FTROPMED

Asia's Leader in Tropical Medicine

Annual Review 2009

Faculty of Tropical Medicine
Mahidol University

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Contents

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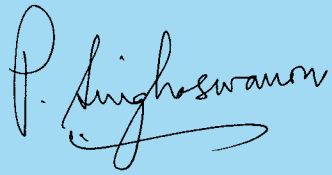
Message from the Dean

We are in the midst of extremely challenging times for medicine in general, and for tropical medicine in particular. Climate change, though apparently still a matter of some debate, implies enlargement of areas affected by tropical diseases. We are already witnessing the incidence of malaria at higher and higher altitudes, and dengue in previously uninfected areas. More recently, we have experienced SARS; avian influenza H1N1 remains a long-term pandemic threat; malaria and tuberculosis are becoming increasingly drug-resistant; we are battling to produce effective influenza, AIDS, malaria, and dengue vaccines; and several diseases that we thought we had beaten are re-emerging. As I write, the world is being widely affected by Pandemic Influenza 2009 (H5N1), with several deaths reported in Thailand, as in other parts of the world. Coupled to this, most countries have been affected to varying degrees by suddenly hard economic times, some compounded further by political and social dislocation, famine, war, and civil unrest. Yes, it is indeed a challenging environment, but we have no option but to rise to the challenge and solve these multifactorial problems using multilateral collaborative approaches.

The Faculty of Tropical Medicine, Mahidol University is committed to the continuous pursuit of excellence in research, education, and healthcare services, and to internationalization, consistent with Mahidol University's strategic plan. To achieve this, we have strived to advance strongly in all 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 saw several substantial infrastructure improvements during the year, with renovations at the Bangkok School of Tropical Medicine and the Hospital for Tropical Diseases. A new Hospital building and accommodation for the Faculty's several Centers of Excellence, will start construction in early 2009.

The following Annual Review 2009 represents an overview of the activities of the Faculty of Tropical Medicine for the calendar year 2008. 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.

I wish to thank the staff of the Faculty, and all of our collaborators and supporters, for their admirable dedication to Tropical Medicine throughout 2008, and am pleased to commend this Annual Review 2009 to you.



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

"The Faculty of Tropical Medicine, Mahidol University is committed to the continuous pursuit of excellence in research, education, and healthcare services, and to internationalization"

In Memoriam

Dedicated to the late Prof. Emeritus Chamlong Harinasuta 17 January 1921 - 4 August 2009

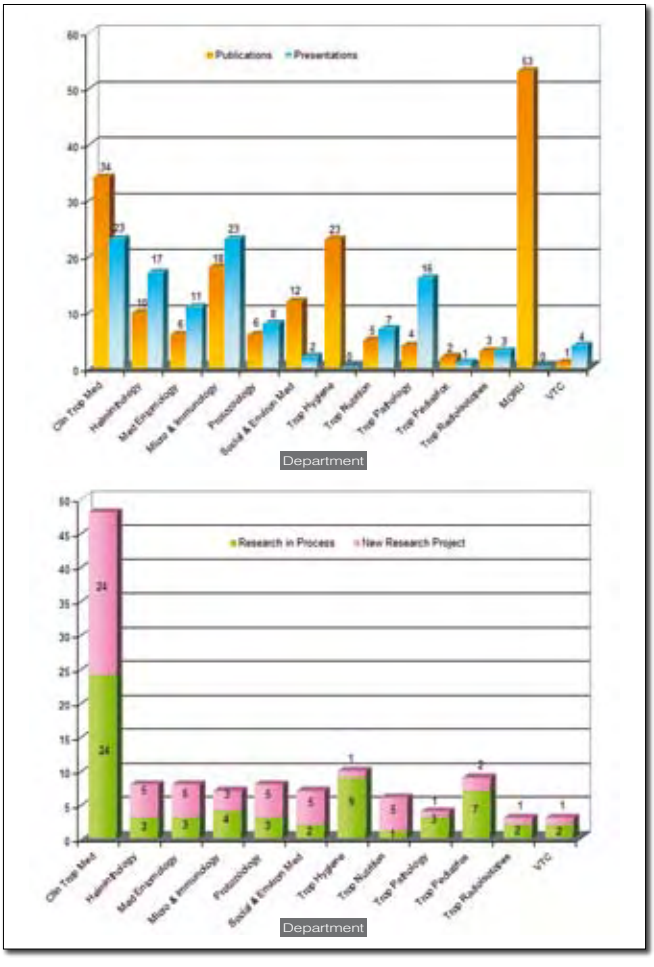
Professor Emeritus
Chamlong Harinasuta
was co-founder of the
Faculty of Tropical Medicine,
Mahidol University, in 1960.
He was also the Faculty's first Dean.
After a remarkably productive life,
devoted to improving the health of
people in tropical regions, he sadly
passed from us on 4 August 2009.
We will always remember him fondly
for his tremendous contributions
to education and research in
tropical medicine, his originality and
intelligence, and his exceptional
kindness.

Research

The Faculty of Tropical Medicine, Mahidol University, has since its inception, been committed to continuously improving the quality of life of people living in tropical regions through basic and applied research. We are pioneers in research into 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 for HIV/AIDS vaccine trials, assessing the efficacy of antiretroviral therapies and treatments for opportunistic infections, epidemiological surveys, and the analysis of social risk factors.

We have established 5 Centers of Excellence

- Biomedical and Public Health Informatics (BIOPHICS),
- Center of Excellence for Food-Borne Parasitic Zoonoses (FBPZ),
- Southeast Asia Influenza Clinical Research Network,
- TropMed Dengue Diagnostic Centre (TDC), and the
- Vaccine Trial centre (VTC)



The Faculty has maintained its research leadership role in Asia, publishing extensive research studies into malaria and other tropical diseases. We continue to encourage, support, and facilitate research in tropical diseases and other related global health problems. In 2008, the Faculty's publication output was very high, with 162 international scientific papers, of which 87.04% (141 papers) were listed in the ISI Web of Science databases. During the year under review, 56% of these publications were cited in ISI 301 citations. In all, the Faculty was conducting 159 research projects in 2008, 55 of which commenced during that year. Forty-seven (47) papers were presented at international conferences and 10 papers at national conferences.

Citation Analysis for Faculty of Tropical Medicine, Mahidol University, Publications
ISI Web of Science (as at 26 Nov. 2009)

Year	No. Articles Published	No. Articles Cited	Number of Times Cited							Total Times Cited
			Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	**Year 2009**	
2009	119	35	0	0	0	0	0	0	76	76
2008	141	79	0	0	0	0	0	64	237	301
2007	152	106	0	0	0	0	69	365	350	784
2006	108	94	0	0	0	61	274	428	272	1,035
2005	107	90	0	0	103	374	537	599	429	2,040
2004	82	78	0	63	361	439	446	476	283	2,067
2003	80	73	23	142	259	218	238	226	145	1,251
Total	**762**	**546**	**23**	**205**	**723**	**1,092**	**1,564**	**2,158**	**1,755**	**7,517**

The Faculty encourages and strengthens research by the development of researchers through Faculty grants, publication rewards, funds to support the costs of publication, and travel awards to assist researchers to present their research work at international conferences. In the year 2008, the Faculty granted 4.5 million Baht in funding to support the continued development and maintenance of the Faculty's research capacity.

The Faculty has a well-established Ethics Committee, which has been recognized by SIDCER/FERCAP as attaining both international and local standards for ethical review. A plaque of recognition was awarded during the 8th FERCAP General Assembly at the Rama Gardens Hotel, Bangkok, Thailand on 26 November 2008. TropMed EC has registered Federal Wide Assurance (FWA) with the Office for Human Research Protection (OHRP). Our FWA number is FWA 00002882, Institutional Review Board/Independent Ethics Committee number (IRB/IEC) is IRB 00005259, and IRB Organization numbers (IORG) is IORG 0002101.

Dr. Pongrama Ramasoota, Deputy Dean for Research & Innovation of the Faculty of Tropical Medicine, and his team--Dr. Chomrach Sirigul, Miss Pannamthip Pitaksajjakul, and Mr. Pholsak Piyatat--were awarded the IFIA Scientific Gold Medal by the International Federation of Inventors Associations (IFIA), and the Silver Prize from the Korea Invention Promotion Association (KIPA), for the invention of "Rapid detection of aerosol MDR-TB and *Legionella* using modified impinger and polymerase chain reaction (PCR)." The award ceremony was held during the "Seoul International Invention Fair (SIIF) 2008", in the Grand Ballroom, COEX Mall, Seoul, South Korea. The fair showcased 479 world-standard inventions from 34 countries. Dr. Pongrama and his team also received the inventor award in the field of medical sciences, from the National Research Council of Thailand (NRCT) for the research work "Rapid detection of *M. tuberculosis* and *Legionella* from indoor air using modified impinger and polymerase chain reaction (PCR) methods", which was supported by research grants from the NRCT and BIOTEC.

Education

The Faculty, through the Bangkok School of Tropical Medicine, offers 6 regular international postgraduate programs--the Diploma in Tropical Medicine and Hygiene (DTM&H), Master of Clinical Tropical Medicine, Master of Clinical Tropical Medicine in 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 2008, we enrolled 35 new DTM&H participants from 10 different countries, 9 Master of Clinical Tropical Medicine participants from 7 countries, 9 Master of Science in Tropical Medicine students from 2 countries, and 17 Doctor of Philosophy in Tropical Medicine students from 3 countries. Therefore, in total, 70 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 15 formal Memoranda of Understanding/ Agreements with international institutes and a host of well-established cooperative and collaborative relationships with individuals, institutes, and other private- and public-sector organizations all around the world.

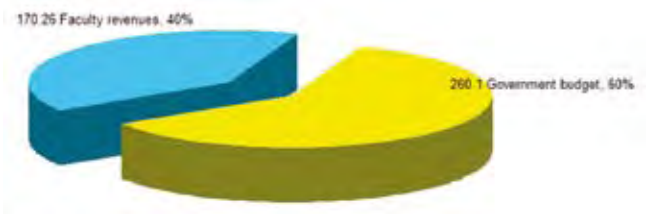
Joint International Tropical Medicine Meeting

The Faculty of Tropical Medicine holds the Joint International Tropical Medicine Meeting annually. In 2008, the Meeting was held 13-14 October at the Imperial Queen's Park Hotel in Bangkok. The main theme

of the Meeting was "Tropical Medicine in the –omics Era". Our Keynote Speaker was Professor Yongyuth Yuthavong, a well known senior researcher into the development of antimalarial drugs at the NSTDA's National Centre for Genetic Engineering and Biotechnology (BIOTEC), to speake on the topic "Trop MoMe - Tropical Molecular Medicine in the -omics Era". This two-day event incorporated a unique scientific program, focusing on a broad range of tropical medicine topics. In 2008, the Meeting attracted 826 registered participants from 24 countries. For more information about the Joint International Tropical Medicine Meeting series, please visit the JITMM website at http://www.jitmm.com

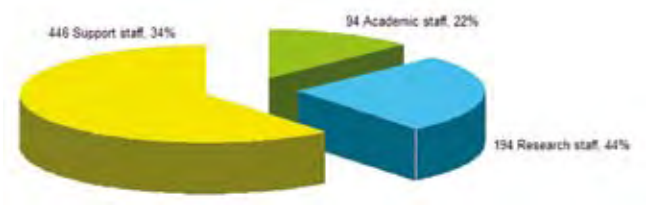
Finance

Total income amounted to 430.36 million Baht, of which 260.10 million Baht were supported by government budget, and 170.26 million Baht from Faculty revenues.



Personnel

In the year 2008, the Faculty had 738 staff, including 94 academic staff, 194 research staff, and 446 support staff. Seventy-five (75) staff (26.04%) had Ph.D. degrees, while 70 (24.3%) had Master Degrees or comparable qualifications. The ratio of Prof. : Assoc. Prof. : Assist. Prof. : Lecturer = 5 : 33 : 22 : 34. Eleven (11) staff retired and five (5) staff opted for early retirement during the year.



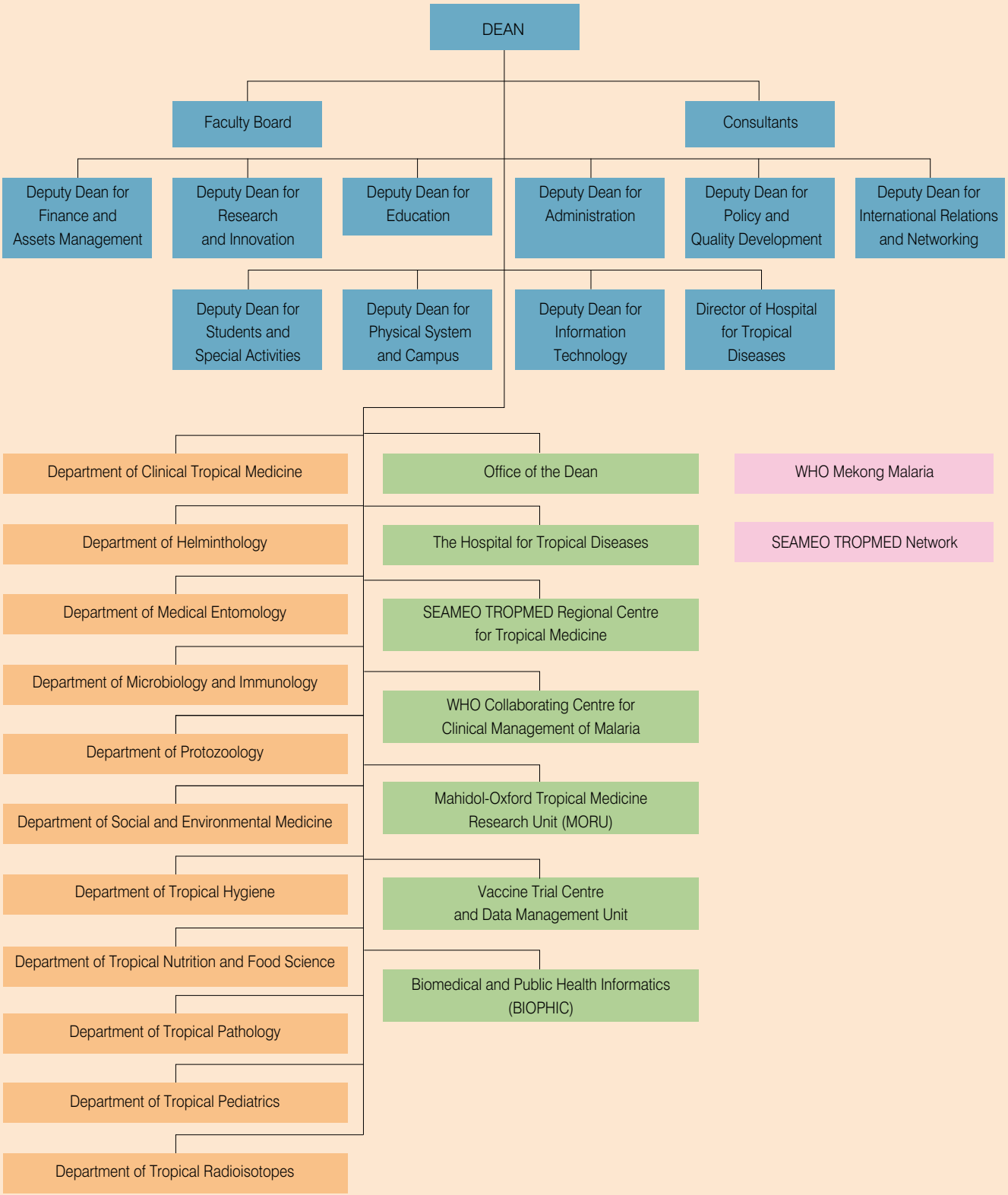
Staff development

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

A new building, "Asia's Center of Excellence for Tropical Diseases", has been under construction since early 2008. The building will serve as a Center of Excellence for clinical trials, research, and the treatment of tropical diseases.

Organization Chart



Administrative Board

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

Assoc.Prof. Jitra Waikagul
Deputy Dean for Administration

Assoc. Prof. Porntip Petmir
Deputy Dean for Finance and Assets Management

Prof. Sasithon Pukrittayakamee
Deputy Dean for International Relations and Networking

Assoc. Prof. Waranya Wongwit
Deputy Dean for Education

Assist. Prof. Chotechuang Panasoponkul
Deputy Dean for Student Affairs and Special Activities (Until 30 September 2008)

Assist. Prof. Pongrama Ramasoota
Deputy Dean for Research and Innovation

Assoc. Prof. Wichit Rojekittikhun
Deputy Dean for Information Technology

Assoc. Prof. Wattana Leowattana
Deputy Dean for Service and Hospital Director (Until 30 September 2008)

Assist. Prof. Udomsak Silachamroon
Director of Hospital for Tropical Diseases

Assist. Prof. Varaporn Suphadtananphongs
Deputy Dean for Policy and Human Resources (Until 30 September 2008)

Assist. Prof. Phanorsri Attanath
Deputy Dean for International Relations (Until 30 September 2008)

Sarunya Kaewprasert
Deputy Dean for Policy and Quality Development

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

Dr. Wichai Ekataksin
Assistant Dean for Education and Information Technology (To 30 September 2008)

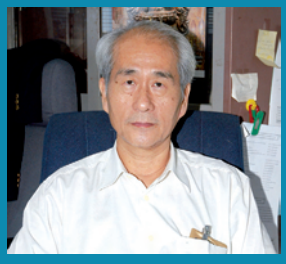
Assoc. Prof. Supatra Thongrungkiat
Assistant Dean for Research (To 30 September 2008)

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. Wijitr Fungladda
Department of Social and Environmental Medicine, and WHO CC for Environmental Management for Disease Vector Control

Assist. Prof. Jaranit Kaewkungwal
Department of Tropical Hygiene, and BIOPHIC

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

Assoc. Prof. Emsri Pongponratn
Department of Tropical Pathology

Prof. Krisana Pengsaa
Department of Tropical Pediatrics

Assist.Prof. Channarong Sanghirun
Department of Tropical Radioisotopes
(To 30 September 2008)

Assoc. Prof. Pratap Singhasivanon
Department of Tropical Radioisotopes

Prof. Polrat Wilairatana
WHO CC for Clinical Management of Malaria

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

Prof. Nicholas Day
Director, MORU

Mr. Somkid Nima
Secretary of the Faculty

Faculty Board

1	Dean	Chair
8	Deputy Deans	Members
2	Assistant Deans	Members
11	Heads of Department	Members
4	Lecturer Representatives	Members
1	Deputy Dean	Member and Secretary

Faculty Senate (2007-2009)

Assoc. Prof. Varee Wongchotigul	Chair (Until December 2008)
Assist. Prof. Watcharee Chokejindachai	Chair (Since January 2009)
Assist. Prof. Pongrama Ramasoota	Vice-Chair (Until September 2008)
Dr. Prapin Thanpoophasiam	Vice-Chair (Since January 2009)
Assoc. Prof. Wichit Rojekittikhun	Lecturer Representative
Assist. 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 Tropical Hygiene
Assist. Prof. Kesinee Chotivanich	Representative, Department of Clinical Tropical Medicine
Dr. Sarunya Kaewprasert	Representative, Department of Tropical Nutrition and Food Science/Secretary General (Until September 2008)
Assist. Prof. Voranuch Wangsuphachart	Representative, Department of Social and Environmental Medicine/Secretary General (Since January 2009)
Assist. Prof. Urai Chaisri	Representative, Department of Tropical Pathology /Assistant Secretary General (Until December 2008)
Dr. Teera Kusolsuk	Representative, Department of Helminthology/Assistant Secretary General (Since January 2009)

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

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, Human Resource Unit, Policy and Planning Unit, Financial and Procurement Unit, Educational Affairs Unit, Information Technology Unit, Educational Technology Unit, International Relations Unit, Asset Management Unit, and Research and Academic Services.

- OFFICE OF THE DEAN
  - Administration & General Affairs Unit
  - Educational Affairs Unit
  - Financial and Procurement Unit
  - Research and Academic Services
  - Policy and Planning Unit
  - Human Resource Unit
  - Educational Technology Unit
  - Information Technology Unit
  - International Relations Unit
  - Asset Management Unit

Mr. Somkid Nima
Secretary of the Faculty

Yaowapa Pratumsuwan
Head, Policy and Planning Unit

Sukanya Ongaree
Head, Human Resource Unit

Kannikarkaew Pinit
Head, Administration & General Affairs Unit

Pornpimon Adams
Head, Research and Academic Services

Keeratiya Nontabutra
Head, International Relations Unit

Duangjai Sahassananda
Head, Information Technology Unit

Chiraporn Praevanit
Head, Educational Affairs Unit

Kesinee Nima
Head, Financial Unit

Prapaiporn Tiacharoen
Head, Procurement Unit

Savek Chomming
Head, Asset Management Unit

Siwaporn Phanphoowong
Head, Educational Technology Unit

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

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

SEAMEO TROPMED Network is a regional cooperative 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.

MOUs/Agreements

No.	Institutes	Duration	Start	Finish	Remarks
1.	Chiangrai Regional Hospital, Thailand	5 yrs	15 June 2004	14 June 2009	MOU
2.	Department of Medical Science, MoPH, Thailand	5 yrs	28 May 2004	27 May 2009	MOU
3.	Canadian Food Inspection Agency, Government of Canada, Saskatoon, Canada	Non-specific	3 Sep. 1998		MOU
4.	University of Leicester, United Kingdom		1 April 2002	present	MOU
5.	Freie Universitat at Berlin, Germany		30 May 1985	present	Agreement
6.	The Swiss Tropical Institute, Basel, Switzerland		3 June 1998	present	MOU
7.	University of Innsbruck, Austria		12 May 1992	present	Agreement
8.	Nagasaki University, Japan	5 yrs	1 Nov. 1999	Automatically extended every 5 yrs	Agreement
9.	University of Shizuoka School of Pharmaceutical Science, Japan	5 yrs	2005	2010	MOU
10.	Ubon Rajathanee University, Thailand	5 yrs	Sep 2004	2008	MOU
11.	The Wellcome Trust-Mahidol University, Oxford Tropical Medicine Research Programme	5 yrs	Sep 2004	2008	MOU
12.	Southeast Asian Ministers of Education Organization (SEAMEO) and the Government of Thailand	-	18 May 1971	-	MOU
13.	The University of Texas Health Science Center at Houston, USA	5 yrs	15 Nov 2005	2009	MOU
14.	Asahikawa Medical College, Japan	5 yrs	February 2008	2013	MOU and Agreement
15.	Malaria Consortium, UK	10 yrs	February 2009	2019	MOU

■ 39th Anniversary of the Royal Bestowal of the name Mahidol University, 3-12 March 2008

■ SEAMEO Service Award 2008. Khun Pinkaew received her award in Malaysia, 13 - 14 March 2008

■ Opening Ceremony for M.Sc. (Trop. Med.) and Ph.D. (Trop.Med.) Courses for The Academic Year 2008, Chalermprakiat conference room, 5th floor, Chalermprakiat Building Faculty of Tropical Medicine, 2 June 2008

■ The Minister for Education, Malaysia, and the President of SEAMEO Council visited the SEAMEO TROPMED Regional Centre for Tropical Medicine (TROPMED/Thailand), 17 June 2008

■ Sports Day / Month at Faculty of Tropical Medicine and Faculty of Dentistry, 14 July - 1 August 2008

■ Training Course on Vector-Borne Disease Control, 28 July - 1 August 2008

■ Ethics Commitee received SIDCER/FERCAP recognition at the International Conference on Empowering Stakeholders in Health Research: Towards Developing an Ethics of Accountability and Responsibility, 24-26 November 2008

■ Epidemiology, Management and Control of Dengue. Organized by the Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, 21 July - 1 August 2008

■ Training Course on Vector-Borne Disease Control at the Faculty of Tropical Medicine, Mahidol University, 28 July - 1 August 2008

■ 6th International Training Course on Management of Malaria 2008 Organized by the WHO Collaborating Centre for the Clinical Management of Malaria, 1-5 September 2008

■ Asst. Prof. Pongrama Ramasoota received the IFIA Scientific Gold Medal at the International Federation of Inventors Associations (IFIA) 14 December 2008

■ Training Course on GIS and Data Management, 4-15 August 2008

■ The School of Assistant Nurses, Faculty of Tropical Medicine, held the 27th Graduation Ceremony (2008) in the Maegraith Hall. Assoc. Prof. Pratap Singhasivanon, Dean of the Faculty, chaired the ceremony and offered his personal congratulations to the 226 assistant nurse graduates, 31 July 2008

■ Joint International Tropical Medicine Meeting 2008: "Tropical Medicine in the -omics Era", at the Imperial Queen's Park Hotel, Bangkok, Thailand, 13-14 October 2008

Department of Clinical Tropical Medicine

Introduction

The Department of Clinical Tropical Medicine was established in 1960, with the founding of the Faculty of Tropical Medicine, to pursue research and training in tropical diseases prevalent in Thailand and thereby contribute to the advancement of knowledge and clinical care in tropical diseases. The Department has a long and proud history of contributions to clinical care and research, especially in malaria, AIDS, melioidosis, leptospirosis, scrub typhus, and avian influenza.

The major focus for malaria is research into complicated and uncomplicated multi-drug resistant falciparum malaria. Combinations of various antimalarials have been evaluated, especially artesunate or artemether followed by mefloquine, which was found to be the drug of choice for an alternative treatment for multidrug-resistant malaria. The Department, as part of the Faculty, was appointed the WHO Collaborating Centre for Clinical Management of Malaria. Departmental staff have also actively performed various research and clinical trials in collaboration with the Ministry of Public Health and various national and international academic institutes, searching for a suitable AIDS vaccine for Thailand over the past 14 years. We are the only group to have conducted two efficacy trials involving rgp120 and vector-based HIV vaccines. Apart from their work on AIDS vaccines, the staff have also participated in multi-centre trials of Quadrivalent HPV vaccine for preventing infection leading to cervical cancer, which was licensed in 2007 last year. Research grants totaled > 60 million Baht in 2007. Staff of the Department are actively involved with major clinical trials for the treatment of melioidosis, scrub typhus, and leptospirosis, in close collaboration with various institutes, including Oxford University, Khon Kaen University, and other hospitals under the Ministry of Public Health, such as Sappasithiprasong and Ubon Ratchathani hospitals. Antiviral drugs, using a variety of new dosages and regimens, have been tested for novel treatments for avian influenza under the SEA Influenza Network. Over the past 20 years, more than 1,000 articles related to the above diseases have been published in international peer-reviewed journals.

Head of Department
Prof Punnee Pitisuttithum
MBBS, DTM&H (Bangkok), Grad Dip Clin Sci (Med), Dip Thai Board of Internal Medicine

List of publications

List of presentations

Department of Helminthology

Head of Department
Assoc Prof Chalit Komalamisra
BSc (Biol), MSc (Trop Med), DAP&E, Dr Med Sci

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 Trop Med) and Doctor of Philosophy in Tropical Medicine (PhD Trop Med).

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.

Training Course

Refresher course on laboratory diagnosis of helminthic infections, 28-29 August 2008 at Kanchanaburi Tropical Disease Research Centre, Mahidol Campus, Saiyok District, Kanchanaburi Province.

Postgraduate program participants

Master of Science in Tropical Medicine	4
Doctor of Philosophy in Tropical Medicine	5
Total	9

Research

On-going Department Research

Angiostrongyliasis

- Angiostrongyliasis: potential fractionated antigens of *Angiostrongylus cantonensis* adult worms for diagnosis using ELISA.
- Analysis of a protein expression from *Angiostrongylus cantonensis* DNA cloning for serodiagnosis of angiostrongyliasis.
- Detection of IgG-subclass for serodiagnosis of angiostrongyliasis.
- *Angiostrongylus cantonensis* in freshwater snails collected from 18 different localities of Thailand: prevalence and parasitic burden, biochemical components, antigenicity and population genetics.

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
- Molecular identification of *Taenia* spp.
- Seroepidemiology for cysticercosis

Echinococcosis

- Mitochondrial DNA sequence of protoscoleces of Echinococcus cysts from four patients.
- Serodiagnosis of suspected echino-coccosis 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.
- The study on life cycle and antigenicity at *Paragonimus pseudoheterotremus* Waikagul, 2007.

Soil-transmitted helminthiasis

- 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.
- Relationship between behavior and soil-transmitted helminthiasis in seaman.
- Soil-transmitted helminthiases control through school-based intervention.

Trichinellosis

- Monoclonal antibody-based competitive ELISA and indirect ELISA for immunodiagnosis of trichinellosis.
- Lipid peroxidation in rats infected with *Trichinella spiralis.*

Fish-borne trematode infections

- Diagnosis of liver and intestinal flukes by PCR.
- Differential serodiagnosis between liver and intestinal trematodes.
- Fish-borne trematodes in Thailand.
- Intestinal parasites isolated from house-flies in the tourist attraction areas in Thailand.
- Coinfection of *Helicobacter pylori* and parasites in healthy people aged 40 years old and over in Kanchanaburi, Nakhon Sri Thammarat and Nan provinces, Thailand.
- Genetic variation and population structure studies of fish-borne trematodes for increasing control impact of opisthorchiasis and cholangiocarcinoma

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

Reference Centre for Food- and Water-borne Parasitic Zoonoses

List of publications

List of presentations

Department of Medical Entomology

Introduction

The Department of Medical Entomology is one of the five departments at the initial stage of establishment of the Faculty of Tropical Medicine in 1960. At present, the Department comprises 7 academic staff, 2 researchers, 3 scientists, 1 medical science associates, 11 medical science assistants, 1 administrative Officer, 1 Business Associate and 2 General Assistants. The Department has been actively involved in education, research and public service. Apart from the regular subjects of Medical Entomology for the Graduate Diploma in Tropical Medicine and Hygiene (DTM&H course), Master of Science Programme in Tropical Medicine [MSc (Trop Med)], Doctor of Philosophy Programme in Tropical Medicine [PhD (Trop Med)], Master of Clinical Tropical Medicine Programme(MCTM) and Doctor of Philosophy Programme in Clinical Tropical Medicine [PhD (CTM)], the Department also provides the training on medically important insects/ arthropods and vector control both for individual or in group upon request. Student dissertation topics span a wide range of issues in molecular and applied medical entomology. Dissertations include basic field work, application of control measures, and biotechnology. Most graduated candidates typically spend no longer than five years taking graduate courses and doing research. The completed graduated students pursue diverse careers. Many take positions in academic institutions or in government organizations as well as the private sector, working in research and development. The main research involves basic and applied knowledge applicable to controlling insect and arthropod vectors of tropical diseases, with emphasis on mosquito-borne diseases. As a reference centre, the Department of Medical Entomology develops the Mosquito Museum and runs the project on application of computer technology on management of biological specimens.

Head of Department
Assoc Prof Chamnarn Apiwathnasorn
BSc (Med Tech), MSc (Trop Med), M Trop Health, PhD (Trop Med)

Highlight activities

EDUCATION: The Department of Medical Entomology teaches several Medical Entomology subjects in the Graduate Diploma in Tropical Medicine and Hygiene (DTM&H) course, Master of Science in Tropical Medicine [MSc (Trop Med)], Doctor of Philosophy in Tropical Medicine [PhD (Trop Med)], the Master of Clinical Tropical Medicine (MCTM) and the Doctor of Philosophy in Clinical Tropical Medicine [PhD (CTM)]

Current Students

Degree Programs	Student Name	Advisor
MSc (Trop Med)	Phubeth Ya-umphan	Assoc Prof Narumon Komalamisra
	Wachiraphan Chittham	Dr Sungsit Sungvornyothin
	Nima Wangdi Gyeltsher	Dr Sungsit Sungvornyothin
PhD (Trop Med)	Pruksa Nawtaisong	Assoc Prof Narumon Komalamisra
	Jakkrawarn Chompoosri	Assoc Prof Narumon Komalamisra
	Raweewan Srisawat	Assoc Prof Narumon Komalamisra
	Suchada Sumroiphon	Assoc Prof Chamnarn Apiwathnasorn
	Parichat Lapcharoen	Assoc Prof Narumon Komalamisra
	Namtip Trongnipat	Assoc Prof Chamnarn Apiwathnasorn
	Chalermpon Kumpitak	Assoc Prof Chamnarn Apiwathnasorn
	Chalalai Rueanghiran	Dr Jiraporn Ruangsittichai
	Kruawan Chotelersak	Dr Jiraporn Ruangsittichai
	Arunrat Taepparat	Assoc Prof Chamnarn Apiwathnasorn

Training Course

Courses	Duration	Total Number of Participants	Places
Entomological Field Survey	28 Jul - 9 Aug 2008	14 Participants From Center of Malariology, Parasitology and Entomology (CMPE), Ministry of Health Lao PDR	Department of Medical Entomology
Epidemiology, Management and Control of Dengue Vector	8-19 Sep 2008	14 Participants From Center of Malariology, Parasitology and Entomology (CMPE), Ministry of Health Lao PDR	Department of Medical Entomology

RESEARCH: The Department has many dynamic research and scholarly activities that are recognized nationally and internationally. "Highlights" include the following.

Epidemiological Investigation of Outbreaks of Vector-borne diseases

This is an important goal to resolve entomological problems in collaboration with the Department of Disease Control, Ministry of Health through research making the results available to other scientists, educators, and the general public to develop effective control measures for the community. As a result of a previous study on the impact of the Asian Tsunami on mosquito vectors in the most affected areas of Phang Nga Province in December 2004, for instance, a small-scale project for controlling mosquito larvae inhabiting swamps and ponds contaminated by seawater using *Bacillus thuringiensis israelensis* (VectoBac® WDG) and application of natural mosquito repellents to prevent the local people from mosquito bites was conducted with a satisfactorily success; its use has been extended to the worst-affected area, Ban Nam Khem Village at present. The department help investigate re-emerging and emerging diseases, e.g., local transmission of malaria in Sa Kaeo

Province and autochthonous visceral leishmaniasis of Thailand and subsequently, a new species of *Leishmania* is proposed. In addition, the department works with local public-health officials to determine 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 (recently, bot flies in travellers), ticks and mites for general hospitals.

Technology to Assist the Public

Studies on insecticidal activities of several species of Thai herbal extracts for controlling mosquito vectors provided various promising oil extracts that showed mosquito repellency effect, such as Qinghao (*Artemisia annua*), May Chang" (*Litsea cubeba*), cloves (*Syzygium aromaticum*), and "Ma Khwaen" (*Zanthoxylum limonella*), and were formulated in forms of cream or gel. The extract of "Thong Pan Chung" (*Rhinacanthus nasutus*) provided high larvicidal activity is prepared as tablet form. The development of mosquito repellent formulation of volatile oils mixture, Qinghao and May Chang has recently been obtained a petty patent with an average protection time longer than 5 hours. Our research group is to transferring low cost and simple production of this patent repellent developed in our laboratory into the communities with malaria- and dengue-high risk transmission through training for their own production and use for the disease prevention with legal permission.

Developing Effective Tools

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 in reduction of man-vector contact and control of dengue-vector populations in Phag Nga Province as an optional tool for DHF control.

Insect Vector Rearing Laboratory

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, flies, bedbugs and ticks are maintained in the insectarium for research and educational purpose.

Mosquito Museum and Reference Center

The Department of Medical Entomology acts as a reference center on mosquito vectors of Thailand through establishment of the Mosquito Museum. The collections include 18 genera of mosquitoes with more than 20,000 specimens (mounted slide and pinned). In addition, DNA of mosquito vectors are extracted and preserved in the museum collections. Academic consultations, especially on mosquito-borne diseases and their control measures are also provided.

Current Research

Molecular biology of insect vectors

The study of vector biology at a molecular level using molecular techniques including gene cloning, creation of cDNA, polymerase chain reaction, gel electrophoresis, various blotting techniques, RFLP analysis, and DNA sequencing. Three currently research areas of medical entomology are: determination of species complexes among insect vectors and their population genetics to incriminate vector species; Molecular differentiation of the morphologically cryptic species: morphological similar species such as *Ae. niveus* subgroup, *Ae.* albopictus, *An. sundaicus* complex, *Mansonia* spp., *An. barbirostris* group and phlebotomine sandflies Identifying insect vector species through DNA barcodes (648-bp stretch of the mitochondrial gene cytochrome *c* oxidase-I) allowing taxonomists to quickly and accurately identify specimens. The specimens include mosquitoes, biting midges, flies, sandflies etc.

Population genetic structure of the mosquito vectors to determine molecular markers related to biting behavior, vectorial capacity, and other characteristics of epidemiological importance determination of the emergence of resistance and insecticide resistance mechanisms in major insect vectors; and vector-parasite interactions in mosquito-borne diseases with emphasis on molecular mechanisms responsible for pathogen destruction, such as melanotic encapsulation and immune peptide production and genetic engineering to generate pathogen-resistant mosquitoes from those that are susceptible.

Mosquito–*Plasmodium* interactions in response to immune activation of the vector.

DNA banking of mosquito vectors of Thailand

Thailand is facing a biodiversity loss owing to environmental changes in extensive magnitude. DNA banks have great potential as tools for biodiversity conservation by secure storage of genetic materials to provide a ready source of material to insure their utility for future generations of researchers. Establishment of DNA banking of mosquito vectors is to collect mosquito samples for the purpose of supporting comparative molecular biology, for instance, phylogeny, population genetics and distribution of species complexes.

Geographic Information System (GIS) is seen primarily as a research tool in the field of vector-borne disease. Mapping efforts are conducted on geographic scales ranging from village to continental levels, and on temporal scales ranging from the duration of an outbreak to multi-year models. GIS serves as a common platform for convergence of multi-disease surveillance activities. Public health resources, specific diseases and other health events can be mapped in relation to their surrounding environment and existing health and social infrastructures. Such information when mapped together creates a powerful tool to analyze the evolution and distribution of parasites and their relationship to different ecological niches, and may dramatically improve our ability to quantify the disease impacts of climatic and ecological changes. However, the use of GIS in decision support has also become an area of growing interest.

GIS Maps of Brugian Filariasis in Narathiwat Province, southern Thailand. A study of Brugian filariasis in Narathiwat Province demonstrated how raster and vector data were used within a GIS framework to produce maps indicating areas of potential risk for filariasis transmission within peat-swamp forest. A risk map was generated by overlay operations of GIS, based on the attribute data distribution of residences of infected people and the boundary of the peat swamp forest conservation zone. The information included in the GIS maps can help identify where current information is lacking, or serve as a stimulus to collect new or additional data at a particular administrative level.

Develop Natural (Plants) Insect Repellents on the basis of cost-effectiveness and safe and finally develop practical applications for the promising formulations in the simplest way to reach the local populations for self protection against a wide variety of insect vectors including mosquitoes, cockroaches, bed bugs, ticks, and flies.

List of Research Projects (Ongoing)

1 Bionomics study of phlebotomine sandflies in Thailand.
2 Dengue virus detection in dark and pale forms of *Aedes aegypti* collected as immature stages from breeding site.
3 DNA Bank of mosquito vectors of Thailand.
4 Dynamic and temporal structure of the troglobitic fauna of medically important insects and arthropods in caves of Kanchanaburi Province.
5 Ecology, behavior and molecular identification of *Anopheles dirus* complex.
6 Effect of heavy metal (Pb2+, Cd2+) on enzymes of *Culex quinquefasciatus.*
7 Efficacy of water dispersible granule formulations of *Bacillus thuringiensis israelensis* (VectoBac®WDG) and *Bacillus sphaericus* (VectoLex® WDG) to control brackish-water mosquitoes, *Anopheles sundaicus* and *Culex sitiens*, in tsunami-affected areas of Phang-nga province, Southern Thailand: monitoring by reduction in larvae density.
8 Field application of *Bacillus thuringiensis israelensis* (VectoBac® WDG) on control of mosquito larvae in the salt-marsh habitats in Ban Nam Khem village, tsunami affected areas, Phang Nga Province, Thailand: monitoring by reduction in adult density.
9 Molecular identification of *Anopheles sundaicus* in natural breeding sources, the coastal area of Andaman and the Gulf of Thailand.
10 Mosquito repellent from medicinal plants.
11 Plant-based insecticides control mosquito vectors.
12 Population dynamic of the dengue vectors and dengue virus infection in *Aedes aegypti,* and *Aedes albopictus,* in urban and suburban areas.
13 Survey and study on geographical distribution of *Aedes albopictus* in Bangkok Metropolitan.
14 Susceptibility to oral infection and transovarial transmission of dengue viruses in *Aedes aegypti* the dark form and the pale form
15 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 of northern, southern and northeastern Thailand.
16 Comparative study knockdown resistance gene (Kdr) in vector of dengue haemorrhagic fever to permethrin.

List of Co-Research Projects (Ongoing)

1 Implementation research of new intervention tools
2 Molecular identification of libellulid dragonflies in rice fields of Central plain, Thailand.
3 Novel methods for dengue prevention by vector control.
4 Population dynamic of the dengue vectors and dengue virus infection in *Aedes aegypti* and *Aedes albopictus*, in urban and suburban areas.
5 Field research on vector-borne infectious diseases, especially to dengue viral analyses in the vector mosquitoes by using molecular biological method in Thailand.

International Linkages

1 Oita University Japan
2 Liverpool School of Tropical Medicine, United Kingdom
3 Institute of Tropical Medicine Nationalestraat 155 B-2000 Antwerpen, Belgium
4 Dainihon Jochugiku Co. Ltd., Japan

National Linkages

1 Department of Disease Control, Ministry of Public Health

Academic Services

1 Identification of arthropods and insects from biological specimens.
2 Determination of filarial worms by Knott's Concentration Technique.
3 Insecticide susceptibility testing service
4 Educational specimens for sale "Mosquito & Important Medical Arthropods"

List of publications

List of presentations

Department of Microbiology and Immunology

Introduction

In the year under review, the Department of Microbiology and Immunology participated in the postgraduate teaching programs offered by the Faculty--the Diploma in Tropical Medicine and Hygiene (DTM&H), Master of Science in Tropical Medicine, and Doctor of Philosophy in Tropical Medicine programs. Concerning research work, 18 research articles were accepted for publication in renowned international journals, with several other manuscripts in preparation. The Department's staffs were also actively involved in international scientific meetings, including both oral and poster presentations. The Department aims to continue its research work focused on various tropical diseases, especially those affecting human health, so that the results can be used for the better health of the Thai people, and those in other regions.

Head of Department
Assoc Prof Manas Chongsa-nguan
BSc, M.P.H., PhD

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 CD_4 and CD_8

International Linkages

The Department has extensive international linkages in Australia, France, India, Japan, Singapore, Sweden, Taiwan, the UK, and the USA.

List of publications

1. Polymorphism patterns in Duffy-binding protein among Thai *Plasmodium vivax* isolates *80*
2. Genome-wide SNP-based linkage analysis of tuberculosis in Thais *80*
3. Genetic diversity and microevolution of *Burkholderia pseudomallei* in the environment *81*
4. Prevalence and sequence diversity of a factor required for actin-based motility in natural populations of *Burkholderia* species .. *81*
5. Rapid detection of the pandemic methicillin-resistant *Staphylococcus aureus* clone ST 239, a dominant strain in Asian hospitals *81*
6. Loop-mediated isothermal amplification method targeting the TTS1 gene cluster for detection of *Burkholderia pseudomallei* and diagnosis of melioidosis. *81*

List of presentations

Department of Protozoology

Departmental/Unit Activities

The Department of Protozoology is one of the 11 departments of the Faculty of Tropical Medicine, Mahidol University. It was one of the first 5 departments created at the founding of the Faculty of Tropical Medicine in 1960. Its responsibilities are teaching, training, research and services in the field of medical protozoa.

Highlight Activities

In response to the Faculty's Mission to be **"Asia Leader's in Tropical Medicine"**, the Department of Protozoology is responsible for the **"Research-based Diagnostic Unit".** We therefore provide diagnostic techniques based on up-to-date research, from enzyme to community levels.

Head of Department
Assoc Prof Yaowalark Sukthana
BSc, DVM, MD (Hons), Dip of Thai Board in Oto Rhino Laryngology, DTM&H, MCTM

Education

The Department teaches Medical Protozoology in the Faculty's regular programs: DTM&H, MSc (TropMed) and PhD (TropMed).
Current postgraduate students majoring in Protozoology

- MSc (TropMed) 2
- PhD (TropMed) 10
- Total 12

Training Courses, Workshops, Meetings

International Level

International Workshop on "Laboratory Diagnosis of Malaria: From Conventional to High Technology" at Faculty of Tropical Medicine, Mahidol University and Kanchanaburi Province, 18-22 Aug 2008; 3 participants

National Level

Workshop on "Intestinal Protozoa: Simple Diagnostic Tips" at Faculty of Tropical Medicine, Mahidol University

Group 1: 20-21 Mar 2008; 58 participants

Group 2: 27-28 Mar 2008; 70 participants

Workshop on "The 6th Annual Diagnosis of Malaria as a Professional" at Faculty of Tropical Medicine, Mahidol University

Group 1: 7-8 Aug 2008; 71 participants

Group 2: 13-14 Aug 2008; 36 participants

Research

Current Research Activities

- Biological contamination-free Thai frozen food
- Studies on DNA replication and DNA repair enzymes 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*.
- Detection of *Trichomonas vaginalis* infection in Thai women by PCR and culture methods.
- Comparison of the detection of *Toxoplasma gondii* antibody by Sabin-Feldman dye test and indirect fluorescent antibody test (IFAT) using *T. gondii* tachyzoites from cell culture and animal inoculation.
- *Toxoplasma gondii* genotyping in domestic and wild felids in Thailand
- PCR assays for detection of *Toxoplasma gondii* in Thai commercial meat products
- Genetic characterization of *Toxoplasma gondii* in Thai free-range chickens

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, Ha Noi, Vietnam.
- Department of Environmental Health Sciences; Division of Environmental Health Engineering, Bloomberg School of Public Health, Johns Hopkins University, USA.

National Linkages

- Inter-laboratory Network for Parasitic Immunology, hosted by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health
- Project of making *Plasmodium* slides to distribute all over the country for laboratory QC at the request of the Department of Medical Sciences, Ministry of Public Health.
- Environment Engineering and Management, Asian Institute of Technology, Thailand.

Laboratory Services

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

List of publications

1. Supression of *Plasmodium falciparum* by serum collected from a case of *Plasmodium vivax* infection *69*
2. Seroprevalence of *Toxoplasma gondii* antibody in Vietnamese villagers *86*
3. Formation, physical stability and *in vitro* antimalarial activity of dihydroartemisinin nanosuspensions obtained by co-grinding method *86*
4. Evidence supporting the zoonotic and non-zoonotic transmission of *Enterocytozoon bieneusi* *86*
5. Inhibitory effects of Thai plants ß-glycosides on *Trichomonas vaginalis* *86*
6. PCR-BASED coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by *Ancylostoma ceylanicum* in temple communities in Bangkok *87*

List of presentations

1. Detection of *Entamoeba dispar* from surface and waste water samples by real-time PCR *137*
2. Understanding centennial *Toxoplasma gondii* by high technology methods *137*
3. Molecular characterization of *Plasmodium falciparum* polynucleotide kinase/phosphatase *138*
4. Detecting *Giardia* contamination in Thai vegetables *138*
5. Comparative study of three laboratories for detecting *Cryptosporidium* in stool samples *138*
6. Large-volume collection: a recommended technique for protozoa detection in water *138*
7. RT-PCR assay: a diagnostic tool for *Toxoplasma* reactivation in immunocompromised host *139*
8. Toxoplasmic encephalitis: the use of stage-specific gene as a diagnostic tool *139*

Department of Social and Environmental Medicine

Education

International Training Course on Environmental Health Impact Assessment (EHIA), 10-25 Nov 2008, at the Faculty of Tropical Medicine, Mahidol University: 51 participants

Highlight

The Department, as part of the WHO Collaborating Centre for Environmental Management for Disease Vector Control in Sustainable Development, co-organized the International Training Course on Environmental Health Impact Assessment (EHIA), November 10-25, 2008, with WHO-SEARO; the SEAMEO TROPMED Network; the Department of Health, Ministry of Public Health, Thailand; and the Office of Natural Resources and Environment Policy and Planning. 51 people participated, from Bangladesh, Bhutan, Indonesia, Lao PDR, Malaysia, the Maldives, Myanmar, the Philippines, Timor Leste, Vietnam, and Thailand.

Services

- Laboratory services for disease vectors and parasites
- Rapid diagnosis of multidrug-resistant *Mycobacterium tuberculosis*
- Producing specimens of *Schistosoma* spp. and *Opisthorchis* sp.

Award-winning work

Assist Prof Pongrama Ramasoota won the Inventor Award in Medical Sciences, National Research Council of Thailand, and the International Federation of Inventors Associations (IFIA) Scientific Gold Medal

"Rapid detection of *M. tuberculosis* and *Legionella* from indoor air using modified impinger and polymerase chain reaction (PCR) methods."

Head of Department
Assoc Prof Wijitr Fungladda
BSc (Med Sc.), MD, DTM&H, MPH, DrPH

List of publications

List of presentations

Department of Tropical Hygiene

Introduction

The Tropical Hygiene Section was formed as one of the original units of the Faculty of Tropical Medicine which was established in 1960. Since then, the section has been responsible for providing lectures and field training as part of the Diploma in Tropical Medicine and Hygiene (DTM&H) curriculum. Research activities of the section were comprised mainly of epidemiological research related to public health problems among rural populations in Thailand. In 1974 along with the rapid growth of the faculty, the Tropical Hygiene Section was upgraded into the Department of Tropical Hygiene. At present, the department is responsible for providing instruction and training to the master and doctoral degree programs in Tropical Medicine in addition to the existing DTM&H course. And continuing its research tradition, activities being carried out at the moment are mainly in the field of epidemiology with the application of Geographical Information System (GIS) on various tropical diseases. The department is also involved in providing specialized short training courses on epidemiology, data analysis, and GIS. Furthermore, the department has direct responsibility in managing the Rajanagarindra Tropical Disease International Centre (RTIC) located in a malaria-endemic rural community near the Thai-Myanmar border in Suan Phung District, Ratchaburi Province. For many years now the RTIC, through its malaria clinic, takes pride in its continuous delivery of free and quality health services to local residents.

Head of Department
Assist Prof Jaranit Kaewkungwal
BSc, MEd, MA, PhD (Applied Statistics and Program Evaluation)

International Training Courses

- Application of Geographical Information Systems in the Epidemiology of Tropical Diseases. Feb 11-22, 2008. Participants: 9
- Statistical Methods in Spatial Epidemiology. Mar 3-12, 2008. Participants: 11

Training Courses

- Data Analysis in Health Science Research - Level 1. August 18-22, 2008. Participants: 23

Current research students

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 on 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

Kwanjai Viputtigul
Polymorphism at the merozoite surface protein-1 (MSP-1) C-terminus of *falciparum* malaria isolated from western border of Thailand

Putri C. Eyanoer
The spatial and temporal distribution of human avian influenza infection in relation to surveillance implementation in Indonesia

Dilok Tongsukh
TB infection and treatment among refugees in Thamhin Camp, Ratchaburi Province, Thailand

Collaborations

1. Dorothée MISSE G.E.M.I., IRD/CNRS Montpellier, France
2. Erik (Rik) Thompson, PhD , 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
3. Chantal Ripoll, INSERM, Immunohistochemistry Unit, Montpellier, France
4. Hans Yssel, INSERM, Immunophysiology Unit, Montpellier, France
5. The Rockefeller Foundation

List of publications

List of Presentations

Department of Tropical Nutrition and Food Science

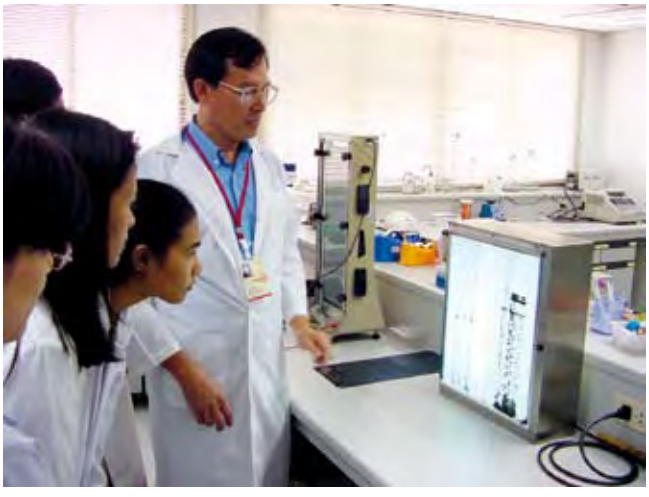
Highlight

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 2008, 7 students in M.Sc and PhD program in Tropical Medicine including 1 PhD student from Faculty of Dentistry, 2 Master of Science program students from Faculty of Public Health are working in the Department. In addition, all staffs are responsible for technical services and the distribution of nutrition information to the community.

Head of Department
Assoc Prof Songsak Petmitr
BEd (Secondary School), MS (Chemistry Ed.),
PhD (Biochemistry)

Training Course

The four-day workshop on "Methods in nutritional assessment and research" was organized by all staff of the Department of Tropical Nutrition and Food Sciences on 22-25, 29 April-2 May 2008, respectively at the Faculty of Tropical Medicine, Mahidol University. 80 participants enrolled in these courses. The objective of this course was to provide knowledge on methodologies in nutrition research, such as anthropometric and biochemical assessment, including computer program for dietary assessment to lecturers, researchers and nutritionists.



