laboratory and field investigations. Efforts are concentrated on disease-oriented problems. In addition to laboratory studies, such as molecular biology of pathogenic organisms, leptospirosis vaccine design, and biology of blood and liver flukes, activities cover environmental epidemiology, environmental health, environmental toxicology and sociobehavioral factors influencing tropical diseases and public health problems of the country, including shigellosis, leptospirosis, DHF, TB, HIV vaccine trial, and HIV epidemiology. The Department offers various kinds of laboratory investigation, for example, circum-oval precipitation test (COPT) for blood fluke infection, identification of medically important mollusks, etc. It is also a home of 'Mollusk Museum' where shell specimens of various medically important snail hosts are collected. This museum is considered one of the most complete mollusk museums in the region. The Department periodically offers training courses on various social and environmental health topics.

Education

The Department teaches in various postgraduate courses leading to the Master of Science and Doctor of Philosophy in Tropical Medicine degrees. Three tracks of specialization include social medicine, environmental health, and environmental toxicology. Fifteen students worked on their theses at the Department in 2007; of these, about half received scholarships from funding agencies. During 2007, three departmental staff were granted Royal Golden Jubilee scholarships to support Ph.D. studies.

Research

Research activities of the Department cover both laboratory and field investigations. This research attracts funding from various organizations, such as the World Health Organization (WHO), Universita Degli Studi Di Brescia, the Thailand Research Fund (TRF), National Research Council of Thailand (NCRT), and funds from the government budget. A highlight in 2007 was the participatory action research (PAR), aiming to solve environmental problems and vector control in an agricultural community by integrating effective microorganisms (EM), Steinernema and chitosan. The Thai Government contributed 9 million Baht funding to the project over 3 years.

The Department offered technical cervices to various organizations. Among them were the Mekong River Committee Secretariat, the Swiss National Center for Competence in Research (NCCR), and SEAMEO TROPMED Network. It also played a major role in the WHO Collaborating Centre for Environmental Management for Disease Vector Control in Sustainable Development (WHO CC No.115).

Laboratory investigations provided by the Department included the Circum-oval Precipitation Test (COPT), rapid detection of multidrug-resistant tuberculosis, and identification of snails of medical importance.

	ist of publications	Page
1.	Multiple mutations in katG and inhA identified in Thai isoniazid-resistant Mycobacterium tuberculosis isolates	113
2.	An empowerment program to enhance women's ability to prevent and control malaria in the community, Chiang Mai Province, Thailand	109
3.	Avian influenza protection knowledge, awareness, and behaviors in a high-risk population in Suphan Buri Province, Thailand	135
4.	Freshwater mollusks at designated areas in eleven provinces of Thailand according to the water resource development projects	135
5.	Intertidal snail-trematode communities on the southern Thailand before and after the South Asia tsunami	135
6.	Effectiveness of DOT for tuberculosis treatment outcomes: a prospective cohort study in Bangkok, Thailand	136
7.	Impacts of pesticide use on semen characteristics among rice farmers in Kienxuong District, Thaibinh Province, Vietnam	136
8.	Evaluation of drinking water treatment and quality in Takua Pa, Thailand	137
L	ist of presentations	
1.	Vivax malaria patients and their drug adherence in Mae Hong Son Province, Northern Thailand	137
2.	Social epidemiological factors affecting DHF between semi-rural and semi-urban dwelling in Chachoengsao Province	137
3.	MTB strains from Thailand	138
4.	A GIS-based estimation of comparative occurrence and fate of waste-related protozoan pathogens in a peri-urban canal network in a tropical	
	environment	138
5.	Occurrence of microbial pathogens in raw and oxidation pond-treated wastewater	139
6.	Fecal contamination of the Mekong River in Chiang Rai and northeastern provinces, Thailand	140
7.	Assessment of microbial infection risks posed by management practices of domestic wastes and urban agriculture activities: case study in	
	peri-urban communities in Thailand	140

Department of Tropical Hygiene

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Assist. Prof. Jaranit Kaewkungwal Head E-mail: tmjkk@mahidol.ac.th

The Department provides instruction and training to master and doctoral degree candidates in Tropical Medicine, and participants in the postgraduate Diploma in Tropical Medicine and Hygiene. Research activities are mainly in the field of epidemiology, with the use of Geographical Information System (GIS) for various tropical diseases. The department is also involved in providing specialized short training courses on epidemiology, data analysis, and GIS, and is responsible for managing the Rajanagarindra Tropical Disease International Centre (RTIC) located in a malaria-endemic rural community near the Thai-Myanmar border (Suan Peung District, Ratchaburi Province). For many years now the RTIC, through its malaria clinic, has provided continuous free and quality health services to local residents.

Current Departmental Research

- A Phase III trial of Aventis Pasteur live recombinant ALVAC-HIV (vCP1521) priming with VaxGen gp120 B/E (AIDSVAX B/E) boosting in HIV-uninfected Thai adults
- Application of GIS in monitoring multi-drug resistant malaria in the Greater Mekong Sub-Region of Southeast Asia III
- Assessment of disability and quality of life in the tsunamiaffected provinces in Thailand.
- Comparative study of dihydroartemisinin (DHA) and artesunate safety in healthy volunteers (Phase 1B)
- Epidemiological study of *Enterobius vermicularis* in preschool children at Tanowsri subdistrict, Suan Phung, Ratchaburi province
- Epidemiological study or asymptomatic malaria parasitemia in endemic area
- Epidemiology and control of malaria in Ratchaburi Province, Thailand
- Epidemiology and health education program and treatment evaluation of *Pediculosis capitis* with leech Lime cream in schoolchildren, Suan Phung district, Ratchaburi province
- Extended evaluation of the virologic, immunologic, and clinical course of volunteers who become HIV-1 infected during participation in a Phase III vaccine trial of ALVAC-HIV and AIDSVAX® B/E
- Field based study of reappearance *Plasmodium vivax* malaria cases in the area near Thai-Myanmar border Suanphung, Ratchaburi Province
- Health behavior and risk factor of soil transmitted helminth and malaria infection in primary school children in Saiyok district, Kanchanaburi province
- Integrated studies of human and animal leptospirosis in endemic areas of Nakorn Rajasima, Thailand
- Monitoring of key health indicators (TRIAMS) at subdistrict health facilities level in Tsunami-affected provinces in Thailand

- Molecular rapid serotypic identification of dengue viruses based on real-time PCR by using SYBR Green I and hybridization probes
- Occurrence of heterophyid metacercariae *Haplorchis* in cyprinoid fish of 2 reservoirs in Tanowsri subdistrict, Suan Phung, Ratchaburi Province
- Relationship of intestinal parasitic infections to malnutrition
- SAGE display application in dengue infected mosquitoes
- Search for genes susceptible to clinical malaria
- Seroprevalence of Rickettsia infection among Thai troops working along Thai-Myanmar border, Suan Phung, Ratchaburi province
- Serotypic assay of dengue viral infection by real-time PCR
- Spatial-temporal pattern of human and animal leptospirosis in endemic areas of Nakhon Ratchasima, Thailand
- Study of health behaviors and factors associated with intestinal parasites infection in the community and air-force unit in Ubolratchatani province
- Study on the ecology of Anopheline larvae in malaria endemic area of 7 hamlets Tanowsri canton, Suan Phung district, Ratchaburi province
- Surrogative protease enzyme markers for severity of dengue infection
- Rapid molecular detection of dengue infected samples
- The effectiveness of rehabilitation program for subacute stroke patients
- The study of hepatitis B among health care workers at Prasat Neurological Institute
- The study of tuberculosis and other occupationally acquired infections in health care workers at Prasat Neurological Institute
- Vb3 related to metastatic property in breast cancer

Master and PhD candidate research Doctor of Philosophy in Tropical Medicine

- Waranee Boonchuayier. Detection of adverse drug reaction signal in the Thai FDA Database: comparison between reporting odds ratio and Bayesian confidence propagation neuron network method
- Tawee Saiwichai. The effect of green tea extract to serum level of pro-inflammatory cytokines in cigarette smoke inhalation rat model
- Jongkol Podang. Burden of head injury due to road traffic injury and cost-effectiveness analysis of intervention promoting helmet use in Thailand
- Tassanee Silawan. The spatiotemporal dynamics of dengue infection in northeastern Thailand
- Rie Takeuchi. Factors related to reappearance of *Plasmodium vivax* malaria
- Putri C. Eyanoer. The spatial and temporal distribution of human avian influenza infection in relation to surveillance implementation in Indonesia

Master of Science in Tropical Medicine

List of publications

- Dilok Tongsukh. TB infection and treatment among refugees in Thamhin Camp, Ratchaburi Province, Thailand
- Wittaya Prachachalerm. Evaluation on reporting serious adverse drug reactions in Thai spontaneous reporting system; a case study of Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)

Training

- Data Analysis in Health Science Research Level 1; Nov. 19-23; Participants: 38.
- Data Analysis in Health Science Research Level 2; Nov. 26-28; Participants: 25.

International Linkages

- Dorothée Misse, IRD/CNRS, France
- Erik Thompson, Head, VBCRC Invasion and Metastasis Unit, University of Melbourne Department of Surgery and St. Vincent's Institute of Medical Research. Director of Research, Bernard O'Brien Institute for Microsurgery, Australia
- Francisco Veas, Director, Viral and Molecular Immunology Lab, France

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• The Rockefeller Foundation

Survival time of HIV-infected patients with cryptococcal meningitis 1. 2. In vitro activity of ferroquine (SSR 97193) against Plasmodium falciparum isolates from the Thai-Burmese border..... 3. Seroprevalence and risk factors of hepatitis B virus infection among health care workers at the Institute of Neurology..... Clinical efficacy of chloroquine versus artemether-lumefantrine for Plasmodium vivax treatment in Thailand..... 4. Predisposing factors for nevirapine toxicity among AIDS patients with low baseline CD4 count..... 5. Potential association of dengue hemorrhagic fever incidence and remote senses land surface temperature, Thailand, 6. 1998..... 7. Increased risk of preterm birth among non- smoking, non-alcohol drinking women with maternal periodontitis..... Importance of collection tube during clinical studies of oseltamivir 8. Efficacy of atovaquone-proguanil for treatment of acute multidrug-resistant Plasmodium falciparum malaria in Thailand 9. 10. Independent evolution of pyrimethamine resistance in *Plasmodium falciparum* isolates in Melanesia..... 11. Acquired haemophilia A in early pregnancy associated with *Plasmodium vivax* malaria and hyperthyroidism..... Pharmacokinetic study of artemether-lumefantrine given once daily for the treatment of uncomplicated multidrug-resistant 12.

 falciparum malaria
 144

 13. In vitro antimalarial activity of azithromycin, artesunate, and quinine in combination and correlation with clinical outcome
 85

 14. How much fat is necessary to optimize lumefantrine oral bioavailability?
 145

 15. Intrahost selection of Plasmodium falciparum pfmdr1 alleles after antimalarial treatment on the northwestern border of
 145

- Thailand
 145

 16. Beyond deworming: the promotion of school-health-based interventions by Japan
 105
- 17. Sexual behaviors of alcohol drinkers and non-drinkers among adolescents and young adults in Nha Trang, Vietnam 146

Department of Tropical Nutrition and Food Science

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The Department of Tropical Nutrition and Food Science was established in 1966, to teach and train postgraduate students, to conduct scientific research, and to provide laboratory services for nutritional disorders. The Department comprises 9 academic staff, 6 consultants, and 2 support staff. The staff teach postgraduate students in the international courses leading to the postgraduate Diploma in Tropical Medicine and Hygiene, Master of Science in Tropical Medicine and Doctor of Philosophy in Tropical Medicine, as well as short training courses, e.g., Food Safety and Food Control, Methods in Nutritional Assessment and Research, etc. They also conduct research in the nutritional sciences. In addition, they are responsible for technical services and the distribution of nutrition information to the community.

Education

The major activities of the Department of Tropical Nutrition and Food Science are teaching and research support for Master of Science and Doctor of Philosophy in Tropical Medicine candidates in the major field of Biochemical Nutrition.

Training

A 4-day workshop on "Methods in nutritional assessment and research" was organized by the Department on 17-20 and 24-27 April 2007, with 84 participants. The objective of the course was to provide training to lecturers, researchers, and nutritionists in nutrition research methodologies, such as anthropometric and bio-chemical assessment, including computer programs for dietary assessment.

Academic activities

Department staff and a volunteer student (Helia Borhani) performed research work on "Health Behaviors and Nutritional status of Tsunami Victims" in PhangNga Province, 4-13 November 2007.

Prof. Florian J. Schweigert, from the Department of Physiology and Pathophysiology, Institute of Nutritional Science, University of Potsdam, Germany, visited the Department in November 2007 and gave a special lecture at the Joint International Tropical Medicine Meeting on the topic "Nutritional proteomics: methods and concepts for research in clinical science".

Miss Andrea Hanze, a German student, conducted a seminar entitled "The importance of the retinol-binding protein 4 (RBP4) in diabetes and the assessment of kidney function" for Departmental staff and students in November 2007.

Research

The research activities of the Department focus on nutritional problems in Thailand in relation to health, especially dislipidemia, coronary heart disease, obesity, and cancer. A health and nutrition survey of Tsunami victims in Southern Thailand was being performed in 2007. Nutrigenomics and molecular carcinogenesis of several cancers in Thai patients, such as breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancer, head and neck cancer, including hepatocellular carcinoma (liver cancer), were investigated. In 2007, studies included:

- Food behavior and micronutrient status in hill-tribe children and women.
- The impact of oxidative stress and antioxidants in to and biochemical parameters to health.
- The association of gene polymorphisms with overweight/ obese Thai.
- Induction of apoptosis and DNA damage by some natural products.
- Determination of novel DNA alterations in several human cancers.
- Protein and gene polymorphism determination in post menopausal of osteoporosis.

International Linkages

The Department of Tropical Nutrition and Food Science, represented by Assoc. Prof. Rungsunn Tungtrongchitr collaborated with 2 external academic professors, Prof. Florian J. Schweigert and Assoc. Prof. Gary Sweeney, Department of Biology, York University, Toronto, Canada, for training in research for Ph.D. students under the Royal Golden Jubilee Ph.D. program.



The Department conducted research entitled "Health behaviors and nutritional status of Tsunami victims" in Phang-Nga Province, funded by the University of Brescia, Italy. Assist. Prof. Karunee Kwanbunjan was the principal investigator, and cooperated with Prof. Michael Krawinkel, Justus Liebig University, Germany on student internships. Dr. Dumrongkiet Arthan collaborated with Prof. Atsuo Kimura of the Faculty of Agriculture, Division of Applied Science, Hokkaido University, Sapporo, Japan, as a postdoctoral fellowship.

- Faculty of Medicine, Khon Kaen University
- Faculty of Medicine, Siriraj Hospital, Mahidol University
- Faculty of Science and Institute of Nutrition, Mahidol University
- Faculty of Medicine and Faculty of Allied Health Sciences, Chulalongkorn University
- Chulabhorn Research Institute
- Provincial sections of the Ministry of Public Health, Phang-Nga Province, etc.

Services

The Department provided laboratory services for the enzymatic determination of Vitamin B1, B2, B6 in red blood cells.

	st of publications	Pag
1.	Novel DNA amplification on chromosomes 6q23-24 and 4p15.2 in breast cancer identified by arbitrarily primed polymerase	
	chain reaction	146
2.	Cytotoxicity, apoptosis and DNA damage induced by Alpinia galangal rhizome extract	147
3.	R219K polymorphism of ATP binding cassette transporter A1 related with low HDL in overweight/ obese Thai male	147
4.	Comparative study of LDL-cholesterol levels in Thai patients by the direct method and using the Friedewald formula	148
5.	Effects of tobacco smoking on Alpha-2-macroglobulin and some biochemical parameters in Thai male	148
6.	Total and high molecular weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic	
	syndrome and liver injury in Thai subjects	149
7.	Prognostic value of DNA alterations on chromosome 17p13.2 for intrahepatic cholangiocarcinoma	149
8.	Partial purification and characterization of DNA polymerase from β -like enzyme from <i>Plasmodium falciparum</i>	131
9.	Genetic instability in cervical cancer detected by arbitrarily primed polymerase chain reaction	150
10.	Novel hMSH2, hMSH6 and hMLH1 gene mutations and microsatellite instability in sporadic colorectal cancer	150
11.	Identification of genetic alterations in Thai breast cancer patients by arbitrarily primed polymerase chain reaction	151
Tex	tbook	
	• Tungtrongchitr R. (editor). Obesity: Biochemical and Nutritional Transition. Bangkok: Chalerndeemunkong Publishing;	
	2007. (Thai version).	
Li	st of presentations	
1.	Partial purification and characterization of DNA polymerase from <i>Plasmodium falciparum</i> and its role on base excision	

	repair	151
2.	Fully automated HPLC assay for total homocysteine and other aminothiols: as a measure of plasma redox status in Thai	
	healthy subjects	152

Local cooperationNational Cancer Institute

• Phramongkutklao Medical College
Department of Tropical Pathology

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Assoc. Prof. Emsri Pongponratn Head E-mail: tmepp@mahidol.ac.th

The Department of Tropical Pathology was originally established as a Pathology Unit under the Department of Tropical Medicine and Hospital for Tropical Diseases, in 1968. During that time Dr. Mario Riganti acted as Head of the Unit. In 1989, it became the Department of Tropical Pathology, and Assoc. Prof. Riganti was the first Head of Department, from 1989-1994. The Department currently undertakes research, education and training, and provides medico-scientific services, in the field of tropical pathology.

The Department is composed of 3 units: the Histopathology Unit, the Electron Microscopy Unit, and the Tissue Culture and Immunocytochemistry Unit.



Education

The Department taught in the Diploma in Tropical Medicine and Hygiene (DTM&H), and the Master of Science and Doctor of Philosophy in Tropical Medicine programs. In 2007, 11 students were working on PhD projects focusing on the pathogenesis and pathology of tropical diseases. Training courses in tropical pathology, electron microscopy and histopathology were provided upon request. **Research**

Research projects focused on histopathology, immunohistochemistry and ultrastructural studies of tropical diseases, especially malaria and other parasitic and infectious diseases.

In country research collaborations

- Faculty of Science, Mahidol University
- Faculty of Veterinary Science, Mahidol University
- Institute of Molecular Biology and Genetics, Mahidol University
- Faculty of Medicine, Siriraj Hospital, Mahidol University
- Faculty of Allied Health Science, Thammasat University, Rangsit Center
- · Faculty of Dentistry, Chulalongkorn University
- Faculty of Dentistry, Srinakarinviroj University
- National Cancer Institute
- Department of Parasitology, Faculty of Medicine, Changmai University
- Department of Pathology, Faculty of Medicine, Khon Kaen University
- Rajavithi Hospital

International linkages

- Division of Electron Microscopy, Department of Cellular Pathology, the John Radcliff Hospital, Oxford University, United Kingdom
- Liverpool School of Tropical Medicine, United Kingdom
- Department of Immunology, The Wenner Gren Institute, Stockholm University, Sweden
- Department of Pathology, Faculty of Medicine, University of Sydney, Australia





Visiting professors, Prof. David Ferguson and Dr. Gareth Turner from Oxford University, UK were appointed official counselors to the Faculty of Tropical Medicine, Mahidol University.

Research Activities

- A murine model of cerebral malaria, with particular emphasis on histopathology, immunohistochemistry and electron microscopy
- Pathology, pathogenesis of severe malaria in human organs: ultrastructural studies and cytokine involvement
- Vascular model for studying pathogenesis of atherosclerosis
- Ig class switching induced by Plasmodium falciparum
- Ig class switching induced by Gnathostoma spinigerum
- Ultrastructural study of malarial parasites and the infected red blood cells after drugs treatment
- Cytokine expression in HIV-positive and AIDS patients
- Proteome of cancerous squamous cells in oral cavity and salivary gland tumor

Books

- · Atlas of Tropical Histopathology
- Manual of Specimen Preparation for Electron Microscopy
 Study
- Special Stain Techniques for Diagnosis

Services

Pathological diagnostic services, including autopsy, were provided for patients at the Hospital for Tropical Diseases and other hospitals. Special specimen services were also provided, i.e. permanent slide for microscopic anatomy and pathology study; electron microscopy service



Awards

- Best Oral Presentation 2007; Mr. Kraisorn Sappayatosok; presented by the Microscopy Society of Thailand, February 2007
- Best Student Presentation Award; Mr. Kraisorn Sappayatosok at the Joint International Tropical Medicine Meeting, November 2007

Centre of Excellence

Reference Centre for Tropical Pathology



List of publications Page A quantitative ultrastructural study of renal pathology in fatal *P falciparum* malaria......malaria.... 152 1. Immunohistochemical study of acute and chronic toxoplasmosis in experimentally infected mice 131 2. High level of soluble expression in Escherichia coli and characterization in the cloned Bacillus thuringiensis Crv4Ba domain 3. 153 III fragment 4. Binding characteristics to mosquito-larval midgut proteins of the cloned domain II-III fragment from the Bacillus thuringiensis Cry 4Ba toxin 153 114 Ompl 1 DNA vaccine cross-protects against heterologous Leptospira spp. challenge 5. List of presentations 1. Expression of iNOS, VEGF, COX-2 in oral squamous cell carcinoma 154 Expression of iNOS, VEGF, COX-2, angiogenesis and their clinico-pathological correlation in oral and para-oral squamous 2. cell carcinoma..... 154 Activation of transcription factor-nuclear factor Kappa B(NF-kB) in endothelial cells upon malaria parasite 3. 155 cytoadherence.....

Department of Tropical Pediatrics

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Assoc. Prof. Krisana Pengsaa Head E-mail: tmkps@mahidol.ac.th

The Department of Tropical Pediatrics has undergone considerable development in its research, teaching, and services during the past 10 years. Collaboration with other research groups, both nationally and internationally, has been a key feature of the Department's activities. Existing malaria and intestinal parasites research areas of expertise have been consolidated by new funding, while new areas of research in vaccine trials, and operational research on diarrheal diseases, have opened up with support from the Faculty and external funding. Staff from the Department actively contribute to the regular academic programs of the Faculty, as well as its short training courses. Since 1994, the Department has been responsible for the Master of Clinical Tropical Medicine (Tropical Pediatrics) program, currently named the Master of Clinical Tropical Pediatrics. The services provided by staff from the Department include 1) patient care at both inpatient and outpatient units of the Hospital for Tropical Diseases, and 2) provision of current knowledge and research information on tropical pediatrics to international visitors and local health personnel in Thailand.

Education

In 2007, the Department taught in the Master of Clinical Tropical Pediatrics and graduate Diploma in Tropical Medicine & Hygiene programs. One student in the Master of Clinical Tropical Pediatrics program completed a thematic paper in tropical pediatrics entitled "Predictive model for diagnosis of neonatal sepsis".

For training, the International Training Course on Common Problems in Tropical Pediatrics: a Practical Approach, was conducted September 17-21, with 31 participants.

Research

The main research activities of the Department were related to dengue infection and dengue and rabies vaccines:

- Epidemiological study of dengue infection in children in Ratchaburi Province
- FavirabTM post-prescription event monitoring
- Safety and immunogenicity of tetravalent dengue vaccine formulations in healthy Thai children: Amendment 3: Evaluation of antibody persistence over an additional follow-up period of 5 years
- Evaluation of long-term immunity against rabies in children vaccinated with different pre-exposure regimens of PCEC (Rabipur,) and immunity after two IM injections of PCEC (Rabipur,) post-exposure in children previously vaccinated with PCEC pre-exposure regimens (149P6 extension)
- Comparison of immunogenicity and adverse reactions after immunization with Japanese encephalitis vaccine

produced by BIKEN and the Government Pharmaceutical Organization (GPO) in healthy Thai children (JE0150)

• Dengue antibody levels and incidence of dengue infection in pre-school-aged children

Cooperation

The Department cooperates with the Center for Vaccine Development, Mahidol University, and the Armed Forces Institute of Medical Sciences for dengue diagnosis, and Sanofi Pasteur for dengue-vaccine research. It also receives support for epidemiological studies of dengue infection from the Pediatric Dengue Vaccine Initiative (PDVI). In 2007, the Department, coordinating with the Tropmed Dengue Diagnosis Center (TDC), was developing a comprehensive laboratory for dengue diagnosis.



List of publications	Page
1. Chronic diarrhea and abnormal serum immunoglobulin levels: a case report	155
2. Congenital malaria in Thailand	156
3. Characterization of atypical lymphocytes and immunophenotypes of lymphocytes in patients with dengue virus infection	117
4. Comparative study of the effectiveness and pharmacokinetics of two rectal artesunate/oral mefloquine combination regimens for the	
treatment of uncomplicated childhood falciparum malaria	156
Textbook	
Textbook of Tropical Pediatrics: common diseases. Editors: Pengsaa K, Sirivichayakul C, Chanthavanich P. (In Thai)	
List of presentations	
1. Symptomatic dengue-infection in Thai children in Ratchaburi cohort, Thailand	156

Department of Tropical Radioisotopes

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Assist.Prof. Channarong Sanghirun Head E-mail: headtmrd@mahidol.ac.th

The Department of Tropical Radioisotopes was established in 1963, three years after the foundation of the Faculty of Tropical Medicine. Its three core functions are research, education, and services related to the use of radioisotopes in tropical diseases.

The main routine work of the Department of Tropical Radioisotopes was assessment of serum vitamin B12, serum folate, and red-blood-cell folate; the folate measurement technique was the most reliable in the region, particularly Southeast Asia. The main research work of the Department was gamma-ray, x-ray, and ultraviolet irradiation to study oxidative stress on macrophage cell lines. Research work on vitamin B12 and folate in Thai foods was almost completed in 2007.

Research

Current research

- Distribution of C¹⁴-labelled arteether after intramuscular injection in mouse kidney and liver
- Studies on radiation effect on mouse macrophage cell line (RAW 264.7) and radio protective effect by various Thai medicinal plants
- Studies on toxicity of heme and oxidative stress after exposure of antimalarial drugs on mouse macrophage cell line (RAW 264.7)
- Determination of vitamin B_{12} and folic acid in Thai foods

Collaborative research

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- Studies on lethal dose LD_{50} of 4 Thai medicinal herbs in mice
- Supplementary hemoglobin in iron-deficiency anemia Wistar rats after feeding of freeze-dried Thai freshwater-crocodile blood (*Crocodylus siamensis*)
- Studies on correlation of serum vitamin B₁₂, serum folic acid, red blood cell folate, plasma homocysteine, micronucleus frequency in human lymphocytes, DNA methylation, and nutritional status in aging and young-adult Thais
- Increased hemoglobin in iron-deficient Wistar rats after eating frozen and thawed crocodile blood
- Effect of oral oxymetholone on lean body mass and insulin resistance in end-stage renal disease patients undergoing maintenance hemodialysis



Services

The Department has two main services (1) measurement of folic acid, and (2) measurement of vitamin B_{12} . Annual service usage, 1 January-31 December 2007, is shown below.

	Vitamin B ₁₂	Serum folate and red-cell folate
	number	number
Adult females	55	396
Adult males	31	329
Children	-	4
Total	86	729



Mahidol-Oxford Tropical Medicine Research Unit (MORU)

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Prof. Nicholas J. White Chairman, SEA E-mail: nickw@tropmedres.ac



Prof. Nicholas Day Director E-mail: nickd@tropmedres.ac

Current Research Activities

The Wellcome Trust-Mahidol University-Oxford Tropical Medicine Research Programme (the 'Thailand Unit') began in 1979 as a research collaboration between the Faculty of Tropical Medicine, Mahidol University and the University of Oxford. The main administrative office and laboratories are embedded within the Faculty, though most clinical studies and much laboratory work takes place in 'up-country' study sites. MORU's main research interests are the epidemiology, diagnosis, pathophysiology and treatment of malaria, scrub typhus, melioidosis, leptospirosis and other tropical infections that impose a substantial disease burden on rural populations throughout this populous region. In addition to SMRU in Mae Sod and the Laos Project, the Unit has study sites in Ubon Ratchatani (melioidosis and cryptococcal meningitis), Udon Thani (scrub typhus and leptospirosis), Mae Sod Hospital (malaria and microbiological support for the Shoklo Malaria Research Unit (SMRU; website: http://www.shoklo-unit.com/), Chittagong Medical College in Bangladesh (severe malaria), and Rourkela in India (severe malaria). The Unit also supervises severe-malaria clinical-trial sites in Burma.

MORU; the "Thailand Unit" - What happens where?





L	ist of publications
1.	Accuracy of Burkholderia pseudomallei identification using the API 20NE systems and a latex aggulutination test
2.	Prevalence of hepatitis E virus antibodies in pigs: implications for human infections in village-based subsistence pig farming
	in the Lao PDR
3.	Prospective study to determine accuracy of rapid serological assays for diagnosis for acute dengue virus infection in Laos
4.	Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: a lack of consensus leads to a lot of confusion
5.	Serolodical and blood culture investigations of Nepalese fever patients
5.	Evaluation of the Panbio dengue virus NS1 antigen detection ang IgM antibody ELISAs for the diagnosis of acute dengue infections in Laos
7.	Accuracy of enzyme-linked immunosorbent assay using crude and purified antigens for the serodiagnosis of melioidosis
3.	Biological relevance of colony morphology and phenotypic switching by Burkholderia pseudomallei
9.	Prospective clinical evaluation of the accuracy of 16S rRNA real-time PCR assay for the diagnosis of melioidosis
10.	The management of patients with severe malaria
11.	The treatment of severe malaria
12.	Levamisole inhibits sequestration of infected red blood cells in patients with falciparum malaria
13.	Contrasting genetic structure in <i>Plasmodium vivax</i> populations from Asia and South America
14.	Relapses of <i>Plasmodium vivax</i> infection usually result from activation of heterologous hypnozoites
15.	Variable presentation of neurological melioidosis in Northeast Thailand
16.	Simultaneous infection with more than one strain of <i>Burkholderia pseudomallei</i> is uncommon in human melioidosis
17.	Validation and application of a liquid chromatographic-mass spectrometric method for determination of artesunate in pharmaceutical samples
18.	Importance of collection tube during clinical studies of oseltamivir
19.	Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of oseltamivir
	and its metabolite oseltamivir carboxylate in plasma, saliva and urine
20.	Effects of malaria heme products on red blood cell deformability
21.	Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum Malaria
22.	Overestimating resistance in field testing of malaria parasites: simple methods for estimating high EC50 values using a
	Bayesian approach
23.	Cardiotoxicity of antimalarial drugs
24.	Clinical diagnosis and geographic distribution of leptospirosis in Thailand
25.	Optimization of <i>Leptospira</i> culture from humans with leptospirosis
26.	Short Report: quantitation of B. pseudomallei in clinical samples

Vaccine Trial Centre (VTC)

Telephone: 66 (0) 2354 9100-19 ext 1501-4 Website: http://www.tm.mahidol.ac.th



Assoc. Prof. Pratap Singhasivanon Director E-mail: tmpsh@mahidol.ac.th

The VTC was established in 1984 and became a fully functioning centre in 1986. The VTC is a joint responsibility of Mahidol University and the Ministry of Public Health, Thailand, and is operated by the Faculty of Tropical Medicine on their behalf. The major purpose of the Centre is to test newly developed vaccines that reach the clinical-trial stage; however, the centre is currently expanding its services into pharmaceutical development, where evaluations with human participants are required. The VTC has extensive experience conducting several Phase I/II and large Phase III trials for various infectious diseases, e.g., diarrheal diseases, malaria, HIV, and other viral infections. Currently, the VTC is involved in the world's largest HIV vaccine community trial, with 16,000 healthy consenting volunteers.

The VTC provides services in the clinical and data management of clinical trials. It is the first and the only facility of its kind in Thailand, in the region, and perhaps also in the developing countries. Since its establishment, the VTC has aimed to ensure that all trials under its responsibility are conducted and documented in accordance with international standards and guidelines, including the International Conference on Harmonisation (ICH) / WHO Good Clinical Practice standards. Each function has pre-established, systematic and written procedures for the organization, conduct, data collection, data management, documentation and verification of the trial. The clinical trials carried out under the management of the VTC are assured of data validity, and the ethical, scientific and technological quality.

The VTC's mission is to collaborate with scientists in national or international institutes, as well as vaccine developers, pharmaceutical companies, and donor agencies. The Centre provides the necessary infrastructure to enable multi-center clinical trials and epidemiological studies meeting international standards for design, conduct, and reporting. The advantage of conducting the trials at VTC is that the Centre is equipped with a validated system, technology and procedures, the professional staff are well-trained, and the Centre has extensive experience in conducting trials. The Centre realizes its mission by planning, implementing, and evaluating the study protocols or concepts through mutual cooperative agreements and contracts.

1. Current projects

 Phase III Trial of Aventis Pasteur Live Recombinant ALVAC-HIV (vCP1521) Priming with VaxGen gp120 B/E (AIDSVAX[™] B/E) Boosting in HIV-uninfected Thai Adults. Total enrollments numbered 16,402; 13,975



Prof. Dr. Punnee Pitisutthithum Principal Investigator E-mail: tmppt@mahidol.ac.th

participants completed vaccination. Serious Adverse Events (SAE) numbered 2,502, deaths 139. 1,758 participants fell pregnant during the vaccination phase and 1,291 participants fell pregnant during follow-up. 504 participants withdrew. The retention rate is 81.31% at month 42 (week 182).

- Immunogenicity and Safety of Quadrivalent HPV (Type 6, 11, 16, 18) L1 Virus Like Particle (VLP) Vaccine in 16 to 23-Year-Old Women With an Immunogenicity Bridge Between the HPV16 component of the Quadrivalent Vaccine and the Monovalent HPV 16 Vaccine Pilot Manufacturing Material—The F.U.T.U.R.E. I Study (Females United to Unilaterally Reduce Endo/ Ectocervical Disease). 134 Thai females aged 16-23 years were enrolled in the HPV vaccine trial. 123 subjects completed the follow-up visit. After the 4-year follow-up visit, 69 placebo subjects were referred to the extension protocol to receive GARDASIL[™] vaccine. The New England Journal of Medicine has reported that GARDASIL[™] vaccine is highly efficacious in preventing certain pre-cancerous conditions.
- Safety, Immunogenicity, and Efficacy of GARDASIL[™] (Human Papillomavirus [Types 6, 11. 16, 18] Recombinant Vaccine) in mid-Adult Women-The FUTURE III (Females United To Unilaterally Reduce Endo-Ecto Cervical Cancer) Study. From November 2004-March 2005, 246 healthy women meeting the eligibility criteria were enrolled into the study. Subjects were randomized in a 1:1 ratio to received either GARDASIL[™] or placebo. The subjects' ages ranged between 24-45 years at first vaccination. Subjects received vaccine or placebo at Day 1, Month 2, and Month 6. For each subject enrolled, the duration of the study is 48 months. Three participants discontinued prior to completion of the protocol; a total of 243 subjects remain in the study. The current visit is month 36.
- Establishment of a *Shigella sonnei* challenge model for evaluation of future vaccine candidates. A total of 48 Thai adults (36 + 12 alternatives) between the ages of 20-40 years will be recruited and separated into 3 groups, each group containing 12 volunteers, to establish a safe and reliable human challenge model for shigellosis. After ingesting sodium bicarbonate buffer, each group of volunteers will be challenged with 100, 400, or 1,600 cfu of *S. sonnei* 53G, respectively. Volunteers will be followed closely to evaluate clinical

symptoms and fecal shedding. On Day 5 postchallenge, unless specific early antibiotic treatment occurs beforehand, the subjects will begin treatment with Ciprofloxacin, Bactrim or Amoxicillin to stop fecal excretion of S. sonnei. They will be released from the ward on Days 8-11. All volunteers will return on Day 14±2 and Day 28±2 for stool and blood specimen collection. All volunteers will be contacted on Day 42+2 by telephone to investigate any complications.

Current Research

- Screening and evaluation of potential volunteers for a Trial in Thailand of a candidate preventive HIV vaccine
- A phase III trial of Aventis Pasteur live recombinant ALVAC-HIV (vCP1521) priming with VaxGen gp120 B/E (AIDSVAX[®]B/E) boosting in HIV-uninfected Thai adults
- Extended evaluation of the virologic, immunologic, and clinical course of volunteers who become HIV-1 infected during participation in a phase III vaccine trial of ALVAC-HIV and AIDSVAX[®] B/E.

L	ist of publications	Page
1. 2. 3.	Outcomes in HIV-infected patients on antiretroviral therapy with tuberculosis Social harms in injecting drug users participating in the first phase III HIV vaccine trial in Thailand Predisposing factors for nevirapine toxicity among AIDS patients with low baseline CD4 count	91 88 91
L	ist of presentations	
1. 2.	Integration of community engagement activities support the prime-boost phase III HIV vaccine trial in Thailand Women's participation in the phase III efficacy trial of ALVAC HIV-1 vaccine priming, AIDSVAX vaccine boosting in Thailand	96 96
3.	Thai prime-boost HIV vaccine phase III trial: update 2007	97
4. 5.	Social impact of participation in a phase III HIV vaccine trial in Thailand Safety and immunogenicity of the MRKAd5 gag HIV-1 vaccine in a worldwide phase I study of healthy adults	98
6.	(Merck V520-018/HVTN 050) HLA class II alleles and neutralizing antibody responses to HIV-1 protein subunit boosting in a Thai population	98 99
7.	Outcome of tuberculosis in advanced HIV-infected patients on antiretroviral therapy	100
8.	Thailand's experiences in conducting HPV vaccine trials and HPV vaccine update	100

Data Management Unit

Telephone: 66 (0) 2354 9100-19 ext 1890 Fax: 66 (0) 2354 9187



Assist. Prof. Jaranit Kaewkungwal Chief E-mail: tmjkk@mahidol.ac.th

The Data Management Unit (DMU) was established in1999 to fulfill the Thailand National AIDS committee requirements for conducting the first large scale HIV vaccine trial in Thailand. Its establishment and early operations were sponsored by VaxGen Inc., Brisbane, CA, U.S.A. Currently, the DMU receives funding and personnel support from the Faculty of Tropical Medicine, as well as the national and international pharmaceutical development industry.

The DMU is structured within the VTC. The major objectives of the DMU include: (1) to establish a datamanagement Centre of Excellence in Thailand and serve as a reference centre for data management for vaccine and clinical trials in the region, (2) to serve as a collaborating centre for high-quality data management for vaccine and clinical trials within the Faculty and for other institutions, including international collaborators, (3) to provide training for Faculty staff and other institutions in clinical data management that complies with international standards and guidelines, and (4) to advise and/or develop data management and analysis for biomedical research and services. The goal of the DMU is to provide data quality for clinical trials or an epidemiological studies, and data management in a regulatory-compliance environment. The DMU mission is realized through collaborative academic and financial agreements among all parties involved. The Faculty renders the necessary administrative and logistical support to enable high-quality performance. The collaborator(s) enjoy the benefits of high-standard data-management practices.

Currently, the DMU plays major roles in data management of the world's largest HIV vaccine trial, being conducted in Thailand. In this trial, all data management processing for over 25,000 volunteers during the 5-year study are being performed by the DMU; this is the first trial in Thailand to maintain a primary database subject to audit by the U.S. FDA, so the DMU has utilized the highest-quality data-management technology ever used in the Region. The DMU is collaborating with other institutions conducting research studies, and provides consultation services and database development, e.g. database development for the quality-control and -assurance processes of the Department of Medical Sciences, Ministry of Public Health, Thailand.

Rajanagarindra Tropical Disease International Centre (RTIC)



The Rajanagarindra Tropical Disease International Centre (RTIC) is located in Huai Muang Subdistrict, Suan Pheung District, Ratchaburi Province, Thailand. Its activities include research on the epidemiology of malaria and various parasitic diseases; field training for students/trainees; provision of health education and free healthcare services, especially for malaria, for the local people. These activities are supported financially by the Tropical Disease Trust Fund, which was initiated on 25 May 1998 under the Honorary Chairmanship of Her Royal Highness Princess Galayani Vadhana Krom Luang Naradhiwas Rajanagarindra. The activities of RTIC are conducted mainly by staff of the Field Research Unit, Department of Tropical Hygiene, Faculty of Tropical Medicine.

Kanchanaburi Tropical Disease Research Centre

Kanchanaburi Tropical Disease Research Centre, at Kanchanaburi Campus, Sai Yok District, Kanchanaburi Province, Thailand, is a research station and field training center for faculty staff, graduate students, and medical students from the Collaborative Project to Increase Production of Rural Doctors, Mahidol University.

Education and Training

Bangkok School of Tropical Medicine

Telephone: 66 (0) 2354 9149 or 66 (0) 2354 9100-19 ext. 1533 or 1532 Fax: 66 (0) 2354 9150 or 66 (0) 2643 5579 E-mail: tmedu@diamond.mahidol.ac.th



Assist. Prof. Usanee Suthisarnsunthorn Deputy Dean for Educational Affairs E-mail: tmuss@mahidol.ac.th 1 Jan 2007-30 Sept 2007



Assist. Prof. Chotechuang Panasoponkul Deputy Dean for Student Affairs and Special Activities E-mail: tmcpn@mahidol.ac.th Acting Deputy Dean for Educational Affairs 1 Oct 2007-31 Dec 2007

The Bangkok School of Tropical Medicine, Mahidol University, offers world-class programs that equip doctors, research workers, medical personnel and professionals concerned with tropical medicine and public health with the skills they need to further their careers in tropical medicine, and the knowledge needed to make a substantial positive difference to healthcare problems in the tropics and elsewhere. Graduates pursue successful careers in public- and private-sector healthcare services; in consulting; in national, regional and international agencies; and in research institutions.

Postgraduate Programs

The Faculty, through the School, offers 6 regular international postgraduate programs, from graduate diploma to doctoral levels, which have attracted participants from over 46 different countries. All programs are taught in the English language. Participants in the Faculty's programs gain practical, hands-on training in a tropical country, and experience the actual patients, diseases, health services, social contexts and environments of a tropical country, along with learning the latest treatments for tropical diseases. Students also experience the cultures and traditions of Thailand, benefiting further from the cultural diversity of their fellow students.

Undergraduate Program

The Faculty also offers 1 undergraduate course, the Certificate program for Nurse Assistants, which is coordinated by the Hospital for Tropical Diseases. In 2007, there were 181 students in this program.

In summary, in 2007, the Faculty had a total of 380 students; among these, 239 were new enrollments, and the majority fee-paying.





New student enrolments 2007

Courses	No. of Students	Nationalities	
Postgraduate			
DTM&H	23	Austria, Germany, Japan, Kenya , Korea, Indonesia,	
6-month course		Myanmar, Pakistan, Sudan, Thailand, Vietnam	
MCTM	12	Austria Japan, Germany, Myanmar, Sudan, Vietnam	
1-year course			
MSc (TM)	10	Lao PDR, Sri Lanka, Thailand, Vietnam, Afghanistan,	
		Cambodia, Indonesia, Philippines	
PhD (TM)	13	Japan, Thailand, Afghanistan, Bangladesh, Indonesia,	
		Thailand, Malaysia	
Subtotal	58		
Undergraduate			
Certificate for Nurse	181	Thailand	
Assistants			
Subtotal	181		
Total	239		

Master of Clinical Tropical Medicine Theses 2007

Department	Name	Title of Thesis
Clinical Tropical Medicine	Dr. Tibor Pfleger	Efficacy of alternative therapies after non-nucleoside reverse transcriptase inhibitor-based (NNRTI-based) human immunodeficiency virus (HIV) 1 treatment failure
Clinical Tropical Medicine	Dr. Sakhr Badawi Omar Elshiekh	Prognostic factors of malaria acute renal failure at the Bangkok Hospital for Tropical Diseases
Clinical Tropical Medicine	Dr. Elsideg Ahmed Mohammed Abdelrhaman	Emergence and clearance of gametocytes in complicated <i>Plasmodium falciparum</i> malaria
Clinical Tropical Medicine	Dr. Andreas Neumayr	Malarial acute renal failure at Mae Sot General Hospital, Thailand: outcome and associated risk factors for death and dialysis
Clinical Tropical Medicine	Dr. Yin Nwe Tun	Prevalence, clinical manifestations and outcomes of opportunistic infections before and after introduction of anti retroviral therapy in HIV patients at Maharat Nakhon Ratchasima Hospital
Clinical Tropical Medicine	Dr. Shinsuke Miyano	Factors related to multidrug-resistant tuberculosis at Bamrasnaradura Hospital
Clinical Tropical Medicine	Dr. Daw Aye Thida	Outcome of cryptococcal meningitis in HIV-infected patients on antiretroviral. therapy.
Clinical Tropical Medicine	Dr. Ho Thi Xuan Ha	The association between helminth infections and severity of <i>Plasmodium falciparum</i> malaria

Master of Clinical Tropical Pediatrics Thesis 2007

Department	Candidate Name	Thesis Title
Tropical Pediatrics	Dr. Dominicus Husada	Predictive model for diagnosis of neonatal sepsis

Master of Science in Tropical Medicine Theses 2007

Department	Candidate Name	Thesis Title
Microbiology & Immunology	Piyanart Chalayon	Expression of leptospiral recombinant protein for serodiagnosis
Clinical Tropical Medicine	Premjit Amornchai	Comparison of latex agglutination test, API Systems and Vitek 2 Compact for the identification of <i>Burkholderia</i> <i>pseudomallei</i>
Protozoology	Sirilak Sukprasert	Detection of <i>Entamoeba dispar</i> and <i>Entamoeba coli</i> in surface and waste water samples by PCR real-time PCR
Microbiology & Immunology	Toungporn Boonchawalit	Expression of leptospiral liproprotein for serodiagnosis
Tropical Hygiene	Wittaya Prachachalerm	Evaluation on reporting serious adverse drug reactions in Thai spontaneous reporting system; a case study of Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN)
Social & Environmental	Jaturong Wonsanit	Risk assessment of nitrate contamination in groundwater using geoinformatics
Clinical Tropical Medicine	Supparat Wannasilp	Study of solubility and efficacy of antimalarial drugs on <i>Plasmodium falciparum</i>
Clinical Tropical Medicine	Juntima Sritabal	Effects of primaquine and its metabolites on the infectivity of <i>P. falciparum</i> gametocyte
Microbiology & Immunology	Sunisa Malijunboa	Identification on genus-and /or species specific antigens of <i>Aeromonas</i> by Western blotting
Microbiology & Immunology	Nattavut Wongdeethai	Application of recombinant outer membrane protein in serodiagnosis of leptospirosis
Microbiology & Immunology	Aucha Sachair	The relationship between MMP and virulence of dengue virus type2 infected cell
Microbiology & Immunology	Ampai Tanganuchitcharnchai	Production of monoclonal antibody to Orientia tsutsugamushi
Microbiology & Immunology	Pornapat Surasombatpattana	Using recombinant NS1 dengue viral protein-based indirect enzyme-linked immunosorbent assay for serodiagnosis of dengue virus infection
Tropical Hygiene Dilok Tongsukh		TB infection and treatment among refugees in Thamhin Camp, Ratchaburi Province
Microbiology & Immunology	Khwananong Youngpakook	Binding characterizations of Southeast Asian dengue 2 virus and American dengue 2 viruses with single E gene amino acid substitutions





Doctor of Philosophy in Tropical Medicine Theses 2007

Department	Candidate Name	Thesis Title
Helminthology	Megumi Ishida	Application of the ribosomal DNA-based PCR to diagnose
т · 1 ц ·		Opisthorchis viverrini infection in human tecal sample
Tropical Hygiene	Archin Songthap	Acceptability of HPV vaccine in adolescents, educators,
		educational tools
Tropical Hygiene	Putri C. Eyanoer	Spatial and temporal distribution of human avian influenza
1 ,0	,	infection in relation to surveillance implementation
		in Indonesia
Protozoology	Panadda Kraijojananan	Molecular and functional characterization of C-type lectin
Halminthalagu	Anishart Vitta	in the midguts of Anopheles dirus for malaria transmission
neminitiology	Apicitari vitta	cantonensis DNA library for immunodiagnosis of
		angiostrongyliasis
Social & Environmental	Nardlada Khantikul	Vivax malaria patients and their drug adherence in northern
		Thailand
Protozoology	Kamlang Chumpolbanchorn	Genetic characterization of Toxoplasma gondii isolates
		from free-range chickens in Thailand
Microbiology & Immunology	Nada Pitabut	Role of NK cells, NKT cells and T cell subpopulations
		through granule exocytosis in response to M. tuberculosis
Social & Environmental	Rungrawee Tipmontree	Health promotion program for malaria prevention:
	nungruvee ripilionalee	utilization of insecticide-treated nets in a multicultural
		community, Kanchanaburi Province
Protozoology	Megha Raj Banjara	Molecular epidemiology of drug-resistant Plasmodium
		falciparum malaria parasite in Nepal
Protozoology	Kritsana Janyapoon	Expression profiles of Plasmodium falciparum in response to
T . 117		pyronaridine by DNA microarray analysis
Tropical Nutrition &	Chutaporn Tongboonchu	Homocysteine and its polymorphism genes in relation to
Food Science		bone mineral density and vitamin status in post menopausal
Protozoology	Ruangrat Buddhirongawatr	Toxonlasma gondii genotyping in domestic and wild felids
1101020010gy	Ruungiut Dudunnongawan	in Thailand
Social & Environmental	Pannamthip Pitaksajjakul	Construction of phage antibody library specific to H5N1
		avian influenza virus
Medical Entomology	Suchada Sumroiphon	Anopheles sundaicus population dynamics, species
		complex and insecticide susceptibility in a coastal area of
Madical Entomology	Parichart Lancharoon	Rayong Province
Medical Entomology	ranchart Lapenaroen	Plasmodium in malaria vector Anonheles dirus
Tropical Pathology	Kasem Somthana	Immunoglobulin class switch DNA recombination in
1 07		human B cell induced by Gnathostoma spinigerum
Tropical Hygiene	Dr. Md. Mushifiqur	The effect of adherence on the efficacy of artemether-
	Rahman	lumefantrine (Coartem) in the treatment of uncomplicated
		Plasmodium falciparum malaria in Bangladesh:
		a randomized controlled trial
Social & Environmental	Patthanasak Khammarnaachan	Social impact assessment of central healthcare waste
Social & Environmental	Somsiri Decharat	Heavy metals exposure of nielloware workers: a health risk
Social & Environmental	Johnshi'i Deenarat	assessment in Muang District. Nakhon Si Thammarat
		Province
Clinical Tropical Medicine Tippawan Sungkapong		Identification of antigenic proteins on the surface of <i>P.vivax</i>
		infected erythrocytes
Tropical Hygiene	Rie Takeuchi	Factors related to reappearance of Plasmodium vivax
		malaria
Clinical Tropical Medicine	Sriwipa Chuangchaiya	Plasmodium vivax alters immunosuppression caused by
		ε ιαςπιθαιαπι jaiciparant

Department	Candidate Name	Thesis Title	
Microbiology & Immunology	Natharinee Horata	Polymorphism and function of PfEMP1 extracellular domains in Thai <i>Plasmodium falciparum</i> isolates causing severe and uncomplicated malaria	
Tropical Pathology Navakanit Sachanonta		The effects of antimalarial drugs induced changes on <i>Plasmodium falciparum</i> and Pe-infected red blood cells ultrastructure	
Medical Entomology	Ruxina Polseela	Population dynamics and bionomics of Phlebotomine sandflies in Sara Buri Province, Thailand	
Clinical Tropical Medicine	Piengchan Sonthayanon	Molecular diagnosis and multilocus sequence typing of scrub typhus	
Medical Entomology	Raweewan Srisawat	The effect of gene mutations to the voltage gated sodium channel in pyrethroid-resistant <i>Aedes aegypti</i>	
Protozoology	Aongart Mahittikorn	Molecular and immunohistochemistry studies on tachyzoite and bradyzoite stage conversion in toxoplasmosis	
Tropical Hygiene	Waranee Boonchuaylier	Detection of adverse drug reaction signal in the Thai FDA database: comparison between Reporting Odds Ratio and Bayesian Confidence Propagation Neuron Network method	
Tropical Hygiene	Pol. Capt. Jongkok Podang	Burden of head injury due to road traffic injury and cost- effectiveness analysis of intervention promoting helmet use in Thailand	
Tropical Hygiene	Tassanee Silawan	The spatio-temporal dynamics of dengue infection in northeastern Thailand	
Tropical Hygiene	Tawee Saiwichai	The effect of green tea extract to serum level of pro inflammatory cytokines in cigarette smoke inhalation rat model	
Tropical Nutrition & Food Science	Kittisak Thawnashom	Gene polymorphisms in homocysteine pathway in relation to homocysteine and vitamin status in overweight/obese Thais	
Microbiology & Immunology	Chutharut Ridruechai	Osteopontin expression and its role in tuberculosis	
Tropical Pathology	Jaiaue Wongtanachai	Effects of antimalarial drugs on <i>Plasmodium falciparum</i> : morphlogy, viability and multiplication potential	
Tropical Radioisotopes	Pachuen Potup	Immunoglobulin class switching induced by Plasmodium falciparum in vitro	
Microbiology & Immunology	Chintana Phawong	Chemokine gene polymorphisms and their susceptibility to tuberculosis	
Tropical Nutrition & Food Science	Naowarat Tanomsing	<i>Plasmodium malariae</i> and <i>Plasmodium ovale:</i> isolation and characterization of the Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) gene	
Protozoology	Jitbanjong Wiengcharoen	Molecular technique for detection of <i>T. gondii</i> and <i>N. caninum</i> in Thai dairy cattle	
Helminthology	Muncharee Tattiyapong	Serodiagnosis of swine trichinellosis using crude somatic, excretory-secretory, surface and purified antigens of <i>Trichinella spiral</i> is infective larvae by indirect ELISA and immunoblot"	
Protozoology	Saranya Sirbal	Molecular characterization of <i>Plasmodium falciparum</i> polynucleotide kinase	
Medical Entomology Jakkrawan Chompoosri		Transformation of <i>Aedes aegypti</i> , vector of dengue hemorrhagic fever, using serine-catalyzed integrases and tyrosine-catalyzed integrases	
Microbiology & Immunology Pornlada Nuchnoi		Molecular variation of TIM gene family in Thai malaria population: association with severe or cerebral malaria	
Medical Entomology Pruksa Nawtaisong		Analysis of ribozyme strategies for suppression of dengue virus in transgenic <i>Aedes albopictus</i>	
Helminthology	Doungrat Riyong	Purification of <i>Dirofilaria immitis</i> antigens for serodiagnosis of Bancroftian filarasis	
Microbiology & Immunology	Suwanna Chaorattanakawee	Malaria genetics: association with severe of cerebral malaria in Thailand	

Department	Candidate Name	Thesis Title	
Protozoology	Somphong Sithiprom	Purification and characterization of DNA topoisomerases from <i>Trichomonas vaginalis</i>	
Microbiology & Immunology	Rongdej Tungtrakanpoung	Mimotope identification from monoclonal antibodies specific to serovar of <i>Leptospira</i> , using phage-displayed random peptide library	
Helminthology	Doungrat Riyong	Paragonimus westermani and paragonimiasis in Tambon Tha Maprang Keang Khoi District, Saraburi Province, Thailand	
Tropical Pathology	Miss Thanida	Immunohistopathological studies of cytokine profiles in	
	Tangwanicharoen	liver tissue of AIDS and HIV infected patients:	
		a necropsy study	
Tropical Nutrition &	Miss Pensri Saelee	Detection of genetic alterations in hepatocellular	
Food Science		carcinomas using arbitrarily primed polymerase chain	
		reaction and gene cloning	
Clinical Tropical Medicine Miss Waraphon		Phenotype analyses of clinical malaria: a family-based	
	Phimpraphi	cohort study	

International Training Courses in Tropical Medicine

Course	No. Attending	Nationalities
Study Tour on Healthcare Financing	6	Vietnam
Epidemiology and Management of HIV/AIDS	9	Japan
Tropical Medicine	6	Lao PDR
Communication: Behavioral Impact	21	Bhutan, Cambodia, Indonesia, the Maldives,
		Sri Lanka, Thailand
Nuclear Medicine Imaging	4	Myanmar
Tropical Medicine	10	Taiwan
Elective Program in Tropical Medicine	15	Australia, Austria, Japan, USA
Global Infectious Diseases	9	Japan
Common Problems in Tropical Pediatrics	29	Indonesia, USA, Canada, Lao PDR, Myanmar,
		Vietnam, Cambodia, Thailand, New Zealand,
		Hong Kong, Japan
Management of Malaria	35	Bhutan, Hong Kong, India, Indonesia, Japan,
		Poland, Sri Lanka, Sudan, Tanzania, Thailand, USA
10 Courses	144	21 Nationalities





Rajvithi Campus Library

Telephone: 66 (0) 2640 9843, ext. 611-612 Fax: 66 (0) 2643 5613 E-mail: lirv@mahidol.ac.th

The Rajvithi Campus Library serves 3 faculties on the Rajvithi Campus of Mahidol University, the Faculty of Tropical Medicine, the Faculty of Public Health, and the Faculty of Dentistry. The library has a total area of 4,800 sq.m. and a seating capacity of 500. Resources cover pure science, applied science, medicine, tropical medicine, dentistry, public health, and related subjects.

Collection Size

861
43,518
14,745
170
2,231
120
271
548
696



TROPMED International House

Tel.: 66 (0) 2354 9100-19, ext. 2119, 2120 Fax: 66 (0) 2643 5607 E-mail: tetep@mahidol.ac.th Website: http://www.tm.mahidol.ac.th

TROPMED International House provides comfortable, secure and conveniently located on-campus accommodation for visitors to the Faculty of Tropical Medicine and course participants. It is particularly popular with international students attending the short training programs and shorter regular programs of the Faculty, such as the 6-month postgraduate Diploma in Tropical Medicine and Hygiene, and the 12-month Master of Clinical Tropical Medicine and Master of Clinical Tropical Pediatrics programs. All rooms (single and double) are fully furnished with air conditioning, refrigerator, hot shower, television, cable TV, internet connection, and wireless internet.





The Hospital for Tropical Diseases

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Assoc. Prof. Wattana Leowattana Director E-mail: dirctm@mahidol.ac.th

The Hospital for Tropical Diseases, founded in 1961, fulfils one of the key functions of the Faculty of Tropical Medicine, by providing expert healthcare service in tropical medicine to the Thai population and to foreign patients. Nowadays, the Hospital has 250 beds, 31 medical doctors, 83 nurses, and 81 nurse assistants. Last year, it served 40,747 out-patients and 2,427 in-patients. Although the Hospital is a center for the tropical diseases, it provides treatment for patients with general medical problems, such as diabetes, hypertension, lung diseases, and liver diseases. The Hospital has many specialist clinics, including clinics for gnathostomiasis, dermatology, travel medicine, chest diseases, nephrology, etc.



Patients treated in the Hospital for Tropical Diseases, by disease, during 2007

Disease	Outpatients	Inpatients
1. Falciparum malaria	408	405
2. Vivax malaria	206	81
3. Mixed falciparum and vivax malaria	3	3
4. Malariae malaria	1	1
5. Unidentified infections	133	133
6. Scrub typhus	4	3
7. Diarrhea	162	18
8. Food poisoning	24	1
9. Hepatitis	992	16
10. Dengue hemorrhagic fever	210	166
11. Taeniasis	23	-
12. Hookworm	1	-
13. Liver flukes	2	-
14. Trichuriasis	-	-
15. Pinworm	4	-
16. Strongyloidiasis	3	-
17. Gnathostomiasis	413	1
18. Opisthorchiasis viverrini	9	-
19. Filariasis	1	-
20. Dermatitis	3,693	3
21. Tuberculosis pulmonary	184	24
22. HIV infections	58	3
23. Hypertension	2,692	47
24. Diabetes mellitus	1,924	35
25. Hyperlipidemia	1,323	-
26. Disease of oral cavity, salivary glands and jaw	377	222
27. Others	27,897	1,392
Total	40,747	2,427

Research at the Hospital for Tropical Diseases, 2007

- 1. *In vivo* bioequivalence study of 16 mg Candesartan tablets preparations in healthy Thai volunteers.
- 2. Bioequivalence study of 40 mg Atorvastatin tablets preparations in healthy Thai male volunteers.
- 3. Bioequivalence study of genetic Glimepiride tablets to innovator Amary[®] (Glimepiride 2 mg) in healthy Thai volunteers.
- 4. Outcome of treatment and durability response of chronic viral hepatitis B: multi-centered study.
- 5. Phase 1, open-label study to evaluate potential pharmacokinetic interactions between orallyadministered Oseltamivir and intravenous Zanamivir in healthy Thai adult subjects.
- 6. Prognostic factors in severe malaria patients.
- 7. White blood cell counts in uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* malaria patients.
- 8. Clinical protocol for a Phase 2b trial of Pafuramidine maleate for the treatment of uncomplicated *P. falciparum* malaria.
- 9. A phase II, randomized, open label, multicentre study to assess antimalarial efficacy and safety of arterolane (RBx 1160) maleate and piperaquine phosphate co-administration and Coartem in patients with acute uncomplicated *Plasmodium falciparum* malaria.
- 10. *In vivo* bioequivalence study of 5 mg Levocetirizine tablet preparations in healthy Thai male volunteers.

















The Travel Clinic specializes in tropical diseases, especially malaria, and travel medicine. The clinic provides comprehensive travel-medicine services, including pre- and post-travel counseling, immunization, prophylaxis, and information on emerging diseases. Apart from prevention and prophylaxis, the clinic provides medical care services. **Services**

- Operate the Travel Clinic; more then 430 travelers attended the clinic
- Public education; interviewed by *Health and Cuisine* magazine
- · Give counseling to military diplomats

Research

- Conduct research in Khaosan Road area of Bangkok on "Backpackers' knowledge, attitudes, and practices towards malaria risk in Southeast Asia"
- Collaboration with GeoSentinel Networks

Education

- Teach in DTM&H and MCTM programs
- Welcome doctors, nurses, medical students, and other healthcare workers to the Travel Clinic





Research in Process 2007 (* = New Project)

No.	Research Title	Grant	Principal Investigator
Departm	ent of Clinical Tropical Medicine		
1	A multicenter, randomized, double-blind, phase II study to evaluate the safety, tolerance and efficacy of multiple doses of SCH 56592 versus fluconazole in the treatment of oropharyngeal candidiasis (OPC) in HIV-positive patients	Schering Plough Research Institute	Prof. Punnee Pitisuttithum
2	Open-label, treatment protocol for the safety and efficacy of SCH 56592 (Oral Suspension) in the treatment of invasive fungal infections	Schering Plough Research Institute	Prof. Punnee Pitisuttithum
3	Observational probe study of <i>in vitro</i> immune response parameters to candidate HIV-1 vaccine antigens among subjects from Thailand	Merck and Co., Inc	Prof. Punnee Pitisuttithum
4	Safety, immunogenicity, and efficacy of quadrivalent HPV (Types 6, 11, 16, 18) L1 virus-like particle (VLP) vaccine in mid-adult women. The FUTURE III)	Merck and Co., Inc	Prof. Punnee Pitisuttithum
5	A Phase III trial of Aventis Pasteur live recombinant ALVAC-HIV (vCP1521) priming with VaxGen gp120 B/E (AIDSVAX B/E) boosting in HIV-uninfected Thai adults (Clinic)	The Henry M. Jackson Foundation for The Advancement of Military Medicine, Inc. and The Ministry of Public Health, Thailand	Prof. Punnee Pitisuttithum
6.	A cross-sectional study to screen for and generate broadly neutralizing monoclonal antibodies from HIV-infected individuals	International AIDS Vaccine Initiative (IAVI)	Prof. Punnee Pitisuttithum
7	A randomized, international, double-blinded (with in-house blinding), controlled study with GARDASIL [™] , tolerability, immunogenicity, and efficacy study of a second generation human papillomavirus (HPV) L1 virus-like particle (VLP) vaccine administered to 16-to-26-vear-old women		Prof. Punnee Pitisuttithum
8	Establishment of a <i>Shigella sonnei</i> challenge model for evaluation of future vaccine candidates	US Army Medical Research and Materiel Command and National Institutes of Allergy and Infectious Diseases	Prof. Punnee Pitisuttithum
*9	A worldwide, phase I, dose-escalating study of the safety, tolerability, and immunogenicity of a three-dose regimen of MRKAd5HIV-1 gag vaccine in healthy adults	Merck & Co., Inc	Prof. Punnee Pitisuttithum
*10	Assessing the psychosocial burden in women with an abnormal pap result after screening interventions of MRKAd5HIV-1 gag vaccine in healthy adults	Merck & Co., Inc	Prof. Punnee Pitisuttithum
11	Liver megaproject Phase I: from basic research to education science and applied technology in clinical study	National Science and Technology Development Agency	Dr.Wichai Ekataksin
*12	Research project for technology transfer of chronic lymphedema treatment targeting medical, public health, and community personnel in Thailand southern border regions	Government Budget	Dr. Wichai Ekataksin
13	Development of molecular technique for evaluation of mefloquine resistance in <i>Plasmodium falciparum</i> in Asia	Commission on Higher Education & The Thailand Research Fund	Assist. Prof. Mallika Imwong

No.	Research Title	Grant	Principal Investigator
Departm	ent of Clinical Tropical Medicine (Continued)		
14	Cell derived microparticles in malaria infection	Wellcome Trust of Great Britain	Assist. Prof. Kesinee Chotivanich
15	Effect of primaquine and its metabolite on the infectivity of <i>P. falciparum</i> gametocytes	Wellcome Trust of Great Britain	Assist. Prof. Kesinee Chotivanich
16	A phase III, randomized, non-inferiority trial, to assess the efficacy and safety of dihydroartemisinin + piperaquine (DHA+PPQ, Artekin) in comparison with artesunate+mefloquine (AS+MQ) in patients affected by acute, uncomplicated <i>Plasmodium falciparum</i> malaria	MMV (Medicine for Malaria Venture) and Sigma Tao	Prof. Sasithon Pukrittayakamee
*17	Phase 1, open-label study to evaluate potential pharmacokinetic interactions between orally-administered oseltamivir and intravenous zanamivir in healthy Thai adult subjects	Glaxo SmithKline	Prof. Sasithon Pukrittayakamee
18	Gnathostomiasis and correlation of skin test with <i>Gnathostoma spinigerum</i> fractionated specific antigen and IgE	Faculty of Tropical Medicine, Mahidol University	Assist. Prof. Wirach Maek- a-Nantawat
19	<i>In vivo</i> bioequivalence study of 5 mg levocertirizine tablet preparations in healthy Thai male volunteers		Assist. Prof. Wirach Maek- a-Nantawat
*20	Bioequivalence study of 4 mg Perindopril tablet preparations in healthy Thai male volunteers	International Bio Service Co., Ltd	Assist. Prof. Weerapong Phumratanaprapin
21	Comparison of the safety and efficacy of a unique intravenous iron preparation (VIT-45) versus oral iron in the treatment of anemia in non- dialysis dependent chronic kidney diseases	Luitpold Pharmaceutical, Inc, USA	Assoc. Prof. Yupaporn Wattanagoon
*22	Bioequivalence study of genetic glimepiride tablets to innovator Amary® (Glimepiride 2 mg) in healthy Thai volunteers	International Bio Service, Co., Ltd.	Assoc. Prof. Yupaporn Wattanagoon
23	Pharmacologic study of oseltamivir in healthy volunteers	National Institutes of Health, USA	Assoc. Prof. Yupaporn Wattanagoon
24	Evaluation of genetic susceptibility to melioidosis		Dr. Wirongrong Chierakul
Departm	eent of Helminthology		
25	Helminthic infection in a tsunami-affected area: soil contamination and infection rates in the population	Brescia University, Italy	Assoc. Prof. Wanna Maipanich
26	Research and development of an application to purify Bithynia snail antigen in serodiagnosis of opisthorchiasis	Government Budget	Assoc. Prof. Jitra Waikagul
27	Study on <i>Paragonimus</i> population: morphology, molecular biology, enzymology and epidemiology aspects	Ministry of Foreign Affairs	Assoc. Prof. Jitra Waikagul
28	Control of soil-transmitted helminths through primary health care activities	Tropical Disease Trust	Assoc. Prof. Jitra Waikagul
29	Development of a school-based approach on emerging infectious disease control with emphasis on avian influenza	International Medicine Center of Influenza in Thailand Japan, Ministry of Health, Labor and Welfare, Japan	Assoc. Prof. Jitra Waikagul

No.	Research Title	Grant	Principal Investigator
Departn	nent of Helminthology (Continued)		
30	Trematode infections in humans of Nghia Phu and Nghia Lac Commune, Nghia Hung, Nam Dinh Province, Vietnam	DANIDA	Assoc. Prof. Jitra Waikagul
31	Discrimination of small liver and intestinal fluke eggs by ribosomal DNA-based PCR	JICA	Assoc. Prof. Jitra Waikagul
*32	The follow-up a trichinellosis case, the reservoir hosts and survey of helminthic infections in the community of Ban Rai District, Uthaithani Province	Government Budget	Assoc. Prof. Jitra Waikagul
*33	Cross-sectional study of parasitic infections and enteric bacteria among food handlers and water source in primary schools and restaurants at Sai-Yok District, Kanchanaburi Province	Faculty of Tropical Medicine	Dr. Teera Kusolsuk
Departn	nent of Medical Entomology		
*34	Dynamic and temporal structure of the troglobitic fauna of medically important insects and arthropods in caves of Kanchanaburi Province	Faculty of Tropical Medicine	Assoc. Prof. Chamnarn Apiwathnasorn
*35	Technology transfer of research and development for essential oils of <i>Lisea cubeba</i> , Qinghao (<i>Artemisia annua</i>) and kaffir lime (<i>Citrus hystrix</i>) as mosquito repellents for control of mosquito-borne diseases to local communities of northern, southern and northeastern Thailand	Government Budget	Assoc. Prof. Chamnarn Apiwathnasorn
*36	Population dynamic of the dengue vectors and dengue virus infection in <i>Aedes aegypti</i> and <i>Aedes albonictus</i> , in urban and suburban areas	Government Budget	Assoc. Prof. Narumon Komalamisra
*37	Dengue virus detection in dark and pale forms of <i>Aedes aegypti</i> collected as immature stages from breeding sites	Government Budget	Assoc. Prof. Supatra Thongrungkiat
38	Efficacy of water dispersible granule formulation of Bacillus thuringiensis israelensis (VECTOBAC® WDG) and Bacillus sphaericus (VECTOLEX® WDG) to control Culex sitiens in brackish water ponds in tsunami-affected areas of Phang-Nga Province, Southern Thailand	Brescia University, Italy	Dr. Yuwadee Trongtokit
39	Field application of <i>Bacillus thuringiensis</i> (VECTOBAC [®] WDG) for the control of mosquito larvae in salt-marsh habitats in tsunami-affected areas, in Phang Nga Province, Thailand	Faculty of Tropical Medicine	Dr. Yuwadee Trongtokit
*40	Molecular identification of <i>Anopheles sundaicus</i> in natural breeding sources, the coastal area of Andaman and the Gulf of Thailand	Faculty of Tropical Medicine	Dr. Jiraporn Ruangsittichai
41	Survey and study on geographical distribution of <i>Aedes albopictus</i> in Bangkok Metropolitan	Faculty of Tropical Medicine	Mr. Yudthana Samung
Departn	nent of Microbiology and Immunology		
42	Analysis of sequence polymorphism of T-cell epitope regions, Th2R and Th3R on <i>Plasmodium falciparum</i> circumsporozoite proteins in Thai isolates	The Thailand Research Fund	Prof. Srisin Khusmith
43	Cytokine gene polymorphism in severe malaria	The Thailand Research Fund	Prof. Srisin Khusmith
44	Relationship between cytokine gene polymorphism and severity of falciparum malaria	The Thailand Research Fund	Prof. Srisin Khusmith

No.	Research Title	Grant	Principal Investigator
Departm	ent of Microbiology and Immunology (Continued)		
45	Polymorphism of Pf EMP1 extracellular domain in Thai isolates causes severe uncomplicated <i>P. falciparum</i> malaria	The Thailand Research Fund	Prof. Srisin Khusmith
46	Chemokine gene polymorphisms and their susceptibility to tuberculoses	The Thailand Research Fund and Commission on Higher Education	Prof. Srisin Khusmith
*47	Clinical and immunological study on difficulty treatment TB in Chiang Rai, Thailand	Japan Health Science Foundation	Prof. Srisin Khusmith
48	Development of strategies for serovar identification of <i>Leptospira</i> sp. based on specific gene sequences within rfb locus	Thailand-Tropical Diseases Research Programmes	Assist. Prof. Thareerat Kalambaheti
49	Production of recombinant leptospiral protein antigen for serodiagnosis	Faculty of Tropical Medicine, Mahidol University	Assist. Prof. Thareerat Kalambaheti
50	Identification of genus- and species-specific antigens of <i>Aeromonas</i> spp. by Western blotting with mouse polyclonal antibodies	Faculty of Tropical Medicine	Assist. Prof. Yuwadee Mahakunkij- charoen
51	Development of a monoclonal antibody test for identification of pathogenic <i>Aeromonas</i> spp	Government Budget	Assist. Prof. Yuwadee Mahakunkij- charoen
52	Influence factors program the development of CD4+ regulatory T cells in autoimmune mouse model	Commission on Higher Education and The Thailand Research Fund	Dr. Pornsawar Leaungwu- tiwong
53	Serodiagnosis of scrub typhus and melioidosis by dual immunoperoxidase test	Faculty of Tropical Medicine, Mahidol University	Assoc. Prof. Varee Wongchotigul
54	Detection of specific antibodies to recombinant dengue virus DEIII/NS1 dengue viral protein using indirect enzyme immunoassay	Faculty of Tropical Medicine, Mahidol University	Dr. Akanitt Jittmittraphap
55	Preparation of human monoclonal antibody that neutralizes tetanus toxin using phage display technology	Commission on Higher Education and The Thailand Research Fund	Dr. Nitaya Intrawattana
*56	Culturing of <i>Brucella</i> sp. from blood, milk, and secretion from uterus in suspected brucellosis animal cases and determine the biochemical properties of isolates	Faculty of Tropical Medicine, Mahidol University	Mrs. Phanita Chanket
Departm	ent of Protozoology		
57	Research and development of biological contamination-free frozen food to the world's kitchens	National Research Council of Thailand	Assoc. Prof. Yaowalark Sukthana
58	<i>Toxoplasma gondii</i> genotyping in domestic and wild felids in Thailand	Commission on Higher Education	Assoc. Prof. Yaowalark Sukthana
59	Molecular characterization of <i>Plasmodium falciparum</i> polynucleotide kinase	TRF (RGJ Program)	Assoc. Prof. Porntip Petmitr
*60	Molecular characterization of <i>Plasmodium falciparum</i> polynucleotide kinase and its role in DNA repair of malaria parasites	Government Budget	Assoc. Prof. Porntip Petmitr

No.	Research Title	Grant	Principal Investigator
Departn	nent of Protozoology (Continued)		
61	PCR assays for detection of <i>Toxoplasma gondii</i> in Thai commercial meat products	Mahidol University	Ms. Rachatawan Chiabchalard
*62	Detection of <i>Trichomonas vaginalis</i> infection in Thai women by PCR and culture methods	Faculty of Tropical Medicine, Mahidol University	Ms. Kanthinich Thima
Departn	nent of Social and Environmental Medicine		
63	Collaborative study to evaluated operational programme of insecticide treated bednets for malaria control in Thailand	WHO	Assoc. Prof. Piyarat Butraporn
64	Modifying mosquito population age structure to eliminate dengue transmission	Gates Foundation	Assoc. Prof. Piyarat Butraporn
65	Construction of monoclonal antibody phage library for developing avian influenza virus diagnostic test	Commission on Higher Education and The Thailand Research Fund	Assist. Prof. Pongrama Ramasoota
66	Mimotope identification from monoclonal antibody leptospiral serovar specific using phage display technique	Commission on Higher Education and The Thailand Research Fund	Assist. Prof. Pongrama Ramasoota
67	Rapid detection of quinoline-resistant Mycobacterium tuberculosis using molecular techniques	Thailand-Tropical Diseases Research Programmes	Assist. Prof. Pongrama Ramasoota
68	Rapid detection of multidrug resistant Mycobacterium tuberculosis (MDR-TB) from indoor air using mol ecular techniques	Thailand-Tropical Diseases Research Programmes	Assist. Prof. Pongrama Ramasoota
*69	Enhancement of diagnostic efficacy for controlling foot-and-mouth disease in pigs and cattle	National Research Council of Thailand	Assist. Prof. Pongrama Ramasoota
*70	Technology transfer on the combination of effective microorganisms (EM), <i>Steinernema</i> <i>carpocapsae</i> , and chitosan for control of the environmental and diseases vectors problems in the agricultural community at Rachaburi Province	Government Budget	Assist. Prof. Pongrama Ramasoota
71	Development of a surveillance system for obesity among school children of high risk groups	WHO	Assist. Prof. Voranuch Wangsuphachart
72	Trans-boundary risk assessment (human health risk, ecological and environmental risk of the Mekong River-surface water)	Mekong River Commission Secretariat: MRCS	Assist. Prof. Voranuch Wangsuphachart
73	Integrated research network on Tachin-Maeklong Basin	Mahidol University	Assist. Prof. Wanchai Phatihattakorn
74	Health watch on emerging fresh- and brackish-water snails that transmit human parasitic diseases, after the tsunami in Phang-Nga Province, southern Thailand	Brescia University, Italy	Mrs. Pusadee Sri-aroon
75	Modifying mosquito age structure to eliminate dengue hemorrhagic fever: community participation in dengue hemorrhagic fever prevention & control		Assoc. Prof. Piyarat Butraporn

No.	Research Title	Grant	Principal Investigator
Depart	ment of Tropical Hygiene		
76	Application of GIS in monitoring multi-drug resistant malaria in Greater Mekong Subregion of Southeast Asia(III)	EC RBM-WHO	Assoc. Prof. Pratap Singhasivanon
77	Integrated GIS disease surveillance (IDS) System in Mekong Region	Rockefeller Foundation	Assoc. Prof. Pratap Singhasiyanon
*78	Avian flu surveillance network in displaced populations on the Thai Burma Border	Center for Disease Control and Prevention	Assoc. Prof. Pratap Singhasivanon
*79	Integrating clinical and laboratory diagnosis for surveillance of emerging infectious diseases in South-East Asia	French Government and Commission on Higher Education, Thailand	Assoc. Prof. Pratap Singhasivanon
*80	A phase III trial of Aventis Pasteur live recombinant ALVAC-HIV (vCP 1521) priming with VaxGen gp120 B/E (AIDSVAX [™] B/E) boosting in HIV-uninfected Thai adults (Data Management)	The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc	Assist. Prof. Jaranit Kaewkungwal
*81	A phase III trial of Aventis Pasteur live recombinant vaccine in Thai adults	Walter Reed Army Institute of Research	Assist. Prof. Jaranit Kaewkungwal
82	Field based study of reappearance of <i>Plasmodium vivax</i> malaria cases in the area near the Thai-Myanmar border, Suanphung, Ratchaburi Province	Faculty of Tropical Medicine, Mahidol University	Mr. Wanchai Maneeboon- young
83	Health behavior and risk factor of soil-transmitted helminth and malaria infections in primary schoolchildren in Saiyok District, Kanchanaburi Province	Faculty of Tropical Medicine, Mahidol University	Ms. Pannamas Maneekarn
84	Epidemiology and health education program and treatment evaluation of Pediculosis capitis with Luch Lime cream in school children, Suan Phung District Ratchaburi Province	Faculty of Tropical Medicine, Mahidol University	Mr. Nipon Thanyavanich
85	Molecular rapid serotypic identification of dengue viruses based on real-time PCR by using SYBR Green I and hybridization probes	Faculty of Tropical Medicine, Mahidol University	Dr. Natthanej Luplerdlop
* 86	Serotype specific of dengue viruses effect on the endothelial integrity trigger through protease enzyme overproduction by infected dendritic cells	The Thailand Research Fund	Dr. Natthanej Luplerdlop
* 87	Rapid dengue stereotypic assay by sing tube - multiplex real-time PCR	WHO, SEARO/TDR	Dr. Natthanej Luplerdlop
* 88	Proteomic characterization of <i>Aedes aegypti</i>	Bourse Scholarship, IRD, France	Dr. Natthanej Luplerdlop
	cells (C6/36)	Medicine, Mahidol University	Yimsamran
Departn	nent of Tropical Nutrition and Food Science		
90	Identification of gene alterations in breast cancer	BIOTEC	Assoc. Prof. Songsak Petmitr
*91	Determination of genes expression profile associated to the prognosis of breast cancer and cholangiocarcinoma using Affymetrix Gene Chip and development of diagnostic kits for prognostic detection of these cancers in Thai patients by real-time PCR technique	Government Budget	Assoc. Prof. Songsak Petmitr
92	Detection of proteins and obesity genes mutation in Thai over weight and obese children	Government Budget	Assoc. Prof. Rungsunn Tungtrongchit
52	Faculty of Tropical Medicine, Mahidol University ANNUAL REVIEW 2008		

No.	Research Title	Grant	Principal Investigator
Departn	nent of Tropical Nutrition and Food Science (Continued)		
93	Development of health behaviors and nutritional status of the tsunami victims in Phang-nga Province	Brescia University, Italy	Assist. Prof. Karunee Kwanbunjan
*94	MTHFR polymorphism of folate metabolic genes and susceptibility to colorectal cancer in Thais	Government Budget	Assist. Prof. Karunee Kwanbunjan
95	Purification and characterization of β-glucosidose specifically hydrolyzing iridoid glucoside from Thai plants	Commission on Higher Education	Dr. Dumrongkiet Arthan
*96	Studies on correlation of serum vitamin B12, serum folic acid, red blood cell folate, plasma homocysteine, micronucleus frequency in human lymphocytes, DNA methylation, and nutritional status in aging and young adult Thai people	Faculty of Tropical Medicine	Dr. Pornrutsami Jintaridhi
Departi	nent of Tropical Pathology		
97	Plasmodium falciparum induce IgE class switching	Swedish International Development Corporation A. (SIDA)	Assoc. Prof. Yaowapa Maneerat
98	A murine model of cerebral malaria, histopathology, immunohistochemistry and electron microscopy	Thailand Center for Excellence of Life Science (TCELS)	Assoc. Prof. Emsri Pongponratn
99	The effects of antimalarial drugs induced changes on <i>Plasmodium falciparum</i> and Pf-infected red blood cells ultrastructure	The Thailand Research Fund (RGJ)	Assoc. Prof. Emsri Pongponratn
100	Immunohistopathological studies of cytokine profiles in liver tissue of AIDS with opportunistic infections and HIV-infected patients: a necropsy study	The Thailand Research Fund (RGJ)	Assoc. Prof. Emsri Pongponratn
*101	Pathogenesis of acute renal failure in severe malaria: ultrastructural and immunopathological study	The Thailand Research Fund (RGJ)	Assoc. Prof. Emsri Pongponratn
102	Proteome of cancerous squamous cells in oral cavity and salivary gland tumor	Faculty of Tropical Medicine	Assist. Prof. Urai Chaisri
Departn	nent of Tropical Pediatrics		
103	A phase II, pilot, randomized, open-label, single-center study to evaluate immunogenicity and safety after PCECV rabies vaccine (Rabipur [®]) administered concomitantly with Japanese encephalitis vaccine as a pre-exposure regimen in 12 to 18 month old toddlers in Thailand	Chiron Vaccines, Co., Ltd.	Prof. Arunee Sabchareon
*104	Epidemiological study of dengue infection in children Ratchaburi Province, Thailand	Pediatric Dengue Vaccine Initiative (PDVI)	Prof. Arunee Sabchareon
105	Evaluation of long-term immunity against rabies in children vaccinated with purified chick embryo cell rabies vaccine (Rabipur®) in different pre-exposure regimens and boosted after one year (Extension of study M49P2)	Tropical Pediatrics	Assoc. Prof. Pornthep Chanthavanich
106	Evaluation of long-term immunity against Japanese encephalitis in children vaccinated with Japanese encephalitis vaccine (Extension of study M49P2)	Department of Tropical Pediatrics	Assoc. Prof. Pornthep Chanthavanich

No.	Research Title	Grant	Principal Investigator
Departm	nent of Tropical Pediatrics (Continued)		
107	Accuracy assessment of using WHO criteria in diagnosis of dengue infection	Department of Tropical Pediatrics	Assoc. Prof. Pornthep Chanthavanich
108	Epidemiological study of dengue infection in children aged 3-10 years in Ratchaburi Province	Ministry of Public Health	Assoc. Prof. Pornthep Chanthavanich
109	Development of surveillance system for under five mortality in Samut Sakhon Province, Thailand	WHO	Assoc. Prof. Chukiat Sirivichayakul
110	Dengue antibodies level and incidence of dengue Infection in preschool-aged children		Assoc. Prof. Krisana Pengsaa
111	Favirab [™] post-prescription event monitoring	Sanofi Pasteur Co., Ltd.	Assoc. Prof. Pornthep Chanthavanich
112	Evaluation of long term immunity against rabies in children vaccinated with different pre-exposure regimens of PCEC (Rabipur [®]) and the immunity after two IM infections of PCEC (Rabipur [®]) post exposure in the children previously vaccinated with PCEC pre-exposure regimens (149P6)		Assist. Prof. Kriengsak Limkittikul
Departm	nent of Tropical Radioisotopes		
113	Studies on radiation effect on mouse macrophage cell line (RAW 264.7) and radioprotective effect by various Thai medicinal plants	Faculty of Tropical Medicine	Ms. Cheeraratana Cheeramakara
114	Studies on heme oxygenase-1 activation in malaria- infected mice after artemisinin derivatives treatment	Department of Tropical Radioisotopes	Ms. Cheeraratana Cheeramakara
*115	Studies on oxidative stress activation by various Thai medicinal plants, radiation, and X-ray <i>in vitro</i>	Government Budget	Ms. Cheeraratana Cheeramakara
116	Studies on vitamin B ₁₂ and folic acid contents in foods	Department of Tropical Radioisotopes	Assist. Prof. Channarong Sanghirun
117	Studies on folic acid contents in fruit juice	Department of Tropical Radioisotopes	Mrs. Wanyarat Thanomsak
118	Studies on vitamin B ₁₂ and folic acid contents in Thai monks	Department of Tropical Radioisotopes	Mrs. Korbkit Cherdchu
119	Comparison of folic acid in humans with assay method and radioassay method	Department of Tropical Radioisotopes	Assist. Prof. Channarong Sanghirun
*120	Studies on toxicity of heme and oxidative stress after exposure of antimalarial drugs on mouse macrophage cell line (RAW264.7)	Faculty of Tropical Medicine, Mahidol University	Ms. Kriyaporn Songmuaeng
121	A phase III trial to determine the efficacy of AIDSVAX [™] B/E Vaccine in intravenous drug users in Bangkok, Thailand	AIDSVAX	Bangkok AIDS Vaccine Evaluation Group (Prof. Punnee Pitisuttithum)

No.	Research Title	Grant	Principal Investigator
Vaccine	Trial Centre		
122	A phase III trial of Aventis Pasteur live recombinant ALVAC-HIV (VCP1521) priming with Vaxgen gp 120 B/E (AIDSVAX B/E) boosting in HIV-uninfected Thai adults	Walter Reed Army Institute of Research	Dr. Supachai Ruekngam (Assoc. Prof. Punnee Pitisuttithum)

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Faculty of Tropical Medicine Publications 2007

Impact Factor from JCR :Science Edition 2006 - ISI Database

No.	Publication	Impact Factor
1.	Amornchai P, Chierakul W, Wuthiekanun V, Mahakhunkijcharoen Y, Phetsouvanh R, Currie BJ, Newton PN, van Vinh Chau N, Wongratanacheewin S, Day NPJ, Peacock SJ. Accuracy of <i>Burkholderia pseudomallei</i> identification using the API 20NE System and a latex agglutination test. <i>J Clin Microbiol</i> 2007:45(11):3774-6.	3.445
2.	Anantaphruti MT, Nuamtanong S, Watthanakulpanich D, Maipanich W, Pubampen S, Sanguankiat S, Kusolsuk T, Muennoo C, Waikagul J. Responses to albendazole treatment for hookworm infection in ethnic Thai and immigrants in west-central Thailand. <i>J Health Sci</i> 2007;53(4):443-9.	0.793
3.	Anantaphruti MT, Waikagul J, Yamasaki H, Ito A. Cysticercosis and taeniasis in Thailand. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(suppl 1):151-8.	-
4.	Anantaphruti MT, Yamasaki H, Nakao M, Waikagul J, Watthanakulpanich D, Nuamtanong S, Maipanich W, Pubampen S, Sanguankiat S, Muennoo C, Nakaya K, Sato MO, Sako Y, Okamoto M, Ito A. Sympatric occurrence of <i>Taenia solium</i> , <i>T. saginata</i> , and <i>T. asiatica</i> , Thailand. <i>Emerg Infect</i> Dis 2007;13:1413-6.	5.094
5.	Anceno AJ, Katayama H, Houpt ER, Chavalitshewinkoon-Petmitr P, Chyluun B, Shipin OV. IMS- free DNA extraction for the PCR-based detection and quantification <i>of Cryptosporidium parvum</i> and <i>Giardia lamblia</i> surface and waste water. <i>Int J Environ Health Res</i> 2007;17;297-310.	0.621
6.	Arreesrisom P, Dondorp AM, Looareesuwan S, Udomsangpetch R. Suppressive effects of the anti- oxidant N-acetylcysteine on the anti-malarial activity of artesunate. <i>Parasitol Int</i> 2007;56:221-6.	1.500
7.	Ashley E.A., Stepniewska K, Lindegardh N, Annerberg A, Kham A, Brockman A, Singhasivanon P, White N.J., Nosten F. How much fat is necessary to optimize lumefantrine oral bioavailability? <i>Trop Med Int Health</i> 2007;12(2):195-200.	2.595
8.	Ashley E.A., Stepniewska K, Lindegardh N, McGready R, Annerberg A, Hutagalung R, Singtoroj T, Hla G, Brockman A, Proux S, Wilahphaingern J, Singhasivanon P, White N.J., Nosten F. Pharmacokinetic study of artemether-lumefantrine given once daily for the treatment of uncomplicated multidrug-resistant falciparum malaria. <i>Trop Med Int Health</i> 2007;12(2):201-8.	2.595
9.	Auewarakul P, Suptawiwat O, Kongchanagul A, Sangma C, Suzuki Y, Ungchusak K, Louisirirotchanakul S, Lerdsamran H, Pooruk P, Thitithanyanout A, Pittayawonganon C, Guo CT, Hiramatsu H, Jampangern W, Chunsutthiwat S, Puthavathana P. An avian influenza H5N1 virus that binds to human-type receptor. <i>J Virol</i> 2007;81(18):9950-5.	5.341
10.	Barends M, Jaidee A, Khaohirun N, Singhasivanon P, Nosten F. <i>In vitro</i> activity of ferroquine (SSR 97193) against <i>Plasmodium falciparum</i> isolates from the Thai-Burmese border. <i>Malar J</i> 2007;6.	2.745
11.	Blacksell SD, Bell D, Kelley J, Mammen MP, Jr., Gibbons RV, Jarman RG, Vaughn DW, Jenjaroen K, Nisalak A, Thongpaseuth S, Vongsouvath M, Davong V, Phouminh P, Phetsouvanh R, Day NP, Newton PN. Prospective study to determine accuracy of rapid serological assays for diagnosis for acute dengue virus infection in Laos. <i>Clin Vaccine Immunol</i> 2007;14:1458-64.	-
12.	Blacksell SD, Bryant NJ, Paris DH, Doust JA, Sakoda Y, Day NP. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard:a lack of consensus leads to a lot of confusion. <i>Clin Infect Dis</i> 2007;44:391-401.	6.186
13.	Blacksell SD, Mammen MP, Thongpaseuth S, Gibbons RV, Jarman RG, Jenjaroen K, Nisalak A, Phetsouvanh R, Newton NP, Day NPJ. Evaluation of the Panbio dengue virus NS1 antigen detection ang IgM antibody ELISAs for the diagnosis of acute dengue infections in Laos. <i>Diag Microbiol Infect Dis</i> 2007;60:43-9.	2.553
14.	Blacksell SD, Myint K, Khounsy S, Phruaravanh M, Mammen MP, Jr., Day NP, Newton PN. Prevalence of hepatitis E virus antibodies in pigs:implications for human infections in village-based subsistence pig farming in the Lao PDR. <i>Trans R Soc Trop Med Hyg</i> 2007;101:305-7.	-
15.	Blacksell SD, Sharma N, Phumratanaprapin W, Jenjaroen K, Peacock SJ, White NJ, Phukrittayakamee S, Day NP. Serolodical and blood culture investigations of Nepalese fever patients. <i>Trans R Soc Trop Med Hyg</i> 2007;101:686-90.	-
16.	Brouwer AE, Siddiqui AA, Kester MI, Sigaloff KCE, Rajanuwong A, Wannapasni S, Chierakul W, Harrison TS. Immune dysfunction in HIV-seronegative, <i>Cryptococcus gattii</i> meningitis. <i>J Infection</i> 2007;54:165-8.	2.037