Research

Current Reserch Activity

1. Determination of gene expression profiles associated with 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.
2. Detection of proteins and obesity gene mutations in Thai overweight and obese children.
3. Development of health behaviors and nutritional status of the Tsunami victims in Phang-nga Province.
4. MTHFR polymorphism of folate metabolic genes and susceptibility to colorectal in Thai.
5. Energy and caffeine intake of Thai adolescents in Bangkok from beverages.
6. Association between genome-wide methylation level and cellular senescence and aging.

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, Department of Physiology and Pathophysiology, Institute of Nutritional Science, University of Postdam, Germany and Assoc Prof Gary Sweeney, Department of Biology, York University, Toronto, Canada, for training PhD students in research under the Royal Golden Jubilee PhD program.

Local cooperation

The Department has cooperated with several local Institutes:

National Cancer Institute of Thailand
Pramongkutklao College of Medicine
Faculty of Medicine, Khon Kaen University
Faculty of Medicine, Siriraj Hospital
Faculty of Science, Institute of Nutrition, Mahidol University
Faculty of Medicine and Faculty of Allied Health Science,
Chulalongkorn University,
Chulabhorn Research Institute
Provincial Section of Public Health Phang-Nga, Phang-Nga.

Academic Service

Laboratory service on "determination of Vitamin B_1, B_2, B_6 in red blood cell by enzymatic method"

List of publications

1. Leptin, soluble leptin receptor, lipid profiles, and *LEPR* gene polymorphisms in Thai children and adolescents *71*
2. Inhibitory effects of Thai plants beta-glycosides on *Trichomonas vaginalis* *86*
3. Food behavior and folate status of hill-tribe schoolchildren and women of childbearing age on the northern border of Thailand *88*
4. Correlation of circulating full-length visfatin (PBEF/NAMPT) with metabolic parameters in subjects with and without diabetes: a cross-sectional study *93*
5. Allelic loss on chromosome 5q34 is associated with poor prognosis in hepatocellular carcinoma *94*

List of presentations

1. Studies on correlation of serum vitamin B_{12}, serum folic acid, red blood cell folate, plasma homocysteine, and micronucleus frequency in human peripheral blood mononuclear cell in aging and adult Thai people *140*
2. A prognostic value of DNA amplification in L2 repeated sequence in cholangio-carcinoma *141*
3. Detection of genetic instabilities in cholangiocarcinoma by arbitrarily primed polymerase chain reaction *141*
4. Health and nutritional status of Hill tribe school children and childbearing age women, north border area of Thailand *141*
5. Are weeds and common plants safe for used in traditional medicine and animal feed? *141*
6. Fully automated HPLC assay for total homocysteine and other aminothiols: as a measure of plasma redox status in *Thai healthy subjects* *140*
7. Fully automated HPLC assay for total homocysteine and other amino-thiols: as a measure of plasma redox status in *Thai adult subjects* *149*

Department of Tropical Pathology

Introduction

The Department of Tropical Pathology is one of 11 departments in the Faculty of Tropical Medicine, responsible for teaching, research, and services. The Department comprises 3 units: the Histopathology Unit, Electron Microscopy Unit, and Tissue Culture and Immunocytochemistry Unit.

Head of Department
Assoc Prof Emsri Pongponratn
DVM, PhD (Trop Med)

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 2008, 10 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.

Current PhD Students (2008)

Student Name	Advisor	Thesis title
Thanida Tangwanicharoen	Assist Prof Urai Chaisri	Immunohistopathological studies of cytokine profiles in liver tissue of HIV-infected patients with opportunistic infections: a necropsy study
Pachuen Potup	Assoc Prof Yaowapa Maneerat	*Plasmodium falciparum* induce Ig class switching
Kraisorn Sappayatosok	Assist Prof Urai Chaisri	Expression of NOS, VEGF, COX-2 and their clinico-pathological correlation in oral and para-oral squamous cell carcinoma
Navakanit Sachanonta	Assoc Prof Emsi Pongponratn	Effects of antimalarial drugs and other stress factors on *Plasmodium falciparum in vivo* and *in vitro*
Jaiaue Wongtanachai	Assist Prof Urai Chaisri	Effect of antimalarial drugs on *Plasmodium falciparum:* morphology, viability, and multiplication rate
Kasem Somthana	Assoc Prof Yaowapa Maneerat	Immunoglobulin class switch DNA recombination in human B-cell induced by *Gnathostoma spinigerum*
Sumate Aumpawong	Assoc Prof Emsi Pongponratn	Experimental cerebral malaria: pathogenesis of brain edema
Ratchanok Kumsiri	Assoc Prof Yaowapa Maneerat	Blood stage *P. falciparum* induce T cell independent B cell activation
Rungrat Nintasen	Assoc Prof Yaowapa Maneerat	Vascular model for studying atherosclerosis by *ex vivo* vein support system
Chuchard Punsawad	Assoc Prof Parnpen Viriyavajakul	Pathogenesis of malaria

Training Courses

Training courses on tropical pathology, electron microscopy and histopathology are provided periodically upon request.

Research Projects

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

Current 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 infected red blood cells after drug treatment
- Cytokine expression in HIV-positive and AIDS patients
- Proteome of cancerous squamous cells in oral cavity and salivary gland tumor

International Linkages

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

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.

List of Publications

List of Presentations

Department of Tropical Pediatrics

Academic activities

Master of Clinical Tropical Medicine in Tropical Pediatrics (International Programme) [MCTM (Trop Ped)]; 2 students, thematic paper titles:

- Dr Khine Lynn Phyu: a retrospective study on clinical features of early neonatal jaundice in term babies at Ratchaburi Hospital.
- Dr Vannyda Namvongsa: Clinical manifestations of DHF/DSS and the clinical risk factors for DSS in patients in Ratchaburi Hospital, Thailand.
- Diploma in Tropical Medicine & Hygiene Course (DTM&H.)
- Training course: Epidemiology, Management, and Control of Dengue, 21 Jul-1 Aug 2008, 14 trainees

Head of Department
Prof Krisana Pengsaa
MD, Dip Thai Board of Pediatrics, DTM&H

Research

Research activities of the Department comprise mainly research related to dengue infection and dengue and rabies vaccines, including:

- 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 five years
- Evaluation of long term immunity against rabies in children vaccinated with different pre-exposure regimens of PCEC (Rabipur®) and the immunity after two IM injections of PCEC (Rabipur®) post-exposure in the children previously vaccinated with PCEC pre-exposure regimens (I49P6 extension)
- The comparison of immunogenicity and adverse reactions after immunization with Japanese encephalitis vaccine produced by BIKEN and Government Pharmaceutical Organization (GPO) in healthy Thai children (JE0150)
- Dengue antibodies level and incidence of dengue infection in pre-school-aged children
- Efficacy and safety of dengue vaccine in healthy children aged 4 to 11 years in Thailand (CYD23) (in preparation)
- Socio-behavioral focus group study of community knowledge and attitudes related to the dengue fever and an up-coming dengue vaccination in Ratchaburi, Thailand

Cooperation

The department has cooperation with Center for Vaccine Development, Mahidol University and Armed Force Institute of Medical Sciences for dengue diagnosis and Sanofi Pasteur for dengue vaccine research. It also receives support for epidemiological study of dengue infection from the Pediatric Dengue Vaccine Initiative (PDVI). The Department, in coordination with the Tropmed Dengue Diagnosis Center (TDC), is developing a comprehensive laboratory for dengue diagnosis.

List of Publications

1. Scientific consultation on immunological correlates of protection induced by dengue vaccines. Report from a meeting held at the World Health Organization 17-18 November 2005 ..... *96*
2. Age-specific prevalence of dengue antibodies in Bangkok infants and children *96*

List of Presentations

1. Epidemiological study of dengue infection in children, Ratchaburi Province, Thailand *147*

Department of Tropical Radioisotopes

Highlight

The most outstanding routine work of the Department of Tropical Radioisotopes are services serum vitamin B12, serum folate, and red blood cell folate, of folate measurement is the most reliable technique in this region, particularly in Southeast Asia. Research deals with irradiation of gamma ray, x-ray, and ultraviolet to study oxidative stress on macrophage cell line. Research work on vitamin B12 and folate in Thai foods is almost complete and ready for publication.

Acting Head of Department
Jan-Sep 2008
Assist Prof Channarong Sanghirun
DVM, MPH

Acting Head of Department
Oct 2008-present
Assoc Prof Pratap Singhasivanon
MBBS, DTM&H, MPH, Dr PH (Epidemiology)

PhD (Trop Med) Students

Name	Title of Thesis	Co-Advisor	Graduation year
Pyanuch Preechapornkul	Quantitative *ex-vivo* studies of genotypic and phenotypic variation in multidrug resistance gene of *Plasmodium falciparum (PFMD1)*	Assist Prof Channarong Sanghirun	2008
Pachuen Potup	Immunoglobulin class switching induced by *Plasmodium falciparum in vitro*		2008

Research

Ongoing Research Projects

- Autoradiography of mouse liver and kidney after intramuscular injection of C14-labeled arteether.
- Studies on radiation effect on mouse macrophage cell line (RAW 264.7) and radioprotective effect by various Thai medicinal plants.
- Pathological examination of vital organs after artemisinin-derivative treatment of mice.
- Studies on oxidative stress activation by Thai medicinal plants, radiation, and X-rays *in vitro*.
- Studies on toxicity of heme, hemoglobin and hemozoin in mouse macrophage cell line (RAW264.7).

Ongoing Collaborative Research Projects

- Study on correlation of serum vitamin B_{12}, serum folic acid, red blood cell folate, plasma homocysteine, DNA methylation status in aging and young-adult Thais
- Characterization of monoclonal antibodies against *Aeromonas* spp.
- Detection of leptin, adiponectin and insulin in Thai overweight and obese children.

Ongoing University Collaborative Research Projects

- Supplementary hemoglobin in iron-deficient anemic Wistar rats after feeding with freeze-dried freshwater crocodile (*Crocodylus siamensis*)

Services and Support

The Department has two projects for services. (1) measurement of folic acid; and (2) measurement of vitamin B12. Annual service usage is shown below.

1 Jan-31 Dec 2008

	Vitamin B12	Serum folate and red cell folate
	number	number
Woman	-	396
Man	-	329
Child	-	4
Total	-	726

List of Publications

1. Distribution of C14-labelled arteether in kidneys and livers of experimental mice after intramuscular injection 66
2. Food behavior and folate status of hill-tribe schoolchildren and women of childbearing age on the northern border of Thailand 88
3. Low serum vitamin B12 in Alzheimer's patients as detected by a solid phase radioimmunoassay 96

List of Presentations

1. Studies on correlation of serum vitamin B12, serum folic acid, red blood cell folate, plasma homocysteine and micronucleus frequency in human peripheral blood mononuclear cell in aging and adult Thai people 140
2. Health and nutritional status of hill tribe school children and childbearing age women, north border area of Thailand 141
3. IgE class switching in purified human B cells induced by blood stage *P. falciparum* 146
4. Study on serum transcobalamin II in patients with murine typhus. 148
5. Acetylcholinesterase and cholinesterase activities in *Giardia lamblia* trophozoites cultured *in vitro* 148
6. Distribution of C14-labelled arteether in kidneys and livers of experimental mice after intramuscular injection 148

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

Prof Nicholas Day
Director, MORU
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. Our main administrative office and laboratories are embedded within the Faculty, though most of our clinical studies and much of our laboratory work takes place in 'up-country' study sites. Our main research interests are the epidemiology, diagnosis, pathophysiology and treatment of malaria, scrub typhus, melioidosis, leptospirosis and other tropical infections which impose a substantial disease burden on rural populations throughout this populous region. In addition to SMRU in Mae Sod and the Laos Project (described below), we have study sites in Ubon Ratchatani (melioidosis and cryptococcal meningitis), Udon Thani (scrub typhus and leptospirosis), Mae Sod Hospital (malaria and microbiological support for SMRU), Chittagong Medical College in Bangladesh (severe malaria), and Rourkela in India (severe malaria). We also supervise severe malaria clinical trial sites in Burma.

List of publications

Current projects

1.1 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.

During the period 2003-2005, 16,402 participants were enrolled; 13,975 of these underwent a complete vaccination course. Serious Adverse Event (SAE) reports noted 2,873 events including 155 deaths. 467 participants were pregnant during the vaccination phase, and 1644 were pregnant during the follow-up phase. 535 participants withdrew from the study. At the final visit (V 20), 11,334 subjects had completed the study.

1.2 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 of these completed the follow-up visit. At 4-year follow-up, 69 placebo subjects were referred to an extension protocol to receive GARDASIL™ vaccine; of these, 54 did receive GARDASIL™ vaccinations. 50 subjects completed the extension protocol on July 31, 2008; 3 discontinued.

1.3 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.

Between November 2004-March 2005, 246 healthy women who met the eligibility criteria were enrolled in the study. Subjects were randomized at a 1:1 ratio to receive either GARDASIL™ or placebo. The ages of the subjects ranged between 24-45 years at first vaccination. Subjects received vaccine or placebo at Day1, Month2, and Month6. For each subject enrolled, the study duration is 48 months. Three participants discontinued prior to completion of the protocol: one withdrew consent, and 2 were lost to follow-up. A total of 243 subjects remain in the study. It is now Month48 (last visit).

1.4 Establishment of a *Shigella sonnei* Challenge Model for Evaluation of Future Vaccine Candidates .

A total of 48 Thai adults (36 + 12 alternatives) aged 20-40 years were recruited and separated into 3 groups. Each group contained 12 volunteers, to establish a safe and reliable human challenge model for shigellosis. After ingesting sodium bicarbonate buffer, each group of volunteers was challenged with 100, 400, or 1,600 cfu of *S. sonnei* 53G, respectively. Volunteers were followed closely to evaluate clinical symptoms and fecal shedding. On Day 5 post-challenge, unless specific early antibiotic treatment occurs beforehand, subjects started ciprofloxacin, Bactrim, or amoxicillin treatment, to eradicate fecal excretion of *S. sonnei*. They will be released from the ward on Day8-11. All volunteers returned on Day14 ± 2 and Day 28 ± 2 for stool and blood specimen collection. All volunteers were contacted by telephone on Day 42 ±2. Enrollment and follow-up in the 3 groups is now complete. 197 subjects were screened and 36 enrolled in the study. No clinically significant adverse events were detected. Most adverse events were potentially associated with the shigella challenge. No unexpected adverse events, toxicities, or unanticipated problems involving risks to subjects occurred throughout the study. Two subjects had increased total bilirubin, classified as grade IV by CBER guideline, with no other associated signs or symptoms at Day14. Liver enzyme (AST and ALT) levels were within normal limits. Total bilirubin level returned to normal without treatment by Day28. Data entry and analysis are in process.

1.5 A Randomized, International, Double-Blinded (With In-House Blinding), Controlled With GARDASIL™, Dose-Ranging, Tolerability, Immunogenicity, and Efficacy Study of a Multivalent Human Papillomavirus (HPV) L1 Virus-Like Particle (VLP) Vaccine Administered to 16- to 26-Year-Old Women.

A total of 55 Thai girls aged 16-26 years were enrolled from October 2008. Day1, Month2 and Month6 are vaccination visits for safety and immunogenicity. Day 1 is complete, while Month2 and Month3 are ongoing.

Abstracts

Expanding Research Capacity and Accelerating AIDS Vaccine Development in Asia. *Southeast Asian J Trop Med Public Health* 2008; 39(4):766-784

The Follow-up Phase of the Phase III Community Trial: Current Activities (Oral Presentation). AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008.

Phase III Trial of HIV Prime-Boost Vaccine Combination in Thailand: Follow up Phase (Abstract). AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008.

Retention Activities of Clinical Sites to Ensure Compliance among Participants in the Phase III Community Trial in Thailand (Abstract). AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008.

Knowledge and Expectations of Participants in the Phase III HIV Vaccine Trial in Preparation for the Results of the Efficacy Interim Analysis (Abstract). AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008.

Expanding research capacity and accelerating AIDS vaccine development in Asia.

Excler JL, Pitisuttithum P, Rerks-Ngarm S, Shao Y, Zhang L, Tamashiro H, Osmanov S; WHO-UNAIDS Regional Consultation.

According to the Joint UN Program on AIDS (UNAIDS), an estimated 4.9 million adults and children are living with HIV in Asia and the Pacific. Refinement and development of existing and new prevention and treatment technologies--including safe, effective, and accessible AIDS vaccines--are urgent public health priorities. The Asian region faces several challenges for AIDS vaccine development. There are multiple genetic variants of HIV-1 driving the epidemic in the region and too few vaccine candidates in the pipeline targeting those subtypes. Low HIV incidence throughout the region means that trial sites must recruit larger numbers of volunteers and shift their focus to higher-risk populations where incidence is higher. Also, the cultural, economic, and political diversity of the region may render collaboration very complex, but also beneficial at a regional level. Recognizing that collaborating as a region could foster and accelerate AIDS vaccine development, participants at the Sapporo International Consultation recommended that an AIDS Vaccine Asian Network (AVAN) be created to facilitate interactions between donors and funding opportunities, increase regional clinical trial and production capacity, support region-specific advocacy and communication strategies, contribute to the Global HIV Vaccine Enterprise Scientific Plan, prepare a regional approach for future vaccine deployment, and develop a regional platform for clinical trials including harmonized legal, regulatory, and ethical frameworks.

Published in: *Southeast Asian J Trop Med Public Health* 2008; 39(4):766-784.

The Follow-up Phase of the Phase III Community Trial: Current Activities

P Pitisuttithum[1], S Rerks-Ngarm[2], J Kaewkungwal[1], S Nitayaphan[3], M Benenson[3], R Paris[3], N Michael[4], J Kim[3,4], C Khamboonruang[2] and P Kunasol[2] for MOPH-TAVEG

[1]Faculty of Tropical Medicine, Mahidol University; [2]Department of Disease Control, Ministry of Public Health; [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [4]Walter Reed Army Institute of Research, Rockville, MD, USA

Background: 16,402 volunteers were enrolled in the phase III community-based trial at the end of 2005. 13,798 (84%) completed all vaccinations. The follow-up period is three years after the last vaccination, with the acquisition endpoint of 50% reduction in relative risk of infection, and virological endpoint. Various activities to ensure the understanding of the participants, to monitor participants' impact events, and to achieve a high follow-up rate are critical for the trial's success.

Methods: Various activities have been put in place. Those activities are at the clinical sites, outside the clinical sites, community engagement and volunteer relation activities to strengthen the understanding of the participants of the importance of retention. Tracking activities, establishing mobile teams and continued monitoring of impact events, have also been implemented. In-depth interviews were conducted to evaluate knowledge gained.

Results: 6,446 volunteers have completed 3 years' follow-up, and 95% retention was achieved at each subsequent visit. The trial is expected to complete by July 2009. During the 18 months (Jun06-Dec07), 35,513 reminder letters were sent out, with 71,583 reminder calls before appointments or calls after missed appointments. 45% could not be contacted by phone, so home visits were made. 2,580 home visits and 2,116 mobile clinical activities were performed. Before the interim analysis of efficacy, in-depth discussions with 134 participants from various demographic backgrounds were conducted. 72% and 22% of respondents said an efficacious vaccine could prevent HIV infection and delay disease progression, respectively. 47% said that the knowledge gained will lead to further vaccine development, despite non-efficacy. Participation Impact Events reported (as of 4 May 2007) were 1.7/100 person-year, 93% reported "minimal impact" and most were resolved. Conclusion: Extensive clinical site activities, tracking, and mobile clinical activities were conducted to achieve a high rate of follow-up with low participation impact events (PIE).

This work was presented at AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008 (p.64)

Phase III Trial of HIV Prime-Boost Vaccine Combination in Thailand: Follow up Phase

S Rerks-Ngarm[1], P Pitisuttithum[2], S Nitayaphan[3], J Kim[3], J Kaewkungwal[2], S Gurunathan[4], M Gurwith[5], N Michael[6], C Khamboonruang[7], P Thongcharoen[7], and P Kunasol[7]

[1]Prime-Boost HIV Vaccine Phase III Trial, Muang Nonthaburi, Nonthaburi, Thailand; [2]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [3]Armed Forces Research of Medical Science (AFRIMS), Bangkok, Thailand; [4]Sanofi Pasteur, Swiftwater, PA, USA; [5]VaxGen Inc, Brisbane, CA, USA; [6]Walter Reed Army Institute of Research, Rockville, MD, USA; [7]Dept. of Disease Control, MOPH, Muang Nonthaburi, Nonthaburi, Thailand

Background: After 10 years of continuing efforts to identify vaccine candidates appropriate to the HIV subtype circulating in Thailand, the prime-boost HIV vaccine combinations passed the "go-no-go" criteria for conducting phase III efficacy trials. The low HIV annual incidence learned from cohort study together with the nature of phase three trial resulted in high number of volunteers and long study duration.

Methods: The world's first efficacy trial of a prime-boost HIV vaccine combination was started in September 2003 to determine if the prime-boost vaccine strategy 1) prevents infection, 2) reduction of set-point viral load in vaccinees who become infected, and 3) is safe. Vaccines were developed specifically for the predominant circulating HIV subtypes in Thailand (CRF01-AE and B) using a recombinant ALVAC-HIV (vCP1521) (Aventis Pasteur) priming and AIDSVAX®gp120 B/E (VaxGen) boosting. This trial is designed for randomized, double-blind, placebo-controlled, community trial.

Results: 16,402 HIV negative Thai adults, aged 18-30 were recruited through the existing Thai MOPH facilities. The study period for each individual is scheduled for 6 months of vaccination period with 3 years follow up. The vaccination was completed in July 2006. In July 2007, the DSMB convened the meeting for an interim efficacy, safety, and futility analysis and recommended the trial be continued. Most of the volunteers are now completed their 18 months follow-up with interval retention rates exceeds 95%. To date, the vaccine has been safe. No death related to vaccination was recorded. Negative social events from volunteers' participation were very few.

Conclusion: The trial required longer time than expected which needs timeliness and more flexible strategies for conducting the trial. Close monitoring and community engagement are essential strategies for the follow-up phase to retain the volunteers. Community engagement is planned for effective communication strategies for the trial result after completion of the follow-up phase in mid-2009.

This work was presented at AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008 (p.176)

Retention Activities of Clinical Sites to Ensure Compliance Among Participants in the Phase III Community Trial in Thailand

Pitisuttithum P[1], Rerks-Ngarm S[2], Bussaratid V[1], Dhitavat J[1], Maekanantawat W[1], Pungpak S[1], Suntharasamai P[1], Vanijanonta S[1], Benenson M[3], and Kim J[3].

[1]Faculty of Tropical Medicine, Mahidol University, [2]Department of Disease Control, Ministry of Public Health, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

Background: 16,402 volunteers were enrolled in the phase III community-based trial by the end of 2005. 13,798 (84%) completed all vaccinations. The follow-up period is three years after the last vaccination so compliance is critical for trial success. The clinical teams implemented various activities in order to ensure compliance and achieve a 95% retention rate at each subsequent visit.

Methods: The retention activities started in early 2005. This data collection was done from July 2006 to December 2007. Apart from extended clinic hours to include one evening per week and Sunday. **Activities at the clinical sites** included provider based care, reminder letters, reminder phone calls, 24 hour service by phone, educational campaigns including interactive games while waiting at the clinical sites and cultural activities all year round. **Activities outside the clinical sites** included tracking activities and mobile clinic activities. These activities were done independently or together with the local health staff.

Results: **Activities at the clinical sites**- 35,513 reminder letters were

sent out, 71,583 reminder calls before appointments or calls after missed appointments. 45% could not be contacted by phone so home visits were made. Of those contacted by phone 69% came to the clinics, 30% postponed visits. Providing mobile phone numbers and 24 hour service resulted in 5,646 incoming calls from volunteers; the majority wanting to postpone appointments. Edutainment was done with poster presentations and games with questions and answers. More than 85% responded correctly. For **outside clinic activities**, 2,580 home visits and 2,116, mobile clinical activities were performed. To date, a retention rate of 95% has been achieved.
Conclusion: Extensive clinical site activities, tracking and mobile clinical activities are crucial for the success of the trial during the follow-up period.

This work was presented at AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008 (p.173)

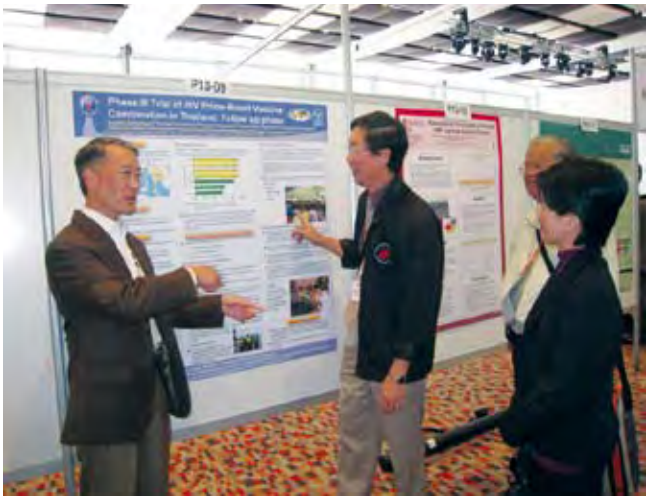
Knowledge and Expectation of Participants in the Phase III HIV Vaccine Trial in Preparation for the Results of the Efficacy Interim Analysis

Liangthorachon C[1], Khowsroy K [1], Boondao R[1], Lothong K[1], Sabmee Y[1], Rerks-Ngarm S[2], Kim J[3], and Pitisuttithum P[1]
[1]Faculty of Tropical Medicine, Mahidol University; [2]Department of Disease Control, Ministry of Public Health; [3]HIV Military Research Program, USA.

Background: The interim analysis for efficacy of the phase III community trial was July 2007. It was critical to assess the knowledge and expectations of the potential results among the participants and community to prepare messages to avoid misconceptions and disappointment.
Methods: In depth discussions with 134 participants from various demographic backgrounds were conducted. Eight standard questions were raised for discussion. These included expectations from the study; what has been gained from participating in the study; the understanding of the word "efficacy"; opinions of the impact on sexual behavior if vaccine is efficacious; the expectations of an efficacious vaccine; the understanding for the word "no efficacy"; the expected impact if the vaccine has no efficacy; and opinions about possible future vaccine trials.
Results: 72% and 22% of respondents said an efficacious vaccine could prevent HIV infection and delay disease progression, respectively. 52% thought if the vaccine was efficacious, there would be an impact by increasing risky behavior (77%). 21% expected that their family would get the vaccine if efficacious. 56% said non-efficacy does not prevent HIV infection. 47% said that the knowledge gained will lead to further vaccine development despite non efficacy. 20% expressed disappointment if the vaccine was not efficacious.70% said that there will be no negative impact to the community if the vaccine has no efficacy. 85% said they would recommend that others participate in future vaccine trials.
Conclusion: There was generally correct knowledge and positive expectations towards trial results. There was a concern about increasing risky behavior if an efficacious vaccine is available. However, defining an efficacious vaccine in terms of delay of disease progression (viral load effect) needs to be strengthened.

This work was presented at AIDS VACCINE 2008, Cape Town, South Africa, October 13-16, 2008 (p.174)

ACTIVITIES



AIDS VACCINE Conference 2008, Cape Town, South Africa

Growing Mangrove Forest, July 11-12, 2008
Kung Krabaen Bay, Chanthaburi Province

Introduction

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.

Deputy Dean for Student Affairs and Special Activities
Acting Deputy Dean for Educational Affairs, 1 Jan-30 Sep 2008
Assist Prof Chotechuang Panasoponkul
BSc, MSc, DAP&E, PhD, Mini-MBA

Deputy Dean for Education
1 Oct 2008-
Assoc Prof Waranya Wongwit
BSc (Biology), MS (Biological Sciences), PhD (Biochemistry)

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 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 fellows 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 2008, there were 206 students in this program.

In summary, in 2008, the Faculty had a total of 409 students; among these, 282 were new enrollments, and the majority fee-paying.

Master of Clinical Tropical Medicine

Student	Advisor	Department	Thematic Paper
Sai Kaung Thar	Wattana Leowattana	Clinical Tropical Medicine	Dynamicity of platelets in patients with *P. falciparum* malaria
Zin Zin Win Ko Ko	Punnee Pitisuttithum	Clinical Tropical Medicine	Outcome of tuberculosis in HIV patient treated with and without ante-retroviral therapy
Win Myint Tun	Sasithon Pukrittayakamee	Clinical Tropical Medicine	The contributing factors of hypertension in HIV-infected patients after receiving antiretroviral therapy (ART) in Chonburi Hospital
Nan Nitra Than	Punnee Pitisuttithum	Clinical Tropical Medicine	Comparison of long-term outcomes between HIV-infected patients with and without HCV co-infection
Hnin Aye Hlaing	Polrat Wilairatana	Clinical Tropical Medicine	Hyperparasitemia in patients with severe *P. falciparum* malaria
Aung Naing Soe	Wirach Maek-a-nantawat	Clinical Tropical Medicine	Long-term outcomes of HIV patients who initiated treatment with NNRTI based ART regimen in year 2004 at Bamrasnaradura Infectious Disease Institute
Saw Ba Oo Gyi	Yupaporn Wattanagoon	Clinical Tropical Medicine	Assessing agreement between routine malaria parasite report and actual parasite count among hospitalized patients at Umphang Hospital, Tak Province, Thailand
Ko Ko Yazar Kyaw Win	Vipa Thanachartwet	Clinical Tropical Medicine	Acute renal failure in severe falciparum malaria patients at Mae Sot General Hospital, Thailand; incidence and risk factors
Khin Myat Wai	Polrat Wilairatana	Clinical Tropical Medicine	Fatal indicators in severe malaria patients
Chen Lei	Udomsak Silachamroon	Clinical Tropical Medicine	Ventilator-associated pneumonia in Intensive Care Unit, Queen Savang Vadhana Memorial Hospital
Dr Rainer Brandl	Punnee Pitisuttithum	Clinical Tropical Medicine	Evaluation of stable sexual relations with characterization of sero-discordant couples in a cohort of HIV patients under highly active antiretroviral therapy
Emanuel Luttersdorfer	Watcharapong Piyaphanee	Clinical Tropical Medicine	Seroprevalence of dengue antibodies among foreign travelers in South East Asia
Tharawit Ouppapong	Supat Chamnanchanunt	Clinical Tropical Medicine	Anemia in adult patients with severe falciparum malaria: outcome and associated factors
Nongnush Marin	Jittima Dhitavat	Clinical Tropical Medicine	Contact tracing outcome in male gonorrhea patients
Nuttapon Ekarakrungreung	Weerapong Phumratanaprapin	Clinical Tropical Medicine	Clinical manifestations and outcome of Russell's viper bite at Somdejprayuparaj Srakaew Hospital, Srakaew Province, Thailand
Chowalit Khumjui	Valai Bussaratid	Clinical Tropical Medicine	Eosinophilia among clients visiting the Hospital for Tropical Diseases in 2008

Master of Clinical Tropical Medicine (Tropical Pediatrics)

Student	Advisor	Department	Thematic Paper
Khine Lynn Phyu (SEAMEO TROPMED)	Kriengsak Limkittikul	Tropical Pediatrics	A retrospective study on clinical features of early neonatal jaundice in term babies at Ratchaburi Hospital
Vannyda Namvongsa (SEAMEO TROPMED)	Chukiat Sirivichayakul	Tropical Pediatrics	Clinical manifestations of DHF/DSS and the clinical risk factors for DSS in patients in Ratchaburi Hospital, Thailand

Master of Science in Tropical Medicine Theses 2008

Department	Student	Thesis Title	Advisor
Microbiology and Immunology	Supaporn Sukkasame	Molecular patterns of recurrent tuberculosis in Thailand	Srisin Khusmith
Clinical Tropical Medicine	Jayathunge Parana Hewage Mangalasiri	Abnormal pap smears and psychosocial burden of abnormal pap smears on HIV-infected women in Chonburi, Thailand	Punnee Pitisuthithum
Social and Environmental Medicine	Chandrasiri Palie Pitiye Arambe Anuruddha	Epidemiological surveillance and burden of diarrheal disease in Klong Nueng of Tambon Klong Luang, Pathumthani Province, Thailand	Voranuch Wangsuphachart
Helminthology	Youthanavanh Vonghachack	*Gnathostoma* and gnathostomiasis in three provinces of Lao PDR	Jitra Waikagul
Microbiology and Immunology	Pham Anh Duc	Characterization and genotyping of hepatitis C virus on core and NS5B regions in Vietnamese blood donors	Pornsavan Leaungwutivong

MSc 3rd year students

Department	Student	Thesis Title	Advisor
Microbiology and Immunology	Piyanart Chalayon	Expression of leptospiral recombinant protein for serodiagnosis	Thareerat Kalambaheti
Medical Entomology	Phubeth Ya-umphan	Seasonal dengue serotypes trans-ovarial transmission and the effects on morphology of *Aedes aegypti* in nature	Narumon Komalamisra
Clinical Tropical Medicine	Chatnapa Duangdee	Surrogative severity marker of *Plasmodium falciparum* infected patients	Polrat Wilairatana
Clinical Tropical Medicine	Sasikrit Theppabutr	Homology modeling of *Plasmodium malariae* and *Plasmodium ovale* DHFRs and development of nested PCR technique to identify *Plasmodium ovale* and *Plasmodium knowlesi* base on *dhfr-ts* linker region	Mallika Imwong
Microbiology and Immunology	Nopporn Tohmee	Production of recombinant *Bacillus anthracis* lethal factor for antibody detection in vaccinated cattle	Pongrama Ramasoota
Tropical Hygiene	Wit Wichaidit	Maternal and child care in low resource setting A case study in Suan Phung district, Ratchaburi Province	Jaranit Kaewkungwal
Microbiology and Immunology	Phakapun Singchai	Evaluation of a simple restriction fragment length polymorphism assay and neutralization for subtyping of coxsackie B viruses	Pornsavan Leaungwutivong
Microbiology and Immunology	Kaewjai Malithao	Validation of electronic air filter for filtrating *Mycobacterium tuberculosis* and virus from contaminated air	Pongrama Ramasoota
Helminthology	Wallop Pakdee	Application of iso-electric focusing *Bithynia* snail antigen for ELISA IgG-subclass detection of human opisthorchiasis	Paron Dekumyoy
Microbiology and Immunology	Thanaporn Nibaddhasobon	Molecular diversity of *Listeria monocytogenes* isolates from raw pork and chicken in various markets	Nitaya Indrawattana
Social and Environmental Medicine	Chalermkwan Kuntawee	Social and behavioral factors related to quality of life among HIV infected children	Wijitr Fungladda
Helminthology	Khemphavanh Manivong	Epidemiology of *Opisthorchis viverrini* metaceracariae in cyprinoid fish in three rivers at Khammouane Province, Lao PDR	Chalit Komalamisara

MSc 4th year students

Department	Student	Thesis Title	Advisor
Protozoology	Sirilak Sukprasert	Detection of human *Entamoeba* spp. from surface and waste water samples by real-time PCR using hybridization probes	Porntip Petmitr
Social and Environmental Medicine	Jaturong Wongsanit	Risk assessment of nitrate contamination in groundwater using geoinformatics	Suwalee Worakhunpiset
Clinical Tropical Medicine	Supparat Wannasilp	Study of solubility and efficacy of antimalarial drugs on *Plasmodium falciparum*	Kesinee Chotivanich
Social and Environmental Medicine	Sirisobha Boonyotha	A survey on vector-borne and water-borne disease in Klong Aae hydropower dam construction project, Surat Thani Province, Thailand	Prapin Thanpoophasiam
Tropical Hygiene	Robert Hutagalung	Dynamics of *Plasmodium* gametocytes in a rural community on the western border of Thailand	Jaranit Kaewkungwal

MSc 5th year students

Department	Student	Thesis Title	Advisor
Microbiology and Immunology	Pornapat Surasombatpattana	Application of recombinant dengue viral protein for serodiagnosis of dengue virus infection	Wipawee Jampangern
Tropical Hygiene	Dilok Tongsukh	TB infection and treatment among refugees in Thamhin Camp, Ratchaburi Province, Thailand	Jaranit Kaewkungwal
Microbiology and Immunology	Khwananong Youngpakool	Binding characterizations of Southeast Asian dengue 2 virus and American dengue 2 viruses with single E gene amino acid substitutions	Wipawee Jampangern

PhD (Trop Med) 2nd year students

Department	Student	Thesis Title	Advisor
Social and Environmental Medicine	Sumonmal Uttayamakul	Gene polymorphism and plasma drug level in HIV/TB patients with antiretroviral therapy	Srisin Khusmith
Helminthology	Megumi Sato	Application of the ribosomal DNA based PCR to diagnose *Opisthorchis viverrini* infection in human fecal sample	Jitra Waikagul

PhD (Trop Med) 3rd year students

Department	Student	Thesis Title	Advisor
Microbiology and Immunology	Wantana Paveenkittiporn	Genotypic diversity and the ability to invade host cell among environmental *Legionella* isolates in Thailand	Thareerat Kalambaheti
Tropical Nutrition & Food Science	Darunee Utennam	Transforming growth factor-beta 1 and osteocalcin polymorphism genes in relation to bone mineral density in post-menopause Thai women	Rungsunn Tungtrongchitr
Tropical Pathology	Rungrat Nintasen	Vascular model for studying atherosclerosis by *ex vivo* vein support system	Yaowapa Maneerat
Tropical Hygiene	Archin Songthap	Assessment and intervention tools facilitating acceptability of HPV vaccine for adolescent vaccination	Punnee Pitisuttithum
Tropical Hygiene	Putri Chairani Eyanoer	Spatial and temporal distribution of human avian influenza infection in relation to surveillance implementation in Indonesia	Jaranit Kaewkungwal
Social and Environmental Medicine	MD. Mokshed Ali	Factors influencing contraceptive use and plan for number of children among couples in Bangladesh	Wijitr Fungladda

PhD (Trop Med) 4th year students

Department	Student	Thesis Title	Advisor
Tropical Hygiene	Kwanjai Viputtigul	Polymorphism at and merozoite surface protein-1 (MSP-1) C-terminus isolated form western border of Thailand	Srivicha Krudsood
Protozoology	Panadda Kraijojananan	Molecular and functional characterization of C-type lectin in the midgets of *Anopheles dirus* for malaria transmission	Porntip Petmitr
Helminthology	Apichat Vitta	Analysis for the expressed protein from *Angiostrongylus cantonensis* DNA library for immunodiagnosis of angiostrongyliasis	Paron Dekumyoy
Medical Entomology	Namtip Trongnipatt	Analysis of transcriptome and proteome of *Plasmodium vivax* sporozoite in *Anopheles dirus* mosquito	Chamnarn Apiwathnasorn
Tropical Clinical	Preeyaporn Monatrakul	Immunological and pharmacological determinants of the therapeutic response to antimalarial drugs *in vitro*	Kesinee Chotivanich
Tropical Pathology	Ratchanok Kumsiri	Blood stage *P. falciparum* antigen induce T cell independent B cell activation	Yaowapa Maneerat
Protozoology	Kamlang Chumpolbanchorn	Genetic characterization of *Toxoplasma gondii* isolates from free-range chickens in Thailand	Yaowalark Sukthana
Microbiology and Immunology	Nada Pitabutr	Role of NK cells, NKT cells and T cell subpopulations through granule exocytosis in response to *M. tuberculosis* infection	Srisin Khusmith
Protozoology	Rachatawan Chiabchalard	Molecular epidemiological study on *Giardia duodenalis* contamination in Thai vegetables	Yaowalark Sukthana
Protozoology	Magha Raj Banjara	Molecular epidemiology of drug resistant *Plasmodium falciparum* in Nepal	Porntip Petmitr

PhD (Trop Med) 5th year students

Department	Student	Thesis Title	Advisor
Protozoology	Kritsana Janyapoon	Expression profiles of *Plasmodium falciparum* in response to pyronaridine by DNA microarray analysis	Porntip Petmitr
Tropical Nutrition & Food Science	Chuthaporn Tongboonchoo	Homocysteine and vitamin status in relation to gene polymorphisms in post-menopausal Thai women	Rungsunn Tungtrongchitr
Protozoology	Ruangrat Buddhirongawatr	*Toxoplasma gondii* genotyping in domestic and wild felids in Thailand	Yaowalark Sukthana
Social and Environmental Medicine	Pannamthip Pitaksajjakul	Construction of phage antibody library specific to H5 N1 avian influenza virus	Pongrama Ramasoota
Medical Entomology	Suchada Sumroiphon	*Anopheles sundaicus* population dynamics, species complex and insecticide susceptibility in a coastal area of Rayong Province, Thailand	Chamnarn Apiwathnasorn
Medical Entomology	Parichat Lapcharoen	The use of gene silencing to study the effects of lysozyme on *Plasmodium* in malaria vector *Anopheles dirus*	Chamnarn Apiwathnasorn
Tropical Pathology	Kasem Somthana	Immunoglobulin class switch DNA recombination in human B cell induced by *Gnathostoma spinigerum*	Yaowapa Maneerat
Social and Environmental Medicine	Patthanasak Khammaneeechan	Social impact assessment of central healthcare waste incinerator project in Yala Province	Kamolnetr Okanurak
Social and Environmental Medicine	Somsiri Decharat	Heavy metals exposure of nielloware workers: a health risk assessment in Muang District, Nakhon Si Thammarat Province	Prapin Thanpoopasiam
Clinical Tropical Medicine	Tippawan Sungkapong	Identification of antigenic proteins on the surface of *P. vivax* infected erythrocytes	Kesinee Chotivanich
Tropical Hygiene	Rie Takeuchi	Factors related to reappearance of *Plasmodium vivax* malaria	Pratap Singhasivanon
Clinical Tropical Medicine	Sriwipa Chuangchaiya	*Plasmodium vivax* alters immunosuppression caused by *Plasmodium falciparum*	Kesinee Chotivanich

PhD (Trop Med) 6th year students

Department	Student	Thesis Title	Advisor
Medical Entomology	Raweewan Srisawat	The effect of gene mutations to the voltage gated sodium channel in resistant pyrethroid *Aedes aegypti*	Narumon Komalamisra
Protozoology	Aongart Mahittikorn	Molecular and immunohistochemistry studies on tachyzoite and bradyzoite stage conversion in toxoplasmosis	Yaowalark Sukthana
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	Pratap Singhasivanon
Tropical Hygiene	Jongkol Podang	Cost-effectiveness of program promote motorcycle helmet use to prevent head to prevent head injury burden in Thailand 2004	Pratap Singhasivanon
Tropical Hygiene	Tawee Saiwichai	The effect of green tea extract to serum level of pro- inflammatory cytokines in cigarette smoke inhalation rat model	Pratap Singhasivanon

PhD (Trop Med) 7th year students

Department	Student	Thesis Title	Advisor
Medical Entomology	Jakkrawarn Chompoosri	Transformation of *Aedes aegypti*, vector of dengue hemorrhagic fever, using serine-catalyzed integrases and tyrosine-catalyzed site-specific integrases	Narumon Komalamisra
Helminthology	Muncharee Tattiyapong	Serodiagnosis of swine trichinellosis using crude somatic excretory-secretory, surface and purified antigens of *Trichinella spiralis* infective larvae by indirect ELISA and immunoblot	Paron Dekumyoy
Protozoology	Saranya Siribal	Molecular characterization of *Plasmodium falciparum* polynucleotide kinase	Porntip Petmitr

PhD (Trop Med) 8th year students

Department	Student	Thesis Title	Advisor
Medical Entomology	Pruksa Nawtaisong	Analysis of ribozyme strategies for suppression of dengue virus in transgenic *Aedes albopictus*	Chamnarn Apiwathnasorn

PhD (Trop Med) 9th year students

Department	Student	Thesis Title	Advisor
Microbiology and Immunology	Onguma Natalang	Exploring the transcriptome of *Plasmodium falciparum* exposed to artesunate	Jintana Patarapotikul
Microbiology and Immunology	Rongdej Tungtrakanpoung	Mimotope identification from monoclonal antibodies specific to serovar of *Leptospira*, using phage-displayed random peptide library	Pongrama Ramasoota

New student enrolments 2008

Course	No. of Students	Name	Nationalities
DTM&H	35	Emanuel Luttersdorfer	Austria
		Maria Parizek	Austria
		Rainer Brandl	Austria
		Shawny Susan John	Australia
		Dipanjali Aich	Bangladesh
		Chen Lei	PR China
		Jani Tapani Anttota	Finland
		Masaki Nakanishi	Japan
		Kazuhiro Omura	Japan
		Hiroko Tsuchiya	Japan
		Yoshimi Osaka	Japan (Attend)
		Antonio Dematteis	Italy
		Khin Myat Wai	Myanmar
		Hnin Aye Hlaing	Myanmar
		Nan Nitra Than	Myanmar
		Zin Zin Win Ko Ko	Myanmar
		Win Thu Hein	Myanmar
		Kyaw Zaw Hein	Myanmar
		Ye Htun Naing	Myanmar
		Sai Kaung Thar	Myanmar
		Kyan Aung	Myanmar
		Zaw Tun Aung	Myanmar
		Ko Ko Yazar Kyan Win	Myanmar
		Saw Ba Oo Gyi	Myanmar
		Win Myint Tun	Myanmar
		Ma Thet Khaing	Myanmar
		Aung Naing Soe	Myanmar
		Vannyda Namvongsa	Lao PDR
		Keobouphaphone	Lao PDR
		Nattapon Ekarakrungeung	Thailand
		Nongnush Marin	Thailand
		Tharawit Ouppapong	Thailand
		Chowalit Khumjui	Thailand
		James Coey	Ireland
		Deborah Lardner	USA

Course	No. of Students	Name	Nationality
MCTM	16	Emanuel Luttersdorfer	Austria
		Rainer Brandl	Austria
		Chen Lei	PR China
		Khin Myat Wai	Myanmar
		Hnin Aye Hlaing	Myanmar
		Nan Nitra Than	Myanmar
		Zin Zin Win Ko Ko	Myanmar
		Sai Kaung Thar	Myanmar
		Ko Ko Yazar Kyan Win	Myanmar
		Saw Ba Oo Gyi	Myanmar
		Win Myint Tun	Myanmar
		Aung Naing Soe	Myanmar
		Nattapon Ekarakrungeung	Thailand
		Nongnush Marin	Thailand
		Tharawit Ouppapong	Thailand
		Chowalit Khumjui	Thailand
MCTM (Tropical Pediatrics)	2	Vannyda Namvongsa	Lao PDR
		Khine Lynn Phyu	Myanmar
MSc (TM)	9	Siriwat Akapirat	Thailand
		Nattaka Chumsang	Thailand
		Apinya Pumpuang	Thailand
		Surachet Benjathummarak	Thailand
		Ussra Ratananikom	Thailand
		Don Buddhinda Rubasinghe	Sri Lanka
		Myo Nyein Aung	Myanmar
		Kinley Wangdi	Bhutan
		Nima Wangdi	Bhutan
PhD (TM)	14	Suda Tunpiboonsak	Thailand
		Ittisak Subrungruange	Thailand
		Uruse Thaenkham	Thailand
		Sirithorn Tangbunsuk	Thailand
		Pikun Thepsuriyanont	Thailand
		Manas Kotepui	Thailand
		Saichon Chimsumang	Thailand
		Kanokkarn Pothong	Thailand
		Duangjai Duangrithi	Thailand
		Saiyud Moolphate	Thailand
		Nyan Win Myint	Myanmar
		Salim Akhter Chowdhury	Bangladesh
		Nan Chen	PR China
		Md. Shamsuzzaman	Bangladesh

Introduction

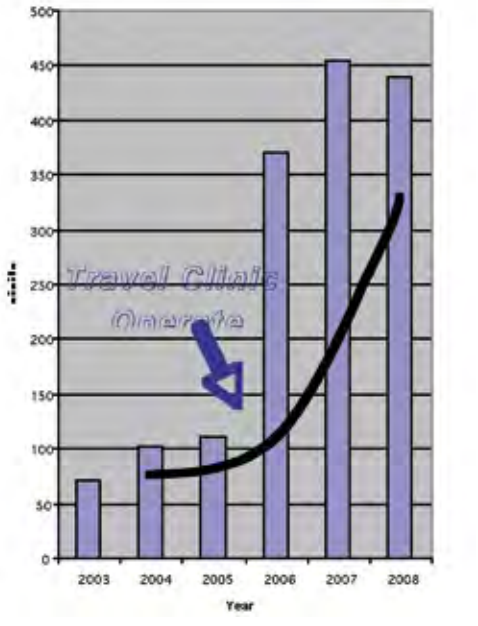
The Hospital for Tropical Diseases, founded in 1961, fulfils one of the key functions of the Faculty of Tropical Medicine, by providing expert healthcare service in tropical medicine to the Thai population and foreign patients. Nowadays, the Hospital has 250 beds, 31 medical doctors, 83 nurses, and 81 nurse assistants. Last year, it served 49,899 out-patients and 2,483 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.

Hospital Director
Assist Prof Udomsak Silachamroon
MD (Hons), Grad Dip Clin Sci (Med), MSc, Dip Thai Board of Internal Medicine, FCCP

Organization of the Hospital for Tropical Diseases

1. Hospital Administration Office
2. Nurse Administration Office
3. Diagnostic Laboratory
   - Microscopy
   - Clinical Chemistry
   - Hematology
4. Radiology
5. Pharmacy
6. Medical Records and Statistics
7. Laundry
8. Supply
9. Nutrition
10. Special Service
    - Thai Traditional Medicine
    - Traditional Chinese Medicine
    - Travel Clinic
    - Laser

Number of Travelers attending the Hospital for Tropical Diseases



In the year 2008, the number of patients treated in the Hospital for Tropical Diseases, classified by disease, were as follows;

	Disease	Out-patients	In-patients
1.	Falciparum malaria	339	371
2.	Vivax malaria	222	104
3.	Mixed falciparum and vivax malaria	7	7
4.	Malariae malaria	2	2
5.	Unidentified infections	223	20
6.	Scrub typhus	6	1
7.	Typhoid fever	1	28
8.	Diarrhea	248	6
9.	Food poisoning	29	12
10.	Hepatitis	956	307
11.	Dengue hemorrhagic fever	263	-
12.	Taeniasis	37	-
13.	Hookworm	2	-
14.	Ascariasis	3	-
15.	Strongyloidiasis	5	-
16.	Gnathostomiasis	411	-
17.	Opisthorchiasis viverrini	4	-
18.	Filariasis	2	1
19.	Dermatitis	3,999	13
20.	Tuberculosis (pulmonary)	225	19
21.	HIV infections	104	4
22.	Hypertension	3,767	32
23.	Diabetes mellitus	2,275	44
24.	Hyperlipidemia	2,323	1
25.	Diseases of oral cavity, salivary gland, and jaw	334	230
26.	Others	34,112	1,279
	Total	**49,899**	**2,483**

Travel Clinic

The Travel Clinic has operated since 2005. It is a specialized clinic that can provide comprehensive care in travel medicine. Services include pre- and post-travel counseling, vaccination and immunization, malaria prevention and prophylaxis, treatment of travel-related diseases. Moreover, the clinic also plays major roles as a training and research base of the Travel Medicine Research Unit.

Services

- The Travel Clinic operates from Mon to Fri, 8.30am to 4.30pm. Last year more than 450 travelers attended our clinic.
- In 2008, the website of the clinic (www.thaitravelclinic.com) had more than 5,500 unique sessions with more than 50,000 page views.
- Collaborate with the Thai Red Cross Society and Ministry of Public Health to prepare medical supplies to help Myanmar people after the Cyclone Nagis disaster.