No.	Publication	Impact Factor
17.	Brouwer AE, van Kan HJ, Johnson E, Rajanuwong A, Teparrukkul P, Wuthiekanun V, Chierakul W, Day N, Harrison TS. Oral versus intravenous flucytosine in patients with Human Immunodeficiency Virus-Associated cryptococcal meningitis. <i>Antimicrob Agents Chemother</i> 2007;51(3):1038-42.	4.153
18.	Chaksangchaichot P, Punyarit P, Petmitr S. Novel hMSH2, hMSH6 and hMLH1 gene mutations and microsatellite instability in sporadic colorectal cancer. <i>J Cancer Res Clin Oncol</i> 2007;133:65-70.	2.469
19.	Chantratita N, Wuthiekanun V, Boonbumrung K, Tiyawisutsri R, Vesaratchavest M, Limmathurotsakul D, Chierakul W, Wongratanacheewin S, Pukritiyakamee S, White NJ, Day NPJ, Peacock SJ. Biological relevance of colony morphology and phenotypic switching by <i>Burkholderia pseudomallei</i> . <i>J Bacteriol</i> 2007;189(3):807-17.	3.993
20.	Chantratita N, Wuthiekanun V, Limmathurotsakul D, Thanwisai A, Chantratita W, Day NP, Peacock SJ. Prospective clinical evaluation of the accuracy of 16S rRNA real-time PCR assay for the diagnosis of melioidosis. <i>Am J Trop Med Hyg</i> 2007;77:814-7.	2.546
21.	Chantratita N, Wuthiekanun V, Thanwisai A, Limmathurotsakul D, Cheng AC, Chierakul W, Day NP, Peacock SJ. Accuracy of enzyme-linked immunosorbent assay using crude and purified antigens for serodiagnosis of melioidosis. <i>Clin Vaccine Immunol</i> 2007;14(1):110-3.	-
22.	Chayaratanasin P, Moonsom S, Sakdee S, Chaisri U, Katzenmeier G, Angsuthanasombat C. 3. High level of soluble expression in <i>Escherichia coli</i> and characterization in the cloned <i>Bacillus thuringiensis</i> Cry4Ba domain III fragment. <i>J Biochem Mol Biol</i> 2007;40(1):58-64.	1.465
23.	Cheng AC, Limmathurotsakul D, Chierakul W, Getchalarat N, Wuthiekanun V, Stephens DP, Day NP, White NJ, Chaowagul W, Currie BJ, Peacock SJ. A randomized controlled trial of granulocyte colony-stimulating factor for the treatment of severe sepsis due to melioidosis in Thailand. <i>Clin Infect Dis</i> 2007;45(3):308-14.	6.186
24.	Chiarakul S, Eunumjitkul K, Vuttiopas S, Vorapimol AR, Kaewkungwal J, Poovorawan Y. Seroprevalence and risk factors of hepatitis B virus infection among health care workers at the Institute of Neurology. <i>J Med Assoc Thai</i> 2007;90(8):1536-45.	-
25.	Chierakul W, Anunnatsiri S, Chaowagul W, Peacock SJ, Chetchotisakd P, Day NP. Addition of trimethoprim-sulfamethoxazole to ceftazidime during parenteral treatment of melioidosis treatment is not associated with long-term outcome benefit. <i>Clin Infect Dis</i> 2007;45:521-3.	6.186
26.	Chottanapund S, Singhasivanon P, Kaewkungwal J, Chamroonswasdi K, Manosuthi W. Survival time of HIV-infected patients with cryptococcal meningitis. <i>J Med Assoc Thai</i> 2007;90 (10):2104-11.	-
27.	Chuensumran U, Wongkham S, Pairojku C, Chauin S, Petmitr S. Prognostic value of DNA alterations on chromosome 17p13.2 for intrahepatic cholangiocarcinoma. <i>World J Gastroenterol</i> 2007;13(21):2986-91.	-
28.	Day NP, Dondorp AM. The management of patients with severe malaria. <i>Am J Trop Med Hyg</i> 2007;77(Suppl 6):29-35.	2.546
29.	De Fost M, Chierakul W, Limpaiboon R, Dondorp A, White NJ, Van Der Poll T. Release of granzymes and chemokines in Thai patients with leptospirosis. <i>Clin Microbiol Infect</i> 2007;13:433-6.	3.254
30.	Digeng H, Boots M, Tamori N, Satoh T, Higashihara J, Okada T, Kato K, Komalamisra N, Ushijima H, Takasaki T, Kurane I, Eshita Y. Effects of food, embryo density and conspecific immatures on hatchability in the dengue vector <i>Aedes albopictus</i> . <i>House and Household Insect Pests</i> 2007;28(2):117-27.	-
31. 32.	Dondorp AM, Day NP. The treatment of severe malaria. <i>Trans R Soc Trop Med Hyg</i> 2007;101:633-4 Dondorp AM, Silamut K, Charunwatthana P, Chuasuwanchai S, Ruangyeerayut R, Krintratun	- 5.363
	S, White NJ, Ho M, Day NP. Levamisole inhibits sequestration of infected red blood cells in patients with falciparum malaria. <i>J Infect Dis</i> 2007;196:460-6.	
33.	Dung DT, De NV, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, Murrell KD. Fishborne zoonotic intestinal trematodes, Vietnam. <i>Emerg Infect Dis</i> 2007;13:1828-33.	5.094
34.	Frincke JM, Stickney DR, Onizuka-Handa N, Garsd A, Reading C, Krudsood S, Wilairatana P, Looareesuwan S. Reduction of parasite levels in patients with uncomplicated malaria by treatment with HE2000. <i>Am J Trop Med Hyg</i> 2007;76(2):232-6.	2.546
35.	Geounuppakul M, Butraporn P, Kunstadter P, Leemingsawat S, Pacheun O. An empowerment program to enhance women's ability to prevent and control malaria in the community, Chiang Mai Province, Thailand. <i>Southeast Asian J Trop Med Public Health</i> 2007 May;38(3):546-59.	-
36.	Gillrie MR, Krishnegowda G, Lee K, Buret AG, Robbins SM, Looareesuwan S, Gowda DC, Ho M. Src-family kinase-dependent disruption of endothelial barrier function by <i>Plasmodium falciparum</i> merozoite proteins. <i>Blood</i> 2007;110:3426-35.	10.370

No.	Publication	Impact Factor
37.	Hananantachai H, Patarapotikul J, Ohashi J, Naka I, Krudsood S, Looareesuwan S, Tokunaga K. Significant association between TNF-a (TNF) promoter allele (21031C, 2863C, and 2857C) and cerebral malaria in Thailand. <i>Tissue Antigens</i> 2007;69(3):277-80.	2.462
38.	Harada M, Sri-aroon P, Lohachit C, Fujimoto C, Hasan AU, Itaki R, Suguri S, Chusongsang Y, Chusongsang P. Intertidal snail-trematode communities on the southern Thailand before and after the South Asia tsunami. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(4):658-62.	-
39.	Imwong M, Nair S, Pukrittayakamee S, Sudimack D, Williams JT, Mayxay M, Newton PN, Kim JR, Nandy A, Osorio L, Carlton JM, White NJ, Day NP, Anderson TJ. Contrasting genetic structure in <i>Plasmodium vivax</i> populations from Asia and South America. <i>Int J Parasitol</i> 2007;37:1013-22.	3.337
40.	Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, Guthmann JP, Nosten F, Carlton J, Looareesuwan S, Nair S, Sudimack D, Day NP, Anderson TJ, White NJ. Relapses of <i>Plasmodium vivax</i> infection usually result from activation of heterologous hypnozoites. <i>J Infect Dis</i> 2007;195:927-33.	5.363
41.	Inpankaew T, Traub R, Thompson RC, Sukthana Y. Canine parasitic zoonoses in Bangkok temples. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(2):247-55.	-
42.	Jampangern W, Vongthoung K, Jittmittraphap A, Worapongpaiboon S, Limkittikul K, Chuansumrit A, Tarunotai U, Chongsa-nguan M. Characterization of atypical lymphocytes and immunophenotypes of lymphocytes in patients with dengue virus infection. <i>Asian Pac J Allergy Immunol</i> 2007;25:27-36.	0.455
43.	Jeamwattanalert P, Mahakunkijcharoen Y, Kittigul L, Mahannop P, Pichyangkul S, Hirunpetcharat C. Long-lasting protective immune response to the 19-kilodalton carboxy-terminal fragment of <i>Plasmodium yoelii</i> merozoite surface protein 1 in mice. <i>Clin Vaccine Immunol</i> 2007;14(4):342-7.	-
44.	Jirakanjanakit N, Leemingsawat S, Thongrungkiat S, Apiwathnasorn C, Singhaniyom S, Bellec C, Dujardin JP. Influence of larval density or food variation on the geometry of the wing of <i>Aedes</i> (<i>Stegomyia</i>) <i>aegypti</i> . <i>Trop Med Int Health</i> 2007;12(2):1-7.	2.595
45.	Khadka DK, Eampokalap B, Panitchakorn J, Ramasoota P, Khusmith S. Multiple mutation in katG and inhA identified in Thai isoniazid resistant <i>Mycobacterium</i> tuberculosis. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(2):376-82.	-
46.	Kihara Y, Satho T, Eshita Y, Sakai K, Kotaki A, Takasaki T, Rongsriyam Y, Komalamisra N, Srisawat R, Lapcharoen P, Sumroiphon S, Iwanaga S, Ushijima H, Endoh D, Miyata T, Sakata A, Kashige N, Miake F, Fukushi S, Saijo M, Kurane I, Morikawa S, Mizutani T. Rapid determination of viral RNA sequences in mosquitoes collected in the field. <i>J Virol Methods</i> 2007;146;372-4.	2.097
47.	Kitjaroentham A, Hananantachai H, Tungtrongchitr A, Pooudong S, Tungtrongchitr R. R219K polymorphism of ATP binding cassette transporter A1 related with low HDL in overweight/ obese Thai male. <i>Arch Med Res</i> 2007;38:834-8.	1.275
48.	Kobayashi J, Jimba M, Okabayashi H, Singhasivanon P, Waikagul J. Beyond deworming:the promotion of school-health-based interventions in Japan. <i>Trends Parasitol</i> 2007;23:25-9.	4.907
49.	Krudsood S, Patel S,N, Tangpukdee N, Thanachartwet W, Leowattana W, Pornpininworakij K, Boggild A,K, Looareesuwan S, Kain K,C. Efficacy of atovaquone-proguanil for treatment of acute multidrug-resistant <i>Plasmodium falciparum</i> malaria in Thailand. <i>Am J Trop Med Hyg</i> 2007;76(4):655-8.	2.546
50.	Krudsood S, Tangpukdee N, Muangnoicharoen S, Thanachartwet V, Luplertlop N, Srivilairit S, Wilairatana P, Kano S, Ringwald P, Looareesuwan S. Clinical efficacy of chloroquine versus artemether-lumefantrine for <i>Plasmodium vivax</i> treatment in Thailand. <i>Korean J Parasitol</i> 2007;45(2):111-4.	-
51.	Larsen RA, Bauer M, Brouwer AE, Sanchez A, Thomas AM, Rajanuwong A, Chierakul W, Peacock SJ, Day NP, White NJ, Rinaldi MG, Harrison TS. <i>In vitro</i> -clinical correlations for amphotericin B susceptibility in AIDS-associated cryptococcal meningitis. <i>Antimicrob Agents Chemother</i> 2007;51(1):343-5.	4.153
52.	Le H,T, Singhasivanon P, Kaewkungwal J, Kaljee L,M, Charoenkul C. Sexual behaviors of alcohol drinkers and non-drinkers among adolescents and young adults in Nha Trang, Vietnam. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(1):152-60.	-
53.	Le H,T,T, Jareinpituk S, Kaewkungwal J, Pitiphat W. Increased risk of preterm birth among non- smoking, non-alcohol drinking women with maternal periodontitis. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(3):586-93.	-
54.	Limmathurotsakul D, Chaowagul W, Wongsrikaew P, Narmwong A, Day NP, Peacock SJ. Variable presentation of neurological melioidosis in Northeast Thailand. <i>Am J Trop Med Hyg</i> 2007;77:118-20.	2.546

Publication **Impact Factor** No. Limmathurotsakul D, Wuthiekanun V, Chantratita N, Wongsuvan G, Thanwisai A, Biaklang 3.445 55. M, Tumapa S, Lee S, Day NP, Peacock SJ. Simultaneous infection with more than one strain of Burkholderia pseudomallei is uncommon in human melioidosis. J Clin Microbiol 2007;45:3830-2. 56. Lindegardh N, Davies GR, Hien TT, Farrar J, Singhasivanon P, Day NP, White NJ. Importance of 4.153 collection tube during clinical studies of oseltamivir. Antimicrob Agents Chemother 2007;51:1835-6 57. Lindegardh N, Dondorp AM, Singhasivanon P, White NJ, Day NP. Validation and application 2.032 of a liquid chromatographic-mass spectrometric method for determination of artesunate in pharmaceutical samples. J Pharm Biomed Anal 2007;45:149-53. 58. Lindegardh N, Hanpithakpong W, Wattanagoon Y, Singhasivanon P, White NJ, Day NPJ. 2.647 Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of oseltamivir and its metabolite oseltamivir carboxylate in plasma, saliva and urine. J Chromatogr B Analyt Technol Biomed Life Sci 2007;859(1):74-83. 59. Liu Y, Retnakaran R, Hanley A, Tungtrongchitr R, Shaw C, Sweeney G. Total and high molecular 5.799 weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in Thai subjects. J Clin Endocrine Metab 2007;92(11):4313-8. Luksameetanasan R, Blacksell SD, Kalambaheti T, Wuthiekanun V, Chierakul W, Chueasuwanchai 60. S, Apiwattanaporn A, Stenos J, Graves S, Peacock SJ, Day NP. Patient and sample-related factors that effect the success of in vitro isolation of Orientia tsutsugamushi. Southeast Asian J Trop Med Public Health 2007;38(1):91-6. Maek-A-Nantawat W, Chantaphakul H, Ruxrungtham K. Skin reaction after desensitization not 8.829 61. predict the outcome of short course sulfa desensitization protocol among AIDS patients. J Allergy Clin Immunol 2007;119:S40. 62. Maneewatch S, Tapchaisri P, Sakolvaree Y, Klaysing B, Tongtawe P, Chaisri U, Songserm T, 0.455 Wongratanacheewin S, Srimanote P, Chongsa-nguan M, Chaicumpa W. OmpL1 DNA vaccine cross-protects against heterologous Leptospira spp. challenge. Asian Pac J Allergy Immunol 2007;25:75-82. Maton T, Butraporn P, Kaewkangwal J, Fungladda W. Avian influenza protection knowledge, 63. _ awareness, and behaviors in a high-risk population in Suphan Buri province, Thailand. Southeast Asian J Trop Med Public Health 2007 May;38(3):560-68. Matsuoka K, Takita C, Koyama T, Miyamoto D, Yingsakmongkon S, Hidari KIPJ, Jampangern 2.538 64. W, Suzuki T, Suzuki Y, Hatano K, Terunuma D. Novel linear polymers bearing thiosialosides as pendant-type epitopes for influenza neuraminidase inhibitors. Bioorg Med Chem Lett 2007;17(14):3826-30. 2.546 65. Mayxay M, Nair S, Sudimack D, Imwong M, Tanomsing N, Pongvongsa T, Phompida S, Phetsouvanh R, White NJ, Anderson TJC, Newton PN. Combined molecular and clinical assessment of Plasmodium falciparum antimalarial drug resistance in the Lao People's Democratic Republic (Laos). Am J Trop Med Hyg 2007 Jul;77(1):36-43. 66. McGready R, Kaveri S.V., Lacroix-Desmazes S, Krudsood S, Newton P.N. Acquired haemophilia 0.835 A in early pregnancy associated with *Plasmodium vivax* malaria and hyperthyroidism. Aust N Z J Obstet Gynaecol 2007;47(1):76-7. 67. Mita T, Tanabe K, Takahashi N, Tsukahara T, Eto H, Dysoley L, Ohmae H, Kita K, Krudsood S, Looareesuwan S, Kaneko A, Bjorkman A, Kobayakawa T. Independent evolution of 4.153 pyrimethamine resistance in Plasmodium falciparum isolates in Melanesia. Antimicrob Agents Chemother 2007;51(3):1071-7. Moonsom S, Chaisri U, Kasinrerk W, Angsuthanasombat C. Binding characteristics to mosquito-1.465 68. larval midgut proteins of the cloned domain II-III fragment from the Bacillus thuringiensis Cry 4Ba toxin. J Biochem Mol Biol 2007;40(5):783-90. 69. Moravec F, Taraschewski H, Anantapruti MT, Maipanich W, Laoprasert T. Heliconema longissimum 0.856 (Ortlepp, 1923) (Nematoda: Physalopteridae) from Pisodonophis boro (Teleostei: Ophichthidae) in Thailand, with remarks on the taxonomy of the Proleptinae Schulz, 1927. Syst Parasitol 2007;66:73-80. 70. Muangnoi P, Lu M, Lee J, Thepouyporn A, Mirzayans R, Le X.C., Weinfeld M, Changbumrung 1.746 S. Cytotoxicity, apoptosis and DNA damage induced by Alpinia galangal rhizome extract. Planta Med 2007;73:748-54. Naka I, Ohashi J, Patarapotikul J, Hananantachai H, Wilairatana P, Looareesuwan S, Tokunaga 2.205 71. K. The genotypes of GYPA and GYPB carrying the MNSs antigens are not associated with cerebral malaria. J Hum Genet 2007;52:476-9.

No.	Publication	Impact Factor
72.	Naoshima-Ishibashi Y, Iwagami M, Kawazu S, Looareesuwan S, Kano S. Analyses of cytochrome b mutations in <i>Plasmodium falciparum</i> isolates in Thai-Myanmar border. <i>Travel Med Infect Dis</i> 2007;5(2):132-4.	-
73.	Ngamjanyaporn P, Janwityanujit S, Dekumyoy P. Anaphylaxis as an initial manifestation of cholangiocarcinoma. <i>J Allergy Clin Immunol</i> 2007;119:S28.	8.829
74.	Nguansangiam S, Day NPJ, Hien TT, Mai NTH, Chaisri U, Riganti M, Dondorp AM, Lee SJ, Phu NH, Turner GDH, White NJ, Ferguson DJP, Pongponratn E. A quantitative ultrastructural study of renal pathology in fatal <i>P. falciparum</i> malaria. <i>Trop Med Int Health</i> 2007;12(9):1037-50.	2.595
75.	Nitatpattana N, Singhasivanon P, Kiyoshi H, Andrianasolo H, Yoksan S, Gonzalez J.P., Barbazan P. Potential association of dengue hemorrhagic fever incidence and remote senses land surface temperature, Thailand, 1998. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(3):427-33.	-
76.	Noedl H, Krudsood S, Leowattana W, Tangpukdee N, Thanachartwet W, Looareesuwan S, Miller R.S., Fukuda M, Jongsakul K, Yingyuen K, Sriwichai S, Ohrt C, Knirsch C. <i>In vitro</i> antimalarial activity of azithromycin, artesunate, and quinine in combination and correlation with clinical outcome. <i>Antimicrob Agents Chemother</i> 2007;51(2):651-6.	4.153
77.	Noranate N, Durand R, Tall A, Marrama L, Spiegel A, Sokhna C, Pradines B, Cojean S, Guillotte M, Bischoff E, Ekala MT, Bouchier C, Fandeur T, Ariey F, Patarapotikul J, Bras J, Trape JF, Rogier C, Mercereau-Puijalon O. Rapid dissemination of <i>Plasmodium falciparum</i> drug resistance despite strictly controlled antimalarial use. <i>PLoS ONE</i> 2007;2(1):139.	-
78.	Nosten F, McGready R, Mutabingwa T. Case management of malaria in pregnancy. <i>Lancet Infect Dis</i> 2007;7:118-25.	111.808
79.	Nuchsongsin F, Chotivanich K, Charunwatthana P, Fausta OS, Taramelli D, Day NP, White NJ, Dondorp AM. Effects of malaria heme products on red blood cell deformability. <i>Am J Trop Med Hyg</i> 2007;77:617-22.	2.546
80.	Nunthawarasilp P, Petmitr S, Chavalitshewinkoon-Petmitr P. Partial purification and characterization of DNA polymerase -like enzyme from <i>Plasmodium falciparum</i> . <i>Mol Biochem Parasitol</i> 2007;154:141-7.	2.641
81.	Ohashi J, Naka I, Kimura R, Natsuhara K, Yamauchi T, Furusawa T, Nakazawa M, Ataka Y, Patarapotikul J, Nuchnoi P, Tokunaga K, Ishida T, Inaoka T, Matsumura Y, Ohtsuka R. FTO polymorphisms in oceanic populations. <i>J Hum Genet</i> 2007;52(12):1031-5.	2.205
82.	Ohta N, Waikagul J. Disease burden and epidemiology of soil-transmitted helminthiases and schistosomiasis in Asia:the Japanese perspective. <i>Trends Parasitol</i> 2007;23(1):30-5.	4.907
83.	Okanurak K, Kitayaporn D, Wanarangsikul W, Koompong C. Effectiveness of DOT for tuberculosis treatment outcomes:a prospective cohort study in Bangkok, Thailand. <i>Int J Tuberc Lung Dis</i> 2007;11(7):762-8.	2.035
84.	Paditaporn R, Riengrojpitak S, Punyarit P, Chindavijak S, Karalak A, Petmitr S. Genetic instability in cervical cancer detected by arbitrarily primed polymerase chain reaction. <i>Asian Pac J Cancer Prev</i> 2007;8:109-12.	-
85.	Pakeetoot T, Punyarit P, Petmitr S. Identification of genetic alterations in Thai breast cancer patients by arbitrarily primed polymerase chain reaction. <i>Asian Pac J Cancer Prev</i> 2007;8(1):83-6.	-
86.	Pakeetoot T, Punyarit P, Petmitr S. Novel DNA amplification on chromosomes 6q23-24 and 4p15.2 in breast cancer identified by arbitrarily primed polymerase chain reaction. J <i>Exp Clin Cancer Res</i> 2007;26(2):253-9.	0.869
87.	Panichakul T, Sattabongkot J, Chotivanich K, Sirichaisinthop J, Cui L, Udomsangpetch R. Production of erythropoietic cells <i>in vitro</i> for continuous culture of <i>Plasmodium vivax</i> . <i>Int J Parasitol</i> 2007;37:1551-7.	3.337
88.	Paris DH, Imwong M, Faiz AM, Hasan M, Yunus EB, Silamut K, Lee SJ, Day NJ, Dondorp AJ. Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria. <i>Am J Trop Med Hyg</i> 2007 Nov;77(5):972-6.	2.546
89.	Pattanakitsakul S-N, Boonnak K, Auethavornanan K, Jairungsri A, Duangjinda T, Puttatesk P, Thongrungkiat S, Malasit P. A new densovirus isolated from the mosquito <i>Toxorhynchites splendens</i> (Wiedemann) (Diptera:Culicidae). <i>Southeast Asian J Trop Med Public Health</i> 2007;38(2):283-93.	-
90.	Pengsaa K. Congenital malaria in Thailand. <i>Ann Trop Paediatr</i> 2007;27:133-9.	0.934
91.	rengsaa K, Sirivichayakui C, Vithayasai N, Senawong S, Luangwedchakarn V. Chronic diarrhea and abnormal serum immunoglobulin levels: A case report. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(3):424-6.	-

No.	Publication	Impact Factor
92.	Phimda K, Hoontrakul S, Suttinont C, Chareonwat S, Losuwanaluk K, Chueasuwanchai S, Chierakul W, Suwancharoen D, Silpasakorn S, Saisongkorh W, Peacock SJ, Day NPJ, Suputtamongkol Y. Doxycycline versus azithromycin for treatment of leptospirosis and scrub typhus. <i>Antimicrob Agents Achemother</i> 2007;51(9):3259-63.	4.153
93.	Pitabut N, Panichakorn J, Mahakunkijcharoen Y, Hirunpetcharat C, Looareesuwan S, Khusmith S. IgG antibody profile to c-terminal region of <i>Plasmodium vivax</i> merozoite surface protein-1 in Thai individuals exposed to malaria. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(1):1-7.	-
94.	Pitisuttithum P, Choopanya K, Bussaratid V, Vanichseni S, van Griensven F, Phonrat B, Martin M, Vimutsunthorn E, Sangkum U, Kitayaporn D, Tappero JW, Heyward W, Francis D. Social harms in injecting drug users participating in the first phase III HIV vaccine trial in Thailand. <i>J Med Assoc Thai</i> 2007 Nov;90(11):2442-8.	-
95.	Piyaphanee W, Issarachaikul R, Soontarach P, Silachamroon U. Concurrent <i>Salmonella</i> bacteremia in <i>P. vivax</i> infectiona report of 2 cases at the Hospital for Tropical Diseases, Thailand. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(4):616-8.	-
96.	Pootong A, Budhirakkul P, Tongtawe P, Tapchairi P, Chongsa-nguan M, Chaicumpa W. Monoclonal antibody that neutralizes pertussis toxin activities. <i>Asian Pac J Allergy Immunol</i> 2007;25:37-45.	0.455
97.	Sakamoto JI, Koyama T, Miyamoto D, Yingsakmongkon S, Hidari K.I.P.J., Jampangern W, Suzuki T, Suzuki Y, Esumi Y, Hatano K, Terunuma D, Matsuoka K. Thiosialoside clusters using carbosilane dendrimer core scaffolds as a new class of influenza neuraminidase inhibitors. <i>Bioorg Med Chem Lett</i> 2007;17(3):717-21.	2.538
98.	Sakolvaree Y, Maneewatch S, Jiemsup S, Klaysing B, Tongtawe P, Srimanote P, Saengjaruk P, Banyen S, Tapchaisri P, Chonsa-nguan M, Chaicumpa W. Proteome and immunome of pathogenic <i>Leptospira</i> spp. revealed by 2DE and 2DE-immunoblotting with immune serum. <i>Asian Pac J Allergy Immunol</i> 2007;25:53-73.	0.455
99.	Sirivichayakul C, Sabchareon A, Pengsaa K, Thaiarporn I, Chaivisuth A, Na-Bangehang K, Wisetsing P, Chanthavanich P, and Pojjaroen-anant C. Comparative study of the effectiveness and pharmacokinetics of two rectal artesunate/oral mefloquine combination regimens for the treatment of uncomplicated childhood falciparum malaria. <i>Ann Trop Paediatr</i> 2007:27;17-24.	0.934
100.	Sri-aroon P, Butraporn P, Limsoomboon J, Kaewpoolsri M, Chusongsang Y, Charoenjai P, Chusongsan P, Numnuan S, Kiatsiri S. Freshwater mollusks at designated areas in eleven provinces of Thailand according to the water resource development projects. <i>Southeast Asian J Trop Med Public Health.</i> 2007 Mar;38(2):294-301.	-
101.	Stepniewska K, Chotivanich K, Brockman A, Day NP, White NJ. Overestimating resistance in field testing of malaria parasites:simple methods for estimating high EC50 values using a Bayesian approach. <i>Malar J</i> 2007;6:4.	2.748
102.	Suriyaprom K, Harnroongroj T, Namjuntra P, Chantaranipapong Y, Tungtrongchitr R. Effects of tobacco smoking on Alpha-2-macroglobulin and some biochemical Parameters in Thai males. <i>Southeast Asian J Trop Med Pub Health</i> 2007;38(5):918-26.	-
103.	Tangpukdee N, Krudsood S, Thanachartwet V, Duangdee C, Paksala S, Chonsawat P, Srivilairit S, Looareesuwan S, Wilairatana P. Predictive score of uncomplicated falciparum malaria patients turning to severe malaria. <i>Korean J Parasitol</i> 2007;45(4):273-82.	-
104.	Tangteerawatana P, Montgomery SM, Perlmann H, Looareesuwan S, Troye-Blomberg M, Khusmith S. Differential regulation of IgG subclasses and IgE antimalarial antibody responses in complicated and uncomplicated <i>Plasmodium falciparum</i> malaria. <i>Parasite Immunol</i> 2007;29:475-83.	2.009
105.	Tangteerawatana P, Pichyangkul S, Hayano M, Kalambaheti T, Looareesuwan S, Troye-Blomberg M, Khusmith S. Relative levels of <i>IL4</i> and IFN-in complicated malaria:association with IL4 polymorphism and peripheral parasitemia. <i>Acta Trop</i> 2007;101:258-65.	2.211
106.	Tanomsing N, Imwong M, Pukrittayakamee S, Chotivanich K, Looareesuwan S, Mayxay M, Dolecek C, Hien TT, Rosario VE, Arez AP, Michon P, Snounou G, White NJ, Day NPJ. Genetic analysis of the dihydrofolate reductase-thymidylate synthase gene from geographically diverse isolates of <i>Plasmodium malariae</i> . <i>Antimicrob Agents Chemother</i> 2007;51(10):3523-30.	4.153
107.	Tansuphaswadikul S, Aung S.E., Phonrat B, Kaewkungwal J, Pitisuttithum P, Maek-a-Nantawat W. Predisposing factors for nevirapine toxicity among AIDS patients with low baseline CD4 count. Asian Pac <i>J Allergy Immunol</i> 2007;25(2-3):147-54.	0.455
108.	Tansuphasawadikul S, Saito W, Kim J, Phonrat B, Dhitavat J, Chamnanchanan S, Pitisuttithum P. Outcomes in HIV-infected patients on antiretroviral therapy with tuberculosis. <i>Southeast Asian J Trop Med Public Health</i> 2007;38(6):1053-60.	-

No. Publication **Impact Factor** 109. 2.037 Tapchaisri P, Na-Ubol M, Jaipaew J, Srimanote P, Chongsa-nguan M, Yamasaki S, Hayashi H, Nair G,B, Kurazono H, Chaicumpa W. Virulence genes of clinical Vibrio cholerae O1 isolates in Thailand and their ribotypes. J Infect 2007;55:557-65. 110. Teerakanchana T, Puavilai W, Suriyaprom K, Tungtrongchitr R. Comparative study of LDLcholesterol levels in Thai patients by the direct method and using the Friedewald formula. Southeast Asian J Trop Med Pub Health 2007;38(3):519-27. 111. Thaenkham U, Visetsuk K, Do TD, Waikagul J. Discrimination of Opisthorchis viverrini from 2.211 Haplorchis taichui using COI sequence marker. Acta Trop 2007;103:26-32. 112. Thaipadungpanit J, Wuthiekanun V, Chierakul W, Smythe LD, Petkanchanapong W, Limpaiboon R, Apiwatanaporn A, Slack AT, Suputtamongkol Y, White NJ, Feil EJ, Day NPJ, Peacock SJ. A dominant clone of Leptospira interrogans associated with an outbreak of human leptospirosis in Thailand. PLoS Negl Trop Dis 2007;1(1):1-6. 113. Thanachartwet V, Phumratanaprapin W, Desakorn V, Sahassananda D, Wattanagoon Y, 1.095 Chaiprasert A, Aimpun P, Supaporn T. Viral hepatitis infections among dialysis patients: Thailand registry report. Nephrology 2007;12:399-405. Tuc VP, Wangsuphachart V, Tasanapradit P, Fungladda W, Trong PV, Nhung NT. Impacts 114. of pesticide use on semen characteristics among rice famers in Kienxuong District, Thaibinh province, Vietnam. Southeast Asian J Trop Med Public Health 2007;38(3):569-75. 115. Udomsangpetch R, Somsri S, Panichakul T, Chotivanich K, Sirichaisinthop J, Yang Z, Cui L, 1.500 Sattabongkot J. Short-term in vitro culture of field isolates of Plasmodium vivax using umbilical cord blood. Parasitol Int 2007;56:65-9. 116. Uhlemann AC, McGready R, Ashley EA, Brockman A, Singhasivanon P, Krishna S, White NJ, 5.363 Nosten F, Price RN. Intrahost selection of Plasmodium falciparum pfmdr1 alleles after antimalarial treatment on the northwestern border of Thailand. J Infect Dis 2007;195(1):134-41. 117. Waikagul J. A new species of Paragonimus (Trematoda:Troglotrematidae) from a cat infected 1.300 with metacercariae from mountain crabs Larnaudia larnaudii. J Parasitol 2007;93(6):1496-500. 118. Waree P, Ferguson DJ, Pongponratn E, Chaisri U, Sukthana Y. Immunohistochemical study of acute and chronic toxoplasmosis in experimentally infected mice. Southeast Asian J Trop Med Public Health 2007;38(2):223-31. 119. White NJ. Cardiotoxicity of antimalarial drugs. Lancet Infect Dis 2007;7:549-58. 11.808 120. Wiengcharoen J, O'Hanley R, Armstrong T, Best W, Sukthana Y, Thompson RCA. Novel drug compounds against Neospora caninum and Toxoplasma gondii in vitro. Southeast Asian J Trop Med Public Health 2007;38(Suppl 1):15-8. Wiersinga WJ, Wieland CW, Dessing MC, Chantratita N, Cheng AC, Limmathurotsakul D, 121. 13.750 Chierakul W, Leendertse M, Florquin S, de Vos AF, White NJ, Dondorp AM, Day NP, Peacock SJ, van der Poll T. Toll-like receptor 2 impairs host defense in gram-negative sepsis caused by Burkholderia pseudomallei (melioidosis). PLoS Med 2007;4(7):1268-80 122. Wongroongsarb P, Chanket T, Gunlabun K, Long DH, Satheanmethakul P, Jetanadee S, 2.068 Thaipadungpanit J, Wuthiekanun V, Peacock S, Blacksell S, Smythe LD, Bulach DM, Kalambaheti T. Molecular typing of Leptospira spp. based on putative O-antigen polymerase gene (WZY), the benefit over 165 rRNA gene sequence. FEMS Microbiol Lett 2007;271:170-9. 123. Wuthiekanun V, Chierakul W, Limmathurotsakul D, Smythe LD, Symonds ML, Dohnt MF, 3.445 Slack AT, Limpaiboon R, Suputtamongkol Y, White NJ, Day NP, Peacock SJ. Optimization of culture of Leptospira from humans with leptospirosis. J Clin Microbiol 2007;45(4):1363-5. Wuthiekanun V, Limmathurotsakul D, Wongsuvan G, Chierakul W, Teerawattanasook N, 124. 2.546 Teparrukkul P, Day NP, Peacock SJ. Quantitation of Burkholderia pseudomallei in clinical samples. Am J Trop Med Hyg 2007;77:812-3. 125. Wuthiekanun V, Sirisukkarn N, Daengsupa P, Sakaraserane P, Sangkakam A, Chierakul W, 5.094 Smythe LD, Symonds ML, Dohnt MF, Slack AT, Day NP, Peacock SJ. Clinical diagnosis and geographic distribution of leptospirosis, Thailand. Emerg Infect Dis 2007;13(1):124-6. Yipp BG, Hickey MJ, Andonegui G, Murray AG, Looareesuwan S, Kubes P, Ho M. Differential 126. 2.405 roles of CD36, ICAM-1, and P-selectin in Plasmodium falciparum cytoadherence in vivo. Microcirculation 2007;14:593-602.


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ACCURACY OF **BURKHOLDERIA PSEUDOMALLEI** IDENTIFICATION USING THE API 20NE SYSTEM AND LATEX AGGLUTINATION TEST

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In an evaluation of the API 20NE for the identification of *Burkholderia* spp., 792/800 (99%) *Burkholderia pseudomallei* and 17/19 (89%) *B. cepacia* isolates were correctly identified but 10 *B. mallei* and 98 *B. thailandensis* isolates were not correctly identified. A latex agglutination test was positive for 796/800 (99.5%) *B. pseudomallei* isolates and negative for 120 other oxidase-positive gram-negative bacilli.

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SUPPRESSIVE EFFECTS OF THE ANTI-OXIDANT N-ACETYLCYSTEINE ON THE ANTI-MALARIAL ACTIVITY OF ARTESUNATE

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The anti-oxidant drug N-acetylcysteine (NAC) has been proposed as adjunctive treatment in severe falciparum malaria. However, this might inhibit the anti-malarial drug action of the artemisinins, which are thought to exert their parasitocidal action through oxidative damage. We studied the interaction between NAC and artesunate as well as quinine in an in vitro drug sensitivity assay. Combination with NAC reduced the parasitocidal effect of artesunate only within the first 6 h of incubation, whereas no interaction was observed with quinine. Pre-incubation of P. falciparum with NAC resulted in a similar inhibitory effect on the anti-malarial activity of artesunate, whereas no inhibition was observed when NAC was added 2 h after parasite exposure to artesunate. Assessment of parasite maturation inhibition by the standard Giemsa's staining was in accordance with the use of a vital staining. The results herein caution the use of adjunctive treatment for malaria infection. Combination of antagonistic drugs may lead to adverse effects.

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SEROLOGICAL AND BLOOD CULTURE INVESTIGATIONS OF NEPALESE FEVER PATIENTS

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Serological testing of paired (i.e. admission and convalescent) sera from 103 fever patients in Kathmandu, Nepal, was performed to estimate the prevalence rates of scrub typhus, murine typhus, *Leptospira* and dengue virus antibodies and to determine their role in the cause of active infections. Blood cultures from 15 patients grew *Salmonella enterica* serovar Typhi, 8 grew *S*. Paratyphi A and 6 grew other bacteria. Diagnostic antibody levels were detected against murine typhus (27/103; 26%), scrub typhus (23/103; 22%), *Leptospira* (10/103; 10%) and dengue virus (8/103; 8%). Nineteen patients (18%) had diagnostically raised antibodies to more than one infectious agent. Seven *S*. Typhi (7/15; 47%) and two *S*. Paratyphi A (2/8; 25%) patients had significant scrub typhus, murine typhus, *Leptospira* or dengue virus IgM antibody titres. This study

confirms the presence of leptospiral, rickettsial and dengue infections in Kathmandu as well as evidence for mixed infections with *S*. Typhi and *Orientia tsutsugamushi* or *Rickettsia typhi*. These infections should be kept in mind when considering the differential diagnoses of fever and empirical treatment options in Nepal. Many patients demonstrated static IgM antibody results between paired serum collections, suggesting recent rather than acutely active infections.

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IMMUNE DYSFUNCTION IN HIV-SERONEGATIVE, CRYPTOCOCCUS GATTII MENINGITIS

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The pathophysiology of meningitis caused by *Cryptococcus gattii* in apparently immunocompetent individuals remains unclear. We measured multiple cytokines in CSF from a HIV-seronegative, apparently immunocompetent, Thai patient with *C. gattii* meningitis, over the first 2 weeks of antifungal therapy. Levels of proinflammatory IFN-g, TNF-a, and IL-6 were very low compared to patients with HIV-related *Cryptococcus neoformans* meningitis and of IL- 10 very high. While patients with *C. gattii* meningitis may be a heterogeneous group, these data suggest in this case a maladapted immune response to cryptococcal exposure had allowed progression to clinical cryptococcal disease.

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ORAL VERSUS INTRAVENOUS FLUCYTOSINE IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED CRYPTOCOCCAL MENINGITIS

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In a randomized controlled trial of amphotericin B-based therapy for human immunodeficiency virus (HIV)-associated cryptococcal meningitis in Thailand, we also compared the mycological efficacy, toxicity, and pharmacokinetics of oral versus intravenous flucytosine at 100 mg/kg of body weight/day for the initial 2 weeks. Half of 32 patients assigned to the two arms containing flucytosine were randomized to oral and half to intravenous flucytosine. Early fungicidal activity was determined from serial quantitative cultures of cerebrospinal fluid (CSF), and toxicity was assessed by clinical and laboratory monitoring. Flucytosine and fluorouracil concentrations in plasma and CSF were measured by high-performance liquid chromatography. No significant bone marrow or hepatotoxicity was seen, there was no detectable difference in bone marrow toxicity between patients on intravenous and those on oral formulation, and no patients discontinued treatment. In patients receiving intravenous flucytosine, the median 24-h area under the concentration-time curve was significantly higher than in the oral group. Despite this difference, there was no difference in early fungicidal activity between patients on intravenous compared with patients on oral flucytosine. The results suggest that either formulation can be used safely at this dosage in a developing country setting, without drug concentration monitoring. The bioavailability of the oral formulation may be reduced in late-stage HIV-infected patients in Thailand. Concentrations of flucytosine with intravenous formulation at 100 mg/kg/day may be in excess of those required for maximal fungicidal activity.

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BIOLOGICAL RELEVANCE OF COLONY MORPHOLOGY AND PHENOTYPIC SWITCHING BY **BURKHOLDERIA PSEUDOMALLEI**

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Melioidosis is a notoriously protracted illness and is difficult to cure. We hypothesize that the causative organism, *Burkholderia pseudomallei*, undergoes a process of adaptation involving altered expression of surface determinants which facilitates persistence in vivo and that this is reflected by changes in colony morphology. A colony morphotyping scheme and typing algorithm were developed using clinical *B. pseudomallei* isolates. Morphotypes were divided into seven types (denoted I to VII). Type I gave rise to other morphotypes (most commonly type II or III) by a process of switching in response to environmental stress, including starvation, iron limitation, and growth at 42°C. Switching was associated with complex shifts in phenotype, one of which (type I to type II) was associated with a marked increase in production of factors putatively associated with in vivo concealment. Isogenic types II and III, derived from type I, were examined using several experimental models. Switching between isogenic morphotypes occurred in a mouse model, where type II appeared to become adapted for persistence in a low-virulence state. Isogenic type II demonstrated a significant increase in intracellular replication fitness following uptake by macrophages in vitro, which was associated with a switch to type II. Mixed *B. pseudomallei* morphologies were common in individual clinical specimens and were significantly more frequent in samples of blood, pus, and respiratory secretions than in urine and surface swabs. These findings have major implications for therapeutics and vaccine development.

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ACCURACY OF ENZYME-LINKED IMMUNOSORBENT ASSAY USING CRUDE AND PURIFIED ANTIGENS FOR SERODIAGNOSIS OF MELIOIDOSIS

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Five enzyme-linked immunosorbent assays developed to detect antibodies to different *Burkholderia pseudomallei* antigen preparations were evaluated as diagnostic tests for melioidosis in northeast Thailand. The highest diagnostic indices were observed for an affinity-purified antigen (sensitivity, 82%; specificity, 72%) and crude *B. pseudomallei* antigen (sensitivity, 81%; specificity, 70%), an improvement over the indirect hemagglutination assay (sensitivity, 73%; specificity, 64%).

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A RANDOMIZED CONTROLLED TRIAL OF GRANULOCYTE COLONY-STIMULATING FACTOR FOR THE TREATMENT OF SEVERE SEPSIS DUE TO MELIOIDOSIS IN THAILAND

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BACKGROUND: Melioidosis is a tropical infectious disease associated with significant mortality. Most deaths occur early and are caused by fulminant sepsis. METHODS: In this randomized, placebo-controlled trial, we assessed the efficacy of lenograstim (granulocyte colony-stimulating factor [G-CSF], 263 mu g per day administered intravenously) in ceftazidime-treated patients with severe sepsis caused by suspected melioidosis in Thailand. RESULTS: Over a 27-month period, 60 patients were enrolled to receive either G-CSF (30 patients, 18 of whom had culture-confirmed melioidosis) or placebo (30 patients, 23 of whom had culture-confirmed melioidosis). Mortality rates were similar in both groups (G-CSF group, 70%; placebo group, 87%; risk ratio, 0.81; 95% confidence interval, 0.61-1.06; P=.2), including among patients with confirmed melioidosis (83% vs. 96%; P=.3). The duration of survival was longer for patients who received G-CSF than for patients who received placebo (33 h vs. 18.6 h; hazard ratio, 0.56; 95% confidence interval, 0.31-1.00; P=.05). CONCLUSIONS: Receipt of G-CSF is associated with a longer duration of survival but is not associated with a mortality benefit in patients with severe sepsis who are suspected of having melioidosis in Thailand. We hypothesize that G-CSF may "buy time" for severely septic patients, but survival is more likely to be improved by management of associated metabolic abnormalities and organ dysfunction associated with severe sepsis.

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RELEASE OF GRANZYMES AND CHEMOKINES IN THAI PATIENTS WITH LEPTOSPIROSIS

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The plasma concentrations of granzymes are considered to reflect the involvement of cytotoxic T-cells and natural killer cells in various disease states. Interferon (IFN)-c-inducible protein-10 (IP-10) and monokine induced by IFN-c (Mig) are members of the non-ELR CXC chemokine family that act on T-cells and natural killer cells. This study revealed that the plasma concentrations of granzyme B (but not granzyme A), IP-10 and Mig were higher in 44 Thai patients with definite or possible leptospirosis than in healthy blood donors. These data suggest that activation of cell-mediated immunity is part of the early host response to leptospirosis.

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REDUCTION OF PARASITE LEVELS IN PATIENTS WITH UNCOMPLICATED MALARIA BY TREATMENT WITH HE2000

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16-Bromoepiandrosterone (HE2000) is a synthetic androstane steroid that has immune effects in preclinical models of malaria, tuberculosis, and infection with human immunodeficiency virus. In pilot studies, 42 patients with confirmed uncomplicated *Plasmodium falciparum* malaria were treated with a seven-day course of HE2000 by either buccal administration or intramuscular injection. Of the 42 patients, 41 showed a 50% reduction in blood levels of parasites, the primary endpoint of the study. Of these, 32 (76%) cleared malaria parasites below detectable levels. All febrile patients became afebrile by

the end of treatment. There was no reduction in gametocyte forms. Adverse events were transient and mild to moderate in intensity. The anti-malarial response was generally similar with either the intramuscular or buccal routes of administration. HE2000 shows a safety profile and pharmacologic activity worthy of further investigation to understand its role in the treatment of malaria, perhaps in combination with anti-malarial agents.

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SRC-FAMILY KINASE-DEPENDENT DISRUPTION OF ENDOTHELIAL BARRIER FUNCTION BY **PLASMODIUM FALCIPARUM** MEROZOITE PROTEINS

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Pulmonary complication in severe *Plasmodium falciparum* malaria is manifested as a prolonged impairment of gas transfer or the more severe acute respiratory distress syndrome (ARDS). In either clinical presentation, vascular permeability is a major component of the pathologic process. In this report, we examined the effect of clinical *P falciparum* isolates on barrier function of primary dermal and lung microvascular endothelium *in vitro*. We showed that parasite sonicates but not intact infected erythrocytes disrupted endothelial barrier function in a Srcfamily kinase-dependent manner. The abnormalities were manifested both as discontinuous immunofluorescence staining of the junctional proteins ZO-1, claudin 5, and VE-cadherin and the formation of interendothelial gaps in monolayers. These changes were associated with a loss in total protein content of claudin 5 and redistribution of ZO-1 from the cytoskeleton to the membrane andthe cytosolicandnuclear fractions. There was minimal evidence of a proinflammatory response or direct cellular cytotoxicity or cell death. The active component in sonicates appeared to be a merozoite-associated protein. Increased permeability was also induced by *P falciparum* glycophosphatidylinositols (GPIs) and food vacuoles. These results demonstrate that parasite components can alter endothelial barrier function and thus contribute to the pathogenesis of severe falciparum malaria.

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SIGNIFICANT ASSOCIATION BETWEEN TNF-A (TNF) PROMOTER ALLELE (21031C, 2863C, AND 2857C) AND CEREBRAL MALARIA IN THAILAND

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We examined a possible association of three single nucleotide polymorphisms (SNPs) of the tumor necrosis factor alpha (TNF) promoter 21031T>C (rs1799964), 2863C>A(rs1800630), and2857C>T (rs1799724) with severe malaria in 466 adult patients having Plasmodium falciparum malaria in northwest Thailand. Four TNF promoter alleles comprising these three SNPs were detected in the studied population. The frequency of the TNF U04 allele designated 21031C, 2863C, and 2857C was found to be significantly greater in patients with cerebral malaria than in patients with mild malaria (12.6%, cerebral malaria vs 5.6%, mild malaria; odds ratio 2.5; P 0.002). The association of U04 with susceptibility to cerebral malaria was not caused by linkage disequilibrium with any specific HLA-B and -DRB1 alleles.

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CONTRASTING GENETIC STRUCTURE IN **PLASMODIUM VIVAX** POPULATIONS FROM ASIA AND SOUTH AMERICA

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Populations of Plasmodium falciparum show striking differences in linkage disequilibrium, population differentiation and diversity, but only fragmentary data exists on the genetic structure of *Plasmodium vivax*. We genotyped nine tandem repeat loci bearing 2-8 bp motifs from 345 P. vivax infections collected from three Asian countries and from five locations in Colombia. We observed 9-37 alleles per locus and high diversity (He = 0.72-0.79, mean = 0.75) in all countries. Numbers of multiple clone infections varied considerably: these were rare in Colombia and India, but > 60% of isolates carried multiple alleles in at least one locus in Thailand and Laos. However, only one or two of the nine loci show >1 allele in many samples, suggesting that mutation within infections may result in overestimation of true multiple carriage rates. Identical nine-locus genotypes were frequently found in Colombian populations, contributing to strong linkage disequilibrium. These identical genotypes were strongly clustered in time, consistent with epidemic transmission of clones and subsequent breakdown of allelic associations, suggesting high rates of inbreeding and low effective recombination rates in this country. In contrast, identical genotypes were rare and loci were randomly associated in all three Asian populations, consistent with higher rates of outcrossing and recombination. We observed low but significant differentiation between different Asian countries (standardized FST = 0.13-0.45). In comparison, we see greater differentiation between collection locations within Colombia (standardized FST = 0.4-0.7), and strong differentiation between continents (standardized FST = 0.48-0.79). The observed heterogeneity in multiple clone carriage rates, linkage disequilibrium and population differentiation are similar in some, but not all, respects to those observed in *P. falciparum*, and have important implications for the design of association mapping studies, and interpretation of P. vivax epidemiology.