Research

- “Rabies exposure among backpackers in Southeast Asia” in Khao San Road area, Bangkok
- “Seroprevalence of Dengue Ab among foreign travelers”
- Collaboration with Geosentinel Surveillance Network

Education

- Our staff have been invited to teach in the DTM&H and MCTM programs
- Opened new elective subject “Practices in Travel Medicine” in DTM&H course
- Composed leaflet “Frequently Asked Questions about Travelers’ Diarrhea”
- Welcome doctors, nurses, medical students, and other healthcare workers to the Travel Clinic

Department of Clinical Tropical Medicine

No.	Research Title	Grant	Principal Investigator
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 *in vitro* immune response parameters to candidate HIV-1 vaccine antigens among subject from Thailand	Merck & 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 & 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 Government of Thailand Ministry of Public Health	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 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
8 ***	Assessing the psychosocial burden in women with An abnormal pap results after screening interventions of MRKAd5HIV-1 gag vaccine in healthy adults	Merck & Co., Inc	Prof. Punnee Pitisuttithum
9 *	Measurement of anogenital wart burden, and cost of illnesses in Bangkok	Merck Research Foundation	Prof. Punnee Pitisuttithum
10 *	A phase 2b/3, randomised, double blind, dose confirming study of the safety, efficacy and tolerability of apricitabine versus lamivudine in treatment-experienced HIV-1 infected patients with the M184V mutation in reverse transcriptase	Avexa Limited, Australia	Prof. Punnee Pitisuttithum
11 *	Measuring herpes zoster and post-herpetic neuralgia associated burden of illness and health care utilization and costs in Thailand	MSD (Thailand)	Prof. Punnee Pitisuttithum
12 *	A bioequivalence study of formulations of 16 mg Candesartan tablets in healthy Thai volunteers	Medica Innova Co., Ltd.	Prof. Punnee Pitisuttithum
13 ***	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 *Plasmodium falciparum* malaria	MMV (Medicine for Malaria Venture) and Sigma Tao	Prof. Sasithon Phukrittayakamee
14 *	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 Phukrittayakamee
15 *	A randomized, open-label, parallel group, single-dose, drug-drug interaction study of the safety and pharmacokinetics of oral doses of amantadine hydrochloride, oseltamivir phosphate, and ribavirin alone and in combination in healthy volunteers		Prof. Sasithon Phukrittayakamee
16 *	A randomized, open-label, cross-over, pilot drug-drug interaction study of the safety and pharmacokinetics of oral doses of amantadine hydrochloride, oseltamivir phosphate, and ribavirin alone and in combination in healthy male and female volunteers under fasted conditions	Adamas Pharmaceuticals, Inc	Prof. Sasithon Phukrittayakamee

* New Project
* * Completed Project
* * * Ongoing Project

Department of Clinical Tropical Medicine

No.	Research Title	Grant	Principal Investigator
17 ***	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
18 *	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
19 ***	Study to evaluate a treatment guideline of twisting tourniquet technique with chronic lymphedema		Dr. Wichai Ekataksin
20 ***	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
21 ***	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
22 ***	Pharmacologic study of oseltamivir in healthy volunteers	National Institutes of Health, USA	Assoc. Prof. Yupaporn Wattanagoon
23 ***	Cell-derived microparticles in malaria infection	Wellcome Trust of Great Britain	Assist. Prof. Kesinee Chotivanich
24 ***	Effect of primaquine and its metabolite on the infectivity of *P. falciparum* gametocyte	Wellcome Trust of Great Britain	Assist. Prof. Kesinee Chotivanich
25 *	Effect of serum lipid concentration on the efficacy of lumefantrine against falciparum malaria *in vitro*	The Thailand Research Fund	Assist. Prof. Kesinee Chotivanich
26 ***	Gnathostomiasis and correlation of skin test with *Gnathostoma spinigerum* fractionated specific antigen and IgE	Faculty of Tropical Medicine, Mahidol University	Assist. Prof. Wirach Maek-a-nantawat
27 *	*In vivo* bioequivalence study of 5 mg levocertirizine tablet preparations in healthy Thai male volunteers		Assist. Prof. Wirach Maek-a- nantawat
28 ***	Bioequivalence study of 4 mg perindopril tablet preparations in healthy Thai male volunteers	International Bio Service Co., Ltd	Assist. Prof. Weerapong Phumratanaprapin
29 ***	Evaluation of genetic susceptibility to melioidosis	The US National Institute of Health	Dr. Wirongrong Chierakul
30 ***	Abnormalities of coagulation and the immune response in diabetic patients with melioidosis	MORU, the Faculty of Tropical Medicine and Amsterdum Medical Centre, Netherlands	Dr. Wirongrong Chierakul
31 ***	Gametocyte clearance in uncomplicated and severe *Plasmodium falciparum* malaria after artesunate-mefloquine treatment in Thailand	Department of Clinical Tropical Medicine	Mr. Noppadon Tangpukdee
32 ***	White blood cell counts in uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* malaria patients		Mr. Noppadon Tangpukdee
33 *	*In Vivo* bioequivalence study of 160 mg fenofibrate film-coated tablet preparation in healthy Thai male volunteers	International Bio Service Co., Ltd	Asst. Prof. Weerapong Phumratanaprapin
34 *	Rabies exposure risk among foreign backpackers from non-ASEAN countries traveling in Southeast Asia		Dr. Watcharapong Piyaphanee
35	Bioequivalence study of 10 mg fenodipine sustained release tablet formulations in healthy Thai male volunteers	International Bio Service Co., Ltd	Dr. Watcharapong Piyaphanee
36 **	Bioequivalence study of genetic filgrastim injection to an innovator Neuprogen® (filgrasm 300 mgc/0.5 ml syringe) in healthy Thai volunteer	International Bio Service Co., Ltd	Assoc. Prof. Wattana Laewwattana
37 *	Comparision of anti-platelet aggregation activity of a generic clopidogrel tablets to the innovator Plavix® in healthy Thai volunteers	International Bio Service Co., Ltd	Assoc. Prof. Wattana Laewwattana
38 *	A phase IV,multi-center, randomized, double blind, placebo controlled study to evaluate the safety of daily inhaled zanamivir 10 mg versus placebo and daily oral oseltamivir 75 mg versus placebo for influenza prophylaxis in healthy volunteers for 16 weeks	SEA Influenza Clinical Research Network	Assoc. Prof. Wattana Laewwattana

Department of Clinical Tropical Medicine

No.	Research Title	Grant	Principal Investigator
39 *	Bioequivalence study of 15 mg pioglitazone tablet preparations in healthy Thai volunteers	International Bio Service Co., Ltd	Assoc. Prof. Wattana Leowattana
40 *	Bioequivalence study of generic clopidogrel tablets to innovator Plavix® (Clopidogrel 75 mg) in healthy Thai male volunteers	International Bio Service Co., Ltd	Assoc. Prof. Wattana Leowattana
41 *	Bioequivalence Study of 70 mg Alendronate Sodium Tablet Preparations in Healthy Thai Volunteers	International Bio Service Co., Ltd	Assoc. Prof. Wattana Leowattana
42 *	Bioequivalence Study of 160 mg Valsartan Tablet Preparations in Healthy Thai Volunteers	International Bio Service Co., Ltd	Assoc. Prof. Wattana Leowattana
43 *	Bioequivalence Study of 50 mg Cilostazol Tablets Preparations in Healthy Thai male Volunteers	International Bio Service Co., Ltd	Assoc. Prof. Wattana Leowattana
44 *	VNTR-based PCR (VNTR Typing for *Plasmodium falciparum* and *Plasmodium vivax*)	Biotech	Assist. Prof. Mallika Imwong
45 ***	Molecular characterization of drug resistance in the human malarias	Intermediate level fellowship, Welcome Trust of Great Britain	Assist. Prof. Mallika Imwong
46 ***	Development of molecular technique for evaluation of mefloquine resistance in *Plasmodium falciparum* in Asia	Commission on Higher Education and The Thailand Research Fund	Assist. Prof. Mallika Imwong
47 *	Pathology of mouse vital organs after injection with artemisinin derivatives	Mahidol-Oxford Tropical Medicine Research Unit	Assist. Prof. Apichart Nontprasert
48 *	Activation of heme oxygenase-1 in human brain endothelial cells *in vitro*	Mahidol-Oxford Tropical Medicine Research Unit	Assist. Prof. Apichart Nontprasert
49 *	A multicenter, randomized, double-blind, double-dummy, phase 3 study of the safety and efficacy of ritonavir-boosted elvitegravir (EVG/r) versus raltegravir (RAL) each administered with a background regimen in HIV-1 infected, antiretroviral treatment-experienced adults	Gilead Sciences	Assist. Prof. Wirach Maek-a- nantawat
50 ***	Early access of MK-0518 in combination with an optimized background antiretroviral therapy (OBT) in highly treatment experienced HIV-1 infected patients with limited to no treatment options	Merck and Co., Inc	Assist. Prof. Wirach Maek-a- nantawat
51 *	Travel information among HIV infected individuals		Assist. Prof. Wirach Maek-a- nantawat

Department of Helminthology

1 ***	Research and development of an application to purify *Bithynia* snail antigen in serodiagnosis of opisthorchiasis	Government Budget	Assoc. Prof. Jitra Waikagul
2 ***	Study on *Paragonimus* population: morphology, molecular biology, enzymology and epidemiology aspects	Ministry of Foreign Affairs	Assoc. Prof. Jitra Waikagul
3 ***	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
4 *	Genetic variation and population structure studies of fish-borne trematodes for increasing control impact of opisthorchiasis and cholangiocarcinoma	The Thailand Research Fund	Assoc. Prof. Jitra Waikagul
5 *	Epidemiological study of *Helicobacter pyroli* and parasitic infection in healthy people	Osaka University, Japan	Assoc. Prof. Chalit Komalamisra
6 *	Intestinal parasites isolated from house-flies in the tourist attraction areas in Thailand	Faculty of Tropical Medicine, Mahidol University	Assoc. Prof. Wanna Maipanich
7 **	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, Mahidol University	Dr. Teera Kusolsuk
8 *	Effects of trichinosis on lipid peroxidation in rat	Faculty of Tropical Medicine, Mahidol University	Mrs. Supaporn Nuamtanong
9 *	The study on life cycle and antigenicity of *Paragonimus pseudoheterotremus* waikagul, 2007	Faculty of Tropical Medicine, Mahidol University	Mrs. Tippayarat Yoonuan

Department of Entomology

No.	Research Title	Grant	Principal Investigator
1 ***	Dynamic and temporal structure of the troglobitic fauna of medically important insects and arthropods in caves of Kanchanaburi Province	Faculty of Tropical Medicine, Mahidol University	Assoc. Prof. Chamnarn Apiwathnasorn
2 *	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 of northern, southern and northeastern Thailand	Government Budget	Assoc. Prof. Chamnarn Apiwathnasorn
3 *	Population dynamics of dengue vectors and dengue virus infection in *Aedes aegypti* and *Aedes albopictus*, in urban and suburban areas	Government Budget	Assoc. Prof. Narumon Komalamisra
4 *	Dengue virus detection in dark and pale forms of *Aedes aegypti* collected as immature stages from breeding sites	Government Budget	Assoc. Prof. Supatra Thongrungkiat
5 **	Efficacy of water dispersible granule formulation of *Bacillus thuringiensis israelensis* (VECTOBAC® WDG) and *Bacillus sphaericus* (VECTOLEX® WDG) to control *Anopheles sundaicus* and *Culex sitiens* in brackish water ponds in Tsunami-affected areas of Phang-Nga Province, Southern Thailand	Faculty of Tropical Medicine, Mahidol University	Dr. Yuwadee Trongtokit
6 **	Field application of *Bacillus thuringiensis israelensis* (VECTOBAC® WDG) on control of mosquito larvae in salt-marsh habitats in Tsunami-affected areas,Phang Nga Province, Thailand: monitoring by reduction in adult density	Brescia University, Italy	Dr. Yuwadee Trongtokit
7 ***	Molecular identification of *Anopheles sundaicus* In natural breeding sources, the coastal area of Andaman and the Gulf of Thailand	Faculty of Tropical Medicine, Mahidol University	Dr. Jiraporn Ruangsittichai
8 ***	Survey and study on geographical distribution of *Aedes albopictus* in Bangkok Metropolitan	Faculty of Tropical Medicine, Mahidol University	Mr. Yudthana Samung
9 *	Comparative study knockdown resistance gene (kdr) in vector of dengue haemorrhagic fever to permethin	Faculty of Tropical Medicine, Mahidol University	Mrs. Raweewan Srisawat
10 *	Feeding behavior, ecological studies, and molecular identification of *Anopheles dirus* complex in man-habitat	Faculty of Tropical Medicine, Mahidol University	Mr. Sungsit Sungwornyothin

Department of Microbiology and Immunology

1 ***	Polymophism of Pf EMP1 extracellular domain in Thai isolates causes severe and uncomplicated *P. falciparum* malaria	The Thailand Research Fund	Prof. Srisin Khusmith
2 ***	Chemokine Th1 cytokines and gene polymorphisms in relation to malaria severity	The Thailand Research Fund and Commission on Higher Education	Prof. Srisin Khusmith
3 ***	Role of NK cells, NKT cells and T cell subpopulations through granule exocytosis in response to *M. tuberculosis* infection	The Thailand Research Fund	Prof. Srisin Khusmith
4 *	Clinical and immunological study on difficulty treatment TB in Chiang Rai, Thailand	Japan Health Science Fundation	Prof. Srisin Khusmith
5 *	Genetic polymorphisms in HIV-1 infected patients receiving antiretroviral therapy	Mahidol University and Ministry of Public Health	Prof. Srisin Khusmith
6 **	Serodiagnosis of scrub typhus and meliodosis by dual immunoperoxidase test	Faculty of Tropical Medicine, Mahidol University	Assoc. Prof. Varee Wongchotigul
7 *	Development of a monoclonal antibody test for identification of pathogenic *Aeromonas* spp.	Government Budget	Assist. Prof. Yuwadee Mahakunkijcharoen
8 **	Identification of genus- and species-specific antigens of *Aeromonas* spp. by Western blotting with mouse polyclonal antibodies	Faculty of Tropical Medicine, Mahidol University	Assist. Prof. Yuwadee Mahakunkijcharoen
9 **	Development of strategies for serovar identification of *Leptospira* sp. Based on specific gene sequences within rfb locus	Thailand-Tropical Diseases Research	Assist. Prof. Tareerat Kalambaheti

Department of Microbiology and Immunology

No.	Research Title	Grant	Principal Investigator
10 **	Production of recombinant leptospiral protein antigen for serodiagnosis	Faculty of Tropical Medicine, Mahidol University	Assist. Prof. Tareerat Kalambaheti
11 **	Influence factors program the development of CD4+regulatory T cells in autoimmune mouse model	Commission on Higher Education and The Thailand Research Fund	Dr. Pornsawan Leaungwutiwong
12 **	Preparation of human monoclonal antibody that neutralizes tetanus toxin using phage display technology	Commission on Higher Education and The Thailand Research Fund	Dr. Nitaya Indrawattana
13 **	Detection of specific antibodies to recombinant dengue virus DEIII/NS1 dengue viral protein using indirect enzyme immunoassay	Faculty of Tropical Medicine, Mahidol University	Miss Akanitt Jittmittraphap
14 ***	Culturing of *Brucella* 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

Department of Protozoology

1 ***	*Toxoplasma gondii* genotyping in domestic and wild felids in Thailand	Commission on Higher Education	Assoc. Prof. Yaowalark Sukthana
2 **	Research and development of biological contamination-free frozen food to world kitchen	National Research Council of Thailand	Assoc. Prof. Yaowalark Sukthana
3 **	Molecular study on toxoplasmosis and neosporidiosis in Thai dairy cows	The Thailand Research Fund	Assoc. Prof. Yaowalark Sukthana
4 **	Molecular and immunohistochemistry studies on stage-specific tachyzoite and bradyzoite of *Toxoplasma gondii*	The Thailand Research Fund	Assoc. Prof. Yaowalark Sukthana
5 *	Genetic characterization of *Toxoplasma gondii* in Thai free-range chickens	The Thailand Research Fund	Assoc. Prof. Yaowalark Sukthana
6 *	Molecular characterization of DNA polymerase õ of *Plasmodium falciparum* and its role in DNA replication and DNA repair	Biotec	Assoc. Prof. Porntip Petmitr
7 *	Molecular characterization of *Plasmodium falciparum* polynucleotide kinase	The Thailand Research Fund	Assoc. Prof. Porntip Petmitr
8 *	Molecular characterization of *Plasmodium falciparum* polynucleotide kinase and its role on DNA repair of malaria parasites	Government Budget	Assoc. Prof. Porntip Petmitr
9 ***	PCR assays for detection of *Toxoplasma gondii* in Thai commercial meat products	Mahidol University	Ms. Rachatawan Chiabchalard
10 ***	Detection of *Trichomonas vaginalis* infection in Thai Women by PCR and culture methods	Faculty of Tropical Medicine, Mahidol University	Ms. Kanthinich Thima
11 *	Comparation of the detection of *Toxoplasma gondii* antibody by Sabin-Feldman Dye Test and Indirect Fluorescent Antibody Test (IFAT) using *T. gondii* from cell culture and animal inoculation	Faculty of Tropical Medicine, Mahidol University	Ms. Ruenruetai Udonsom
12 **	Detection of protozoal viability; contamination of water used in Thai frozen food industry by cell culture PCR technique	Faculty of Tropical Medicine, Mahidol University	Ms. Chantira Suthikornchai

Department of Social and Environmental Medicine

1 ***	Modifying mosquito population age structure to eliminate dengue transmission	Gates Foundation	Assoc. Prof. Piyarat Butraporn
2 ***	Modifying mosquitoes age structure to eliminate dengue haemorrhagic fever: community participation in dengue haemorrhagic fever		Assoc. Prof. Piyarat Butraporn
3 *	Technology transfer on the combination of effective microorganisms (EM) and chitosan for control of environmental and disease vector problems using participatory action research	Government Budget	Assist. Prof. Pongrama Ramasoota
4 **	Construction of monoclonal antibody phage library for developing avian influenza virus diagnostic test	The Thailand Research Fund, Commission on Higher Education and Faculty of Tropical Medicine, Mahidol University	Assist. Prof. Pongrama Ramasoota

Department of Social and Environmental Medicine

No.	Research Title	Grant	Principal Investigator
5 **	Enhancement of diagnostic efficacy for controlling Foot and mouth disease in pig and cattle	National Research Council of Thailand	Assist. Prof. Pongrama Ramasoota
6 **	Factors associating with completion and default among DOTS and self-administered therapy (SAT) for treatment of TB		Assoc. Prof. Kamolnetr Okanurak
7 *	Evaluation of the performance of migrant health worker and migrant health volunteer	Ministry of Public Health	Assoc. Prof. Kamolnetr Okanurak
8 *	Effect of Global fund on the treatment system for HIV/AIDS	Ministry of Public Health	Assoc. Prof. Kamolnetr Okanurak
9 **	Development of a suveillance system for obesity among school children of high risk groups	WHO	Assist. Prof. Voranuch Wangsuphachart
10 **	Trans-boundary risk assessment (human health risk, ecological and environmental risk of the Mekong River-surface water)	Mekong River Comission Secretariat:MRCS	Assist. Prof. Voranuch Wangsuphachart
11 **	Integrated research network on Tachin- Maeklong Basin	Mahidol University	Assist. Prof. Wanchai Phatihattakorn
12 *	Assessment of urinary cotinine as exposure biomonitoring to active and secondhand smoking and biomarkers of cardiovascular disease risk	The Thailand Research Fund and Commission on Higher Education	Dr. Prapin Tharnpoophasiam
13 *	A surveillance of snail intermediate host of Schistosomiasis: *Schistosoma mekongi* (*Neotricula aperta* ß-race) using the geographic information system in the area of Mun river, Ubon-Ratchatani Province	Faculty of Tropical Medicine, Mahidol University	Mr. Peerapol Chusongsaeng

Department of Tropical Hygiene

1 ***	Avian flu surveillance network in displased populations on the Thai Burma Border	Center for Disease Control and Prevention	Assoc. Prof. Pratap Singhasivanon
2 ***	A phase III trial of Aventis Pasteur live recombinant ALVAC-HIV (vCP 1521) priming with VaxGen gp120 B/E (AIDSVAXTM B/E) Boosting in HIV-uninfected Thai-Adults (Data Management)	The Henry M. Jackson Foundation for Military Medicine, Inc.	Assist. Prof. Jaranit Kaewkungwal
3 ***	A phase III trial of Aventis Pasteur live recombinant Thai adults	Walter Reed Army Institute of Research	Assist. Prof. Jaranit Kaewkungwal
4 ***	A phase II, randomized, open label, multicentre study to assess the antimalarial efficacy and safety of arterolane (RBx11160) maleate and piperaquine phosphate coadministration and Coartem in patients with acute uncomplicated *Plasmodium falciparum* malaria	Ranbaxy Laboratories Ltd., India	Assoc. Prof. Srivicha Krudsood
5 *	Evaluation of Fosmidomycin, when administered concurrently to adult subjects with acute uncomplicated *Plasmodium* malaria	Jomaa Pharma GmbH, Hamburg, Germany	Assoc. Prof. Srivicha Krudsood
6 ***	Serotype specific of dengue viruses affected to the endothelial integrity trigger through the protese enzyme over-production by infected dendritic cells	The Thailand Research Fund	Dr. Natthanej Luplerdlop
7 ***	Proteomics characterization of *Aedes aegypti*	Bourse Scholarship, IRD, France (Researcher exchange Thai-Montpellier)	Dr. Natthanej Luplerdlop
8 ***	Spatial-temporal distribution and determinants of malaria along the Thai-Myanmar border	University of Maryland, Baltimore and Mahidol University	Dr. Saranath Lawpoolsri
9 ***	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
10 ***	Field based study of reappearance *Plasmodium vivax* malaria cases in the area near Thai- Myanmar border Suanphung Ratchaburi Province	Faculty of Tropical Medicine, Mahidol University	Mr. Wanchai Maneeboonyoung
11 *	Study of specific T-cell immune response against dengue viral infection		Ms. Pannamas Maneekan

Department of Tropical Nutrition and Food Science

No.	Research Title	Grant	Principal Investigator
1 *	Determination of gene 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
2 **	Identification of gene alterations in breast cancer	Biotec	Assoc. Prof. Songsak Petmitr
3 *	Detection of proteins and obesity gene mutations in Thai overweight and obese children	Government Budget	Assoc. Prof. Rungsunn Tungtrongchitr
4 ***	Development of health behaviors and nutritional status of the Tsunami victims in Phang-nga Province	Brescia University, Italy	Assist. Prof. Karunee Kwanbunjan
5 *	MTHFR polymophism of folate metabolic genes and susceptibility to colorectal cancer in Thai	Government Budget	Assist. Prof. Karunee Kwanbunjan
6 *	Energy and caffeine intake of Thai adolescents in Bangkok from beverages	Mahidol University International College	Assist. Prof. Karunee Kwanbunjan
7 **	Purification and characterization of ß-glucosidose, specifically hydrolyzing iridoid glucoside from Thai plants	The Thailand Research Fund and Commission on Higher Education	Dr. Dumrongkiet Arthan
8 **	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, Mahidol University	Dr. Pornrutsami Jintaridth
9 *	Association between genome-wide methylation level and cellular senescence and aging	The Thailand Research Fund	Dr. Pornrutsami Jintaridth
10 **	Study of methylenetetrahydrofolate reductase (MTHFR) and cystathionnine ß-synthase (CBS) gene polymorphisms in relation to homocysteinemia: cardiovascular risk factors in Thai smoker subjects	Faculty of Tropical Medicine, Mahidol University	Ms. Apanchanid Thepouyporn

Department of Tropical Pathology

1 ***	A murine model of cerebral malaria, histopathology, immunohistochemistry and electron microscopy	Thailand Center of Excellence, for Life Sciences (TCELS)	Assoc. Prof. Emsri Pongponratn
2 **	The effects of antimalarial drugs induced changes on *Plasmodium falciparum* and Pf-infected red blood cells Ultrastructure	The Thailand Research Fund	Assoc. Prof. Emsri Pongponratn
3 **	Pathogenesis of acute renal failure in severe malaria: ultrastructural and immunoapathological	The Thailand Research Fund	Assoc. Prof. Emsri Pongponratn
4 ***	Immunohistopathological studies of cytokine profiles in liver tissue of AIDS with opportunistic infections and HIV-infected patients: a necropsy study	The Thailand Research Fund	Assoc. Prof. Parnpen Viriyavejakul
5 ***	Proteome of cancerous squamous cells in oral cavity and salivary gland tumor	Faculty of Tropical Medicine, Mahidol University	Assist. Prof. Urai Chaisri
6 **	*Plasmodium falciparum* induce IgE class switching	Swedish International Development Corporation A. (SIDA)	Assoc. Prof. Yaowapa Maneerat
7 *	Blood stage *P. falciparum* antigens induce T cell	SEAMEO NETWORK	Assoc. Prof. Yaowapa Maneerat
8 **	Time consuming and penetration efficacy of dengue virus type 2 (D2-16681) pass through the peritrophic membrane to midgut epithelial cell in *Aedes aegypti* mosquitoes	Faculty of Tropical Medicine, Mahidol University	Ms. Supattra Suwanmanee

Department of Tropical Pediatrics

No.	Research Title	Grant Principal	investigator
1 ***	Epidemiological study of dengue infection in children, Ratchaburi Province, Thailand	Pediatric Dengue Vaccine Initiative (PDVI)	Prof. Arunee Sabchareon
2 **	A phase II, pilot, randomized, open-label, single-center study to evaluate immunogenicity and safety after PCECV rabies vaccine (Rabipur®) administered concomitantly with Japanesse encephalitis vaccine as a pre-exposure regimen in 12 to 18 month old toddlers in Thailand	Chiron Vaccines Co., Ltd., Italy	Prof. Arunee Sabchareon
3 *	A controlled study of the safety and immunogenicity of ChimericVax™ Japanese encephalitis vaccine in Thai toddlers and children	Sanofi Pasteur Co., Ltd.	Prof. Arunee Sabchareon
4 **	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	Department of Tropical Pediatrics	Assoc. Prof. Pornthep Chanthavanich
5 **	Development of surveillance system for under five mortality in Samut Sakhon Province, Thailand	WHO	Assoc. Prof. Chukiat Sirivichayakul
6 ***	Evaluation of long-term immunity against Japanese encephalitis in children vaccinated with Japanese encephalitis vaccine	Department of Tropical Pediatrics	Assoc. Prof. Pornthep Chanthavanich
7 ***	Accuracy assessment of using WHO criteria in diagnosis of dengue infection	Department of Tropical Pediatrics	Assoc. Prof. Pornthep Chanthavanich
8 ***	Epidemiological study of dengue infection in children age 3-10 years in Ratchaburi Province	Ministry of Public Health	Assoc. Prof. Pornthep Chanthavanich
9 ***	Favirab™ post prescription event monitoring	Sanofi Pasteur Co., Ltd.	Assoc. Prof. Pornthep Chanthavanich
10 ***	The comparison of immunogenicity and adverse reactions after immunization with Japanese encephalitis vaccine produced by BIKEN and Government Pharmaceutical Organization (GPO) in healthy Thai children (JE0150)	Government Pharmaceutical Organization	Assoc. Prof Pornthep Chanthavanich
11 ***	Evaluation of long term immunity against rabies in children vaccinated with different pre-esposure regimens of PCEC (Rabipur®) and the exposure in the children previously vaccinated with PCEC pre-exposure regimens (I49P6)	Department of Tropical Pediatrics	Assist. Prof. Kriengsak Limkittikul
12 *	Socio-behavioral focus group study of community knowledge and attitudes related to dengue fever and up-coming dengue vaccination in Ratchaburi, Thailand.	Pediatric Dengue Vaccine Initiative (PDVI), International Vaccine Institute, Seoul, Korea	Dr. Alfred Pach, Dr. Linda Kaljee, Assoc. Prof. Chukiat Sirivichayakul

Department of Tropical Radioisotopes

1 ***	Studies on vitamin B12 and folic acid contents in foods	Department of Tropical Radioisotopes	Assist. Prof. Channarong Sanghirun
2 *	Studies on oxidative stress activation by various Thai medicinal plants, radiation, and X- ray *in vitro*	Government Budget	Ms. Cheeraratana Cheeramakara
3 **	Studies on radiation effect on mouse macrophage cell line (RAW 264.7) and radioprotective effect by various Thai medicinal plants	Faculty of Tropical Medicine, Mahidol University	Ms. Cheeraratana Cheeramakara
4 ***	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

Vaccine Trial Centre

1 **	A phase III trial to determine the efficacy of AIDSVAX™ B/E vaccine in intravenous drug users in Bangkok, Thailand	AIDSVAX	Prof. Punnee Pitisuttithum
2 ***	Establishment of a *Shigella sonnei* challenge model for evaluation of future vaccine candidates	US Army Medical Research and Meterial Command and the National Institue of Allergy and Infectious Diseases (NIAID)	Prof. Punnee Pitisuttithum
3 *	A randomized, international, double-blinded (with in-house blinding), controlled with GARDASIL™,dose-ranging, tolerability, immunogenicity, and efficacy study of a multivalent human papillomavirus (HPV) L1 virus-like particle (VLP) vaccine administered to 16 to 26 year old women	Merck & Co., Inc	Prof. Punnee Pitisuttithum
4 ***	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 (Prof. Punnee Pitisuttithum)

Research Reports 2008

Faculty of Tropical Medicine, Mahidol University

ABSTRACTS OF PUBLICATIONS

THE TRANSCRIPTOME OF *PLASMODIUM VIVAX* REVEALS DIVERGENCE AND DIVERSITY OF TRANSCRIPTIONAL REGULATION IN MALARIA PARASITES

Bozdech Z, Mok S, Hu G, Imwong M, Jaidee A, Russell B, Ginsburg H, Nosten F, Day NP, White NJ, Carlton JM, Preiser PR

Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Bangkok, Thailand
School of Biological Sciences, Nanyang Technological University, Singapore 637551

Plasmodium vivax causes over 100 million clinical infections each year. Primarily because of the lack of a suitable culture system, our understanding of the biology of this parasite lags significantly behind that of the more deadly species *P. falciparum*. Here, we present the complete transcriptional profile throughout the 48-h intraerythrocytic cycle of three distinct *P. vivax* isolates. This approach identifies strain specific patterns of expression for subsets of genes predicted to encode proteins associated with virulence and host pathogen interactions. Comparison to *P. falciparum* revealed significant differences in the expression of genes involved in crucial cellular functions that underpin the biological differences between the two parasite species. These data provide insights into the biology of *P. vivax* and constitute an important resource for the development of therapeutic approaches.

Published in: *Proc Natl Acad Sci U S A* 2008 Oct 21;105(42):16290-5.

DISTRIBUTION OF C^{14}-LABELLED ARTEETHER IN KIDNEYS AND LIVERS OF EXPERIMENTAL MICE AFTER INTRAMUSCULAR INJECTION

Cheeratana Cheeramakara[1], Vasant Khachonsaksumet[2], Nopachai Suthisat[1], Wanyarat Nakosiri[1], Kriyaporn Songmuaeng[1], Channarong Saenghirun[1], Duangduen Phienpicharn[1], and Apichart Nontprasert[3]

[1]Department of Tropical Radioisotopes, [2]Department of Tropical Pathology, [3]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

To study the distribution and localization of oil-soluble arteether in experimental mice, we injected C^{14}-labelled arteether (20μCi/kg body weight) intramuscularly and measured radioactivity in the blood, kidney, and liver. The labeled arteether distributed and localized more to the kidney (819, 180.4 ± 34,134 dpm/cm^3) than the liver (288,628.9 ± 54,954 dpm/cm^3) 4 hours post-injection. The main localization of labeled arteether was in the kidney cortex rather than the medulla ($p<0.05$). However, the distribution of radioactivity was homogeneous in the liver. The terminal half-life of labeled arteether in the blood was 1.8 hours. The blood : kidney : liver ratio was 1 : 5 : 2. these findings show that labeled arteether was distributed quickly and localized in the cytoplasmic cortex of the kidney and homogeneously in the liver.

Published in: *Southeast Asian J Trop Med Public Health* 2008;39(2):195-9.
Presented at: The 17th International Congress of Tropical Medicine and Malaria on September 29 – 3 October 2008

CONSENSUS GUIDELINES FOR DOSING OF AMOXICILLIN-CLAVULANATE IN MELIOIDOSIS

Cheng AC, Chierakul W, Chaowagul W, Chetchotisakd P, Limmathurotsakul D, Dance DA, Peacock SJ, Currie BJ

Department of Clinical Tropical Medicine, Faculty of Tropical Medicine Mahidol University, Bangkok, Thailand
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Melioidosis is an infectious disease endemic to northern Australia and Southeast Asia. In response to clinical confusion regarding the appropriate dose of amoxicillin-clavulanate, we have developed guidelines for the appropriate dosing of this second-line agent. For eradication therapy for melioidosis, we recommend 20/5 mg/kg orally, three times daily.

Published in: *Am J Trop Med Hyg* 2008 Feb;78(2):208-9.

ACTIVATION OF THE COAGULATION CASCADE IN PATIENTS WITH LEPTOSPIROSIS

Chierakul W, Tientadakul P, Suputtamongkol Y, Wuthiekanun V, Phimda K, Limpaiboon R, Opartkiattikul N, White NJ, Peacock SJ, Day NP

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BACKGROUND: Disseminated intravascular coagulation (DIC) is common among patients with sepsis. Leptospirosis is an important cause of sepsis in tropical areas, and pulmonary hemorrhage associated with thrombocytopenia is the major cause of death, but the coagulopathy in severe leptospirosis has not been further characterized. The aim of this study was to evaluate coagulation factors and the presence of DIC in patients with leptospirosis in northeast Thailand.

METHODS: We measured plasma concentrations of fibrinogen, D-dimer, thrombin-antithrombin III complexes, and prothrombin fragment 1,2 and evaluated the DIC score in 79 patients with culture-confirmed and/or serologically confirmed leptospirosis and in 33 healthy Thai control subjects.

RESULTS: The median concentrations of fibrinogen, D-dimer, thrombin-antithrombin III complexes, and prothrombin fragment 1,2 were significantly elevated in a cohort of 79 patients with leptospirosis, compared with healthy control subjects ($P<$or$=.001$ for all tests). Patients with leptospirosis had significantly longer prothrombin times, longer activated partial thromboplastin times, and lower platelet counts. Thrombocytopenia was present in 38% of case patients and occurred more frequently among patients with culture-negative leptospirosis; in multivariate analysis, it was the only hemostasis factor independently associated with clinical bleeding. Patients who were culture-negative for Leptospira species had higher Acute Physiology and Chronic Health Evaluation II and Sepsis-Related Organ Failure Assessment scores and more bleeding complications. Nearly one-half of patients with leptospirosis had overt DIC as defined by an International Society on Thrombosis and Hemostasis DIC score.

CONCLUSIONS: Activation of the coagulation system is an important feature of leptospirosis. Thrombocytopenia is an indicator of severe disease and risk of bleeding.

Published in: *Clin Infect Dis* 2008 Jan 15;46(2):254-60.

TREATMENT-SEEKING BEHAVIORS AND IMPROVEMENT IN ADHERENCE TO TREATMENT REGIMEN OF TUBERCULOSIS PATIENTS USING INTENSIVE TRIAD-MODEL PROGRAM, THAILAND

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The aims of this study were to determine tuberculosis patients' treatment-seeking behaviors, to describe knowledge of TB among TB patients, how that knowledge affected their treatment-seeking behaviors, and to develop an appropriate model to strengthen the existing DOTS program. A cross-sectional study was conducted in all government TB clinics in Samut Prakan Province, Thailand during November 2005-May 2006. The triad model, which emphasizes the role of a triad of persons (the healthcare provider, the TB patient, and his/her treatment supporter), can improve patient adherence to TB treatment regimen. The results showed that only about a quarter (27.7%) of TB patients chose the hospitals with TB clinic for first treatment, while others chose alternative healthcare modes, including self-care and purchasing drugs from drugstores. The rate of successful treatment was higher for the experimental group (96.0%) than the control group (84.9%) ($p = 0.057$). The confirmed cure rate was also significantly higher in the experimental group (95.3% vs 78.9%, $p = 0.02$). The program could be utilized to strengthen the existing DOTS program.

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HEALTH RISKS IN TRAVELERS TO CHINA: THE GEOSENTINEL EXPERIENCE AND IMPLICATIONS FOR THE 2008 BEIJING OLYMPICS

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Selected data collected for travelers to China from 1998 through November 2007 by the GeoSentinel Surveillance Network were used to provide an evidence base for prioritizing recommendations for Olympic and other future travelers to China. Respiratory illness and injuries were common among patients seen during their travel; acute diarrhea and dog bites were common among those seen after travel. Tropical and parasitic diseases were rare. Pre-travel consultation for China travelers should be individualized according to these findings.

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EXPANDING RESEARCH CAPACITY AND ACCELERATING AIDS VACCINE DEVELOPMENT IN ASIA

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According to the Joint UN Program on AIDS (UNAIDS), an estimated 4.9 million adults and children are living with HIV in Asia and the Pacific. Refinement and development of existing and new prevention and treatment technologies--including safe, effective, and accessible AIDS vaccines--are urgent public health priorities. The Asian region faces several challenges for AIDS vaccine development. There are multiple genetic variants of HIV-1 driving the epidemic in the region and too few vaccine candidates in the pipeline targeting those subtypes. Low HIV incidence throughout the region means that trial sites must recruit larger numbers of volunteers and shift their focus to higher-risk populations where incidence is higher. Also, the cultural, economic, and political diversity of the region may render collaboration very complex, but also beneficial at a regional level. Recognizing that collaborating as a region could foster and accelerate AIDS vaccine development, participants at the Sapporo International Consultation recommended that an AIDS Vaccine Asian Network (AVAN) be created to facilitate interactions between donors and funding opportunities, increase regional clinical trial and production capacity, support region-specific advocacy and communication strategies, contribute to the Global HIV Vaccine Enterprise Scientific Plan, prepare a regional approach for future vaccine deployment, and develop a regional platform for clinical trials including harmonized legal, regulatory, and ethical frameworks.

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PLASMODIUM VAVAX RESISTANCE TO CHLOROQUINE IN DAWEI, SOUTHERN MYNMAR

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Objective: To assess the efficacy of chloroquine in the treatment of *Plasmodium vivax* malaria in in Dawei District, southern Myanmar.

Methods: Enrolled patients at Sonsinphya clinic >6 months of age were assessed clinically and parasitologically every week for 28 days. To differentiate new infections from recrudescence, we genotyped pre- and post-treatment parasitaemia. Blood chloroquine was measured to confirm resistant strains.

Results: Between December 2002 and April 2003, 2661 patients were screened, of whom 252 were included and 235 analysed. Thirty-four per cent (95% CI: 28.1–40.6) of patients had recurrent parasitaemia and were considered treatment failures. 59.4% of these recurrences were with a different parasite strain. Two (0.8%) patients with recurrences on day 14 had chloroquine concentrations above the threshold of 100 ng/ml and were considered infected with chloroquine resistant parasites. 21% of failures occurred during the first 3 weeks of follow-up: early recurrence and median levels of blood chloroquine comparable to those of controls suggested *P. vivax* resistance.

Conclusions: *Plasmodium vivax* resistance to chloroquine seems to be emerging in Dawei, near the Thai-Burmese border. While chloroquine remains the first-line drug for *P. vivax* infections in this area of Myanmar, regular monitoring is needed to detect further development of parasite resistance

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GENE AMPLIFICATION OF THE MULTIDRUG RESISTANCE 1 GENE OF *PLASMODIUM VIVAX* ISOLATES FROM THAILAND, LAOS, AND MYANMAR

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Plasmodium vivax mdr1 gene amplification, quantified by real-time PCR, was significantly more common on the western Thailand border (6 of 66 samples), where mefloquine pressure has been intense, than elsewhere in southeast Asia (3 of 149; P =0.02). Five coding mutations in pvmdr1, independent of gene amplification, were also found.

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PLASMODIUM VIVAX PARASITES ALTER THE BALANCE OF MYELOID AND PLASMACYTOID DENDRITIC CELLS AND THE INDUCTION OF REGULATORY T CELLS

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Immunity induced by *Plasmodium vivax* infections leads to memory T-cell recruitment and activation during subsequent infections. Here, we investigated the role of regulatory T cells (Treg) in coordination with the host immune response during *P. vivax* infection. Our results showed a significant increase in the percentage of FOXP3+ Treg, IL-10-secreting Type I Treg (Tr1) and IL-10 levels in patients with acute *P. vivax* infection as compared with those found in either naive or immune controls. The concurrent increase in the Treg population could also be reproduced *in vitro* using peripheral blood mononuclear cells from naive controls stimulated with crude antigens extracted from *P. vivax*-infected red blood cells. Acute *P. vivax* infections were associated with a significant decrease in the numbers of DC, indicating a general immunosuppression during *P. vivax* infections. However, unlike *P. falciparum* infections, we found that the ratio of myeloid DC (MDC) to plasmacytoid DC (PDC) was significantly lower in acute *P. vivax* patients than that of naive and immune controls. Moreover, the reduction in PDC may be partly responsible for the poor antibody responses during *P. vivax* infections. Taken together, these results suggest that *P. vivax* parasites interact with DC, which alters the MDC/PDC ratio that potentially leads to Treg activation and IL-10 release.

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HIGH-DOSE PRIMAQUINE REGIMENS AGAINST RELAPSE OF *PLASMODIUM VIVAX* MALARIA

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Plasmodium vivax causes debilitating but usually non-lethal malaria in most of Asia and South America. Prevention of relapse after otherwise effective therapy for the acute attack requires a standard daily dose of primaquine administered over 14 days. This regimen has < 90% efficacy in Thailand, and is widely regarded as ineffective because of poor compliance over the relatively long duration of dosing. We evaluated the efficacy, safety, and tolerability of alternative primaquine dosing regimens combined with artesunate among 399 Thai patients with acute, symptomatic *P. vivax* malaria. Patients were randomly assigned to one of six treatment groups: all patients received artesunate, 100 mg once a day for 5 days. Groups 1-5 then received primaquine, 30 mg a day for 5, 7, 9, 11, and 14 days, respectively. Group 6 received primaquine, 30 mg twice a day for 7 days. The 28-day cure rates were 85%, 89%, 94%, 100%, and 96%, respectively. Treatment of *P. vivax* malaria with artesunate for 5 days followed by high-dose primaquine, 30 mg twice a day for 7 days, was highly effective, well-tolerated, and equivalent or superior to the standard regimen of primaquine therapy.

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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 glycine 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.

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DEFECTIVE ERYTHROPOIETIN PRODUCTION AND RETICULOCYTE RESPONSE IN ACUTE *PLASMODIUM FALCIPARUM* MALARIA-ASSOCIATED ANEMIA

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To elucidate the relationship between falciparum malaria-associated anemia and serum erythropoietin (Epo) levels and reticulocyte response during acute malaria infection, 87 adults aged 18-65 years presenting with acute, uncomplicated malaria were examined on enrollment and for 28 days of follow-up. The 87 patients were divided into 2 groups: those with anemia (n = 45) and those without (n = 42). Serum samples were taken on admission (Day 0), then on Days 7, 21, and 28, to measure the reticulocyte count, absolute reticulocyte count, reticulocyte hemoglobin content, and erythropoietin level (Epo). The absolute reticulocyte counts for the anemic patients were significantly higher than for those without anemia on Days 0, 7, 21 and 28. The serum Epo levels for the anemic patients were significantly higher than the non-anemic group only on Day 0 (44.39 +/- 4.06vs 25.91 +/-4.86 mlU/ml, $p < 0.001$). Inadequate Epo production was found in 31.03% (27/87) of patients on Day 0, 37.93% (33/87) on Day 7, 43.67% (38/87) on Day 21, and 39.08% (34/87) on Day 28. These results indicate defective Epo production and reticulocyte response in adult patients suffering from acute *P. falciparum* malaria, which differs from pediatric patients. Our findings may provide the basis for further study into the choice of therapeutic strategies to treat acute *P. falciparum* malaria-associated anemia with recombinant human Epo to correct refractory anemia due to malaria.

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SAFETY AND EFFICACY OF CKBM-A01, A CHINESE HERBAL MEDICINE, AMONG ASYMPTOMATIC HIV PATIENTS

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Complementary remedies represent a potential alternative treatment for chronic diseases, including HIV/AIDS cases not suited to highly active antiretroviral therapy (HAART). This study evaluated the safety and efficacy of CKBM-A01, a Chinese herbal medicine, and patients' quality of life (QoL). Asymptomatic HIV patients with CD4 counts of 250-350 cells/?L were recruited into this open trial. Liquid CKBM-A01 was prescribed for a 36-week period. Study participants recorded all symptoms themselves in diary cards. Study parameters, including CD4 cell count, HIV viral load, and blood chemistry, were periodically monitored and questionnaires used to assess QoL, and for risk reduction. 18 volunteers, mean age (? SD) 32.07 (?6.88) years had a median (IQR) baseline CD4 count of 292 (268.50-338.25) cells/?L. No serious drug-related adverse events were detected. Only 27.8% experienced possibly drug-related adverse events, including increased bowel movement, dizziness, sore throat/heartburn, nausea/vomiting, and somnolence. Regarding efficacy, no significant changes in log viral load or CD4 cell count were observed at the end of the study.72.2% expressed satisfaction with CKBM-A01 and had a positive perception. Common colds and nasal symptoms were reportedly significantly reduced post-treatment ($p=0.019$) CKBM-A01 appeared to be safe and may improve QoL, with less morbidity, for asymptomatic HIV patients not suited to standard therapy

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SUPPRESSION OF *PLASMODIUM FALCIPARUM* BY SERUM COLLECTED FROM A CASE OF *PLASMODIUM VIVAX* INFECTION

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BACKGROUND: It has frequently been reported that *Plasmodium vivax* suppressed *Plasmodium falciparum* and ameliorated disease severity in patients infected with these two species simultaneously. The authors investigate the hypothesis that immunological responses stimulated by *P. vivax* may play a role in suppressing co-infecting *P. falciparum*.

METHODS: Sera, taken sequentially from one of the authors (YN) during experimental infection with *P. vivax*, were added to in vitro cultures of *P. falciparum*. Cross-reactive antibodies against *P. falciparum* antigens, and cytokines were measured in the sera.

RESULTS: Significant growth inhibitory effects upon *P. falciparum* cultures (maximally 68% inhibition as compared to pre-illness average) were observed in the sera collected during an acute episode. Such inhibitory effects showed a strong positive temporal correlation with cross-reactive antibodies, especially IgM against *P. falciparum* schizont extract and, to a lesser degree, IgM against Merozoite Surface Protein (MSP)-119. Interleukin (IL)-12 showed the highest temporal correlation with *P. vivax* parasitaemia and with body temperatures in the volunteer. CONCLUSION: These results suggest the involvement by cross-reactive antibodies, especially IgM, in the interplay between plasmodial species. IL-12 may be one of direct mediators of fever induction by rupturing *P. vivax* schizonts, at least in some subjects. Future studies, preferably of epidemiological design, to reveal the association between cross-reactive IgM and cross-plasmodial interaction, are warranted.

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QUANTITATION OF CELL-DERIVED MICROPARTICLES IN PLASMA USING FLOW RATE BASED CALIBRATION

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Activation of vascular endothelium and blood cells can result in the formation of microparticles (MPs), which are membrane vesicles with a diameter < 1 microm which can play a pathogenetic role in a variety of infectious and other diseases. In this study, we validated a modified quantitative method called "flow rate based calibration", to measure circulating MPs in plasma of healthy subjects and malaria patients using FACSCalibur flow cytometry. MPs counts obtained from "flow rate based calibration" correlated closely with the standard method (R2 = 0.9, p = 0.001). The median (range) number of MPs in healthy subjects was 163/microl (81-375/microl). We demonstrated a flow rate based calibration for the quantitation of MPs in *P. falciparum* malaria-infected patients. The median (range) number of MPs was 2,051/microl (222-6,432/microl), n = 28 in patients with falciparum malaria. The number of MPs in plasma from patients with severe falciparum malaria was significantly higher than in uncomplicated falciparum malaria (2,567/microl (366-6,432/microl), n = 18 versus [1,947/microl (222-4,107/microl), n = 10, p < 0.01]. Cellular origin of MPs in malaria patients were mainly derived from red blood cells (35%), platelets (10%), and endothelial cells (5%). There was no significant correlation between the total number of MPs and parasitemia. Flow rate based calibration is a simple, reliable, reproducible method and more affordable to quantitate MPs.

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THE ABSOLUTE COUNTING OF RED CELL-DERIVED MICROPARTICLES WITH RED CELL BEAD BY FLOW RATE BASED ASSAY

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BACKGROUND: Activation of red blood cell is associated with the formation of re d cell-derived microparticles (RMPs). Analysis of circulating RMPs is becoming more refined and clinically useful. A quantitative Trucounttrade mark tube method is the conventional method uses for quantitating RMPs. In this study, we validated a quantitative method called "flow rate based assay using red cell bead (FCB)" to measure circulating RMPs in the peripheral blood of healthy subjects.

METHODS: Citrated blood samples collected from 30 cases of healthy subjects were determined the RMPs count by using double labeling of annexin V-FITC and anti-glycophorin A-PE. The absolute RMPs numbers were measured by FCB, and the results were compared with the Trucounttrade mark or with flow rate based calibration (FR). Statistical correlation and agreement were analyzed using linear regression and Bland-Altman analysis.

RESULTS: There was no significant difference in the absolute number of RMPs quantitated by FCB when compared with those two reference methods including the Trucounttrade mark tube and FR method. The absolute RMPs count obtained from FCB method was highly correlated with those obtained from Trucounttrade mark tube (r(2) = 0.98, mean bias 4 cell/mul, limit of agreement [LOA] -20.3 to 28.3 cell/mul), and FR method (r(2) = 1, mean bias 10.3 cell/mul, and LOA -5.5 to 26.2 cell/mul).

CONCLUSION: This study demonstrates that FCB is suitable and more affordable for RMPs quantitation in the clinical samples. This method is a low cost and interchangeable to latex bead-based method for generating the absolute counts in the resource-limited areas. (c) 2008 Clinical Cytometry Society.

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HIV VACCINE RESEARCH IN THAILAND: LESSONS LEARNED

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Thailand is the only country that has conducted two Phase III efficacy trials and ten other Phase I/II studies using various HIV-1 vaccine constructs. This outstanding record is the result of strong efforts and commitment among various partners, including policy makers, regulators, researchers and foreign collaborators. Recently, it has become apparent that hardly any new HIV/AIDS candidate vaccines are in the pipeline reaching the stage of clinical testing, especially a candidate vaccine that is suitable for Thailand and the surrounding region. However, many lessons learned can be utilized or modified for other vaccine trials, particularly vaccines against other infectious diseases prevalent in developing countries.

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AIDS VACCINES RESEARCH PROJECT

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[no abstract]

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LEPTIN, SOLUBLE LEPTIN RECEPTOR, LIPID PROFILES, AND *LEPR* GENE POLYMORPHISMS IN THAI CHILDREN AND ADOLESCENTS

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OBJECTIVE: To evaluate the relationships between leptin, soluble leptin receptor, lipid profiles, and LEPR gene polymorphisms in child and adolescent Thai subjects. DESIGN: Cross-sectional study of Thai children and adolescents.

SUBJECTS: 116 male and 65 female at risk for overweight/ overweight child and adolescent Thai subjects, and 33 male and 62 female healthy child and adolescent Thai subjects (age: 5-19 years). MEASUREMENTS: Leptin levels, soluble leptin receptor levels, lipid profiles, LEPR gene polymorphisms. RESULTS: Significantly higher levels of cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and leptin levels were observed in at risk for overweight/overweight group. On the other hand, high-density lipoprotein cholesterol (HDL-C) and soluble leptin receptor levels were significantly lower in the same group. Serum soluble leptin receptor levels were significantly negatively correlated with leptin. The at risk for overweight/overweight subjects with the Lys656Lys homozygous wild type LEPR gene had significantly higher cholesterol and LDL-C levels than those with Lys656Asn heterozygous and Asn656Asn homozygous mutant type. In contrast, subjects with Lys656Lys homozygous wild type had significantly lower leptin levels than those with Lys656Asn heterozygous and Asn656Asn homozygous mutant type. There was a statistically significant association between body mass index (BMI) and hyperleptinemia (odds ratio; OR = 2.49, p = 0.000) and females had more increased risk of hyperleptinemia than males (OR = 15.74, p = 0.004) in adolescent Thai subjects.

CONCLUSION: The present study is the first report of Lys656Asn polymorphism of the LEPR gene associated with cholesterol, LDL-C, and leptin levels in Thai children and adolescents. Serum leptin levels were significantly higher in the at risk for overweight/overweight. In contrast, there were significantly lower soluble leptin receptor levels in the same group. In addition, there was a statistically significant association between BMI, sex, and hyperleptinemia in adolescent Thai subjects.

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EFFECTS OF DIFFERENT ANTIMALARIAL DRUGS ON GAMETOCYTE CARRIAGE IN *P. VIVAX* MALARIA

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The gametocytocidal and asexual stage activities of eight antimalarial and eight antibiotic-containing regimens were evaluated in 349 adult patients with *P. vivax* malaria. Gametocytemia was found in 63% of patients (22% before and 41% after treatment). The median (range) gametocyte clearance time was 24 hours (range, 2-504 hours) and correlated with asexual parasite clearance time ($r = 0.52$, $P < 0.001$). Gametocytemia in vivax malaria was more common in patients with admission parasitemia > 10,000/microL and after treatment with drugs which have weak antimalarial activity, and was also associated with an increased rate of vivax reappearance (29.4% versus 14.1%, $P = 0.002$). Sexual stage activities corresponded with asexual stage activity for all tested regimens. Treatment with potent antimalarial drugs reduces the transmission potential of *P. vivax*.

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ADHERENCE AND EFFICACY OF SUPERVISED VERSUS NON-SUPERVISED TREATMENT WITH ARTEMETHER/ LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN BANGLADESH: A RANDOMISED CONTROLLED TRIAL

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As artemether/lumefantrine is now deployed as the first-line treatment for uncomplicated falciparum malaria in Bangladesh, information on its efficacy and adherence to its use is important. A randomised controlled non-inferiority trial comparing directly observed treatment (DOT) and non-directly observed treatment (NDOT) was conducted in 320 patients with uncomplicated falciparum malaria in Bandarban Hill Tract District, Bangladesh. Both regimens showed similar high levels of PCR-corrected 42-day parasitological and clinical cure rates (99.3% in the NDOT group and 100% in the DOT group; P=0.49). Survival analysis for the time to recurrence of infection showed no difference between treatment groups (log rank, P=0.98). Adherence, as assessed by counting remaining tablets and oral interviews, was 93% in the NDOT group and was confirmed by Day 7 lumefantrine concentrations. Adherence was independent of educational level. Patients with plasma lumefantrine concentrations < 280 ng/ml at Day 7 were at greater risk for re-infection (relative risk 5.62; P=0.027). The efficacy of artemether/lumefantrine for the treatment of uncomplicated falciparum malaria in Bangladesh is high and is similar for DOT and NDOT. Adherence to therapy is high.