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RELAPSES OF **PLASMODIUM VIVAX** INFECTION USUALLY RESULT FROM ACTIVATION OF HETEROLOGOUS HYPNOZOITES

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BACKGROUND: Relapses originating from hypnozoites are characteristic of *Plasmodium vivax* infections. Thus, reappearance of parasitemia after treatment can result from relapse, recrudescence, or reinfection. It has been assumed that parasites causing relapse would be a subset of the parasites that caused the primary infection. METHODS: Paired samples were collected before initiation of antimalarial treatment and at recurrence of parasitemia from 149 patients with vivax malaria in Thailand (n=36), where reinfection could be excluded, and during field studies in Myanmar (n=75) and India (n=38). RESULTS: Combined genetic data from 2 genotyping approaches showed that novel *P. vivax* populations were present in the majority of patients with recurrent infection (107 [72%] of 149 patients overall [78% of patients in Thailand, 75% of patients in Myanmar {Burma}, and 63% of patients in India]). In 61% of the Thai and Burmese patients and in 55% of the Indian patients, the recurrent infections contained none of the parasite genotypes that caused the acute infection. CONCLUSIONS: The *P. vivax* populations emerging from hypnozoites commonly differ from the populations that caused the acute episode. Activation of heterologous hypnozoite populations is the most common cause of first relapse in patients with vivax malaria.

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EFFICACY OF ATOVAQUONE-PROGUANIL FOR TREATMENT OF ACUTE MULTIDRUG-RESISTANT **PLASMODIUM FALCIPARUM** MALARIA IN THAILAND

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A combination of atovaquone-proguanil (Malarone ; GlaxoSmithKline, Research Triangle Park, NC) was previously shown to be highly effective in the treatment of uncomplicated *Plasmodium falciparum* malaria. However, there are only limited recent efficacy data, particularly from regions of multidrug resistance. In this study, we examined the efficacy of atovaquone-proguanil for the treatment of uncomplicated *P. falciparum* malaria on the Thailand-Myanmar border. Patients were given directly observed atovaquone-proguanil (1,000 mg/400 mg) once a day for three days and followed-up for four weeks in a non-transmission area. Of 140 eligible patients enrolled in this open-label study, 97.8% (95% confidence interval _ 95.4-100%) responded to therapy and remained clear of parasitemia at follow-up. Mean parasite clearance time was 41.9 hours and mean fever clearance time was 37.1 hours. On the basis of genotyping, three cases of treatment failure were identified (1 RIII and 2 RI). These data indicate that atovaquone-proguanil remains highly efficacious for the treatment of multidrug-resistant *P. falciparum* malaria in Thailand.

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CLINICAL EFFICACY OF CHLOROQUINE VERSUS ARTEMETHER-LUMEFANTRINE FOR **PLASMODIUM VIVAX** TREATMENT IN THAILAND

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Chloroquine remains the drug of choice for the treatment of vivax malaria in Thailand. Mixed infections of falciparum and vivax malaria are also common in South-East Asia. Laboratory confirmation of malaria species is not generally available. This study aimed to find alternative regimens for treating both malaria species by using falciparum antimalarial drugs. From June 2004 to May 2005, 98 patients with *Plasmodium vivax* were randomly treated with either artemether-lumefantrine (n = 47) or chloroquine (n = 51). Both treatments were followed by 15 mg of primaquine over 14 days. Adverse events and clinical and parasitological outcomes were recorded and revealed similar in both groups. The cure rate was 97.4% for the artemether-lumefantrine treated group and 100% for the chloroquine treated group. We concluded that the combination of artemether-lumefantrine and primaquine was well tolerated, as effective as chloroquine and primaquine, and can be an alternative regimen for treatment of vivax malaria especially in the event that a mixed infection of falciparum and vivax malaria could not be ruled out.

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IN VITRO-CLINICAL CORRELATIONS FOR AMPHOTERICIN B SUSCEPTIBILITY IN AIDS-ASSOCIATED CRYPTOCOCCAL MENINGITIS

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Reliable measures of antifungal drug susceptibility are needed. We tested the susceptibility of *Cryptococcus neoformans* from patients treated with amphotericin B. In vitro susceptibility employed a modified broth macrodilution method. We demonstrate a strong correlation between the quantitative measures of in vitro amphotericin B susceptibility and the quantitative response observed in patients.

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DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHIC TANDEM MASS SPECTROMETRIC METHOD FOR DETERMINATION OF OSELTAMIVIR AND ITS METABOLITE OSELTAMIVIR CARBOXYLATE IN PLASMA, SALIVA AND URINE

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A bioanalytical method for the analysis of oseltamivir (OP) and its metabolite oseltamivir carboxylate (OC) in human plasma, saliva and urine using off-line solid-phase extraction and liquid chromatography coupled to positive tandem mass spectroscopy has been developed and validated. OP and OC were analysed on a ZIC-HILIC column (50mm2.1 mm) using a mobile phase gradient containing acetonitrile-ammonium acetate buffer (pH 3.5; 10 mM) at a flow rate of 500 _L/min. The method was validated according to published FDA guidelines and showed excellent performance. The lower limit of quantification for OP was determined to be 1, 1 and 5 ng/mL for plasma, saliva and urine, respectively and for OC was 10, 10 and 30 ng/mL for plasma, saliva and urine, respectively. The upper limit of quantification for OP was determined to be 600, 300 and 1500 ng/mL for plasma, saliva and urine, respectively and for OC was 10,000, 10,000 and 30,000 ng/mL for plasma, saliva and urine, respectively. The within-day and between-day precisions expressed as R.S.D., were lower than 5% at all tested concentrations for all matrices and below 12% at the lower limit of quantification range. Matrix effects were thoroughly evaluated both graphically and quantitatively. No matrix effects were detected for OP or OC in plasma or saliva. Residues from the urine matrix (most likely salts) caused some ion suppression for both OP and its deuterated internal standard but had no effect on OC or its deuterated internal standard. The suppression did not affect the quantification of OP.

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PATIENT AND SAMPLE-RELATED FACTORS THAT EFFECT THE SUCCESS OF IN VITRO ISOLATION OF ORIENTIA TSUTSUGAMUSHI

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Orientia tsutsugamushi is the causative agent of scrub typhus infection, a major cause of human disease in rural areas of Southeast Asia. Twenty-six blood samples collected from patients with serologically proven scrub typhus during a six month period were sent to Bangkok (535 km from the clinical site) by road at ambient temperature (average daily temperature range: 27.1-29.1 degrees C) for attempted *in vitro* isolation in Vero cells. O. tsutsugamushi was isolated from 12 samples (sensitivity 46.7%) with the time to isolation ranging from 16 to 37 days [median 27 days, inter-quartile range (IQR) 22.5-33.5 days]. Patient factors such as days of fever and *O. tsutsugamushi* IgM antibody titer, transport factors such as transit time, and isolate genotype (Karp and Gilliam/Kawasaki) were assessed to determine their influence on the outcome of in vitro isolation. None of the factors significantly influenced the isolation outcome. This study demonstrates that *O. tsutsugamushi* can often be isolated *in vitro* from the blood of scrub typhus patients when transported at ambient tropical temperatures for many days.

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SKIN REACTION AFTER DESENSITIZATION NOT PREDICT THE OUTCOME OF SHORT COURSE SULFA DESENSITIZATION PROTOCOL AMONG AIDS PATIENTS

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RATIONALE: HIV/AIDS is known as a risk factor of sulfa hypersensitivity, commonly reported in drug allergy. Though avoidance of a culprit and use of alternative drugs can be an easier approach to intolerable patients, still sulfa is necessary in some conditions. Short course protocol of sulfa desensitization can be an option to augment continuing tolerability of sulfa in case of need.

OBJECTIVE: To assess the outcome of using the developed 6 and a half hour desensitization protocol for sulfa allergy among HIV/AIDS.

RESULTS: The desensitization protocol for sulfa allergy has been considered to shorten the duration of graded challenge and hospitalization. 6 HIV/AIDS who previously developed generalized maculopapular rash after trimethoprimsulfamethoxazole (TMP-SMX) exposure were again indicated for sulfa treatment. Median (range) of age and CD4 count were 31 (22-40) and 111 (70-181), respectively. No adverse event was found during challenge following the protocol, however, 5/6 developed cutaneous burning sensation followed by generalized maculopapular rash with a median onset after the last dose of 12 hours. Only antihistamines were used. The symptoms resolved within 6 days (median of 2 days, range 1-6 days). All can continue daily TMP-SMX up to 3 months or until no indication.

CONCLUSION: Eventually, rash and fever have been found after TMPSMX challenge. Early adverse event may not persist and predict the longterm tolerability. In summary, a six and a half hour graded challenge is safe and effective to enable more sulfa allergy experienced patients to take TMP-SMX.

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COMBINED MOLECULAR AND CLINICAL ASSESSMENT OF **PLASMODIUM FALCIPARUM** ANTIMALARIAL DRUG RESISTANCE IN THE LAO PEOPLE'S DEMOCRATIC REPUBLIC (LAOS)

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Molecular markers provide a rapid and relatively inexpensive approach for assessing antimalarial drug susceptibility. We collected 884 *Plasmodium falciparum*-infected blood samples from 17 Lao provinces. Each sample was genotyped for 11 codons in the chloroquine resistance transporter (*pfcrt*), dihydrofolate reductase (*pfdhfr*), and dihydropteroate synthase (*pfdhps*) genes. The samples included 227 collected from patients recruited to clinical trials. The *pfcrt K76T* mutation was an excellent predictor of treatment failure for both chloroquine and chloroquine plus sulfadoxine-pyrimethamine, and mutations in both *pfdhfr* and *pfdhps* were predictive of sulfadoxine-pyrimethamine treatment failure. In multivariate analysis, the presence of the *pfdhfr* triple mutation (51 + 59 + 108) was strongly and independently correlated with sulfadoxine-pyrimethamine failure (odds ratio_9.1, 95% confidence interval_1.4-60.2, *P*_0.017). Considerable geographic heterogeneity in allele frequencies occurred at all three loci with lower frequencies of mutant alleles in southern than in northern Laos. These findings suggest that chloroquine and sulfadoxinepyrimethamine are no longer viable therapy in this country.

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INDEPENDENT EVOLUTION OF PYRIMETHAMINE RESISTANCE IN PLASMODIUM FALCIPARUM ISOLATES IN MELANESIA

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Pyrimethamine resistance in *Plasmodium falciparum* has previously been shown to have emerged once in Southeast Asia, from where it spread to Africa. Pyrimethamine resistance in this parasite is known to be conferred by mutations in the gene encoding dihydrofolate reductase (*dhfr*). We have analyzed polymorphisms in *dhfr* as well as microsatellite haplotypes flanking this gene in a total of 285 isolates from different regions of Melanesia (Papua New Guinea, Vanuatu, and the Solomon Islands) and Southeast Asia (Thailand and Cambodia). Nearly all isolates (92%) in Melanesia were shown to carry a *dhfr* double mutation (CNRNI [underlining indicates the mutation]) at positions 50, 51, 59, 108, and 164, whereas 98% of Southeast Asian isolates were either triple (CIRNI) or quadruple (CIRNL) mutants. Microsatellite analysis revealed two distinct lineages of *dhfr* double mutants in Melanesia. One lineage had the same microsatellite haplotype as that previously reported for Southeast Asia and Africa, suggesting the spread of this allele to Melanesia from Southeast Asia. The other lineage had a unique, previously undescribed microsatellite haplotype, indicative of the *de novo* emergence of pyrimethamine resistance in Melanesia.

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THE GENOTYPES OF **GYPA** AND **GYPB** CARRYING THE MNSs ANTIGENS ARE NOT ASSOCIATED WITH CEREBRAL MALARIA

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The Japan Society of Human Genetics and Springer 200

Plasmodium falciparum invades erythrocytes via several routes using different red blood cell receptors that include glycophorin A (GYPA) and glycophorin B (GYPB). *GYPA* has two codominant alleles, i.e., *M* and *N*, that correspond to the M and N antigens, which differ by two amino acids (S1L, G5E); the codominant alleles of *GYPB*, i.e., *S* and *S*, correspond to the S and s antigens, which differ by a single amino acid (T29M). If these antigens influence the efficiency of erythrocyte invasion by malaria parasites, the MNSs phenotype may be associated with the severity of malaria. To examine this, the *GYPA* and *GYPB* genotypes carrying the MNSs antigens were analyzed in 109 and 203 Thai patients with cerebral malaria and mild malaria, respectively. Neither the genotype nor allele frequencies at each locus were statistically different between the cerebral and mild malaria patients. Thus, we conclude that the MNSs antigens do not reveal the difference in susceptibility to cerebral malaria.

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ANALYSES OF CYTOCHROME B MUTATIONS IN **PLASMODIUM FALCIPARUM** ISOLATES IN THAI-MYANMAR BORDER

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BACKGROUND: The combination of atovaquone and proguanil (Malarone) has been established as a drug of choice to prevent and treat multi-drug resistant *Plasmodium (P) falciparum* malaria in travelers. However, several cases of resistance against Malarone have been reported in some parts of Africa, and many of the cases are believed to be associated with mutations at the codon 268 of cytochrome b gene in mitochondria of *P falciparum*. The aim of the study was to estimate the effectiveness of Malarone in treatment and prophylaxis for the travelers to Thai-Myanmar border where multi-drug resistant malaria is highly endemic. **METHODS:** Seventy *P falciparum* samples obtained from patients from Thai-Myanmar border were sequenced to detect mutations around the codon 268. The same samples were also sequenced to detect *P. falciparum* chloroquine resistance transporter mutation (PfCRT K76T). **RESULTS:** All the 70 samples showed no mutations at the codon 268 of cytochrome b gene. Whereas, 50 samples, whose *pfcrt* genes were sequenced successfully, had an identical genotype for K76T mutation. **CONCLUSION:** In Asian countries, even in the multi-drug resistant areas in the great Mekong region, no case of Malarone resistance has been reported clinically or genetically thus far. In this study, all the *P. falciparum* parasites tested successfully were shown to be chloroquine resistant but atovaquone susceptible genetically. The more the usefulness of Malarone increases for both treatment and prophylaxis, the wider the drug-resistance against Malarone may spread in the region. Although the total number of samples examined is not large, it is concluded from these findings that Malarone should be recommended for prophylaxis of malaria for travelers to the Mekong region.

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IN VITRO ANTIMALARIAL ACTIVITY OF AZITHROMYCIN, ARTESUNATE, AND QUININE IN COMBINATION AND CORRELATION WITH CLINICAL OUTCOME

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Azithromycin when used in combination with faster-acting antimalarials has proven efficacious in treating Plasmodium falciparum malaria in phase 2 clinical trials. The aim of this study was to establish optimal combination ratios for azithromycin in combination with either dihydroartemisinin or quinine, to determine the clinical correlates of in vitro drug sensitivity for these compounds, and to assess the cross-sensitivity patterns. Seventy-three fresh P. falciparum isolates originating from patients from the western border regions of Thailand were successfully tested for their drug susceptibility in a histidine-rich protein 2 (HRP2) assay. With overall mean fractional inhibitory concentrations of 0.84 (95% confidence interval [CI] 0.77 to 1.08) and 0.78 (95% CI _ 0.72 to 0.98), the interactions between azithromycin and dihydroartemisinin, as well as quinine, were classified as additive, with a tendency toward synergism. The strongest tendency toward synergy was seen with a combination ratio of 1:547 for the combination with dihydroartemisinin and 1:44 with quinine. The geometric mean 50% inhibitory concentration (IC50) of azithromycin was 2,570.3 (95% CI _ 2,175.58 to 3,036.58) ng/ml. The IC50s for mefloquine, quinine, and chloroquine were 11.42, 64.4, and 54.4 ng/ml, respectively, suggesting a relatively high level of background resistance in this patient population. Distinct correlations ($R_0.53$; $P_0.001$) between quinine in vitro results and parasite clearance may indicate a compromised sensitivity to this drug. The correlation with dihydroartemisinin data was weaker (R = 0.34; P = 0.038), and no such correlation was observed for azithromycin. Our in vitro data confirm that azithromycin in combination with artemisinin derivatives or quinine exerts additive to synergistic interactions, shows no cross-sensitivity with traditional antimalarials, and has substantial antimalarial activity on its own.

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EFFECTS OF MALARIA HEME PRODUCTS ON RED BLOOD CELL DEFORMABILITY

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In falciparum malaria, the deformability of the entire erythrocyte population is reduced in proportion to disease severity, and this compromises microcirculatory blood flow through vessels partially obstructed by cytoadherent parasitized erythrocytes. The cause of rigidity of uninfected erythrocytes in not known but could be mediated by malaria heme products. In this study, we show that red blood cell deformability (RBC-D), measured by laser-assisted optical rotational cell analyzer, decreased in a dose-dependent manner after incubation with hemin and hydrogen peroxide but not with hemoglobin or _-hematin. Hemin also reduced mean red cell volume. Albumin decreased and *N*-acetylcysteine (NAC) both prevented and reversed rigidity induced by hemin. Hemin-induced oxidative damage of the membrane seems to be a more important contributor to pathology than cell shrinkage because the antioxidant NAC restored RBC-D but not red blood cell volume. The findings suggest novel approaches to the treatment of potentially lethal malaria

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PRODUCTION OF ERYTHROPOIETIC CELLS IN VITRO FOR CONTINUOUS CULTURE OF PLASMODIUM VIVAX

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Plasmodium vivax cannot be maintained in a continuous culture. To overcome this major obstacle to *P vivax* research, we have developed an *in vitro* method to produce susceptible red blood cell (RBC) precursors from freshly isolated human cord hematopoietic stem cells (HSCs), which were activated with erythropoietin to differentiate into erythroid cells. Differentiation and maturation of erythroid cells were monitored using cell surface markers (CD71, CD36, GPA and Fy6). Duffy+ reticulocytes appeared after 10 days of erythroid cell culture and exponentially increased to high numbers on days 14-16. Beginning on day 10 these erythroid cells, referred to as growing RBCs (gRBCs), were co-cultured with *P. vivax*-infected blood directly isolated from patients. Parasite-infected gRBCs were detected by Giemsa staining and a P. vivax-specific immunofluorescence assay in 11 out of 14 *P. vivax* isolates. These *P. vivax* cultures were continuously maintained for more than 2 weeks by supplying fresh gRBCs; one was maintained for 85 days before discontinuing the culture. Our results demonstrate that gRBCs derived *in vitro* from HSCs can provide susceptible Duffy+ reticulocytes for continuous culture of *P. vivax*. Of particular interest, we discovered that parasites were able to invade nucleated erythroid cells or erythroblasts that are normally in the bone marrow. The possibility that *P. vivax* causes erythroblast destruction and hence inflammation in the bone marrow needs to be addressed.

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LOOP-MEDIATED ISOTHERMAL PCR (LAMP) FOR THE DIAGNOSIS OF FALCIPARUM MALARIA

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A recently described loop-mediated isothermal polymerase chain reaction (LAMP) for molecular detection of *Plasmodium falciparum* was compared with microscopy, PfHRP2-based rapid diagnostic test (RDT), and nested polymerase chain reaction (PCR) as the "gold standard" in 115 Bangladeshi in-patients with fever. DNA extraction for LAMP was conducted by conventional methods or simple heating of the sample; test results were either assessed visually or by gel electrophoresis. Conventional DNA extraction followed by gel electrophoresis had the highest agreement with the reference method (81.7%, 0.64), with a sensitivity (95% CI) of 76.1% (68.3-83.9%), comparable to RDT and microscopy, but a specificity of 89.6% (84.0-95.2%) compared with 100% for RDT and microscopy. DNA extraction by heat treatment deteriorated specificity to unacceptable levels. LAMP enables molecular diagnosis of falciparum malaria in settings with limited technical resources but will need further optimization. The results are in contrast with a higher accuracy reported in an earlier study comparing LAMP with a non-validated PCR method.

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DOXYCYCLINE VERSUS AZITHROMYCIN FOR TREATMENT OF LEPTOSPIROSIS AND SCRUB TYPHUS

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Leptospirosis and scrub typhus are important causes of acute fever in Southeast Asia. Options for empirical therapy include doxycycline and azithromycin, but it is unclear whether their efficacies are equivalent. We conducted a multicenter, open, randomized controlled trial with adult patients presenting with acute fever (<15 days), without an obvious focus of infection, at four hospitals in Thailand between July 2003 and January 2005. Patients were randomly allocated to receive either a 7-day course of doxycycline or a 3-day course of azithromycin. The cure rate, fever clearance time, and adverse drug events were compared between the two study groups. A total of 296 patients were enrolled in the study. The cause of acute fever was determined for 151 patients (51%): 69 patients (23.3%) had leptospirosis; 57 patients (19.3%) had scrub typhus; 14 patients (4.7%) had murine typhus; and 11 patients (3.7%) had evidence of both leptospirosis and a rickettsial infection. The efficacy of azithromycin was not inferior to that of doxycycline for the treatment of both leptospirosis and scrub typhus, with comparable fever clearance times in the two treatment arms. Adverse events occurred more frequently in the doxycycline group than in the azithromycin group (27.6% and 10.6%, respectively; $P_0.0.2$). In conclusion, doxycycline is an affordable and effective choice for the treatment of both leptospirosis and scrub typhus. Azithromycin was better tolerated than doxycycline but is more expensive and less readily available.

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IgG ANTIBODY PROFILE TO C-TERMINAL REGION OF **PLASMODIUM VIVAX** MEROZOITE SURFACE PROTEIN-1 IN THAI INDIVIDUALS EXPOSED TO MALARIA

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Naturally acquired immune response to C-terminal region of *Plasmodium vivax* merozoite surface protein1 (PvMSP1) in 200 individuals with recent clinical episodes of malaria from malaria endemic areas along Thai-Myanmar border in the west and Thai-Cambodia border in the east of Thailand was evaluated by enzyme-linked immunosorbent assay (ELISA). The anti-PvMSP1-IgG antibody was observed in 110 individuals (55%). Among IgG responders, IgG1 coexpressed with IgG3 were the predominant subclasses. The levels of anti-PvMSP1 total IgG, IgG1 and IgG3 antibody response seem to be increased with age although no detectable significant correlation was found (r = 0.004, p = 0.484 for total IgG; r = 0.035, p = 0.386 for IgG1; r = -0.600, p = 0.142 for IgG2; r = 0.077, p = 0.227 for IgG3; r = 0.664, p = 0.051 for IgG4). However, the mean level of specific total IgG was highest in the age group of >40 years. These levels of either specific total IgG, IgG1 and IgG3 antibody response related with the lower of parasitemia density was observed although no significant correlation was found. Our data indicate that individuals exposed to vivax malaria in Thailand developed antibodies to the potential candidate vaccine antigen, PvMSP1 (C-terminal).

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SOCIAL HARMS IN INJECTING DRUG USERS PARTICIPATING IN THE FIRST PHASE III HIV VACCINE TRIAL IN THAILAND

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OBJECTIVE: To study related social harms due to identification with a group of participants in an HIV-1 vaccine trial who are potentially high risk for HIV/AIDS. MATERIAL AND METHOD: Two thousand, five hundred forty-six injecting drug users (IDU) were enrolled in a 36-month vaccine trial. Volunteers received education and risk reduction counseling at every six-month study visit. Social harms were not actively solicited, but volunteers were encouraged to report any during the process of counseling at every six-month visit. If a social harm was reported, a questionnaire was administered and the harm was tracked If necessary, clinic staff assisted in resolving the social harm. RESULTS: Thirty-nine social harms were reported by 37 participants; 33 (84.6%) were disturbances in personal relationships, three (7.7%) in employment, one (2.6%) was medically related, one (2.6%) was related to admission in the military and one (2.6%) was related with misbelieve about the vaccine. The most common reason for disturbances in personal relationships was suspicion of HIV infection (n=20). The impact of these harms on quality of life was characterized as minimal by 31 (79.5%) participants, as moderate by seven (17.9%), and as major by one (2.6%). All social harms were documented to be resolved by the end of the study. CONCLUSION: A few participants reported study-related social harms during the course of the trial. Most harm had minimal impact and all could be resolved by the end of the present study.

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CONCURRENT **SALMONELLA** BACTEREMIA IN **P. VIVAX** INFECTION--A REPORT OF 2 CASES AT THE HOSPITAL FOR TROPICAL DISEASES, THAILAND

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Malaria and concurrent bacteremia has been described in many reports, most of them with *P. falciparum*. Concurrent bacteremia with *P. vivax* infected patients is very rare. We reported 2 cases of salmonella bacteremia with *P. vivax* infection. Both patients presented with fever and the diagnosis of *P. vivax* was confirmed microscopically. The first patient presented with fever, jaundice, shock and renal failure which rarely occurs with *P. vivax* infection. The second patient had no clinical response after receiving standard antimalarial drugs. Hemoculture was positive for Salmonella spp in both cases. They recovered completely after appropriate antibiotics and antimalarial treatment.

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OVERESTIMATING RESISTANCE IN FIELD TESTING OF MALARIA PARASITES: SIMPLE METHODS FOR ESTIMATING HIGH EC₅₀ VALUES USING A BAYESIAN APPROACH

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Conventional methods of assessing *in-vitro* antimalarial drug-concentration effect relationships in field testing of fresh isolates assess each parasite isolate individually. This leads to systematic overestimation of EC_{50} values for the most resistant isolates, and thus overestimation of the degree of resistance. In antimalarial drug-susceptibility studies conducted on the north-western border of Thailand the overestimation of EC_{50} for the most resistant isolate ranged from 15% for artesunate to 43% for mefloquine. If isolates cannot be stored for re-testing, more accurate estimations of the degree of resistance can be obtained using a Bayesian approach to data analysis which is described here.

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PREDICTIVE SCORE OF UNCOMPLICATED FALCIPARUM MALARIA PATIENTS TURNING TO SEVERE MALARIA

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In acute uncomplicated falciparum malaria, there is a continuum from mild to severe malaria. However, no mathematical system is available to predict uncomplicated falciparum malaria patients turning to severe malaria. This study aimed to devise a simple and reliable model of Malaria Severity Prognostic Score (MSPS). The study was performed in adult patients with acute uncomplicated falciparum malaria admitted to the Bangkok Hospital for Tropical Diseases between 2000 and 2005. Total 38 initial clinical parameters were identified to predict the usual recovery or deterioration to severe malaria. The stepwise multiple discriminant analysis was performed to get a linear discriminant equation. The results showed that 4.3% of study patients turned to severe malaria. The MSPS = 4.38 (schizontemia) + 1.62 (gametocytemia) + 1.17 (dehydration) + 0.14 (overweight by body mass index; BMI) + 0.05 (initial pulse rate) + 0.04 (duration of fever before admission) - 0.50 (past history of malaria in last 1 year) - 0.48 (initial serum albumin) - 5.66. Based on the validation study in other malaria patients, the sensitivity and specificity were 88.8% and 88.4%, respectively. We conclude that the MSPS is a simple screening tool for predicting uncomplicated falciparum malaria patients turning to severe malaria. However, the MSPS may need revalidation in different geographical areas before utilized at specific places.

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DIFFERENTIAL REGULATION OF IgG SUBCLASSES AND IgE ANTIMALARIAL ANTIBODY RESPONSES IN COMPLICATED AND UNCOMPLICATED **PLASMODIUM FALCIPARUM** MALARIA

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The aim of this study was to assess the immunoglobulin (Ig)-subclass distribution of antimalarial antibody responses in 110 and 169 Thai patients with complicated and uncomplicated *Plasmodium falciparum* malaria, respectively. Antimalarial plasma IgG subclasses and IgE antibody levels against a crude malaria blood stages, and antigen preparation were determined using enzyme-linked immunosorbent assay (ELISA). On admission, the levels of anti-*P. falciparum* IgG1, IgG2 and IgG3 were significantly lower in patients with complicated malaria than uncomplicated malaria (IgG1, P< 0.0001; IgG2, P < 0.0001; IgG3, P < 0.0001). The levels of antimalarial IgE were slightly lower, but not statistically significant (P =0.389) in the complicated malaria. After adjusting all antibody levels and age, anti-*P. falciparum* IgG3 levels remained significantly associated with complicated malaria. None of the other antibody concentrations showed statistically significant associations with complicated malaria. The anti-*P. falciparum* IgG3 levels were related to the IgG1 as well as IgG2 levels. A correlation between anti-*P. falciparum* IgG2 and IgE was observed in the complicated malaria group, and this may indicate their roles in the severity of disease. Our data suggest that anti-*P. falciparum* IgG3 is associated with a reduced risk of complicated malaria and that antimalarial Ig-subclasses are differently regulated in patients with complicated and uncomplicated malaria.

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RELATIVE LEVELS OF IL4 AND IFN- γ IN COMPLICATED MALARIA: ASSOCIATION WITH **IL4** POLYMORPHISM AND PERIPHERAL PARASITEMIA

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Functional *IL4*-590 C/T polymorphisms and the relative amounts of IL4 and IFN- γ were investigated in relation to severity of malaria in 110 and 169 Thai patients with complicated and uncomplicated malaria, respectively. The plasma IL4 and IFN- γ levels were determined by ELISA and the *IL4*-590 C/T polymorphisms were genotyped. The IFN- γ levels were significantly elevated in patients with complicated malaria in the initial stage of the disease before treatment compared to the levels found with uncomplicated malaria (231 pg/ml versus 150 pg/ml, p = 0.0029), while the IL4 levels were significantly elevated 7 days after treatment (167 pg/ml versus 81 pg/ml, p = 0.0003). Our study did not reveal any association between the *IL4*-590 C/T transition and the severity of malaria. However, a significant difference in the IL4 to IFN- γ ratio between patients with complicated malaria was observed only in patients with *IL4*-590 T allele homozygosity (geometric mean: 0.321 versus 0.613, p = 0.0087 for TT allele). A significant inverse correlation between IL4 to IFN- γ ratio and peripheral parasitemia was observed only in complicated malaria patients carrying TT genotype (r = -0.283, p = 0.046). These results suggest that the *IL4*-590 C/T polymorphism may play a role in the balance between IL4 and IFN- γ , as well as in the control of parasitemia, which in turn may alter the severity of malaria.

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GENETIC ANALYSIS OF THE DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE GENE FROM GEOGRAPHICALLY DIVERSE ISOLATES OF **PLASMODIUM MALARIAE**

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Plasmodium malariae, the parasite responsible for quartan malaria, is transmitted in most areas of malaria endemicity and is associated with significant morbidity. The sequence of the gene coding for the enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS) was obtained from field isolates of *P. malariae* and from the closely related simian parasite *Plasmodium brasilianum*. The two sequences were nearly 100% homologous, adding weight to the notion that they represent genetically distinct lines of the same species. A survey of polymorphisms of the *dhfr* sequences in 35 isolates of *P. malariae* collected from five countries in Asia and Africa revealed a low number of nonsynonymous mutations in five codons. In five of the isolates collected from southeast Asia, a nonsynonymous mutation was found at one of the three positions known to be associated with antifolate resistance in other *Plasmodium* species. Five isolates with the wild-type DHFR could be assayed for drug susceptibility in vitro and were found to be sensitive to pyrimethamine (mean 50% inhibitory concentration, 2.24 ng/ml [95% confidence interval, 0.4 to 3.1]).

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OUTCOMES IN HIV-INFECTED PATIENTS ON ANTIRETROVIRAL THERAPY WITH TUBERCULOSIS

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HIV-infected patients with active tuberculosis (TB) having CD4 counts <100/mm³ and who were antiretroviral therapy (ART) naïve were reviewed retrospectively to determine the outcomes of their tuberculosis infection. All patients received ART at or after receiving anti-TB treatment. Clinical manifestations, treatment regimens and outcomes were analyzed. Of 101 patients, 62 (61.4%) completed TB treatment. Of these, 53.2% were treated with a 6-month standard TB regimen, while the rest were treated with prolonged TB regimens. The median interval between anti-TB treatment and ART was 68 days (range: 0-381). Among the clinically cured patients 66.1% received rifampin concomitantly with nevirapine, and 32.3% received rifampin concomitantly with efavirenz. The treatment success rate was 75.6%, with a mortality rate of 6.1%. The risk factors for death were resistant TB (p=0.03) and poor compliance (p<0.05). Seven point nine percent had multi-drug resistant TB. Possible or probable immune reconstitution inflammatory syndrome (IRIS) was seen in 15 cases (14.9%). No life-threatening IRIS was reported, and it did not affect disease outcome (p=0.5). A shorter time between anti-TB treatment and ART onset was associated with the occurrence of IRIS (31 days vs 90 days; p<0.05). Regarding adverse drug effects, 44.6% had side effects due either to anti-TB drugs or ART. Sixty-six point one percent of them occurred within the first 2 months of TB treatment, and 43 (76.8%) had to stop or change either anti-TB treatment or ART. The mortality rate with TB and HIV on ART was low and the occurrence of IRIS did not carry any additional mortality.

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PREDISPOSING FACTORS FOR NEVIRAPINE TOXICITY AMONG AIDS PATIENTS WITH LOW BASELINE CD4 COUNT

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The objective of the study was to determine the predisposing factors and incidence of toxicity among AIDS patients treated with a nevirapine (NVP)-based regimen in clinical practice. A retrospective cohort study of representative samples of AIDS patients treated with a NVP-based regimen was performed. A total of 206 adult HIV/AIDS cases with median age (IQR) 33 years (range, 29-38 years), 51% male, treated between January 2004-December 2005, were included. Most (92.2%) of the patients were naïve to antiretroviral drug. The incidence of NVP toxicity was 1.09/100 person-months. The median onset time was 4 weeks post NVP initiation (2.57 weeks for skin toxicity and 12.43 weeks for hepatic toxicity). History of drug allergy and NVP toxicity were significantly associated (p = 0.006), as were sulfamethoxazole allergy and toxicity (p = 0.015). Regarding concomitant medication, concurrent anti-tuberculosis drugs significantly increased the risk of NVP associated liver toxicity (p = 0.001). Therefore, it is important to monitor adverse events from NVP, including liver function tests among HIV/AIDS patients with history of drug allergy, especially against sulfamethoxazole, and those concurrently treated with antituberculosis drugs.

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A DOMINANT CLONE OF **LEPTOSPIRA INTERROGANS** ASSOCIATED WITH AN OUTBREAK OF HUMAN LEPTOSPIROSIS IN THAILAND

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Background: A sustained outbreak of leptospirosis occurred in northeast Thailand between 1999 and 2003, the basis for which was unknown.

Methods and Findings: A prospective study was conducted between 2000 and 2005 to identify patients with leptospirosis presenting to Udon Thani Hospital in northeast Thailand, and to isolate the causative organisms from blood. A multilocus sequence typing scheme was developed to genotype these pathogenic Leptospira. Additional typing was performed for Leptospira isolated from human cases in other Thai provinces over the same period, and from rodents captured in the northeast during 2004. Sequence types (STs) were compared with those of Leptospira drawn from a reference collection. Twelve STs were identified among 101 isolates from patients in Udon Thani. One of these (ST34) accounted for 77 (76%) of isolates. ST34 was Leptospira interrogans, serovar Autumnalis. 86% of human Leptospira isolates from Udon Thani corresponded to ST34 in 2000/2001, but this figure fell to 56% by 2005 as the outbreak waned (p = 0.01). ST34 represented 17/24 (71%) of human isolates from other Thai provinces, and 7/8 (88%) rodent isolates. By contrast, 59 STs were found among 76 reference strains, indicating a much more diverse population genetic structure; ST34 was not identified in this collection.

Conclusions: Development of an MLST scheme for Leptospira interrogans revealed that a single ecologically successful pathogenic clone of L. interrogans predominated in the rodent population, and was associated with a sustained outbreak of human leptospirosis in Thailand.

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VIRAL HEPATITIS INFECTIONS AMONG DIALYSIS PATIENTS: THAILAND REGISTRY REPORT

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Background: Patients on dialysis are at high risk of acquiring viral hepatitis infections. However, there were only few data from Thailand. The aim of the present study was to assess the prevalence, incidence and associated risk factors of viral hepatitis infections among dialysis patients.

Methods: A retrospective study was conducted to evaluate 5179 medical records of dialysis patients from the Thailand Renal Replacement Therapy Registry.

Results: In 2002, the seroprevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were 6.3% (n = 2454) and 4.8% (n = 2167), respectively. HBV and HCV seroprevalence became 6.5% (n = 2585) and 4.3% (n = 2399) in 2003. The incidence of HBV and HCV infections were 1.5 and 2.4 cases per 1000 patientyears, respectively. Logistic regression analysis showed that age and gender were significant risk factors for HBV infection, but not for HCV infection. *Conclusion:* In Thailand, it was not uncommon for dialysis patients to acquire viral hepatitis infections. However, our prevalence is similar to reports from some other South-East Asian countries.

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SHORT-TERM IN VITRO CULTURE OF FIELD ISOLATES OF PLASMODIUM VIVAX USING UMBILICAL CORD BLOOD

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Plasmodium vivax research has been hampered by the lack of technology for culturing this parasite. Culturing *P. vivax* is difficult because the parasite selectively invades reticulocytes. Here we describe a modified procedure to establish and maintain short-term cultures of freshly collected *P. vivax* parasites using reticulocyte-enriched cord blood. Using this method, parasites could be cultured for a month. Manipulation of the culture allowed procurement of synchronized stages of the parasite. This short-term culture method can be easily adapted to study various aspects of the parasite biology.

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TOLL-LIKE RECEPTOR 2 IMPAIRS HOST DEFENSE IN GRAM-NEGATIVE SEPSIS CAUSED BY **BURKHOLDERIA PSEUDOMALLEI** (MELIOIDOSIS)

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Background: Toll-like receptors (TLRs) are essential in host defense against pathogens by virtue of their capacity to detect microbes and initiate the immune response. TLR2 is seen as the most important receptor for gram-positive bacteria, while TLR4 is regarded as the gram-negative TLR. Melioidosis is a severe infection caused by the gram-negative bacterium, Burkholderia pseudomallei, that is endemic in Southeast Asia. We aimed to characterize the expression and function of TLRs in septic melioidosis.

Methods and Findings: Patient studies: 34 patients with melioidosis demonstrated increased expression of CD14, TLR1, TLR2, and TLR4 on the cell surfaces of monocytes and granulocytes, and increased CD14, TLR1, TLR2, TLR4, LY96 (also known as MD-2), TLR5, and TLR10 mRNA levels in purified monocytes and granulocytes when compared with healthy controls. In vitro experiments: Whole-blood and alveolar macrophages obtained from TLR2 and TLR4 knockout (KO) mice were less responsive to B. pseudomallei in vitro, whereas in the reverse experiment, transfection of HEK293 cells with either TLR2 or TLR4 rendered these cells responsive to this bacterium. In addition, the lipopolysaccharide (LPS) of B. pseudomallei signals through TLR2 and not through TLR4. Mouse studies: Surprisingly, TLR4 KO mice were indistinguishable from wild-type mice with respect to bacterial outgrowth and survival in experimentally induced melioidosis. In contrast, TLR2 KO mice displayed a markedly improved host defenses as reflected by a strong survival advantage together with decreased bacterial loads, reduced lung inflammation, and less distant-organ injury.

Conclusions: Patients with melioidosis displayed an up-regulation of multiple TLRs in peripheral blood monocytes and granulocytes. Although both TLR2 and TLR4 contribute to cellular responsiveness to B. pseudomallei in vitro, TLR2 detects the LPS of B. pseudomallei, and only TLR2 impacts on the immune response of the intact host in vivo. Inhibition of TLR2 may be a novel treatment strategy in melioidosis.

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OPTIMIZATION OF CULTURE OF LEPTOSPIRA FROM HUMANS WITH LEPTOSPIROSIS

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A prospective study of 989 patients with acute febrile illness was performed in northeast Thailand to define the yield of *Leptospira* from four different types of blood sample. Based on a comparison of the yields from whole blood, surface plasma, deposit from spun plasma, and clotted blood samples from 80 patients with cultureproven leptospirosis, we suggest a sampling strategy in which culture is performed using whole blood and deposit from spun plasma.

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SHORT REPORT: QUANTITATION OF B. PSEUDOMALLEI IN CLINICAL SAMPLES

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We undertook a prospective study to quantitate *Burkholderia pseudomallei* in blood, pus, respiratory secretions, and urine obtained from 414 patients with melioidosis. The median was count 1.1, 1.5×104 , 1.1×105 , and 1.1×107 CFU/ mL in these sample types, respectively. This provides important insights into the likely feasibility of future studies such as expression microarray analysis using clinical material.

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CLINICAL DIAGNOSIS AND GEOGRAPHIC DISTRIBUTION OF LEPTOSPIROSIS, THAILAND

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We defined the positive predictive accuracy of a hospital-based clinical diagnosis of leptospirosis in 9 provinces across Thailand. Of 700 suspected cases, 143 (20%) were confirmed by laboratory testing. Accuracy of clinical diagnosis varied from 0% to 50% between the provinces and was highest during the rainy season. Most confirmed cases occurred in the north and northeast regions of the country.

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DIFFERENTIAL ROLES OF CD36, ICAM-1, AND P-SELECTIN IN PLASMODIUM FALCIPARUM CYTOADHERENCE IN VIVO

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Cytoadherence of *Plasmodium falciparum*-infected red blood cells (IRBCs) on human microvascular endothelium is mediated by synergistic adhesive interactions with different adhesion molecules *in vitro*. Here, the authors used a unique human/severe combined immunodeficient (SCID) mouse chimeric model to directly visualize IRBC-endothelial interactions in an intact human microvasculature *in vivo*. Stimulation of human skin grafts with 100 ng TNF- α for 4 h led to a dramatic reduction in the distance rolled by IRBCs before arrest, so that the majority of IRBCs adhered directly to the endothelium with a 1.8-fold increase in the number of adherent cells. The decrease in rolling distance and increase in adhesion could be reversed by anti-ICAM-1. More importantly, the effect of TNF- α could be seen only in the presence of CD36. A further increase in adhesion by 4.9-fold was observed after 24 h of TNF- α stimulation. The increase could be reversed by anti-ICAM-1. In histamine-stimulated grafts, the rolling flux fraction and adhesion increased by 2.8- and 1.6-fold, respectively. The increases were attributable to P-selectin as an inhibitory anti-P-selectin antibody abrogated both the increased rolling flux fraction and firm adhesion. These findings indicate that in addition to CD36, ICAM-1, and P-selectin are major contributors to the dynamic process of IRBC adhesion by different mechanisms in vivo.

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CLONING AND CHARACTERIZATION OF **PLASMODIUM VIVAX** SERINE HYDROXYMETHYLTRANSFERASE

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Serine hydroxymethyltransferase (SHMT), which catalyzes the reversible reaction of serine and tetrahydrofolate to alanine and methylenetetrahydrofolate, is one of the three enzymes in dTMP synthesis pathway that is highly active during cell division and has been proposed as a potential chemotherapeutic target in infectious diseases and cancer. This is the first study to describe nucleotide and amino acid sequences of SHMT from the malaria parasite Plasmodium vivax. Sequencing of 12 P. vivax isolates revealed limited polymorphisms in 3 noncoding regions. Its biological function is also reported. [©]2007 Elsevier Ireland Ltd. All rights reserved.

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INTEGRATION OF COMMUNITY ENGAGEMENT ACTIVITIES SUPPORT THE PRIME-BOOST PHASE III HIV VACCINE TRIAL IN THAILAND

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Background: A prime-boost phase III HIV Vaccine trial has been underway in Thailand since Sept 2003. The trial screened over 26,000 potential volunteers and enrolled over 16,400 volunteers by December 2005. Community engagement activities were used to provide understanding and support for the trial and to foster support for volunteers' participation.

Objective: To demonstrate the pattern and model of the volunteer relation activities and networks in this HIV vaccine trial

Methods: The key target groups for communication were identified: the volunteers and the community thought leaders. The appropriate activities were designed and assigned to each group. A two-way communication technique was used for group discussions. The volunteers were invited to join in "Volunteer Relation Activities" facilitated by health staff and an NGO team. This specially-designed activity was initiated and conducted mainly by the volunteers, which enhanced their sense of ownership and participation in the trial. Representative community leaders were invited to participate in community health forums and provided the opportunity to ask questions and make comments about the trial. The actively involved participants were selected to form the core of the community network.

Lesson Learned: In 2006, 38 volunteer clubs were established in the areas where screening had taken place. These are linked to each other and are facilitated by the district health office, MOPH, and NGOs. The number of volunteer clubs continues to increase due to active participation of the volunteers. Volunteer relation activities include: community service, peer HIV/AIDS education, religious and cultural events, sports activities and a motorcycle safety rally. Volunteer network between volunteer clubs has been encouraged sharing information and strengthening volunteer sense of ownership of the trial. Approximately 400 community health forums were conducted by the trained health staff. Community networking among active participants from various community health forums and volunteer relation activities has been initiated. Community networks have been established in both provinces, the network leaders have become community advisory board members.

Conclusion: Community support is a crucial part of field research. It is important to have multiple channels of activities to reach the target population and create the sense that community and researchers are in partnership for trial success.

Presented at: AIDS VACCINE 2007, Seattle, USA, August 20-23, 2007 (p.85).

WOMEN'S PARTICIPATION IN THE PHASE III EFFICACY TRIAL OF ALVAC HIV-1 VACCINE PRIMING, AIDSVAX VACCINE BOOSTING IN THAILAND

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Background: Developing an effective HIV vaccine is important to combat the growing number of HIV infections worldwide. Nearly half of the adults (15+) living with HIV in 2006 are women. In order for HIV vaccine trials to be implemented successfully, investigators need to consider gender issues and gender sensitive approaches to recruitment and enrollment. This may be difficult because of cultural and social norms. We attempted to determine whether there were differences in participant impact events (PIE) between men and women enrolled in the Phase III trial.

Methods: Randomized, double-blind placebo controlled trial involving eligible volunteers 18-30 years old. The study has completed enrollment and vaccination and is now in the follow up phase. General demographic characteristics, reasons for enrolment, follow up rate and participant impact events (PIE) in women were analyzed.

Results: 6334 out of 16402 (38.6%) participants were female. 66% were married. 20% were single, and the rest were divorced, widow or separated. 33% had primary education, 37% had junior secondary education, and 28% had senior

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secondary or higher education. Only 2% reported of no education. Among female participants, 76% were employed, 12% unemployed, and 12% housewives. Regarding reasons for interest in vaccine trial, 79% reported "altruism." Only 4.6 % (297) of women reported higher participant impact events which is significantly different from men (p< 0.01). Marital status was significantly associated with PIE among woman with PIE (p<0.01). 85.2% of PIE were related to personal relationship problems, and almost all PIE (92.4%) were resolved satisfactorily. 17.4% of women reporting a PIE withdrew from participation in spite of resolved events. There was no significant difference of withdrawal rate between male and female (p= 0.17). **Conclusion**: Recruitment of women in the Phase III trial was good (38.6%),

but there may be additional factors that contribute to lower enrollment than men. Women recruited into the trial were well educated and typically employed. Married women reported higher rates of PIE than the other status group. Resolution of PIE was satisfactory in most cases.

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THAI PRIME-BOOST HIV VACCINE PHASE III TRIAL: UPDATE 2007

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Background: Thai Ministry of Public Health launched the Phase III HIV-1 Prime boost trial in late 2003 collaboratively with Mahidol University, Armed Forces Research Institute of the Medical Sciences, NIAID-NIH and WRAIR.

Objective: To determine if the prime-boost (ALVAC HIV + AIDSVAX B/E) strategy (1) prevents HIV infection and (2) reduces viral load at set point after breakthrough infection

Methods: Community engagement activities were conducted widely in Chon Buri and Rayong provinces to recruit and enroll the potential volunteers. Community recruitment teams were trained to perform outreach activities; community leaders were informed, and various local and national media were used. Screened volunteers were randomly assigned to receive ALVAC HIV canary pox vector vaccine (or placebo) prime at 0, 4, 12, and 24 weeks and an AIDSVAX B/E gp120 (or placebo) boost at 12 and 24 weeks. After vaccination, volunteers returned to clinic at 6 month intervals for 3 years for HIV testing, safety, counseling, and elicitation of social impact events.

Result: 26,675 volunteers were screened and 16,402 were enrolled in the vaccine trial by the end of 2005. A variety of strategies were launched to accelerate screening and enrollment activity by strengthening community engagement activities and recruiting teams. "Word of mouth" from other volunteers, local health staff and recruitment team played a major role in the success of enrollment. Tracking activities were launched in April 2004 to track volunteers who missed appointments, and community engagement activities were conducted simultaneously and continuously. 13,978 (85%) volunteers completed full vaccination. Local and systemic post-injection reactions (PIR) were common but generally mild, consistent with expectations. The pattern and intensity of reactogenicity does not appear to contribute significantly to discontinuation of immunization. The overall performance of retention and tracking activity resulted in retention rates that exceed 95% in each 6 month period.

Conclusion: The Thai Phase III HIV vaccine trial is on track for completion in 2009. The collaborators have gained experience, and lessons learned during this trial will help future Phase IIB and III HIV vaccine trials.

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SOCIAL IMPACT OF PARTICIPATION IN A PHASE III HIV VACCINE TRIAL IN THAILAND

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Background: A community-based, phase III HIV vaccine trial in Thailand has enrolled 16,400 young adult volunteers. Volunteers have experienced favorable or unfavorable social events associated with their participation. Periodic review of such participation impact events (PIE) is carried out by the investigators, sponsors, IRBs, and the independent Data and Safety Monitoring Board.

Methods: At each study visit, a standard elicitation question identifies potential PIEs, and the volunteer is referred to a counselor to characterize the reported event, describe its impact, and track until resolution. Education and counseling are directed towards prevention and alleviation of such events, and intervention from study staff is provided when necessary. **Results:** There were a total of 530 PIEs reported as of 2 Dec 2006 with 26,162 person years of follow-up, an overall rate of 20 PIE/1000 person-years (py). During the vaccination phase, the rate was 30 PIE/1000 py, while during the follow-up phase the rate dropped to 10/1000 py. Overall 83% were coded as personal relationship problems. Most PIEs resolve with minimal impact. Sixty-one percent (324 cases) were coded as "no or minimal change to normal daily living" and 170 (32%) reported "short term change to normal daily living". Seven percent (36 cases) reported a "long term or permanent change to normal daily living". Eighty-three percent continued in the protocol, while 90 cases (17%) withdrew as a result of the PIE. Of those who withdrew, 15 later decided to resume participation. Only 23 cases (4%) reported the outcome of the PIE as unsatisfactory.

Conclusion: Both positive and negative PIEs are uncommon, with more occurring during the vaccination phase than during the follow-up phase. The majority are resolved satisfactorily with minimal impact. This may be due to effective counseling and community education prior to and during the trial. It is important that interventions, including outreach to employers, family, and friends, are made available during HIV vaccine trials to address participation impact events as they occur.

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SAFETY AND IMMUNOGENICITY OF THE MRKAd5 GAG HIV-1 VACCINE IN A WORLDWIDE PHASE I STUDY OF HEALTHY ADULTS (MERCK V520-018/HVTN 050)

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Background: Phase I studies indicate the monovalent MRKAd5 HIV-1 gag vaccine is well tolerated and immunogenic in N. American populations. High prevalence of preexisting immunity to adenovirus type 5 (Ad5) may affect vaccine response rates. **Aim:** We analyzed the preliminary data through Week 30 of this international Phase I study testing the safety and immunogenicity of an Ad5 HIV vaccine candidate.

Methods: Healthy adults aged 18-50 at low risk for HIV infection were randomized 1:3:3 to receive placebo, 1x109 or 1x1010 viral particles (vp) of the MRKAd5 HIV-1 gag vaccine at Day 1, Week 4 and Week 26 in a dose-escalating staged study in 24 centers in Africa, Asia, Caribbean, N. and S. America. Enrollment was not stratified by baseline Ad5 titer. Adverse events (AE) and lab values were assessed after each dose. Immunogenicity was evaluated using an IFN-γ ELISPOT gag 15-

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mer assay. Positive ELISPOT responses were defined as >55 SFC/106 PBMC and ≥4-fold over mock control.

Results: 360 people (55% male, median age 30) were enrolled (87 each in Asia and N. and S.America; 75 in the Caribbean; 24 in Africa). The vaccine was generally well tolerated at both doses. The most common AEs were injection site reaction, headache, fever, and diarrhea. At Week 30, pooled ELISPOT responses were 57/133 (43%) in the 1x109 vp group and 108/139 (78%) in the 1x1010 vp group. Overall, responses to 1x1010 vp were 85% and 68% in subjects with low (\leq 200, n=75) and high (>200, n=62) baseline Ad5 titers, respectively. Response rates among subjects with high baseline Ad5 titers who received 1x1010 vp were: 23/26 (88%) in Asia, 2/7 (29%) in N. America, 10/13 (77%) in S.America, 7/12 (58%) in the Caribbean, and 0/4 in Africa.

Conclusion: The MRKAd5 HIV-1 gag vaccine was generally well tolerated and immunogenic in diverse world regions. The 1x1010 vp dose was generally more immunogenic than 1x109 vp. Although there may be a modest effect of high baseline Ad5 titers on ELISPOT responses, overall most subjects with high levels of preexisting Ad5 immunity had positive ELISPOT responses to 1x1010 vp. These data indicate that the MRKAd5 HIV-1 gag vaccine may be immunogenic in regions with high prevalence of Ad5 immunity. This international study of the MRKAd5 gag vaccine supports ongoing Phase II test-of-concept trials of a next generation MRKAd5 trivalent gag/pol/nef vaccine.

Presented at: AIDS VACCINE 2007, Seattle, USA, August 20-23, 2007 (p.59).

HLA CLASS II ALLELES AND NEUTRALIZING ANTIBODY RESPONSES TO HIV-1 PROTEIN SUBUNIT BOOSTING IN A THAI POPULATION

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Background: Lack of neutralizing antibodies after subunit vaccination (hepatitis B, influenza) has been associated with certain HLA Class II alleles.

Objective: To examine the association of HLA class II alleles and neutralizing antibody (NAb) responses to CRF01_AE after a prime-boost vaccine regimen among HIV-seronegative Thai volunteers.

Methods: HLA class II polymorphisms were typed on volunteers from two phase II trials of ALVAC-HIV (vCP1521) boosted with oligomeric gp160 (n=23) or two different rgp120 candidate vaccines (n=22 and n=88). One rgp120 was tested at two different doses, 100mcg (n=43) and 300 mcg (n=45). Neutralizing antibody responses were assessed in standard T-cell line-based assays against CRF01_AE TCLA isolates, NPO3 and CM244. The association between the frequency of neutralization responses and alleles of DRB1 (combined by serologic classification), DRB3, DRB5, DQA1, and DQB1 was assessed.

Results: Statistically significant associations were observed for non-response to the 300 mcg dose of rgp120 and DRB1*11 (p=0.003, Fisher's exact) and RB1*1602 (0/4 vs. 32/41, p=0.005), though these associations were not robust to multiplicity adjustment. These associations were not observed with the 100 mcg dose, but neutralization occurred less frequently with this dose (47% vs. 71% of the 300 mcg group). There were no associations observed among the smaller groups boosted with the other rgp120 and ogp160.

Conclusion: Neutralizing antibody responses to HIV-1 subunit vaccines may be associated with class II alleles. Larger, phase II immunogenicity studies should include HLA genotyping to evaluate potential non-responders with an adequate sample size.

Presented at: AIDS VACCINE 2007, Seattle, USA, August 20-23, 2007 (p.135).

OUTCOME OF TUBERCULOSIS IN ADVANCED HIV-INFECTED PATIENTS ON ANTIRETROVIRAL THERAPY

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Objective: To determine the outcome of tuberculosis in advanced HIV patients with antiretroviral therapy. **Methods:** 115 HIV-infected patients with active TB and CD4 counts <100/mm³ were reviewed retrospectively. All received anti-TB therapy and ART concomitantly. Clinical manifestations, treatment regimens and outcome were analyzed.

Results: 101 received ART after TB diagnosis or at the same time as anti-TB treatment (TB-ART group). 14 patients were diagnosed with TB after receiving antiretroviral therapy (TB -ART group). 114/115 (99.2%) patients were treated with non-nucleoside reverse transcriptase inhibitor-based ART (nevirapine 75.7%, efavirenz 23.5%). The median interval between anti-TB treatment and ART was 68 days (range: 0-381) in the TB-ART group). Of 115 patients, 48.6% of cases were treated with a 6-month standard TB regimen, while the rest were treated with prolonged TB regimens. 62 cases (53.9%) received rifampin concomitantly with nevirapine, and 21 cases (18.3%) received rifampin concomitantly with efavirenz. Treatment success rate was 72.9%, with a mortality rate of 6.3%. The risk factors for death included resistant TB (p=0.03) and poor compliance (p<0.05). 8.7% developed multi-drug resistant TB. Possible or probable IRIS was seen in 16 cases (13.9%). No life-threatening IRIS was reported, and it did not affect disease outcome (p=0.5). A shorter time between anti-TB treatment and ART was a risk factor for the occurrence of IRIS (31 days vs.90 days; p<0.05). Regarding drug adverse effects, 42.6% of cases had side effects due either to the anti-TB drugs or ART. Thirty-one patients (63.2%) who experienced adverse drug reactions were given anti-TB treatment and ART concomitantly. 65% of adverse drug reactions occurred within the first 2 months of TB treatment, and 47 patients (40.9%) had to stop or change either anti-TB treatment or ART.

Conclusions: The mortality rate in TB with HIV and ART is low. The occurrence of IRIS did not confer additional mortality.

Presented at: IAS 2007, Sydney, Australia, July 22-25, 2007.

THAILAND'S EXPERIENCES IN CONDUCTING HPV VACCINE TRIALS AND HPV VACCINE UPDATE

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Cervical cancer is the most common cancer affecting women in developing countries. It has been estimated to have been responsible for almost 260,000 deaths in 2005, of which about 80% occurred in developing countries. About 70% of cervical cancer is caused by human papillomavirus (HPV) types 16 and 18. HPV (especially genotypes 6 and 11) can also cause genital warts, a common benign condition of the external genitalia that causes significant morbidity.

Two VLP vaccines: a quadrivalent (Merck) and a bivalent (GSK), have been assessed in phase II and large phase III efficacy trials. More than 20,000 women, aged 16-26 have been included in the trials for the quadrivalent vaccine and more than 27,000 women, aged 15-25, in trials of the bivalent vaccine. Regions included in the trials were North and Latin America, Europe and Asia-Pacific. The vaccines have been evaluated using different study designs, but with many common features. Phase III studies for the quadrivalent vaccine and interim analyses of phase III studies for bivalent vaccine have been published. Different analysis populations have been included in the published studies.

Results form those trials have shown that both vaccines have a high efficacy against HPV type-related endpoints among females naïve to the relevant vaccine type: In the per protocol populations: once study of the quadrivalent vaccine showed an efficacy of 98% (95% CI 86-100%) for the prevention of HPV 16/18 related CIN 2/3 or AIS; another study found 100% for the prevention of condyloma (95% CI 92-100%) and VIN or VaIN grade 2+ (95% CI 49-100%). GSK has not published a per protocol analysis of their phase III trial data.

The quadrivalent vaccine trial included 818 participants form Asian-pacific countries, from which 120 were Thais. Sub group analysis for efficacy in this population yielded similar results. Acceptability of participants who enrolled in the HPV vaccine trials in Thailand were excellent with high rate of long term follow up. The efficacy trial in mid adults using quadrivalent vaccine is being conducted and now is in third year of follow up.

Presented at: National Cancer Conference, Bangkok, Thailand, December 12-14, 2007 p.47.

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SUIDASIA DERMATITIS: A REPORT OF THREE CASES

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Suidasia medanensis (pontifica) is an acarid mite that is cosmopolitan in distribution. The mite has been found on rice bran, groundnuts, cowpeas, dead mosquitoes, peanuts, milk confectionery, mild powder, and dried seafood products. It is also a common house dust mite and sensitization to its allergens has been reported. We describe 3 cases of localized dermatitis caused by *S. medanensis (pontifica)*. The mites were identified from scrapings of the skin lesions. All three patients were treated successfully with gammabenzene hexachloride gel.

Diagnosing suidasia dermatitis can be quite a challenge. Dermatologists observing patients with unexplained dermatitis should consider the possibility of suidasia dermatitis.

Presented at: 21st World Congress of Dermatology, Buenos Aires, Argentina, September 30-October 5, 2007.

A NEW SPECIES OF **PARAGONIMUS** (TREMATODA: TROGLOTREMATIDAE) FROM A CAT INFECTED WITH METACERCARIAE FROM MOUNTAIN CRAB **LARNAUDIA LARNAUDII**

Jitra Waikagul

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The identification of *Paragonimus* spp. lung flukes is based on the morphology of both the metacercaria and the adult. A very small *Paragonimus* sp. metacercaria was found in a freshwater crab caught in Kanchanaburi Province, West Thailand, an area where metacercariae of *Paragonimus heterotremus* had not been found. The metacercariae cysts were 180-204 µm in diameter, which was smaller than metacercariae of *P. heterotremus*. The coefficient of difference in body size between this metacercaria and *P. heterotremus* was 1.69, which was greater than a subspecific difference. Adults recovered from the lungs of a cat fed with the metacercariae were morphologically similar to, but smaller than, *P. heterotremus* dissected from the lungs of a feline experimental host. The tegumental spines of the worm in this study were singly spaced in arrangement, which is similar to, but larger than, the spines of *P. heterotremus*. Therefore, this *Paragonimus pseudoheterotremus* is proposed as a new species.

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SYMPATRIC OCCURRENCE OF TAENIA SOLIUM, T. SAGINATA, AND T. ASIATICA, THAILAND

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We confirmed the sympatric occurrence of *Taenia solium*, *T. saginata*, and *T. asiatica* in western Thailand. DNA analysis of morphologically identified *T. saginata*, in a dual infection with *T. solium*, indicated it was *T. asiatica*. To our knowledge, this report is the first report of *T. asiatica* and a dual *Taenia* infection from Thailand.

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RESPONSES TO ALBENDAZOLE TREATMENT FOR HOOKWORM INFECTION IN ETHNIC THAI AND IMMIGRANT IN WEST-CENTRAL THAILAND

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Ethnic Thai and immigrant schoolchildren and villagers of Bo-ong, a village in Pilok sub-district, Thong Pha Phum District, Kanchanaburi Province, western Thailand, were investigated for helminth infections in September 2003 and July 2004. Among the 143 schoolchildren, total cumulative hookworm prevalence in both surveys was 58.7%, with 47.6% for Thais and 63.4% for immigrants, while among the 183 villagers, it was 69.4% (Thais: 60.6%; immigrants: 75.0%). The efficacy of 400 mg single-dose albendazole among different hookworm-infected racial-ethnic groups was analyzed 21 days' post-treatment. Kato-Katz and polyethylene tube cultivation methods were used for stool examination. Among the 211 hookworm-positive cases in both surveys, only 82 cases from the last survey were followed up. By Kato-Katz technique, for the schoolchildren and villagers combined, the cure rate tended to be higher among the immigrants (65.0%) than the Thais (54.6%) (p = 0.445). By Sasa's modified Harada-Mori culture technique, the cure rates also differed by racial grouping, and were higher (46.3%) among the immigrants and lower (27.8%) among the Thais (p = 0.269). However, similar egg reduction rates were found for the two racial groups, at 96.0%, respectively. In addition, a higher intensity of hookworm infection tolerated albendazole therapy, lower cure rates were obtained in moderate-to-heavy infections (56.3%) and higher rates for light infections (63.6%) among the total population. There were no significant differences in drug efficacy among the 2 ethnic groups of Thai and immigrants (p > 0.05) in Kanchanaburi Province, Thailand.

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CYSTICERCOSIS AND TAENIASIS IN THAILAND

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Taeniasis is a common food-borne parasitic zoonosis in Thailand. Infection rates by stool examination vary, depending on the place and time of examination, and have been reported as 0.2-7.0%. Nationwide data indicate that most cases occur in the north and northeast (approximately 1.2%). By molecular analysis, *T. asiatica* infection was first discovered in Thailand in 25% (6/24) of taeniasis cases. The identifications were based on either multiplex PCR, base excision sequence scanning thymine-base (BESS T-base) analysis, and DNA sequencing of PCR products using cytochrome coxidase subunit 1 and cytochrome b genes. DNA sequencing showed that the *T. solium* in these patients were the Asian genotype. By molecular identification, a dual infection of *T. solium* and *T. asiatica* was first found in Thailand, which was also the first in Asia. The usefulness of molecular analysis for identifying human taeniid cestode infections is stressed. Cysticercosis, a potentially fatal chronic disease in humans, is caused by *C. cellulosae* developed in humans by ingestion of *T. solium* eggs. From 1947 to 2004, approximately 500 cases of human cysticercosis have been reported in Thailand, while the actual number of cases is speculated to be several time more.

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DISCRIMINATION OF **OPISTHORCHIS VIVERRINI** FROM **HAPLORCHIS TAICHUI** USING COI SEQUENCE MARKER

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This study aimed to discriminate infections of two common fish-borne trematodes in Thailand, *Opisthorchis viverrini* from *Haplorchis taichui*, based on mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Designed primers (COI-OV-Hap F&R primers) amplified partial COI fragments of *O. viverrini* and *H. taichui* with high sensitivity in different developmental stages (adult, metacercaria, and egg). Polymerase chain reaction (PCR) amplicons were generated with low genomic DNA concentration (\approx 10⁻⁴ng) of *O. viverrini* and *H. taichui* at 50 and 56 °C, annealing temperatures, respectively. At 50 °C, COI fragments of *Clonorchis sinensis* and *H. taichui* were also obtained, but this was less sensitive than *O. viverrini*. At 56 °C, only *H. taichui* could be amplified and discriminated from *H. pumilo*, *H. yogokawai*, *O. viverrini*, and *C. sinensis*. Between 50 and 56 °C, the PCR amplicons of *H. pumilio* and *H. taichui* were distinguished by PCR-RFLP method. The PCR-RFLP profiles might be useful for diagnosing mixed *O.viverrini* and *H. tachui* infections in endemic areas, and for detecting metacercariae of *O. viverrini*, *C. sinensis* and *H. taichui* in epidemiological surveys of infections in fish hosts.

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FISH-BORNE ZOONOTIC INTESTINAL TREMATODES, VIETNAM

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Although fishborne zoonotic trematodes that infect the liver are well documented in Vietnam, intestinal fishborne zoonotic trematodes are unreported. Recent discoveries of the metacercarial stage of these flukes in wild and farmed fish prompted an assessment of their risk to a community that eats raw fish. A fecal survey of 615 persons showed a trematode egg prevalence of 64.9%. Infected persons were treated to expel liver and intestinal parasites for specific identification. The liver trematode *Clonorchis sinensis* was recovered from 51.5%, but ≥ 1 of 4 intestinal species of the family Heterophyidae was recovered from 100%. The most numerous were *Haplorchis* spp. (90.4% of all worms recovered). These results demonstrate that fishborne intestinal parasites are an unrecognized food safety risk in a country whose people have a strong tradition of eating raw fish.

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DISEASE BURDEN AND EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHIASES AND SCHISTOSOMIASIS IN ASIA: THE JAPANESE PERSPECTIVE

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The disease burden due to soil-transmitted helminthiases (STH) and schistosomiasis is not well documented in Asia. Both STH and schistosomiasis are chronic diseases but case detection is not easy because of the absence of clinical symptoms. STH and schistosomiasis are, however, endemic in Asia and their burden is significant. At the preparatory meeting for the Hashimoto Initiative in Japan in 1997, STH and schistosomiasis were categorized as Group 2 diseases. Parasitic infections in this category were well understood at the time but sophisticated control strategies were lacking. Japan has promoted comprehensive collaborative projects to reduce the burden of STH and schistosomiasis throughout Asia, creating an international network to collect epidemiological information and to implement and improve disease control, thus extending the school-based control method that had proved so successful in Japan.

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HELICONEMA LONGISSIMUM (ORTLEPP, 1923) (NEMATODA: PHYSALOPTERIDAE) FROM PISODONOPHIS BORO (TELEOSTEI: OPHICHTHIDAE) IN THAILAND, WITH REMARKS ON THE TAXONOMY OF THE PROLEPTINAE SCHULZ, 1927

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Physalopterid nematodes identified as *Heliconema longissimum* (Ortlepp, 1923) were collected from the stomach of rice-paddy eels *Pisodonophis boro* (Hamilton) (Anguilliformes: Ophichthidae) from two brackish-water localities (mangroves) in Thailand: one in Phan-Nga Province, southwestern Thailand, northeast of Phuket Island, and one in Ranong Province, near the border with Myanmar. Study of the morphology of this hitherto insufficiently known nematode species, including its first SEM examination, enabled a detailed redescription of *H. longissimum*. Present taxonomic problems in the subfamily Proleptinae Schulz, 1927 are discussed, where a new delimitation of *Proleptus* Dujardin, 1845, *Heliconema* Travassos, 1919 and *Paraleptus* Wu, 1927 is proposed based on the cephalic dentation. *H. minnanensis* [sic] Damin & Heqing, 2001 is transferred to *Paraleptus* Wu, 1927 as *P. minnanensis* (Damin & Heqing 2001) n. comb. and *Paraleptus chiloscyllii* Yin & Zhang, 1983 transferred by Damin & Heqing (2001) to *Heliconema*, is retained in *Paraleptus*. *H. ahiri* Karve, 1941 is considered a junior synonym of *H. longissimum* (Ortlepp, 1923). The present finding of *H. longissimum* in *Pisodonophis boro* represents the first host record and the first record of this nematode from Thailand.

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BEYOND DEWORMING: THE PROMOTION OF SCHOOL-HEALTH-BASED INTERVENTIONS BY JAPAN

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Deworming bestows a variety of health and socioeconomic benefits and has been embraced by developing countries. To extend the beneficial impact of deworming, the Asian Centre of International Parasite Control (ACIPAC) project has carried out activities to link deworming with health-promoting school programs in the Greater Mekong Subregion (Cambodia, Laos, Myanmar, Thailand and Vietnam). ACIPAC has also conducted an integrated school-health-based program, including deworming and malaria education, under the umbrella of the health-promoting schools initiative. Implementing 'beyond-deworming' efforts is now a practical challenge in the subregion.

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EVALUATION OF HELMINTH CONTROL PROGRAMME IN NORTHERN THAILAND

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Nan Province, located in northern Thailand, is hyperendemic for parasite infections; the helminth infection rate in school children was 60.0% in 2001. Mass anthemintic chemotherapy has been conducted with schoolchildren, and selective treatment has been given to people in the community, from 2002. The prevalence and intensity of helminth infections in Chalerm Prakiat District were measured once a year during 2002-2004. The prevalence of helminthic infections decreased slowly from 60.0 to 40.3% in schoolchildren and from 70.8 to 60.0% in the older age. Three parasite species were common; hookworm, *Ascaris* and *Haplorchis*-an intestinal trematode. Hookworm presented throughout the whole district. *Ascaris* infection occurred at high rates in some villages, while in some village none was found. The villages where *Ascaris* infection was nil had high rates of *Haplorchis* infection, and vice versa. Heavy intensity infection was found in 20.0% of *Ascaris* cases examined. Mass anthelminthic treatment should be continued.

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PARAGONIMIASIS HETEROTREMUS IN SARABURI PROVINCE, THAILAND

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Saraburi Province was claimed an endemic area of paragonimiasis twenty years ago. The metacercariae of two human species, *P. westermani* and *P. heterotremus*, were still abundantly infected crabs collected from Chet Khot Waterfall, Saraburi Province. 91 individuals who lived near the waterfall were interviewed and examined. Immunoblot was performed with sera of 23 villagers suspected of having lung fluke infections, due either to clinical sign of chronic cough or history of eating raw crabs. 11 cases reacted positively at diagnostic bands 35, 33, and 32.5 kDa, of *P. heterotremus* crude antigen. These bands are specific for paragonimiasis heterotremus. The result of immunodiagnosis suggested no paragonimiasis westermani case in this village. Rats, hamsters and a cat were fed with metacercariae of *P. westermani*; no cyst or worm appeared in the rats and hamsters, but young adults were found from the cat 150 days'post-infection. Further study is needed to confirm that *P. westermani* is unable to infect humans in Thailand.

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CYSTICERCOSIS AND TAENIASIS IN THAILAND

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Cysticercosis, a potentially fatal disease in humans is caused by the larval stage of *Taenia solium*, *Cysticercus cellulosae*. There have been reported approximately 500 human cases in Thailand since 1947 to 2004, while the number of the presumptive cases is estimated to be several times more. In contrast taeniasis is one of common food-borne parasitic zoonoses caused by the infection of adudlt *Taenia* spp. In Thailand, nationwide infection rates of the disease by stool examination have been indicated higher (1.2%) in the north and northeast regions than other regions. However, individual surveys show different infection rates (0.6-6.0%) depending on the locality and time of examination. Many reports on taeniasis have shown infections with *Taenia saginata* rather than *T. solium*. In recent investigation conducted in Kanchanaburi Province, situated in west part of Thailand, 24 taeniasis patients were found: 11(46%) of which were *T. solium* infection, 7(29%) were T. saginata and 6(25%) were due to *T. asiatica* infections by mitochondrial DNA analysis of the expelled proglottids and or morphological characters of scolices. More interestingly, a dual infection with *T. solium* and *T. asiatica* was found in the area, which was the first case in Asia.

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MOLECULAR PHYLOGENETIC RELATIONSHIP OF PARAGONIMUS PSEUDOHETEROTREMUS

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The partial mitochondrial cytochrome *c* oxidase subunit I (COI) gene and the nuclear ribosomal DNA second internal transcribed spacer 2 (ITS2) of a newly described lung fluke, *Paragonimus pseudoheterotremus* were sequenced and compared with *P. heterotremus* -the species with similar morphology. The pairwise distances of the COI sequences revealed a genetic difference between *P. heterotremus* and *P. pseudoheterotremus*, with nucleotide differences of 10.6%. The constructed phylogenic tree with high bootstrap proportion suggested *P. pseudoheterotremus* to be a sister species of *P. heterotremus*.

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DIFFERENTIAL SERODIAGNOSIS OF BRUGIAN AND BANCROFTIAN FILARIASES USING ANTIGENIC PRODUCTS FROM **DIROFILARIA IMMITIS** ADULT WORMS

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Antibody detection of Brugian filariasis is not available in Thailand and a good test has not been developed for this detection in the world as immunochromatographic test (ICT) and Og4C3-ELISA for Bancroftian filariasis. In the present study, excretory-secretory antigens, either non-delipidized (NDLP) or ether-delipidized (DLP) extracts of *Dirofilaria immitis* female and male adult worms were primarily evaluated by IgG-ELISA for the detection. Sera of Brugian filariasis (36 cases), other infections (181) and negative controls (54) encountered with those antigens. The sensitivity and specificity using male ES antigen were 100% and 96.2% at cut-off value 0.587, respectively, which can differentiate Brugian filariasis from Bancroftian filariasis. Under the same conditions, ES female antigen produced 100% sensitivity and 81.1% specificity of test at cut-off value, 0.616. By immunoblot comparison between positive and negative pool sera using NDLP- and DLP-extracts, NDLP-male and DLP-female antigens were selected for full scale ELISA. Test using NDLP- male antigen gave 100% sensitivity and 46.8% specificity, at cut-off value 0.097. Several serum samples of all twenty seven heterologous diseases and negative controls showed false positives 53.2% (125/235) with NDLP-male antigen, and 54.5% (128/235) with DLP-female antigen. This study showed that ES male antigen can provide the differential diagnosis between Brugian and Bancroftian filariases and perhaps serodiagnosis of Brugian filariasis. For obtaining higher specificity, antigenic products should be continued using different purification techniques.

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CAPILLARIASIS: A CHRONIC WATERY DIARRHEA NOT ONLY FROM MICROORGANISMS; WHAT DO YOU THINK?

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A 54-year-old male Thai patient from Prachinburi Province, presented with history of chronic watery diarrhea for many years. He passed stool five to ten times a day with occasionally colicky pain, abdominal distension, nausea and vomiting. He had visited hospitals and private clinic for his suffering and treated with some medications but not improved. He came to Hospital for Tropical Disease, Bangkok, Thailand. On physical examinations, moderated dehydration, fatique, abdominal distension and gurgling stomach were found. The eggs, larvae and adult worms of *Capillaria philippinensis* were found from stool examination. The patient was admitted and treated with Mebendazole 400 mg for 20 days, clinical was recovered after treated.

Two months ago, he had history of ingested raw small fresh-water fish dish called "Pra-Pla Siw/Soi". Some small freshwater fish near by the patient's home were collected and examined at the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, for *Capillaria philippinensis* larvae but showed negative results. The conclusion of this case, chronic watery diarrhea was not only from the microorganisms, some parasitic infections such as *Capillaria philippinensis*, also a causation and its can cause of serious clinical problems.

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EFFECT OF ALBENDAZOLE ON LIPID PEROXIDATION IN RAT INFECTED WITH TRICHINELLA SPIRALIS

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This study aimed to investigate the lipid peroxidation in the course of experimental trichinellosis and the effect of albendazole on lipid peroxidation in day 7 post-infection. Melondialdehyde (MDA) level were determined in heart, kidney, liver, diaphragm, skeletal muscle and duodenal tissue homogenate and in plasma of rats orally infected with *Trichinella spiralis* (400 larvae/rat) on day 7 and 35 of the post-infection period (7 and 35 dpi). A single dose of 20 mg albendazole was administered 48 hours after larva administration per os. It is found that MDA was increased significantly in heart, kidney, liver, diaphragm and skeletal muscle tissue of 7 dpi rats. These elevation were remained in only kidney and muscle tissue of 35 dpi rats. Albendazole treatment did not alter these increased MDA level in heart, kidney and liver of 7 dpi rats. However, albendazole made the level of MDA increased even more in diaphragm and skeletal muscle of 7 dpi rats. These results suggested that lipid peroxidation occurred in trichinellosis and skeletal muscle and kidney were more oxidative damaged than other organs. Albendazole may obviously potentiate the oxidative stress in the body of Trichinellosis rats at least in the skeletal muscle tissue.

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ANAPHYLAXIS AS AN INITIAL MANIFESTATION OF CHOLANGIOCARCINOMA

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RATIONALE: Although *Opisthorchis viverini*[OV] is commonly associated with cholangiocarcinoma in Thailand, it has never been report as a cause of anaphylaxis.

CASE REPORT: A 57-year old female was admitted to hospital after developing angioedema, urticaria, wheezing and hypotension. Immediate treatment included adrenaline, chlorpheniramine, ranitidine, dexamethasone and intubation. During admission, she developed urticarial rash after almost every meal. There was no rash if she was starving. Investigation showed normal CBC and mild transaminitis. Total IgE level was 1,306 kIU/L. Specific IgE to aeroallergens and food were all negative. After 1 week of investigation for cause of anaphylaxis, she developed progressive jaundice. Ultrasonography of upper abdomen showed inhomogeneous hyperechoic lesion at posteroinferior segment of right hepatic lobe and prominent common bile duct[CBD] with abrupt narrowing. Endoscopic retrograde cholangiopancreatography[ERCP] showed marked dilatation of both intrahepatic duct[IHD] and CBD with abrupt narrowing at distal part. Sphincterotomy was done and stent was applied, resulting in good drainage of bile and also diminution of urticarial symptom. Bile cytology demonstrated *Opisthorchis* ova. Diagnosis of cholangiocarcinoma was made based on ERCP finding. She developed two times of ascending cholangitis and expired four months after diagnosis. Her serum was sent for identification of specific IgE to *Opisthorchis viverrini*. By ELISA, IgE antibody to *OV* and snail antigens gave titers at 1:1,600 and 1:6,400. By immunoblot assay, patient's and prototype serum demonstrated identical band of specific IgE to OV and snail antigens.

CONCLUSIONS: This is a first case report of *OV* inducing both cholangiocarcinoma and IgE mediated anaphylaxis.

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EFFECTS OF FOOD, EMBRYO DENSITY AND CONSPECIFIC IMMATURES ON HATCHABILITY IN THE DENGUE VECTOR **AEDES ALBOPICTUS**

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Aedine mosquitoes, well-known to exhibit asynchronous birth and development patterns, are subject to complex interactions between developmental stages that can interfere with hatchability. We explored the hatching responses of embryos from the dengue vector *Aedes albopictus* (Diptera: Culicidae) to food, embryo abundance and to different densities of conspecific larval stages. The breeding regimen resulted in females with little size variation which is to suggest a similar fitness in experimental embryos. Food presence exerted a significant consistently positive impact on hatching, which was drastically depressed in its absence. Hatch success correlated well with embryo density showing almost total hatching at low densities and low hatching at the highest density. Immature stages significantly impacted hatching responses. First instars larvae exerted an inhibition to hatching, but this impact was less perceptible at the highest density. Our findings suggest that *Ae. albopictus* embryos may actually be able to assess habitats with respect to suitability for larval development completion. This study emphasizes the potential intraspecific interactions within floodwater *Aedes* populations and has important implications for population maintenace.

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AN EMPOWERMENT PROGRAM TO ENHANCE WOMEN'S ABILITY TO PREVENT AND CONTROL MALARIA IN THE COMMUNITY, CHIANG MAI PROVINCE, THAILAND

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Paulo Freire's theory was modified to empower a women's group in Chiang Mai Province, Thailand, to prevent and control malaria. This study conducted an intervention in Mueang Na Wan Village, Mueang Na sub-district, Chiang Dao District, Chiang Mai Province, where 45 women were systematically recruited into the study cohort. Navail Village was selected as a control village because it resembled the intervention village. The empowerment program emphasized enhancement of malaria preventive levels, using insecticide-treated bed nets, self-esteem, and self confidence expectation to prevent and control malaria. Intensive training was conducted and activities performed among the women's group, with 10 participatory meetings in all. Data collection was conducted for the pre-test in month 1, and post-intervention in mounths 3, 6, 9 and 12. The qualitative methods used were focus-group discussions, non-participant observations, and in-depth interviews with housewives, their husbands, and youths at risk for malaria. The results showed that, post-intervention, there were significantly increased levels for malaria preventive behaviors, behaviors of using insecticide-treated nets, self-esteem, and self confidence expectations, in the intervention village compared with the control village. Insecticide-treated net usage and insecticide-treated net usage behaviors increased in the intervention village more than before and more than that in the control village. The women's group in the intervention village created the following plans, which were crucial to malaria prevention: (1) a family protection plan, (2) providing malaria education to community members, (3) a mosquito-control campaign, (4) scaling-up insecticide-impregnated bed nets, and (5) malaria control among foreign laborers. Finally, the empowered women's group performed sustainable activities. Between malaria-prevention activities, they conducted a joint program to raise income for their families.

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A NEW DENSOVIRUS ISOLATED FROM THE MOSQUITO **TOXORHYNCHITES SPLENDENS** (WIEDEMANN) (DIPTERA: CULICIDAE)

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A new densovirus was isolated and characterized in laboratory strains of *Toxorhynchites splendens*. The virus was detected by polymerase chain reaction (PCR) from mosquitoes reared in our laboratory. PCR fragments from each mosquito were compared by single strand conformation polymorphism (SSCP) assay and found to be indistinguishable. Thus, it is likely the densoviruses from these mosquitoes contain homologous nucleotide sequences. The PCR fragment corresponding to a 451 bp densovirus structural gene segment from each of 5 mosquitoes had 100% identical nucleotide sequences. Phylogenetic analysis of the structural gene sequence suggests the newly isolated densovirus is more closely related to *Aedes aegypti* densovirus (*AaeDNV*) than to *Aedes albopictus* densovirus (*Aa/DNV*). Analysis of offspring and predated larvae suggests that vertical and horizontal transmission are responsible for chronic infections in this laboratory strain of *Toxorhynchites splendens*. The viruon DNA is 4.2 kb in size, is closely related to, but distinct from, known densoviruses in the genera *Brevidensovirus* and *Contravirus*. The virus is tentatively named *Toxorhynchites splendens* densovirus (*TsDNV*).

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INFLUENCE OF LARVAL DENSITY OR FOOD VARIATION ON THE GEOMETRY OF THE WING OF AEDES (STEGOMYIA) AEGYPTI

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Background and method: Variation in wing length among natural populations of *Aedes (Stegomyia) aegypti* (L.) (Diptera: Culicidae) is associated with diffeent vectorial capacities. Geometric morphometrics allowed us to use a more powerful estimator of wing size ('centroid size'), as well as to visualize the variation of wing shape, to describe the effects of density or food variation at larval stage on 20 anatomical landmarks of the wing of *A. aegypti*.

Results: Almost perfect correlations between (centroid) size and larval density or size and larval food were observed in both sexes: a negative correlation with increasing density and positive one with increasing amount of food. The allometric component of shape change was always highly significant, with stronger contribution of size to shape under food effects. Within each experiment, either food or density effects, and exculding extreme conditions, allometric trends were similar among replicates and sexes. However, they differed between the two experiments, suggesting different axes of wing growth.

Conclusion: *Aedes aegypti* size is highly sensible to food concentration or population density acting at larval stages. As larger individuals could be better vectors, and because of the stronger effect of food concentration on size, vector control activities should pay more attention in elimination containers with rich organic matter, Furthermore, as simple reduction in larval density could significantly increase the size of the survivors, turning them into potentially better vectors, the control activities should try to obtain a complete elimination of the domestic populations.

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RAPID DETERMINATION OF VIRAL RNA SEQUENCES IN MOSQUITOES COLLECTED IN THE FIELD

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A method for rapid determination of viral RNA sequences (RDV) was applied to homogenates of *Aedes aegypti* collected in Thailand in an area in which dengue fever (dengue hemorrhagic fever) is endemic, using the mosquito cell line C6/36. Nucleic acid sequences of dengue virus type 4 and cell fusing agent virus were detected. This RDV method has the potential to become a standard method for detection of both known and newly emerging, unknown mosquito-borne viruses.

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THE GEOMETRY OF THE WING OF **AEDES (STEGOMYIA) AEGYPTI** IN ISOFEMALE LINES THROUGH SUCCESSIVE GENERATIONS

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Under a common laboratory environment, three isofemale lines of *Aedes (Stegomyia) aegypti* were used to score metric properties (size and shape) of the wings during 10 generations. Since the number of generations was much higher than the number of founders in each line, genetic drift was expected to occur.

Size tended to slightly increase with time, but its variation among the successive generations did not show any detectable information specific to each line. Shape could discriminate among lines in females. Males of lines A and B were not discriminated before generation 8, after which they became completely separated.

For each line at each generation, the variance of size and the metric disparity index (as an estimate of shape variance) were higher in females than in males. From one generation to another, the within line shape variance decreased in both sexes, while shape similarity progressively and consistently decreased between males of lines A and B. At each generation, in both sexes, shape variance of the pooled lines was higher than those for each line separately, a pattern not observed for size variance.

In conclusion, shape, as a metric property, was closer to a genetic character than size: (i) its showed modifications compatible with the hypothesis of genetic drift and (ii) its variation was related to the complexity of the sample.

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EFFECTIVENESS OF **BACILLUS THURINGIENSIS** H-14 AGAINST TEMEPHOS-RESISTANT AEDES AEGYPTI

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Aedes aegypti is an important disease bearing mosquito in Thailand. Recently, temephos have been commonly used in the control of mosquito larvae, especially *Ae. aegypti*. Its widespread use has resulted in temephos resistance; posing a serious problem for the control of this species. *Bacillus thuringiensis* H-14 (Bti) can be used form control *Ae. aegypti* that have become resistant to temephos. Our research was performed to determine the activity of Bti as an alternative larvicide for the control of *Ae. aegypti*. A sample of *Ae. aegypti* from Buriram province, Thailand with a high level of temephos resistance ($LC_{90} = 0.03521$ ppm., $RR_{90} = 14.37$) and Bora Bora (control) strain were subjected to the study. Insecticide resistance mechanisms were detected. Biochemical tests, polyacrylamide gel electrophoresis (PAGE) and inhibition studies revealed the presence of elevated esterase activity which is associated with temephos resistance. The effectiveness of Bti against the mosquito resistance was observed when mosquito larvae were sequentially exposed to temephos and Bti. The results showed that temephos-resistant larvae showed no resistance to Bti. In conclusion, Bti can be used to eliminate temephos-resistant *Ae. aegypti* mosquitoes.

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MOLECULAR IDENTIFICATION OF COASTAL MALARIA VECTOR, ANOPHELES SUNDAICUS, IN NATURAL BREEDING SOURCES, THE COASTAL AREA OF ANDAMAN AND THE GULF OF THAILAND

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Anopheles (Cellia) sundaicus is an important malaria vector on islands and coastal areas of Southeast Asia. In Thailand, there are many cases of malaria patients occur in the coastal areas and *Anopheles sundaicus* is suspected as malaria vector. *Anopheles sundaicus* infers to the Sundaicus complex because of its ecological and ethological differences. Morphology identity may be confused in case of the Sundaicus complex. So molecular identity is used for investigating this species complex. For this study, the *Anopheles* mosquitoes were collected in three provinces, Phang-Nga, Chanthaburi and Rayong along the coastal area. We performed PCR amplification and nucleotide sequence analysis for internal trancribed spacer 2 (ITS 2) and domain-3 (D3) of 28S rRNA. The 660 bp of ITS 2 and 400 bp of D3 from 28S rRNA were amplified in high intensity. Multiple alignments of these rRNA sequences were done to compare with *Anopheles sundaicus* in other coastal areas of Vietnam, Malasia, Indonesia and India. The differences of nucleotide sequence alignment from these rRNA sequences can be informed the molecular identification of the Sundaicus complex mosquitoes.

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POSSIBILITY OF DOMESTIC SAND FLIES AS A VECTOR OF LEISHMANIASIS IN THAILAND

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Recently there was an increase in cases of autochthonous leishmaniasis with visceral involvement in Thailand as reported in Surat Thani, Nan, Phang Nga and Nakhon Si Thammarat. Incrimination of the vectors investigation of responsible sand flies for transmission of *Leishmania* were carried out in all houses of the patients by means of CDC light traps, cow-baited traps, aspirators and human landing catches. The species composition and relative abundance of phlebotomine sand flies at the different collecting sites were similar indicating common characteristics of their habitats. They were collected in low number with few *Phlebotomus* species including *Phlebotomus argentipes* and *P. stantoni* and common species of *Sergentomyia*; *S. gemmea, S. silvatica, S. iyengari* and *S. barraudi*. Although no evidence exists to link the presence of sand flies to *Leishmania* infection, reservoir hosts with their specific species of sand flies corresponding to the domestic environments might implicate disease transmission. Longitudinal studies of temporal and spatial distribution of sand flies in relation to their environments are essential to clarify vector status of sand fly species.

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GEOMETRY OF **AEDES (STEGOMYIA) AEGYPTI** WINGS UNDER THE INFLUENCE OF LARVAL DENSITY AND FOOD SUPPLY

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Geometric morphometric analysis was applied to explore a more powerful estimator of size and to visualize the variation of wing shape under the effects of density or food variation at larval stage. Both size and shape were based on twenty anatomical landmarks of *Aedes (Stegomyia) aegypti* wings. Size had a negative correlation with increasing density and a positive one with increasing amount of food in both sexes. Geometric shape change was mainly driven by size variation, presenting major modification in the central part of the wing. However, the direction of shape changes was statistically different according to the cause of variation, either food or density, and the difference was visible at seven landmarks delimiting the posterior border of the wing. A laboratory model of field conditions would help in understanding the natural metric variation in *Ae. aegypti* which could have the impact on their fitness and vector competency.

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MULTIPLE MUTATIONS IN katG AND inhA IDENTIFIED IN THAI ISONIAZID-RESISTANT MYCOBACTERIUM TUBERCULOSIS ISOLATES

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A total of 29 Thai multi-drug-resistant/isoniazid-resistant *Mycobacterium tuberculosis* isolates were analyzed for mutations in *katG* from codons 254 to 549, *inhA* promoter and *inhA* open reading frame by DNA sequencing and single strand conformation polymorphism. Twenty five multi-drug resistant isolates exhibited single point mutations (17 isolates at Ser315Thr plus Arg463Leu, 1 at Thr308Pro plus Arg463Leu, 7 at either Ser315Thr or Arg463Leu) while the other 4 isoniazid-resistant isolates had single point mutation only at Arg463Leu. Seven of 25 multi-drug-resistant isolates (4 at C(-15)T, 1 at T(-8)C; 1 at C(-15)T plus Ser94Ala and 1 at Ile21Val) and 2 of 4 isoniazid-resistant isolates (1 at C(-15)T, 1 at C(-15)T plus Ile21Thr) had mutations in *inhA* promoter and open reading frame, while the other 20 isolates had no mutation at any position. No frame shift mutation was observed in any tested isolates. This is the first report of two mutations, Trp308Pro of *katG* and T (-8)C of *inhA* in *Mycobacterium tuberculosis* isolates

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PROTEOME AND IMMUNOME OF PATHOGENIC **LEPTOSPIRA** SPP. REVEALED BY 2DE AND 2DE-IMMUNOBLOTTING WITH IMMUNE SERUM

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In this study, proteomes of two pathogenic Leptospira spp., namely L. interrogans, serogroup Ictero-haemorrhagiae, serovar Copenhageni and L. borgpetersenii, serogroup Tarassovi, serovar Tarassovi, were revealed by using two dimensional gel electrophoresis (2DE)-based-proteomics. Bacterial cells were disrupted in a lysis buffer containing 30 mM Tris, 2 M thiourea, 7 M urea, 4% CHAPS, 2% IPG buffer pH 3-10 and protease inhibitors and then subjected to sonication in order to solubilize as much as possible the bacterial proteins. The 2DE-separated components of both *Leptospira* homogenates were blotted individually onto membranes and antigenic components (immunomes) were revealed by probing the blots with immune serum of a mouse readily immunized with the homogenate of L. interrogans, serogroup Icterohaemorrhagiae, serovar Copenhageni. The immunogenic proteins of the two pathogenic *Leptospira* spp. could be grouped into 10 groups. These are: 1) proteins involved in the bacterial transcription and translation including beta subunit transcription anti-termination protein of DNA polymerase III, elongation factors Tu and Ts, and tRNA (guanine-N1)-methyltransferase; 2) proteins functioning as enzymes for metabolisms and nutrient acquisition including acetyl-Co-A acetyltransferase, putative glutamine synthetase, glyceraldehyde-3-phospahte dehydrogenase, NifU-like protein, 3-oxoacyl-(acyl-carrier-protein) reductase, oxidoreductase, sphingomyelinase C precursor, spermidine synthase, beta subunit of succinyl-CoA synthetase, and succinate dehydrogenase iron-sulfur subunit; 3) proteins/enzymes necessary for energy and electron transfer, i.e. electron transfer flavoprotein, and proton-translocating transhydrogenase; 4) enzymes for degradation of misfolded proteins, i.e. ATP-dependent Clp protease; 5) molecular chaperone, i.e. 60 kDa chaperonin; 6) signal transduction system, i.e. response regulator; 7) protein involved in immune evasion in host, *i.e.* peroxiredoxin; 8) cell structure proteins including MreB (cytoskeletal) and flagellin/ periplasmic flagellin; 9) lipoproteins/outer membrane proteins: LipL32, LipL41, LipL45 and OmpL1; and 10) various hypothetical proteins. Many immunogenic proteins are common to both Leptospira spp. These proteins not only are the diagnostic targets but also have potential as candidates of a broad spectrum leptospirosis vaccine especially the surface exposed components which should be vulnerable to the host immune effector factors.