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HIGH *ORIENTIA TSUTSUGAMUSHI* DNA LOADS ARE ASSOCIATED WITH MORE SEVERE DISEASE IN ADULTS WITH SCRUB TYPHUS

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Orientia tsutsugamushi, the cause of scrub typhus, is a major pathogen in the Asia-Pacific region. Severity of infection ranges from mild features to multi-organ failure and death. The aim of this prospective study was to define the *O. tsutsugamushi* loads in the blood of patients with scrub typhus on the day of hospital admission, and to determine whether this was associated with disease severity. Quantitation was performed using a real-time PCR assay targeting the 16S ribosomal RNA gene of *O. tsutsugamushi*. A total of 155 patients with a confirmed diagnosis of scrub typhus had a median (interquartile range (IQR), range) *O. tsutsugamushi* DNA load in blood of 13 (0-334, 0-310,253) copies/mL. This included 74 patients who had an undetectable bacterial load. An analysis of bacterial load versus clinical features for all 155 patents demonstrated that duration of illness ($p<0.001$), presence of eschar ($p=0.004$) and the concentration of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase ($p<0.001$ for all three) were positively correlated with bacterial load. Patients who died had a significantly higher bacterial load than those who survived (mean (SD); 17154 (+/-12.68) vs 281(+/-5.21) copies/mL, $p<0.001$). This study has demonstrated a relationship between bacterial load and disease severity in adults with scrub typhus.

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DIFFERENCES IN GENETIC POPULATION STRUCTURES OF *PLASMODIUM FALCIPARUM* ISOLATES FROM PATIENTS ALONG THAI-MYANMAR BORDER WITH SEVERE OR UNCOMPLICATED MALARIA

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BACKGROUND: There have been many reports on the population genetic structures of *Plasmodium falciparum* from different endemic regions, but few studies have examined the characteristics of isolates from patients with different clinical outcomes. The population genetic structures of *P. falciparum* isolates from patients with either severe or uncomplicated malaria were examined.

METHODS: Twelve microsatellite DNA loci from *P. falciparum* were used to assess the population genetic structures of 50 isolates (i.e., 25 isolates from patients with severe malaria and 25 from patients with uncomplicated malaria) collected in the Thai-Myanmar border area between 2002 and 2005.

RESULTS: Genetic diversity and effective population sizes were greater in the uncomplicated malaria group than in the severe malaria group. Evidence of genetic bottlenecks was not observed in either group. Strong linkage disequilibrium was observed in the uncomplicated malaria group. The groups demonstrated significant genetic differentiation ($P < 0.05$), and allele frequencies for 3 of the 12 microsatellite loci differed significantly between the two groups.

CONCLUSION: These findings suggest that the genetic structure of *P. falciparum* populations in patients with severe malaria differs from that in patients with uncomplicated malaria. The microsatellite loci used in this study were presumably unrelated to antigenic features of the parasites, but, these findings suggest that some loci may influence the clinical outcome of malaria.

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GAMETOCYTE CLEARANCE IN UNCOMPLICATED AND SEVERE *PLASMODIUM FALCIPARUM* MALARIA AFTER ARTESUNATE-MEFLOQUINE TREATMENT IN THAILAND

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Artemisinin-based combination therapy (ACT) is currently promoted as a strategy for treating both uncomplicated and severe falciparum malaria, targeting asexual blood-stage Plasmodium falciparum parasites. However, the effect of ACT on sexual-stage parasites remains controversial. To determine the clearance of sexual-stage *P. falciparum* parasites from 342 uncomplicated, and 217 severe, adult malaria cases, we reviewed and followed peripheral blood sexual-stage parasites for 4 wk after starting ACT. All patients presented with both asexual and sexual stage parasites on admission, and were treated with artesunate-mefloquine as the standard regimen. The results showed that all patients were asymptomatic and negative for asexual forms before discharge from hospital. The percentages of uncomplicated malaria patients positive for gametocytes on days 3, 7, 14, 21, and 28 were 41.5, 13.1, 3.8, 2.0, and 2.0%, while the percentages of gametocyte positive severe malaria patients on days 3, 7, 14, 21, and 28 were 33.6, 8.2, 2.7, 0.9, and 0.9%, respectively. Although all patients were negative for asexual parasites by day 7 after completion of the artesunate-mefloquine course, gametocytemia persisted in some patients. Thus, a gametocytocidal drug, e.g., primaquine, may be useful in combination with an artesunate-mefloquine regimen to clear gametocytes, so blocking transmission more effectively than artesunate alone, in malaria transmission areas.

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EFFICACY OF ARTEQUICK VERSUS ARTESUNATE-MEFLOQUINE IN THE TREATMENT OF ACUTE UNCOMPLICATED FALCIPARUM MALARIA IN THAILAND

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To determine the efficacy, safety and tolerability of an alternative short-course, artemisinin-based combination therapy for acute uncomplicated Plasmodium falciparum malaria, we compared Artequick--a fixed-dosed combination of artemisinin (80 mg), piperaquine (400 mg), and primaquine (4 mg), per tablet--with a standard regimen of artesunate-mefloquine. A total of 130 patients were randomly assigned to treatment with an orally administered, once-daily, 3-day regimen of either Artequick (Group A: 3.2 mg/Kg/day of artemisinin, 16 mg/Kg/day of piperaquine, and 0.16 mg/Kg/day of primaquine) or artesunate-mefloquine (Group B: artesunate, 4 mg/Kg/day, with mefloquine, 8 mg/Kg/day). Patients receiving each regimen had a rapid clinical and parasitological response. All treatments were well tolerated, and no serious adverse effects occurred. No significant differences were found in fever- and parasite-clearance times between the two study groups. The 28-day cure rates were similarly high, at 98.5% and 100%, in groups A and B, respectively. We conclude that Artequick was as effective and well tolerated as artesunate-mefloquine and could be used as an alternative treatment for multidrug-resistant *Plasmodium falciparum* malaria in Southeast Asia.

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DYNAMIC CHANGES IN WHITE BLOOD CELL COUNTS IN UNCOMPLICATED *PLASMODIUM FALCIPARUM* AND *P. VIVAX* MALARIA

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Total and differential white blood cell (WBC) counts are basic and essential indicators in any type of illness resulting from infection. In malaria, WBC counts are generally characterized as low to normal during treatment. WBC-counts data, before and during treatment with artemisinin derivatives, was gathered for patients with either *Plasmodium falciparum* or *Plasmodium vivax* infection (at 28-day follow-up), to investigate dynamic changes in WBC count. We analyzed and compared the WBC counts of 1,310 inpatients presenting with uncomplicated *P. falciparum* and *P. vivax* malaria at the Hospital for Tropical Diseases, in Bangkok, Thailand. Before-treatment, a statistically significant negative correlation was found between initial WBC count and highest temperature on admission. Before and during treatment, WBC counts were significantly lower in *P. falciparum* than *P. vivax* infection on days 0 and 7, but the numerical difference was small. We also found clinically significantly low WBC counts during the acute stages of both types of malaria, which subsequently normalized by day 28 follow-up. This finding has important clinical implications for the conventional method of estimating parasitemia using an assumed WBC count of 8,000 cells/microL. The most significant finding in our analysis is that WBC counts in acute *P. falciparum* and *P. vivax* malaria are significantly lower than previously assumed for estimating malaria-parasite density. However, these abnormalities returned to normal within several weeks after artemisinin-derivative-based treatment.

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RHEUMATOLOGICAL MANIFESTATIONS IN PATIENTS WITH MELIOIDOSIS

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Melioidosis, an infection caused by the bacterium Burkholderia pseudomallei, has a wide range of clinical manifestations. Here, we describe rheumatological melioidosis (involving one or more of joint, bone or muscle), and compare features and outcome with patients without rheumatological involvement. A retrospective study of patients with culture-confirmed melioidosis admitted to Sappasithiprasong Hospital, Ubon Ratchathani during 2002 and 2005 identified 679 patients with melioidosis, of whom 98 (14.4%) had rheumatological melioidosis involving joint (n=52), bone (n = 5), or muscle (n = 12), or a combination of these (n=29). Females were over-represented in the rheumatological group, and diabetes and thalassemia were independent risk factors for rheumatological involvement (OR; 2.49 and 9.56, respectively). Patients with rheumatological involvement had a more chronic course, as reflected by a longer fever clearance time (13 vs 7 days, $p = 0.06$) and hospitalization (22 vs 14 days, $p < 0.001$), but lower mortality (28% vs 44%, $p = 0.005$). Patients with signs and symptoms of septic arthritis for longer than 2 weeks were more likely to have extensive infection of adjacent bone and muscle, particularly in diabetic patients. Surgical intervention was associated with a survival benefit, bur not a shortening of the course of infection.

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ANALYSIS OF POLYMORPHISMS IN THE 5'-FLANKING REGION OF THE APOLIPOPROTEIN(A) [APO(A)] GENE IN THAI SUBJECTS WITH CORONARY ARTERY DISEASES

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Lipoprotein(a) [Lp(a)] is a complex lipoprotein particle in human plasma. It is composed of apolipoprotein B (Apo B)-100 and apolipoprotein(a) which are linked by a disulfide bond. Plasma levels

of the Lp(a) vary greatly (over 1,000 folds) among individuals. Elevated plasma levels of the Lp(a) have been shown to be an independent risk factor for coronary artery diseases (CAD). The level of Lp(a) is controlled by a single gene, the Apo(a) gene, with multiple alleles; each encodes different concentrations of the Lp(a). Previous studies revealed the presence of polymorphisms in the 5'-flanking region (FL) of the Apo(a) gene at 3 positions: G or A (-914), C or T(-49), and G or A (-21), which can be detected by cleavage of PCR-amplified DNA products with TaqI, MaeII and HhaI, respectively. The 5'-FL genotypes of the Apo(a) gene can be classified by the combination of the presence (+) or absence (-) of these restriction sites into 5 types; type A, +++, type B, -++, type C, -+-, type D, --+ and type E, +-+. In the present study, the authors analyzed the 5' FL types of the Apo(a) gene by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) in 100 healthy control subjects, 26 CAD patients with [Lp(a)] < or = 30 mg/dL, and 94 CAD patients with [Lp(a)] > 30 mg/dL. The authors found that the genotype frequencies of the Apo(a) gene were 53, 16, 27 and 4%, for types A, B, C and D respectively in normal healthy controls. In CAD patients with [Lp(a)] < or = 30 mg/dL, the distribution of the genotype frequencies were 53.8, 11.5, 30.8 and 3.9% for types A, B, C and D, respectively. Additionally in CAD patients with [Lp(a)] > 30 mg/dL, the genotype frequencies were 60.6, 11.7, 21.3 and 6.4% for types A, B, C and D, respectively. The present study might shed some light to understand CAD at the molecular level.

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HYPONATRAEMIA AND HYPOKALAEMIA IN ADULTS WITH UNCOMPLICATED MALARIA IN THAILAND

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In a retrospective study of 1415 patients aged 15 and over, we determined the incidence of clinically important hyponatraemia and hypokalaemia in adults with uncomplicated malaria. On admission, serum concentrations of sodium (135-145 mmol/L) and potassium (3.5-5.0 mmol/L) were found outside these reference ranges in 81% of patients. Severe hypokalaemia (K+ <3.0 mmol/L) and severe hyponatraemia (Na+ <125 mmol/L occurred in 4.4% and 0.6% of the patients, respectively. For hypokalaemia (43%) and hyponatraemia (37%), hypovolaemia, blood urea to creatinine ratio and high serum glucose (>100 mg/dL) were all independent factors (P < 0.001). Other independent predictors for hypokalaemia were *Plasmodium vivax* infection, female gender; and for hyponatraemia, *P. falciparum* infection, male gender, concentrations of G-6-PD and serum bicarbonate.

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BURKHOLDERIA PSEUDOMALLEI GENOME PLASTICITY ASSOCIATED WITH GENOMIC ISLAND VARIATION

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BACKGROUND: *Burkholderia pseudomallei* is a soil-dwelling saprophyte and the cause of melioidosis. Horizontal gene transfer contributes to the genetic diversity of this pathogen and may be an important determinant of virulence potential. The genome contains genomic island (GI) regions that encode a broad array of functions. Although there is some evidence for the variable distribution of genomic islands in *B. pseudomallei* isolates, little is known about the extent of variation between related strains or their association with disease or environmental survival.

RESULTS: Five islands from *B. pseudomallei* strain K96243 were chosen as representatives of different types of genomic islands present in this strain, and their presence investigated in other *B. pseudomallei*. In silico analysis of 10 *B. pseudomallei* genome sequences provided evidence for the variable presence of these regions, together with micro-evolutionary changes that generate GI diversity. The diversity of GIs in 186 isolates from NE Thailand (83 environmental and 103 clinical isolates) was investigated using multiplex PCR screening. The proportion of all isolates positive by PCR ranged from 12% for a prophage-like island (GI 9), to 76% for a metabolic island (GI 16). The presence of each of the five GIs did not differ between environmental and disease-associated isolates ($p > 0.05$ for all five islands). The cumulative number of GIs per isolate for the 186 isolates ranged from 0 to 5 (median 2, IQR 1 to 3). The distribution of cumulative GI number did not differ between environmental and disease-associated isolates ($p = 0.27$). The presence of GIs was defined for the three largest clones in this collection (each defined as a single sequence type, ST, by multilocus sequence typing); these were ST 70 (n = 15 isolates), ST 54 (n = 11), and ST 167 (n = 9). The rapid loss and/or acquisition of gene islands was observed within individual clones. Comparisons were drawn between isolates obtained from the environment and from patients with melioidosis in order to examine the role of genomic islands in virulence and clinical associations. There was no reproducible association between the individual or cumulative presence of five GIs and a range of clinical features in 103 patients with melioidosis.

CONCLUSION: Horizontal gene transfer of mobile genetic elements can rapidly alter the gene repertoire of *B. pseudomallei*. This study confirms the utility of a range of approaches in defining the presence and significance of genomic variation in natural populations of *B. pseudomallei*.

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CULTIVATION OF *PLASMODIUM VIVAX*

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Establishment of a continuous line of *Plasmodium vivax* parasite is crucial to understand the parasite's biology; however, this has not yet been achieved. Beginning in the 19th century, there were several efforts to cultivate this malaria parasite but without much success until the late 1980S. In addition, to date, only minor modifications of the methodology have been investigated, which has resulted in extending the cultivation period to around four weeks by supplying reticulocytes obtained from normal blood or rare hemochromatotic blood. However, the use of laboratory-produced erythroblasts to cultivate *P. vivax* enables maintenance of a continuous line of the parasite stably in the laboratory. Here, we summarize and compare the available methodologies and conditions for the in vitro cultivation of *P. vivax*.

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PHARMACOKINETICS OF HIGH DOSE OSELTAMIVIR IN HEALTHY VOLUNTEERS

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The effects of loading doses and probenecid coadministration on oseltamivir pharmacokinetics were evaluated in groups of 8 healthy adult Thai volunteers at four increasing dose levels (125 individual series). Doses up to 675 mg were well tolerated. The pharmacokinetics were dose-linear. Oseltamivir phosphate (OS) was rapidly and completely absorbed and converted (median; 93%) to the active carboxylate (OC) metabolite. Median (95% CI) elimination half-lives were OS; 1.0 (0.9-1.1) h and OC; 5.1(4.7-5.7). One subject repeatedly showed markedly reduced OS to OC conversion indicating constitutionally impaired carboxylesterase activity. Coadministration of probenecid resulted in a mean (95% CI) contraction in OC apparent volume of distribution of 40% (37% - 44%) and reduction in renal elimination of 61% (58 to 62%), thereby increasing the median (range) OC AUC by 154% (71% - 278%). The salivary OC AUC increase was approximately three times less than the plasma AUC increase. A loading dose 1.25 times the maintenance dose should be given in severe influenza pneumonia. Probenecid coadministration may allow considerable dose saving of oseltamivir but more information on OC penetration into respiratory secretions is needed to devise appropriate dose regimens.

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QUANTITATION OF *B. PSEUDOMALLEI* IN CLINICAL SAMPLES

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We undertook a prospective study to quantitate Burkholderia pseudomallei in blood, urine, respiratory secretions, and pus [corrected] obtained from 414 patients with melioidosis. The median was count 1.1, 1.5 x 10(4), 1.1 x 10(5), and 1.1 x 10(7) 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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DIPHYLLOBOTHRIUM LATUM INFECTION IN A NON-ENDEMIC COUNTRY: CASE REPORT

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Diphyllobothriasis, which is rarely described in Brazil, was reported initially as a travelers' disease and as an accidental infection in individuals who ate raw freshwater fish. This report aims to present the case of a 20-year-old patient with confirmed *Diphyllobothrium latum* infection.

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MALARIA EDUCATION FROM SCHOOL TO COMMUNITY IN OUDOMXAY PROVINCE, LAO PDR

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School-based malaria education has been shown to be effective for improving the knowledge, attitudes, and practices of school children toward malaria control. However, little has been reported about the effect of such education on communities in developing countries. To evaluate the influence of school-based malaria education on the knowledge, attitudes, and practices of people in the community toward malaria, we conducted a school-based intervention in Oudomxay province, Lao PDR, and compared scores obtained before and after the intervention. Participants were 130 school children in grades 3-5 at two primary schools, 103 guardians of these children, and 130 married women who did not have children in the target grades. The intervention included presentation of a flipchart at home and a 1-day campaign conducted by the school children and aimed at the community. The flipchart presentation was conducted at villages where school children of both primary schools resided. The 1–day campaign was, however, conducted only at one village. Before and after the intervention, we conducted a questionnaire-based survey of community women that pertained to malaria. Our main finding was that, in married women without children in the target grades, particularly those who were presented with the flipchart and participated in the campaign, the scores of the mean knowledge, attitudes and practices were significantly increased 1 month after the intervention. In conclusion, our results suggest that school children can act as health information messengers from schools to communities for malaria control in Lao PDR.

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PARASITOLOGICAL MONITORING OF HELMINTH CONTROL PROGRAM IN NORTHERN THAILAND

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Nan Province, located in northern Thailand, is hyperendemic for parasite infections; the helminthic infection rate in 1,010 schoolchildren was 60.0% in 2001. Mass anthelminthic chemotherapy has been conduceted with schoolchildren, and selective treatment has been given to people in the community, from 2002. The modified cellophane thick smear method was used to examine the prevalence and intensity of helminth infections in schoolchildren and community people once a year during the period 2002-2004. The prevalence of helminth infections decreased slowly from 60.0 to 40.3% in schoolchildren and from 70.8 to 60.0% in the older age population. 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 villages none was found. The villages where *Ascaris* infection was nil had high rates of *Haplorchis* infection, and vice versa. Most hookworm and *Trichuris* infections were of light intensity. Heavy intensity infection was found in 12.8-18.1% of *Ascaris* cases examined. Parasite infection rates in Chaloem Phra Kiat District can be classified as low prevalence.

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SCHOOL-BASED HEALTH EDUCATION FOR THE CONTROL OF SOIL-TRANSMITTED HELMINTHIASES IN KANCHANABURI PROVINCE, THAILAND

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Soil-transmitted helminthiases are major parasitic diseases that cause health problems world-wide. School-based health education is one of several basic interventions currently recommended by the World Health Organization for the control of these infections. A 3-year programme of health education for the control of soil-transmitted helminthes (STH) has recently been completed in four primary schools in the Hauykayeng subdistrict of Thong Pha Phum district, in the Kanchanaburi province of Thailand. Overall, the percentage of the schoolchildren found infected with STH increased between the start of year 1 of the intervention (16.6%) and the end of year 2 (23.8%) but showed signs of falling by the end of year 3 (19.4%). Although none of these year-on-year changes in overall prevalence was statistically significant, some significant trends were detected when the six school grades (i.e. age groups) were considered separately. The grade showing the highest prevalence of STH infection changed, from grade 6 (representing the oldest children investigated) at the start of year 1 (when grade-1 children were excluded from the survey) to grade 1 (representing the youngest children) at the ends of year 2 and year 3. By the end of year 3, the children in grades 5 and 6 had significantly lower prevalences of infection than the grade-1 subjects. The prevalence of STH infection in the grade-1 children was significantly higher than that in any of the older grades at the end of year 2 and significantly higher than that in grades 3-6 at the end of year 3. These results indicate that the health education had a greater impact on the children in the higher grades (who, presumably had better levels of understanding and practised better, personal, infection prevention) than on the younger children. Although school-based interventions can serve as a useful entry point for parasite control, more effort, including anthelminthic treatment, may be required among the youngest children. The activities need to sustainable and supported by appropriate school-health policies.

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APPLICATION OF TOXOCARA CANIS EXCRETORY-SECRETORY ANTIGENS AND IGG SUBCLASS ANTIBODIES (IGG1-4) IN SERODIAGNOSTIC ASSAYS OF HUMAN TOXOCARIASIS

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A major problem in the serodiagnosis of human toxocariasis in tropical countries is cross-reaction with antibodies to other helminthic diseases and a lack of sensitivity. The majority of tests currently available use total IgG and, in this study, the use of peroxidase conjugated anti-human IgG subclass antibodies (IgG1-4) was compared with total IgG for the diagnosis of human toxocariasis by using Toxocara excretory-secretory (TES) antigens in an enzyme-linked immunosorbent assay (ELISA) format. All four IgG subclass antibodies gave approximately 10-fold increases in optical density (OD) values for 50 toxocariasis patients compared to 29 healthy normals; this was significantly greater than the approximate doubling of OD values seen in the total IgG-ELISA format. IgG2 gave by far the greatest sensitivity (values: IgG, 50%, IgG1, 60%; IgG2, 98%; IgG3, 78%, IgG4, 64%). Significant cross-reactivity using all IgG subclasses in the TES ELISA was seen with 141 serum samples from patients with 10 other helminthic infections. However, IgG3 gave the best specificity (values: IgG, 73%; IgG1, 76%; IgG2, 71%; IgG3, 81%; IgG4, 71%). Thus, of the IgG subclass antibodies, IgG2 appeared best and employing this subclass can improve the serodiagnosis of human toxocariasis since it recognises carbohydrate epitopes of TES antigens.

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MOLECULAR PHYLOGENETIC RELATIONSHIP OF *PARAGONIMUS PSEUDOHETEROTREMUS*

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A part of the mitochondrial cytochrome **c** oxidases 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 a similar morphology. Pairwise distance of COI sequences revealed a genetic difference between *P. heterotremus* and *P. pseudoheterotremus* with a nucleotide difference of COI sequences between these two species of 10.6%. The constructed phylogenic tree with high bootstrap proportion suggested that *P. pseudoheterotremus* is a sister species of *P. heterotremus*.

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PARAGONIMIASIS PREVALENCES IN SARABURI PROVINCE, THAILAND, MEASURED 20 YEARS APART

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Saraburi Province, Central Thailand has been a paragonimiasis –endemic area since 1956. This study compared the prevalences of human paragonimiasis in two villages near Chet Khot Waterfall, Kaeng Khoi District, investigated in 1984-1985 and 2005. The results from the 1980s showed 6.3% and 1% of villagers were positive for *Paragonimus* eggs in sputum and stool, respectively. In 2005, *Paragonimus* eggs were not found in feces or sputum. An IgG-ELISA for paragonimiasis was conducted on 33 serum samples collected in the 1980s, 23 collected in 2005 and 25 diagnosed with other parasitic infections. Ninety percent of the samples from the eighties were positive for paragonimiasis, and 43% from 2005 were positive, equivalent to 10.9% and 4.9% of the total population examined in the 1980s and 2005, respectively. Serodiagnosis is currently the best method for detecting paragonimiasis. The positive cases in the 1980s were age 10-60 years and in 2005 were age 34-67 years old. The prevalence and intensity of *Paragonimus* metacercariae in fresh waterfall crabs collected from Chet Khot Waterfall were significantly lower in the 1980s than in 2005. The prevalence of paragonimiasis in this endemic area has decreased to the level that no egg-producing cases were detected. No infections were found in villagers age <30 years, despite the high density of metacercariae in the crabs, indicating a change in the habit of eating raw food among the younger people.

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CAPILLARIASIS: CHRONIC WATERY DIARRHEA NOT ONLY FROM MICROORGANISMS

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A 54-year-old male Thai patient from Prachin Buri Province presented with a history of chronic watery diarrhea for many years. He passed stool five to ten times per day with occasionally colicky pain, abdominal distension, nausea and vomiting. He had visited hospitals and private clinics and received treatment but with no improvement. He presented to the Hospital for Tropical Diseases, Bangkok, Thailand, where on physical examination, he had moderate dehydration, weakness, abdominal distension and a gurgling abdomen. The eggs, larvae and adult worms

of *Capillaria philippinensis* were found on stool examination. The patient was admitted and treated with Mebendazole for 20 days, whereupon his symptoms resolved. Two months previously, he had ingested a raw small fresh-water fish dish called *"Phra Pla Siw/Soi"*. Small fresh-water fish near the patient's home were collected and examined for *Capillaria philippinensis* larva. The results were negative for parasitic organisms.

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HOUSE FLIES: POTENTIAL TRANSMITTERS OF SOIL-TRANSMITTED-HELMINTH INFECTIONS IN AN UNSANITARY COMMUNITY

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This study aimed to determine an appropriate technique for isolating helminthic objects from the exteriors of the bodies of flies, and to investigate helminth transmission rates among flies in an unsanitary community. The study area was Ban Nam Khem Village, Takua Pa District, Phang-nga Province, Thailand. In 2006, the prevalence of soil-transmitted-helminth (STH) infections in the community was 34.9%. Soil contamination in the swamp areas, where human feces were observed, ranged between 41.2-100% in the period February 2005-May 2006. Flies were abundant in defecation areas and around houses.

One year after treatment and health education, the prevalence decreased to 22.5%. While the infection rate among the schoolchildren decreased, the rate among the villagers increased to 50.0%. In June 2007, the soil contamination rate was 13.3%. The 567 houseflies in the study were all *Chrysomya megacephala*. Hookworm and *Trichuris trichiura* eggs on the body surfaces for the flies were isolated using an ultrasonic cleaner. The helminth transmission rate for flies in the defecation area was 25.9%, and in the household surroundings 11.8%. The average number of eggs on the body surfaces of flies in the defecation area was 0.4. After feeding on human excreta, 508 resting flies left 0.5 g of feces with pathogens in the surroundings. Anthelminthic treatment and health education were repeated to improve the helminth infection situation in the community.

Manual shaking and ultrasonic-cleaner techniques provided equal detection rates (80%), but ultrasonic cleaning retrieved more eggs.

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ENTEROBIASIS: A NEGLECTED INFECTION IN ADULTS

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In this study, adult patients were treated with praziquantel to expel intestinal flukes. Unexpectedly, dozens of adult *Enterobius vermicularis* worms with disfigured morphology, which had not been detected on fecal examination using Kat's modified thick-smear technique, were expelled from 6 of 33 patients.

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EXPRESSION OF THREE SERINE PROTEASE GENES FROM THE SOUTH EAST ASIAN MALARIA VECTOR, *ANOPHELES DIRUS*, IN RELATION TO BLOOD FEEDING AND PARASITE INFECTION

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Three serine protease cDNA clones were isolated from *Anopheles dirus*, a major vector of malaria in Southeast Asia. Transcript abundance was examined following infection by malaria in Southeast Asia. Transcript abundance was examined follwing infection by *Plasmodium falciparum* by RT-PCR analysis. *SerF3* exhibited increased transcript abundance in the whole body at 10 days post-infection with *P. falciparum*. All three genes are candidates for further investigation to determine their roles in mosquito immune responses.

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A SUSPECTED NEW SPECIES OF *LEISHMANIA*, THE CAUSATIVE AGENT OF VISCERAL LEISHMANIASIS IN A THAI PATIENT

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A suspected new species of *Leishmania* is described as the causative agent of the third reported case of autochthonous visceral

leishmaniasis in a Thai man living in Southeast Thailand. The results of PCR-restriction fragment length polymorphism and sequence analysis of the internal transcribed spacer 1 of ssrRNA and the mini-exon genes were different from those of previously reported ***Lishmania*** species. A direct agglutination test (DAT) revealed that antibody against ***Leishmania*** infection was detected in nine domestic cats. No potential vectors could be identified. A large-scale epidemiological survey of leishmaniasis should be urgently conducted since visceral leishmaniasis is considered an emerging disease of public health concern in Thailand.

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CONTROL OF MOSQUITO VECTORS OF TROPICAL INFECTIOUS DISEASES: (1) BIOEFFICACY OF MOSQUITO COILS CONTAINING SEVERAL PYRETHROIDS AND A SYNERGIST

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The bioefficacy of mosquito colls containing several pyrethroids were tested in a 25m room against *Culex pipiens quinquefasciatus, Aedes aegypti* and *Anopheles dirus*. The test results were compared with tests against *Culex pipiens pallens* in Japan. Based on the KT values (the 50% knockdown time) of mosquito coils containiing *dl, d* –T80-allethrin, *d,d*-T-prallethrin and methoxymethyl-tetramethyl-cyclopropanecarboxylate (K-3050) at doses of 0.05-0.5%(w/w) with or without a synergist, the pyrethroid susceptibility of the four mosquito species was as follows; *Cx. p. quinquefasciatus* was several times more tolerant to pyrethroids than *Cx. p. pallens, Ae. aegypti* was a further several times more tolerant than *Cx. p. quinquefasciatus*, and *An. dirus* was more susceptible than *Cx .p. pallens* (KT value about half of *Cx. p. pallens*). The order of their susceptibilities is common for pyrethroids. Mosquito coils containing *d, d*-T-prallethrin and K-3050 at doses of 0.05-0.2%(w/w) and N(-2-ethylhexyl) bicycle-[2,2,1]-hept -5-ene-2, 3-dicarboxyimide as a synergist at a ratio of 2 times the active ingredient were highly effective against *Ae. aegypti*, the most important mosquito vector for dengue fever.

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CONTROL OF MOSQUITO VECTORS OF TROPICAL INFECTIOUS DISEASES; (2) PYRETHROID SUSCEPTIBILITY OF *AEDES AEGYPTI* (L) COLLECTED FROM DIFFERENT SITES IN THAILAND

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Four strains (SS, BS, A and B) of *Aedes aegypti* collected from different sites in Bangkok and at different times were examined for their pyrethroid susceptibility. Mosquito coils containing *di, d*–T80-Alletrin *d, d*-T-prallethrin and methoxymethyl-tetrafluorobenzyl tetramethyl-cyclopropanecarboxylate (K-3050)with or without a synergist were tested by the 25 m^3 semi-field test method. One strain (SS) was the most susceptible with KT_{50} values of about <30 minutes for all mosquito coils, while the other three strains (BS, A and B)were found to be around 10 to 20 times more tolerant to pyrethroids than the SS strain. A similar tendency for the pyrethroid susceptibility of the four strains was obtained with tests by topical application method. In field efficacy tests, mosquito coils with *d, d*–T-prallethrin 0.20% plus N(2-ethylhexyl) bicycle –~(2,2,1)-hept –5 –ene-2,3-dicarboxyimide as a synergist exhibited a repellent effect of about 85%, while those with K-3050 0.10% plus the synergist exhibited a greater repellent effect of about 90%. In contrast, the repellent effect of commercial *dl, d*-T80-allethrin0.20% coils was as low as about 50% .The ***d,d***-Tprallethrin and K-3050 coils with the synergist were confirmed to be highly effective in repelling *Ae. aegypti.*

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DEVELOPMENT AND APPLICATION OF A SIMPLE COLORIMETRIC ASSAY REVEALS WIDESPREAD DISTRIBUTION OF SODIUM CHANNEL MUTATIONS IN THAI POPULATIONS OF *AEDES AEGYPTI*

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Dengue fever and its more serious complications dengue haemorrhagic fever and dengue shock syndrome are growing public health problems in tropical and subtropical countries. In the absence of a vaccine, most dengue control programmes rely heavily on the use of insecticides to target the *Aedes* mosquito vectors. As a limited number of insecticides are routinely used in control, monitoring for the presence of resistance is an essential component of dengue prevention programmes. The pyrethroid insecticides target the voltage-gated sodium channel on the insects' neurons. Substitutions at residue 1016 of this

protein have been associated with pyrethroid and DDT resistance in *Aedes aegypti* populations from Latin America and Asia. Here we report on the development of a simple colorimetric assay to detect these mutations in individual mosquitoes. Evaluation of this diagnostic assay on 180 *Ae. aegypti* individuals from Thailand revealed the presence of high frequencies of the Val1016Gly mutation throughout the country. The assay requires no specialised equipment and will enable monitoring for insecticide resistance associated alleles to be routinely incorporated into dengue surveillance operations.

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POLYMORPHISM PATTERNS IN DUFFY-BINDING PROTEIN AMONG THAI *PLASMODIUM VIVAX* ISOLATES

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Background: The Duffy-binding protein II of *Plasmodium vivax* (PvDBPII) has been considered as an attractive target for vaccine-mediated immunity despite a possible highly polymorphic nature. Among seven PvDBP domains, domain II has been shown to exhibit a high rate of nonsynonymous polymorphism, which has been suggested to be a potential immune (antibody binding) evasion mechanism. This study aimed to determine the extent of genetic polymorphisms and positive natural selection at domain II of the PvDBP gene among a sampling of Thai *P. vivax* isolates.

Methods: The PvDBPII gene was PCR amplified and the patterns of polymorphisms were characterized from 30 Thai *P. vivax* isolates using DNA cloning and sequencing. Phylogenetic analysis of the sequences and positive selection were done using DnaSP ver 4.0 and MEGA ver 4.0 packages.

Results: This study demonstrated a high rate of nonsynonymous polymorphism. Using Sal I as the reference strain, a total of 30 point-mutations were observed in the PvDBPII gene among the set of Thai *P. vivax* isolates, of which 25 nonsynonymous and five synonymous were found. The highest frequency of polymorphism was found in five variant amino acids (residues D384G, R390H, L424I, W437R, I503K) with the variant L424I having the highest frequency. The difference between the rates of nonsynonymous and synonymous mutations estimated by the Nei and Gojobori's method suggested that PvDBPII antigen appears to be under selective pressure. Phylogenetic analysis of PvDBPII Thai *P. vivax* isolates to others found internationally demonstrated six distinct allele groups. Allele groups 4 and 6 were unique to Thailand.

Conclusions: Polymorphisms within PvDBPII indicated that Thai vivax malaria parasites are genetically diverse. Phylogenetic analysis of DNA sequences using the Neighbour-Joining method demonstrated that Thai isolates shared distinct alleles with *P. vivax* isolates from different geographical areas. The study reported here will be valuable for the development of PvDBPII-based malaria vaccine.

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GENOME-WIDE SNP-BASED LINKAGE ANALYSIS OF TUBERCULOSIS IN THAIS

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Tuberculosis, a potentially fatal infectious disease, affects millions of individuals annually worldwide. Human protective immunity that contains tuberculosis after infection has not been clearly defined. To gain insight into host genetic factors, nonparametric linkage analysis was performed using high-throughput microarray-based single nucleotide polymorphism (SNP) genotyping platform, a GeneChip array comprised 59 860 bi-allelic markers, in 93 Thai families with multiple siblings, 195 individuals affected with tuberculosis. Genotyping revealed a region on chromosome 5q showing suggestive evidence of linkage with tuberculosis (Z(lr) statistics=3.01, logarithm of odds (LOD) score=2.29, empirical P-value=0.0005), and two candidate regions on chromosomes 17p and 20p by an ordered subset analysis using minimum age at onset of tuberculosis as the covariate (maximum LOD score=2.57 and 3.33, permutation P-value=0.0187 and 0.0183, respectively). These results imply a new evidence of genetic risk factors for tuberculosis in the Asian population. The significance of these ordered subset results supports a clinicopathological concept that immunological impairment in the disease differs between young and old tuberculosis patients. The linkage information from a specific ethnicity may provide unique candidate regions for the identification of the susceptibility genes and further help elucidate the immunopathogenesis of tuberculosis.

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GENETIC DIVERSITY AND MICROEVOLUTION OF *BURKHOLDERIA PSEUDOMALLEI* IN THE ENVIRONMENT

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BACKGROUND: The soil dwelling Gram-negative pathogen Burkholderia pseudomallei is the cause of melioidosis. The diversity and population structure of this organism in the environment is poorly defined.

METHODS AND FINDINGS: We undertook a study of *B. pseudomallei* in soil sampled from 100 equally spaced points within 237.5 m(2) of disused land in northeast Thailand. *B. pseudomallei* was present on direct culture of 77/100 sampling points. Genotyping of 200 primary plate colonies from three independent sampling points was performed using a combination of pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Twelve PFGE types and nine sequence types (STs) were identified, the majority of which were present at only a single sampling point. Two sampling points contained four STs and the third point contained three STs. Although the distance between the three sampling points was low (7.6, 7.9, and 13.3 meters, respectively), only two STs were present in more than one sampling point. Each of the three samples was characterized by the localized expansion of a single *B. pseudomallei* clone (corresponding to STs 185, 163, and 93). Comparison of PFGE and MLST results demonstrated that two STs contained strains with variable PFGE banding pattern types, indicating geographic structuring even within a single MLST-defined clone.

CONCLUSIONS: We discuss the implications of this extreme structuring of genotype and genotypic frequency in terms of micro-evolutionary dynamics and ecology, and how our results may inform future sampling strategies.

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PREVALENCE AND SEQUENCE DIVERSITY OF A FACTOR REQUIRED FOR ACTIN-BASED MOTILITY IN NATURAL POPULATIONS OF *BURKHOLDERIA* SPECIES

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Actin-based motility of the melioidosis pathogen *Burkholderia pseudomallei* requires BimA. We report a high degree of conservation of *bimA* in 99 *B. pseudomallei* isolates from the area of endemicity. A geographically restricted subset of *B. pseudomallei* isolates harbored a *B. mallei*-like *bimA* allele (12.1%), confounding a differential diagnostic test based on amplification of species-specific *bimA* regions.

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RAPID DETECTION OF THE PANDEMIC METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CLONE ST 239, A DOMINANT STRAIN IN ASIAN HOSPITALS

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We describe and validate a novel PCR assay to detect the pandemic hospital-acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA) lineage ST 239. Results based on previously uncharacterized isolates from a hospital in northeast Thailand support the view that at least 90% of HA-MRSA isolates in mainland Asia correspond to ST 239 or close relatives.

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LOOP-MEDIATED ISOTHERMAL AMPLIFICATION METHOD TARGETING THE TTS1 GENE CLUSTER FOR DETECTION OF *BURKHOLDERIA PSEUDOMALLEI* AND DIAGNOSIS OF MELIOIDOSIS

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Melioidosis is a severe infection caused by *Burkholderia pseudomallei*. The timely implementation of effective antimicrobial treatment

requires rapid diagnosis. Loop-mediated isothermal amplification (LAMP) targeting the TTS1 gene cluster was developed for the detection of *B. pseudomallei*. LAMP was sensitive and specific for the laboratory detection of this organism. The lower limit of detection was 38 genomic copies per reaction, and LAMP was positive for 10 clinical *B. pseudomallei* isolates but negative for 5 *B. thailandensis* and 5 *B. mallei* isolates. A clinical evaluation was conducted in northeast Thailand to compare LAMP to an established real-time PCR assay targeting the same TTS1 gene cluster. A total of 846 samples were obtained from 383 patients with suspected melioidosis, 77 of whom were subsequently diagnosed with culture-confirmed melioidosis. Of these 77 patients, a positive result was obtained from one or more specimens by PCR in 26 cases (sensitivity, 34%; 95% confidence interval [CI], 23.4 to 45.4%) and by LAMP in 34 cases (sensitivity, 44%; 95% CI, 32.8 to 55.9%) ($P = 0.02$). All samples from 306 patients that were culture negative for *B. pseudomallei* were negative by PCR (specificity, 100%; 95% CI, 98.8 to 100%), but 5 of 306 patients (1.6%) were positive by LAMP (specificity, 98.4%; 95% CI, 96.2 to 99.5%) ($P = 0.03$). The diagnostic accuracies of PCR and LAMP were 86.7% (95% CI, 82.9 to 89.9%) and 87.5% (95% CI, 83.7 to 90.6%), respectively ($P = 0.47$). Both assays were very insensitive when applied to blood samples; PCR and LAMP were positive for 0 and 1 of 44 positive blood cultures, respectively. The PCR and LAMP assays evaluated here are not sufficiently sensitive to replace culture in our clinical setting.

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EVALUATING *BURKHOLDERIA PSEUDOMALLEI* BIP PROTEINS ASVACCINES AND BIP ANTIBODIES AS DETECTION AGENTS

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Burkholderia pseudomallei is a biothreat agent and an important natural pathogen, causing melioidosis in humans and animals. A type III secretion system (TTSS-3) has been shown to be critical for virulence. Because TTSS components from other pathogens have been used successfully as diagnostic agents and as experimental vaccines, it was investigated whether this was the case for BipB, BipC and BipD, components of *B. pseudomallei*'s TTSS-3. The sequences of BipB, BipC and BipD were found to be highly conserved among *B. pseudomallei* and *B. mallei* isolates. A collection of monoclonal antibodies (mAbs) specific for each Bip protein was obtained. Most recognized both native and denatured Bip protein. *Burkholderia pseudomallei* or *B. mallei* did not express detectable BipB or BipD under the growth conditions used. However, anti-BipD mAbs did recognize the TTSS needle structures of a Shigella strain engineered to express BipD. The authors did not find that BipB, BipC or BipD are protective antigens because vaccination of mice with any single protein did not result in protection against experimental melioidosis. Enzyme-linked immunosorbent assay (ELISA) studies showed that human melioidosis patients had antibodies to BipB and BipD. However, these ELISAs had low diagnostic accuracy in endemic regions, possibly due to previous patient exposure to *B. pseudomallei.*

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IMMUNE RESPONSES OF SELECTED PHAGOTOPES FROM MONOCLONAL ANTIBODIES OF *BURKHOLDERIA PSEUDOMALLEI*

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Random peptide libraries displayed by bacteriophage T7 and M13 were employed to identify mimotopes from 4 monoclonal antibodies (MAbs) specific to *Burkholderia pseudomallei.* Insert DNA sequences of bound phages selected from four rounds of panning with each MAb revealed peptide sequences corresponding to *B. pseudomallei* K96243 hypothetical protein BPSL2046, hypothetical protein BpseP_02000035, *B. pseudomallei* K96243 hypothetical protein BPSS0784, *B. pseudomallei* 1710b hypothetical protein BURPS1710b_1104, and *B. cenocepacia* H12424 TonB-dependent siderophore receptor, all located at the outer membrane. The immune responses from all selected phagotopes were significantly higher than that of lipopolysaccharide. The study demonstrates the feasibility of identifying mimotopes through screening of phage-displayed random peptide libraries with *B. pseudomallei* MAbs.

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A SIMPLE SCORING SYSTEM TO DIFFERENTIATE BETWEEN RELAPSE AND RE-INFECTION IN PATIENTS WITH RECURRENT MELIOIDOSIS

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BACKGROUND: Melioidosis is an important cause of morbidity and mortality in East Asia. Recurrent melioidosis occurs in around 10% of patients following treatment either because of relapse with the same strain or re-infection with a new strain of *Burkholderia pseudomallei.* Distinguishing between the two is important but requires bacterial genotyping. The aim of this study was to develop a simple scoring system to distinguish re-infection from relapse.

METHODS: In a prospective study of 2,804 consecutive adult patients with melioidosis presenting to Sappasithiprasong Hospital,

NE Thailand, between1986 and 2005, there were 141 patients with recurrent melioidosis with paired strains available for genotyping. Of these, 92 patients had relapse and 49 patients had re-infection. Variables associated with relapse or re-infection were identified by multivariable logistic regression and used to develop a predictive model. Performance of the scoring system was quantified with respect to discrimination (area under receiver operating characteristic curves, AUC) and categorization (graphically). Bootstrap resampling was used to internally validate the predictors and adjust for over-optimism.

FINDINGS: Duration of oral antimicrobial treatment, interval between the primary episode and recurrence, season, and renal function at recurrence were independent predictors of relapse or re-infection. A score of <5 correctly identified relapse in 76 of 89 patients (85%), whereas a score >/=5 correctly identified re-infection in 36 of 52 patients (69%). The scoring index had good discriminative power, with a bootstrap bias-corrected AUC of 0.80 (95%CI: 0.73-0.87).

CONCLUSIONS: A simple scoring index to predict the cause of recurrent melioidosis has been developed to provide important bedside information where rapid bacterial genotyping is unavailable.

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SIGNIFICANT ASSOCIATION BETWEEN TIM1 PROMOTER POLYMORPHISMS AND PROTECTION AGAINST CEREBRAL MALARIA IN THAILAND

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Summary Although cerebral malaria is a major life-threatening complication of Plasmodium falciparum infection, its pathophysiology is not well understood. Prolonged activation of the T helper type 1 (Th1) response characterized by the production of pro-inflammatory cytokines such as IFN-gamma and TNF-alpha has been suggested to be responsible for immunopathological process leading to cerebral malaria unless they are downregulated by the anti-inflamatory cytokines produced by the Th2 response. The T cell immunoglobulin and mucin domain (TIM) family of proteins are cell surface proteins involved in regulating Th1 and Th2 immune responses. In this study, the possible association between the polymorphisms of TIM1, TIM3, and TIMD4 genes and the severity of malaria was examined in 478 adult Thai patients infected with P. falciparum malaria. The TIM1 promoter haplotype comprising three derived alleles, -1637A (rs7702919), -1549C (rs41297577) and -1454A (rs41297579), which were in complete linkage disequilibrium, was significantly associated with protection against cerebral malaria (OR = 0.41; 95% CI = 0.24-0.71; P= 0.0009). Allele-specific transcription quantification analysis revealed that the level of mRNA transcribed from TIM1 was higher for the protective promoter haplotype than for the other promoter haplotype (P= 0.004). Engagement with TIM1 in combination with T cell receptor stimulation induces anti-inflammatory Th2 cytokine production, which can protect the development of cerebral malaria caused by overproduction of pro-inflammatory Th1 cytokines. The present results suggest that the higher TIM1 expression associated with the protective TIM1 promoter haplotype confers protection against cerebral malaria.

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DYNAMIC RNA PROFILING IN *PLASMODIUM FALCIPARUM* SYNCHRONIZED BLOOD STAGES EXPOSED TO LETHAL DOSES OF ARTESUNATE

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Background : Translation of the genome sequence of *Plasmodium sp*. into biologically relevant information relies on high throughput genomics technology which includes transcriptome analysis. However, few studies to date have used this powerful approach to explore transcriptome alterations of *P. falciparum* parasites exposed to antimalarial drugs.

Results : The rapid action of artesunate allowed us to study dynamic changes of the parasite transcriptome in synchronous parasite cultures exposed to the drug for 90 minutes and 3 hours. Developmentally regulated genes were filtered out, leaving 398 genes which presented altered transcript levels reflecting drug-exposure. Few genes related to metabolic pathways, most encoded chaperones, transporters, kinases, Zn-finger proteins, transcription activating proteins, proteins involved in proteasome degradation, in oxidative stress and in cell cycle regulation. A positive bias was observed for over-expressed genes presenting a subtelomeric location, allelic polymorphism and encoding proteins with potential export sequences, which often belonged to subtelomeric multi-gene families. This pointed to the mobilization of processes shaping the interface between the parasite and its environment. In parallel, pathways were engaged which could lead to parasite death, such as interference with purine/pyrimidine metabolism, the mitochondrial electron transport chain, proteasome-dependent protein degradation or the integrity of the food vacuole.

Conclusion : The high proportion of over-expressed genes encoding proteins exported from the parasite highlight the importance of extra-parasitic compartments as fields for exploration in drug research which, to date, has mostly focused on the parasite itself rather than on its intra and extra erythrocytic environment. Further work is needed to clarify which transcriptome alterations observed reflect a specific response to overcome artesunate toxicity or more general perturbations on the path to cellular death.

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LACK OF ASSOCIATION OF THE *HBE* VARIANT WITH PROTECTION FROM CEREBRAL MALARIA IN THAILAND

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Hemoglobin E (*HbE*; β26Glu → Lys) is the most common variant of the β-globin gene in Southeast Asia; it has been suggested that it confers resistance against *Plasmodium falciparum* malaria. In this study 306 adult patients with *P. falciparum* malaria (198 mild and 108 cerebral malaria patients) living in northwest Thailand were investigated to examine whether the *HbE* variant is associated with protection from cerebral malaria. Our results revealed that the sample allele frequency of *HbE* was not significantly different between mild (7.3%) and cerebral malaria (7.4%) patients. Thus, the *HbA*/*HbE* polymorphism would not be a major genetic factor influencing the onset of cerebral malaria in Thailand.

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LINKAGE DISEQUILIBRIUM STRUCTURE OF THE 5Q31-33 REGION IN A THAI POPULATION

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A number of loci related to the immune response are located on human chromosomal region 5q31-33, and polymorphisms in this region have been reported to be associated with autoimmune and infectious diseases. In Southeast Asian populations, no systematic survey with dense SNP markers has been performed for the 5q31-33 region. In this study, the LD and haplotype structures for a 472-kb region on 5q31 were investigated in a Thai population to provide useful information for association studies. In addition, the LD structure in Thais was compared with that of the CHB and JPT HapMap populations (CHB + JPT) to evaluate the transferability of tagging SNPs from CHB + JPT for Thais. We show that the minor allele frequency, pattern of LD block, and genetic structure in the 5q31-33 region were highly concordant between Thais and CHB + JPT. A high transferability of tagging SNPs from CHB + JPT for Thais was observed. Our results suggest that tagging SNPs from CHB + JPT (Northeast Asians) can efficiently capture common variants in Southeast Asians, and that the HapMap data are useful for association studies in Southeast Asian populations.

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RECOMBINANT *LIGA* FOR LEPTOSPIROSIS DIAGNOSIS AND *LIGA* AMONG THE *LEPTOSPIRA* SPP. CLINICAL ISOLATES

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Rapid diagnosis for differentiation of leptospirosis from other pyrogenic infections prevailing in the same locality is imperative for proper treatment. During infection, the pathogenic *Leptospira* spp. express virulence factors which induce antibody responses in the infected host. In this study, 50 referenced *Leptospira* spp. belonging to six genomospecies and 10 *L. interrogans* clinical isolates were studied for the presence of a gene encoding an in vivo expressed, surface exposed, immunoglobulin-like protein, LigA, by using PCR and southern hybridization specific to the 5′ terminus sequence of the DNA. LigA was also detected in the *Leptospira* spp. whole cell homogenates by a direct ELISA using a mouse antiserum to the C-terminal portion of recombinant LigA (cLigA) as a detection reagent. All pathogenic *Leptospira* spp. except one of the two strains of *L. santorasai* were positive for the gene and its phenotype while all of the *L. borgpetersenii* and *L. biflexa* strains were negative. Recombinant cLigA was used as an antigen in ELISAs for detecting IgM and IgG in the sera of leptospirosis patients and in the sera of patients with other febrile illnesses and healthy subjects. When acute phase sera were tested by the cLigA IgM- and IgG-ELISAs, 92% and 100% of the MAT-positive sera were positive, respectively. The diagnostic sensitivity was 100% when both IgM- and IgG-ELISAs were performed on the same acute phase sera and the results were combined. Acute and convalescence sera of patients who were *Leptospira* culture positive but MAT/IgM-dipstick negative gave 88% and 100% positives by combined cLigA IgM/IgG ELISAs. The diagnostic specificities for the cLigA IgM- and IgG-ELISAs were 98% and 100%, respectively. Our cLigA based-serology has a high potential for early diagnosis of leptospirosis especially when the culture and MAT results are not yet available

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A MURINE MODEL OF ALLERGY CAUSED BY AMERICAN COCKROACH (CR), *PERIPLANETA AMERICANA*

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SUMMARY An animal model resembling the human immuno-pathological features of CR allergy is needed for CR allergy

research, e.g., measuring allergenicity of novel allergens, testing immunotherapeutic efficacies of drugs and vaccines. In this study we develop a murine model of American CR, *P. americana* allergy. BALB/c mice, 6 weeks old, were individually intraperitoneally injected with three doses (days 0, 7 and 14) of alum adjuvanted-crude extract of *P. americana*. On days 21 and 23, they were given crude CR extract in PBS intranasally (10 μl) and aerosolically (10 ml) via an air-pressure nebulizer, respectively. Mice received alum alone and PBS instead of the CR extract served as non-allergenic controls. All mice were bled twenty four hours after the nebulization and sacrificed. Their serum samples, broncho-alveolar lavage fluids (BALF), and lung tissues were collected. BALF of all allergen-treated mice had marked cellular infiltration notably neutrophils, eosinophils and lymphocytes. The average total cell count in BALF of the allergenic mice was 1.9 x 10^5cells/ml which out-numbered those of the non-allergenic con-trols (8 x 10^4 cells/ml). The eosinophil infiltration was pronounced in lungs of the allergen-treated mice. Specific serum IgE to the CR extract elevated in serum samples of all allergen treated mice and nil in the sera of the controls. None of the mice showed detectable level of IgG2a to the CR extract. RT-PCR revealed that all allergen-treated mice had marked increase of IL-13, IL-4 and TNF-α gene expressions, slight increase of IL-5 gene expression, and absence of detectable IFN-γ gene expression in comparison to the non-allergenic controls. None of the allergen-treated mice and 50% of the non-allergenic controls had IL-12 gene expression as detected by RT- PCR. One al-lergen treated-mouse (25%) had subpar level of the IL-18 gene expression compared to the controls. Results of the quantitative real-time PCR conformed to those of the RT-PCR. A murine model of *P. americana* resembling human allergic manifestations was successfully developed.

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MOLECULAR TYPING OF *VIBRIO CHOLERAE* O1 ISOLATES FROM THAILAND BY PULSED-FIELD GEL ELECTROPHOESIS

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The aim of the present study was to genotypically characterize *Vibrio cholerae* strains isolated from cholera patients in various provinces of Thailand. Two hundred and forty *V. cholerae* O1 strains, isolated from patients with cholera during two outbreaks, i.e. March 1999-April 2000 and December 2001-February 2002, in Thailand, were genotypically characterized by NotI digestion and pulsed-field gel electrophoresis (PFGE). In total, 17 PFGE banding patterns were found and grouped into four Dice-coefficient clusters (PF-I to PF-IV). The patterns of V. cholerae O1, El Tor reference strains from Australia, Peru, Romania, and the United States were different from the patterns of reference isolates from Asian countries, such as Bangladesh, India, and Thailand, indicating a close genetic relationship or clonal origin of the isolates in the same geographical region. The Asian reference strains, regardless of their biotypes and serogroups (classical O1, El Tor O1, O139, or O151), showed a genetic resemblance, but had different patterns from the strains collected during the two outbreaks in Thailand. Of 200 Ogawa strains collected during the first outbreak in Thailand, two patterns (clones)--PF-I and PF-II--predominated, while other isolates caused sporadic cases and were grouped together as pattern PF-III. PF-II also predominated during the second outbreak, but none of the 40 isolates (39 Inaba and 1 Ogawa) of the second outbreak had the pattern PF-I; a minority showed a new pattern--PF-IV, and others caused single cases, but were not groupable. In summary, this study documented the sustained appearance of the pathogenic *V. cholerae* O1 clone PF-II, the disappearance of clones PF-I and PF-III, and the emergence of new pathogenic clones during the two outbreaks of cholera. Data of the study on molecular characteristics of indigenous *V. cholerae* clinical isolates have public-health implications, not only for epidemic tracing of existing strains but also for the recognition of strains with new genotypes that may emerge in the future.

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MURINE MONOCLONAL ANTIBODIES NEUTRALIZING THE CYTOTOXIC ACTIVITY OF DIPHTHERIA TOXIN

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In this study, murine monoclonal antibodies that specifically bound to the A and B subunits of diphtheria toxin (DT) were produced by conventional hybridoma technology using the spleens of BALB/c mice immunized with diphtheria DTP vaccine and CRM197. Monoclonal antibodies specific to the A subunit, i.e. clone AC5, as well as those specific to the B subunit, i.e. clone BB7, could neutralize the DT-mediated cytotoxicity to Vero cells in microcultures. The DT neutralizing mechanisms have yet to be determined. The MAbBB7 is hypothesized to either interfere with the DT receptor binding or with the pore forming function of the T domain of the B subunit. The MAbAC5 could neutralize the DT mediated cytotoxicity when mixed with the DT before adding to the Vero cell culture thus suggesting that the antibody interfered with the translocation of the A subunit. The A subunit-antibody complex might be too large to pass through the membrane channel formed by the T domain and thus prevent the accessibility of the A subunit to the cytosolic target. It is also possible that the MAb AC5 blocked the enzymatic active site of the enzyme catalytic subunit. While further experiments are needed to localize the epitopes of the two MAbs on the holo-DT in order to reveal the DT neutralizing mechanisms, both MAbs in their humanized forms have a high potential as human therapeutic antibodies for diphtheria.

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SEROPREVALENCE OF *TOXOPLASMA GONDII* ANTIBODY IN VIETNAMESE VILLAGERS

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Toxoplasmosis caused by *Toxoplasma gondii* is a protozoan infection found worldwide. It usually produces non-specific symptoms, but in pregnant women and immunocompomised individuals, it may cause severe and fatal illness. Many serological studies have been done in various parts of the world, but information is lacking for Vietnam. A seroprevalence study of *T. gondii* antibodies in Vietnamese villagers (n = 650) was performed using the Sabin-Feldman dye test. The average seroprevalence was 4.19% (95% CI = 1.78-4.62), including 6.36% (95% CI = 3.22-11.09), 4.73% (95% CI = 1.92-9.50) and 1.09% (95% CI = 0.23-3.15) from Nghe An, Lao Cai and Tien Giang provinces, respectively. This study confirmed the low prevalence of toxoplasmosis in Vietnam similar to other countries in the region. Further studies are necessary in order to provide a complete picture for the country.

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

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The purpose of this study was to investigate the formation of drug nanoparticles from binary and 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 give 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 were 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 *Plasmodium 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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EVIDENCE SUPPORTING THE ZOONOTIC AND NON-ZOONOTIC TRANSMISSION OF *ENTEROCYTOZOON BIENEUSI*

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[No abstract available]

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INHIBITORY EFFECTS OF THAI PLANTS B-GLYCOSIDES ON *TRICHOMONAS VAGINALIS*

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Trichomonasis is now an important health problem in developing countries. Although metronidazole has so far been widely used to treat this disease, the prevalence of metronidazol-resistant protozoa and unpleasant adverse effects have been found. In this study, natural products purified from Thai plants were, therefore, investigated for their effectiveness against *Trichomonas vaginalis*. The minimal inhibitory concentrations for all β-glycosides against *Trichomonas vaginalis* at 24 h were in a range of 6.25-12.5 μM. In addition, torvoside A and H were found to be more potent than their corresponding aglycones, deglucosylated torvoside A and H, while other β-glycosides were generally as active as their corresponding aglycones. The cytotoxicity of these compounds was also determined. Except for dalcochinin, none of the tested compounds showed cytotoxicity against Vero and cancer call lines (KB and MCF-7), having IC_{50} values greater than 50 μg/ml. In conclusion, β-glycosides and several aglycones showed selective inhibition against *Trichomonas vaginalis* without harmful effect to mammalian cells.

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PCR-BASED COPRODIAGNOSTIC TOOLS REVEAL DOGS AS RESERVOIRS OF ZOONOTIC ANCYLOSTOMIASIS CAUSED BY *ANCYLOSTOMA CEYLANICUM* IN TEMPLE COMMUNITIES IN BANGKOK

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A survey of gastrointestinal parasites of dogs and humans from temple communities in Bangkok revealed that 58% of dogs and 3.4% of humans, among those sampled, were infected with hookworms utilising faecal flotation techniques and microscopy. A previously established polymerase chain reaction (PCR)-RFLP approach was utilised to determine the species of hookworms infecting dogs found positive for hookworm eggs. Single infections with *Ancylostoma ceylanicum* and *Ancylostoma caninum* were recorded in 77% and 9% of hookworm positive dogs, respectively and mixed infections with both species of *Ancylostoma* were recorded in 14% of dogs. A single-step PCR for the multiplex detection of *Ancylostoma* species and *Necator americanus* DNA in human faeces was developed and applied to characterize the species of hookworms in microscopy positive individuals. Single infection with N. americanus was recorded in five and *A.ceylanicum* infection in two, out of seven individuals positive for hookworm. This study demonstrates that humans are at risk of acquiring infection with *A. ceylanicum* in communities where this species of hookworm is endemic in dogs.

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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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WATER MONITORING AND TREATMENT FOR DRINKING PURPOSES IN 2004 TSUNAMI AFFECTED AREA-BAN NAM KHEM, PHANG NGA, THAILAND

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University of Brescia and Mahidol University of Bangkok developed a project in Phang Nga province (Thailand), the most damaged by 2004 tsunami. In particular,the study, performed between April and May 2006, dealt with the surface and ground water monitoring in Ban Nam Khem village and the experimental evaluation of possible drinking treatment alternatives. The monitoring highlighted that saline content in the tsunami affected area is decreasing but still very high (conductivity presented values up to 2,600 and 6,230 muS/cm in ground and surface water, respectively); hence, advanced and complicated processes such as reverse osmosis should be adopted to treat such water for drinking purposes. Waiting for ground water salinity to assume acceptable values, activities for the reduction of its organic and microbiological contamination will be started. However, it has to be underlined that the diffusion of drinking water to a greater part of population can be obtained only through the realization of new centralized treatment plants and the improvement of existing ones (serving at the moment about 20% of inhabitants).

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GENDER DIFFERENCES IN KAP RELATED TO HIV/AIDS AMONG FRESHMEN IN AFGHAN UNIVERSITIES

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This cross-sectional study aimed to describe the level of knowledge, perception/ attitude, and practices related to HIV among 1,054 freshmen students in four Afghan universities differences between genders. A probability, two stage sampling method was used. Data were collected by a self administered structured questionnaire. SPSS software was used for data analysis. Descriptive and inferential statistics were performed. Most of respondents were male (72.1%), their average age was 20.1 +/- 2 years, and most were unmarried (93.4%). The

majority (90.8%) were aware of HIV but only 28.3% had a good level of knowledge. Around one-third (35.6%) had a positive level of attitude toward HIV. Approximately 30% had at least one risk practice; therefore, they were counted as high-risk behavior group members. Females were statistically more knowledgeable than males, and high-risk behaviors were significantly more prevalent among males; p = 0.01 and p = 0.001, respectively. However, general awareness, and attitude were not statistically different between genders. A considerable proportion of students (14.6%), as compared to peer-countries, were sexually active. A very high level of sharing injecting needles (4.5%) and shaving sets (20.8%) were also reported among informants.

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FOOD BEHAVIOR AND FOLATE STATUS OF HILL-TRIBE SCHOOLCHILDREN AND WOMEN OF CHILDBEARING AGE ON THE NORTHERN BORDER OF THAILAND

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An insight into the folate nutritional status of the population is important from a public health perspective. The protective effect of folate against neural tube defects (NTDs) is widely recognized. To assess the health and nutritional status, especially folate status, of vulnerable hill-tribe groups, a cross-sectional study was conducted on 197 schoolchildren and 136 women of childbearing age in Chaloem Phra Kiat District, Nan Province, Thailand. The nutritional status of the study group was investigated by dietary survey, and blood samples were taken to determine hematocrit, protein, and serum and red blood cell folate. Anthropometric measurements were taken to assess body size, composition and nutritional indexes. The health and nutritional status of the hill-tribe schoolchildren and women of childbearing age were found to be unacceptable, particularly inregard to folate status, which was indicated by low folate levels found in the blood samples, and in the intake of this micronutrient.

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FACTORS CONTRIBUTING TO TREATMENT SUCCESS AMONG TUBERCULOSIS PATIENTS: A PROSPECTIVE COHORT STUDY IN BANGKOK

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SETTING: Chest Clinic, Ministry of Public Health and health care centres, Bangkok Metropolitan Administration.
OBJECTIVE: To determine patient factors predicting successful tuberculosis (TB) treatment. DESIGN: A prospective cohort was conducted during May 2004 to November 2005. Newly diagnosed TB patients aged > or = 15 years were recruited after giving informed consent. Three sets of questionnaires were used to collect data from the patients three times. Data were also gathered from treatment cards.

RESULTS: Of 1241 patients, 81.1% were successfully treated. Bivariate analysis indicated that patients' sex, education, occupation, level of knowledge about TB and adverse effects were associated with treatment success. Unconditional logistic regression analysis showed that females had a higher success rate than males (OR = 1.9, 95%CI 1.2-2.9). Patients with regular incomes had twice the likelihood of success of the unemployed (OR = 2.0, 95%CI 1.1-3.5). Patients with high knowledge levels were more likely to complete treatment (OR = 2.0, 95%CI 1.2-3.4), while those with adverse effects were less likely to adhere (OR = 0.6, 95%CI 0.4-0.9).

CONCLUSION: The current low treatment success rate may be partly due to inadequate knowledge about TB among patients. Improvements in health education and early detection and management of adverse effects should be prioritised by the National Tuberculosis Programme.

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DETECTION OF *OPISTHORCHIS VIVERRINI* IN INFECTED BITHYNID SNAILS BY REAL-TIME FLUORESCENCE RESONANCE ENERGY TRANSFER PCR-BASED METHOD AND MELTING CURVE ANALYSIS

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A real-time fluorescence resonance energy transfer (FRET) PCR combined with melting curve analysis was developed for the detection of *Opisthorchis viverrini* in experimentally infected bithynid snails, its first intermediate hosts. The test is based on the fluorescence melting curve analysis of a hybrid between an amplicon from the pOV-A6-specific probe sequence, a 162-bp repeated sequence specific to *O. viverrini* and specific fluorophore-labeled probes. The real-time FRET PCR could detect as little as a single cercaria artificially introduced in a pool of 30 non-infected snails. The *O. viverrini*-infected snails were discriminated from non-infected snails and from genomic DNA of other parasite DNAs by their melting temperatures. Sensitivity and specificity of this method were both 100%. Melting curve analysis is a sensitive alternative for the specific detection of *O. viverrini*-infected snails; it is rapid, allows a high throughput, and can be done on small samples. The assay not only has a high potential for epidemiological surveys of *O. viverrini*-infected bithynid snails, but also for the detection of cercariae infestations of natural waterways when monitoring transmission sites.

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REAL-TIME FLUORESCENCE RESONANCE ENERGY TRANSFER PCR WITH MELTING CURVE ANALYSIS FOR THE DETECTION OF *OPISTHORCHIS VIVERRINI* IN FISH INTERMEDIATE HOSTS

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A real-time fluorescence resonance energy transfer (FRET) PCR combined with a melting curve analysis was developed for the detection of Opisthorchis viverrini in its fish intermediate host, cyprinoid fishes. Real-time FRET PCR is based on a fluorescence melting curve analysis of a hybrid between an amplicon generated from a family of repeated DNA elements, the pOV-A6 specific probe sequence (Genbank Accession No. S80278), a 162 bp repeated sequence specific to O.viverrini, and specific fluorophore-labeled probes. The real-time FRET PCR could detect as little as a single metacercaria artificially inoculated in 30 fishsamples. The O. viverrini infected fishes were distinguished from non-infected fishes and from the genomic DNA of other parasites by their melting temperature. Sensitivity and specificity of this method were both 100% in the laboratory setting and it outperformed the microscopic method on field-collected samples as well. Melting curve analysis is a rapid, accurate, and sensitive alternative for the specific detection of O. viverrini infected fishes. It allows a high throughput and can be performed on small samples. The assay has not only great potential for epidemiological surveys of fish intermediate hosts but it could also be adapted as screening tool for a range of foodborne parasites in freshwater fishes.

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DIFFERENCES OF SEXUAL BEHAVIOR PREDICTORS BETWEEN SEXUALLY ACTIVE AND NONACTIVE FEMALE ADOLESCENTS IN CONGESTED COMMUNITIES, BANGKOK METROPOLIS

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OBJECTIVE: To test the differences among the predictors between sexually active and non-active female adolescents. STUDY DESIGN: Descriptive research. MATERIAL AND METHOD: The participants included 581 Thai female adolescents: 262 sexually non-active and 319 sexually active (average age = 19.7 years). They completed questionnaires measuring self-discrepancy, depression, power in relationships(decision making dominance and relationship control), sexual self-efficacy (ability to say no, assertiveness, precaution), cognitive strategies (gain thinking: relationship, development, curiosity; punishment avoidance thinking: negative consequence, ethical-related, fear-related), and sexual behavior. The t-test and the Hierarchical Regression were employed for data analyses.

RESULTS: Among the sexually active, 68.8% had vaginal or anal sexual intercourse (11.7%) without using a condom. Significant enabling predictors among the sexually active included sexual self-efficacy (precaution), and gain thinking (relationship), whereas punishment avoidance thinking (negative consequence) had a negative influence: it accounted about 11.0%. Among sexually non-active, alcohol consumption, power in a relationship (decision making dominance), and gain thinking (relationship) accounted for 26.9% of the variance in explaining sexual behavior.

CONCLUSION: A specific link between sexual self-efficacy and cognitive strategies will be drawn to develop a program for the sexually active. Implications for behavioral modification addressing alcohol drinking and power in a relationship should be discussed among the sexually non-active.

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MIMOTOPE IDENTIFICATION FROM MONOCLONAL ANTIBODIES OF *BURKHOLDERIA PSEUDOMALLEI* USING RANDOM PEPTIDE PHAGE LIBRARIES

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Summary This study used random peptide libraries, displayed by bacteriophage T7 and M13, to identify mimotopes from four monoclonal antibodies (mAbs) specific to *Burkholderia pseudomallei.* Bound phages, selected from fourth-round panning with each mAb, were tested for binding specificity with each mAb using ELISA, before being further amplified and checked for phage peptide sequence using PCR and DNA sequencing. Overall, 75 of 90 phages (83.3%) were ELISA-positive with each mAb. Mimotopes from all 75 phages (100%) were found to match protein sequences of *Burkholderia* spp. from GenBank. The predominant mimotopes were TPGRTRVT found in 13.3%, LTPCGRTxD (8%), AREVTLL (6.7%), NxVxKVVSR (5.3%), PCAPRSS (4%), LGRVLAN (4%), RNPKKA (2.7%) and CPYPR (2.7%). The following GenBank-matched proteins (Le. the hypothetical proteins) were located at the outer membrane of *Burkholderia* spp.: BPSL2046 of *B. pseudomallei* K96243 (matched with mimotope CGRTxD), BpseP _02000035 (matched with LGRVLAN), BPSS0784 of *B. pseudoma{{ei* K96243 (matched with CPYPR), BURPS1710b_1104 of *B. pseudomallei* 1710b (matched with CARQY) and TonB-dependent siderophore receptor of *B. cenocepacia* H12424 (matched with CVRxxLTPC and TPCRxRT). These phage mimotopes and matched proteins may have the potential for further use as diagnostic reagent and immunogen against melioidosis. These results demonstrate that phage-display technique has the potential for rapidly identifying phage mimotopes that interact with *B. pseudomallei* mAbs.

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EPIDEMIOLOGICAL STUDY OF STRONGYLOIDIASIS IN SOUTHERN THAILAND, 2007

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A survey of *Strongyloides stercoralis* infection was conducted in 11 southern provinces of Thailand by agar-plate culture technique. A total of 1,308 stool samples were collected by 30-cluster sampling technique during July-August 2007. The results revealed that the overall prevalence of *S.stercoralis* in the study areas was 20.6% and the intensity was mostly low. The highest infection rate was found in Phatthalung Province (29.9%), and the lowest in Phuket Province (7.5%). The highest positive finding was among the group aged 60 years and over (28.5%), and the rate of infection was comparatively high among males (25.4%). At cluster or village level, the highest infection rate was 51.9% in Ban Don Gun Village, Nakhon Si Thammarat Province, and the lowest 2.3% in Ban Yang Mark Village, Trang Province.

The results of the surveillance showed that a majority of the population had high strongyloidiasisrelated risk behaviors–poor hygiene and inappropriate footwear behaviors, such as wearing open sandals, so that 89.9% had an enhanced risk of infection, and only 22.2% wore casual shoes. Only 24.1% habitually wore boots when they worked in the paddy field, with the remaining 75.9% at higher risk of infection. However, it was also instructive to learn that the proportion of people who routinely washed fresh vegetables thoroughly before eating, to prevent larval contamination, was quite high (94.4%). Almost all (96.0%) of the people habitually defecated in a sanitary latrine when at home; however, when they worked in the field, the rate was far lower, at only 37.7%. It was interesting to find that 11.6% of the people habitually defecated outside a latrine, on the ground, while about 50.1% sometimes defecated on the ground, which would also result in a higher risk of the spread of disease.

The information obtained from the behavioral survey also showed that high-level risk behaviors for the transmission of strongyloidiasis in southern Thailand, where potential hosts do exist, higher than the previously reported data about the prevalence of *S. stercoralis* infection. Proactive health education and empowerment of the community are recommended to control strongyloidiasis, and so prevent health problems among the people in such areas.

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INFECTION RISK ASSESSMENT OF DIARRHEA-RELATED PATHOGENS IN A TROPICAL CANAL NETWORK

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A quantitative microbial risk assessment (QMRA) of *Cryptosporidium*, Giardia and diarrhegenic Escherichia coli (DEC) infection was performed using Monte Carlo simulations to estimate the human health risks associated with the use of canal water for recreational purposes, unrestricted and restricted irrigation in a tropical peri-urban area. Three canals receiving municipal, agricultural, and, predominantly, industrial wastewater were investigated. Identification of pathogenic protozoans revealed the major presence of *Cryptosporidium hominis* and both assemblages A and B of *Giardia lamblia.* The highest individual infection risk estimate was found to be for *Giardia* in an exposure scenario involving the accidental ingestion of water when swimming during the rainy season, particularly in the most polluted section, downstream of a large wholesale market. The estimated annual risks of diarrheal disease due to infection by the protozoan parasites were up to 120-fold greater than the reported disease incidence in the vicinity of the studied district and the entire Thailand, suggesting a significant host resistance to disease beyond our model's assumptions. In contrast, annual disease risk estimates for DEC were in agreement with actual cases of diarrhea in the study area.

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A LIQUID CHROMATOGRAPHIC-TANDEM MASS SPECTROMETRIC METHOD FOR DETERMINATION OF ARTESUNATE AND ITS METABOLITE DIHYDROARTEMISININ IN HUMAN PLASMA

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A bioanalytical method for the analysis of artesunate and its metabolite dihydroartemisinin in human plasma using high throughput solid-phase extraction in the 96-wellplate format and liquid chromatography coupled to positive tandem mass spectroscopy has been developed and validated. The method was validated according to published FDA guidelines and showed excellent performance. The within-day and between-day precisions expressed as RSD, were lower than 7% at all tested concentrations including the lower limit of quantification. Using 50mul plasma the calibration range was 1.19-728ng/ml with a limit of detection at 0.5ng/ml for artesunate and 1.96-2500ng/ml with a limit of detection at 0.6ng/ml for dihydroartemisinin. Using 250mul of plasma sample the lower limit of quantification was decreased to 0.119ng/ml for artesunate and 0.196ng/ml dihydroartemisinin. Validation of over-curve samples in plasma ensured that accurate estimation would be possible with dilution if samples went outside the calibration range. The method was free from matrix effects as demonstrated both graphically and quantitatively.

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MAJOR PITFALLS IN THE MEASUREMENT OF ARTEMISININ DERIVATIVES IN PLASMA IN CLINICAL STUDIES

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A bioanalytical method for the analysis of artesunate (ARS) and its metabolite dihydroartemisinin (DHA) in human plasma using protein precipitation and liquid chromatography coupled to positive tandem mass spectroscopy was developed. The method was validated according to published US FDA-guidelines and showed excellent performance. However, when it was applied to clinical pharmacokinetic studies in malaria, variable degradation of the artemisinins introduced an unacceptable large source of error, rendering the assay useless. Haemolytic products related to sample collection and malaria infection degraded the compounds. Addition of organic solvents during sample processing and even low volume addition of the internal standard in an organic solvent caused degradation. A solid phase extraction method avoiding organic solvents eliminated problems arising from haemolysis induced degradation. Plasma esterases mediated only approximately 20% of ex vivo hydrolysis of ARS into DHA. There are multiple sources of major preventable error in measuring ARS and DHA in plasma samples from clinical trials. These various pitfalls have undoubtedly contributed to the large inter-subject variation in plasma concentration profiles and derived pharmacokinetic parameters for these important antimalarial drugs.

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AUDITORY ASSESSMENT OF PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA TREATED WITH THREE-DAY MEFLOQUINE-ARTESUNATE ON THE NORTH-WESTERN BORDER OF THAILAND

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BACKGROUND: The use of artemisinin derivatives has increased exponentially with the deployment of artemisinin combination therapy (ACT) in all malarious areas. They are highly effective and are considered safe, but in animal studies artemisinin derivatives produce neurotoxicity targeting mainly the auditory and vestibular pathways. The debate remains as to whether artemisinin derivatives induce similar toxicity in humans.

METHODS: This prospective study assessed the effects on auditory function of a standard 3-day oral dose of artesunate (4 mg/kg/day) combined with mefloquine (25 mg/kg) in patients with acute uncomplicated falciparum malaria treated at the Shoklo Malaria Research Unit, on the Thai-Burmese border. A complete auditory evaluation with tympanometry, audiometry and auditory brainstem responses (ABR) was performed before the first dose and seven days after initiation of the antimalarial treatment.

RESULTS: Complete auditory tests at day 0 (D0) and day 7 (D7) were obtained for 93 patients. Hearing loss (threshold > 25 dB) on admission was common (57%) and associated with age only. No patient had a threshold change exceeding 10 dB between D0 and D7 at any tested frequency. No patient showed a shift in Wave III peak latency of more than 0.30 msec between baseline and D7. CONCLUSION: Neither audiometric or the ABR tests showed clinical evidence of auditory toxicity seven days after receiving oral artesunate and mefloquine.

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DEVELOPMENT AND VALIDATION OF A HIGH-THROUGHPUT ZWITTERIONIC HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY SOLID-PHASE EXTRACTION-LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR DETERMINATION OF THE ANTI-INFLUENZA DRUG PERAMIVIR IN PLASMA

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An assay for the analysis for the quantification of the anti-influenza drug peramivir in human plasma using high-throughput zwitterionic (ZIC) hydrophilic interaction liquid chromatography (HILIC) solid-phase extraction (SPE) in a 96-wellplate format and liquid chromatography coupled to positive tandem mass spectroscopy has been developed and validated. The ZIC-HILIC SPE efficiently removed sources of interference present in the supernatant after protein precipitation of plasma proteins. The main advantage of the ZIC-HILIC SPE sample preparation step was that it allowed load and elution conditions to be optimised to extract only peramivir and minimize co-extraction of lipophilic phospholipids. The method was validated according to published US Food and Drugs Administration guidelines and showed excellent performance. The assay was validated over two calibration ranges (0.952-500 and 50-50,000ng/mL) to support analysis of peramivir after intra-venous administration. The lower limit of quantification for peramivir in plasma was 1ng/mL and the upper limit of quantification was 50,000ng/mL. The within-day and between-day precisions expressed as RSD, were lower than 8% at all tested quality control concentrations and below 11% at the lower limit of quantification. Validation of over-curve samples ensured that it would be possible with dilution if samples went outside the calibration range.

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THROMBOCYTOPAENIA IN PREGNANT WOMEN WITH MALARIA ON THE THAI-BURMESE BORDER

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BACKGROUND: Haematological changes associated with malaria in pregnancy are not well documented, and have focused predominantly on anaemia. Examined here is thrombocytopaenia in pregnant women infected with *Plasmodium falciparum* or *Plasmodium vivax* in a low transmission area on the north-western border of Thailand.

METHODS: In this observational study we reviewed the platelet counts from routine complete blood count (CBC) in a cohort of healthy and malaria infected Karen pregnant women attending weekly antenatal clinics. A platelet count of 75,000/microL was the threshold at 2 standard deviations below the mean for healthy pregnant women used to indicate thrombocytopenia. Differences in platelet counts in non-pregnant and pregnant women were compared after matching for age, symptoms, malaria species and parasitaemia.

RESULTS: In total 974 pregnant women had 1,558 CBC measurements between February 2004 and September 2006. The median platelet counts (/microL) were significantly lower in patients with an episode of falciparum 134,000 [11,000-690,000] (N = 694) or vivax malaria 184,000 [23,000-891,000] (N = 523) compared to healthy pregnant women 256,000 [64,000-781,000] (N = 255), P < 0.05 for both comparisons. *Plasmodium falciparum* and *P. vivax* caused a 34% (95% CI 24-47) and 22% (95% CI 8-36) reduction in platelet count, respectively. Pregnant compared to non pregnant women were at higher risk OR = 2.27 (95%CI 1.16-4.4) P = 0.017, for thrombocytopaenia. Platelets counts were higher in first compared with subsequent malaria infections within the same pregnancy. Malaria associated thrombocytopaenia had a median [range] time for recovery of 7 234567891011121314 days which did not differ by antimalarial treatment (P = 0.86), or species (P = 0.63) and was not associated with active bleeding.

CONCLUSION: Pregnant women become more thrombocytopenic than non-pregnant women with acute uncomplicated malaria. Uncomplicated malaria associated thrombocytopaenia is seldom severe. Prompt antimalarial treatment resulted in normalization of platelet counts within a week.

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CHLOROQUINE PHARMACOKINETICS IN PREGNANT AND NONPREGNANT WOMEN WITH VIVAX MALARIA

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PURPOSE: We compared the pharmacokinetics of chloroquine in pregnant and nonpregnant women treated for *Plasmodium vivax* malaria.

METHODS: Twelve pregnant women and 15 nonpregnant women of child-bearing age with acute *P. vivax* malaria were treated with 25 mg chloroquine base/kg over 3 days on the northwestern border of Thailand. Blood concentrations of chloroquine and desethylchloroquine were measured using hydrophilic interaction liquid chromatography coupled with fluorescence detection. Twenty-five women completed the pharmacokinetic study.

RESULTS: Although increasing gestational age was associated with reduced chloroquine AUC0-->infinity, there was no significant difference overall in the pharmacokinetics of chloroquine between pregnant and nonpregnant women. Fever was associated with lower chloroquine AUC0-->infinity values. Desethylchloroquine area under the curve (AUC) values were not significantly affected by pregnancy.

CONCLUSIONS: Pregnancy did not significantly affect blood concentrations of chloroquine or its metabolite, desethylchloroquine, in women with *P. vivax* malaria.

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MMP CELLULAR RESPONSES TO DENGUE VIRUS INFECTION-INDUCED VASCULAR LEAKAGE

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Dengue hemorrhagic fever and dengue shock syndrome, the major life-threatening outcomes of severe dengue disease, are the consequence of plasma leakage in the vascular areas. We previously demonstrated that dengue virus (DV)-infected dendritic cells (DC) trigger vascular leakage through matrix metalloproteinase (MMP)-9 overproduction, however little is known concerning the consequences of direct infection of macrovascular endothelial cells (MVEC) by DV. In this study, we show that infection of primary human MVEC results in overproduction of MMP-2 and to a lesser extent of MMP-9, leading to enhanced endothelial permeability. This permeability was associated with loss of expression of the vascular endothelium-cadherin cell-cell adhesion. The MMP response to DV infection is strikingly different between DC and MVEC. Therefore, our results demonstrated that endothelial cells are an important target for DV infection, and that DV-induced MMP-2 overproduction by direct infection of endothelial cells may contribute to the pathogenesis of severe dengue infection.

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LONGITUDINAL STUDY OF *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN A KAREN POPULATION IN THAILAND

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BACKGROUND: Clinical case treatment of malaria infections where *Plasmodium falciparum* and *Plasmodium vivax* are sympatric has achieved effective reductions in *P. falciparum* prevalence and incidence rates, but has been less successful for *P. vivax*. The high transmissibility of *P. vivax* and its capacity to relapse have been suggested to make it a harder parasite species to control.

METHODS: A clinical malaria case treatment programme was carried out over a decade in a Karen community composed of seven hamlets on the Thai-Myanmar border.

RESULTS: From 1994 to 2004, prevalence rates of both *P. falciparum* and *P. vivax* decreased by 70-90% in six of the seven study hamlets, but were unchanged in one hamlet. Overall, incidence rates decreased by 72% and 76% for *P. falciparum* and *P. vivax* respectively over the period 1999-2004. The age-incidence and prevalence curves suggested that *P. vivax* was more transmissible than *P. falciparum* despite a greater overall burden of infection with *P. falciparum*. Male gender was associated with increased risk of clinical presentation with either parasite species. Children (< 15 years old) had an increased risk of presenting with *P. vivax* but not *P. falciparum*.

CONCLUSION: There was a considerable reduction in incidence rates of both *P. vivax* and *P. falciparum* over a decade following implementation of a case treatment programme. The concern that intervention methods would inadvertently favour one species over another, or even lead to an increase in one parasite species, does not appear to be fulfilled in this case.

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TEMPORAL PATTERNS AND FORECAST OF DENGUE INFECTION IN NORTHEASTERN THAILAND

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This study aimed to determine temporal patterns and develop a forecasting model for dengue incidence in northeastern Thailand. Reported cases were obtained from the Thailand national surveillance system. The temporal patterns were displayed by plotting monthly rates, the seasonal-trend decomposition procedure based on loess (STL) was performed using R 2.2.1 software, and the trend was assessed using Poisson regression. The forecasting model for dengue incidence was performed in R 2.2.1 and Intercooled Stata 9.2 using the seasonal Autoregressive Integrated Moving Average (ARIMA) model. The model was evaluated by comparing predicted versus actual rates of dengue for 1996 to 2005 and used to forecast monthly rates during January to December 2006. The results reveal that epidemics occurred every two years, with approximately three years per epidemic, and that the next epidemic will take place in 2006 to 2008. It was found that if a month increased, the rate ratio for dengue infection decreased by a factor 0.9919 for overall region and 0.9776 to 0.9984 for individual provinces. The amplitude of the peak, which was evident in June or July, was 11.32 to 88.08 times greater than the rest of the year. The seasonal ARIMA (2, 1, 0) (0, 1, 1)12 model was model with the best fit for regionwide data of total dengue incidence whereas the models with the best fit varied by province. The forecasted regional monthly rates during January to December 2006 should range from 0.27 to 17.89 per 100,000 population. The peak for 2006 should be much higher than the peak for 2005. The highest peaks in 2006 should be in Loei, Buri Ram, Surin, Nakhon Phanom, and Ubon Ratchathani Provinces.

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DIHYDROARTEMISININ-PIPERAQUINE RESCUE TREATMENT OF MULTIDRUG-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA IN PREGNANCY: A PRELIMINARY REPORT

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Dihydroartemisinin-piperaquine (DHA-PPQ) is a promising new artemisinin combination treatment. There are no published data on the intentional use of the drug in pregnancy. Between June 2006 and January 2007, 50 Karen pregnant women with recurrent *P. falciparum* infections, despite 7-day treatments with quinine or artesunate (+/- clindamycin) or both, were treated with DHA-PPQ. This rescue treatment was effective and well tolerated and there was no evidence of toxicity for the mothers or the fetus. The PCR adjusted cure rate by Kaplan Meier analysis at day 63 was 92.2% (95% CI: 76.9-97.4).

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CORRELATION OF CIRCULATING FULL-LENGTH VISFATIN (PBEF/NAMPT) WITH METABOLIC PARAMETERS IN SUBJECTS WITH AND WITHOUT DIABETES: A CROSS-SECTIONAL STUDY

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Objective Here we use a novel ELISA that is specific for full-length visfatin (PBEF/NAMPT), compare it with the existing

C-terminal based assay and use it to Investigate associations of visfatin with metabolic parameters.

Design, patients and measurements

We established the specificity and full-length visfatin with clinical, anthro- Pometric and metabolic parameters in a cross sectional study of 129 Thai subjects, consisting of 50 outpatients with type 2 diabetes and 79 healthy volunteers.

Results The new ELISA accurately recovered full-length recombinant visfatin and detected visfatin secreted by primary human and rat adipocytes. We found serum full-length visfatin was significantly higher in subjects with diabetes compared to their nondiabetic peers (median 2.75 vs. 2.22 ng/ml, $P = 0.0142$). After adjustment for age, gender and traditional metabolic risk factors, adjusted mean visfatin remined signifycantly higher in the diabetes group (3.80 vs.2.10 ng/ml, $P = 0.0021$). On spearman vnivariate correlation analysis, visfatin was significantly associated with Resistin ($r = 0.30$m $P = 0.0011$), but not with any other anthropometric or metabolic variables, Including adiponectin multimers. On multiple linear regression analysis, the only covariates independently associated with visfatin were diabetes ($t = 3.11$, $P = 0.0024$) and log resistin ($t = 2.68$, $\boldsymbol{P} = 0.0086$).

Conclusions Circulating visfatin is independently associated with diabetes and resistin concentration, but is not related to adiponectin multimers or other metabolic covariates. These data are suggestive of a potential role visfatin in sub-clinical inflammatory states.

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ALLELIC LOSS ON CHROMOSOME 5Q34 IS ASSOCIATED WITH POOR PROGNOSIS IN HEPATOCELLULAR CARCINOMA

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Purpose: To identify and characterize novel genetic alterations in hepatocellular Carcinoma (HCC).

Methods: DNA was extracted from 29 HCC and corresponding normal tissues and amplified with 59 different 10 base arbitrary primers. A 550 bp DNA fragment amplified using primer Q-9 and which was present in 19 of 29 cases (66%) was cloned, sequenced, and compared with known nucleotide sequences deposited in Genome database, and Quantified by real-time PCR.

Results: DNA alterations were found on chromosomes 5q34, 6p25.2 and 8q12.1 in 11 of 29 cases (38%), 7 of 29 cases (24%), and 12 of 29 cases (41%), respectively. Multivariate analysis showed that the allelic loss on chromosome 5q34 was an independent prognostic factor for poor survival of HCC patients, with the median survival time of 19 weeks for allelic loss versus 109 weeks for no allelic loss ($P = 0.001$).

Conclusions: This study indicates that allelic loss on chromosome 5q34 may be involved in the development of HCC and could be used as a prognostic indicator in HCC patients.

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HOST VASCULAR ENDOTHELIAL GROWTH FACTOR IS A TROPHIC FACTOR FOR *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS

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Plasmodium falciparum, the protozoan parasite responsible for severe malaria infection, undergoes a complex life cycle including intraerythrocytic development in the human host. Infected red blood cells (iRBC) sequestered in host cerebral microvessels show positive staining for Vascular Endothelial Growth Factor (VEGF) using immunohistochemistry on post mortem brain samples. Confocal microscopy of *in vitro* cultured PRBC revealed concentration of VEGF within the parasitophorous vacuole. iRBC cultured in serum free media failed to release VEGF. Confocal microscopy showed increased host VEGF-R1 and -R2 expression on the surface of infected compared to uninfected control RBC, but no concentration of VEGF-R within the parasitophorous vacuole. Addition of VEGF to parasite cultures showed a trophic effect on parasite growth, which was significantly increased when rescuing drug treated parasites. These effects were abrogated when parasites were grown in serum free media, suggesting a requirement for soluble VEGF-R from serum. As the parasite produces no homologues to human VEGF or VEGF-R, we conclude that *P. falciparum* iRBC show increased VEGF-R expression on the red cells membrane, and concentrate host VEGF within the parasitophorous vacuole. This may have a trophic effect on parasite growth or represent a stress response to drug treatment.

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Poster presentation at: 17th International Congress for Tropical Medicine and Malaria, Jeju, korea, 29 September-3 October 2008.

IMMUNOCHEMICAL DETECTION OF GROWTH HORMONE AND PROLACTIN CELLS IN THE PITUITARY GLAND OF THE MOUNTAIN FROG (*RANA BLYTHII*)

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Immunocytochemistry and immunogold techniques were employed to classify and localize the cell producing growth hormone (GH) and prolactin (PRL) in the pars distalis of the mountain frog (*Rana blythii*) pituitary. The pituitary glands obtained from mature frogs during the breeding season (November-February) were fixed in Bouin's fluids for an immunocytochemical study, and in 0.2% glutaraldehyde, 2% paraformaldehyde, and 0.5% picric acid in 0.05M Millonig's phosphate buffer pH 7.4 at 4°C for an immunogold study. By immunocytochemical study, GH immunoreactive cells appeared as round or oval shaped,

concentrated mainly in the dorso-posterior region of pars distalis. PRL immunoreactive cells were elongated in shape and scattered throughout the pars distalis. By immunogold technique, GH cells were identified by the presence of lipid droplets and contained three types of granules; round-shaped (356.2±4.1 nm in diameter), rod-shaped (196.1±4.1 nm in width and 345.6±5.3 nm in length), and irregular shaped (139.5-310.8 nm in width and 223.3-459.7 nm in length). PRL cells exhibited round granules of moderate electron density (355.2±5.3 nm in diameter).

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EXPRESSION OF PRO-INFLAMMATORY PROTEIN, INOS, VEGF AND COX-2 IN ORAL SQUAMOUS CELL CARCINOMA (OSCC), RELATIONSHIP WITH ANGIOGENESIS AND THEIR CLINICO-PATHOLOGICAL CORRELATION

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One main etiology for oral squamous cell carcinoma (OSCC) is inflammation. Inducible nitric oxide synthase (iNOS), 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 objective of this research was to study the expression and relationship amon 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 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 metastasis and tumor staging (TNM) of the patients and angiogenesis. VEGF shows correlation with tumor grading, tumor staging and angiogenesis. COX-2 shows correlation with cervical lymph node metastasis. In conclusion, the expression of iNOS, VEGF and COX-2 exists in OSCC. The data provided show the expression of these chemical mediators associated with carcinogenesis and angiogenesis in OSCC. This could constitute a primary database before using anti-angiogenesis drug against these mediators for OSCC treatment.

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GASTROINTESTINAL AND LIVER INVOLVEMENT IN FALCIPARUM MALARIA

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Nausea and vomiting are common symptoms found in falciparum malaria, particularly in children with high fever. Vomiting may be provoked by antimalarial drug treatment. While diarrhea has been reported from some parts of the world, it appears to be uncommon in others. Although malabsorption of amino acids, sugars, fats, chloroquine and quinine have been documented, in most studies the absorption of oral antimalarials in uncomplicated malaria has been normal in even the most seriously ill patients. In the acute phase of severe falciparum malaria, patients show the greatly reduced absorption of those sugars that rely on mediated mechanisms and unmediated diffusion. Absorption returns to normal in convalescence. Gastric emptying is normal in uncomplicated falciparum malaria. Biopsies of the gut show parasite sequestration in the vascular bed which presumably interferes with the process of absorption. Gastrointestinal permeability is increased during severe and uncomplicated falciparum malaria but reverts to normal in convalescence. Endotoxemia may also originate in the gut. Gram-negative bacteria or endotoxin may shift from the gut lumen; the normal hepatic clearance mechanisms may fail.

Hyperbilirubinemia is attributable to intravascular hemolysis of parasitized erythrocytes and to hepatic dysfunction and possibly to an element of microangiopathic hemolysis associated with disseminated intravascular coagulation. Impairment of hepatic function is common in severe malaria. Unfortunately, assessment of liver function by measurement of blood concentrations of bilirubin and liver-related enzymes is notoriously imprecise, particularly in the presence of coexisting hemolysis. Jaundice is more common in adults with severe malaria than in children. The measurable consequences of hepatic dysfunction are coagulation abnormalities resulting from failure of clotting factor syntheses, hypoalbuminemia, and reduced metabolic clearance of many substances, including alanine, lactate, and antimalarial drugs. The biotransformation of drugs, particularly those metabolized by hepatic microsomal enzymes, is reduced in proportion to disease severity. Hepatic blood flow is reduced during acute malaria and is significantly lower in severe malaria than in uncomplicated malaria. It returns to normal during convalescence. Hepatic microsomal metabolism is also apparently slow in severe falciparum malaria but reverts to normal in convalescence. Liver metabolic function does not appear to be significantly affected in uncomplicated malaria.

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HIGH PREVALENCE OF MICROSPORIDIUM INFECTION IN HIV-INFECTED PATIENTS

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Sixty-four patients confirmed to be infected with the human immunodeficiency virus (HIV) participated in the study to determine

the opportunistic enteric pathogens and compare with the pertinent clinical status. The leading prevalence of opportunistic enteric pathogens was microsporidium (81.25%), followed by *Cryptosporidium parvum* (20.31%), *Candida albicans* (12.50%) and *Blastocystis hominis* (10.94%). Less frequently found pathogens were *Giardia intestinalis* (6.25%), *Cyclospora* (4.69%), *Opishorchis viverrini* ova (3.12%), *Strongyloides stercoralis* larvae (3.12%) and hookworm ova (1.56%). There was no statistical significance between the clinical data and the presence of enteric pathogens. The study found a high prevalence rate of the emergence microsporidium based on microscopic examination. This confirms worldwide importance of microsporidium in HIV-infected/AIDS patients and the necessity for stool evaluation in all HIV-infected patients.

CASE REPORT UNDIAGNOSED AMOEBIC BRAIN ABSCESS

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We report a case of amoebic brain abscess due to *Entamoeba histolytica*. The patient was a 31 year old man who presented with amoebic liver abscess. With proper treatment and drainage, his clinical course still deteriorated. He developed delirium, became lethargic and expired. With the history of heroin addiction, withdrawal syndrome from heroin was suspected. At autopsy, amoebic abscesses were detected in the liver, large intestine, meninges and brain. A huge amoebic liver abscess, measuring 19 cm in diameter was seen at the right lobe of the liver. Amoebic brain abscess, 4 cm in diameter was located at the right occipital lobe. Microscopically, the tissue sections from the affected organs were confirmed with the presence of degenerated *E. histolytica* trophozoites. Involvement of the brain in amoebic liver abscess should be suspected in patients with neurological changes.

AGE-SPECIFIC PREVALENCE OF DENGUE ANTIBODIES IN BANGKOK INFANTS AND CHILDREN

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Seroprevalence of dengue antibodies were determined in healthy children in Bangkok. At 9, 12, and 18 months of age 23%, 9%, and 17% of children respectively had neutralizing antibodies against one or more serotypes. DEN1 was the most prevalent, followed by DEN3, DEN2, and DEN4. Twelve children had serologic evidence of dengue infection. The nadir of dengue antibodies in children was at 12 months of age. In highly endemic areas, dengue vaccination could be needed at an early age.

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SCIENTIFIC CONSULTATION ON IMMUNOLOGICAL CORRELATES OF PROTECTION INDEUCED BY DENGUE VACCINES. REPORT FROM A MEETING HELD AT THE WORLD HEALTH ORGANIZATION 17-18 NOVEMBER 2005

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Several dengue vaccine candidates have been evaluated in early clinical phase, and some are scheduled for efficacy testing in population-based studies. Given the advancements in dengue vaccine development, there is an increased interest in identifying immunological correlates of protection for these vaccines in order to facilitate their evaluation, further refinement, production and registration. To this end, the WHO Initiative for Vaccine Research (IVR) convened a consultation on primary and secondary immunological correlates of protection induced by dengue vaccines. The meeting was held on the 17th and 18th of November, 2005 at WHO headquarters in Geneva. The consultation was a first dedicated review of the available data in support of establishing correlates. It is concluded that it is not yet possible to define one specific set of correlates, the consultation concluded in recommendations that should help to gather the missing evidence in conjunction with future vaccine trials.

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LOW SERUM VITAMIN B12 IN ALZHEIMER'S PATIENTS AS DETECTED BY A SOLID PHASE RADIOIMMUNOASSAY

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Objective: to determine the relationship between serum levels of vitamin B12 and folate in AD and other types of dementia in Thai patents.

Methods: One hundred and eleven Thai subjects were classified into 3 groups: 32 AD patients, 43 non-AD dementia patients and 36 age-matched controls. Serum concentrations of vitamin B12 and folate were measured using a solid phase radioimmunoassay.

Results: Serum vitamin B12 levels were found to be significantly lower in AD and non-AD dementia patients than in age-matched controls. There is a significant relationship between Mini-Mental State Examination score and vitamin B12 level in AD and non-AD dementia groups. However, there is no significant difference in serum folate in AD and non-AD dementia groups when compared to age-matched controls.

Conclusion: These findings suggest the need for vitamin B12 supplementation in AD and non-AD dementia patients.

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EVALUATION OF THE PANBIO DENGUE VIRUS NONSTRUCTURAL 1 ANTIGEN DETECTION AND IMMUNOGLOBULIN M ANTIBODY ENZYME-LINKED IMMUNOSORBENT ASSAYS 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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GENETIC TYPING OF THE 56-KDA TYPE-SPECIFIC ANTIGEN GENE OF CONTEMPORARY *ORIENTIA TSUTSUGAMUSHI* ISOLATES CAUSING HUMAN SCRUB TYPHUS AT TWO SITES IN NORTH-EASTERN AND WESTERN THAILAND

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Orientia tsutsugamushi is the causative agent of scrub typhus, a major cause of febrile illness in the rural areas of Southeast Asia. Twenty-three strains of *O. tsutsugamushi* were isolated from patients with scrub typhus in north-east (Udorn Thani province) and western Thailand (Tak province) between 2003 and 2005. The isolates were characterized by sequencing the entire ORF of the 56-kDa-type-specific antigen gene, followed by phylogenetic analysis. The majority (15/23) of isolates clustered with the Karp-type strain, six with a Gilliam-type strain and one each with the T.

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CLINICAL SIGNIFICANCE OF SEQUESTRATION IN ADULTS WITH SEVERE MALARIA

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Reduced microcirculatory flow is a fundamental feature in the pathophysiology of severe *Plasmodium falciparum* malaria and sequestration of red blood cells containing mature parasites is considered a central cause of this. Direct microscopic observation of the microcirculation in the living patient with severe malaria has enabled us to quantify this phenomenon and link it to severity of disease, supporting the findings of pathology studies. Moreover, the sequestered parasite biomass, calculated from parasite derived plasma PfHRP2 concentrations, strongly correlates with disease severity. Artesunate prevents sequestration by killing ring form parasites, aborting their maturation, which can explain the mortality benefit of this drug compared to quinine in the treatment of adult severe malaria. Levamisole is currently tried as adjunctive treatment in severe malaria targeting sequestration.

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DIRECT *IN VIVO* ASSESSMENT OF MICROCIRCULATORY DYSFUCTION IN SEVERE FALCIPARUM MALARIA

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***BACKGROUND*:** This study sought to describe and quantify microcirculatory changes in the mucosal surfaces of patients with severe malaria, by direct in vivo observation using orthogonal polarization spectral (OPS) imaging.

METHODS: The microcirculation in the rectal mucosa of adult patients with severe malaria was assessed by use of OPS imaging, at admission and then daily. Comparison groups comprised patients with uncomplicated falciparum malaria, patients with bacterial sepsis, and healthy individuals.

RESULTS: Erythrocyte velocities were measured directly in 43 adult patients with severe falciparum malaria, of whom 20 died. Microcirculatory blood flow was markedly disturbed, with heterogeneous obstruction that was proportional to severity of disease. Blocked capillaries were found in 29 patients (67%) and were associated with concurrent hyperdynamic blood flow (erythrocyte velocity, >750 mm/s) in adjacent vessels in 27 patients (93%). The proportion of blocked capillaries correlated with the base deficit in plasma and with the concentration of lactate. Abnormalities disappeared when the patients recovered. In healthy individuals and in patients with uncomplicated malaria or sepsis, no stagnant erythrocytes were detected, and, in patients with sepsis, hyperdynamic blood flow was prominent.

CONCLUSION: Patients with severe falciparum malaria show extensive microvascular obstruction that is proportional to the severity of the disease. This finding underscores the prominent role that microvascular obstruction plays in the pathophysiology of severe malaria and illustrates the fundamental difference between the microvascular pathophysiology of malaria and that of bacterial sepsis

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THE RELATIONSHIP BETWEEN AGE AND THE MANIFESTATIONS OF AND MORTALITY ASSOCIATED WITH SEVERE MALARIA

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BACKGROUND: The reported case-fatality rate associated with severe malaria varies widely. Whether age is an independent risk factor is uncertain.

METHODS: In a large, multicenter treatment trial conducted in Asia, the presenting manifestations and outcome of severe malaria were analyzed in relation to age.

RESULTS: Among 1050 patients with severe malaria, the mortality increased stepwise, from 6.1% in children (age, <10 years) to 36.5% in patients aged >50 years (P<0.001). Compared with adults aged 21-50 years, the decreased risk of death among children (adjusted odds ratio, 0.06; 95% confidence interval, 0.01-0.23; P<0.001) and the increased risk of death among patients aged >50 years (adjusted odds ratio, 1.88; 95% confidence interval, 1.01-3.52; P<0.001) was independent of the variation in presenting manifestations. The incidence of anemia and convulsions decreased with age, whereas the incidence of hyperparasitemia, jaundice, and renal insufficiency increased with age. Coma and metabolic acidosis did not vary with age and were the strongest predictors of a fatal outcome. The number of severity signs at hospital admission also had a strong prognostic value.

CONCLUSION: Presenting syndromes in severe malaria depend on age, although the incidence and the strong prognostic significance of coma and acidosis are similar at all ages. Age is an independent risk factor for a fatal outcome of the disease.

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THE RELATIONSHP BETWEEN THE HAEMOGLOBIN CONCENTRATION AND THE HAEMATOCRIT IN *PLASMODIUM FALCIPARUM* MALARIA

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Background: Malaria is a very important cause of anaemia in tropical countries. Anaemia is assessed either by measurement of the haematocrit or the haemoglobin concentration. For comparisons across studies, it is often necessary to derive one measure from the other.

Methods: Data on patients with slide-confirmed uncomplicated falciparum malaria were pooled from 85 antimalarial drug trials conducted in 25 different countries, to assess the haemoglobin/haematocrit relationship at different time points in malaria. Using a linear random effects model, a conversion equation for haematocrit was derived based on 3,254 measurements from various time points (ranging from day 0 to day 63) from 1,810 patients with simultaneous measurements of both parameters. Haemoglobin was also estimated from haematocrit with the commonly used threefold conversion.

Results: A good fit was obtained using Haematocrit = 5.62 + 2.60 * Haemoglobin. On average, haematocrit/3 levels were slightly higher than haemoglobin measurements with a mean difference (± SD) of -0.69 (± 1.3) for children under the age of 5 (n = 1,440 measurements from 449 patients).

Conclusion: Based on this large data set, an accurate and robust conversion factor both in acute malaria and in convalescence was obtained. The commonly used threefold conversion is also valid.