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OMPL1 DNA VACCINE CROSS-PROTECTS AGAINST HETEROLOGOUS **LEPTOSPIRA** SPP. CHALLENGE

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Available leptospirosis vaccines made up of inactivated bacteria or their membrane components elicit immunity which is serovar specific and unsatisfactory immunological memory. A vaccine that protects across *Leptospira* serogroups/serovars, *i.e.* broad spectrum, and induces long-lasting memory is needed for both human and veterinary uses. In this study, a plasmid DNA vaccine was constructed from cloning gene encoding a trans-membrane porin protein, OmpL1, of pathogenic *Leptospira interrogans*, serogroup Icterohaemorrhagiae, serovar Copenhageni into a mammalian expression vector pcDNA3.1(+). The protective efficacy of the *ompL1*-pcDNA3.1(+) plasmid DNA vaccine was studied by immunizing hamsters intramuscularly with three doses of the vaccine (100 µg per dose) at two week intervals. The empty pcDNA3.1(+) and PBS were used as mock as negative vaccine controls, respectively. All animals were challenged with the heterologous *Leptospira interrogans*, serogroup Pomona, serovar Pomona (10 LD50), at one week after the last vaccine booster. The *ompL1*-pcDNA3.1(+) plasmid DNA vaccine rescued some vaccinated animals from the lethal challenge and delayed death time, reduced morbidity, *e.g.*

fever, and/or the numbers of *Leptospira* in the tissues of the vaccinated animals. While the results are encouraging, further studies are needed to optimize the immunization schedule, vaccine dosage and formulation in order to maximize the efficacy of the vaccine.

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MONOCLONAL ANTIBODY THAT NEUTRALIZES PERTUSSIS TOXIN ACTIVITIES

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Pertussis or whooping cough is a disease with high mortality among infants and small children. The disease is caused by infection of the respiratory tract by a gram negative bacterium, Bordetella pertussis. The superficial colonized bacteria produce a myriad of toxins which enter the circulation causing various patho-physiologicalal changes in the host. Although antimicrobial therapy reduces the number of the coughed out bacteria and also the infectious time of the infected host, but it is not effective in amelioration of the clinical manifestations as the pertussis morbidity is due principally to the pertussis toxin (PT). Antibody based-therapy is frequently practiced in conjunction with other supportive measure to resuscitate the patient. Nevertheless, human derived antiserum against PT is of the limited supply and the ethical concern. Thus in this study a hybridoma clone, i.e. clone PT6-2G6, secreting monoclonal antibody (MAb) specific to the S1 subunit, the active enzyme of the PT that intracellularly ADP-ribosylates the host Gi-protein, was produced. The MAbPT6-2G6 inhibited the in vitro hemagglutination of chicken erythrocytes which is the activity of the B oligomer of PT; thus we hypothesize that the MAb bound to its epitope on the S1 subunit and stereologically hinders the binding sites of the B subunits. The MAb also inhibited ex vivo Chinese hamster ovarian cell clustering and neutralized the in vivo leucocytosis- promotion in mice which are usually mediated by intracellular S1 subunit. The large molecular nature of the intact MAb and its molecular hydrophilicity led us to speculate that the observed PT neutralizing activities of the MAb were due to interfering with the cellular entry of the S1 rather than the intracellular enzyme neutralizing activity per se. While further experiments are needed to pinpoint the MAb neutralizing activity and to identify the amino acid sequence and location of the MAbPT6-2G6 epitope, our findings indicate that this murine MAb, in its humanized-version, should have high therapeutic potential for pertussis.

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VIRULENCE GENES OF CLINICAL **VIBRIO CHOLERAE** O1 ISOLATES IN THAILAND AND THEIR RIBOTYPES

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To determine virulence associated-genes and ribotypes of *Vibrio cholerae* epidemic strains isolated from cholera patients in Thailand.

Method: A total of 240 *V. cholerae* El Tor, O1 strains, isolated from patients with cholera in Thailand during two different periods, i.e. 1999-2000 (200 strains; 193 Ogawa and 7 Inaba) and 2001-2002 (40 strains; all Inaba), were analyzed for the presence of virulence genes, namely *ctxA*, *ctxB*, *zot*, *ace*, *toxR*, *tcpA*, *hlyA*, *nanH* and *ninT* by PCR. For ribotyping, genomic DNA segments of the 240 strains and 10 reference *V. cholerae* strains isolated before 1999 from Thailand and elsewhere were digested with *Bg*II endonuclease, subjected to a 0.8% agarose gel electrophoresis, blotted onto a nylon membrane and probed with enzyme-labeled *Escherichia coli* rRNA. The DNA bands were visualized by autoradiography.

Results: Genes encoding the A and B subunits of CT, Zot, Ace, ToxR, TcpA, HlyA, NanH and NinT could be mplified from all of the 10 *V. cholerae* O1 reference strains and from 239 of the 240 studied isolates. One Inaba isolate of 2001-2002 gave only amplicons of *toxR* and *hlyA*. For ribotyping, the 10 reference strains revealed six different patterns designated A to F. None of the 240 strains isolated in Thailand during the two periods had the A-C, E and F ribotypes. The isolates of 1999-2000 revealed ribotype D and three other ribotypes, designated G, H and I. The majority of the isolates of 2001-2002 showed ribotype G. The remaining showed other, J and K.

Conclusions: The clinical *V. cholerae* isolates of two epidemics from Thailand showed a sustained appearance of one epidemic *V. cholerae* clone, and a constant, but gradual and minor change in the genetic constituent of the other *V. cholerae* strains as indicated by the change of the ribotypes of the strains in the two study periods. Moreover, we found that a *V. cholerae* strain which cannot produce CT, Zot, Ace, TcpA, NanH and NinT can still cause symptomatic cholera.

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NOVEL LINEAR POLYMERS BEARING THIOSIALOSIDES AS PENDANT-TYPE EPITOPES FOR INFLUENZA NEURAMINIDASE INHIBITORS

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A conventional synthesis of α -thioglycoside of sialic acid as a glycomonomer was accomplished. Radical copolymerization of the glycomonomer with vinyl acetate proceeded smoothly to afford a new class of glycopolymers having thiosialoside residues, in which all protection was removed by a combination of transesterification and saponification to provide a watersoluble thiosialoside cluster. The results of a preliminary study on biological responses against influenza virus neuraminidases using the thiosialoside polymer as a candidate for a neuraminidase inhibitor showed that the glycopolymer has potent inhibitory activity against the neuraminidases.

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THIOSIALOSIDE CLUSTERS USING CARBOSILANE DENDRIMER CORE SCAFFOLDS AS A NEW CLASS OF INFLUENZA NEURAMINIDASE INHIBITORS

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An efficient synthesis of a series of carbosilane dendrimers uniformly functionalized with α -thioglycoside of sialic acid was accomplished. The results of a preliminary study on biological responses against influenza virus sialidases using thiosialoside clusters showed that some of the glycodendrimers have inhibitory potencies against the sialidases.

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CHARACTERIZATION OF ATYPICAL LYMPHOCYTES AND IMMUNOPHENOTYPES OF LYMPHOCYTES IN PATIENTS WITH DENGUE VIRUS INFECTION

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To characterize the immunophenotypes of lymphocytes in patients with dengue infection, we performed flow cytometric analysis of peripheral blood mononuclear cells collected from 49 dengue hemorrhagic fever (DHF), 25 dengue fever (DF), and 26 dengue-like syndrome (DLS) cases. The mean total atypical lymphocytes in DHF (916.1 ± 685.6 cells/µl) and DF (876.2 ± 801.9 cells/µl) were higher than those of DLS (310.5 ± 181.4 cells/µl). An atypical lymphocyte count of 10% or higher was a good indicator of dengue infection (sensitivity 50% and specificity 86%). Flow cytometric studies showed that the percentages of atypical lymphocytes correlated with those of CD19⁺ B lymphocytes and inversely correlated with the percentages of CD69⁺ lymphocytes. The mean absolute counts of atypical lymphocytes and CD19⁺ cells on the discharge day were significantly higher than those on the admission day. Low percentages of TdT⁺ cells were found in all groups of patients. We concluded that atypical lymphocyte and CD19⁺ cell counts may be a useful diagnostic tool for dengue infection and the recovery from the disease could be judged when numbers of both cell types are significantly elevated.

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AN AVIAN INFLUENZA H5N1 VIRUS THAT BINDS TO A HUMAN-TYPE RECEPTOR

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Avian influenza viruses preferentially recognize sialosugar chains terminating in sialic acid- α 2,3-galactose (SA α 2,3Gal), whereas human influenza viruses preferentially recognize SA α 2,6Gal. A conversion to SA α 2,6Gal specificity is believed to be one of the changes required for the introduction of new hemagglutinin (HA) subtypes to the human population, which can lead to pandemics. Avian influenza H5N1 virus is a major threat for the emergence of a pandemic virus. As of 12 June 2007, the virus has been reported in 45 countries, and 312 human cases with 190 deaths have been confirmed. We describe here substitutions at position 129 and 134 identified in a virus isolated from a fatal human case that could change the receptor-binding preference of HA of H5N1 virus from SA α 2,3Gal to both SA α 2,3Gal and SA α 2,6Gal. Molecular modeling demonstrated that the mutation may stabilize SA α 2,6Gal in its optimal *cis* conformation in the binding pocket. The mutation was found in approximately half of the viral sequences directly amplified from a respiratory specimen of the patient. Our data confirm the presence of H5N1 virus with the ability to bind to a human-type receptor in this patient and suggest the selection and expansion of the mutant with human-type receptor specificity in the human host environment.

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LONG-LASTING PROTECTIVE IMMUNE RESPONSE TO THE 19-KILODALTON CARBOXY-TERMINAL FRAGMENT OF **PLASMODIUM YOELII** MEROZOITE SURFACE PROTEIN 1 IN MICE

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Merozoite surface protein 1 (MSP1) is the major protein on the surface of plasmodial merozoite and its carboxy terminus, the 19-kDa fragment (MSP1₁₉), is highly conserved and effective in induction of a protective immune response against malaria parasite infection in mice and monkeys. However, the duration of the immune response has not been elucidated. As such, we immunized BALB/c mice with a standard four-dose injection of recombinant *Plasmodium yoelii* MSP1₁₉ formulated with Montanide ISA51 and CpG oligodeoxynucleotide (ODN) and monitored the MSP1₁₉-specific antibody levels for up to 12 months. The antibody titers persisted constantly over the period of time without significant waning, in contrast to the antibody levels induced by immunization with Freund's adjuvant, where the antibody levels gradually declined to significantly lower levels 12 months after immunization. Investigation of immunoglobulin *G* (IgG) subclass longevity revealed that only the IgG1 antibody level (Th2 type driven response) decreased significantly by 6 months, while the IgG2a antibody level (Th1 type driven response) due to the approach after immunization, but that the boosting effect was seen in the IgG1 antibody responses but not in IgG2a antibody responses. After challenge infection, all immunized mice survived with negligibly patent parasitemia. These findings suggest that protective immune responses to MSP1₁₉ following immunization using oil-based Montanide ISA51 and CpG ODN as an adjuvant are very long-lasting and encourage clinical trials for malaria vaccine development.

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MOLECULAR TYPING OF **LEPTOSPIRA** SPP. BASED ON PUTATIVE O-ANTIGEN POLYMERASE GENE (wzy), THE BENEFIT OVER 165 rRNA GENE SEQUENCE

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Molecular typing of leptospiral strains based on variation within putative O-antigen polymerase gene (*wzy*) was determined among reference strains and those isolated from patients. Using the PCR primers designed from the flanking gene of *wzy* derived from *Leptospira interrogans* serovar Copenhageni, all *L. interrogans* serovars as well as human and rodent leptospiral isolates from Thailand could be amplified. The size of PCR product ranged from 1 to 1.5 kb. The limitation of these primer pairs was the inability to amplify those strains whose sequences differ in the region of the primers, these included *Leptospira biflexa* (serovar Patoc), *Leptospira borgpetersenii* (serovar Tarassovi) and *Leptospira kirschneri* (serovar Bim, Bulgarica, Butembo). Notably, amplification was not limited to *L. interrogans* as demonstrated by the amplification of some strains from *L. kirschneri*, *Leptospira meyeri*, *Leptospira noguchii*, *Leptospira santarosai*, *L. borgpetersenii* and *Leptospira weilii*. The phylogenetic tree of *wzy* sequence, inferred by posterior probability of the Bayesian, enabled the categorization of leptospiral serovars into seven genetically related group, of which its differentiation power was better than that of the more highly conserved 16S rRNA gene, which is used extensively for genotyping.

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RAPID DISSEMINATION OF **PLASMODIUM FALCIPARUM** DRUG RESISTANCE DESPITE STRICTLY CONTROLLED ANTIMALARIAL USE

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Background: Inadequate treatment practices with antimalarials are considered major contributors to Plasmodium falciparum resistance to chloroquine, pyrimethamine and sulfadoxine. The longitudinal survey conducted in Dielmo, a rural Senegalese community, offers a unique frame to explore the impact of strictly controlled and quantified antimalarial use for diagnosed malaria on drug resistance. Methodology/Principal Findings. We conducted on a yearly basis a retrospective survey over a ten-year period that included two successive treatment policies, namely quinine during 1990-1994, and chloroquine (CQ) and sulfadoxine/pyrimethamine (SP) as first and second line treatments, respectively, during 1995-1999. Molecular beaconbased genotyping, gene sequencing and microsatellite analysis showed a low prevalence of Pfcrt and Pfdhfr-ts resistance alleles of Southeast Asian origin by the end of 1994 and their effective dissemination within one year of CQ and SP implementation. The Pfcrt resistant allele rose from 9% to 46% prevalence during the first year of CQ reintroduction, i.e., after a mean of 1.66 CQ treatment courses/person/year. The Pfdhfr-ts triple mutant rose from 0% to 20% by end 1996, after a mean of 0.35 SP treatment courses/person in a 16-month period. Both resistance alleles were observed at a younger age than all other alleles. Their spreading was associated with enhanced in vitro resistance and rapidly translated in an increased incidence of clinical malaria episodes during the early post-treatment period. Conclusion/Significance. In such a highly endemic setting, selection of drug-resistant parasites took a single year after drug implementation, resulting in a rapid progression of the incidence of clinical malaria during the early post-treatment period. Controlled antimalarial use at the community level did not prevent dissemination of resistance haplotypes. This data pleads against reintroduction of CQ in places where resistant allele frequency has dropped to a very low level after CQ use has been discontinued, unless drastic measures are put in place to prevent selection and spreading of mutants during the post-treatment period.

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FTO POLYMORPHISMS IN OCEANIC POPULATIONS

Ohashi J, Naka I, Kimura R, Natsuhara K, Yamauchi T, Furusawa T, Nakazawa M, Ataka Y, Patarapotikul J, Nuchnoi P, Tokunaga K, Ishida T, Inaoka T, Matsumura Y, Ohtsuka R

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It has been suggested that Neel's "thrifty genotype" model may account for high body weights in some Oceanic populations, which presumably arose in modern times. In European populations, common variants (rs1421085-C, rs17817449-G, and rs9939609-A) in the fat mass and obesity (FTO associated) were recently found to be associated with body mass index (BMI) or obesity. In this study, we investigated the population frequencies of these variants in six Oceanic populations (Melanesians, Micronesians, and Polynesians) and tested for an association with BMI. Unlike European populations, the Oceanic populations displayed no significant association between the FTO polymorphisms and BMI. These variants were in strong linkage disequilibrium. The population frequencies ranged between 4.2 and 30.3% in the six Oceanic populations, and were similar to those in southeast and east Asian populations. Our study of the FTO polymorphisms has generated no evidence to support the thrifty genotype hypothesis for Oceanic populations.

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REGULATION OF IMMUNE RESPONSE IN COMPLICATED MALARIA: BALANCE BETWEEN TH1 AND TH2 CYTOKINES AND CYTOKINE GENE POLYMORPHISM

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Complicated malaria caused by P. falciparum is characterized by multiple organ dysfunction. The pathogenesis of complicated malaria involves complex host-parasite interactions that include polarized cytokine responses. Complicated malaria is associated with differential elevations of certain pro- and anti-inflammatory cytokines such as TNF- α , IFN γ , IL-1, Il-6, Il-4, IL-10 and IL-12 etc. Over production of $INF-\alpha$, IL-1 and IL-6 has been shown to implicate in severity and pathogenesis of cerebral malaria. The IL-10 and IL-12 may function as immune modulators in *P. falciparum* infection by the finding of the elevation of IL-10 and IL-12 levels in plasma of individuals with complicated malaria. The dramatic increase of IL-10 was noted in the initial stage of the disease, followed by a reduction towards control values and closely paralleled parasite clearance; while IL-12, modest but persistence increases were noted over the entire stage of the disease that did not correlate with parasitaemia. The balance between Th1 like cytokines such as TNF- α and IFN- γ and Th2 like cytokines such as IL-4 and IL-10 involved in modulating the immune response and determined the severity of malaria. The IL-10 to TNF- α ratios were significantly lower in patients with hyperparasitaemias associated with anemia suggesting that higher IL-10 production reduced anemia by dampening the inflammatory effect of TNF- α . The IFN- γ levels were significantly elevated in patients with complicated malaria in the initial stage while the IL-4 levels were significantly elevated during the intermediate and late stages of the disease. The relative level of IL-4 and IFN- γ in association with IL-4 polymorphism and peripheral parasitaemia were investigated. A significant inverse correlation between the IL-4 to IFN- γ ratio and parasitaemia was found only in complicated malaria. The IL-4 expression could be regulated by host IL-4-590 gene polymorphism, which was determined by the counter regulation between IL-4 and IFN- γ . This could also modulate and regulate the production of anti-P. falciparum IgG, IgG subclasses and IgE antibodies which could be linked to the control of parasitaemia and affect the clinical outcome.

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POLYMORPHISM OF THE GENE ENCODING DUFFY BINDING PROTEIN OF **PLASMODIUM VIVAX** FROM THAI PATIENTS

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Plasmodium vivax Duffy binding protein (*Pv*DBP) has been considered to be a promising malaria vaccine candidate. Several studies have shown considerable genetic diversity in the cysteine rich region II of *Pv*DBP. We investigated the sequence variation of DBP gene at region II in 30 Thai isolates. Our analysis revealed a greater polymorphic rate in the region II than earlier studies. Haplotype analysis demonstrated 25 different haplotypes in 30 Thai isolates. A total of 25 and 5 nonsynonymous and synonymous mutations were found within region II of *Pv*DBP, respectively. Phylogenetic analysis based on measurement of nucleotide diversity between and within the different geographic populations illustrated the shared derived characters of *Pv*DBPII alleles between populations.

Presented at: The American Society of Tropical Medicine and Hygiene (ASTMH) 56th Annual Meeting, November 4-8, 2007, Philadelphia, Pennsylvania, USA.

DETECTION OF DENGUE NS1 ANTIGEN FOR EARLY DIAGNOSIS OF DENGUE VIRUS INFECTIONS

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The aim of this study is to determine dengue virus NS1 antigen for early acute dengue. Acute serum samples within 5 days after onset of symptoms were obtained from 65 patients consisting of 47 dengue cases and 18 non-dengue cases. In addition, 20 samples from blood donors as negative controls. The samples were tested for the presence of dengue antigen by using Dengue NS1 antigen-capture ELISA and dengue RNA by using RT-PCR. Dengue NS1 antigen was detected in 22 (46.8%) samples from dengue cases but none in samples from non-dengue cases and blood donors. Among negative results with dengue IgM (<40 units) and IgG (<100 units), dengue antigen was detected in 21 of 47 (44.7%) samples for IgM and in 14 of 21 (66.7%) for IgG. The results indicate that dengue NS1 antigen detection is valuable for early diagnosis and for using in a combination with serological test.

Presented at: Third Asian Regional Dengue Research Network Meeting, 21-24 August, 2007. The Grand Hotel, Taipei, Taiwan.

THE ENHANCING ANTIBODIES OF DENGUE VIRUS INFECTION IN THAI PATIENTS' SERA

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Dengue virus (DENV) can cause a wide spectrum of diseases, from undifferentiated febrile illness to dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Children with secondary infection with a different dengue virus serotype may have more severe DHF/DSS, which can be fatal. Antibody-dependent enhancement of infection (ADE) is widely accepted as central to the development of these clinical entities. Non-neutralizing anti-dengue antibody bound to the dengue virus infection, we used sensitive flow cytometry to detect dengue virus-infected K562 cells; a serial 3 to 9 – fold of patient serum was diluted and mixed with dengue virus, incubated with K562 cells, and then the percentage infected cells was determined by cytoplasmic staining with anti-NS1 antibody. The titer of enhancement was determined as the reciprocal of the serum dilution that ceased to provide enhancing antibodies increased gradually post-dengue virus infection; The convalescent phase had higher titers of enhancing antibodies than the acute phase. Furthermore, The Thai dengue patients' sera contained high titers of enhancing antibodies against their own dengue virus isolate, and the titers were similar to other serotypes of dengue virus. It was concluded that anti-dengue antibody would enhance dengue virus infection in a concentration-dependent, but serotype-independent manner.

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DENGUE VIRUS INFECTION RATES IN AEDES ALBOPICTUS RELATED TO ENVIRONMENTAL FACTORS

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Aedes albopictus is an important dengue-vector mosquito in forests, rural, and urban area and is a serious health problem in many tropical and subtropical regions, especially in Thailand. Environmental factors highly influence to dengue abundance and the feedding behavior of mosquitoes while the effect of temperature and relative humidity are possible contributing factors for engue hemorrhagic fever. The objective of this study is to examine the relationship of dengue virus infection rates in Ae. albopictus to environmental factors. The sensitivity and specificity of NASBA comparing to RT-PCR was 100 % and 90.62 %, respectively. The sensitivity of RT-PCR for detection of DENV-1 and DENV-3 were at 10 PFU/ml while DENV-2 and DENV-4 were at 100 PFU/ml. The growth rates of dengue virus were detected with NASBA and RT-PCR where the highest was in a part of the thorax and the head that was more amplified than the abdomen at day 0, day 7, and day 14 at 105-1 PFU/ml, respectively. One thousand, four hundred and twenty-one female Ae. albopictus mosquitoes were collected from dengue patients' house and control from Nakhonsithammarat Province in the rainy season (June to October, 2004) and in the dry season (March to April, 2005). Dengue virus 16 pools were positive with NASBA and while 4 pools and 4 individual mosquitoes were positive with RT-PCR. Identified dengue serotype was found in 3 of DENV-1 and only one of DENV-3 with RT-PCR and ELISA. Dengue virus infection rates in Ae. albopictus mosquitoes were 0.5% in rainy season, and no infection in dry season. Multiple regression for the dengue virus infection rates in Ae. albopictus mosquitoes indoor houses were positive correlation with humidity but not correlation with temperature and biting rate (r2 =0.054, p< 0.05). Multiple regression for the dengue infection rates in Ae. albopictus mosquitoes outdoor houses were not correlated with temperature, humidity, and biting rate (r2 =0.034, p> 0.05). The environmental factors and dengue infection rates had beneficial in controlling dengue viral distribution in the future for the Ministry of Public Health.

Presented at: Third Asian Regional Dengue Research Network Meeting, : 21-24 August, 2007. Venue: The Grand Hotel, Taipei, Taiwan.

AVIAN INFLUENZA-RELATED KABP AMONG SCHOOL STUDENTS, PARENTS AND TEACHERS IN THAILAND

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Since 2003, highly pathogenic avian influenza (HPAI) H5N1 has spread among human and poultry across the world. Up to the end of August 2007, 25 confirmed cases in humans and 17 deaths have been reported in Thailand, too. Among them, 3 confirmed cases were reported in Suphanburi Province, one of whom has died. We conducted this cross-sectional study to examine avian influenza-related knowledge, attitudes, beliefs and practices (KABP) among primary school student grade 4-6, their parents and teachers in Suphan buri Province, Thailand, in May 2007.

Most of the students, parents and teachers had high level of knowledge regarding transmission, risk group, symptom, prevention and control methods of avain influenza. Most of their attitudes and beliefs about avian influenza were also appropriate. However, some of these respondents presented relatively risky practices especially regarding control method; less than 30% of the respondents neither immediately report to the local authority/parents when one see dead poultry, nor immediately go and see doctors when they get common cold with high fever (38C) and cough. Mass media, such as television, newspaper and radio appear to have been effective at reaching the information of avian influenza to the people in the province.

These findings suggest that high level of knowledge does not necessary lead to behaviour change and that more interventions are necessary to improve the participants' risky behaviours. Since Thailand has well-established school heath system, health education intervention utilizing this system would induce their behavioural change.

Presented at: The Joint International Tropical Medicine Meeting (JITMM) 2007, 29-30 November 2007, Imperial Queen's Park Hotel, Bangkok, Thailand.

DENGUE VIRUS INFECTION RATES IN **AEDES ALBOPICTUS** RELATED TO ENVIRONMENTAL FACTORS

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Aedes albopictus is an important dengue-vector mosquito in forests, rural, and urban area and is a serious health problem in many tropical and subtropical regions, especially in Thailand. Environmental factors highly influence to dengue abundance and the feeding behavior of mosquitoes while the effect of temperature and relative humidity are possible contributing factors for Dengue hemorrhagic fever. The objective of this study is to examine the relationship of dengue virus infection rates in Ae. albopictus to environmental factors. The sensitivity and specificity of NASBA comparing to RT-PCR was 100 % and 90.62 %, respectively. The sensitivity of RT-PCR for detection of DENV-1 and DENV-3 were at 10 PFU/ml while DENV-2 and DENV-4 were at 100 PFU/ml. The growth rates of dengue virus were detected with NASBA and RT-PCR where the highest was in a part of the thorax and the head that was more amplified than the abdomen at day 0, day 7, and day 14 at 10⁵-1 PFU/ml, respectively. The 1,421 of female Ae. albopictus mosquitoes were collected from dengue patients' house and control from Nakhonsithammarat Province in the rainy season (June to October, 2004) and in the dry season (March to April, 2005). Dengue virus 16 pools were positive with NASBA and while 4 pools and 4 individual mosquitoes were positive with RT-PCR. Identified dengue serotype was found in 3 of DENV-1 and only one of DENV-3 with RT-PCR and ELISA. Dengue virus infection rates in Ae. albopictus mosquitoes were 0.5% in rainy season, and no infection in dry season. Multiple regression for the dengue virus infection rates in Ae. albopictus mosquitoes indoor houses were positive correlation with humidity but not correlation with temperature and biting rate (r^2 =0.054, p< 0.05). Multiple regression for the dengue infection rates in Ae. albopictus mosquitoes outdoor houses were not correlated with temperature, humidity, and biting rate (r^2 =0.034, p> 0.05). The environmental factors and dengue infection rates had beneficial in controlling dengue viral distribution in the future for the Ministry of Public Health.

Presented at: The Joint International Tropical Medicine Meeting (JITMM) 2007, 29 -30 November 2007, Imperial Queen's Park Hotel Bangkok, Thailand.

DENGUE INFECTION RATES IN AEDES AEGYPTI, PETCHABUN PROVINCE

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Dengue hemorrhagic fever is the most important arthropod-borne viral disease, and is one of the major public health problems in Thailand. The most important vector of the disease is *Aedes aegypti*. Environmental factors affect dengue virus infection, such as temperature, relative humidity, the number of *Aedes* sp. mosquitoes, biting rate, and infection rate. In this study, *Ae. aegypti* mosquitoes were collected in Amphur Muang, Petchabun Province. The mosquitoes were collected from 16 villages (16 patients' houses and 64 neighboring houses), and from 20 houses with no incidence of dengue infection during the past 3-5 years. Of the 1,574 *Aedes* sp., 1,433 (91.04%) were *Ae. aegypti* and 141 (8.96%) were *Ae. albopictus*. Indoor biting rates of *Ae. aegypti* were 14.87 times higher than outdoor, and biting times of 09.00-10.00, 10.00-11.00, 11.00-12.00 yielded 1.77, 1.46, 0.68 mosquitoes/man-hour, respectively. The infection rate was 1.76% and most were found in neighbors' houses; the probability to detect dengue virus in mosquitoes at the neighbors' houses was 1.25 times, especially where distances between neighboring houses and patients' houses were less than 50 meters. The relative humidity in dengue-infected mosquitoes was significantly higher than villages that free from dengue-infected mosquitoes (Mean±SD = 82.55±5.5% VS 78.93±7.67%, p < 0.05). The results of this study will be useful to the Ministry of Public Health for controlling dengue transmission efficiently in the future.

Presented at: The Joint International Tropical Medicine Meeting (JITMM) 2007, 29-30 November 2007, Imperial Queen's Park Hotel Bangkok Thailand.

A COMPARATIVE EVALUATION OF FOUR TECHNIQUES FOR THE SEROLOGICAL DIAGNOSIS OF SCRUB TYPHUS

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This study was performed to compare the performance of four techniques namely the indirect immunoperoxidase test (IIP), Weil Felix test (WF), latex agglutination test (LAT) and the indirect immunofluorescence antibody test (IFA). Among 545 Pyrexia of unknown origin (PUO) cases from two hospitals, the IFA confirmed that 27.5% of 295 cases from Burirum Hospital and 35.6% of 250 cases from Surin Hospital were scrub typhus. The scrub typhus, non-scrub typhus and additional 180 negative control sera were further evaluated with IIP, WF and LAT compared to the IFA as a reference standard. The IIP at cut-off titer \geq 1:400 had a sensitivity of 98.8% and specificity of 99.5% while the sensitivity of the WF test at cut-off titer \geq 1:160 was only 37.1% and the specificity was 93.5%. In addition, the LAT at cut-off titer \geq 1:16 gave the optimal 86.5% sensitivity and 95.5% specificity. The data indicated that IFA, IIP and LAT were superior to the WF. However, the IIP and IFA require a trained technician and laboratory equipment while the LAT is easy to perform but it need to use antigen from *O. tsutsugamushi* which is difficult to culture. Development of a similar kind of LAT by using recombinant protein antigens would be desirable.

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SERODIAGNOSIS OF SCRUB TYPHUS AND MELIOIDOSIS BY DUAL IMMUNOPEROXIDASE TEST

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The dual immunoperoxidase test (dual IIP) was developed for a simple and rapid detection of antibody against *Orientia tsutsugamushi* and *Burkholderia pseudomallei* in serum specimens. The test was evaluated by using 300 serum samples from known cases of scrub typhus, melioidosis, other febrile diseases, and healthy control and compared with the indirect immunofluorescence antibody (IFA) test as a reference method. In this study, a titer of \geq 1:400 was selected as a cut-off titer for scrub typhus and melioidosis diagnosis. When compared with the IFA, it showed 100% sensitivity and specificity for scrub typhus detection. With the same test it showed 98.1% sensitivity and 98% specificity for the diagnosis of melioidosis. The performance of the dual IIP test was further evaluated with 475 clinical specimens collected from Buriram Hospital and Takao Pa District. In conclusion, this study found that the dual IIP test exhibits a good potential as a replacement for the IFA test. Furthermore, this test can be used for the concomitant diagnosis of both scrub typhus and melioidosis.

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IDENTIFICATION OF GENUS- AND/OR SPECIES-SPECIFIC ANTIGENS OF **AEROMONAS** BY WESTERN BLOTTING

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Aeromonas spp. are important intestinal and extra-intestinal pathogens of humans and other animals. However, classification of genus Aeromonas has been troublesome due to changing taxonomy, reporting of new species, and unavailability of a universal accepted identification scheme. A pragmatic approach is therefore important to identify Aeromonas spp. of clinical relevance. Immunological system is one of choice based on the principle of specificity, simplicity and rapidity. Prior to conduct the process, specific antigens are thus needed to be identified. This study, aimed to identify genus-and/or species-specific antigens of Aeromonas. The whole cells lysates of 123 Aeromonas spp. and other closely related bacteria were examined by western blotting with mouse polyclonal antisera raised against whole cells of each Aeromonas spp. (A. hydrophila, A. sobria, A. caviae, A. trota, A. jandaei, A. veronii, A. media). The results showed that antibodies against all seven species of Aeromonas reacted to an antigen with molecular mass about 14 kDa. This indicated that the band could presumptively be genus-specific antigen of Aeromonas. However, it was difficult to determine species-specific antigens because the reaction patterns of the antibodies and homologous antigens appeared as a smear or ladder or both. Moreover, comparison of the reactions to the other isolates of the same species revealed highly heterogeneity. In an attempt to study this heterogeneity, the reaction patterns of antibodies to each species were identified from 3-26 isolates of each and grouped by the UPGMAgrouping system (SynGene software). Data obtained in analysis with Jaccard's coefficient ≥ 0.50 demonstrated that 26 isolates of A. hydrophila, A. sobria, A. caviae, A. trota, contained 12, 11, 11 and 13 groups, respectively. While 8 of A. jandaei contained 6 groups, 7 of A. veronii contained 3 groups and all 3 A. media were classified into 3 different groups.

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PRODUCTION OF MONOCLONAL ANTIBODY AGAINST AEROMONAS VERONII

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Aeromonas veronii is a Gram-negative bacterium and is potentially pathogenic to humans. Serological diagnosis for detection of *A. veronii* has not been well established. This study, therefore, aimed to produce monoclonal antibodies (MAbs) against *A. veronii*. Spleen cells from mice immunized with crude antigens of *A. veronii* 03086 were fused to myeloma cells and five hybridomas, i.e., 72A5, 73G7, 76D12, 75D5 and 73B3, were obtained and characterized. They were not reactive to other Gram-negative bacteria as tested by ELISA. The isotypes of these MAbs were of lgG1, except clone 75D5 that produced both lgG1 and lgG3. They recognized the antigen on outer membrane protein (Omp) of molecular mass approximately 40 and 48 kDa. These MAbs limitedly reacted to different strains of *A. veronii*; all MAbs reacted to 2 of 7 *A. veronii* strains tested and three of them were able to react to *A. hydrophila* 03054.

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CORRELATION OF CD4+CD25+ REGULATORY T CELLS AND ACTIVE AUTOIMMUNITY IN C4KO MICE

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Classical complement component C4 deficiency is the high risk factor for autoimmune disease including systemic lupus erythematosus (SLE) in Angio-Irish, North American, Black American Mexican American, Australian, and Japanese including Thai populations. Our previous study, we found that 5.93% of C4 null genes (C4A/ C4B) were found in Thai patients by touchdown PCR and its C4 deletion and/or point mutations have been revealed by SSP-PCR. From those deficiencies, all SLE patients who had C4A and/ or C4B deficiencies exhibited more than 5 ACR criterias including malar rash, oral ulcers, renal disorder, immunological disorder including anti-nuclear antibody. Most studies shown that thymus-derived CD4+CD25+ regulatory T cell (Treg) are essential for the maintenance of immunological self-tolerance. To understand the correlation between autoantibody production and Treg cells expression, our study had been focused to quantity of Treg cell in active autoimmunity (high ELISA autoantibody titre) in various tissues (spleen, bone marrow and blood) by using Flow Cytometry in C4KO mice comparison with normal mice in different ages (2, 4, 6 and 8 months). Our results shown that the percentage of CD4+CD25+ cells in C4 knockout mice were higher than normal mice in all age groups. Furthermore, CD4+CD25+ Treg cells were highest found in blood when compared with bone marrow and spleen in both C4 knockout mice and normal mice. Also, real time qualification PCR was used to study of transcription factors (*FoxP3* and TGF-b) of Treg cell to confirm the Treg expression in mouse tissues.

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CONSTRUCTION OF SINGLE CHAIN ANTIBODY LIBRARY SPECIFIC TO H5N1 AVIAN INFLUENZA VIRUS

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Objective: To construct scFv antibody phage library specific to H5N1 avian influenza virus using phage display technique.

Methods: For antibody library construction specific to H5N1 avian influenza virus, First, the attenuated H5N1 antigen were used to immunized the white leg horn chicken. After the high titer immunity to H5N1 antigen were obtained tested by ELISA, three chicken spleens were isolated and pooled together. Total RNA were then isolated from these chicken spleens using TRIZol reagent. After that, complementary DNA (cDNA) were reverse transcribed from the RNA. cDNA were further used as template for antibody gene amplification specific to variable heavy chain (V_{H}) and variable light chain (V_{I}) . These V_{H} and V₁ were linked together by second PCR (Overlap extension PCR) with peptide linker to generate scFv which composed of V_1 -linker- V_{H} . This scFv were then digested with restriction enzyme Sfi1 and ligated to phagemid vector pComb3XSS that is also previously cut with the same enzyme. The recombinant DNA were further transformed into XL1-Blue E. coli host cell, and co-infected with wild type helper phage VCSM13. Currently, scFv antibody libraries were successfully constructed $(1.9 \times 10^8 \text{ different clones})$, by which scFv sequence were displayed as fusion protein with the major coat protein of phage particles. The H5N1 specific antibody sequence will be further selected from this library by affinity selection depending on the interaction between antigen and antibody in panning step. In this step, the H5N1 antigen will be immobilized on solid support (microtiter plate). The antibody library will be incubated with the antigen on the plate. After incubation, the unbound phage with non-specific sequence will be washed off. Then, the bound phage will be eluted and used for further selection in the next panning step. This panning will be performed around 3-5 times until the high specific clones of H5N1 were selected.

Result: scFv antibody library with 1.9 x 10⁸ diversities were successfully produced from non-immunized chicken. Moreover, scFv antibody library from H5N1 immunized chicken are also under construction.

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CONSTRUCTION OF ANTIBODY PHAGE LIBRARY SPECIFIC TO H5N1 AVIAN INFLUENZA VIRUS

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Avian influenza virus H5N1 strain has caused adverse impact on the social and economic of both developed and developing countries. So, high sensitivity and specificity diagnostic reagent of this deadly disease is needed. In this study, it was aim to construct single chain antibody library specific to H5N1 avian influenza virus originated from H5N1 immunized chicken, for further use as diagnostic reagent. Moreover, antibody library from non-immunized chicken, were also constructed. Three chicken spleens from immunized, and non-immunized chicken were isolated and were pooled together in each library. Total RNA were then isolated and complementary DNA (cDNA) were reverse transcribed. Variable heavy chain (V_H) and variable light chain (V_L) were further amplified. These VH and VL were linked together by second PCR (Overlap extension PCR) with peptide linker for scFv construction (V_L -linker- V_H). This scFv were then digested with restriction enzyme *Sf*₁1 and ligated to phagemid vector pComb3XSS. The recombinant DNA were transformed in to XL1-Blue *E. coli* host cell, and displayed on phage surface by co-infected with wild type helper phage VCSM13. Currently, two scFv antibody libraries were successfully produced, 1st library from non-immunized chicken with 1.9 x 10⁸ diversity and 2nd library from immunized chicken with 3.1 x 10⁹. These libraries will be further selected for the H5N1 avian influenza virus specific antibody clone in panning step.

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RECOMBINANT TROPOMYOSIN OF AMERICAN COCKROACH, PERIPLANETA AMERICANA

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Background: American cockroach (CR), *Periplaneta americana* is an important source of indoor allergens among Thais. Currently, screening and monitoring allergic status of allergic patients are performed by skin prick test using crude CR extract which varies in the allergenic composition from batch-to-batch. Thus, recombinant CR allergens may be a better standardized alternative. Thus, in this study, we produced recombinant tropomyosin (Per a7) which is one of the *P. americana* major allergens. The protein was tested for its ability to bind to IgE in sera of CR allergic Thai patients.

Objectives: To produce recombinant tropomyosin of American cockroach, and to determine the IgE binding ability of the recombinant allergen

Materials & Methods: Sera of patients with CR allergy who gave positive skin prick test to CR extract and sera of nonallergic counterparts were collected. Total RNA was extracted from adult *P. americana* and first strand cDNA was produced by RT-PCR. Gene encoding tropomyosin was amplified using oligonucleotide primers and the cDNA as a template. The amplified sequences were cloned into a cloning vector and a protein expression vector. The latter was used to transfect an *Escherichia coli* host. *E. coli* transformant was grown and production of tropomyosin protein was induced by IPTG. The protein was extracted and purified from the bacterial lysate.

Results: Gene encoding American cockroach tropomyosin (450 bp) was amplified and cloned into a cloning vector, *i.e.* pGEM T and a protein expression vector, *i.e.* pET 20 b+. Amplified sequence showed 100% homology to the tropomyosin gene sequence in the database. Recombinant tropomyosin was produced and purified from whole cell lysis of an *E. coli* transformant grown under IPTG induced-condition. The protein (~16 kDa) was found to bind IgE in sera of allergic Thai patients.

Conclusion and Clinical Relevant: Recombinant tropomyosin of *P. americana* was successfully produced in pure form. The protein binds IgE in sera of CR allergic Thai patients. Thus, the protein may be used as a standard reagent for screening and monitoring of the allergic status of patients.

Presented at: World Allergy Congress 2007, Queen Sirikit National Convention Center (QSNCC), Bangkok. Thailand. December 2-6, 2007. Abstract page no. 80.

DEVELOPMENT OF NOVEL COCKROACH ALLERGY MODEL

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Background: Allergy mouse model is used for investigation the mechanisms of inhalational tolerance to antigens or of allergic airway sensitization. However, these responses have typically been assessed isolation from each other. Thus, in this study, we developed a murine cockroach allergy model by intraperitoneally immunized mice with crude American CR extract mixed with alum and mice were given the crude CR extract in phosphate buffered saline (PBS) in aerosol, using a home-made mouse nebulizer.

Objectives: To develop a cockroach allergy model in mice

Materials & Methods: Individual BALB/c mice were injected intraperitoneally (i.p.) with three doses of crude CR extract emulsified in aluminum hydroxide (Alum) on days 0, 7, and 14. They were challenged on days 21 and 22 with 10 ml nebulized crude CR extract (1.0% w/v in PBS), using an air-pressure nebulizer. On day 23 mice were sacrificed by cervical dislocation; and serum, broncho-alveolar lavage fluid (BALF) and lungs were collected for further examination. Mice received Alum alone which served as controls were challenged with PBS and were sacrificed as for the test group.

Results: All mice were found to be allergic to the CR allergen. Their BALF contained inflammatory cells that outnumbered those of the non-allergic counterparts as determined by differential cell counts after cyto-spinning and staining of the BALF with Hematoxylin-Eosin dyes. Levels of serum IgE specific to the American CR extract rose significantly above those of the controls. Inflammatory cell infiltration, most were eosinophils, was pronounced in lung tissues of the allergenic mice and was negligible in the controls.

Conclusion: A murine cockroach allergy model was successfully development. It can use for study immunopathology of allergy and determine the allergenicity of the protein of interest.

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PREPARATION OF HUMAN MONOCLONAL ANTIBODY THAT NEUTRALIZES TETANUS TOXIN USING PHAGE DISPLAY TECHNOLOGY

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Introduction: Current mainstay for treatment of tetanus is by passively giving an anti-tetanus toxin serum to the patient. Most of the therapeutic preparations are from heterologous sources. Complications usually develop in the serum treated-recipients, such as IgE-mediated type 1 (immediate) hypersensitivity or serum sickness. Other limitations in using the animal derived immune serum include prolonged immunization procedure, risk of the recipient to certain zoonosis, and variation in the serum therapeutic efficacy. Fully human tetanus toxoid specific antibody should be a suitable alternative of the animal derived immune serum.

Objective: To produce immunotherapeutic human monoclonal antibodies in the form of single chain antibodies (ScFv) against tetanus toxin by using phage display human antibody library

Methods: Tetanus toxoid was recovered from the commercially available tetanus vaccine by removal of the alum adjuvant. The purified toxoid was used in a bio-panning process to select a population of phages displaying toxoid specific ScFv on their surface. High antigen binding affinity phage clones were further selected by using a phage ELISA. Individual phages were used to transfect non-suppressor *E. coli* bacteria for production of the soluble ScFv.

Results: Purified toxoid was successfully recovered from the tetanus vaccine after centrifugation and dialysis to remove the alum adjuvant. The purified toxoid was used in a "single-round" phage bio-panning against an established phage display human antibody library and the tetanus toxin specific phage population was obtained. Six individual phage clones with

high binding affinity ScFv to the tetanus toxoid were obtained after a phage ELISA using the toxoid as an antigen to coat a microtiter ELISA plate and the bovine serum albumin as a negative antigen control. These clones were used individually to transfect HB2151 *E. coli*. Transformants carrying *scFv*-phagemid were obtained.

On-going work: Soluble ScFv specific to the tetanus toxoid will be prepared and purified from the appropriate transformed *E. coli* clone(s). Antigenic specificity of the ScFv will be determined. Individual ScFv or their cocktail will be tested for protective efficacy to tetanus toxin by *in vitro* neutralization using neuron cell line.

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DEVELOPMENT OF NOVEL MULTIPLEX REVERSE TRANSCRIPTASE-PCR ASSAYS FOR RAPID DETECTION OF ARBOVIRUSES

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This study was designed to develop a highly specific, sensitive and cost-effective molecular diagnostic assay for rapid detection of Dengue virus sero-types (DEN 1-4), Chikungunya virus (CHIK), Japanese encephalitis virus (JEV) and West Nile viruses (WNV) from the clinical sample (serum), infectious culture fluid (ICF) and mosquitoes, by a single-tube-single-step multiplex RT-PCR (mRTPCR I & II) assays. Specificity and sensitivity of the mRT-PCRs and uniplex RT-PCR (uRT-PCR) have been compared and evaluated using 6 different cross combinations of Reverse transcriptases (RTAce and AMV-RT) and DNA-polymerases (LA-Taq, rTaq and Tth). Of the 6 combinations, the AMV-RT and LA Taq was found superior in terms of sensitivity and specificity compare to other. Genome detection limit of mRT-PCR I for DEN-1, 1; DEN-2, 20 ; DEN-3, 0.1; DEN-4, 10 and CHIK was 10 FFU (focus forming unit) and the mRT-PCR II for JEV and WNV was 10 and 1 FFU, respectively. The primers designed specifically for these mRT-PCRs did not show cross-reactivity within the sero-types of Flavi and Alpha viruses. Therefore, these two mRT PCRs assay could be used as a highly sensitive, reliable, rapid and cost-effective diagnostic tools for the diagnosis and molecular epidemiological surveillance of the viruses.