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STRONGER ACTIVITY OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 PROTEASE INHIBITORS AGAINST CLINICAL ISOLATES OF *PLASMODIUM VIVAX* THEN AGAINST THOSE OF *P. FALCIPARUM*

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Recent studies using laboratory clones have demonstrated that several antiretroviral protease inhibitors (PIs) inhibit the growth of *Plasmodium falciparum* at concentrations that may be of clinical significance, especially during human immunodeficiency virus type 1 (HIV-1) and malaria coinfection. Using clinical isolates, we now demonstrate the in vitro effectiveness of two HIV-1 aspartic PIs, saquinavir (SQV) and ritonavir (RTV), against *P. vivax* (n = 30) and *P. falciparum* (n = 20) from populations subjected to high levels of mefloquine and artesunate pressure on the Thailand-Myanmar border. The median 50% inhibitory concentration values of *P. vivax* to RTV and SQV were 2,233 nM (range, 732 to 7,738 nM) and 4,230 nM (range, 1,326 to 8,452 nM), respectively, both within the therapeutic concentration range commonly found for patients treated with these PIs. RTV was fourfold more effective at inhibiting *P. vivax* than it was at inhibiting *P. falciparum*, compared to a twofold difference in SQV sensitivity. An increased *P. falciparum* mdr1 copy number was present in 33% (3/9) of isolates and that of *P. vivax* mdr1 was present in 9% of isolates (2/22), but neither was associated with PI sensitivity. The inter-*Plasmodium* sp. variations in PI sensitivity indicate key differences between *P. vivax* and *P. falciparum*. PI-containing antiretroviral regimens may demonstrate prophylactic activity against both vivax and falciparum malaria in HIV-infected patients who reside in areas where multidrug-resistant *P. vivax* or *P. falciparum* is found

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DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHIC-TANDEM MASS SPECTROMETRIC METHOD FOR DETERMINATION OF PIPERAQUINE IN PLASMA STABLE ISOTOPE LABELED INTERNAL STANDARD INTERNAL STANDARD DOES NOT ALWAYS COMPENSATE FOR MATRIX EFFECTS

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A bioanalytical method for the analysis of piperaquine in human plasma using off-line solid-phase extraction and liquid chromatography coupled to positive tandem mass spectroscopy has been developed and validated. It was found that a mobile phase with high pH (i.e. 10) led to better sensitivity than mobile phase combinations with low pH (i.e. 2.5–4.5) despite the use of positive electrospray and a basic analyte. The method was validated according to published FDA guidelines and showed excellent performance. The within-day and between-day precisions expressed as R.S.D., were lower than 7% at all tested concentrations (4.5, 20, 400 and 500 ng/mL) and below 10% at the lower limit of quantification (LLOQ) (1.5 ng/mL). The calibration range was 1.5–500 ng/mL with a limit of detection (LOD) at 0.38 ng/mL. Validation of over-curve samples ensured that it would be possible with dilution if samples went outside the calibration range. Matrix effects were thoroughly evaluated both graphically and quantitatively. Matrix effects originating from the sample clean-up (i.e. solid-phase extraction) procedure rather than the plasma background were responsible for the ion suppression seen in this study. Salts remaining from the buffers used in the solid-phase extraction suppressed the signals for both piperaquine and its deuterated internal standard. This had no effect on the quantification of piperaquine. Triethylamine residues remaining after evaporation of the solid-phase extraction eluate were found to suppress the signals for piperaquine and its deuterated internal standard differently. It was found that this could lead to an underestimation of the true concentration with 50% despite the use of a deuterated internal standard.

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CLINICALLY UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA WITH HIGH SCHIZONTAEMIA: A CASE REPORT

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BACKGROUND: The treatment options for acute *Plasmodium falciparum* malaria are based on the clinician classifying the patient as uncomplicated or severe according to the clinical and parasitological findings. This process is not always straightforward.

CASE PRESENTATION: An adult male presented to a clinic on the western border of Thailand with a physical examination and *P. falciparum* trophozoite count (1.2% of infected red blood cells, IRBC) from malaria blood smear, consistent with a diagnosis of uncomplicated *P. falciparum* infection. However, the physician on duty treated the patient for severe malaria based on the reported *P. falciparum* schizont count, which was very high (0.3% IRBC), noticeably in relation to the trophozoite count and schizont:trophozoite ratio 0.25:1. On intravenous artesunate, the patient deteriorated clinically in the first 24 hours. The trophozoite count increased from 1.2% IRBC at baseline to 20.5% IRBC 18 hours following the start of treatment. By day three, the patient recovered and was discharged on day seven having completed a seven-day treatment with artesunate and mefloquine.

CONCLUSION: The malaria blood smear provides only a guide to the overall parasite biomass in the body, due to the ability of *P. falciparum* to sequester in the microvasculature. In severe malaria, high schizont counts are associated with worse prognosis. In low transmission areas or in non-immune travelers the presence of schizonts in the peripheral circulation is an indication for close patient supervision. In this case, an unusually high schizont count in a clinically uncomplicated patient was indicative of potential deterioration. Prompt treatment with intravenous artesunate is likely to have been responsible for the good clinical outcome in this case

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ADAPTIVE COPY NUMBER EVOLUTION IN MALARIA PARASITES

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Copy number polymorphism (CNP) is ubiquitous in eukaryotic genomes, but the degree to which this reflects the action of positive selection is poorly understood. The first gene in the Plasmodium folate biosynthesis pathway, GTP-cyclohydrolase I (gch1), shows extensive CNP. We provide compelling evidence that gch1 CNP is an adaptive consequence of selection by antifolate drugs, which target enzymes downstream in this pathway. (1) We compared gch1 CNP in parasites from Thailand (strong historical antifolate selection) with those from neighboring Laos (weak antifolate selection). Two percent of chromosomes had amplified copy number in Laos, while 72% carried

multiple (2-11) copies in Thailand, and differentiation exceeded that observed at 73 synonymous SNPs. (2) We found five amplicon types containing one to greater than six genes and spanning 1 to >11 kb, consistent with parallel evolution and strong selection for this gene amplification. gch1 was the only gene occurring in all amplicons suggesting that this locus is the target of selection. (3) We observed reduced microsatellite variation and increased linkage disequilibrium (LD) in a 900-kb region flanking gch1 in parasites from Thailand, consistent with rapid recent spread of chromosomes carrying multiple copies of gch1. (4) We found that parasites bearing dhfr-164L, which causes high-level resistance to antifolate drugs, carry significantly ($p = 0.00003$) higher copy numbers of gch1 than parasites bearing 164I, indicating functional association between genes located on different chromosomes but linked in the same biochemical pathway. These results demonstrate that CNP at gch1 is adaptive and the associations with dhfr-164L strongly suggest a compensatory function. More generally, these data demonstrate how selection affects multiple enzymes in a single biochemical pathway, and suggest that investigation of structural variation may provide a fast-track to locating genes underlying adaptation.

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SIMPLE, RAPID AND SENSITIVE DETECTION OF *ORIENTIA TSUTSUGAMUSHI* BY LOOP-ISOTHERMAL DNA AMPLIFICATION

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We present a loop-mediated isothermal PCR assay (LAMP) targeting the groEL gene, which encodes the 60kDa heat shock protein of Orientia tsutsugamushi. Evaluation included testing of 63 samples of contemporary in vitro isolates, buffy coats and whole blood samples from patients with fever. Detection limits for LAMP were assessed by serial dilutions and quantitation by real-time PCR assay based on the same target gene: three copies/mul for linearized plasmids, 26 copies/mul for VERO cell culture isolates, 14 copies/mul for full blood samples and 41 copies/mul for clinical buffy coats. Based on a limited sample number, the LAMP assay is comparable in sensitivity with conventional nested PCR (56kDa gene), with limits of detection well below the range of known admission bacterial loads of patients with scrub typhus. This inexpensive method requires no sophisticated equipment or sample preparation, and may prove useful as a diagnostic assay in financially poor settings; however, it requires further prospective validation in the field setting.

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DIFFERENTIAL PATTERNS OF ENDOTHELIAL AND LEUCOCYTE ACTIVATION IN 'TYPHUS-LIKE' ILLNESSES IN LAOS AND THAILAND

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Scrub typhus is responsible for a large proportion of undifferentiated fevers in south-east Asia. The cellular tropism and pathophysiology of the causative agent, Orientia tsutsugamushi, remain poorly understood. We measured endothelial and leucocyte activation by soluble cell adhesion molecule enzyme-linked immunosorbent assays in 242 Lao and Thai patients with scrub or murine typhus, leptospirosis, dengue, typhoid and uncomplicated falciparum malaria on admission to hospital. Soluble E-selectin (sE-selectin) levels were lowest in dengue, sL-selectin highest in scrub typhus with a high sE-selectin to sL-selectin ratio in leptospirosis patients. In scrub typhus patients elevated sL-selectin levels correlated with the duration of skin rash ($P = 0.03$) and the presence of eschar ($P = 0.03$), elevated white blood cell (WBC) count ($P = 0.007$), elevated lymphocyte ($P = 0.007$) and neutrophil counts ($P = 0.015$) and elevated levels of sE-selectin correlated with the duration of illness before admission ($P = 0.03$), the presence of lymphadenopathy ($P = 0.033$) and eschar ($P = 0.03$), elevated WBC ($P = 0.005$) and neutrophil counts ($P = 0.0003$). In comparison, soluble selectin levels in murine typhus patients correlated only with elevated WBC counts ($P = 0.03$ for sE-selectin and sL-selectin). Soluble intercellular adhesion molecule-1 and soluble vascular adhesion molecule-1 levels were not associated significantly with any clinical parameters in scrub or murine typhus patients. The data presented suggest mononuclear cell activation in scrub typhus. As adhesion molecules direct leucocyte migration and induce inflammatory and immune responses, this may represent O. tsutsugamushi tropism during early dissemination, or local immune activation within the eschar.

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REAL-TIME MULTIPLEX PCR ASSAY FOR DETECTION AND DIFFERENTIATION OF RICKETTSIAE AND ORIENTIAE

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The high incidence of rickettsial diseases in Southeast Asia necessitates rapid and accurate diagnostic tools for a broad range of rickettsial agents, including Orientia tsutsugamushi (scrub typhus) and Rickettsia typhi (murine typhus), but also spotted fever group infections, which are increasingly reported. We present an SYBR-Green-based, real-time multiplex PCR assay for rapid identification and differentiation of scrub typhus group, typhus group and spotted fever group rickettsiae using 47kDa, gltA and ompB gene targets. Detection limits for amplification of these genes in reference strains ranged from 24 copies/microl, 5 copies/microl and 1 copy/microl in multiplex and 2 copies/microl, 1 copy/microl and 1 copy/microl in single template format, respectively. Differentiation by melt-curve analysis led to distinct melt temperatures for each group-specific amplicon. The assay was subjected to 54 samples, of which all cell-culture and 75% of characterised clinical buffy coat samples were correctly identified. Real-time PCR has the advantage of reliably detecting and differentiating rickettsial and orientia cell-culture isolates in a single-template assay, compared with the more time-consuming and laborious immunofluorescence assay. However, further optimisation and validation on samples taken directly from patients to assess its clinical diagnostic utility is required.

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APPLICATION OF CARBOHYDRATE MICROARRAY TECHNOLOGY FOR THE DETECTION OF *BURKHOLDERIA PSEUDOMALLEI, BACILLUS ANTHRACIS AND FRANCISELLA TULARENSIS* ANTIBODIES

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We developed a microarray platform by immobilizing bacterial 'signature' carbohydrates onto epoxide modified glass slides. The carbohydrate microarray platform was probed with sera from non-melioidosis and melioidosis (*Burkholderia pseudomallei*) individuals. The platform was also probed with sera from rabbits vaccinated with *Bacillus anthracis* spores and *Francisella tularensis* bacteria. By employing this microarray platform, we were able to detect and differentiate *B. pseudomallei*, *B. anthracis* and *F. tularensis* antibodies in infected patients, and infected or vaccinated animals. These antibodies were absent in the sera of naý¨ve test subjects. The advantages of the carbohydrate microarray technology over the traditional indirect hemagglutination and microagglutination tests for the serodiagnosis of melioidosis and tularemia are discussed. Furthermore, this array is a multiplex carbohydrate microarray for the detection of all three biothreat bacterial infections including melioidosis, anthrax and tularemia with one, multivalent device. The implication is that this technology could be expanded to include a wide array of infectious and biothreat agents.

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MANAGEMENT OF ACCIDENTAL LABORATORY EXPOSURE TO *BURKHOLDERIA PSEUDOMELLEI* AND *B. MALLEI*

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The gram-negative bacillus *Burkholderia pseudomallei* is a saprophyte and the cause of melioidosis. Natural infection is most commonly reported in northeast Thailand and northern Australia but also occurs in other parts of Asia, South America, and the Caribbean. Melioidosis develops after bacterial inoculation or inhalation, often in relation to occupational exposure in areas where the disease is endemic. Clinical infection has a peak incidence between the fourth and fifth decades; with diabetes mellitus, excess alcohol consumption, chronic renal failure, and chronic lung disease acting as independent risk factors. Most affected adults (approximately 80%) in northeast Thailand, northern Australia, and Malaysia have >/=1 underlying diseases. Symptoms of melioidosis may be exhibited many years after exposure, commonly in association with an alteration in immune status. Manifestations of disease are extremely broad ranging and form a spectrum from rapidly life-threatening sepsis to chronic low-grade infection. A common clinical picture is that of sepsis associated with bacterial dissemination to distant sites, frequently causing concomitant pneumonia and liver and splenic abscesses. Infection may also occur in bone, joints, skin, soft tissue, or the prostate. The clinical symptoms of melioidosis mimic those of many other diseases; thus, differentiating between melioidosis and other acute and chronic bacterial infections, including tuberculosis, is often impossible. Confirmation of the diagnosis relies on good practices for specimen collection, laboratory culture, and isolation of *B. pseudomallei*. The overall mortality rate of infected persons is 50% in northeast Thailand (35% in children) and 19% in Australia.

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PUBLIC HEALTH IMPACT OF ESTABLISHING THE CAUSE OF BACTERIAL INFECTIONS IN RURAL ASIA

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Recent studies delineating bacterial causes of fever in rural Asia indicate a major role for several previously under-recognized pathogens, including Rickettsia and Leptospira. The use of blood culture for the first time to investigate patients with febrile illness in rural Asia has also revealed some unexpected findings, e.g. Staphylococcus aureus is the major cause of bacteraemia in children aged <1 year in Laos. The spread of antimicrobial-resistant pathogens such as MRSA into rural Asia has already occurred and requires monitoring. These factors have major implications for empirical therapy of fever. Initiatives are urgently needed to strengthen the infrastructure of microbiology in rural Asia.

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SPREAD OF ANTIMALARIAL DRUG RESISTANCE: MATHEMATICAL MODEL WITH IMPLICATIONS FOR ACT DRUG POLICIES

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Background: Most malaria-endemic countries are implementing a change in anti-malarial drug policy to artemisinin-based combination therapy (ACT). The impact of different drug choices and implementation strategies is uncertain. Data from many epidemiological studies in different levels of malaria endemicity and in areas with the highest prevalence of drug resistance like borders of Thailand are certainly valuable. Formulating an appropriate dynamic data-driven model is a powerful predictive tool for exploring the impact of these strategies quantitatively.

Methods: A comprehensive model was constructed incorporating important epidemiological and biological factors of human, mosquito, parasite and treatment. The iterative process of developing the model, identifying data needed, and parameterization has been taken to strongly link the model to the empirical evidence. The model provides quantitative measures of outcomes, such as malaria prevalence/incidence and treatment failure, and illustrates the spread of resistance in low and high transmission settings. The model was used to evaluate different anti-malarial policy options focusing on ACT deployment.

Results: The model predicts robustly that in low transmission settings drug resistance spreads faster than in high transmission settings, and treatment failure is the main force driving the spread of drug resistance. In low transmission settings, ACT slows the spread of drug resistance to a partner drug, especially at high coverage rates. This effect decreases exponentially with increasing delay in deploying the ACT and decreasing rates of coverage. In the high transmission settings, however, drug resistance is driven by the proportion of the human population with a residual drug level, which gives resistant parasites some survival advantage. The spread of drug resistance could be slowed down by controlling presumptive drug use and avoiding the use of combination therapies containing drugs with mismatched half-lives, together with reducing malaria transmission through vector control measures.

Conclusion: This paper has demonstrated the use of a comprehensive mathematical model to describe malaria transmission and the spread of drug resistance. The model is strongly linked to the empirical evidence obtained from extensive data available from various sources. This model can be a useful tool to inform the design of treatment policies, particularly at a time when ACT has been endorsed by WHO as first-line treatment for falciparum malaria worldwide.

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THE CORE AND ACCESSORY GENOMES OF *BURKHOLDERIA PSEUDOMALLEI:* IMPLICATIONS FOR HUMAN MELIOIDOSIS

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Natural isolates of Burkholderia pseudomallei (Bp), the causative agent of melioidosis, can exhibit significant ecological flexibility that is likely reflective of a dynamic genome. Using whole-genome Bp microarrays, we examined patterns of gene presence and absence across 94 South East Asian strains isolated from a variety of clinical, environmental, or animal sources. 86% of the Bp K96243 reference genome was common to all the strains representing the Bp "core genome", comprising genes largely involved in essential functions (eg amino acid metabolism, protein translation). In contrast, 14% of the K96243 genome was variably present across the isolates. This Bp accessory genome encompassed multiple genomic islands (GIs), paralogous genes, and insertions/deletions, including three distinct lipopolysaccharide (LPS)-related gene clusters. Strikingly, strains recovered from cases of human melioidosis clustered on a tree based on accessory gene content, and were significantly more likely to harbor certain GIs compared to animal and environmental isolates. Consistent with the inference that the GIs may contribute to pathogenesis, experimental mutation of BPSS2053, a GI gene, reduced microbial adherence to human epithelial cells. Our results suggest that the Bp accessory genome is likely to play an important role in microbial adaptation and virulence.

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ANALYSES OF VACCINATION PROTOCOLS FOR *LEPTOSPIRA INTERROGANS* SEROVAR AUTUMNALIS IN HAMSTERS

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Leptospirosis, caused by Leptospira spp., is a zoonotic disease found worldwide. Killed whole cell leptospiral vaccines have been used as effective vaccines to elicit specific antibodies for protection. However, the involvement of cytokine responses after vaccination is not well characterized. Hamsters were immunized with killed L. interrogans serovar Autumnalis before challenge to study cytokine mRNA expression levels (interferon [IFN]-gamma, tumor necrosis factor [TNF]-alpha, interleukin [IL]-10, and IL-4). Vaccinated groups showed 92-100% survival rates, whereas control hamsters died within 6-10 days. However, live organisms were detected in vaccinated groups, and mild to moderate pathology was observed early in infection. IFN-gamma and TNF-alpha mRNA expression levels correlated with the severity of infection and lung pathology, whereas IL-4 and IL-10 expression levels were significantly higher in vaccinated groups. In summary, commonly used vaccines changed the cytokine profiles and protected hamsters from death but failed to stimulate sterile immunity and were unable to prevent the occurrence of pathology.

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PHARMACOKINETIC DETERMINANTS OF THE WINDOW OF SELECTION FOR ANTIMALARIAL DRUG RESISTANCE

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The selection and spread of antimalarial drug resistance pose enormous challenges to the health of people living in tropical countries. Most antimalarial drugs are slowly eliminated and so, following treatment in areas of endemicity, provide a gradient of concentrations to which newly acquired parasites are exposed. There is a variable period during which a new blood-stage infection with resistant malaria parasites can emerge from the liver and subsequently produce gametocyte densities sufficient for transmission while reinfection by sensitive parasites is still suppressed. This "window of selection" drives the spread of resistance. We have examined the factors which determine the duration of this window and, thus, the resistance selection pressure. The duration ranges from zero to several months and is dependent on the degree of

parasite resistance, the slope of the concentration-effect relationship, and the elimination kinetics of the antimalarial drug. The time at which the window opens and the duration of opening are both linear functions of the terminal elimination half-life. Because of competition from sibling susceptible parasites, the greater risks of extinction with low starting numbers, and opening of the window only when blood concentrations have fallen below the MIC, the window of selection for de novo resistance is narrower than that for resistance acquired elsewhere. The windows were examined for the currently available antimalarials. Drugs with elimination half-lives of less than 1 day, such as the artemisinins and quinine, do not select for resistance during the elimination phase.

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AMPLIFICATION OF PVMDR1 ASSOCIATED WITH MULTIDRUG-RESISTANT *PLASMODIUM VIVAX*

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BACKGROUND: Multidrug-resistant strains of *Plasmodium vivax* are emerging in Southeast Asia.

METHODS: *In vitro* drug susceptibility and *pvmdr1* genotype were determined in P. vivax field isolates from Indonesia and Thailand.

RESULTS: Increased *pvmdr1* copy number was present in 21% of isolates from Thailand (15/71) and none from Indonesia (0/114; [Formula: see text]). Compared with Indonesian isolates, the median IC(50) of Thai isolates was lower for chloroquine (36 vs. 114 nmol/L; [Formula: see text]) but higher for amodiaquine (34 vs. 13.7 nmol/L; [Formula: see text]), artesunate (8.33 vs. 1.58 nmol/L; [Formula: see text]), and mefloquine (111 vs. 9.87 nmol/L; [Formula: see text]). In 11 cryopreserved Thai isolates, those with increased *pvmdr1* copy number had a higher IC(50) for mefloquine (78.6 vs. 38 nmol/L for single-copy isolates; [Formula: see text]). Compared with isolates with the wild-type allele, the Y976F mutation of *pvmdr1* was associated with reduced susceptibility to chloroquine (154 nmol/L [range, 4.6-3505] vs. 34 nmol/L [range, 6.7-149]; [Formula: see text]) but greater susceptibility to artesunate (1.8 vs. 9.5 nmol/L; [Formula: see text]) and mefloquine (14 vs. 121 nmol/L; [Formula: see text]).

CONCLUSIONS: Amplification of *pvmdr1* and single-nucleotide polymorphisms are correlated with susceptibility of *P. vivax* to multiple antimalarial drugs. Chloroquine and mefloquine appear to exert competitive evolutionary pressure on *pvmdr1*, similar to that observed with *pfmdr1* in *Plasmodium falciparum*.

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OSELTAMIVIR IS ADEQUATELY ABSORBED FOLLOWING NASOGASTRIC ADMINISTRATION TO ADULT PATIENTS WITH SEVERE H5N1 INFLUENZA

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In the absence of a parenteral drug, oral oseltamivir is currently recommended by the WHO for treating H5N1 influenza. Whether oseltamivir absorption is adequate in severe influenza is unknown. We measured the steady state, plasma concentrations of nasogastrically administered oseltamivir 150 mg bid and its active metabolite, oseltamivir carboxylate (OC), in three, mechanically ventilated patients with severe H5N1 (male, 30 yrs; pregnant female, 22 yrs) and severe H3N2 (female, 76 yrs). Treatments were started 6, 7 and 8 days after illness onset, respectively. Both females were sampled while on continuous venovenous haemofiltration. Admission and follow up specimens (trachea, nose, throat, rectum, blood) were tested for RNA viral load by reverse transcriptase PCR. *In vitro* virus susceptibility to OC was measured by a neuraminidase inhibition assay. Admission creatinine clearances were 66 (male, H5N1), 82 (female, H5N1) and 6 (H3N2) ml/min. Corresponding AUC_{0-12} values (5932, 10,951 and 34,670 ng.h/ml) and trough OC concentrations (376, 575 and 2730 ng/ml) were higher than previously reported in healthy volunteers; the latter exceeded 545 to 3956 fold the H5N1 IC_{50} (0.69 ng/ml) isolated from the H5N1 infected female. Two patients with follow-up respiratory specimens cleared their viruses after 5 (H5N1 male) and 5 (H3N2 female) days of oseltamivir. Both female patients died of respiratory failure; the male survived. 150 mg bid of oseltamivir was well absorbed and converted extensively to OC. Virus was cleared in two patients but two patients died, suggesting viral efficacy but poor clinical efficacy.

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PHARMACOKINETICS AND METABOLISM OF THE ANTIMALARIAL PIPERAQUINE AFTER INTRAVENOUS AND ORAL SINGLE DOSES TO THE RAT

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This study aimed to evaluate the pharmacokinetic properties of piperaquine in the rat after intravenous and oral administration, and to identify and characterize the main piperaquine metabolites in rat plasma, urine, faeces and bile after intravenous administration. Male Sprague-Dawley rats were administered piperaquine as an emulsion orally or as a short-term intravenous infusion. Venous blood for pharmacokinetic evaluation was frequently withdrawn up to 90 h after dose. Urine, bile and faeces were collected after an infusion in rats kept in metabolic cages or in anesthetized rats. Pharmacokinetic characterization was done by compartmental modeling and non-compartmental analysis using WinNonlin. Piperaquine disposition was best described by a 3-compartment model with a rapid initial distribution phase after intravenous administration. The pharmacokinetics of piperaquine was characterized by a low clearance, a large volume of distribution and a long terminal half-life. Piperaquine displayed a low biliary clearance and less than 1% of the total dose was recovered in urine. The absolute oral bioavailability was approximately 50%. The main metabolite after intravenous administration of piperaquine was a carboxylic acid product identical to that reported in humans. The similarity with results in humans indicates the rat to be a suitable species for nonclinical *in vivo* piperaquine studies.

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QUANTIFICATION OF THE ANTIMALARIAL PIPERAQUINE IN PLASMA

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Malaria is one of the most common parasitic diseases in the world, with up to three million deaths a year. Piperaquine is an antimalarial drug that was extensively used in China during the 1980s and has recently received renewed interest as a partner drug in artemisinin-based combination therapy. Despite extensive use, the first bioanalytical method was published in 2003. In total there are eight previously published methods for quantification of piperaquine in different biological matrices using HPLC with UV or tandem mass spectrometric detection. Five of these allow for quantification of piperaquine in plasma and are discussed in this paper.

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POPULATION PHARMACOKINETICS OF PIPERAQUINE AFTER TWO DIFFERENT TREATMENT REGIMENS WITH DIHYDROARTEMISININ-PIPERAQUINE IN PATIENTS WITH *PLASMODIUM FALCIPARUM* MALARIA IN THAILAND

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The population pharmacokinetics of piperaquine in adults and children with uncomplicated *Plasmodium falciparum* malaria treated with two different dosage regimens of dihydroartemisinin-piperaquine were characterized. Piperaquine pharmacokinetics in 98 Burmese and Karen patients aged 3 to 55 years were described by a two-compartment disposition model with first-order absorption and interindividual random variability on all parameters and were similar with the three- and four-dose regimens. Children had a lower body weight-normalized oral clearance than adults, resulting in longer terminal elimination half-lives and higher total exposure to piperaquine (area under the concentration-time curve from 0 to 63 days [AUC day 0-63]). However, children had lower plasma concentrations in the therapeutically relevant posttreatment prophylactic period (AUC day 3-20) because of smaller body weight-normalized central volumes of distribution and shorter distribution half-lives. Our data lend further support to a simplified once-daily treatment regimen to improve treatment adherence and efficacy and indicate that weight-adjusted piperaquine doses in children may need to be higher than in adults.

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QINGHAOSU (ARTEMISININ): THE PRICE OF SUCCESS

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Artemisinin and its derivatives have become essential components of antimalarial treatment. These plant-derived peroxides are unique among antimalarial drugs in killing the young intraerythrocytic malaria parasites, thereby preventing their development to more pathological mature stages. This results in rapid clinical and parasitological responses to treatment and life-saving benefit in severe malaria. Artemisinin combination treatments (ACTs) are now first-line drugs for uncomplicated falciparum malaria, but access to ACTs is still limited in most malaria-endemic countries. Improved agricultural practices, selection of high-yielding hybrids, microbial production, and the development of synthetic peroxides will lower prices. A global subsidy would make these drugs more affordable and available. ACTs are central to current malaria elimination initiatives, but there are concerns that tolerance to artemisinins may be emerging in Cambodia.

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SIMPLIFIED ANTIMALARIAL THERAPEUTIC MONITORING: USING THE DAY-7 DRUG LEVEL?

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The blood concentration profiles of most antimalarial drugs vary considerably between patients. The interpretation of antimalarial drug trials evaluating efficacy and effectiveness would be improved considerably if the exposure of the infecting parasite population to the antimalarial drug treatment could be measured. Artemisinin combination treatments are now recommended as first-line drugs for the treatment of falciparum malaria. Measurement of the blood, serum or plasma concentration of the slowly eliminated partner antimalarial drug on day 7 of follow-up is simpler and might be a better determinant of therapeutic response than the area under the concentration-time curve. Measurement of the day-7 drug level should be considered as a routine part of antimalarial drug trials.

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HOW ANTIMALARIAL DRUG RESISTANCE AFFECTS POST-TREATMENT PROPHYLAXIS

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Slowly eliminated antimalarial drugs suppress malaria reinfections for a period of time determined by the dose, the pharmacokinetic properties of the drug, and the susceptibility of the infecting parasites. This effect is called post-treatment prophylaxis (PTP). The clinical benefits of preventing recrudescence (reflecting treatment efficacy) compared with preventing reinfection (reflecting PTP) need further assessment. Antimalarial drug resistance shortens PTP. While blood concentrations are in the terminal elimination phase, the degree of shortening may be estimated from measurements of *in-vitro* susceptibility and the terminal elimination half-life. More information is needed on PTP following intermittent preventive treatments, and on the relationship between the duration of PTP and immunity, so that policy recommendations can have a firmer evidence base.

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NEW MEDICINES FOR TROPICAL DISEASES IN PREGNANCY: CATCH-22

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There were an estimated 536,000 maternal deaths in the world in 2005, of which 533,000 (99%) occurred in developing countries [1]. Maternal and perinatal conditions are a major contributor to the global burden of disease, yet the pipeline of new drugs specifically for maternal health is alarmingly small [2]. Only 17 drugs are under active development for maternal health indications—less than 3% of the pipeline in cardiovascular health. Since the disaster of thalidomide 50 years ago, the medical profession has been rightfully very cautious about giving newly developed drugs to pregnant women, for fear that they might damage the unborn baby. Particular caution has been exercised in the first trimester to avoid teratogenicity during organogenesis.

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BURKHOLDERIA PSEUDOMALLEI ANTIBODIES IN CHILDREN, CAMBODIA

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Antibodies to *Burkholderia pseudomallei* were detected in 16% of children in Siem Reap, Cambodia. This organism was isolated from 30% of rice paddies in the surrounding vicinity. Despite the lack of reported indigenous cases, melioidosis is likely to occur in Cambodia.

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ACCESS TO ARTEMISININ COMBINATION THERAPY FOR MALARIA IN REMOTE AREAS OF CAMBODIA

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BACKGROUND: Malaria-endemic countries are switching antimalarial drug policy to artemisinin combination therapies (ACTs) and the global community are considering the setting up of a global subsidy mechanism in order to make them accessible and affordable. However, specific interventions may be needed to reach remote at-risk communities and to ensure that they are used appropriately. This analysis documents the coverage with ACTs versus artemisinin monotherapies, and the effectiveness of malaria outreach teams (MOTs) and Village Malaria Workers (VMWs) in increasing access to appropriate diagnosis and treatment with ACTs in Cambodia, the first country to switch national antimalarial drug policy to an ACT of artesunate and mefloquine (A+M) in 2000.

METHODS: A cross-sectional survey was carried out in three different types of intervention area: with VMWs, MOTs and no specific interventions. Individuals with a history of fever in the last three weeks were included in the study and completed a questionnaire on their treatment seeking and drug usage behaviour. Blood was taken for a rapid diagnostic test (RDT) and data on the household socio-economic status were also obtained.

RESULTS: In areas without specific interventions, only 17% (42/251) of respondents received a biological diagnosis, 8% (17/206) of respondents who received modern drug did so from a public health facility, and only 8% of them (17/210) received A+M. Worryingly, 78% (102/131) of all artemisinin use in these areas was as a monotherapy. However, both the VMW scheme and MOT scheme significantly increased the likelihood of being seen by a trained provider (Adjusted Odds Ratios (AOR) of 148 and 4 respectively) and of receiving A+M (AORs of 2.7 and 7.7 respectively).

CONCLUSION: The coverage rates of appropriate diagnosis and treatment of malaria were disappointingly low and the use of artemisinin monotherapy alarmingly high. This reflects the fragmented nature of Cambodia's health system in remote areas and the reliance placed by these communities on informal vendors from whom artemisinin monotherapies are widely available. However VMWs in particular are an effective means of improving access to malaria diagnosis and treatment. The VMW scheme and the social marketing of RDTS and blister-packaged artesunate and mefloquine have both been scaled up nationally. Case management in the public sector has also reportedly improved. Given recent concerns regarding the development of artemisinin drug resistance on the Thai-Cambodia border, the effectiveness of these measures in reducing the use of artemisinin monotherapy needs to be urgently re-evaluated.

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COST OF INCREASING ACCESS TO ARTEMISININ COMBINATION THERAPY: THE CAMBODIAN EXPERIENCE

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Background: Malaria-endemic countries are switching antimalarial drug policy from cheap ineffective monotherapies to artemisinin combination therapies (ACTs) for the treatment of *Plasmodium falciparum* malaria and the global community are considering setting up a global subsidy to fund their purchase. However, in order to ensure that ACTs are correctly used and are accessible to the poor and remote communities who need them, specific interventions will be necessary and the additional costs need to be considered.

***Methods*:** This paper presents an incremental cost analysis of some of these interventions in Cambodia, the first country to change national antimalarial drug policy to an ACT of artesunate and mefloquine. These costs include the cost of rapid diagnostic tests (RDTs), the cost of blister-packaging the drugs locally and the costs of increasing access to diagnosis and treatment to remote communities through malaria outreach teams (MOTs) and Village Malaria Workers (VMW).

***Results*:** At optimum productive capacity, the cost of blister-packaging cost under $0.20 per package but in reality was significantly more than this because of the low rate of production. The annual fixed cost (exclusive of RDTs and drugs) per capita of the MOT and VMW schemes was $0.44 and $0.69 respectively. However because the VMW scheme achieved a higher rate of coverage than the MOT scheme, the cost per patient treated was substantially lower at $5.14 compared to $12.74 per falciparum malaria patient treated. The annual cost inclusive of the RDTs and drugs was $19.31 for the MOT scheme and $11.28 for the VMW scheme given similar RDT positivity rates of around 22% and good provider compliance to test results.

***Conclusion*:** In addition to the cost of ACTs themselves, substantial additional investments are required in order to ensure that they reach the targeted population via appropriate delivery systems and to ensure that they are used appropriately. In addition, differences in local conditions, in particular the prevalence of malaria and the pre-existing infrastructure, need to be considered in choosing appropriate diagnostic and delivery strategies.

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EXPRESSION OF TOLL-LIKE RECEPTORS ON ANTIGEN-PRESENTING CELLS IN PATIENTS WITH FALCIPARUM MALARIA.

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The continuous release of blood-stage malaria parasites and their products can activate components of the innate immune system and induce the production of proinflammatory cytokines. Toll-like receptors (TLRs) have emerged as pattern-recognition receptors, residing on/in innate immune cells whose function is recognizing specific conserved components on different microbes. The aim of this study was to determine the expression of TLR2, TLR4 and TLR9 on antigen-presenting cells (APCs) in patients with mild and severe forms of falciparum malaria. Healthy individuals were used as controls. Peripheral blood mononuclear cells (PBMCs) were stained with specific monoclonal antibodies (mAbs) to investigate the percentage and the level of TLR expression by flow cytometry. Patients with severe and mild malaria showed increased surface expression of TLR2 and TLR4 on CD14(+)monocytes and myeloid dendritic cells (MDCs) and decreased intracellular expression of TLR9 on plasmacytoid dendritic cells (PDCs), compared to those of healthy controls. A significant decrease in the percentage of circulating CD14(+)monocytes and MDCs expressing TLR2 was found in both severe and mild malaria patients. These findings suggested that TLRs might play role in innate immune recognition in which the differential expression of TLRs on APCs could be regulated by the *P. falciparum* parasite.

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ABSTRACTS OF PRESENTATIONS

KNOWLEDGE, ATTITUDES AND PRACTICES AMONG FOREIGN BACKPACKERS TOWARDS MALARIA RISK IN SOUTHEAST ASIA

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Objective: Malaria is still prevalent in Southeast Asia where large numbers of backpackers visited each year. This study aimed to assess the knowledge, attitude and practices among foreign backpackers towards malaria risk in Southeast Asia.

Method: Questionnaires were administered to foreign backpackers in Bangkok, Thailand. They were asked about their general background, their attitude to malaria risk and their preventive measure against malaria. Their knowledge about malaria was assessed by ten true-false questions in the questionnaires.

Result: In total, 434 questionnaires were evaluated. Fifty-five percent of travellers were male and the median age was 28 years old. The main reason for travel was tourism (91%). Almost all travellers (94%) aware the risk of malaria. 22% of them would take antimalarial prophylaxis, 33% would use measure against mosquito bite, but nearly 40% had "no prevention" at all. Mean knowledge score was only 5.52 of 10. Most backpackers (92%) knew that malaria is a serious disease and sometime fatal, 74% knew that some travellers could develop malaria after they return. However, up to 35% believed that taking dirty food could lead to malaria infection. And 49% believed that malaria could be 100% prevented by antimalarial medication. In backpackers who had traveled in the forest (n=65), only 54% use insect repellent regularly. Among those who had taken antimalarial prophylaxis, nearly 30% had stopped the medication prematurely.

Conclusion: Although most backpackers perceive the risk of malaria in Southeast Asia, they have some misunderstandings about malaria and tend to comply poorly with mosquito bite prevention and chemoprophylactic strategies.

Presented at: Asia Pacific International Conference on Travel Medicine, Melbourne, Australia February 24-27, 2008

HERPES ZOSTER AND POST HERPETIC NEURALGIA-THE THAILAND PERSPECTIVE

Pitisuttithum P

[no abstract]

Presented at: 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia, June 19-22, 2008

ALTERED PLASMA CHOLESTEROL PATTERN IN SEVERE MALARIA PATIENTS: A WEEKLY SERIAL FOLLOW-UP STUDY FOR 28 DAYS

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Severe malaria infection had been found to have abnormal cholesterol level and the level was changed during and after treatment. Although there have been some reports of the changing of cholesterol profiles in severe malaria patients, the serial cholesterol profiles for 28 days follow-up of severe malaria patients have not been fully investigated. To investigate the changing in cholesterol levels in severe malaria patients, the cholesterol levels were monitored in 60 patients who had severe falciparum malaria infection and 60 healthy controls were matched by age in the day of admission, seventh, fourteenth and twenty-eighth. The decrease of cholesterol level (119.05 mg/dL) was found in the first day of admission compare to the control (218.35 mg/dL) with significantly observed, $p<0.001$. The results also showed that the cholesterol level was rapid increase on day 7 after received the antimalarial drug treatment (169.30 mg/dL) and become normal level when compared with healthy controls on day 28 of treatment (217.70 mg/dL), $p=0.632$. The mechanism of decline in cholesterol may remain obscure but it may be explained by increasing of IL and TNF. The raising of cholesterol after treatment may be useful as an additional sign of treatment successful.

Presented at: The XVIIth International Congress for Tropical Medicine and Malaria, Jeju, Korea, 29 September – 3 October 2008

MINOR LIVER DYSFUNCTIONS IN PATIENTS INFECTED WITH *PLASMODIUM VIVAX*, *P. MALARIAE* AND *P. OVALE*

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Liver dysfunction has been a common finding in malaria patients. Although there have been some reports of the liver dysfunction in malaria observed using modern clinical laboratory instruments, the serial liver profile for 28 days follow-up of patients infected with *Plasmodium vivax*, *P. malariae* and *P. ovale* have not been fully investigated. To investigate the changes of the hepatic biochemical indices in those patients before and after treatment with artemisinin derivatives, we performed liver function tests in 61 vivax, 54 malariae and 15 ovale malaria patients who were admitted to Bangkok Hospital for Tropical Diseases, Thailand. On admission and prior to treatment, hepatic dysfunction was found among the 3 groups. Serum liver function tests and physical examinations were performed weekly during the 28-day follow-up period. Initially elevated serum bilirubin and diminished albumin returned to normal within 2 weeks of treatment. Serum alkaline phosphatase and aminotransferases returned to within normal limits within 3 weeks. We conclude that patients with *Plasmodium vivax*, *P. malariae* and *P. ovale* infections had slightly elevated serum bilirubin, aminotransferase and alkaline phosphatase levels, and hypoalbuminemia. These minor abnormalities returned to normal within a few weeks after treatment with therapies based on artemisinin derivatives.

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PREDICTIVE OF OUTCOME IN ACUTE UNCOMPLICATED FALCIPARUM MALARIA PATIENTS

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In acute uncomplicated *Plasmodium falciparum* malaria infection, there is a continuum from mild to severe malaria. However, there is no mathematical system 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 with adult patients infected with acute uncomplicated *Plasmodium falciparum* malaria admitted to the Bangkok Hospital for Tropical Diseases between 2000 and 2007. 38 initial clinical parameters that may potentially affect the severity outcome of disease were identified to predict normal recovery or deterioration into severe malaria. Stepwise multiple discriminant analysis was performed to ascertain a linear discriminant equation. Based on data analyses, 4.3% of study patients turned to severe malaria. The MSPS = 4.38 (presenting schizontemia) + 1.62 (presenting gametocytemia) + 1.17 (presenting dehydration) + 0.14 (overweight by Body Mass Index) + 0.05 (initial pulse rate) + 0.04 (duration of fever before admission) – 0.50 (previous history of malaria in the last year) – 0.48 (initial serum albumin) – 5.66. In the MSPS validation study, sensitivity and specificity were 88.8 and 88.4%, respectively, with an accuracy of 88.4%. It was concluded that MSPS is an appropriate simple screening tool for predicting severity of outcome in acute uncomplicated *P. falciparum* malaria patients. The development of the MSPS was intended as a pilot for the further development of a suitable model for prognostic purpose. The score may require revalidation in other geographical areas, for subsequent utilization at specific sites.

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RISK FACTORS FOR ACUTE RENAL FAILURE IN SEVERE MALARIA PATIENTS AT BANGKOK HOSPITAL FOR TROPICAL DISEASES

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Severe malaria is almost exclusively caused by *Plasmodium falciparum* malaria. It occurs most often in non-immune or persons who have low immunity to malaria. Acute renal failure (ARF) is one of the serious complications of malaria and could be life threatening. The objectives of this retrospective study were to identify the risk factors for ARF in severe malaria patients. We reviewed the hospital records of 189 adult severe falciparum malaria patients admitted into the Hospital for Tropical Diseases, Bangkok, Thailand between January 2000 and December 2006. The results of bi-variate analysis revealed, increased WBC count, high AST, high ALT, hyperbilirubinemia, hypoalbuminemia, impaired consciousness, abdominal pain, liver enlargement, pallor, high body mass index, long duration of fever, urine volume < 400 ml per 24 hrs and residency in a non-endemic malaria area, to be risk indicators for ARF. However, to substantiate the initial results obtained, all associated factors from bi-variate analysis were subjected to the stepwise logistic regression analysis. After adjusted for all variables, we found that only 2 variables were found to be significant for the logistic model, 1.) urine volume < 400 ml per 24 hrs (adjusted odds ratio [AOR] = 6.69, 95% CI = 5.09-8.29, p< 0.05) and 2.) residency in a non-endemic malaria area (adjusted odds ratio [AOR] = 6.04, 95% CI = 4.76-7.32, p< 0.05). Although our results were different when compared to other previous studies, the possible significance of these observations is discussed.

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PHARMACOKINETICS OF DIHYDROARTEMISININ AND PIPERAQUINE IN ADULT THAI PATIENTS WITH UNCOMPLICATED *P. FALCIPARUM* MALARIA: NINETY DAYS OF FOLLOW UP

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Adult Thai males (mean 26.3 y, 51.4 kg) with acute uncomplicated *P. falciparum* malaria (n=25) received combination film-coated oral tablets containing 40 mg of dihydroartemisinin (DHA) and 320 mg piperaquine (PQ) phosphate as a single daily dose (three tablets) for three consecutive days. PCR-corrected cure rate at Day 90 was 100% (ITT and PP population). Blood samples were collected intensively over the first 24 hours (16 samples), after 36 and 48 hours and on days 3, 7, 14, 21, 28, 56 and 90. Plasma DHA and PQ were measured by LC-MS/MS. Non-compartmental max max) of 752 ng/mL a T of 2.14 h, a half-life DHA pharmacokinetics analysis (after the first dose only) revealed a mean maximum concentration (C of 1.04 h, an apparent oral clearance (CL/F) of 1.34 L/h/kg and a volume of distribution (V/F) of 2.08 L/kg. The plasma DHA profile was described by a one compartment population model with a residual variability of 9.2%. PQ pharmacokinetics was described by a three-compartment population model with two input functions accounting for variable absorption. Population mean CL/F and V/F values were 1.12 L/h/kg and 721 L/kg, respectively, and the half-life of PQ was 576 h (24 days). The residual model variability was 28.1%. The long half-life of PQ can be linked to extensive distribution of PQ outside of a central compartment (total V/F was about 27 times greater than the central volume of 26.7 L/kg). Our data support the clinical evidence that DHA+PQ is effective in curing uncomplicated *P. falciparum* malaria allowing also a prolonged prophylactic effect against new infections.

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GENETIC DIVERSITY OF *ORIENTIA TSUTSUGAMUSHI* FROM SCRUB TYPHUS PATIENTS IN RURAL THAILAND

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Scrub typhus is rickettsial infection that caused by *Orientia tsutsugamushi*, an obligate intracellular bacterium. To study *O. tsutsugamushi* population genetic structure from patients with scrub typhus in Udon Thani, Thailand, we performed a novel multilocus sequence typing scheme. Seven housekeeping genes (*gpsA, mdh, nrdB, nuoF, ppdK, sucD, sucB*) selected from partial sequence of a Thai isolate (strain UT76) were successfully amplified and sequenced from DNA extracted from blood taken from 84 Thai scrub typhus patients and from a further 20 Thai isolates cultured *in vitro*. The results demonstrated high allelic diversity (17-22 alleles/locus); a high number of sequence types per isolate (0.58 ST/isolate), and high index of diversity (Simpson's index at 95% CI = 0.96 (0.94-0.98)). These suggested that *O. tsutsugamushi* population genetic were genetically diverse. The diversification of this bacteria occurred by recombination rather than mutation due to the high recombination to mutation ratio (r/m) in this setting (14:1 at the allelic level; 103:1 at the nucleotide level). Comparing MLST method with antigenic typing based on 56-kDa-protein gene in 23 scrub typhus isolates from *in vitro* culture, the discrimination index of MLST was 0.96 (0.91-0.99) while the latter was 0.53 (0.34-0.71). There was evidence of clonal expansion, with some patients from the same geographical area infected with bacteria with identical sequence types. In addition, eleven percent of patients in this study were simultaneously infected with multiple sequence types, suggesting that multiple strains co-existing within individual mites.

Presented at the XVIIth International Congress for Tropical Medicine and Malaria, Jeju, Korea, 29 Sep – 3 Oct 2008

EFFECTS OF SERUM LIPID CONCENTRATIONS ON THE *IN VITRO* EFFICACY OF LUMEFANTRINE AND ATOVAQUONE AGAINST FALCIPARUM MALARIA

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Lumefantrine and atovaquone are highly efficacious antimalarial drugs. These drugs are highly lipophilic. Plasma lipids (i.e. triglyceride-rich plasma lipoproteins) might decrease the fraction of active free drug in the blood and affect *in vivo* therapeutic responses. This study aimed to test the efficacy of lumefantrine and atovaquone *in vitro* cultures of *P. falciparum* (Thai isolates, N=10) containing 10% serum with different concentrations of lipids. Serum was obtained from healthy volunteers under fasting conditions and at various times after ingestion of a standard fatty meal. There was a 2-fold increase in the 50% inhibition concentration (IC_{50}) for lumefantrine (from 191 ng/mL to 465 ng/mL) and 20- fold increase in IC_{50} for atovaquone (from 0.5 ng/mL to 12 ng/mL), with a 2 fold increase in plasma triglyceride level (from 106 mg/dL to 200 mg/dL). The IC_{50} of chloroquine diphosphate, a hydrophilic drug used as a control was independent of triglyceride concentration. This result suggests that altered plasma lipoproteins profiles influence the efficacy of lipophilic antimalarial drugs and should be considered when interpreting their pharmacodynamic profiles.

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THE CORRELATION BETWEEN CLINICAL COURSES AND THE FIRST-DAY COAGULATION LABORATORY VALUE IN GREEN PIT VIPER BITES

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The snake bite is most common in Thailand. The most prevalence snake bite in Bangkok is green pit viper. Although most patients have experience, only mild to moderate coagulation defects, but also a dilemma in clinical management. Additionally, potentially fatal allergic reactions to the antivenom preclude its universal usage. Therefore, decisions for antivenom infusion rely upon prolonged observation and repeated coagulation tests, resulting in economic loss from absence from work as well as the hospital and transportation expense. Initial laboratory values (prothrombin time (PT), thrombin time (TT), fibrinogen, antiplasmin levels) are proposed as potential predictors for subsequently severe coagulopathy. Coagulation laboratories were assayed in 185 patients within 24 hours after bites to correlate with their clinical courses. The study was done in King Chulalongkorn Memorial hospital, Bangkok, from 2003 to 2005.The TT was the most sensitive test for the severe coagulopathy. If TT was used as a initial screening test, the patients needed follow-up will be reduced by 56% and the possibility of requirement of antivenom in 72 hours, if test was negative, was only 0.97%(95% confident interval 0-2.9%). Fibrinogen and antiplasmin levels were found to most predictive for prolonged venous clotting time (VCT) and thrombocytopenia. PT was not helpful in predicting severe coagulopathy, because of low sensitivity. This data give us deeper insight in pathogenesis of coagulopathy and suggest TT as a screening test to save cost and prevent inconvenience incurred by this common problem.

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AIDS VACCINE RESEARCH IN THAILAND

Pitisuttithum P

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Thailand was affected by the HIV epidemic, especially in 1992-1994. With the launch of the National Plan for HIV/AIDS Vaccine Development and Evaluation in 1992, ten Phase I/III trials and two Phase III efficacy trials were approved and conducted. The result of the first phase III efficacy trial of rgp 120 vaccine subtype B/E, among 2,545 injecting drug users, showed no efficacy. However, intravenous drug use in the study cohort declined by a third (from nearly 100 to 66.5 %) and the rate of needle-sharing decreased from 33.0 % at the beginning of the trial to 17.5% by the end of the study. The second Phase III study, using canary pox-based vaccine and rgp 120 as priming and boosting vaccines, respectively, is being conducted in Chonburi and Rayong communities and involves 16,402 participants. Interim efficacy was analyzed by the Data Safety Monitoring Board in July 2007, and the trial was continued. The results will be available in late 2008, or early 2009. This outstanding performance is the result of strong efforts and commitment by various partners, including policy-makers, regulators, researchers, and international collaborators.

Presented at: 21st Joint Meeting of the AIDS Panels, Tokyo, Japan, 10-12 September 2008.

THE ADHESION RECEPTORS OF *P. VIVAX*-INFECTED RED CELLS

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The cytoadherence of *P. falciparum* infected red cells resulting in sequestration has been well documented. We present the results of a study of adhesion in *P. vivax* isolates (N=50). None of the parasites adhered to immobilized CD36, ICAM-1, or TSP, the major receptors for *P. falciparum* adhesion. However, all parasite isolates adhered to immobilized chondroitin sulfate A (CSA) and hyaluronic acid (HA). Adhesion was observed when the parasites had developed to the large ring stage (about 16 hours after invasion into the red blood cells (RBCs), with a peak of adhesion at 32 hours post-invasion. The adhesion to the immobilized CSA and HA could be inhibited by preincubating the infected RBCs (IRBCs) with soluble CSA and HA, demonstrating the specificity of adhesion. Pretreating the IRBCs with trypsin totally inhibited adhesion. CSA and HA are the major carbohydrate molecules expressed in placenta, and infection of *P. vivax* during pregnancy is known to be associated with the low birth weight, particularly in multigravidae (Nosten et al, 1999). These findings suggest that the adhesion of *P. vivax*-infected RBCs to the placenta may be involved in the disease pathogenesis.

Presented at: Joint International Tropical Medicine 2008, Bangkok, Thailand 13-14 October 2008

SIMPLE TOOL CAN CURE DIFFICULT DISEASE PROPOSING A SELF-MANAGABLE TWISTING TOURNIQUET TECHNIQUE© FOR ROUTINE ADOPTION TO LYMPHEDEMA PATIENTS WITH FILARIASIS AND BEYOND

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Lymphedema is a chronic swelling of extremity(s) seen commonly as a longterm sequela of filariasis, or as an iatrogenic disease after surgery or radiotherapy of breast or cervical malignancy, or the like. Lymphedema can also be encountered as an independent clinical entity as in primary lymphedema praecox/tarda, the onset of which interestingly is often related to a small joint/bone trauma or a minor skin infection, and is rapidly worsened by an incautious intake of food containing animal products like cartilage, bone, or skin of poutry and pork, meat of cattles, or flesh of fish and seafood. Still lymphedema is manifested in syndromes like Milroy disease, Aagenaes syndrome, Klippel Trenaunay syndrome, venous insufficiency syndrome, von Recklinghausen disease, and lymphangiomatosis. In newborns lymphedema is expressed especially when switched from breast milk to bottle (formulated cow) milk, or when food ingestion involves ingredients of animal origin such as chicken. Manifestation can affect one single upper or lower limb, bilateral, unilateral (whole body half-sided), diagonal (ipsilateral of upper against lower), or even zigzagged (crossing the midline between face-to-arm and arm-to-leg).

It appears from our clinical experience that the actual suffering of patients is more complicated than generally recognized by medical profession. The World Health Organization reported an estimate of 250 million population worldwide are at risk of filarial infection, and 120 million people already positive for filariasis, 40 million of which have severe form of elephantiasis. On the other hand, data of patients with non-filarial lymphedema are less documented. Approximately one million individuals are in the USA, and about 0.3 million in Japan. Based on Thailand Cancer Registry it is estimated that 100,000 patients with lymphedema are still alive; putting into consideration those other etiologies, 1.5 times could be existing.