Presented at: Third Asian Regional Dengue Research Network Meeting. 21-24 August, 2007. The Grand Hotel, Taipei, Taiwan.

DETECTION OF SPECIFIC ANTIBODIES TO RECOMBINANT DENGUE VIRAL PROTEIN USING IMMUNOBLOT ASSAY

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The four serotypes of dengue virus, a member of the Family *Flaviviridae* of positive-sense single-stranded RNA viruses, cause a broad spectrum of clinical manifestations in humans, ranging from acute febrile illness DF to the life-threatening DHF/DSS. Laboratory diagnosis of dengue virus infection relies on the detection of specific anti-dengue IgM and IgG antibodies by using various kinds of antigen. Recombinant DNA technology is a method used to produce novel proteins in prokaryotic and eukaryotic expression system. The prM-M protein plays important role in immune response and is the major target of neutralizing antibodies. In this study, the prM-M gene was cloned into a bacterial expression vector of pRSET-B, and was expressed in *E. coli* BL21(DE3)pLysS. The expressed protein was found in the insoluble form (inclusion bodies) of the cell lysates. This protein was then solubilized with 8 M urea, before subjected to purify by Ni-NTA affinity chromatography. The purified recombinant prM-M protein (~18 kDa) could be detected for Histidine tag fusion protein. However, there were a few smaller bands of protein in addition to the recombinant protein, which indicated some impurity. These may also be caused by the degradation products of the recombinant protein. The partially purified prM-M protein could react

to antibodies in sera sample from patients infected with dengue virus and could react to some sera from patients infected with scrub typhus. The efficacy and sensitivity to use immunoblot for diagnosis was low.

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DETECTION OF DENGUE NS1 ANTIGEN FOR EARLY DIAGNOSIS OF DENGUE VIRUS INFECTIONS

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The aim of this study is to determine dengue virus NS1 antigen for early acute dengue. Acute serum samples within 5 days after onset of symptoms were obtained from 65 patients consisting of 47 dengue cases and 18 non-dengue cases. In addition, 20 samples from blood donors as negative controls. The samples were tested for the resence of dengue antigen by using Dengue NS1 antigen-capture ELISA and dengue RNA by using RT-PCR. Dengue NS1 antigen was detected in 22 (46.8%) samples from dengue cases but none in samples from non-dengue cases and blood donors. Among negative results with dengue IgM (<40 units) and IgG (<100 units), dengue antigen was detected in 21 of 47 (44.7%) samples for IgM and in 14 of 21 (66.7%) for IgG. The results indicate that dengue NS1 antigen detection is valuable for early diagnosis and for using in a combination with serological test.

Presented at: The Seventeenth Scientific Annual Meeting of The Virology Association (Thailand), 16 November 2007, The Twin Tower Hotel, Bangkok, Thailand.

CANINE PARASITIC ZOONOSES IN BANGKOK TEMPLES

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Fecal samples were collected from 204 humans and 229 dogs from 20 different temples in Bangkok, as well as communities in the surrounding temple ground areas. Human and dog stool samples were examined for intestinal parasites including Giardia using zinc sulfate flotation and microscopy. Hookworms were the most common parasite in dogs (58.1%) followed by *Trichuris* (20.5%), *Isospora* (10%), *Giardia* (7.9%), *Toxocara* (7.4%), *Dipylidium caninum* (4.4%) and *Spirometra* (3.1%). *Blastocystis hominis* (5.9%) was the most common parasite in humans followed by hookworms (3.4%), *Giardia* (2.5%), *Strongyloides* (2%) and *Cryptosporidium* (1.5%). All samples microscopy-positive for *Giardia* were genotyped. The majority of *Giardia* isolated from the dog population was placed in Assemblage A, followed by Assemblages D, B and C, respectively, while human isolates were placed in Assemblages A and B. Therefore, dogs in temple communities posed a potential zoonotic risk to humans for transmission of hookworms, *Giardia* (especially Assemblage A genotypes) and *Toxocara canis*.

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IMMUNOHISTOCHEMICAL STUDY OF ACUTE AND CHRONIC TOXOPLASMOSIS IN EXPERIMENTALLY INFECTED MICE

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Acute and chronic Toxoplasma infections were evaluated in mice using stage specific antibodies and immunocytochemistry. Mice with acute toxoplasmosis were less active, had erectile body hair and seldom took food or water resulting in weight loss. All mice died within 7 days post-inoculation. The immunohistochemical technique enhanced visualization of parasites allowing their distribution to be accurately followed. Following intraperitoneal infection, tachyzoites were initially identified on the surface of the liver and spleen. There was a rapid increase in the number of tachyzoites associated with invasion from the surrounding connective tissue into the organs with formation of inflammatory lesions in the liver. The focal inflammatory lesions showed increasing numbers of tachyzoites with the period post-inoculation. Similar increases in tachyzoites were observed for the spleen. In contrast, only a few individual tachyzoites were seen in the brain at the final time point. In chronic infections, the mice were asymptomatic but tissue cysts containing large numbers of bradyzoites were observed in all brains with the average number of 295 tissue cysts per half brain and the average cystic size of 46.02 +/- 5.08 micron. By histology and immunostaining, the tissue cysts were readily identifiable along with a mild inflammatory cell infiltration into the meninges and perivascular cuffing. Double immunocytochemical labelling confirmed the exclusive presence of tachyzoites during the acute phase and bradyzoites during the chronic phase.

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NOVEL DRUG COMPOUNDS AGAINST NEOSPORA CANINUM AND TOXOPLASMA GONDII IN VITRO

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Neospora caninum has recently been identified as an important cause of abortion in cattle worldwide. This parasite is closely related to *Toxoplasma gondii*. To identify the drug compounds for potential use against both parasites *in vitro*, nine novel drug compounds were incubated with either parasite on microtiter plate. The number of extracellular tachyzoites and the quantities of Vero cells left in the wells after incubating with those nine drugs were compared to the conventional drug control, a combination of sulfadiazine 25g/ml and pyrimethamine 0.1g/ml. The most effective drugs against both *N. caninum* and *T. gondii* in this study were trifluralin analogues.

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PARTIAL PURIFICATION AND CHARACTERIZATION OF DNA POLYMERASE BETA-LIKE ENZYME FROM PLASMODIUM FALCIPARUM

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DNA polymerases play crucial roles not only in DNA replication, transcription and recombination but also in DNA repair in order to maintain the integrity of the cell's genome. In *Plasmodium falciparum*, only three types of DNA polymerases, namely DNA polymerase- α , γ and δ have previously been characterized whereas DNA polymerase β , the major enzyme operating during base excision repair in eukaryotes has yet to be isolated and characterized. In this study, DNA polymerase β -like activity was detected in crude extract of *P. falciparum* trophozoites. Partial purification of *P. falciparum* DNA polymerase

β-like enzyme was performed using fast protein liquid chromatography, with a yield of 2.8% and 825-fold purification. The partially purified enzyme was highly resistant to aphidicolin and N-ethylmaleimide, as in other eukaryotic enzymes, but was also resistant to 2', 3'-dideoxythymidine-5'-triphosphate and to other synthetic nucleoside analogs. The parasite enzyme showed low processivity. Using UG mismatch substrate to investigate base excision repair, the *P. falciparum* DNA polymerase β-like enzyme was able to repair a patch size of 3 to 5 nucleotides, indicative of involvement in a long patch repair pathway, the first evidence of such a property in DNA polymerase of a malaria parasite.

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IMS-FREE DNA EXTRACTION FOR THE PCR-BASED QUANTIFICATION OF CRYPTOSPORIDIUM PARVUM AND GIARDIA LAMBLIA IN SURFACE AND WASTE WATER

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Extremely limited knowledge exists on the occurrence of protozoan pathogens in surface and waste water in the developing world. The article addresses one of the main reasons for this: a prohibitively costly immunomagnetic separation and commercial DNA extraction kits required for the pathogen detection. As the presence of inhibitory substances critically impedes the PCR-based detection of *Cryptosporidium* and *Giardia* in environmental samples, several direct DNA extraction methods based on the combination of physico-chemical means were evaluated in terms of reducing the impact of PCR inhibitors present in (oo)cyst-spiked water concentrates. Modifications that included the use of guanidine thiocyanate as a lysis agent and a sonication step were found to be more efficient in extracting DNA from (oo)cysts, while treatment with Chelex 100 chelating resin at post-lysis proved to be effective in the removal of the PCR inhibitors rather than the inclusion of the PCR facilitators during thermocycling. Direct DNA extraction protocol at a substantially reduced cost is proposed for the use in the PCR-based detection/quantification of the pathogens.

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BIOLOGICAL CONTAMINATION-FREE THAI FROZEN FOOD

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The globalization of Free Trade Agreement (FTA) on food products increases the possibility of more extensive transmission of biological contamination. Hazard Analysis Critical Control Point (HACCP) system was introduced as a tool to prevent protectionistic trade. As a major seafood exporter to various regions of the world, Thailand is considering to include the HACCP system for the frozen food industries. Hazard identification of protozoa was performed in raw materials, food products and water used in the industry in 3 Provinces, namely Samut Sakon, Ranong and Songkla. Raw and treated water samples were collected by large volume technique through a filter with 1 µm nominal porosity. Immunomagnetic separation (IMS) for *Giardia* and *Cryptosporidium* was then applied to eluted and concentrated water. Identifications were individually performed by immunofluorescent and PCR techniques. In raw water, *Giardia* was found in 10, 10 and 60% whilst *Cryptosporidium* was found in 10, 20 and 35% in Samut Sakon, Ranong and Songkla Provinces, respectively. However, no contamination in treated water samples was found. Coli form bacteria was also determined by culture technique, but was not found in any treated water. *Giardia* was also identified in raw materials and food products. The viability of recovered protozoa was analyzed by the reverse transcriptase polymerase chain reaction (RT-PCR). The giardin beta-subunit mRNA gene was selected as the target. Positive RT-PCR for *Giardia* recovered from raw materials and food products indicates viability. The effect of ultra violet light with the concentration of 10 mWs/cm² for 20-100 seconds inactivated viable recovered protozoa, but the sun light exposure was unable to control those viable biohazards.

Information gathering from this study suggested that protozoa are important biological hazard recovered not only from water system, but also from raw materials and final frozen food products. We recommended that the critical control points

should be applied in all steps including water system by using ultra violet short exposure time as well as raw material and food product systems by controlling human contamination. Further studies concerning tracing the sources of contamination and an alternative methods, which are more sensitive but simple to carry out on an industrialized scale, are needed.

This project was financially supported by the National Research Council of Thailand.

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GIARDIA AND CRYPTOSPORIDIUM AS BIOHAZARD IN FROZEN FOOD PRODUCTS

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Giardia and *Cryptosporidium* commonly cause food and water-borne disease outbreaks worldwide. They occur in both normal and immunocompromised hosts causing both health and economic burdens. The globalization of Free Trade Agreement (FTA) on food products increases the possibility of more extensive transmission. Hazard Analysis Critical Control Point (HACCP) system at present covers only conventional bacteria. As a major seafood exporter to various regions of the world, Thailand is considering to include those protozoa into the HACCP for the frozen food industries.

Hazard identification of protozoa was performed in raw materials, food products and water used in the industry. Raw and treated water samples were collected by large volume technique through a filter with 1 micrometer (µm) nominal porosity. Immunomagnetic separation (IMS) for *Giardia* and *Cryptosporidium* was then applied to eluted and concentrated water. Identifications were individually performed by immunofluorescent and PCR techniques. In raw water, *Giardia* was found in 42.8-60% whilst *Cryptosporidium* was found in 20-35%, but no contamination in treated water samples was found. *Giardia* was also identified in raw materials and food products. The viability of recovered protozoa was analyzed by the reverse transcriptase polymerase chain reaction (RT-PCR). The giardin beta-subunit mRNA gene was selected as the target. Positive RT-PCR for *Giardia* recovered from raw materials and food products indicates viability. The effect of various concentrations of ultra violet light and difference in duration of solar exposure to control those viable recovered biohazards was studied.

This project was financially supported by the National Research Council of Thailand.

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DETECTION OF FOOD-BORNE PATHOGENIC PROTOZOA CONTAMINATED IN THAI VEGETABLES

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Contaminated protozoa such as *Giardia*, *Cryptosporidium* and *Cyclospora* are causative agents of food-borne diseases resulting in health and economic burdens. Thai famous dish called "Somthum", includes the following vegetables papaya, chili, tomato, morning glory and cabbage. There is no report about protozoa contamination in Thai vegetables. The aim of our study is to detect *Cryptosporidium*, *Cyclospora* and *Giardia* in famous Thai dish ingredients. Total of 50 vegetable samples were collected from 10 different markets that represented each region of Bangkok. Elution by Tween20 solution, then floatation technique with sucrose were performed. By using Modified Acid Fast staining, Giemsa staining method and *Cryptosporidium/Giardia* by those methods. No positive sample for *Cyclospora* was found. Even though the contamination in fresh vegetables was low, thoroughly cleansing is recommended for preventing food-borne diseases.

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DETECTION OF **ENTAMOEBA** SPP. FROM SURFACE AND WASTE WATER SAMPLES BY PCR USING GENUS-SPECIFIC PRIMERS

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Infections with *Entamoeba* spp. can result in either a harmless colonization of the intestine or in invasion of the colon wall and damage of other host tissues such as the liver, lung, and brain (amoebiasis). The occurrence of *Entamoeba* spp. in surface and waste water in many countries including Thailand has not been known and investigated. Based on the limitation of this knowledge, detection of *Entamoeba* spp. from surface and waste water in Thailand was performed by using PCR assay. In this study DNA of 137 samples from surface and waste water in Pathumthani province, Thailand were extracted and examined for *Entamoeba* spp by PCR using genus-specific primers. These primers can amplify DNAs of *Entamoeba* spp including *E. polecki, E. chattoni, E. dispar, E. histolytica, E. hartmani*, and *E. coli*. The results showed that 37 (27%) samples were positive for *Entamoeba* spp. Such as *Entamoeba histolytica, Entamoeba dispar* and *Entamoeba moshkovskii*, however they are all negative for these three human *Entamoeba*. This study showed for the first time an existence of *Entamoeba* spp. in surface and waste water from Thailand and it may indicate the possible route of transmission of these amoeba to humans. Their species should also be further investigated by using more sensitive method such as real-time PCR and specific primers for *E. polecki, E. chattoni, E. doil* should be designed for future work.

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FORMATION, PHYSICAL STABILITY AND IN VITRO ANTIMALARIAL ACTIVITY OF DIHYDROARTEMISININ NANOSUSPENSION OBTAINED BY CO-GRINDING METHOD

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The purpose of this study was to investigate the formation of drug nanoparticles from binaryand ternary mixtures consisting of dihydroartemisinin (DHA), a poorly water-soluble antimalarial drug, with water-soluble polymer and/or surfactant. Binary mixtures of drug/polyvinyl pyrrolidone K30 (PVP K30), binary mixtures of drug/sodium deoxycholate (NaDC) and ternary mixtures of drug/PVP K30/NaDC were prepared at different weight ratios and then ground by vibrating rod mill to obtain ground mixtures. Nanosuspension was successfully formed after dispersing ternary ground mixtures or DHA/NaDC ground mixtures in water. The ternary ground mixtures did not gave superior nanosuspension in terms of particle size reduction and recovery of drug nanoparticles but they provided more physically stable nanosuspensions than DHA/NaDC ground mixtures. The size of drug nanoparticles was decreased with increasing grinding time and lowering amount of PVP K30 and NaDC. About 95% of drug nanoparticles was found in the nanosuspension from ternary ground mixtures. Zeta potential measurement suggested that stable nanosuspension was attributable to adsorption of NaDC and PVP K30 onto surface of drug particles. Atomic force microscopy and transmission electron microscopy with selected area diffraction indicated that DHA in nanosuspension was existed as nanocrystals. The obtained nanosuspensions had higher *in vitro* antimalarial acitivity against *P. falciparum* than microsuspensions. The results suggest that co-grinding of DHA with PVP K30 and NaDC seems to be a promising method to prepare DHA nanosuspension.

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AVIAN INFLUENZA PROTECTION KNOWLEDGE, AWARENESS, AND BEHAVIORS IN A HIGH-RISK POPULATION IN SUPHAN BURI PROVINCE, THAILAND

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Avian influenza (Al) had outbreaks in Thailand from January 2004 to December 2005, which resulted in 22 human cases, and 14 deaths. Three confirmed cases were reported in Suphan Buri Province in 2004, one of whom died. A cross-sectional study aimed to investigate knowledge, attitudes, and practices about AI in Song Phi Nong District of Suphan Buri Province. Most of the respondents had moderate levels of knowledge. Most of their attitudes towards and practices of the prevention and control of AI were also appropriate. However, the peoples' knowledge about major signs and symptoms of AI was limited. The study suggested that those who had received information from media had better attitudes towards and practices of AI prevention and control, compared with those who had not received information from media. Therefore, the media played an important role in improving knowledge, attitudes, and behaviors; but for the better protection from AI, continuing health education will be necessary in Thailand.

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FRESHWATER MOLLUSKS AT DESIGNATED AREAS IN ELEVEN PROVINCES OF THAILAND ACCORDING TO THE WATER RESOURCE DEVELOPMENT PROJECTS

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The study was conducted at 75 collecting loci in 15 districts of 11 provinces in Thailand during 1999-2004. A total of 12,079 live mollusks were collected, 11,874 were snails and 205 were clams. The snails were comprised of 39 species and classified into 9 families: Ampullariidae, Bithyniidae, Buccinidae, Potamiopsidae, Stenothyridae, Thiaridae, Viviparidae, Planorbidae and Lymnaeidae. The clams were comprised of 14 species classified into 2 families: Amblemidae and Corbiculidae. Fifteen species were medically important snails: Pomacea canaliculata, Pila ampullacea, P. pesmei, P. polita, Bithynia (Digoniostoma) funiculata, B. (D.) siamensis goniomphalos, B. (D.) s. siamensis, Filopaludina (Siamopaludina) martensi martensi, F. (Filopaludina) sumatrensis polygramma, Melanoides tuberculata, Tarebia granifera, Helicorbis umbilicalis, Gyraulus convexiusculus, Indoplanorbis exustus and Radix rubiginosa. Of these 3 snail species harbored trematode cercariae. I. exustus harbored Echinostoma malayanum, Xiphidio and Schistosoma spindale, and R. rubiginosa and B. (D.) siamensis goniomphalos harbored Xiphidio and intestinal flukes, respectively.

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INTERTIDAL SNAIL-TREMATODE COMMUNITIES ON THE SOUTHERN THAILAND BEFORE AND AFTER THE SOUTH ASIA TSUNAMI

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Intertidal snail-trematode communities in southern Thailand were examined before and after the South Asia tsunami. Infection rates and species diversity of cercaria in the host snail *Cerithidea* in tidal zones did not change significantly from one year before to one month after the tsunami. However, the host snails *C. quadrata, C. alata and C. obtusa* disappeared from greatly damaged sites. It is important to follow up on the intertidal snail-trematode community recovery process after destruction of the intertidal ecosystem.

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EFFECTIVENESS OF DOT FOR TUBERCULOSIS TREATMENT OUTCOMES: A PROSPECTIVE COHORT STUDY IN BANGKOK, THAILAND

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SETTING: All health care centres under the Department of Health, Bangkok Metropolitan Administration. OBJECTIVES: To investigate patterns of drug administration for tuberculosis (TB) patients and to determine whether these patterns affect treatment success rates. DESIGN: In a prospective cohort study conducted during May 2004 to November 2005, newly diagnosed TB patients aged >/=15 years were enrolled after giving informed consent. The cohort was followed until treatment outcome. Structured questionnaires were used to interview patients three times: at the first visit, at the end of the intensive phase and at treatment completion. Data were also collected from treatment cards.

RESULTS: Five patterns of drug administration were used in the health centres: centre-based directly observed treatment (DOT), family-based DOT, self-administered treatment (SAT), centre-based DOT + SAT and centre- + family-based DOT. The pattern of drug administration had a significant impact on treatment success (P < 0.001). Using unconditional binary multiple logistic regression controlling for confounding factors, centre- + family-based DOT had the highest success rates compared with centre-based DOT (OR 20.9, 95%CI 5.0-88.3).

CONCLUSION: The pattern of drug administration impacted on treatment success. Centre- + family-based DOT, family-based DOT and centre-based DOT + SAT achieved higher rates of treatment success than the World Health Organization target. Centre-based DOT had the lowest success.

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IMPACTS OF PESTICIDE USE ON SEMEN CHARACTERISTICS AMONG RICE FARMERS IN KIENXUONG DISTRICT, THAIBINH PROVINCE, VIETNAM

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This case-control study assessed the effects of pesticide use on semen characteristics among rice farmers of Kienxuong District, Thaibinh Province, Vietnam. Semen samples of 1,036 rice farmers were obtained by manual masturbation and screened at Commune Health Stations. Of these, 156 abnormal semen samples were identified; 314 rice farmers with normal semen were recruited as controls. The semen characteristics (volume, sperm concentration, total sperm count, motility, vitality and morphology) of the cases were considerably poorer than the controls. Factors associated with abnormal semen after adjusting for age, smoking and alcohol drinking by logistic regression were: distance of less than 300 meters from household to rice fields and duration of work over 10 years as a farmer (adjusted OR = 3.16, 95% Cl: 1.97-5.05 and adjusted OR = 3.98, 95% Cl: 2.20-7.21, respectively). Rice farmers without personal protective equipment (PPE) when spraying pesticides and without pesticide training (adjusted OR = 3.05, Cl: 1.92-4.85 and adjusted OR = 1.90, Cl: 1.14-3.16, respectively) were also at risk for abnormal semen compared to controls. These findings showed the strength of association between pesticide use and abnormal semen characteristics among rice farmers in Kienxuong District, Thaibinh Province, Vietnam.

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EVALUATION OF DRINKING WATER TREATMENT AND QUALITY IN TAKUA PA, THAILAND

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After 2004 Indian Ocean Tsunami, which hit and devastated several Countries in Southeast Asia, University of Brescia and Mahidol University started a project on water monitoring and treatment for drinking purposes in Takua Pa district (Thailand), the most damaged by the tsunami. In particular, this paper presents the results of a study conducted to evaluate the effectiveness of Takua Pa drinking water treatment plant and to identify actions that could be adopted to improve its performances. The results show that, even if the effluent usually meets Thai guide values, except for pH which is already too acid in the influent, the plant needs several structural and managerial improvements, such as filtration and sedimentation upgrade, coagulation/flocculation and final disinfection re-organization, use of proper registers to better plan and control employees activities. Moreover, it was determined that water quality in the distribution network is characterized by turbidity and organic matter values higher than those evaluated in the plant effluent.

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VIVAX MALARIA PATIENTS AND THEIR DRUG ADHERENCE IN MAE HONG SON PROVINCE, NORTHERN THAILAND

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Vivax malaria is a significant cause of malaria morbidity in Northern Thailand, accounting for approximately 50% of all malaria cases. The objective of this study is to determine behavioral factors associated with adherence to the standard 14 day course of chloroquine and primaquine prescribed from malaria clinics among vivax malaria patients. We conducted a cross-sectional study among 206 patients living in Muang and Mae Sa Riang Districts, Mae Hong Son Province in northern Thailand. Data on adherence and potential behavioral factors related to adherence were collected using a structured interviewer-administered questionnaire and supplemented with qualitative data from focus group interviews. The results indicated that 76.21% of vivax malaria patients did not intend to complete the medication course. The patient's adherence were associated with knowledge scores of malaria (adjusted OR=2.22, 95%CI=1.10-4.45) and accessing drug prescription scores (aOR=5.58, 95%CI=2.06-15.14). Therefore, further effort is needed to educate and encourage vivax malaria patients to comply with the treatment.

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SOCIAL EPIDEMIOLOGICAL FACTORS AFFECTING DHF BETWEEN SEMI-RURAL AND SEMI-URBAN DWELLING IN CHACHOENGSAO PROVINCE

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The study was to determine the differences of knowledge, attitudes and practices and their related factors to presence of immature *Aedes* mosquitoes in sampled numbers of 239 and 232 dwellers in the semi-rural and semi-urban communities, respectively, in Chachoengsao province. The semi-rural setting is located in Plaengyao district and the semi-urban is in Phanom Sarakarm. The study conducted a cross-sectional method to collect data by interview with the head of household with a structured questionnaire before an observation of immature *Aedes* mosquitoes in all water containers. The results

revealed that the respondents in both the settings had higher knowledge about dengue vectors that was significantly related to the higher educational level (P=0.001) and the higher score of attitudes (P=0.003). The semi-rural dwellers knew about disease prevention and control method better than those in the semi-urban (P=0.003). The type of households and the dwellers living in the semi-rural community had higher indices of immature mosquitoes (P=0.000). However, dwellers living and working in the semi-urban setting had regularly practiced in reducing density of immature mosquitoes better than the dwellers living in the semi-rural area. The data serves to the need of an implementation to reduce the disease vectors in different types of the communities.

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MTB STRAINS FROM THAILAND

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Novel mutations in the rpoB gene are reported for 70 rifampicin-resistant (RIFr) MTB strains from Thailand. Sequence analysis of these strains revealed mutations in a 435 base-pair region of the rpoB gene. Twenty-eight strains (40%) had single mutations, and 26 of those strains had mutations at positions never before reported, of which, just one had a substitution at Val-432 (Asp), and the remaining 25, a silent mutation at Gln-517. All other strains had multiple mutations, of which 24(34%) had mutations at two positions; 9(13%), at three pos.itions; 2(3%), at five positions; and 1(1%) at six positions. Five strains (7%), reported to have the RIFr phenotype, contained no mutation in the examined region of the rpoB gene. Surprisingly, one RIFr strain had silent mutations at 29 positions. By far the dominant mutation was the silent mutations at Gln-517 (86%). This investigation demonstrates that mutations in the rpoB gene of MTB strains from Thailand are more varied than previously reported for R1Fr MTB strains. Screening by means of PCR-SSCP clearly separated RIFr strains from RIF susceptible strains.

A total of 29 Thai multi-drug-resistant (MDR) lisoniazid-resistant MTB strains were analyzed for mutations in katG from codons 254 to 549, inhA promoter and inhA open reading frame using DNA sequencing and PCR-SSCP. Twenty-five MDR-TB strains exhibited single point mutations (17 isolates at Ser315Thr plus Arg463Leu, 1 at Thr308Pro plus Arg463Leu, 7 at either Ser315Thr or Arg463Leu) while the other 4 isoniazid-resistant isolates had single point mutation only at Arg463Leu. Seven of 25 MDR-TB strains [4 at C(-15)T, 1 at T(-8)C; 1 at C(-15)T plus Ser94Ala and 1 at Ile21Val] and 2 of 4 isoniazid-resistant isolates [1 at C(-15)T, 1 at C(-15)T plus Ile21 Thr] had mutations in inhA promoter and open reading frame, while the other 20 strains had no mutation at any position. This is the first report of two mutations, Trp308Pro of katG and T (-8)C of inhA in MTB strains.

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A GIS-BASED ESTIMATION OF COMPARATIVE OCCURRENCE AND FATE OF WASTE-RELATED PROTOZOAN PATHOGENS IN A PERI-URBAN CANAL NETWORK IN A TROPICAL ENVIRONMENT

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The role of extensive Thai canal network (16–21 km in length) is scrutinized and evaluated in terms of wastewater treatment, pathogen removal and pollution mitigation. It is a pioneering effort for a tropical country. Three different canals, namely: (i) one receiving municipal wastewater, (ii) one situated in agricultural area, and (iii) one receiving nearly almost only industrial wastewater were investigated. Hydraulic regimes and pollution loads were studied using conventional environmental techniques, as well as geographic information systems (GIS). The study demonstrated that during rainy

season the canals are free-flowing water bodies that can be compared to rivers, while in dry season they behave as ponds. The field concentrations of the protozoan pathogens (*Cryptosporidium* and *Giardia*) were monitored by realtime PCR for more than 12 months. Despite the highly polluted status of the canals, predominantly caused by point pollution sources (0.04 kg $BOD/m^3/day$), a 70% BOD_5 and 4 log_{10} pathogen removal have been achieved. The main removal factor was sedimentation and, to an extent, solar irradiation. *Cryptosporidium* removal appeared to be accomplished by other mechanisms (potentially, predation by protozoa and rotifers) since no oocysts were detected in the sediments. Genotyping studies revealed the presence of both human and animal genotypes of the protozoan pathogens. Quantitative microbial risk analysis and Monte Carlo risk simulations showed that single exposures to the canal waters present risks of infection greater than both the yearly accepted risk by the World Health Organization and the yearly reported risk in Thailand.

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OCCURRENCE OF MICROBIAL PATHOGENS IN RAW AND OXIDATION POND-TREATED WASTEWATER

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Wastewater treatment plants (WWT) are an obvious high risk source of pathogens both in terms of number and strain of pathogens. The occurrence of microbial pathogens in the raw and oxidation pond-treated wastewater was studied in the context of various biogeochemical factors and climatic conditions over the period of June 2006 to February 2007. The wastewater samples collected from two stabilization pond systems in Thailand (AIT ponds in Pathumthani province, and Kamphaengphet in Kamphaengphet province) The poor performance of the AIT WWT system was found in this study period which the removal efficiency was lower than that of Kamphaengphet WWT plant and has deteriorated substantially in comparison to that of previous study in 2005 - 2006. The lower performance values at present are attributed to the lack of desludging of the ponds over many years and the treated wastewater from the AIT WWT plant was found to be significantly polluted in terms of the BOD as compared to the surface water standards of Thailand. The coliform bacteria concentrations in the treated wastewater were higher than the concentration in this receiving water body and not achieved the WHO guidelines for the restricted irrigation. Furthermore, G. lamblia cysts were found in the treated wastewater which can pose a threat to public health while no oocysts of C. parvum were discharged. The possible removal mechanisms for the protozoa in the AIT WWT plant appeared to be time-dependent sedimentation, absorption of (oo)cysts onto settable solids and a result of cumulative effect of High Retention Time (HRT) with abundant of solar radiation for G. lamblia cysts. Moreover, great significance of seasonal effect on the concentrations of waterborne pathogens in the AIT WWT plant was found. On the other hand, there was found no significant impact of physical-chemical parameters and seasonal factors on the concentration of the coliform bacteria, the possible removal mechanisms would be the sedimentation of solid-associated bacteria. However, the occurrence of pathogens (G. lamblia and C. parvum) did not correlate significantly with the presence of the bacterial indicator parameters in the AIT WWT plant.

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FECAL CONTAMINATION OF THE MEKONG RIVER IN CHIANG RAI AND NORTHEASTERN PROVINCES, THAILAND

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There is no study or spatial data available for the existence of the bio-pathogens in the Mekong River through seasonal changes. This study collected water samples from twenty two sampling points along the Mekong River which runs from Chiang Rai (10), Loei (3), Nong Khai (5) to Nakorn Phanom (4) provinces during August 2006-September 2007. The sampling point started from the Nam Ruk at the golden Triangle to Ban Jam Pong, before it runs into Laos PDR. In Chiang Khan, Loei province, is the first point which the Mekong River runs back to Thailand again from Laos PDR after Ban Jam Pong in Chiang Rai. The last sampling point was in That Phanom (border road). Fourteen water samples were on the Mekong River, the rest were at the discharges into the Mekong River. Physical (pH, temperature, conductivity, DO and turbidity) and biological (total and fecal coliform, E.coli) parameters were analyzed. Concentrations of all the parameters increased significantly according to the seasonal changes (dry to rainy season) both in Chiang Rai and in all three northeastern provinces. In Chiang Rai, the averaged pH, conductivity and turbidity increased from 6.5 to 7.5; 40 to 180 mS/cm; 60 to 280 NTU respectively. The averaged total colifom and E.coli increased from 1.0E+03 to 2.0E+04 MPN/100mL, and 20 to 100 MPN/100 mL respectively. For the discharged canals, they showed the same trend as the Mekong River. Averaged pH, conductivity, total coliform and E.coli changed from 6 to 7, 25 to 100 mS/cm, 5.0E+03 to 1.0E+04 MPN/100 mL and 20 to 100 MPN/100 mL respectively. In some sampling points (rainy season), the number of E.coli went up from 2.7E+02 to 2.6E+03 MPN/100 mL, reflecting high contamination from domestic wastewater of the nearby localities. Improper or lack of sanitation system may suggest that bio-pathogens in the Mekong River can pose possible risk to human health. The DO concentrations in the Mekong River were quite high as compared to the natural river water, 4.67-5.45 mg/L and 5.46-6.30 mg/L during the dry and rainy seasons, respectively. Concentration of all the parameters in the three northeastern provinces also increased with seasonal changes, showing similar trends to that of Chiang Rai with top rank of fecal colifom concentration of 1.6E+05 MPN/100 mL. The concentration of the total coliforms and the E. coli were quite high after flowing through urban areas and were decreased by time. These parameters were easily changed due to the condition of the environments and the die-off rate. But if the contamination went beyond the capacity of the water bodies, the concentration of contaminants would be extremely presented along the Mekong River. With reference to the bio-parameters of the surface water quality standard by the Thai National Environmental Board (1994), the Mekong River can be classified as Class 1 or 2 when running through the rural area and Class 3 when running through the urban area.

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ASSESSMENT OF MICROBIAL INFECTION RISKS POSED BY MANAGEMENT PRACTICES OF DOMESTIC WASTES AND URBAN AGRICULTURE ACTIVITIES: CASE STUDY IN PERI-URBAN COMMUNITIES IN THAILAND

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Due to rapid urbanization in the past decades, a number of peri-urban areas in Thailand are facing inadequate sanitation systems for management of solid waste, wastewater and faecal sludge, resulting in spreading of waterborne pathogen. The objective of this study is to estimate health risks due to contamination of waterborne pathogen from management practices of domestic waste and urban agriculture activities. *E. coli* and *Salmonella* spp. were selected as faecal-contaminated indicator and waterborne pathogen, respectively. This study applies the Quantitative Microbial Risk Assessment (QMRA) for assessment of the infection risks of Klong Luang municipality, Pathumthani province (representative of a peri-urban community in Thailand). Results from the field observations showed that average concentrations of *E. coli* and *Salmonella* spp. were 2.2E+05 and 8.0E+03 MPN/100 mL in grey water, and 2.3E+05 and 1.0E+04 MPN/100 mL in seepage at 1-m distance from cesspool, respectively. Likely due to the discharge of domestic wastewater into canals, *E. coli* and *Salmonella* spp. concentrations were

1.8E+04 and 1.0E+03 MPN/100 mL, respectively. In the kitchen waste piles, the *E. coli* and *Salmonella* spp. concentrations were found to be 2.0E+04 and 6.4E+04 MPN/g (dry weight), respectively. After that contaminated canal water is irrigated onto farmland, it could result in the remaining *E. coli* and *Salmonella* spp. concentrations at the furrow and vegetable (lettuce) of 2.3E+02, 2.1E+02 MPN/100mL and 6.5E+04, 3.0E+04 MPN/100 g, respectively. Based on the above mentioned of pathogenic concentration, the yearly risks by *E. coli* were 2.1E-03, 1.2E-05, 6.9E-06 and 3.5E-05 due to the activities of swimming in canal, contacts of kitchen wastes, irrigation of canal water and raw vegetable consumption, respectively. Yearly risks by *Salmonella spp*. were 1.0E-03, 3.4E-02, 1.8E-02 and 6.7E-03 due to the similar activities. It can be postulated that the pathogenic contamination is caused by the poor management practices of domestic wastes. Compared with the annual acceptable risk of 1E-04, proposed by USEPA, the infection risks from *Salmonella* spp. in this study is higher in every activity. The infection risk due to *E. coli* is higher than the acceptable risk only at the swimming in canal. However, the result of risk assessment could not identify the pathogen loads from each pollution source. Pathogen Flow Analysis (PFA) is suggested to integrate with QMRA for identification of critical control points and the planning of health and environmental sanitation.

Presented at: NCCR North South Program Synthesis Workshop 1-3, October 2007, AIT, Thailand.

SURVIVAL TIME OF HIV-INFECTED PATIENTS WITH CRYPTOCOCCAL MENINGITIS

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Objective: To study survival time and risk factors of mortality among HIV-infected patients who had cryptococcal meningitis. Design: Retrospective cohort study. Material and Method: Patients' medical records of those who had HIVinfection with newly diagnosed cryptoccocal meningitis between January 2002 and December 2004 were reviewed. Each patient was classified into one of two groups, according to their anti-retroviral status (ART). Results: Five hundred and forty nine patients enrolled in the present study: 281 (51.2%) in the ART+ group and 268 (48.8%) in the ART-group. The mean age was 33.4 ± 6.9 years old in the ART + group and 33.6 ± 7.0 years old in the ART-group. There were more male in both groups: 207 males and 74 females in the ART+ group, and 195 males and 73 females in the ART-group. Baseline CD4 cell count of both groups was 20 (6-74) cells/ mL and 24 (9-72) cells/ ml. About 30% of both groups of patients experienced major opportunistic infection before cryptococcal meningitis. All patients were treated by standard amphotericin B for a 2-week duration followed by fluconazole for an additional 8 weeks. There were no differences of baseline characteristics between the two groups (p > 0.05). The survival rates at 12, 24, and 36 months were 92.8%, 87.4%, and 85.4% in the ART+ group and 55.3%, 42.2%, and 36.8% in the ART- group, respectively (p < 0.01). The median survival time in the ART- group was 15 months. From the Cox regression model, the hazard ratio for "not received ART" was 4.87 (95%CI = 2.48-9.44, p < 0.01). Conclusion: The present study demonstrated the substantial increasing of survival time of HIV-infected patients with cryptococcal meningitis by initiated ART, even in a resource limited setting (no flucytosine, local combined antiretroviral drugs with NVP based regimens).

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IN VITRO ACTIVITY OF FERROQUINE (SSR 97193) AGAINST PLASMODIUM FALCIPARUM ISOLATES FROM THE THAI-BURMESE BORDER

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Background. On the borders of Thailand, Plasmodium falciparum has become resistant to nearly all available drugs, and there is an urgent need to find new antimalarial drugs or drug combinations. Ferroquine (SSR97193) is a new 4aminoquinoline antimalarial active against chloroquine resistant and sensitive P. falciparum strains in vivo and in vitro. This antimalarial organic iron complex (a ferrocenyl group has been associated with chloroquine) is meant to use the affinity of Plasmodium for iron to increase the probability for encountering the anti-malarial molecule. The aim of the present study was to investigate the activity of ferroquine against P. falciparum isolates from an area with a known high multi-drug resistance rate. Methods. Parasite isolates were obtained from patients with acute falciparum malaria attending the clinics of SMRU. In vitro cultures of these isolates were set-up in the SMRU-laboratory on pre-dosed drug plates, and grown in culture for 42 hours. Parasite growth was assessed by the double-site enzyme-linked pLDH immunodetection (DELI) assay. Results. Sixtyfive P. falciparum isolates were successfully grown in culture. The ferroquine mean IC50(95% CI) was 9.3 nM (95% C.I.: 8.7 - 10.0). The mean IC50 value for the principal metabolite of ferroquin, SR97213A, was 37.0 nM (95% C.I.: 34.3 - 39.9), which is four times less active than ferroquine. The isolates in this study were highly multi-drug resistant but ferroquine was more active than chloroquine, quinine, mefloquine and piperaquine. Only artesunate was more active than ferroquine. Weak but significant correlations were found between ferroquine and its principal metabolite (r2 = 0.4288), chloroquine $(r^2 = 0.1107)$ and lumefantrine $(r^2 = 0.2364)$. Conclusion. The results presented in this study demonstrate that the new ferroquine compound SSR97193 has high anti-malarial activity in vitro against multi-drug resistant P. falciparum. © 2007 Barends et al; licensee BioMed Central Ltd.

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SEROPREVALENCE AND RISK FACTORS OF HEPATITIS B VIRUS INFECTION AMONG HEALTH CARE WORKERS AT THE INSTITUTE OF NEUROLOGY

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Objectives: To define the seroepidemiology of Hepatitis B virus (HBV) infection among health care workers (HCWs) in the Institute of Neurology, and to evaluate the risk factors of HBV markers. Material and Method: Blood samples were taken from 548 HCWs for HBV profiling (HBsAg, anti-HBs and anti-HBc) by Microparticle Enzyme Immunoassay (MEIA) methods. Questionnaires of demographics, type, and duration of work, history of blood exposure, HBV vaccination, and non-occupational risks of HBV infection were interviewed. Results: Twenty-nine (5.3%) HCWs were HBsAg positive, 135 (24.6 %) had anti-HBc with anti-HBs suggesting immunity acquired from a previous HBV infection, 232 (42.3 %) had totally negative profiles, 40 (7.3 %) had anti-HBc only, 105 (19.2%) had protective levels of anti-HBs, 7 (1.3 %) had low anti-HBs levels. The significant risk factors included not having received the hepatitis B vaccine, male gender, past history of jaundice, viral hepatitis, family history of hepatoma, spouse with hepatitis B, and duration of employment in a clinical environment exceeding 5 years. No significant risk factors of hepatitis B virus infection among HCWs, these findings will help implement effective measures aimed at preventing HBV infection.

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POTENTIAL ASSOCIATION OF DENGUE HEMORRHAGIC FEVER INCIDENCE AND REMOTE SENSES LAND SURFACE TEMPERATURE, THAILAND, 1998

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A pilot study was designed to analyze a potential association between dengue hemorrhagic fever (DHF) incidence and, temperature computed by satellite. DHF is a mosquito transmitted disease, and water vapor and humidity are known to have a positive effect on mosquito life by increasing survival time and shortening the development cycle. Among other available satellite data, Land Surface Temperature (LST) was chosen as an indicator that combined radiated earth temperature and atmospheric water vapor concentration. Monthly DHF incidence was recorded by province during the 1998 epidemic and obtained as a weekly combined report available from the National Ministry of Public Health. Conversely, LST was calculated using remotely sensed data obtained from thermal infrared sensors of NOAA satellites and computed on a provincial scale. Out of nine selected study provinces, five (58.3%) exhibited an LST with a significant positive correlation with rainfall (p < 0.05). In four out of nineteen surveyed provinces (21.3%), LST showed a significant positive correlation with DHF incidence (p < 0.05). Positive association between LST and DHF incidence was significantly correlated in 75% of the cases during non-epidemic months, while no correlation was found during epidemic months, Non-climatic factors are supposed to be at the origin of this discrepancy between seasonality in climate (LST) and DHF incidence during epidemics.

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INCREASED RISK OF PRETERM BIRTH AMONG NON-SMOKING, NON-ALCOHOL DRINKING WOMEN WITH MATERNAL PERIODONTITIS

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The aim of this case-control study was to examine the association between periodontitis and preterm birth among non-smoking, non-alcohol drinking women, The cases were 130 women who delivered a live singleton newborn before 37 weeks gestation. A random sample of 260 women who delivered a normal child on the same day as the cases were selected as controls. Periodontal examinations were performed during 24-hour period postpartum at bedside. Other related information was collected by structured questionnaire and medical records. Multiple logistic regression analysis was performed controlling for age, ethnicity, place of residence, education, occupation, income, pre-pregnancy body mass index (BMI), weight gain, antenatal care (ANC), parity, systematic infections, genitourinary infections, antibiotics used, and history of periodontal treatment. Periodontitis (defined as presence of at least 4 teeth having ≤ 1 site with a probing depth (PD) ≥ 4 mm, clinical attachment loss (CAL) ≥ 3 mm and bleeding on probing (BOP) after 10 seconds at the same site) was diagnosed in 33.9% of cases and 10.4% of controls. Periodontitis was significantly associated with preterm birth (adjusted OR = 4.47, 95%CI= 2.43, 8.20). These findings suggest that periodontitis may increase the risk of preterm delivery even among women who do not smoke or drink.

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IMPORTANCE OF COLLECTION TUBE DURING CLINICAL STUDIES OF OSELTAMIVIR

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Ex vivo conversion of the anti-influenza drug oseltamivir to its active metabolite can be inhibited by the esterase inhibitor dichlorvos or by using commercial fluoride-oxalate tubes. Oseltamivir and its active metabolite remain intact in plasma samples during a proposed virus heat inactivation step: incubation at 60°C for 45 min.

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PHARMACOKINETIC STUDY OF ARTEMETHER-LUMEFANTRINE GIVEN ONCE DAILY FOR THE TREATMENT OF UNCOMPLICATED MULTIDRUG-RESISTANT FALCIPARUM MALARIA

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Background: Adherence to antimalarial drug regimens is improved by simple dosing. If the fixed antimalarial drug combination artemether-lumefantrine (AL) could be given once daily, this should improve adherence and thus effectiveness and lower the risk of selecting for resistance. Methods: In an open randomized study, 43 patients with uncomplicated falciparum malaria were given equivalent doses of AL with 200 ml flavoured milk either as the conventional twice-daily regimen or as a single daily dose for 3 days. The primary end point was a comparison of the areas under the plasma lumefantrine concentration-time curves (AUC). Secondary end points were the day 42 polymerase chain reaction (PCR)-adjusted cure rates and the tolerability profiles. Results: Lumefantrine pharmacokinetic profiles were obtained for 36 patients. The AUC $(0 \rightarrow \infty)$ of the once-daily regimen was 30% lower than that in the conventional regimen (P = 0.011) with a median (range) value of 306 (114-5781) µg/ml h, compared with 432 (308-992) µg/ml h. There was no significant difference in the peak plasma concentrations reached. PCR-adjusted cure rate estimates at day 42 of follow-up were 94% (95% CI: 84-100) in the six-dose arm and 85% (70-100) in the three-dose arm (P = 0.3). Conclusion: Artemether-lumefantrine efficacy is reduced by once-daily dosing, because absorption of lumefantrine is dose limited. At currently recommended doses, this antimalarial should be given twice daily in a 3-day regimen, with food containing fat. © 2007 Blackwell Publishing Ltd.

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ACQUIRED HAEMOPHILIA A IN EARLY PREGNANCY ASSOCIATED WITH **PLASMODIUM VIVAX** MALARIA AND HYPERTHYROIDISM

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HOW MUCH FAT IS NECESSARY TO OPTIMIZE LUMEFANTRINE ORAL BIOAVAILABILITY?

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Background: Artemether-lumefantrine (AL) is the only fixed, artemisinin-based combination antimalarial drug which is registered internationally and deployed on a large scale. Absorption of the hydrophobic lipophilic lumefantrine component varies widely between individuals and is greatly increased by fat coadministration; but patients with acute malaria are frequently nauseated and anorexic, making dietary advice difficult to comply with. The aim of this study was to describe the dose-response relationship between coadministration of fat and relative lumefantrine bioavailability, in order to determine the minimum amount of fat necessary to optimize absorption. Method: We conducted a multiple crossover pharmacokinetic study in 12 healthy volunteers. This compared the area under the plasma concentration-time curve (AUC) for lumefantrine after administration of a single dose of AL in the fasting state given with 0, 10, 40, 150 and 500 ml of soya milk corresponding to 0, 0.32, 1.28, 4.8 and 16 g of fat. All volumes of milk supplements were tested in all subjects with a 3- to 4-week washout period in-between. Results: A dose-response relationship was demonstrated between the volume of soya milk administered and lumefantrine bioavailability. AL administration with soya milk increased the lumefantrine AUC more than five fold. The population mean estimated volume of soya milk required to obtain 90% of maximum effect (in terms of lumefantrine AUC) was 36 ml (corresponding to 1.2 g of fat). Conclusions: Coadministration of artemether-lumefantrine with a relatively small amount of fat (as soya milk) was required to ensure maximum absorption of lumefantrine in healthy adult volunteers.