Following the discovery of the honeycomb architecture (Ekataksin et al., 2000) in prelymphatic system, and the endotheliofibroblast (Ekataksin et al., 2005), a new cell type responsible for lymphangiogenesis, we have developed a series of simple tools for reducing the sizes of the swelling limbs. These are patented under a *Twisting Tourniquet Technique*© (Ekataksin *et al*., 2006), and used primarily in the Lymphedema Day Care Center and Lymphedema Clinic. During the last 2.5 years, 666 patients, 6 months of age to 99 years old, from a dozen of countries including Thailand, Japan, England, Wales, Germany, Belgium, Netherlands, Bangladesh, Australia, Taiwan, Italy, and USA, have used it with satisfactory results, ranging from minormajor improvement to complete or near-complete cure. Duration of use is a few days to several months depending on severity grading. Effectiveness is witnessed from the first day of use; no single individual ever claimed of non-effective nor without reducing limb size.

Since the tool, with a proper instruction and training, can be conveniently operated by the patient or family members, no matter s/he is living at a hilltop or in a megacity, at home or on the go, we are proposing this as a routine adoption to the society and community where specialty care is unaffordable.

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ANTIMALARIAL DRUG RESISTANCE IN *P. VIVAX:* MOLECULAR MARKERS

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Plasmodium vivax lines resistant to antimalarial drugs have now emerged. Over the last two decades, there have been documented cases of high level resistance to chloroquine (CQ) and/or sulfadoxine-pyrimethamine (S-P) in some geographic locations. There is an urgent need to understanding better the mechanisms of (multi-) drug resistance in order to monitor and guide effective antimalarial chemotherapy regimens. Here we review recent advances in the identification of molecular markers of S-P, and candidate genes of CQ such as *Pvmdr1* (*P. vivax* multi-drug resistant gene 1) and *Pvcg10* (equivalent to *Pfcrt*) are implicated. The relationship between mutations in *Pvdhfr* and *Pvdhps* (dihydrofolate reductase and dihydropteroate synthase; implicated in resistance to S-P) and clinical response to S-P have been clearly demonstrated in numerous previous studies whereas further studies are needed in CQ resistance mechanism.

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SAFETY AND EFFICACY OF CKBM-A01, A CHINESE HERBAL MEDICINE, AMONG ASYMPTOMATIC HIV PATIENTS

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Complementary remedies represent a potential alternative treatment for chronic diseases, including HIV/AIDS cases not suited to highly active antiretroviral therapy (HAART). This study evaluated the safety and efficacy of CKBM-A01, a Chinese herbal medicine, and patients' quality of life (QoL). Asymptomatic HIV patients with CD4 counts of 250-350 cells/μL were recruited into this open trial. Liquid CKBM-A01 was prescribed for a 36-week period. Study participants recorded all symptoms themselves in diary cards. Study parameters, including CD4 cell count, HIV viral load, and blood chemistry, were periodically monitored and questionnaires used to assess QoL, and for risk reduction. 18 volunteers, mean age (± SD) 32.07 (±6.88) years had a median (IQR) baseline CD4 count of 292 (268.50-338.25) cells/μL. No serious drug-related adverse events were detected. Only 27.8% experienced possibly drug-related adverse events, including increased bowel movement, dizziness, sore throat/heartburn, nausea/vomiting, and somnolence. Regarding efficacy, no significant changes in log viral load or CD4 cell count were observed at the end of the study. 72.2% expressed satisfaction with CKBM-A01 and had a positive perception. Common colds and nasal symptoms were reportedly significantly reduced post-treatment (p=0.019). CKBM-A01 appeared to be safe and may improve QoL, with less morbidity, for asymptomatic HIV patients not suited to standard therapy

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MULTILOCUS SEQUENCE TYPING (MLST) OF *ORIENTIA TSUTSUGAMUSHI* FROM SCRUB TYPHUS PATIENTS

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Scrub typhus is a major cause of acute febrile illness in Asia Pacific region. The disease caused by an obligate intracellular bacterium, Orientia tsutsugamushi. To study the population genetics of this causative organism, a multilocus sequence typing scheme based on 7 housekeeping genes (gpsA, mdh, nrdB, nuoF, ppdK, sucD, sucB) from a Thai isolate (strain UT76) was developed. These genes were successfully amplified and sequenced from DNA extracted from blood taken from 84 Thai scrub typhus patients and from a further 20 Thai patient isolates cultured in vitro. The results showed high allelic diversity (17-22 alleles/locus); a high number of sequence types per isolate (0.58 ST/isolate), and high index of diversity [Simpson's index at 95% CI = 0.96 (0.94-0.98)]. These are indicative of a genetically diverse bacterial population. The recombination to mutation ratio in closely related O. tsutsugamushi bacteria in this setting was 14:1 at the allelic level and 103:1 at the nucleotide level, indicating that the diversification of O. tsutsugamushi is characterized by recombination rather than mutation. Despite this there was evidence of clonal expansion, with some patients from the same geographical area infected with bacteria with identical sequence types. In addition eleven percent of patients in this study were simultaneously infected with multiple sequence types, suggesting either multiple mite bites or multiple strains co-existing within individual mites; that the strains are often related suggests the latter.

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RETENTION ACTIVITIES OF CLINICAL SITES TO ENSURE COMPLIANCE AMONG PARTICIPANTS IN THE PHASE III COMMUNITY TRIAL IN THAILAND

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Background: 16,402 volunteers were enrolled in the phase III community-based trial by the end of 2005. 13,798 (84%) completed all vaccinations. The follow-up period is three years after the last vaccination so compliance is critical for trial success. The clinical teams implemented various activities in order to ensure compliance and achieve a 95% retention rate at each subsequent visit.

Methods: The retention activities started in early 2005. This data collection was done from July 2006 to December 2007. Apart from extended clinic hours to include one evening per week and Sunday, activities at the clinical sites included provider based care, reminder letters, reminder phone calls, 24 hour service by phone, educational campaigns including interactive games while waiting at the clinical sites and cultural activities all year round. Activities outside the clinical sites included tracking activities and mobile clinic activities. These activities were done independently or together with the local healthstaff.

Results: Activities at the clinical sites- 35,513 reminder letters were sent out, 71,583 reminder calls before appointments or calls after missed appointments. 45% could not be contacted by phone so home visits were made. Of those contacted by phone 69% came to the clinics, 30% postponed visits. Providing mobile phone numbers and 24 hour service resulted in 5,646 incoming calls from volunteers; the majority wanting to postpone appointments. Edutainment was done with poster presentations and games with questions and answers. More than 85% responded correctly. For outside clinic activities, 2,580 home visits and 2,116, mobile clinical activities were performed. To date, a retention rate of 95% has been achieved.

Conclusion: Extensive clinical site activities, tracking and mobile clinical activities are crucial for the success of the trial during follow-up period.

Presented at: AIDS VACCINE 2008, Cape Town, South Africa**,** 13-16 October 2008 p.173

KNOWLEDGE AND EXPECTATION OF PARTICIPANTS IN THE PHASE III HIV VACCINE TRIAL IN PREPARATION FOR THE RESULTS OF THE EFFICACY INTERIM ANALYSIS

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Background: The interim analysis for efficacy of the phase III community trial was July 2007. It was critical to assess the knowledge and expectations of the potential results among the participants and community to prepare messages to avoid misconceptions and disappointment.

Methods: In depth discussions with 134 participants from various demographic backgrounds were conducted. Eight standard questions were raised for discussion. These included expectations from the study; what has been gained from participating in the study; the understanding of the word "efficacy"; opinions of the impact on sexual behavior if vaccine is efficacious; the expectations of an efficacious vaccine; the understanding for the word "no efficacy"; the expected impact if the vaccine has no efficacy; and opinions about possible future vaccine trials.

Results: 72 % and 22 % of respondents said an efficacious vaccine could prevent HIV infection and delay disease progression respectively. 52 % thought if the vaccine was efficacious, there would be an impact by increasing risky behavior (77%). 21% expected that their family would get the vaccine if efficacious. 56% said non efficacy does not prevent HIV infection. 47% said that the knowledge gained will lead to further vaccine development despite non efficacy. 20% expressed disappointment if the vaccine was not efficacious.70% said that there will be no negative impact to the community if the vaccine has no efficacy. 85% said they would recommend that others participate in future vaccine trials.

Conclusion: There was generally correct knowledge and positive expectations towards trial results. There was a concern about increasing risky behavior if an efficacious vaccine is available. However, defining an efficacious vaccine in terms of delay of disease progression (viral load effect) needs to be strengthened.

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PHASE III TRIAL OF HIV PRIME-BOOST VACCINE COMBINATION IN THAILAND: FOLLOW UP PHASE

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Background: After ten years of continuing efforts to identify vaccine candidate appropriate to HIV subtype circulating in Thailand, the prime-boost HIV vaccine combinations were passed the "go-no-go" criteria for phase III efficacy trial to be conducted. The low HIV annual incidence learned from cohort study together with the nature of phase three trial resulted in high number of volunteers and long study duration.

Methods: The world's first efficacy trial of a prime-boost HIV vaccine combination was started in September 2003 to determine if the prime-boost vaccine strategy 1) prevents infection, 2) reduction of set-point viral load in vaccinees who become infected, and 3) is safe. Vaccines were developed specifically for the predominant circulating HIV subtypes in Thailand (CRF01-AE and B) using a recombinant ALVAC-HIV (vCP1521) (Aventis Pasteur) priming and AIDSVAX®gp120 B/E (VaxGen) boosting. This trial is designed for randomized, double-blind, placebo-controlled, community trial.

Results: 16,402 HIV negative Thai adults, aged 18-30 were recruited through the existing Thai MOPH facilities. The study period

for each individual is scheduled for 6 months of vaccination period with 3 years follow up. The vaccination was completed in July 2006. In July 2007, the DSMB convened the meeting for an interim efficacy, safety, and futility analysis and recommended the trial be continued. Most of the volunteers are now completed their 18 months follow-up with interval retention rates exceeds 95%. To date, the vaccine has been safe. No death related to vaccination was recorded. Negative social events from volunteers' participation were very few.

Conclusion: The trial required longer time than expected which needs timeliness and more flexible strategies for conducting the trial. Close monitoring and community engagement are essential strategies for the follow-up phase to retain the volunteers. Community engagement is planned for effective communication strategies for the trial result after completion of the follow-up phase in mid-2009.

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THE FOLLOW-UP PHASE OF THE PHASE III COMMUNITY TRIAL: CURRENT ACTIVITIES

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Background: 16,402 volunteers were enrolled in the phase III community-based trial at the end of 2005. 13,798 (84%) completed all vaccinations. The follow-up period is three years after the last vaccination, with the acquisition endpoint of 50% reduction in relative risk of infection, and virological endpoint. Various activities to ensure the understanding of the participants, to monitor participants' impact events, and to achieve a high follow-up rate are critical for the trial's success.

Methods: Various activities have been put in place. Those activities are at the clinical sites, outside the clinical sites, community engagement and volunteer relation activities to strengthen the understanding of the participants of the importance of retention. Tracking activities, establishing mobile teams and continued monitoring of impact events, have also been implemented. In-depth interviews were conducted to evaluate knowledge gained.

Results: 6,446 volunteers have completed 3 years' follow-up, and 95% retention was achieved at each subsequent visit. The trial is expected to complete by July 2009. During the 18 months (Jun06-Dec07), 35,513 reminder letters were sent out, with 71,583 reminder calls before appointments or calls after missed appointments. 45% could not be contacted by phone, so home visits were made. 2,580 home visits and 2,116 mobile clinical activities were performed. Before the interim analysis of efficacy, in-depth discussions with 134 participants from various demographic backgrounds were conducted. 72% and 22% of respondents said an efficacious vaccine could prevent HIV infection and delay disease progression, respectively. 47% said that the knowledge gained will lead to further vaccine development, despite non-efficacy. Participation Impact Events reported (as of 4 May 2007) were 1.7/100 person-year, 93% reported "minimal impact" and most were resolved.

Conclusion: Extensive clinical site activities, tracking, and mobile clinical activities were conducted to achieve a high rate of follow-up with low participation impact events (PIE).

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PSYCHOSOCIAL BURDEN OF WOMEN WITH ABNORMAL PAP SMEARS

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Cervical cancer remains one of the leading causes of cancer deaths among women worldwide' however, it is highly curable when detected early. This cross-sectional study aimed to describe the psychosocial burden of women with abnormal pap-smear results during the 3 months after recruitment into the study. All participants were in good general health, willing to provided signed informed consent, and self-complete a study-related questionnaire. Seventy-five women negative for intraepithelial lesions and 76 women with epithelial cell abnormalities were recruited. The two study groups did not differ in baseline demographic characteristics or gynecological history. However, the mean Health Impact Profile (HIP) scores were higher for the women negative for intraepithelial lesions [68.18 ± 14.22 and 57.74 ± 16.29, respectively ($p < 0.001$)], who were mostly concerned about getting cancer, pain during the visit to the gynecologist, and that having sex with their partner may give them an infection ($p < 0.001$). There were no statistically significant differences in mean scores for SDS, WPAI, HUI, HSS, and HADS, between the two study groups. However, there was a significant difference in mean scores for HSS within the younger age group (18-28 years) [(75.00 ± 13.64, n=19 and 59.72 ± 19.13, n=18, respectively)] ($p = 0.008$). In conclusion, the provision of information, counseling, and advice, support services and clinician consultation times, need strengthening, to help alleviate women's concerns about infection, and their worries, anxiety or depression, following an abnormal Pap result.

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ABNORMAL PAP SMEARS AND PSYCHOSOCIAL BURDEN OF ABNORMAL PAP SMEARS ON HIV INFECTED WOMEN IN CHONBURI HOSPITAL, CHONBURI, THAILAND

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This study was carried out to assess the prevalence and characteristics of abnormal Pap smears and the psychosocial burden of those on HIV infected women. Existing literature contain only studies done on relatively less vulnerable, non-immunesuppressed patients.

172 Subjects were recruited from women attending anonymous clinic at Chonburi hospital who had undergone Pap smear screening during last one-year period. A comparison was made between women with positive Pap smear results and negative Pap smear results. Psychosocial burden was assessed using 3 questionnaires (PEAPS-Q, HADS Scale, and EURO-Qol Thermometer).The demographic and characteristic data were abstracted from 480 medical records.

Results indicated that women with abnormal Pap smear test results had significantly higher scores on PEAPS-Q ($p<0.01$) HADS Scale ($p<.05$) and EURO-Qol Thermometer ($p<.001$) than women with normal Pap smear results. The prevalence of pre-cervical cancer lesions was

17.9% (ASCUS 3.1%, LSIL 3.9%, HSIL 7.5%, Squamous cell carcinoma 1.7% and Atypical glandular cells including Adenocarcinoma 1.7%)

The findings suggest that testing positive in Pap screening adds significant psychosocial burden with increased anxiety, distress and concern about body image and sexual relationships. Supportive counseling and education about significance of lesions and the importance in adhering to treatment are recommended.

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EFFECTS OF PRIMAQUINE AND ITS METOBOLITES ON THE INFECTIVITY OF *P. FALCIPARUM* GAMETOCYTE

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The infectivity of *P. falciparum* gametocytes (TM036) to *Anopheles dirus* after in vitro exposure to primaquine and its metabolites were assessed. To assess the effects of gametocyte infectivity of primaquine and its metabolites three volunteers were given primaquine orally (45 mg single dose). Serial serum samples from each volunteer incubated with gametocytes at varying time periods. Percent infectivity to Anopheles of gametocytes incubated with serum from the 3 volunteers (A, B, C) ranged from 37 to 88%. There was no significant change compared to control serum in the number of oocysts in the mosquito midgut after gametocytes incubation with serum from volunteer A. However, the mean number of oocysts after incubation with serum from volunteer B at 1 hour after drug administration was significantly decreased ($P<0.01$). There was a 4 times reduction in the mean number of oocysts after gametocyte incubation with serum from volunteer C at 2, 4, 8 and 24 hours after drug administration ($P<0.01$). The peak plasma level of primaquine in the three volunteer was observed within 1 to 2 hours after drug administration mean Cmax (SD) = 132 (23) ng/ml. The highest level of carboxyprimaquine was observed after 8 hours of primaquine administration mean Cmax (SD) = 1,232 (157) ng/ml. These results indicate that primaquine or its metabolites have effects on the infectivity of *P. falciparum* gametocyte in vitro. The mechanisms underlying reduced *P. falciparum* gametocyte infectivity needs further study.

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HISTOPATHOLOGY OF LYMPHEDEMA: A NEW LOOK AT PRELYMPHATIC SYSTEM OF THE SKIN AND CLINICAL CORRELATION

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[3] Lymphedema Day Care Center, Faculty of Tropical Medicine, Mahidol University, Bangkok, ThailandBackground: lymphedema is the most obvious manifestation of lymphatic filariasis. Although it is barely recognized as a life-threatening disease, it causes

Lymphedema is the most obvious manifestation of lymphatic filariasis causing debilitating condition with no known complete cure. Following the discovery of honeycomb architecture in prelymphatics (Ekataksin et al., 2000) and endotheliofibroblast (Ekataksin et al., 2005), we studied pathohistology of prelymphatic and lymphatic system of lymphedematous skins. Skins from 7 lymphedema patients and normal skins from 2 autopsy cases were investigated by light microscope of 5-8-μm-thick paraffin-embedded and frozen sections. Paraffin sections were stained with H&E; frozen sections were stained for 5'-nucleotidase and alkaline phosphatase. Lymphedematous skins were thickened by edematous fluid and collagen deposition in dermis, plus fat deposition in hypodermis. Lymphatic capillaries were markedly dilated in contrast with compressed ones in normal skin. Newly formed lymphatic capillaries and lymphatic plexus were abundant. In mild fibrosis (stage II), collagenous bundles were loosely arranged upon edematous interstitium. In progressive stage (stage III), collagen were densely packed, therefore edematous fluid was limited in perivascular adventitia. The latter revealed potential spaces of micro chambers resembling honeycomb compartments. The latter formed conduit-like structure that emptied into true lymphatic capillaries. Evidences showed preferential pathway for the cell trafficking and fluid movement along adventitia of pre-existing vessels. We demonstrated lymphangiogenesis that is possibly originated from honeycomb compartments. Nevertheless, such compensatory mechanism seems to exacerbate the severity of lymphedema. Clinical challenge may focus on the control of lymphangiogenesis of impaired lymphatic vessels and restoration of their function. Supported by Reverse Brain Drain SP Grant 2005-2007; Thai Government Research Grant for Transferring of Technology to Remote Communities 2008.

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A STUDY OF TAENIASIS AND CYSTICERCOSIS IN KANCHANABURI PROVINCE, THAILAND

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A field study was performed in a village in Thong Pha Phum District, Kanchanaburi Province, Thailand, in 2006. A total of 265 stool samples were examined by Kato-Katz technique, 28.7% (76) were positive for intestinal helminthic infections. Among these, hookworm was the most prominent, with an infection rate of 26.4% (70). The others were minor infections, including 2.3% *Trichuris trichiura*, 0.8% *Ascaris lumbricoides* and 0.4% *Enterobius vermicularis*. Two (0.8%) patients were egg-positive for *Taenia* sp. Interviews with community members revealed that 3 had a history of discharging worm segments in stool. All 5 *Taenia*-infected patients were treated. One patient expelled long strobila without scolex. The other 3 patients evacuated worms with one scolex each, of which one scolex had armed hooklets, and the other 2 did not. One other *Taenia* egg-positive patient was negative post-treatment. Molecular identification of the expelled proglottids showed that one sample was *Taenia solium*, one was *T. saginata* and 2 were *T. asiatica*.

The seroprevalence for cysticercosis among 114 residents of this community was investigated; 5 (4.4%) were ELISA-positive with native *T. solium* antigen. Immunoblot using native and recombinant antigens confirmed that 4 (3.5%) cases were cysticercosis. Taeniasis infection in this study was less prevalent, when diagnosed by microscopic stool examination. However, a relatively moderate rate of cysticercosis was found by seroepidemiological investigation. *Taenia solium* carriers must be investigated and treated to prevent the spread of cysticercosis.

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INTESTINAL TREMATODE INFECTIONS IN THAILAND

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Seven families of intestinal trematodes have been found to infect humans in Thailand, comprising 5 species of Heterophyidae, 7 of Echinostomatidae, 3 each of Plagiorchiidae and Lecithodendriidae, and 1 each of Fasciolidae, Paramphistomatidae, and Anchitrematidae. Among these families, the Heterophyiidae are the most common, and metacercariae have been found in several species of fresh- and brackish-water fish throughout the country. The prevalence of the Echinostomatidae, Plagiorchiidae and Lecithodendriidae is low, and they are mostly distributed innortheastern Thailand. The Paramphistomatidae and Fasciolidae had not been reported in humans for more than 10 years. However, recently, one *Anchitrema sanguineum* adult was removed from the caecum of a man in Bangkok. In Thailand, *Anchitrema* have been found in rats, and a puppy on only one occasion. This is the first record of this worm in a human.

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PRELIMINARY ANALYSIS OF PREPARATIVE COMPONENTS OF *TAENIA SOLIUM* METACESTODE-DELIPIDIZED EXTRACTS

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The adult and intermediate stages of helminths have complex biochemical structures. Therefore, an antigen should be prepared using a techniquethat can obtain good antigenic properties, yielding high sensitivity and specificity for research and routine diagnostic application. Six different antigens of *Taenia solium* metacestodes were prepared by distilled water and 1% strengthening concentrations of Tween 20, NP40, mercaptoethanol (ME), SDS and deoxychlorate (DOC), and finally by ether-delipidization. Non-delipidized and delipidized extracts were compared using Coomassie brilliant blue. Only delipidized extracts were analyzed by Concanavalin A and antibody reactions with normal control (NS), echinococcosis (Ecc), and neurocysticercosis (Ncc). By Coomassie brilliant blue staining, delipidized-Tween 20 and NP40 extracts were denser at 16.5 kDa, and 30, 28, and 27 kDa, respectively. Meanwhile, 35 and 36 kDa of the non-delipidized NP40 extract were stronger than the delipidized extract. The ME and DOC extracts did not contain 100 or 18.5 kDa. DOC extracts showed 4 defined bands (18, 17.5, 16 and 15.5 kDa), which were not shown in the others. A 45 kDa band of all extracts was a carbohydrate component detected by Concanavalin A. The majority of extracts were glycoproteins, except 46, 43, 30, 29, 18.5 and < 14.4 kDa, being proteins. Serum samples showed similar reactions. Only Ncc serum reacted with DW, Tween 20, NP40 extracts at 52.5 kDa, and at 64 kDa for the ME and DOC extracts. Ncc antibody bound only with 97.4 kDa of the ME extract, and frequently with 16.5 kDa of all extracts, but not with NS or Ecc. Different extractions appear useful choices for antigen preparation, including deglycosylation, and thus merit further research.

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HOUSE FLIES: POTENTIAL TRANSMITTERS OF SOIL-TRANSMITTED HELMINTHIC INFECTIONS IN AN UNSANITARY COMMUNITY OF THAILAND

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This study investigated the helminth transmission rates of flies in an unsanitary community, i.e. Ban Namkhem Village, Takua Pa District, Phangnga Province. In this community, flies were abundant in the household surroundings and around a mangrove swamp, where piles of fecal matter were observed. The community prevalence of soil-transmitted helminths (STH) was determined by Katz's thick smear technique. Fecal samples in the swamp area were examined. The degree of soil contamination with helminth eggs was detected by sugar flotation method. Flies in the defecation area and in the community were trapped for study. The results showed that the villagers residing near the

contaminated mangrove swamp area had an infection rate of 50.0%. The soil contamination rate was 13.3%. The 567 houseflies trapped in the study belonged to one species, *Chrysomya megacephala*. Hookworm and *Trichuris trichiura* eggs were isolated on their body surfaces and were detected in fecal samples from the swamp area. The helminth-transmission rate of flies in the defecation area was 25.9%, and in the household surroundings 11.8%. The average number of eggs on the body surface of one fly in the defecation area was 0.4. After feeding on human excreta, 508 resting flies left 0.5 gm of feces with pathogens in the surroundings. These findings, and the discovery of *Ascaris lumbricoides* eggs from 2 flies in the swamp area, supported the view that *C. megacephala* was a potential transmitter of soil-transmitted helminths.

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MODIFICATION OF POLYETHYLENE TUBE CULTURE TECHNIQUE IN CULTIVATION OF HOOKWORM LARVAE

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Cultivation is a good technique for obtaining hookworm larvae from a fecal sample. Polyethylene tube cultivation is practical for field studies, since the materials and the prepared samples are easily handled during transportation. However, one limitation is the high cost of filter paper. If a suitable alternative can replace it, and clean drinking water can be used in the process, this method can be used in remote areas where laboratory supplies are scarce. Four kinds of paper--filter paper, copy paper, paper towel, and absorbent paper, were tested for efficacy in harvesting hookworm larvae in normal saline, distilled water, and bottled drinking water. In each test, 400 mg of feces from a hookworm-infected person were smeared onto the paper and retained in the polyethylene tube with the designated water solution. The presence and number of larvae were observed at 5, 7, and 10 days. The results showed that cultivation with copy paper and distilled water yielded the highest number of larvae at day 10. There was no statistical difference for number of larvae by culture with filter paper or absorbent paper. A low survival rate was noticed at day 10. Therefore, for identification, or for the purpose of further study, we recommend observing or collecting cultured hookworm larvae at day 7.

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SIX-SUCKER *TAENIA SAGINATA*: THREE CASES REPORTED IN THAILAND

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The Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, conducts teaching and research activities related to the medically important helminths. To understand the problem of helminth infection in Thailand, our mobile team often travels to remote country areas for epidemiological studies, and to provide medical services. In rural areas, people with worm infections visit our field station seeking treatment. In September 2006 and March 2007 the team visited Nan Province in northern Thailand. Fifteen persons who had passed anal tapeworm segments sought treatment. After administration of nicosamide 600 mg/tab 4 tablets per oral, they were given a purgative, and the whole stool from 3-4 movements was collected for examination for any expelled worms. After collection, the worm parts were preserved for morphological study and fixed for staining. Permanent stained slides of scolex, mature and gravid segments were studied morphologically. All mature and gravid segments recovered agreed morphologically with *Taenia saginata*, but among 17 scolices found, 3 had 6 suckers rather than the normal 4. DNA was extracted from parts of all scolices, COI sequencing confirmed that all tapeworms were indeed *T. saginata*. The 6-sucker type is an abnormal form of *T. saginata*, not a typical form of a new species.

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ACCIDENTAL FINDING OF *ANCHITREMA SANGUINEUM* DURING COLONOSCOPY OF PATIENT WITH CHRONIC ABDOMINAL DISTURBANCE: CASE REPORT

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A 46-year-old male Thai patient, a resident of Bangkok, presented with chronic abdominal pain and disturbance for many years. He had visited several hospitals and received medication, but had not improved. In November 2007, he visited a private hospital in Bangkok for physical examination. He had mild abdominal pain, especially around his perineum, defecation and urination normal, no abdominal distension, no fever, pallor, or jaundice. Colonoscopy revealed a small parasite attached to the caecum mucosa. The worm was removed and sent to the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, for identification. The specimen was the middle section of a trematode; the anterior and posterior ends were missing. However, the remaining part contained specific organs that enabled identification to species by comparison with a complete specimen. The worm was identified as *Anchitrema sanguineum*, a trematode reported in the reptile, *Chamaeleo vulgaris*, from Egypt and the intestines of rats in Thailand. The patient was treated with praziquantel, but no more worms or worm parts were expelled. Symptoms improved slightly. It is not clear whether the chronic abdominal disturbance was caused by this trematode infection. This is the first report of *Anchitrema sanguineum* infection in humans.

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ONCOMELANIA SNAILS AS POTENTIAL FIRST-INTERMEDIATE HOSTS FOR *PARAGONIMUS PSEUDOHETEROTREMUS* IN THE LABORATORY

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Currently, 6 species of *Paragonimus* have been reported in Thailand- *P. westermani, P. heterotremus, P. harinasutai, P. bangkokensis, P. siamensis*, and *P. macrorchis*. The life cycles have not yet been clearly demonstrated, since no natural first intermediate host was found, other than for *P. siamensis*. In 2007, very small metacercariae were found in crabs collected in Kanchanaburi Province. The metacercariae were developed in a cat to adult stage and were described as *P. pseudoheterotremus*, a new *Paragonimus* species. Fully-grown miracidia were observed in the eggs 2 months after the eggs were discharged. Experimental first-intermediate hosts in the laboratory, *Oncomelania* snails (*O. nosophora* and *O. quadrasi*), were exposed to the hatched miracidia. Cercariae escaped from *O. nosophora* on day 60 post-infection. *O. quadrasi* produced cercariae at day 77 post-infection. *O. nosophora* showed much higher susceptibility to miracidial infection than *O. quadrasi*, at 100 and 16.6%, respectively. It was concluded that *O. nosophora* is a better host for *P. pseudoheterotremus* than *O. quadrasi*.

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AQUATIC SNAILS AS NEW PARATENIC HOSTS OF *GNATHOSTOMA SPINIGERUM*

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Aquatic snails, *Pomacea canaliculata* and *Pila polita* were found experimentally to be suitable paratenic hosts for third-stage larvae (L3) of thenematode *Gnathostoma spinigerum*, the causative parasite of gnathostomiasis in human. *G. spinigerum* (L3) were found to be encapsulated in the tissue of snail's foot. This is the first evidence to reveal that not only vertebrates but also invertebrates (snails) can serve as paratenic hosts to this parasite.

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ELISA VALUES OF SERUM ANTIBODIES FROM HEALTHY THAI INDIVIDUALS AGAINST ANTIGENS OF TISSUE HELMINTHS

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This study was conducted to determine the ELISA values of serum antibodies against crude and partially purified somatic antigens of five tissue helminths: *Gnathostoma spinigerum, Angiostrongylus cantonensis, Trichinella spiralis, Toxocara canis* and *Cysticercus cellulosae*. Serum samples were obtained from 25 positive controls corresponding to each parasite (group A). Group B was 100 healthy individuals free of intestinal parasites. Group C was 400 healthy Thai individuals (blood donors without screening for parasitic infections; 1. students, 2. government officials, 3. employees, 4. laborers). When crude antigens were used in the ELISA, cut-off values were calculated from group B at X+1 SD. When partially purified antigens were used, cut-off values were drawn at mean group B plus 1 SD to 4 SD, as follows: *G. spinigerum* peaks 2, 3 (X+2 SD; 0.349, 0.336), *A. cantonensis* peak 2 (X+4 SD, 0.577), *T. spiralis* peak 1 (X+3 SD, 0.352), *T. spiralis* peak 2 (X+2 SD, 0.310), *T. canis* peak 1 (X+2 SD, 0.472) and *C. cellulosae* peak 1 (X+2 SD, 0.425). The sensitivities and specificities of the ELISA using crude antigens of the above five helminthes were 64.0 and 54.2%, 44.0 and 97.2%, 92.0 and 82.0%, 12.0 and 91.8%, and 20.0 and 88.6%, respectively. When partially purified antigens were used, the sensitivity and specificity of the test were both 100% for all helminths. Purified antigens may be suitable for use in indirect ELISA as a screening test for tissue helminth infections.

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ALBENDAZOLE POTENTIATES LIPID PEROXIDATION IN RAT INFECTED WITH *TRICHINELLA SPIRALIS*

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Melondialdehyde (MDA) levels as an indicator of lipid peroxidation were determined in six homogenated tissues including heart, kidney, liver, diaphragm, skeletal muscle and intestine and in plasma of experimental rats. In the course of experimental trichinellosis (400 larvae per os/rat), MDA level increased in the above six tissues in day 7 post infection (7 dpi) rats and were maintained only in kidney and skeletal muscle of 35 dpi rats. This indicated lipid peroxidation occurred in trichinellosis and the kidney and skeletal muscle were more oxidative damaged than the other organs. Seven days after single oral administration of an anthelmintic drug, albendazole, (10 and 20 mg/kg) in control rats, MDA level significantly increased in a dose dependent manner in all tissues except intestine. This result suggested that albendazole treatment increases lipid peroxidation in the pivotal organs. After 48 hour of larva inoculation, administration of a single dose of albendazole (20 mg/kg) did not alter the increased MDA level in heart, kidney and liver of the 7 dpi rats. However, albendazole made the level of MDA increased even more in diaphragm and skeletal muscle of 7 dpi rats. It is concluded that albendazole treatment in trichinellosis rats potentiates the oxidative stress in the skeletal muscle tissue.

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SECOND OUTBREAK OF TRICHINELLOSIS PAPUAE IN THAILAND

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In July 2006, the first outbreak of trichinellosis papuae occurred in Ban-Rai Distrcit, Uthaithani Province, Thailand, involving 19 hospitalized patients and 14 sero-positive villagers. The second outbreak occurred in September 2007, in the same area previously reported, involving 23 patients. They were treated with mebendazole (100 mg) twice daily for 3 days, followed by 200 mg twice daily for 10 days, plus prednisolone. Two seriously ill patients were admitted to Ban-Rai District Hospital. One was a 34-year-old male, who presented with low-grade fever, muscle pain and edema at the legs and arms. Clinical symptoms began one month after eating under-cooked wild-boar meat with neighbors. One patient was referred to Uthaithani Provincial Hospital for muscle biopsy and proper management. A muscle biopsy and 13 serum samples from some patients were sent to the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, in Bangkok, Thailand. One non-encapsulated *Trichinella* larva was found in a muscle biopsy and COI-PCR confirmed the worm to be *T. papuae*. Immunoblot was positive for trichinellosis at 109 kDa. Follow-up at 4 months found the cases to be healthy, with no muscle pain or edema, and only minimal symptoms of fatigue. In conclusion, early diagnosis and prompt treatment using the standard regimen of mebendazole and prednisolone were useful in saving the life of the patient with *T. papuae* infection.

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POSSIBLE ANTIGENIC DISPLAY OF ADULT *PARAGONIMUS PSEUDOHETEROTREMUS* EXTRACT TO PARAGONIMIASIS ANTIBODIES FROM THAILAND, LAO PDR, AND ECUADOR

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The adult *Paragonimus pseudoheterotremus* worm is reportedly akin to *Paragonimus heterotremus*, the main causative agent of human paragonimiasis in Thailand and neighboring countries. Based on a life cycle, *P. pseudoheterotremus* can develop in rats. An alternative antigen from *P. pseudoheterotremus* is expected to replace *P. heterotremus* antigen. Therefore, basic analysis should be conducted, following simple structural components and antigenicity. Adult worm extracts of both species showed two major proteins, 28.5 and 17 kDa, with Coomassie brilliant blue (CBB). Several weak bands of *P. heterotremus* extract were found at 84-44.5 kDa, and others. In contrast, *P. pseudoheterotremus* extract gave two smear bands from 28.5-17 and 17-< 6.4 kDa. All bands of both extracts showed stronger detection by silver stain than CBB. With ConcanavalinA, four bands of 195.7-71.5 kDa of *P. heterotremus*, and of 100-48.5 kDa with several minor bands *P. pseudoheterotremus* extract carried moiety of glucose and mannose. In immunoblot using *P. pseudoheterotremus* antigen, all paragonimiasis sera from Thailand and Lao PDR, and Ecuador (*P. ecuadoriensis*) reacted with common bands (30, 26.5 kDa), including healthy controls. Paragonimiasis ecuadoriensis gave very weak reactions on the entire strips. The majority of Thai and Lao patient antibodies reacted with 195.7-26.5 kDa, which are partly composed of carbohydrates. It was concluded that *P. pseudoheterotremus* antigen should be used to detect paragonimiasis heterotremus in both Thailand and Lao PDR, and glycoprotein antigens are useful for further ELISA detection.

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MOLECULAR CHARACTERIZATION OF A FULL-LENGTH CDNA SEQUENCE ENCODING SMALL MUTS RELATED (SMR) DOMAIN-CONTAINING PROTEIN OF *ANGIOSTRONGYLUS CANTONENSIS*

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Angiostrongylus cantonensis, the rat lung worm, is the primary cause of eosinophilic meningitis in humans. Here, we describe identification, sequence analysis and expression of a Smr domain-containing protein from adult *A. cantonensis*. Immunoscreening of a female worm ZAP expression cDNA library using pooled positive sera from patients who were diagnosed with angiostrongyliasis yielded several positive clones, including one encoding a Smr domain-containing protein. This clone contained a 2,736 bp insert with a 2,136 bp open reading frame encoding a predicted protein that exhibits significant sequence similarity to a Smr domain-containing protein of *Brugia malayi*. The full-length protein was successfully expressed in *Escherichia coli* BL21(DE3) cells and is now being produced in large-scale for further purification. It is anticipated that the recombinant Smr domain-containing protein may be useful as an antigen for serodiagnosis of human angiostrongyliasis.

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A PILOT STUDY ON *GNATHOSTOMA* AND GNATHOSTOMIASIS IN CHAMPASACK PROVINCE, SOUTHERN OF LAOS

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Gnathostomiasis - a migratory swelling syndrome caused by *Gnathostoma* nematode, is a food-borne parasitic zoonosis, endemic in some Asian and Latin American countries, but very little known about this disease is known in Lao PDR. This study aimed to investigate human gnathostomiasis and distribution of gnathostome in Lao PDR starting a pilot study in Nongthearnoy village, Phonthong district, Champasack Province. The total of 131 serum samples was collected among them, 51(38.9%) sera were positive with *Gnathostoma* antibody on 24 kDa, the diagnostic band using immunoblot technique. Fourteen dropped stools of dogs were collected from the village surrounding, 2 of them were positive with *Gnathostoma* eggs, and 3 of 16 frogs from the village were found harboring 9 advanced third stages of gnathostome larvae. The result confirms that Nongthearnoy village is the endemic area of gnathostomiasis. Transmission occurs in the village, dog is reservoir host and frog is one of the source of infection to human. This is the first endemic area of gnathostomiasis demonstrated in Lao PDR with high prevalence. In addition, 9 of 28 and 10 of 16 frogs were found *Spirometra* spp. larvae in markets and village, respectively. High infection rate of *Spirometra* spp. larvae was noted within intermediate hosts in the village which may have a chance to get disease to people. More study is needed in other provinces before distribution of gnathostomiasis inside Lao PDR is clearly understood and control policy can be decided.

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DETECTION OF TRICHINELLOSIS IN LIVE SWINE USING ANTIGENIC PRODUCTS FROM *TRICHINELLA SPIRALIS* MUSCLE LARVAE

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Since 1971, human trichinellosis in Thailand has been reportedly caused by *Trichinella* species, mainly *Trichinella spiralis*, including one outbreak of *T. pseudospiralis* and two of *T. papuae*. Mostly, the patients had consumed raw or improperly cooked meat of hill-tribe or domestic pigs containing muscle larvae. Larval detection is suitable only for carcasses, so that pigs from farms, or pigs for live export, should be examined serologically for trichinellosis. The current study used crude and excretory-secretory antigens (CAg and ESAg) to evaluate their potential use in indirect ELISA. Pig-serum samples were collected from 5 pigs experimentally infected with *T. spiralis* (pTs), 147 positive sera of 8 other infections, 9 mixed infections of other parasitic organisms, and 35 normal controls. The result by ESAg was better than CAg: sensitivity, specificity, positive and negative predictive values; ESAg, 100, 92.1, 62.5 and 100%, respectively, at cut-off value 0.835; CAg, 100, 67.3, 22.7 and 100%, respectively at cut-off value 0.332. False positives to ESAg showed OD values close to 0.835 for coccidiasis (1), mixed infections; GI nematode-coccidiasis (1), and -Ascaris (1). For CAg, trichuriasis (2), coccidiasis (5), and 5 mixed infections (8) gave cross-reactions, but some of these samples had OD values far above 0.332. Increasing antibody levels with the collection of consecutive serum samples from 5 pTs resulted in trichinellosis detection by ESAg (at 0.835) on days 24-38. It was concluded that ESAg may be used to detect antibody to *T. spiralis* in pigs at least 24 days' post-infection.

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SECOND OUTBREAK AND IMPLEMENT OF AN EDUCATION PROGRAMME ON THE CONTROL OF TRICHINELLOSIS PAPUAE IN BAN-RAI DISTRICT, UTHAITHANI PROVINCE, THAILAND

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Trichinellosis has been described as an emerging and/or re-emerging disease in Europe and the world. *Trichinellosis papuae* is one of eight of *Trichinella* spp., the infection has been reported only in animals. In Thailand, the first outbreak of trichinellosis papuae occurred in July 2006 in Uthaithani Province. One year later, the second outbreak occurred again in September 2007, in the same subdistrict but different village, about 30 km far from the previously reported, involving 43 patients. They were treated with mebendazole (100mg) twice daily for 3 days, followed by 200mg twice daily for 10 days, plus prednisolone. For two seriously ill patients, they were hospitalized at Ban-Rai hospital, one of them was referred to Provincial hospital for muscle biopsy and proper management. Follow-up at 4 months found the cases to be healthy, with no muscle pain or edema, and only minimal symptoms of fatigue. These results suggest that early diagnosis and prompt treatment using the standard regimen of mebendazole and prednisolone were useful in saving the life of the patient with *T. papuae* infection. An education programme on the control of trichinellosis was implemented to the schoolchildren and community via health officers and community's leaders. After the health education, they gained knowledge on the disease, source of the transmission and how to prevent its. They interested to change their food preparation method for a food safety.

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EFFECTIVE SUPPRESSION OF DENGUE FEVER VIRUS IN MOSQUITO CELL CULTURES USING RETROVIRAL TRANSDUCTION OF HAMMERHEAD RIBOZYMES TARGETING THE VIRAL GENOME

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Outbreaks of Dengue impose a heavy economic burden on developing countries in terms of vector control and human morbidity. Effective vaccines against all four serotypes of Dengue are in development, but population replacement with transgenic vectors unable to transmit the virus might ultimately prove to be an effective approach to disease suppression, or even eradication. A key element of the refractory transgenic vector approach is the development of transgenes that effectively prohibit viral transmission. In this report we test the effectiveness of several hammerhead ribozymes in suppression DENV in lentivirus transduced mosquito cells in an attempt to mimic the transgenic use of the these effector molecules in mosquitoes. A lentivirus vector that expresses these ribozymes as a fusion RNA molecule using an *Ae. aegypti* tRNAval promoter and terminating with a 60A tail insures optimal expression, localization, and activity of the hammerhead ribozyme against the DENV genome. Among the 14 hammerhead ribozymes designed to attack the DENV genome, several appear to be relatively effective in reducing virus production from transduced cells by as much as 2 logs. Among the sequences targeted are 10 that are conserved among all DENV serotype 2 strains. Our results confirm that hammerhed ribozymes can be effective in suppressing DENV in a transgenic approach, and provide an alternative or supplementary approach to proposed siRNA strategies for DENV suppression in transgenic mosquitoes.

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INSECTICIDE TREATED CURTAINS TO PREVENT DENGUE: COMPARISON OF 2 DISTRIBUTION MODELS, THAILAND

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Background: The efficacy of Insecticide Treated Curtains (ITC) to reduce dengue fever (DF) vector densities was demonstrated recently. Notwithstanding, the operational effectiveness of a new tool depends not only on efficacy, but equally on implementation process, coverage and user-acceptance.

Objective: To test different strategies for delivering ITC and to assess the attained coverage, acceptance and effectiveness.

Methods: In a cluster randomized trial in (sub)urban areas of Laem Chabang, Chon Buri province, Thailand, 22 clusters of 70-100 houses were randomly allocated to 2 intervention arms. In the vertical model arm (VM), ITC were distributed through the routine DF control program and in the partnership model arm (PM), through partnerships of community volunteers, organized and trained by the researchers. We conducted baseline surveys in October 2006. ITC, together with information on their use and possible side-effects, were distributed in March 2007. After two and five months, we assessed ITC distribution coverage, acceptability and use through household (HH) surveys. Aedes infestation was monitored through entomological surveys every six months.

Results: At baseline 97.3% of interviewees had correct knowledge on DF transmission and 90% of HH were storing water in containers. Abate was present in only 11% of the HH. The ITC distribution coverage reached 94.5% (VM: 95.4%; PM: 93.5%; $P = 0.335$), with a median of 3 ITC/covered HH. 85.5% of the HH in VM were still using ITC two months after distribution, against 80.2% in PM ($p = 0.10$). This dropped, after 5 months, to 78.6% against 70.5% respectively ($p<0.01$). Only 17.3% of HH that ever used ITC had washed them, and 78.4% found them effective against mosquito nuisance (no significant differences between models). In October 2007 (7 months after distribution) all entomological indices had decreased in comparison to October 2006; e.g. the Container Index from 15.6% to 10.3% in PM ($p = 0.05$) and from 18.2% to 8.6% in VM ($p<0.05$). The reduction of Pupal Index was 60% higher ($p<0.01$) in VM (decrease of 0.27 pupae/inhabitant) than in PM (decrease of 0.17 pupae/inhabitant).

Conclusions: In both models excellent distribution coverage was reached. Five-months-uptake remained fair, but was, surprisingly, significantly higher in VM than in PM. This difference was reflected in a more pronounced Aedes infestation decrease. Ongoing surveys will permit to evaluated the long term effectiveness and efficiency of both models.

Presented at: the Second International Conference on Dengue and Dengue Haemorrhagic Fever "Global Innovation to Fight Dengue". Phuket, Thailand, October 15th –17th, 2008.

MOLECULAR IDENTIFICATION OF COASTAL MALARIA VECTOR, *ANOPHELES SUNDAICUS*, IN NATURAL BREEDING SOURCES 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 *An. sundaicus* is suspected as malaria vector. *An. sundaicus* infers to species complex because of its ecological and ethological differences. Morphology identification may be confusing in case of *An. sundaicus* complex. So, molecular identification is used to investigate this species complex. In this study, *Anopheles* mosquitoes were collected in three provinces, Phang-Nga, Chanthaburi and Rayong, along the coastal area of Andaman and the Gulf of Thailand. We performed PCR amplification and the nucleotide sequence analysis of ITS2, D3 of 28s rRNA and COI were done to compare with *An. sundaicus* in other coastal areas of Vietnam, Malaysia, and India. These analyses confirm *An. sundaicus* from Thailand is *An. epiroticus*. The sequence alignment of ITS2 of *An.*

sundaicus from 3 areas of Thailand showed identical with *An. sundaicus* A from Vietnam but different from *An. sundaicus s.s* from Malaysia. While, the D3 of 28s rRNA of *An. sundaicus* from Thailand showed identical with *An. sundaicus* D from India. Moreover the sequence alignment of COI of *An. sundaicus* from 3 areas of Thailand showed some nucleotide variation. In conclusion, *An. sundaicus* (*An. epiroticus*) from Thailand can be confirmed speciation and reveal the possibility to use the molecular marker, ITS2 and COI, for molecular identification of *An. sundaicus* complex mosquitoes.

Presented at: the XVIIth International Congress for Tropical Medicine and Malaria, Jeju Island, Korea, 29 September – 3 October 2008.

THE USE OF INSECTICIDE TREATED MATERIALS TO CONTROL DENGUE VECTORS IN PHANG NGA, THAILAND: A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Background: Dengue fever is currently the fastest spreading arboviral disease worldwide. In the absence of a vaccine, *Aedes aegypti* vector control remains the most effective strategy to prevent transmission. Initial studies have suggested that insecticide treated materials (ITMs) can impact on dengue vector populations and potentially on dengue virus transmission.

Objectives: This is the first large-scale trial of ITMs for dengue vector control in Southeast Asia.

Methods: ITMs were deployed as window curtains and indoors (as door curtains, room dividers and wardrobe covers) in a cluster randomized trial in approximately 2000 households in the Krasom and Khok Kloi subdistricts, Takuatung district of Phang Nga province, southern Thailand. A total of 26 clusters where included in the study. Clusters were stratified based on numbers of houses within each cluster and randomly allocated to either receive the ITM intervention or act as a control.

Results: The trial will end in late 2008. Preliminary data will be presented.

Conclusions: The future potential of ITMs to control dengue vectors in SE Asia and recommendations for further study will be discussed.

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ALTERNATIVE MOSQUITO REPELLENT

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In Thailand, many species of mosquito carry disease causing agents such as *Aedes aegypti*, *Anopheles dirus*, *Mansonia* spp. and *Culex quinquefasciatus* (lymphatic filariasis), etc. Many ways exist for protection against these diseases such as eliminating mosquito breeding areas, using chemical protective clothes or bed netting and chemical skin repellent creams. However, using synthetic chemical repellents over long periods may cause health problems. Chemical evaporation may cause dysfunction or destroy chromosomes. Other possible side effects from synthetic chemical repellents include rash, burning, pimples and itching. Thus natural chemical repellents, which are safe and produce no side effects, have grown in popularity as an alternative. Various Thai natural aromatic products repel mosquitoes and other insects, such as coconuts, lemon grass, chilli, turmeric, chrysanthemums, bergamot, lavender, tobacco Citronella Grass and others. Because these natural products are strong and easily cause irritation, extraction processes for commercial purposes are usually considered prohibitively expensive. We have performed a research to develop a mosquito repellent product in both cream and balm form that is safe to use, with no side effects or skin irritation. The active ingredient is qinghao oil (*Artemisia annua*), along with may chang oil (*Litsea cubeba*) and kaffir lime oil (*Citrus hystric*). In essence, the product works by changing the human odor to distract mosquitoes by absorbing into skin it causes chemical changes to create a substance that covers the human odor naturally attractive to mosquitoes. The protection time was longer than 3 hour in *Ae. aegypti* and *An. dirus*

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MOSQUITO FAUNA OF PEAT SWAMP FORESTS OF THAILAND

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Entomological surveys (2001-2005) were carried out in Surat Thani and Narathiwat Province to determine mosquito fauna in addition to the biodiversity record of the peat swamp forest. Mosquitoes were collected by several conventional methods as follows: animal-baited traps (cow and cat); human-landing catches; light traps, and immature collections by ovitraps and direct search from breeding places. Among the 430 mosquito species belonging to 23 genera known in Thailand, 54 species belonging to 13 genera were identified from 837 larval specimens and 2,923 adult mosquitoes, including major vectors of *Brugian fillariasis, Mansonia annulata, Ma. bonneae, Ma. dives, Ma. uniformis, Ma. indiana* and *Anopheles letifer. Ma. annulata* and *An. letifer* were reported for the first time in Thailand as lymphatic filariasis vectors with high biting density during the day and at night. In addition, some rare species of mosquitoes, for instance, *An. letifer* and *Toxorhynchites manopi, Zeugnomyia gracilis* were collected in small to large number from natural breeding sites which were different from those previously reported. For example, larvae of *Ze. gracilis* and *Tripteroides* tenax were collected from pitcher plants (*Nepenthes gracilis*). The bionomics of certain vector species is discussed.

Presented at: the XVIIth International Congress for Tropical Medicine and Malaria, Jeju Island, Korea, 29 September – 3 October 2008.

OCCURRENCE, DISTRIBUTION AND PREVALENCE OF *AEDES ALBOPICTUS* IN BANGKOK METROPOLITAN

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Bangkok is among top ten cities of Thailand with highest dengue cases which transmitted mainly by *Aedes aegypti*. Recently we collected *Ae. albopictus* in the vicinity of Bangkok. *Ae. albopictus* ranks second to *Ae. aegypti* in importance to man as a vector of dengue and dengue hemorrhagic fever. In addition, it is considered a competent vector for many pathogens in both field and laboratory conditions. Initially sylvatic, *Ae. albopictus* has shown a remarkable capacity to adapt to human environments, allowing it to replace *Ae. aegypti* population in many places. The role of the species as an actual or potential vector of dengue haemorrhagic fever in Bangkok is questionable. Therefore, the present study is to initially determine the occurrence, distribution and characteristics of breeding habitats of *Ae. albopictus* in Bangkok metropolitan. From December 2006 to October 2007, 1,364 ovitraps were placed in 341 households with vegetation around the houses. The ovitraps were collected weekly and kept under laboratory conditions to allow the eggs to develop into the adult mosquitoes for species confirmation. It was found that albopictus mosquitoes occurred both indoors and mostly outdoors in small proportion (7-10%) with varied numbers of eggs ranging from 6-81 eggs per ovitrap. *Ae. albopictus* has shown from our study a capacity to adapt itself to urbanization. In addition, it was observed that vegetation was an important factor for *Ae. albopictus* to thrive in the urban environments. Further study on its population dynamics and vector competence is under investigation. Poster Sessions

Presented at: the XVIIth International Congress for Tropical Medicine and Malaria, Jeju Island, Korea, October 2 (Thursday) - October 3 (Friday) 2008.