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INTRAHOST SELECTION OF **PLASMODIUM FALCIPARUM pfmdr1** ALLELES AFTER ANTIMALARIAL TREATMENT ON THE NORTHWESTERN BORDER OF THAILAND

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Background: Increased *pfmdr1* copy number is associated with reduced susceptibility to structurally unrelated antimalarial drugs. We assessed how administration of different antimalarial drugs altered *pfmdr1* polymorphism in parasites from patients who experienced treatment failure. Methods. In studies conducted on the northwestern border of Thailand, amplifications and single-nucleotide polymorphisms in *pfmdr1* were compared before and after antimalarial drug treatment. Results. Intrahost changes in *pfmdr1* copy number were observed in 20% (26/132) of patients with recurrent infections. Among infections that recrudesced after mefloquine-containing regimens, increases in *pfmdr1* copy number occurred in 68% (95% confidence interval [CI], 46%-85%), and decreases occurred in 2% (95% CI, 0.4%-11%) of isolates; corresponding proportions after artemether-lumefantrine were 25% (2/8) and 11% (2/19); after quinine, 50% (1/2) and 40% (4/10); and after artemisinins alone, 0% (0/10) and 19% (3/16) of isolates (overall P < .001). Conclusions. Intrahost selection based on *pfmdr1* copy number occurs frequently in parasite populations within individual patients. Amplification confers multidrug resistance but probably imposes a significant fitness cost to the parasites.

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SEXUAL BEHAVIORS OF ALCOHOL DRINKERS AND NON-DRINKERS AMONG ADOLESCENTS AND YOUNG ADULTS IN NHA TRANG, VIETNAM

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A cross-sectional quantitative survey was conducted during August to November 2005 with 880 youths (16-24 yearsold), including 412 males and 468 females in Nha Trang City, Vietnam. It aimed to examine the association between alcohol use and sexual behaviors by gender difference. The data revealed that the majority of respondents (65.9%) had consumed alcohol, 25.8% had sexual touching with boy/girl friends, and 10.1% of respondents had engaged in sexual experiences including vaginal sex, anal sex, and/or oral sex. Young men were significantly more likely to drink than young women were (p<0.001), and alcohol use was significantly associated with engagement in sexual experiences (p<0.001). There was a strong significant different between sexual touching and alcohol drinking among males (p<0.001) and females (p<0.001). Forty percent of young men who did not use condom in last sex and 45% of young men who had multiple sex partners were drinkers compared to 4.8% and 1.6% of non-drinkers, respectively. These significant findings will be baseline data for integrating and adapting into intervention programs for alcohol and HIV among Vietnamese youth.

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NOVEL DNA AMPLIFICATION ON CHROMOSOMES 6q23-24 AND 4P 15.2 IN BREAST CANCER IDENTIFIED BY ARBITRARILY PRIMED POLYMERASE CHAIN REACTION

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Breast cancer is the most common cancer among women worldwide. The molecular basis of breast cancer has not yet been fully elucidated. In this report, novel DNA amplification on chromosomes 6q23-24 and 4p 15.2 were identified by arbitrarily primed polymerase chain reaction, gene cloning, nucleotide sequencing and identified by comparison with known sequences in genome data base, and quantitated by real-time PCR. Results revealed that 25 of 32 (78.1%) breast cancer cases harbored DNA amplification on chromosomes 6q23-24 and 4p 15.2. There was a significant association between increase in tumor size (>3 cm) and DNA amplification on chromosome 6q23-24 (Odds ratio = 13.75, 95% CI = 1.26-35.38, P = 0.018). The results indicated that DNA amplification on chromosome 6q23-24 may be involved in the progression of breast cancer.

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CYTOTOXICITY, APOPTOSIS AND DNA DAMAGE INDUCED BY ALPINIA GALANGAL RHIZOME EXTRACT

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Alpinia galangal, or galangal, has been a popular condiment used in Thai and Asian cuisine for many years. However, relatively little is known of the potential beneficial or adverse health effects of this spice. This study was conducted to analyze the capacity of galangal extract to induce cytotoxicity and DNA damage in six different human cell lines including normal and p53-inactive fibroblasts, normal epithelial and tumour mammary cells and a lung adenocarcinoma cell line. We deliberately focused on treatlung adenocarcinoma cell line. We deliberately focused on treatment with the crude aqueous extract of galangal rhizomes, rather than compounds extracted into an organic solvent, to more closely reflect the mode of dietary consumption of galangal. The cell lines displayed a broad range of cytotoxicity. There was no evidence for preferential cytotoxicity of tumour cells, but there was an indication that p53-active cell lines may be more sensitive than their p53-inactive counterparts. The contribution of apoptosis to total cell killing was only appreciable after exposure to 300 μ g/ml of extract. Apoptosis appeared to be independent of p53 expression. Exposure to as little as 100 μ g/ml galangal extract generated a significant level of DNA single-strand breaks as judged by the single-cell gel electrophoresis technique (comet assay). The three major UV-absorbing compounds in the aqueous extract were identified by mass spectrometry as 1'-acetoxychavicol acetate and its deacetylated derivatives. However, when tested in A549 human lung adenocarcinoma cells, these compounds were not responsible for the cytotoxicity induced by the complete aqueous extract.

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R219K POLYMORPHISM OF ATP BINDING CASSETTE TRANSPORTER A1 RELATED WITH LOW HDL IN OVERWEIGHT/OBESE THAI MALES

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Background: ATP binding cassette transporter A1 (ABCA1) plays a role in the initial stage of removing cholesterol from the body via cholesterol efflux. Muta-tions of this gene cause wide-ranging HDL deficiency, as evident in Tangier disease and familial hypoalphalipoproteinemia. The aim of this study was to elucidate whether the presence of ABCA1 gene polymorphism could be a risk factor for overweight/obesity. Methods. The presence of R219K and I883M genetic variant was determined by PCRRFLP analysis in 112 overweight/obese and 117 control subjects of both sexes. Statistical analysis was performed to find an association between polymorphism and lipid data. Results. Overweight/obese men carrying the mutant allele of R219K had lower level of HDL than the control (p= 0.006). However, no positive association was observed using bivariate logistic regression analysis. On the contrary, there was no difference in HDL level among genotypes in I883M polymorphism. Both polymorphisms appeared to be common in Thai ethnic groups. No difference was detected in genotype frequency between the two populations for both polymorphisms. Conclusions. Although the lower level of HDL in overweight/obese men carrying R219K in comparison to the control suggests the possible involvement of this gene with obesity, further investigations are needed to prove the influence of ABCA1 gene polymorphism on HDL level and to determine whether it could be a genetic determinant of obesity.

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COMPARATIVE STUDY OF LDL-CHOLESTEROL LEVELS IN THAI PATIENTS BY THE DIRECT METHOD AND USING THE FRIEDEWALD FORMULA

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In this study, low-density lipoprotein cholesterol (LDL-C) levels by direct measurement and estimation using the Friedewald formula, were compared among 1,016 Thai patients. The study assessed blood samples from out-patients sent to the Clinical Chemistry Laboratory, Department of Clinical Pathology, Rajvithi Hospital, Ministry of Public Health, for measurement of total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels, January 2004-December 2005. Patients' ages ranged 8-89 years, 573 (56.4%) were females. Linear regression analysis showed the two methods had highly significant correlation coefficients (p<0.001). Upon comparing the two methods, at TG levels of 151-200 mg/dl, bias was 18.3 mg/dl; and for TG levels of 201-300 mg/dl, bias was lower at 11.4 mg/dl; for TG levels of 301-400 mg/dl, bias increased to 20.9 mg/dl. The direct assay meets currently established analytical performance targets and may be useful for the diagnosis and management of hyperlipidemic patients. The Friedewald formula did not give a homogeneous performance when estimating LDL-C levels in samples with different TG levels.

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EFFECTS OF TOBACCO SMOKING ON ALPHA-2-MACROGLOBULIN AND SOME BIOCHEMICAL PARAMETERS IN THAI MALE

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This cross-sectional study was carried out among smokers and nonsmokers from suburban and urban residential areas in Bangkok, Thailand. One hundred eighty-six smokers and 102 nonsmokers, who voluntarily participated in the study, were investigated. The levels of alpha-2-macroglobulin (A2M), albumin, total protein, and other biochemical and hematological parameters as well as body mass index (BMI) measurements were taken. The levels of A2M, BUN and WBC counts were significantly higher in smokers than nonsmokers. Total protein and albumin concentrations were significantly lower in smokers than nonsmokers, but the levels of other biochemical parameters did not differ between the two groups. The relationship between BMI and median A2M levels in the smoker and nonsmoker groups showed the higher the BMI, the lower the serum A2M levels. Smokers had a higher percentage of hyperalpha-2-macroglobulinemia than nonsmokers. A2M concentrations correlated inversely with BMI, BUN, albumin, total cholesterol, triglycerides, and the quantity of cigarette pack-years were the most closely related variables to A2M concentrations among smokers. These findings suggest cigarette smoking affects inflammation markers, increasing A2M and WBC and decreasing albumin. This effect may be the mechanism responsible for the development of chronic disease states associated with smoking since cigarette smoke contains many toxic compounds harmful to health.

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TOTAL AND HIGH MOLECULAR WEIGHT BUT NOT TRIMERIC OR HEXAMERIC FORMS OF ADIPONECTIN CORRELATE WITH MARKERS OF THE METABOLIC SYNDROME AND LIVER INJURY IN THAI SUBJECTS

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CONTEXT/OBJECTIVE: Decreased total adiponectin has been associated with metabolic disorders, including obesity, diabetes, fatty liver, and the metabolic syndrome. Although circulating adiponectin is composed of trimers, hexamers, and high molecular weight (HMW) multimers, there has been limited study of the specific metabolic correlates of these isoforms in humans. Thus, our objective was to evaluate the associations of these adiponectin isoforms with metabolic and anthropometric parameters. DESIGN/PARTICIPANTS/SETTING: A total of 53 diabetic and 68 nondiabetic subjects attending outpatient clinics underwent cross-sectional metabolic characterization. Circulating levels of HMW, hexameric, and trimeric adiponectin were measured using a multimeric adiponectin ELISA based upon selective protease-mediated digestion. RESULTS: On Spearman univariate analysis, both total and HMW adiponectin levels were inversely associated with body mass index, fasting glucose, homeostasis model of assessment of insulin resistance, triglycerides, and alanine aminotransferase (ALT) (all $|r| \ge 0.22$; P < 0.05), with the HMW isoform also positively correlated with high-density lipoprotein cholesterol (r = 0.19; P = 0.036). In contrast, hexameric and trimeric adiponectin were significantly associated with only body mass index (r = -0.23; P = 0.0102) and mid-upper arm circumference (r = 0.21; P = 0.039), respectively. On separate forward stepwise multiple linear regression analyses, fasting glucose and ALT emerged as independent, negative covariates of both total and HMW adiponectin, whereas no independent covariates of hexameric and trimeric adiponectin were identified. Furthermore, after adjustment for age, gender, and diabetes, mean ALT was highest in subjects in the lowest tertile of HMW adjoonectin, followed in turn by the middle and highest tertiles, respectively (trend P = 0.028).

CONCLUSIONS: HMW adiponectin, but not hexameric or trimeric, tracks with the metabolic correlates of total adiponectin. Furthermore, an independent inverse association exists between ALT and HMW adiponectin.

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PROGNOSTIC VALUE OF DNA ALTERATIONS ON CHROMOSOME 17p13.2 FOR INTRAHEPATIC CHOLANGIOCARCINOMA

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AIM: To characterize and evaluate DNA alterations among intrahepatic cholangiocarcinoma (ICC) patients. METHODS: DNA from tumor and corresponding normal tissues of 52 patients was amplified with 33 arbitrary primers. The DNA fragment that alters most frequently in ICC was cloned, sequenced, and identified by comparison with known nucleotide sequences in the genome database (www.ncbi.nlm.nih.gov). The DNA copy numbers of the allelic alterations in cholangiocarcinoma were determined by quantitative real-time PCR and interpreted as allelic loss or DNA amplification by comparison with the reference gene. Associations between allelic imbalance and clinicopathological parameters of ICC patients were evaluated by chi2-test. The Kaplan-Meier method was used to analyze survival rates. RESULTS: From 33 primers, an altered DNA fragment (518 bp) amplified from BC17 random primer was found frequently in the tumors analyzed and mapped to chromosome 17p13.2. Sixteen of 52 (31%) cases showed DNA amplification, while 7 (13%) showed allelic loss. Interestingly, DNA amplification on chromosome 17p13.2 was associated with a good prognosis, median survival time (wk) of amp vs no amp was 44.14 vs 24.14, P = 0.002; whereas allelic loss of this DNA sequence corresponded with a poor prognosis, median survival time (wk) of loss vs no loss was 18.00 vs 28.71, P = 0.019). Moreover, Kaplan-Meier curves comparing the DNA alterations with survival depicted highly significant separation that the median survival time equal to DNA amplification, allelic loss, and normal was 44.14 wk, 18.00 wk, and 24.29 wk, respectively (P = 0.005). CONCLUSION: Alterations in the DNA sequence on chromosome 17p13.2 may be involved in cholangio-carcinogenesis, and could be used as a prognostic marker in the treatment of ICC patients.

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GENETIC INSTABILITY IN CERVICAL CANCER DETECTED BY ARBITRARILY PRIMED POLYMERASE CHAIN REACTION

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The genetic instability in 54 Thai cervical cancer tissues were analyzed by Arbitrarily Primed Polymerase Chain Reaction (AP-PCR). The band alterations produced from 54 arbitrary primers were compared between the DNA finger printing from the patients and their corresponding normal cervical tissues. Results revealed 7 arbitrary primers provided DNA alteration patterns. Of these, an allelic loss in tumor DNA was found in DNA fingerprinting obtained from primers F-2 (64.8%), F-11 (68.5%), U-8 (51.9%), AE-3 (75.9%), AE-11 (53.7%), respectively. Moreover, DNA amplification was exhibited in patterns with primers B-12 (42.6%), J-16 (24.1%) and U-8 (70.4%). When genetic instability was investigated for associations with clinicopathological features, only the DNA amplified fragment with primer U-8 was significantly associated with stage II (P=0.030). Likewise, allelic loss amplified from arbitrary primer AE-3 showed significantly associate with age lower than 50 years old (P=0.003). Our findings suggest that the DNA alteration fragments produced from arbitrary primers of U-8 and AE-11 might be relevant to the pathogenesis of cervical cancer in Thai patients.

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NOVEL hMSH2, hMSH6 AND hMLH1 GENE MUTATIONS AND MICRO-SATELLITE INSTABILITY IN SPORADIC COLORECTAL CANCER

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PURPOSE: To detect the hMSH2, hMSH6 and hMLH1 DNA mismatch repair gene mutations and microsatellite instability in somatic colorectal cancer. PATIENTS AND METHODS: The mutations of hMSH2, hMSH6, and hMLH1 genes, including microsatellite instability of BAT-26, BAT-40, D2S123, D5S346 and D17S250 were analyzed in 31 patients with colorectal. RESULTS: The results revealed that eight cases (25.8%) harbored mutations in DNA mismatch repair genes. Of these, five novel mutations including I237V in exon 4 of hMSH2, ins T at codon 1196 in exon 7 of hMSH6, and ins G at codon 154 in exon 6, N158H in exon 6, and del A at codon 257 in exon 9 of hMLH1 were identified. Moreover, several intronic polymorphisms, including c-g transversion at IVS-1 nt211 + 9 of hMSH2, del T in poly T track at IVS-6 nt3559-5, ATCT duplicate in IVS-7 nt 3642 + 35 and t-g transversion at IVS-10 nt4080 + 185 of hMSH6 were demonstrated in these patients. In addition, seven cases (22.5%) exhibited microsatellite instability (MSI). CONCLUSION: These results suggested that the inactivation of DNA mismatch repair genes and microsatellite instability may play a minor role in somatic colorectal cancer development.

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IDENTIFICATION OF GENETIC ALTERATIONS IN THAI BREAST CANCER PATIENTS BY ARBITRARILY PRIMED POLYMERASE CHAIN REACTION

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Breast cancer is the most common cancer among women worldwide. Genetic alterations prevalent in breast cancer are still being elucidated. In this report, changes in 30 breast cancer tissues, in comparison with normal tissues from Thai patients, were analyzed by arbitrarily primed polymerase chain reaction (AP-PCR). Genetic instability was detected by DNA fingerprinting obtained with 13 of 60 random primers. Of these, at least one amplification band, the incidence ranging from 27 to 80%, was observed in DNA amplified with 8 primers, whereas a band loss was exhibited with from 6 primers, the incidences ranging from 23 to 40%. Likewise, an amplification band amplified from primer D15 was observed in 80% of this patient group and a band loss produced from primer B12 presented in 40% of all cases. These results showed that AP-PCR is effective for the detection of genetic alterations in breast cancer tissues.

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PARTIAL PURIFICATION AND CHARACTERIZATION OF DNA POLYMERASE FROM **PLASMODIUM FALCIPARUM** AND ITS ROLE ON BASE EXCISION REPAIR

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OBJECTIVES: This study aims to purify and characterize *Plasmodium falciparum* DNA polymerase β including its role on base excision repair. The result will lead to exploit-tation of the development of drugs that specifically target this enzyme.

METHODS: *Plasmodium falciparum* parasites were cultivated by a large scale cultiva-tion method. Partial purification of *Plasmodium falciparum* DNA polymerase β was performed using fast protein liquid chromatography. Crude extract was loaded onto Resource Q, Hitrap Heparin, Hitrap Blue HP and ssDNA columns. In a presence of aphidicolin and N-ethylmaleimide (NEM) DNA polymerase β activity could be moni-tored during purification step. Molecular weight of the partially purified enzymes was determined by SDS PAGE. Partial purified DNA polymerase β from *P. falciparum* was tested with some inhibitors such as aphidicolin, NEM and 2', 3'-dideoxythymidine -5-triphosphate (ddTTP). Poly (dT) 200. oligo (dA) 12-18 was used as template/primer to investigate the enzyme processitivity. Repair activity of *P. falciparum* DNA pol β was identified by detecting repaired products using a 28-mer with a UG mismatch located in the middle of the sequence.

RESULTS: In this study, DNA polymerase β , the major enzyme operating during base excision repair in eukaryote, was detected in crude extracts of *P. falciparum* trophozoites and partial purification of enzyme showed a yield of 2.8% and 825-fold. Approximetely six dense bands including 15, 17, 27, 37, 52 and 64 kDa of partially purified *P. falciparum* DNA polymerase β were observed on SDs-PAGE. *P. falciparum* DNA polymerase β was highly resistant to aphidicolin and *N*-ethylmaleimide, as seen in other eukaryotic enzymes, but was resistant to 2', 3'-dideoxythymidine-5'-triphosphate and to other synthetic nucleoside analogs. The parasite enzyme showed low processivity. *P. falciparum* DNA polymerase β was able to repair a patch size of 3-5 nucleotides.

CONCLUSION: This study has shown the existence of *P* falciparum DNA poly-merase β . It differs from human enzyme in its resistance to ddTTP. Differences in sensitivity of the parasite enzyme to known inhibitor of mammalian enzymes should allow exploitation of the development of drugs that specifically target *P* falciparum DNA polymerase β . Moreover, the parasite enzyme indicates its roles in a long patch repair pathway, the first evidence of such a property in malaria parasite DNA.

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FULLY AUTOMATED HPLC ASSAY FOR TOTAL HOMOCYSTEINE AND OTHER AMINOTHIOLS: AS A MEASURE OF PLASMA REDOX STATUS IN THAI HEALTHY SUBJECTS

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Metabolism of aminothiols has been implicated in human pathology, so the measurement of thiol and disulfide concentrations in human body fluids is of interest. Most plasma thiols are metabolically related such that homocysteine (Hcy) exhibited prooxidative properties and glutathione antioxidative, and there is extensive interconversion between these metabolites. Numerous studies have indicated that total Hcy (tHcy) concentrations consistently increased in patients with arteriosclerosis, renal failure and other diseases, but cysteine and glutathione metabolism in these conditions were reported only rarely. In this study, determination of the concentrations of total homocysteine and other aminothiols were done in 78 healthy Thai adult subjects (range 26-57 years). The total plasma aminothiol profile was determined by HPLC with fluorescence detection after derivatization with ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (ABDF). Disulfide and protein-bound aminothiols were reduced by tri-*n*-butylphosphine (the TBP method). Separation was performed by reversed-phase HPLC using a precolumn ODS Hypersil 5 μ m 125×4 mm; mobile phase: 0.1mol/l KH₂PO₄ with 10% acetonitrile, adjusted to pH 2.1 with ortho-phosphoric acid, flow rate 1.0 ml/min. The total concentrations of the most important thiols in plasma-cysteine, cystenylglycine, homocysteine, and glutathione were 185.86±34.65 μ mol/l (range 110.65-276.98 μ mol/l), 27.94±6.77 μ mol/l (range 14.77-51.23 μ mol/l), 8.42±2.3 μ mol/l (range 4.06-14.65 μ mol/l), 4.83±2.03 μ mol/l (range 1.72-9.69 μ mol/l), respectively. Total analysis time was 6 min. The described method is well suited for analysis of thiols in blood specimens, since it is convenient and rapid.

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A QUANTITATIVE ULTRASTRUCTURAL STUDY OF RENAL PATHOLOGY IN FATAL **P. FALCIPARUM** MALARIA

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Acute renal failure is a serious complication of severe falciparum malaria in adults. The incidence and clinical course of malaria-associated renal failure (MARF) is variable and the pathophysiology of MARF not well characterised.

Electron microscopy was used to examine the role of parasitized red blood cell (PRBC) sequestration in the pathogenesis of acute renal failure in severe falciparum malaria. Ultrastructural pathological examination of renal tissues from Southeast Asian adults (n=63) who died from severe falciparum malaria. Qualitative and quantitative determination of the major pathological features of disease, including PRBC and leukocyte sequestration. Clinico-pathological correlation with the premortem clinical picture and peripheral parasite count.

There was a high incidence of MARF in this population (> 40%) and a correlation between the incidence of MARF, severe malarial anaemia and shock. Pathological features included PRBC sequestration in glomerular and tubulo-interstitial vessels, acute tubular damage and mild glomerular hypercellularity resulting from the accumulation of host monocytes within glomerular capillaries. No evidence for an immune complex mediated glomerulonephritis was found. There was a correlation between parasite sequestration in the kidney and pre-mortem renal failure, although overall levels of sequestration were relatively low. Levels of sequestration (Knob+ PRBC) were significantly higher in MARF compared with fatal cases without renal failure (P=0.005).

In conclusion, MARF is a common and serious complication of severe *P. falciparum* malaria in this population, associated with acute tubular injury rather than glomerulonephritis, and linked to localisation of host monocytes in the kidney as well as sequestration of PRBC.

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HIGH LEVEL OF SOLUBLE EXPRESSION IN **ESCHERICHIA COLI** AND CHARACTERIZATION OF THE CLONED **BACILLUS THURINGIENSIS** Cry4Ba DOMAIN III FRAGMENT

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Similar to the other known structures of *Bacillus thuringiensis* Cry4Ba δ -endotoxins, the crystal structure of the 65-kDa activated Cry4Ba toxin comprises three domains which are, from the N- to C-terminus, a bundle of α -helices, a three- β -sheet domain, and a β -sandwich. To investigate the properties of the C-terminal domain III in isolation from the rest of the toxin, the cloned Cry4Ba-domain III was over-expressed as a 21-kDa soluble protein in *Escherichia coli*, which cross-reacted with anti-Cry4Ba domain III monoclonal antibody. A highly-purified domain III was obtained in a monomeric form by ion-exchange and size-exclusion FPLC. Circular dichroism spectroscopy indicated that the isolated domain III fragment distinctly exists as a β -sheet structure, corresponding to the domain III structure embodied in the Cry4Ba crystal structure. *In vitro* binding analysis *via* immuno-histochemical assay revealed that the Cry4Ba-domain III protein was able to bind to the apical microvilli of the susceptible *Stegomyia aegypti* larval midguts, albeit at lower-binding activity when compared with the full-length active toxin. These results demonstrate for the first time that the C-terminal domain III of the Cry4Ba mosquito-larvicidal protein, which can be isolated as a native folded monomer, conceivably participates in toxin-receptor recognition.

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BINDING CHARACTERISTICS TO MOSQUITO-LARVAL MIDGUT PROTEINS OF THE CLONED DOMAIN II-III FRAGMENT FROM THE **BACILLUS THURINGIENSIS** Cry4Ba TOXIN

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Receptor binding plays an important role in determining host specificity of the *Bacillus thuringiensis* Cry δ -endotoxins. Mutations in domains II and III have suggested the participation of certain residues in receptor recognition and insect specificity. In the present study, we expressed the cloned domain II-III fragment of Cry4Ba and examined its binding characteristics to mosquito-larval midgut proteins. The 43-kDa Cry4Ba-domain II-III protein over-expressed in *Escherichia coli* as inclusion bodies was only soluble when carbonate buffer, pH 10.0 was supplemented with 4 M urea. After renaturation *via* stepwise dialysis and subsequent purification, the refolded domain II-III protein, which specifically reacts with anti Cry4Ba-domain III monoclonal antibody, predominantly exists as a β -sheet structure determined by circular dichroism spectroscopy. *In vitro* binding analysis to both histological midgut tissue sections and brush border membrane proteins prepared from susceptible *Aedes aegypti* mosquito-larvae revealed that the isolated Cry4Ba-domain II-III protein showed binding functionality comparable to the 65-kDa full-length active toxin. Altogether, the data present the 43-kDa Cry4Ba fragment comprising domains II and III that was produced in isolation was able to retain its receptor-binding characteristics to the target larval midgut proteins.

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EXPRESSION OF iNOS, VEGF, COX-2, IN ORAL SQUAMOUS CELL CARCINOMA

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In Thailand, squamous cell carcinoma in oral and maxillofacial regions still ranks as one of the highest causes of death from cancer. Inflammation and angiogenesis has been proved to play important roles in carcinogenesis. The objective of this research is to study the expression of inducible nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) which are the inducible inflammation mediators showing close relationship not only to carcinogenesis but also angiogenesis of oral squamous cell carcinoma (OSCC). In this study, standard indirect immunohistochemical technique using polyclonal antibodies specific to human iNOS, VEGF and COX-2 was performed in formalin-fixed paraffin-embedded tissue sections of normal oral mucosa and sixty OSCC tissue samples. The results showed that the squamous epithelium of normal oral mucosa showed negative stain for iNOS, VEGF and COX-2 while the epithelial components of squamous cell carcinoma as well as the inflammatory cells infiltrating near the tumor interface demonstrated moderate to intense staining for all of these proteins. In conclusion, the expression of iNOS, VEGF and COX-2 exists in OSCC of Thai patients. The data provided are the first to show the expression of these chemicals associated with angiogenesis in OSCC treatment in Thai population.

Presented at: 24th Annual Conference of The Microscopy Society of Thailand to Commemorate His Majesty The King's 60th Anniversary of His Accession to the Throne, 14-16 February, 2007 at The Golden Jubilee Building, Kasetsart University, Bangkok, Thailand.

This work was financially supported by Collaborative Research Network (CRN) Scholarship, Faculty of Dentistry, Srinakarinwirot University.

EXPRESSION OF iNOS, VEGF, COX-2, ANGIOGENESIS AND THEIR CLINICO-PATHOLOGICAL CORRELATION IN ORAL AND PARA-ORAL SQUAMOUS CELL CARCINOMA

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In Thailand, squamous cell carcinoma in oral and maxillofacial regions still ranks as one of the highest causes of death from cancer. One main etiology for oral and para-oral squamous cell carcinoma (OSCC) is inflammation. Nitric oxide synthase (NOS), vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2) are the important molecules showing close relation to not only inflammation but also carcinogenesis and angiogenesis. Angiogenesis is defined as the formation of new blood vessels from existing vasculature. It is necessary for tumor growth and progression and also involved in metastasis. The problem in using anti-angiogenesis drug against cancer is that cancer must express angiogenic markers. Moreover, genetic differences are another factor influencing drug efficiency. The objective of this research was to study the expression and relationship among iNOS, VEGF, COX-2, angiogenesis and their clinico-pathological correlation in OSCC. In this study, standard indirect immunohistochemical technique using polyclonal antibodies specific to human iNOS, VEGF, COX-2 and CD31 was performed in formalin-fixed paraffin-embedded tissue sections of 66 OSCC samples. The staining patterns and intensity are measured and analyzed statistically. The amount of iNOS, VEGF and COX-2 expression was

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interpreted according to the intensity of the positive immunoreactivity. The results showed that epithelial components of squamous cell carcinomas demonstrated moderate to intense staining for iNOS, VEGF and COX-2. iNOS shows correlation with cervical lymph node status and tumor staging (TNM) of the patients and angiogenesis. VEGF shows correlation with tumor grading, tumor staging and angiogenesis. COX-2 shows correlation with lymph node status of patients. In conclusion, the expression of iNOS, VEGF and COX-2 exists in OSCC of Thai patients. The data provided are the first to show the expression of these chemical mediators associated with carcinogenesis and angiogenesis in OSCC of Thai population. It can be the primary database before using angiogenesis drug against these mediators for OSCC treatment in Thai population.

Presented at: Joint International Tropical Medicine Meeting, 29-30 November, 2007, Imperial Queen's Park Hotel, Bangkok, Thailand.

This work was financially supported by the Collaborative Research Network (CRN) Scholarship, Faculty of Dentistry, Srinakarinwirot University.

ACTIVATION OF TRANSCRIPTION FACTOR-NUCLEAR FACTOR KAPPA B (NF-κB) IN ENDOTHELIAL CELLS UPON MALARIA PARASITE CYTOADHERENCE

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The work is focused on post-adhesive signaling events that follow cytoadherence of malaria-infected red blood cells to endothelial cells. We aimed at generating new findings that will provide a better understanding of the pathogenesis of severe malaria. Nuclear factor kappa B (NF- κ B) is an important transcription factor sequestered in the cytoplasm by I κ B family of inhibitor proteins. Following stimulation, NF- κ B is cleaved from inhibitor proteins and is translocated from the cytoplasm to the nucleus where NF- κ B can eventually regulate gene expression. NF- κ B is signaling molecule has been known to play an important role in apoptosis. We have previously documented the occurrence of apoptosis in endothelial cells co-cultured with *P. falciparum*. In this study, we further investigate the role of NF- κ B in endothelial cell-malaria parasite cytoadherence. Employing molecular techniques of endothelial cells transfections to pNF- κ B-Luc vector (Stratagene, UK) and co-culture of malaria-infected red blood cells to endothelial cells, we reported activation of NF- κ B in transfected endothelial cells using Luciferase Assay. It can be concluded that NF- κ B activation in endothelial cells induced by cytoadherence of malaria-infected red blood cells could be one signaling molecule responsible for the endothelial changes and some aspects of the subsequent severe manifestation of severe malaria.

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CHRONIC DIARRHEA AND ABNORMAL SERUM IMMUNOGLOBULIN LEVELS: A CASE REPORT

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A 15-year-old Thai boy with multiple episodes of chronic diarrhea caused by giardiasis with hypogammaglobulin M and IgG_4 subclass deficiency (but normal antibody response to rabies vaccine) is reported. Immune status follow-up is necessary for a definite diagnosis and proper management.

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CONGENITAL MALARIA IN THAILAND

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Congenital malaria, although considered to be relatively rare, is not uncommon now, and prevalence rates vary between geographical regions. Fifteen cases of congenital malaria admitted from various parts of Thailand are reviewed. Most came from Thai border areas.

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SYMPTOMATIC DENGUE INFECTION IN THAI CHILDREN IN RATCHABURI COHORT, THAILAND

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Background: Thailand is an endemic area of dengue infection. However, only few epidemiological studies have been conducted.

Objective: To prospectively study symptomatic dengue infection in term of incidence and clinical manifestations from a dengue epidemiology cohort.

Methods: A school-based cohort study has been conducted among 3066 children aged 3-11 years in Ratchaburi Province, Thailand whose estimated dengue-naive sero-prevalence was 48%. The children who had fever were bled for dengue IgM/IgG ELISA test and viral isolation. Detailed clinical manifestations were recorded.

Results: During the year 2006, there were 51 serologically confirmed dengue infection (incidence 1943 per 100000 person-year). Five and 46 cases had primary infection and secondary infection, respectively. Dengue viruses were isolated in 34(66.7%) cases including 17, 1, 3, and 13 cases of DEN1, 2, 3, and 4, respectively. Infections occurred all year-round but were more common in rainy season. Undifferentiated viral syndrome, dengue fever, and dengue hemorrhagic fever were diagnosed in 19, 23, and 9 patients, respectively. The mean(SD) duration of fever and peak body temperature were 5.7(1.6) days and 39.4(1.0) degree celsius, respectively. Common clinical manifestations included anorexia (79%), nausea and vomiting (75%), headache (70%), positive tourniquet test (69%), myalgia (47%), and convalescent rash (41%). However, only rash and retro-orbital pain (26%) were found to be significantly related to sero-positive patients. The percentage of atypical lymphocyte was also significantly higher in dengue infected cases.

Conclusion: Most of the symptomatic dengue infections in the cohort were secondary infections. Most of the infections were mild and difficult to differentiate from other non-specific infections clinically.

Presented at: 3rd Asian Regional Dengue Research Network Meeting, 22-24 August 2007, Grand Hotel, Taipei, Taiwan **Funding:** Pediatric Dengue Vaccine Initiative (PDVI), Thai Ministry of Public Health.

COMPARATIVE STUDY OF THE EFFECTIVENESS AND PHARMACOKINETICS OF TWO RECTAL ARTESUNATE/ORAL MEFLOQUINE COMBINATION REGIMENS FOR THE TREATMENT OF UNCOMPLICATED CHILDHOOD FALCIPARUM MALARIA

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Background: Rectal artesunate has been shown to be an effective treatment for falciparum malaria and is useful in patients who cannot take medicine orally or when parenteral medication is inconvenient. A combination with mefloquine can decrease the duration of treatment, increase compliance and delay development of resistance. There are no clear data

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on whether a higher dosage of rectal artesunate results in a better clinical response.

Aim: To assess two rectal artesunate/oral mefloquine regimens for treating uncomplicated multi-drug-resistant childhood falciparum malaria.

Methods: Seventy children aged 1-14 years with uncomplicated falciparum malaria were randomly assigned to receive either 10 (range 8-12) or 20 (range 16-24) mg/kg/day rectal artesunate for 3 days followed by 25 mg/kg oral mefloquine. The study endpoints were fever clearance time, parasite clearance time and proportion of patients with recrudescence. Serum levels of artesunate and dihydro-artemisinin were measured after the first dose of rectal artesunate in 16 subjects. *Results.* Both regimens were safe and effective. The cure rate was 100% in the 53 patients who completed 28-day follow-up. All of the study endpoints were comparable between both treatment groups.

Conclusion: A regimen of rectal artesunate 10 mg/kg/day for 3 days followed by mefloquine 25 mg/kg is optimal for the treatment of uncomplicated falciparum malaria. There was no definite benefit from increasing the dosage of rectal artesunate from 10 to 20 mg/kg/day.

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PREVALENCE OF HEPATITIS E VIRUS ANTIBODIES IN PIGS: IMPLICATIONS FOR HUMAN INFECTIONS IN VILLAGE-BASED SUBSISTENCE PIG FARMING IN THE LAO PDR

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We report a high seroprevalence of hepatitis E virus (HEV) in pigs in the Lao PDR. HEV seroprevalence was 51.2% (300/586) amongst abattoir pigs and 15.3% (46/301) amongst village pigs. The age distribution suggested previous invillage HEV pig infections. These findings suggest a zoonotic risk associated with village-based smallholder pig farming.

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PROSPECTIVE STUDY TO DETERMINE ACCURACY OF RAPID SEROLOGICAL ASSAYS FOR DIAGNOSIS OF ACUTE DENGUE VIRUS INFECTION IN LAOS

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There is an urgent need for accurate and simple dengue virus infection diagnostic assays in limited-resource settings of dengue endemicity, to assist patient management. Using a panel of reference samples (S. D. Blacksell, P. N. Newton, D. Bell, J Kelley, M. P. Mammen, D. W. Vaughn, V. Wuthiekanun, A. Sungkakum, A. Nisalak, and N. P. Day, *Clin. Infect. Dis.* 42:1127-1134, 2006), we recently evaluated eihgt commercially available immunochromatographic rapid diagnostic

tests (RDTs) designed to detect dengue virus-specific immunoglobulin M (IgM) and/or IgG. We found that 6/8 RDTs had sensitivities of less than 50% (range, 6 to 65%), but specificities were generally high. Here, in conjuction with dengue virus serotyping by reverse transcriptase PCR and in the limited-resource setting of Laos, where dengue virus is endemic, we evaluated the same eight RDTs against a previously validated dengue IgM/IgG enzyme-linked immunosorbent assay for diagnosis of acute dengue virus infection. Paired serum samples were collected from 87 patients, of whom 38 had confirmed dengue virus infections (4 had primary infections, 33 had secondary infections, and 1 had an infection of indeterminate status). RDT sensitivity was low, with 7/8 RDTs having admission sample sensitivities of less than 20% (range, 4 to 26%). The majority (6/8) of the RDTs, demonstrated high specificity (>95%). Kappa statistic values ranged from 6 to 54% for the RDTs, demonstrating poor to moderate variation between three operators. No RDT adequately differentiated between primary and secondary dengue virus infections. The findings of this study suggest that currently available RDTs based on the detection of IgM antibodies for the diagnosis of acute dengue virus infections are unlikely to be useful for patient management.

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SCRUB TYPHUS SEROLOGIC TESTING WITH THE INDIRECT IMMUNOFLUORESCENCE METHOD AS A DIAGNOSTIC GOLD STANDARD: A LACK OF CONSENSUS LEADS TO A LOT OF CONFUSION

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A review was performed to determine the evidence base for scrub typhus indirect immunofluorescence assay (IFA) methodologies and the criteria for positive results. This review included a total of 109 publications, which comprised 123 eligible studies for analysis (14 publications included 2 substudies). There was considerable underreporting of the IFA methodology and seropositivity criteria used, with most studies using a defined cutoff titer rather than an increase in the titer in paired samples. The choice of positivity cutoff titer varied by country and purpose of the IFA test. This variation limits the comparability of seroprevalence rates between studies and, more seriously, raises questions about the appropriateness of the cutoffs for positive IFA results chosen for diagnosis of acute scrub typhus infection. We suggest that the diagnosis of scrub typhus using IFA should be based on a > or =4-fold increase in the titer in paired serum samples and should only be based on a single sample titer when there is an adequate local evidence base.

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EVALUATION OF THE PANBIO DENGUE VIRUS NS1 ANTIGEN DETECTION AND IGM ANTIBODY ELISAS FOR THE DIAGNOSIS OF ACUTE DENGUE INFECTIONS IN LAOS

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We evaluated 2 commercial enzyme-linked immunosorbent assays (ELISAs) for the diagnosis of dengue infection; one a serologic test for immunoglobulin M (IgM) antibodies, the other based on detection of dengue virus nonstructural 1 (NS1) antigen. Using gold standard reference serology on paired sera, 41% (38/92 patients) were dengue confirmed, with 4 (11%) acute primary and 33 (87%) acute secondary infections (1 was of indeterminate status). Sensitivity of the NS1-ELISA was 63% (95% confidence interval [CI], 53–73) on admission samples but was much less sensitive (5%; 95% CI, 1–10) on

convalescent samples. The IgM capture ELISA had a lower but statistically equivalent sensitivity compared with the NS1-ELISA for admission samples (45%; 95% CI, 35–55) but was more sensitive on convalescent samples (58%; 95% CI, 48–68). The results of the NS1 and IgM capture ELISAs were combined using a logical OR operator, increasing the sensitivity for admission samples (79%; 95% CI, 71–87), convalescent samples (63%; 95% CI, 53–73), and all samples (71%; 95% CI, 65–78).

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PROSPECTIVE CLINICAL EVALUATION OF THE ACCURACY OF 16S rRNA REAL-TIME PCR ASSAY FOR THE DIAGNOSIS OF MELIOIDOSIS

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The accuracy of a *Burkholderia pseudomallei* 16s rRNA real-time PCR assay was evaluated against culture for the diagnosis of melioidosis in Thailand. A total of 846 samples were obtained from 383 patients with suspected melioidosis. One or more specimens were PCR positive for 47 of 77 patients with culture-proven melioidosis (sensitivity 61.0%, 95% CI: 49.2–72.0%). PCR was negative for all 306 patients who were culture negative for *B. pseudomallei* (specificity 100%, 95% CI: 98.8–100%). Diagnostic sensitivity of PCR was 22.7% for patients who were culture positive for blood only, compared with 79.4% for patients who were culture positive for samples other than blood. The median (interquartile range) *B. pseudomallei* colony count in blood for 44 of 77 patients with positive blood cultures was 2.4 CFU/ml (0.2–13.5 CFU/ml); this may explain the low sensitivity of PCR for this specimen. The PCR assay described here is not sufficiently sensitive to replace culture in our clinical setting.

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THE MANAGEMENT OF PATIENTS WITH SEVERE MALARIA

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Severe malaria is a global problem, claiming at least 1 million lives annually. Few adequately powered clinical studies have been directed at improving the management of severe malaria over the years, but this situation is slowly changing. The antimalarial treatment of severe disease is being transformed by the development and deployment of the water-soluble artemisinin derivative artesunate. Parenteral artesunate is now the treatment of choice in low-transmission areas and in the 2nd and 3rd trimesters of pregnancy, and research is underway into whether it should replace quinine as the treatment of choice in African children. Development of good manufacturing practice (GMP) formulations should make parenteral artesunate more widely available in the near future. The development of artesunate suppositories offers another exciting prospect, the ability to treat patients with severe disease in remote rural settings, delaying the evolution of disease and buying them time to reach a health care facility. No adjunctive therapy has been shown to improve the outcome of severe malaria, but most studies have been underpowered. Future trials of interventions shown to be promising in pilot studies should be large and adequately powered. This will require multi-center designs and necessitate close collaboration between groups, as well as agreement on the research agenda. We suggest a list of candidate interventions for debate.

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THE TREATMENT OF SEVERE MALARIA

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In the SEAQUAMAT trial, parenteral artesunate was shown to be associated with a considerably lower mortality than quinine, and is now the recommended treatment for severe malaria in low-transmission areas and in the second and third trimesters of pregnancy. A trial is underway to establish its role in African children. The development of artesunate suppositories may provide the means to treat patients with severe disease in remote rural settings, potentially buying the time needed to reach a health care facility. The increasing availability of basic intensive care facilities in developing countries also has the potential to further reduce mortality.

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LEVAMISOLE INHIBITS SEQUESTRATION OF INFECTED RED BLOOD CELLS IN PATIENTS WITH FALCIPARUM MALARIA

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Background: Sequestration of infected red blood cells (iRBCs) in the microcirculation is central to the pathophysiology of falciparum malaria. It is caused by cytoadhesion of iRBCs to vascular endothelium, mediated through the binding of Plasmodium falciparum erythrocyte membrane protein-1 to several endothelial receptors. Binding to CD36, the major vascular receptor, is stabilized through dephosphorylation of CD36 by an alkaline phosphatase. This is inhibited by the alkaline phosphatase-inhibitor levamisole, resulting in decreased cytoadhesion.

Methods: Patients with uncomplicated falciparum malaria were randomized to receive either quinine treatment alone or treatment with a single 150-mg dose of levamisole as an adjunct to quinine. Peripheral blood parasitemia and parasite stage distribution were monitored closely over time.

Results: Compared with those in control subjects, peripheral blood parasitemias of mature P. falciparum parasites increased during the 24 h after levamisole administration (n=21; P=.006). The sequestration ratio (between observed and expected peripheral blood parasitemia) of early trophozoite and midtrophozoite parasites increased after levamisole treatment, with near complete prevention of early trophozoite sequestration and >65% prevention of midtrophozoite sequestration.

Conclusion: These findings strongly suggest that levamisole decreases iRBC sequestration in falciparum malaria in vivo and should be considered as a potential adjunctive treatment for severe falciparum malaria.

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VARIABLE PRESENTATION OF NEUROLOGICAL MELIOIDOSIS IN NORTHEASTERN THAILAND

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We describe three instructive cases of neurologic melioidosis that demonstrate the variable nature of clinical manifestations and disease pathology. The appropriate duration and choice of parenteral and oral antimicrobial therapy for neurologic melioidosis are also discussed.

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SIMULTANEOUS INFECTION WITH MORE THAN ONE STRAIN OF **BURKHOLDERIA PSEUDOMALLEI** IS UNCOMMON IN HUMAN MELIOIDOSIS

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A prospective study was performed to determine the rate at which patients with melioidosis are infected with more than one strain of *Burkholderia pseudomallei*. Genotyping of 2,058 bacterial colonies isolated from 215 samples taken from 133 patients demonstrated that mixed infection is uncommon (2/133 cases [1.5%; 95% confidence interval, 0.2 to 5.3%]).

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VALIDATION AND APPLICATION OF A LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC METHOD FOR DETERMINATION OF ARTESUNATE IN PHARMACEUTICAL SAMPLES

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A simple and rapid liquid chromatographic-mass spectrometric assay for the evaluation of artesunate in vials for injection has been developed and validated. The content of each vial was dissolved in 3.0 mL of methanol using a SGE analytical syringe (1.0 mL). Each sample was diluted to a theoretical concentration of 1000 ng/mL and analysed in triplicate. Three replicates of calibration standards at concentrations 500, 1000 and 1500 ng/mL were used to construct a calibration curve. Artesunate was analysed by liquid chromatography with atmospheric pressure chemical ionisation (APCI) mass spectrometric (MS) detection on a Hypersil Gold column (100 mm x 4.6 mm) using a mobile phase containing methanol-ammonium acetate 10 mM pH 5.3 (70:30, v/v) at a flow rate of 1 mL/min. The assay was implemented for the analysis of artesunate for injection purchased from Guilin Pharmaceutical Company in China.

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CARDIOTOXICITY OF ANTIMALARIAL DRUGS

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There are consistent differences in cardiovascular state between acute illness in malaria and recovery that prolong the electrocardiographic QT interval and have been misinterpreted as resulting from antimalarial cardiotoxicity. Of the different classes of antimalarial drugs, only the quinolines, and structurally related antimalarial drugs, have clinically significant cardiovascular effects. Drugs in this class can exacerbate malaria-associated orthostatic hypotension and several have been shown to delay ventricular depolarisation slightly (class 1c effect), resulting in widening of the QRS complex, but only quinidine and halofantrine have clinically significant effects on ventricular repolarisation (class 3 effect). Both drugs cause potentially dangerous QT prolongation, and halofantrine has been associated with sudden death. The parenteral quinoline formulations (chloroquine, quinine, and quinidine) are predictably hypotensive when injected rapidly, and cardiovascular collapse can occur with self-poisoning. Transiently hypotensive plasma concentrations of chloroquine can occur when doses of 5 mg base/kg or more are given by intramuscular or subcutaneous injection. At currently recommended doses, other antimalarial drugs do not have clinically significant cardiac effects. More information on amodiaquine, primaquine, and the newer structurally related compounds is needed.

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