POPULATION DYNAMICS, BITING ACTIVITY, AND MOLECULAR IDENTIFICATION OF *ANOPHELES DIRUS* COMPLEX IN HUMANMADE CONTAINERS

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Population dynamic of *Anopheles dirus* complex was preliminary study in a dhamma retreated temple in Kanchanaburi Province, Thailand, as a result from a discovery of unusual type of *An. dirus* breeding site. The characteristic of breeding place was a brick and cement constructed well exposed to the full sunlit with light intensity over 1000 lux. Larval density was 3.7 per dip in average. The larvae were found to associate with *Culex* larvae, dragonfly naiads, and tadpoles. Adult female mosquitoes were collected from human landing methods during 18:00 hr-24:00 hr. The species composition of the collected comprised of 13 species, namely *An. dirus* complex, *Aedes albopictus, Aedes aegypti, Aedes annandalei, Aedes vittatus, Aedes niveus* group, *Aedes desmotes, Ochlerotatus chrysolineatus, Culex quinquefaciatus, Culex ascocephala, Armigeres magnus, Armigeres subalbatus*, and *Armigeres malayi*. The predominant species of collected mosquito were *Aedes albopictus* followed by *An. dirus* complex. Regarding the rapid polymerase chain reaction based method, *An. dirus* complex was suspected to be *An. dirus* or *An. baimaii*. Confirmation of its species is on trial. Female *An. dirus* complex start to bite from 19:00 hr with a sharp peak during 19:00-20:00 hr. The highest biting density was 6 mosquitoes per man per hour at 19:00-20:00 hr. Seasonal abundance of *An. dirus* complex will be completed by January, 2009. This is the first report of *An. dirus* complex breeding in artificial container in Thailand.

Presented at: the XVIIth International Congress for Tropical Medicine and Malaria, Jeju Island, Korea, 29 September – 3 October 2008.

RESIDUAL EFFECT OF MOSSMAN 100 (PERMETHRIN 10% EC) IMPREGNATED BED NET AND ITS IMPACT ON MALARIA VECTOR AND MALARIA CASES

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The objective of this field trial was to assess the residual effectiveness for malaria control of mosquito nets treated with Mossman100 (permethrin 10% EC) compared to untreated nets. The study was carried out between July and December 2007 in the Pong-Nam-Ron district of Chantaburi province, Thailand. Mosquito population densities were assessed by landing catch technique. They were collected between 18:00 and 24:00 hours. Additionally standard WHO bioassay tests were carried out monthly using *Anopheles dirus* mosquitoes reared in the insectary of Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. The results showed that the population densities of *Anopheles* spp., including the malaria vector *Anopheles minimus*, were unaffected in the study area where mosquito nets treated with Mossman 100 (permethrin 10% EC) at 300 mg/sq. m were used. WHO bioassay tests showed that the nets treated with Mossman 100 remained biologically effective against *An. dirus* for up to six months. Indigenous cases of malaria were reduced by 27.7% in the site in which nets treated with Mossman 100 (permethrin 10% EC) were used but was not change of malaria cases at the control site.

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IDENTIFICATION OF MOSQUITOES THROUGH DNA BARCODES, A CASE STUDY

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Many molecular technologies are developed for identifying mosquitoes from field collection. Because of species complexes, DNA barcoding has been used to solve and remains to be developed continuously as high throughput technology. For this study, many species of mosquitoes in Thailand are classified as group taxon of DNA barcodes. Fourteen genera of mosquitoes were collected, extracted DNA and amplified DNA barcode sequence, a short fragment of cytochrome oxidase I (COI) region from mitochondria. Each mosquito species are differentiated as mosquito genus groups after taxon tree analysis. Sequence divergence was analyzed a 639-base pair fragment of COI from each mosquitoes species. The percentage of sequence divergence in

each mosquito species calculated average 12.9% (2-14%). It is indicated that DNA barcode displays an important molecular tools for identification of mosquito species.

Presented at: the Joint International Tropical Medicine Meeting 2008, at Imperial Queen's Park, Bangkok, Thailand, 13-14 October 2008.

ADULT SIZE FO *AEDES AEGYPTI* (DIPTERA: CULICIDAE) COLLECTED AT DIFFERENT DENGUE TRANSMISSION SEASONS IN NAKHON RATCHASIMA, THAILAND

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Adult mosquito size may be related to longevity, feeding frequency, and other factors that impact vectorial capacity, as well as response to repellents. It has been reported that more of the larger *Ae. aegypti* mosquitoes were infected than the medium or small mosquitoes. However, in other study no evidence for significant interaction between body size and DENV dissemination was observed. Although, is noteworthy that most of these studies relied on artificially derived variation in body size. Hence, we aim to ascertain the seasonality of vertically transmitted dengue serotypes, and to investigate the relation between dengue virus infection and mosquito size in *Ae. aegypti* natural populations. We measured the wing size of adult females that emerged from larvae fourth instars and pupae collected from a dengue hyperendemic area at Muang District in Nakhon Ratchasima Province. L_4 and pupae were collected from inside and around houses with reported dengue patients during low (February), beginning (June), during (August), and end (November) of dengue transmission seasons in 2007. So far, we have observed that mean wing size low and end seasons than that of the beginning and during the transmission seasons. We expect that our findings provide new insights on the overall natural maintenance of virus in nature and the seasonality of the dengue serotypes in hyperendemic areas, as well as on the eventual effects of dengue virus infection on the development of its main vector, *Ae. aegypti*.

Presented at: the Joint International Tropical Medicine Meeting 2008, Imperial Queen's Park Hotel, Bangkok, Thailand, 13-14 October 2008.

EXPRESSION OF TOLL-LIKE RECEPTOR 2 ON PERIPHERAL BLOOD MONOCYTES AND MYELOID DENDRITIC CELLS IN FALCIPARUM MALARIA PATIENTS

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Toll-like receptors (TLRs) have emerged as a pattern recognition receptor residing on innate immune cells, whose function is responsible for recognizing specific conserved component of microbes. In mouse model, Glycosylphosphatidylinositol (GPI) anchor of *Plasmodium falciparum* can be recognized by TLR2 which induce the proinflammatory cytokine production and cause transient pyrexia and hypoglycemia. The aims of this study were to determine the TLR2 expression on peripheral blood monocytes and myeloid dendritic cells (MDC) and their correlation to the level of plasma cytokines in patients with mild and severe form of falciparum malaria. Healthy individuals were used as controls. An increase in the surface expression of TLR2 on peripheral blood monocytes and MDC were found in patients with severe and mild malaria compared to healthy controls. The percentage of circulating monocytes and MDC expressing TLR2 was significantly decreased in both patients with severe and mild malaria. Additionally, the increase of TLR2 expression on monocytes and MDC in patients with severe and mild malaria was significantly correlated with IL-12p70 and IFN-γ cytokines. These finding suggested that *P. falciparum* parasite could modulate TLRs expression in human falciparum malaria infection.

Presented at: Keystone symposium on Malarai: Immunology, Pathogenesis and Vaccine Perspectives (part of the Keystone Global health Series) June 8-13, Alphach Congress Centrum, Alphach, Austria

POLYMORPHISMS IN THE GENE ENCODING INTERFERON- AND INTERFERON- RECEPTOR 1 IN PATIENTS WITH FALCIPARUM MALARIA FROM NORTHWESTERN THAILAND

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Introduction and Objectives: Roles of IFNG and IFNGR1 gene polymorphisms in susceptibility to severe malaria had been studied

in Africans. This study aimed to determine an association between the polymorphisms and susceptibility to severe malaria and relation to hyperparasitemia as well plasma IFN- levels.

Methods: This case-control study involved 103 cases (patients with severe malaria), and 252 controls (patients with uncomplicated malaria). Analyses of IFNG-1616A/G, IFNG+2200A/G and IFNGR1-56A/G were performed by TaqMan® assays. Association studies between polymorphisms and susceptibility to severe malaria or hyperparasitemia and comparisons between the polymorphisms and parasite density and plasma IFN- levels were performed.

Results: No associations were observed between the polymorphisms and susceptibility to severe malaria or hyperparasitemia. However, relations between IFNG+2200 and IFNGR1-56 and hyperparasitemia (250,000/ l) as well as plasma IFN- levels were seen. IFNG+2200 heterozygosity and IFNGR1-56 G homozygosity in cases were significant higher parasite density compared to controls ($p<0.001$ and $p<0.001$). In addition, IFNGR1-56 G homozygosity in cases had a significant higher IFN- levels compared to controls ($p=0.0397$ and $p=0.0371$, respectively).

Conclusions: Our data suggest that IFNG+2200AG and IFNGR1-56GG might be high-risk variants for severe malaria in population living in northwestern Thailand. However, further study with a larger sample size is required to make conclusive evidence on association of IFNG and IFNGR1 alleles with susceptibility to severe malaria. Functional studies both in vivo and in vitro are essential in order to clarify the role of the polymorphisms influencing susceptibility to severe malaria.

Presented at: RGJ Congress IX, April 4-6 2008, Jomthien Pattaya, Chonburi

MAJOR ADHESION HAPLOTYPES OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHOCYTES ISOLATED FROM PATIENTS WITH SEVERE AND UNCOMPLICATED MALARIA IN THAILAND

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Introduction and Objective: The sequestration of *P. falciparim* infected erythrocytes in small vessel endothelium leading to specific organ dysfunctions. Are there any different adhesion behaviors between *P. falciparum* infected erythrocytes isolated from severe and uncomplicated malaria?

Methods: Fifty three out of seventy nine parasite isolates were successfully primarily cultured up to 24-36 h. The rosette formation and the binding capacity to CD36, ICAM-1^{ref}, ICAM-1^{Kilifi} and ICAM-$1^{S22/A}$ using static proteins binding were assayed. The binding capacity of ICAM-1^{kilifi} and ICAM-$1^{S22/A}$ in comparison to ICAM-1^{ref} were coded into adhesion "haplotype": "a" for increase/no difference, "b" for minor decrease and "c" for moderate/major decrease in adhesion (A4 = ca; ItG = ac; JDP8 = ab).

Results: Among the cultured parasites, the number of parasite infected red cells (PRBC) of isolates from patients with severe malaria adhered to CD36 (mean ± SD = 82.73±92.75) were higher than those isolates from uncomplicated malaria (mean ± SD = 47.10±74.82). Seventy nine percent of clinical isolates (23 and 19 isolates from patients with severe and uncomplicated malaria, respectively) showed rosette formation. The percentage of rosette formation in isolates from severe malaria (mean ± SD=19.74±19.85) was higher than those from uncomplicated malaria (mean ± SD=11.30±12.69). A significant correlation between the binding capacity to CD36 and high concentration ICAM-1^{ref} condition was observed ($r=0.351$, $p=0.009$). However, no correlation between the binding capacity to CD36 or high concentration ICAM-1^{ref} condition and the percentage of rosette formation ($r=0.193$, $p=0.159$ and $r=0.110$, $p=0.422$, respectively) were found. For ICAM-1 proteins, the major adhesion haplotype of isolates were "aa" and "cc" in both ICAM-1 concentration conditions. Some parasite isolates showed A4 haplotype (7%), ItG haplotype (7%) and JDP8 haplotype (4%) at high ICAM-1 concentration condition. The A4 haplotype and "bb" haplotype could not be found at the low ICAM-1 concentration condition. There was no significant association in adhesion haplotypes of isolates from patients with severe and uncomplicated malaria ($p=0.065$).

Conclusion: Clinical isolates varied in their adhesion to ICAM-1 proteins, although the major adhesion haplotypes were "aa". A significant correlation between the binding capacity to CD36 and high concentration ICAM-1^{ref} condition was observed, therefore, ICAM-1 and CD36 worked synergistically in mediating adhesion to endothelium cells. There were no significant correlations in the binding capacity to CD36, ICAM-1 and rosette formation between isolates of severe and uncomplicated malaria.

Presented at: RGJ Congress IX, April 4-6 2008, Jomthien Pattaya, Chonburi

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

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Complicated malaria is associated with differential elevations of certain pro- and anti-inflammatory cytokines. Over production of TNF , IL-1 and IL-6 implicates in severity and pathogenesis of cerebral malaria. The IL-10 and IL-12 may function as immune modulators by their elevated levels in plasma of complicated malaria patients. 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 IL-10 to TNF 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 . The IFN level was significantly elevated in the initial stage while IL-4 levels was elevated during the intermediate and late stages of the disease. There was no association between the IL-4 -590 CT transition and the severity of malaria. A significant inverse correlation between IL-4 to IFN ratio and parasitaemia was found only in complicated malaria patients carrying TT genotype. This could also modulate and regulate the production of anti-*P. falciparum*-IgG, -IgG subclasses and -IgE antibodies linking to the control of parasitaemia and affect the clinical outcome.

Presented at: International Congress of Tropical Medicine and Malaria (ICTM) 2008, Jeju, Korea, 29 September-3 October, 2008

A LOW COST HIV-1 VIRAL LOAD DETECTION USING A COMMERCIAL REAL-TIME PCR REAGENT AND LIGHT CYCLER 1.2 INSTRUMENT

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Objective: Although plasma HIV-1 Viral load is the most effective marker for evaluation of anti-retroviral therapy in HIV-1-infected individuals, HIV-1 viral load detection usually costs very high and is frequently omitted in resource-limiting situations. The aim of the present study is to develop plasma HIV-1 Viral load detection system with a reasonable cost.

Materials and Methods: Commercial TaqMan real-time PCR reagent was modified and applied on Light Cylcer 1.2 for quantifying HIV-1 RNA in plasma and compared with the reference method; COBAS AmpliPrep/ COBAS Amplicor HIV-1 monitor test version 1.5. Three hundred and eight frozen and fresh plasma samples were used for evaluation. Sequential specimens were also tested for follow-up cases.

Results: The correlation between HIV-1 RNA values obtained by reference and modified method with automated and manual sample preparation were significant with $r=0.916$ and 0.908 ($p<0.001$, $p<0.001$) respectively with similar agreement log of mean bias (0.5 versus 0.48). High degree of correlation and agreement were observed between the assays in blind fresh plasma, $r= 0.953$ ($p<0.001$) with 0.15 log difference in HIV-1 RNA level. Among follow-up samples, both methods gave 100% concordant results.

Conclusion: This modified protocol can be used for monitoring efficacy of anti-retroviral therapy in HIV-1-infected individuals in Thailand. The authors would like to transfer this low-cost technique to other laboratories that have Light Cycler (LC) machine from the Global Fund.

Presented at: The 8th Awaji International Forum on Infection and Immunity (AIFII8), Awaji Island, Japan, September 7- 11, 2008

DEVELOPMENT OF CEFTAZIDIME RESISTANT *BURKHOLDERIA PSEUDOMALLEI* DURING HUMAN MELIOIDOSIS ASSOCIATED WITH MULTIPLE GENE DELETION

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Ceftazidime is a treatment of choice for melioidosis. We identified six patients unrelated by place or time who presented with culture-proven melioidosis caused by ceftazidime-susceptible *B. pseudomallei* who initially responded to ceftazidime but then deteriorated after 16-27 days of therapy. *B. pseudomallei* strains isolated at this point were unable to grow in commercial blood culture bottles or on most routine laboratory media and grew slowly on Ashdowns agar, due to the presence of glycerol. All six treatment failure strains were highly resistant to ceftazidime. Gram-stain of the primary isolates demonstrated Gram-negative rods, but all six 'failure' strains appeared as long bacterial filaments. Multilocus sequence typing confirmed that each of the six patients was infected with a different clone, but that each strain pair from a given patient belonged to the same clone. Comparison of strain pairs by pulsed-field gel electrophoresis demonstrated a change in banding pattern for each patient-pair. Array-based comparative genomic hybridization of primary and failure strains identified a recurrently deleted genomic region of between 50-75 genes with a conserved deletion of 50 genes in all six strains. This region contained genes putatively involved in peptidoglycan synthesis and a range of metabolic functions. This novel mechanism of ceftazidime resistance may be grossly under-recognised because of failure of these variants to grow on commonly used media. Melioidosis is notoriously difficult to cure, and this report represents the first explanatory mechanism for some cases of treatment failure.

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A MURINE MODEL OF ALLERGY CAUSED BY AMERICAN COCKROACH (CR), *PERIPLANETA AMERICANA*

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SUMMARY An animal model resembling the human immuno-pathological features of CR allergy is needed for CR allergy research, e.g., measuring allergenicity of novel allergens, testing immunotherapeutic efficacies of drugs and vaccines. In this study we develop a murine model of American CR, *P. americana* allergy. BALB/c mice, 6 weeks old, were individually intraperitoneally injected with three doses (days 0, 7 and 14) of alum adjuvanted-crude extract of *P. americana*. On days 21 and 23, they were given crude CR extract in PBS intranasally (10 μl) and aerosolically (10 ml) via an air-pressure nebulizer, respectively. Mice received alum alone and PBS instead of the CR extract served as non-allergenic controls. All mice were bled twenty four hours after the nebulization and sacrificed. Their serum samples, broncho-alveolar lavage fluids (BALF), and lung tissues were collected. BALF of all allergen-treated mice had marked cellular infiltration notably neutrophils, eosinophils and lymphocytes. The average total cell count in BALF of the allergenic mice was 1.9 x 10^5cells/ml which out-numbered those of the non-allergenic con-trols (8 x 10^4 cells/ml). The eosinophil infiltration was pronounced in lungs of the allergen-treated mice. Specific se-rum IgE to the CR extract elevated in serum samples of all allergen treated mice and nil in the sera of the controls. None of the mice showed detectable level of IgG2a to the CR extract. RT-PCR revealed that all allergen-treated mice had marked increase of IL-13, IL-4 and TNF-α gene expressions,

slight increase of IL-5 gene expression, and absence of detectable IFN-γ gene expression in comparison to the non-allergenic controls. None of the allergen-treated mice and 50% of the non-allergenic controls had IL-12 gene expression as detected by RT- PCR. One allergen treated-mouse (25%) had subpar level of the IL-18 gene expression compared to the controls. Results of the quantitative real-time PCR conformed to those of the RT-PCR. A murine model of *P. americana* resembling human allergic manifestations was successfully developed.

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NOVEL *PERIPLANETA AMERICANA* ALLERGEN, GLUTATHIONE S-TRANSFERASE

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American cockroach (CR), *Periplaneta americana* is an important source of indoor allergens among Thais. Diagnosis and therapy of cockroach allergy are complicated by the presence of allergen isoforms and panallergens. Thus, in this study, we produce recombinant glutathione S-transferase (GST) which is major insect panallergens. The protein was tested for its ability to bind to IgE in sera of CR allergic Thai patients. Total RNA was extracted from adult *P. americana* and first strand cDNA was produced by RT-PCR. Gene encoding American cockroach GST (651 bp) was amplified and cloned into a cloning vector, *i.e.* pKRX and a protein expression vector, *i.e.* pET 20b+. Amplified sequence showed 100% homology to the GST gene sequence in the database. Recombinant GST was produced and purified from whole cell lysis of an *E. coli* transformant grown under IPTG induced-condition. The protein was found to bind IgE in sera of allergic Thai patients. This report show GST as a new American cockroach allergen among CR allergic Thais and this protein may be used as a standard reagent for screening and monitoring of the allergic status of patients.

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GIARDIA DUODENALIS IN THAILAND: GENOTYPE IDENTIFICATION AND CLINICAL RELEVANCE ACCORDING TO HOST AGE

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This study investigated *G. duodenalis* cysts by PCR- based genotyping. The cysts were collected from the stool samples of 61 Thai subjects from Bangkok and from rural communities with and without gastro-intestinal symptoms. The assemblages of the cysts were identified by single step PCR of the 148- and 81- bp *tpi* gene segments. Among the 61 cyst preparations, 5 (8%) belonged to assemblage A only, 25 (41%) to a mixture of assemblages A + B, and 31 (51%) to assemblage B only. Based on the restriction fragment length polymorphism (RFLP) of the 384 bp –giardin gene segments, 12% and 88% of the assemblage A cysts belonged to groups AI and AII, respectively. For the B sub-assemblages based on the RFLP of the 492 *gdh* gene segments, 45.5 and 54.5% belonged to groups BIII and BIV, respectively. AII and 70% of the subjects with AI and BIII sub-assemblages, respectively, had gastro-intestinal symptoms. Eighty percent of the symptomatic Bangkok residents were adults/elderly while 85% of the giardiasis cases from the rural communities were young children. From our data we conclude that both assemblages have pathogenic potential and that the sub-assemblages that can cause disease are less frequent than non-pathogenic ones in general, but with a higher prevalence in the rural area compared to the city.

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MOLECULAR DIVERSITY OF *LISTERIA MONOCYTOGENES* ISOLATES FROM DAIRY PRODUCT IN VARIOUS MARKET

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Listeriosis, a food-borne disease is causes by *Listeria monocytogenes*. This bacterium can cause septicemia, encephalitis and abortion in man and animals. *L. monocytogenes* is commonly found in the enveironments and in foods such as dairy products. Contamination of bacteria can occurred during or after food process and transportation. This study aims to characterize and investigate the diversity of isolated *Listeria monocytogenes* in raw pork and chicken from market in Bangkok and Pathum-thani provice during April-November 2007. *Listeria spp*. was isolated from one hundred and ten samples by using conventional culture technique as gold standard. The isolated *L. monocytogenese* were characterized the virulence gene (*hlyA, actA, iap* and *flaA*) by using PCR. The result showed thirty two (29%) *L. monocytogenese* were isolated. From isolated *L. monocytogenese* Twenty seven samples were further analysis by virulence gene amplification. Fifteen percents of selected samples were positive for all virulence genes, 26% were positive for *hlyA, actA* and *flaA,* 4% were positive for *actA,* and *flaA, 15%, 11%, 3%* were positive for *actA,* and *flaA, 7%* were positive for *hlyA, actA* and *iap,* respectively. Our study provided the baseline information on the molecular diversity of *L. monocytogenese* isolated from food samples.

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

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Current mainstay for treatment of tetanus is by passively giving and 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 ot cetain 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. This study aims to produce immunotherapeutic human monoclonal antibodies in the form of single chain antibodies (ScFv) against tetanus toxin by using phage display human antibody library. Tetanus toxoid was recovered from the commercially available tetanus vaccine by removing 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. Individual phages were used to transfect non-suppressor *E. coli* bacteria for production of the human soluble ScFv. Transformants carrying scFv-phagemids were obtained. RFLP assay was showed different restricted DNA pattern of individual scFv-phagemids. ScFv was purified by anti-E tag column. The efficiency of ScFv against tetanus toxin activity was inverstigated by immunological assay.

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CHARACTERISTICS OF ANTIGEN RECOGNIZED BY 111E8-3G1, AN *AEROMONAS* GENUS-SPECIFIC MONOCLONAL ANTIBODY

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Aeromonas is an infectious bacterium causing gastroenteritis, wound and other organ infections. Laboratory diagnosis by culture can identify the bacteria but man and time consuming, Serological test would be more useful but not well established. Our initial data showed that *Aeromonas*-immunized mouse sera reacted to a low molecular weight antigen of various *Aeromonas* spp., but not to the antigen of other Gram-negative bacteria, suggesting of the presence of a genus-specific antigen. We then produced monoclonal antibodies against *Aeromonas* spp. and a clone, namely 111E8-3G1, is genus-specific. By ELISA, this antibody reacted to crude antigen prepared from different species of *Aeromonas*, but not to crude antigen from other Gram-negative bacteria. Western blot with 5-20% gradient polyacrylamide gel electrophoresis of crude antigens showed that this antibody reacted to a single antigen band of 8.5 kDa. Treatment of crude antigen with proteinase K or boiling did not abrogate the antibody binding reactivity. These findings indicated that the genus-specific antigen is heat stable and present in non-protein low molecular weight component of *Aeromonas* bacteria.

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GENUS-SPECIFIC ANTIGEN AND INTRA-SPECIES VARIATION OF *AEROMONAS*

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Aeromonas bacteria are natural habitat in environment, some being pathogenic for animals and humans by causing gastrointestinal diseases and other serious infections of various organ systems. Its classification has still been argued. This study aimed to evaluate genus- and/or species-specific antigens of this bacterium using Western blot analysis. Groups of three mice were immunized with whole cell extracts of *A. hydrophila, A. sobria, A. caviae, A. trota, A. jandaei, A. veronii,* and *A. media*. Results showed that all immunized sera reacted to the antigen of molecular mass of 8.5 kDa of all 123 *Aeromonas* isolates of those species, but not reacted to the antigen from other irrelevant Gram-negative bacteria, indicating that this antigen could be *Aeromonas* genus-specific. Species-specific antigens were not found in this study. Heterogeneity within species was also evaluated using UPGMA-grouping system (SynGene software) and Jaccard's coefficient (Sj) $\geq$0.5 and showed that 26 isolates each of *A. sobria, A. caviae, A. trota* can be classified into 11, 11, and 13 groups, respectively, 27 isolates of *A. hydrophila* into 12 groups, 8 isolates of *A. jandaei* into 6 groups, 7 isolates of *A. veronii* into 3 groups, and 3 isolates of *A. media* into 3 different individuals. These findings would be helpful in development of serological diagnostic test and for further study in group-related pathogenesis of *Aeromonas* genus.

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IDENTIFICATION OF *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR* BY PCR ASSAY AMONG FAECAL SPECIMENS FROM THAI/MYANMAR BORDER

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Due to the indistinguishable morphology between *Entamoeba histolytica* (pathogenic) and *Entamoeba dispar* (non pathogenic), the further analysis was required to distinguish between them. The correct identification would support the medical treatment and controlling the disease. Based on microscopy, the suspected *Entamoeba* cells were detected in 30 out of 455 analyzed faeces samples (6.6%), and those feces containing *Entamoeba* cell were then subjected to DNA extraction procedure. The presence of either *E. histolytica* or *E. dispar* was verified by the polymerase chain reaction (PCR) assay. The target genes for the PCR amplification included the genes encoding small subunit rRNA (SSU-rRNA), chitinase and serine rich protein. PCR primers derived from SSU-rRNA were expected to amplify both *E. histolytica* and *E. dispar* with expected size of 1,080 bp, and this primer pair was less sensitive which tun positive in 3 out of 30 (10%). PCR primers derived from chitenase gene were expected to specifically amplifed *E. histolytica*, with expected product of 500 and 1200 bp, and the samples were positive in 12 out of 30 samples (40%). Due the high variation of genes encoding serine rich protein between *E. histolytica* and *E. dispar*, two specific sets of primers were designed, SREH-primer set was specific to *E. histolytica*, and the expected PCR products were 524 and 700 bp. The SREH primer set was quite sensitive and could amplify 21 out of 30 sample (70%). Lastly, the SED-primer set was specific to *E. dispar* and liberate the product size of 557 bp, in addition the nested primers were designed internally from the product and yield the product of 477 bp. These nested - PCR primers detected 15 out of 30 as *E. dispar* (53.33%). Detection of single and mixed infection of the 2 *Entamoeba* species could be effectively demonstrated directly from DNA-extracted from feces without culturing the parasites.

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CONSTRUCTION AND SELECTION OF FAB ANTIBODY LIBRARY SPECIFIC TO H5N1 AVIAN INFLUENZA VIRUS

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Objective : Construction and selection Fab antibody phage library specific to H5N1 avian influenza virus using phage display technique.

Methods : For construction Fab antibody library specific to H5N1 avian influenza virus, the attenuated H5N1 vaccine were immunized to the white leg horn chicken. After the high titer immunity to H5N1 antigen was obtained tested by ELISA, three chicken spleens were harvested 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 chicken variable heavy chain (V_H) and light chain (Vλ), and human constant heavy chain (C_H1) and light chain (Cκ-pelB) were also amplified from pComb3XTT vector. After that, three pairs of overlap extension primer were used for Fab amplification. The first pair was used for the *heavy chain (Fd)* overlap extension PCR of chicken VH PCR product and human C_H1 PCR product (VH- C_H1). The second primer was used to amplify **chimeric light chain** by fusing the chicken light-chain variable region PCR product (Vλ) with human Cκ-pelB for (Vλ – Cκ) fragment amplification. Then, the final overlap extension PCR were performed to combine the and chimeric light chain-pelB fragment and Fd fragment for the construction of chicken chimeric Fab libraries. This Fab were then digested with restriction enzyme *Sfi*1 and ligated to phagemid vector pComb3XSS that is also previously cut with the same enzyme. The purified ligation DNA were further transformed in to ER2738 *E. coli* host cell, and rescue with wild type helper phage VCSM13. Currently, Fab antibody libraries were successfully constructed (1.0×10^7 different clones), by which Fab 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 H5 antigen was firstly immobilized on solid support (microtiter plate). The antibody library was incubated with this antigen on the microtiter plate. After incubation, the unbound phages with non-specific sequence were washed off. Then, the bound phages were eluted and used for further selection in the next panning step. This panning was performed around 3-5 times until the high specific clones of H5 antigen were selected.

Result : Fab antibody library from H5N1 immunized chicken with 1.0×10^7 transformants were successfully constructed and used for selection in panning. From panning, the specific clones were enriched in consecutive round tested by phage pool ELISA. Among 46 picked phages from the 3rd and 4th round of panning with stringency increased condition, one phage was found to be positive with H5 antigen, using Fab ELISA. Among 20 picked phages from non stringency increased condition, two phages were found to be positive with H5 antigen, using Fab ELISA. The amino acid sequences of antibody genes from these three specific binders were aligned and analyzed. There have some variation in the CDR1, CDR2, with the most variation in the CDR3 region, the most important region for antigen binding.

Keywords : Fab antibody library, H5N1 Avian influenza virus**,** Phage Display

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CONSTRUCTION AND SELECTION OF FAB ANTIBODY LIBRARY SPECIFIC TO H5N1 AVIAN INFLUENZA VIRUS USING PHAGE DISPLAY TECHNIQUE

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Avian Influenza Virus is becoming the public health problem in Thailand since the first emergent on 2003-2004. Development of diagnostic kit for H5N1 avian influenza virus was set as high priority for national disease control policy. Monoclonal antibody has been well known as diagnostic reagent. To produce the monoclonal antibody, both traditional hybridoma technology and phage display technology can be applied, however, the latter is considered to be a much more feasible and cheaper. So, the *objective* of this study is to construct and select Fab antibody phage library specific to H5N1 avian influenza virus using phage display technique.

Methods – Three white leg horn chickens were immunized with the dead H5N1. The chicken spleens were harvested and pooled together. Total RNA were isolated from these spleens using TRIZol reagent. Then, CDNA were reverse transcribed from the RNA. cDNA were further used as template for antibody gene amplification specific to chicken variable heavy chain (V_H) and light chain (Vλ). Moreover, human constant heave chain (C_H1) and light chain (Cκ) were also amplified from pComb3XTT vector. After that, overlap extension PCR were performed to amplify the heavy chain Fd (VH- C_H1), Chimeric light chain (Vλ – Cκ), and final Fab fragment, This Fab were then digested with restriction enzyme S***fi***I and ligated to phagemid vector pComb3XSS that is also previously cut with the same enzyme. The recombinant DNA was transformed in to ER2738 *E. coli* host cell, and rescue with wild type helper phage VCSM13. According to panning strategy with immobilized antigen, the hemagglutinin-specific antibodies were selected from a repertoire of this chimeric Fab library.

Result- Fab antibody library from H5N1 immunized chicken with a library size at 1.0×10^7 was successfully constructed and used for selection in panning. From panning, the specific phage clones were enriched in consecutive round tested by phage pool ELISA. Totally 86 clones were selected for single clone Fab ELISa. Among these clones, we found 3 clones were found to give positive result from ELISA. All binders were further analyzed by DNA-sequencing and tested for their specificity by western blotting. Sequencing result showed that all binders have 93% homology with chicken immunoglobulin gene, and it was found that there have high variations in CDR region. For westem blot analysis, binder 1 and 3 were specifically bind with H5 antigen.

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RELATIONSHIP BETWEEN MMP EXPRESSION AND VIRULENCE OF DENGUE VIRUS TYPE 2 INPECTED CELL

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The severity of dengue virus infection is correlated with the increasing of soluble factors such as TNF-α and IL-8, which can play an important role in inducing the MMPs secretion. Moreover, the function of MMPs is increasing vascular permeability. Therefore, these soluble factors have the relationship with the pathogenesis and can be the markers of the severity in dengue virus infection. There have been many efforts to develop vaccine combat this infection. However, the vaccine strategies are in progress and an effective vaccine has not been available. In process of testing, the vaccine candidates will be tested for the safety by identifying biological markers in cell culture. These markers play role in the relationship between virulence, and vaccine candidate. The secretion of MMP-2 and MMP-9 was measured by the zymogram gelatinolytic activity and determined the amount of these enzymes by measuring the optical density. Among 3 groups of the dengue type-2 strain, the amounts of MMP-2 and MMP-9 were found predominantly in strain 16681 from both of LLC/MK2 and C6/36 infected cell line, while the lower amount of MMP-2 and MMP-9 were observed in strain NGC and PKD53, respectively. Our results suggested that the amounts of MMPs related to the virulence of the dengue virus strain, and MMPs may be used as cell surrogative marker testing for safety of vaccine candidate.

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INFLUENCE FACTORS PROGRAM THE DEVELOPMENT OF THE CD4+ REGULATORY T CELLS IN AUTOIMMUNE MOUSE MODEL

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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, 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 criteria's 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 study the correlation of Treg cells and autoimmune disease, we used C4 knockout mice as animal model and normal mice as control. We quantified the amount of Treg cells in blood circulation including in secondary lymphoid tissues e.g. spleen bone marrow and blood. Our results from mouse serum showed that the Anti-ANA antibody of normal mice was similar to that of C4KO mice. However, Anti-ANA antibody was significantly found in old ages mice (6-8 months) in both normal and C4KO mice. The result from FACS showed that percentage of Treg cells in C4KO mice were lower than normal mice in all age groups and were highest found in bone marrow when compared with spleen and blood in both C4KO mice and normal mice. Furthermore, the expression of FoxP3 and TGF-b were predominant found in normal mice than in C4KO mice in all kinds of tissue ($n=10$; $P<0.05$).

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PERFORMANCE ASSESSMENT OF THE FLOW CYTOMETRIC ASSAY AND THE OPTIMAL® DIPSTICK TEST FOR RAPID DETECTION OF MALARIA PARASITES FROM CLINICAL BLOOD SAMPLES

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Rapid diagnosis and prompt treatment of cases are the main objectives of management of malaria in malaria endemic areas. In this study, we used the rapid flow cytometric (FCM) assay to detect the malaria parasites and compare to the OptiMAL® dipstick test by using the microscopic examination as a standard test. After the determination of 453 clinical blood samples, the FCM assay detected malaria parasites with a sensitivity of 91.26%, specificity of 86.28%, PPV of 66.20%, NPV of 97.11% and accuracy of 87.42%, while the OptiMAL® dipstick test had sensitivity, specificity, PPV, NPV and accuracy of 97.09%, 94.57%, 84.03%, 99.10% and 95.14%, respectively. In addition, the efficiency of the FCM assay and the OptiMAL® dipstick test for malaria parasites at different levels of parasitemia were also evaluated.

Presented at:

- The 32nd Annual Meeting of the Medical Technologist Association of Thailand. May 6-9, 2008 Ambassader city Jomthian Hotel Chonburee, Thailand.
- 17th International Congress on Tropical Medicine and Malaria. September 29-October 3, 2008, Jeju Island, Korea.

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 NS1 antigen for early laboratory acute dengue. Acute serum samples within 5 days after onset of symptoms were obtained from 66 patients consisting of 49 dengue cases and 17 non-dengue cases. In addition, samples from 7 hepatitis cases and 36 healthy subjects as negative controls. All cases were diagnosed dengue by dengue IgM- and IgG-capture ELISA. Single acute serum samples were tested for the presence of dengue antigen by using Dengue NS1 antigen-capture ELISA and dengue RNA by using RT-PCR. Dengue virus NS1 antigen was detected in 21/42 (50%) samples from dengue cases but none in samples from non-dengue cases. Among negative results with dengue-capture ELISA of IgM (<40 units) and of IgG (<100 units), dengue antigen was detected in 20 of 41 (42%) samples for IgM antibodies and in 20 of 36 (47%) for IgG antibodies. The results indicate that Dengue NS1 antigen-capture ELISA can be particularly used in serum samples within 5 days after onset of symptoms for rapid and early diagnosis of dengue virus infections. In addition, dengue antigen detection test can increase frequency of dengue cases in laboratory diagnosis. The best method for dengue diagnosis is a combination of dengue antigen and serological tests.

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APPLICATION OF RECOMBINANT DENGUE VIRAL PROTEIN FOR SERODIAGNOSIS OF DENGUE VIRUS INFECTION

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The four serotypes of dengue virus, a member of the Family *Flaviviridae* of positive-sense single-stranded RNA viruses, cause broad spectrum of clinical manifestations in humans, ranging from acute febrile illness DF to life-threatening DHF/DSS. Laboratory diagnosis of dengue virus infection relies on the detection of specific anti-dengue IgM and IgG antibodies. During the export viral particles through the secretory pathway, the precursor to the M structural protein (prM), is cleaved by the proprotein convertase furin and can not found on surface of mature flaviviruses particle, except dengue virus. In this study to produce dengue virus prM-M recombinant proteins by using *Escherichia coli* expression system, aimed to use them as antigens in development of immunodiagnosis test. The prM-M gene of DENV-2 strain 16681 was cloned into bacterial expression vector of pRSET-B, and was expressed in *E. coli* BL21(DE3) pLysS. This insoluble protein was solubilized with 8 M urea, before subjected to purify by Electro-Eluter. The purified

protein was tested for the immunogenicity with the dengue and Japanese encephalitis patients' sera by western blot analysis and the indirect ELISA assay. The purified recombinant prM-M protein (~18 kDa) could be detected for Histidine tag fusion protein. The sensitivity of indirect ELISA was 41% and relatively low in western blot assay, with specificity of 82% and 100% respectively. Sera from patient infected with Japanese encephalitis showed little and no reactivity.

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- Joint International Tropical Medicine Meeting 2008, "Tropical Medicine in the-omics Era" 13-14 October 2008, The Imperial Queen's Park Hotel, Bangkok, Thailand.
- The Second International Conference on Dengue and Dengue Haemorrhagic Fever Global Innovation to Fight Dengue, 15-17 October 2008, Hilton Arcadia Phuket, Thailand

BINDING CHARACTERIZATIONS OF SOUTHEAST ASIAN DENGUE 2 VIRUS AND AMERICAN DENGUE 2 VIRUSES WITH SINGLE E GENE AMINO ACID SUBTITUTIONS

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Dengue viruses (DENV) are the most important arboviral pathogens in tropical and subtropical regions. Previous studies have reported that Southeast Asian and American strains of dengue viruses are genetically different and infection results in distinct clinical manifestations at most mild disease without hemorrhagic feature. Viral factors are suggested to be involved in progressive dengue hemorrhagic fever. The phenotypic functional activity of DENV-2 American mutants carrying a single amino acid substitution of Southeast Asian strain was determined. Sucrose gradient ultracentrifuge-purified viruses, DENV-2 Asian prototype (D2-NGC), one American strains (D2/QT2913 prototype) and four American strains with specific mutagenic sites; Tonga 74, 81, 162, and 390 were prepared. The inhibition of virus infection by heparin or MAbs (3H5, 4G2 and 2H2), competitive inhibition of virus infection between heparin VS MAbs and end-point binding activities were demonstrated. Neutralizing activities of 3H5 and 4G2 were more than 80%, while 2H2 shown 30-50% for D2/QT2913 and Tonga derivatives. Heparin (0-2,000 μg/ml) could not inhibit the infection of 3H5 of Tonga derivatives were greater than those of prototypes. Heparin inhibits virus entry of D2(NGC) but not D2/QT2913 and Tonga derivatives into LLC-MK2 cells. Tonga derivatives with Southeast Asian single amino acid substitutions do not show phenotypic difference of MAb neutralizing activity or heparin inhibition of infection of Southeast Asian strain.

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- The Second International Conference on Dengue and Dengue Haemorrhagic Fever Global Innovation to Fight Dengue, 15-17 October 2008, Hilton Arcadia Phuket, Thailand

SURVEILLANCE OF DENGUE VIRUS INFECTION IN 3 PROVINCES, THAILAND

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BACKGROUND: Dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the re-emerging infectious diseases caused by the four serotypes of dengue (DEN) virus, type 1 to 4, belonging to the family Flaviviridae and genus Flavivirus. In Thailand during 2002-2003, more than 150,000 cases of dengue virus infection and more than 100 deaths were reported by Office of Dengue Control Ministry of Public Health.

OBJECTIVES: To determine the seroprevalence and the serotypes of dengue virus infection in pediatric patients of Petchabun, Srisaket, Nakhon SiThammarat provinces by using IgM capture enzyme-linked immunosorbent assay (Mc-ELISA) and reverse transcriptase-polymerase chain reaction (RT-PCR), respectively.

METHODS: Paired sera samples were collected from patients of Petchabun, Srisaket, Nakhon SiThammarat provinces; who have the clinical diagnosis as dengue virus infection and patients who have clinical diagnosis as pyrexia of unknown origin (PUO) in 2004. The historical background of all patients was collected. All serum samples were subjected for determining the dengue virus infection by Mc-ELISA and RT-PCR.

RESULTS: The seroprevalence and the serotypes of dengue virus infection in pediatric patients of Petchabun, Srisaket, Nakhon SiThammarat provinces were analyzed in 670 patients during May to September 2004. The illness was caused by DEN-2, DEN-1, DEN-3 and DEN-4 in the percentage of 38, 29, 18 and 15, respectively. Almost all of the DHF cases caused by DEN-2 and DEN-4 were in secondary infection, while approximately 25% of the DHF cases caused by DEN-1 and DEN-3 were in primary infection. Male: female ratio and age distribution were not different among four serotypes in primary and secondary infections.

CONCLUSIONS: The prevalence of dengue virus and the serotypes of virus causing it will show the indication of dengue epidemiology in the area. This will help in focusing the preventive measures being applied in high-risk zone.

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DETECTION OF *ENTAMOEBA DISPAR* FROM SURFACE AND WASTE WATER SAMPLES BY REAL-TIME PCR

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E. dispar and *E. moshkovskii* are morphological appearance indistinguishable from *E. histolytica* at both cyst and trophozoite stages. It was estimated that E. histolytica may infect half billion people world wide annually. However, by considering the existence of a species complex of *Entamoeba*, current data indicates that *E. dispar* is perhaps 10 times more common than *E. histolytica* worldwide. Although, previous studies showed that *E. dispar* was considered to be nonpathogenic parasite but the intestinal symptoms in patients infected with this species have been reported. Due to the *Entamoeba* species infect human through fecal-oral route transmission, water is common in developing countries where much of the water supply for drinking is untreated and contaminated with feces. Using of human feces for fertilizer is also an important source of infection. Cysts can survive days to weeks or month especially under damp conditions in the external environment and are responsible for transmission. In this study, 137 surface and waste water samples collected from Pathumthani Province, Thailand were examined for *E. histolytica*, *E. dispar* and *E. moshkovskii* by using multiplex real-time PCR assay. Specific fluorescence-labeled hybridization probes were designed to detect and differentiate the species of these three human *Entamoeba*. This assay was able to detect as little as 0.2 pg of E. histolytica DNA and 2 pg each for *E. dispar* and *E. moshkovskii* DNA. The result showed that out of 137 surface and waste water samples, 18 were positive for *E. dispar*. When 137 samples were examined by PCR using Entamoeba genus-specific primers, 37 samples showed 550-bp positive products. Therefore, a group of *Entamoeba* spp. positive samples will be further investigated for their species by using new assays with specific probes and primers for *E. polecki*, *E. chattoni*, *E. hartmani*, and *E. coli*. Our finding indicates firstly an existence of *Entamoeba dispar* in surface and waste water from Thailand and it shows the possible route of transmission of this amoeba to humans in this area of Thailand.

Presented at: Joint International Tropical Medicine Meeting, 13-14 October 2008, Bangkok, Thailand

UNDERSTANDING CENTENNIAL *TOXOPLASMA GONDII* BY HIGH TECHNOLOGY METHODS

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Toxoplasma gondii, a protozoan known to humans for a hundred years, is highly successful once infected and harbored in the host life-long. Rapidly dividing tachyzoites cause acute infection with non-specific and often unnoticed clinical manifestations, then they transform into the bradyzoite stage soon after causing unrecognized chronic infection. Toxoplasmic encephalitis (TE) occurs in immune suppressed individuals due to reactivation of quiescent bradyzoites. Up to now, the diagnosis require both clinical and laboratory criteria. Immunohistochemistry and RT-PCR assay were developed to detect tachyzoite/bradyzoite stage-specific expressions during acute, chronic and immunosuppressed periods in experimental *Toxoplasma* infected mice. The immunohistochemical technique enhanced visualization of parasites enabling their number and distribution to be accurately measured. In addition, double immunocytochemical labelling confirmed the exclusive presence of tachyzoites during the acute phase and bradyzoites during the chronic phase. RT-PCR assay showed that tachyzoites were transformed from bradyzoites since the first week of suppression period and were more apparent at the second and third weeks in the cerebrum, cerebellum, eye, heart, lung, diaphragm, liver, spleen and kidney. Bradyzoites were also found in nearly all organs at the end of this study. Results obtained from our study suggested that during suppression period, bradyzoites were not only transformed to tachyzoites, but also caused new bradyzoite development.Both high technology immunohistochemistry and RT-PCR could be further developed as alternative methods for the prognosis and diagnosis of the centennial *Toxoplasma gondii* infection leading to better and appropriate chemoprophylaxis or treatment.

Presented at: International Congress for Tropical Medicine and Malaria, 29 September – 3 October 2008, JeJu, Korea

MOLECULAR CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* POLYNUCLEOTIDE KINASE/PHOSPHATASE

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Polynucleotide kinase (PNK)/phosphatase is a bifunctional enzyme that can phosphorylate 5'-OH termini and dephosphorylate 3'-phosphate termini of DNA. It is a DNA repair enzyme involved in the processing of strand break termini to a form suitable for other proteins to complete the replacement of missing nucleotides and strand rejoining. PNK is implicated in the repair of both single-strand breaks and double-strand breaks. Mammalian PNK is a key component of both the base excision repair (BER) and nonhomologous end-joining (NHEJ) DNA repair pathways. In *Plasmodium falciparum*, little is known about its DNA repair including roles of PNK on parasite DNA repair. In this study, PNK of *Plasmodium falciparum* strain K1 (PfPNK) was successfully cloned and expressed. The purified GST-PfPNK showed a band at ~ 81.5 KDa on SDS/PAGE. MALDI-TOF analysis gave an enzyme mass of 55584 Da, which is in good agreement with the calculation based on its sequence. Surprisingly, its 3' phosphatase activity was detected whereas 5' kinase activity was not found. The 3' phosphatase activity was inhibitd by 150 mM NaCl. The recombinant enzyme was active in the presence of 10 mM MgCl2 or MnCl2 or CaCl2. Moreover, we have employed site directed mutagenesis to change two amino acids involved in DNA binding that may recover 5' kinase activity. We found that substitutions of aspartic acid for Glu330 or threonine substitutions for Val357 couldn't activate the 5' kinase, without effecting 3' phosphatase. In addition, double mutation did not support both 5' kinase and 3' phosphatase activities.

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DETECTING GIARDIA CONTAMINATION IN THAI VEGETABLES

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The protozoa *Giardia duodenalis* is an important pathogenic parasite causing food-borne diseases which result in health and economic burden throughout the world. Apart from water, food is an important vehicle that transmits such protozoan to human. Fresh papaya, chili and tomato are important ingredients of "Somthum" or "Papaya Salad", a Thai famous dish. Other two vegetables including raw morning glory and cabbage are usually eaten with Somthum. The aim of this study is to detect *G. duodenalis* in five famous Thai dish ingredients since there is no report about protozoan contamination in Thai vegetables. A total of 100 vegetable samples were collected from 10 different markets representing each region of Bangkok during wet and dry seasons. Elution by Tween20 solution followed by floatation technique with sucrose were performed. Giemsa staining and PCR techniques are then applied. Six samples (6%) was found positive for *G. duodenalis* by those methods. Even though the contamination in fresh vegetables is less, thorough cleansing is recommended for preventing food-borne diseases.

Presented at: Presented at: International Congress for Tropical Medicine and Malaria, 29 September – 3 October 2008, JeJu, Korea

COMPARATIVE STUDY OF THREE LABORATORIES FOR DETECTING CRYPTOSPORIDIUM IN STOOL SAMPLES

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Cryptosporidium parvum is one of the leading causes of opportunistic infections among HIV patients. Due to its small oocysts size, detected of this protozoan require special staining and laboratory expertise. In this study, we compare the effectiveness of three leading parasitology laboratories in Mahidol University, Thailand. Serial concentrations of *Cryptosporidium parvum* from 5 to 5x10 4 oocysts per 10 μl were added to stool samples and distributed to the studied laboratories. Conventional microscopy and special staining using dimethyl sulfoxide (DMSO) modified acid-fast stain and modified cold kinyoun acid-fast techniques were applied. The sensitivity of both techniques was similar. All three laboratories could detect at less as 5 oocysts per 10 μl. Either staining method could be applied at any laboratories serving opportunistic infection in HIV patients. Training laboratory technician to acquire such expertise and standardization are necessary. Effective laboratory diagnosis would be very helpful for physician leading to patients' appropriate management.

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LARGE-VOLUME COLLECTION: A RECOMMENDED TECHNIQUE FOR PROTOZOA DETECTION IN WATER

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Giardia and *Cryptosporidium* are waterborne protozoa which cause problems to many water supplies leading to water-borne outbreak to both human and animal worldwide since the 1980s. Though they usually contaminate water in low number, they cause diseases with low infective doses. Moreover, they survive for months under optimal environment and are resistant to adverse condition. Thus large volume of water is needed for detection. We collected raw and treated water by filtering through the 1 ?m nominal porosity activated carbon block filter with the rate of 3-4 L/min for 3-5 hours. Five hundred to one thousand liters of 80 raw and treated water samples from the central part of Thailand were collected. Elution by Tween solution followed by concentration and purification with Immunomagnetic Separation (IMS) were performed. Contaminated protozoa were identified by Immunofluorescence and PCR techniques.

We found no contamination in treated water but 20% (16 out of 80) of raw water samples were c ontaminated with *Giardia*, whilst 9 out of 80 (11.25%) were contaminated with *Cryptosporidium* and 3 (3.75%) were affected by both. The large-volume water collection technique is cumbersome but is recommended for increasing the possibility of finding both protozoa due to the low concentration in water.

Presented at: International Congress for Tropical Medicine and Malaria, 29 September – 3 October 2008, JeJu, Korea

RT-PCR ASSAY: A DIAGNOSTIC TOOL FOR *TOXOPLASMA* REACTIVATION IN IMMUNOCOMPROMISED HOST

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Although *Toxoplasma gondii* infects human and warm-blooded animal worldwide, there is non specific sign and symptom. Majority of the cases thus always unrecognized such infection, whilst the bradyzoites maintain in infected host life long. However, when host immunity is impaired the quiescence bradyzoites will be reactivated into rapidly dividing tachyzoites causing Toxoplasmic encephalitis (TE), a life threatening disease. Definite diagnosis could not be made by only one method. Presumptive diagnosis is mainly based on central nervous system manifestations, suggestive neuro-imaging features, sero- positive *T. gondii* antibodies and therapeutic response to anti-toxoplasmic drugs.

Over three decades, PCR-based techniques have been introduced and found to be useful for the diagnosis of parasitic infections including *Toxoplasma*. However, it could not differentiate between bradyzoite and tachyzoite state. Recently, many stage specific genes of *T. gondii* have been identified along with the development of molecular technologies.

Our laboratory have developed the Reverse transcriptase-polymerase chain reaction (RT-PCR) in order to detect the expression of *Toxoplasma* bradyzoite (BAG1) and tachyzoite (SAG1) specific genes during stage conversion in immunocompromised patients. It was found that RT-PCR is an efficient assay which high sensitivity and specificity. In addition, this assay could identify the process of re-differentiation from bradyzoites into tachyzoites at the earliest stage, enabling the prophylaxis or treatment of patients in time before the occurrence of severe clinical manifestations. Moreover, the duplex RT-PCR could be further developed to offer a rapid, sensitive, easy-to-handle and reproducible method. Thus the RT-PCR technique may serve as an alternative tool to diagnose TE in severely immuno-compromised patients in the next decade.

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TOXOPLASMIC ENCEPHALITIS: THE USE OF STAGE-SPECIFIC GENE AS A DIAGNOSTIC TOOL

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Toxoplasmic encephalitis (TE), a life threatening disease in immuno-compromised hosts, is caused by reactivation of dormant bradyzoites into rapidly dividing tachyzoites of *Toxoplasma gondii*. Definite diagnosis could not be made by only one method. Presumptive diagnosis is mainly based on central nervous system manifestations, suggestive neuro-imaging features, sero- positive *T. gondii* antibodies and therapeutic response to anti-toxoplasmic drugs.

Over three decades, PCR-based techniques have been introduced and found to be useful for the diagnosis of parasitic infections including *Toxoplasma*. However, it could not differentiate between bradyzoite and tachyzoite state. Recently, many stage specific genes of *T. gondii* have been identified along with the development of molecular technologies.

Reverse transcriptase-polymerase chain reaction (RT-PCR) is an efficient assay to detect the expression of bradyzoite (BAG1) and tachyzoite (SAG1) specific genes during stage conversion. In addition, this assay could identify the process of re-differentiation from bradyzoites into tachyzoites at the earliest stage, enabling the prophylaxis or treatment of patients in time before the occurrence of severe clinical manifestations. Moreover, the duplex RT-PCR could be further developed to offer a rapid, sensitive, easy-to-handle and reproducible method. Thus the RT-PCR technique may serve as an alternative tool to diagnose TE in severely immuno-compromised patients in the next decade.

Presented at: Joint International Tropical Medicine Meeting, 13-14 October 2008, Bangkok, Thailand

RAPID DETECTION OF MDR-TB FROM INDOOR AIR USING AN IN HOUSE MODIFIED IMPINGER AND NESTED PCR

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The aerosol transmissions of multidrug resistant *M. tuberculosis* (MDR-TB) among health care workers and patients have been increasing. Inhalation only 2 active MTB bacilli can cause disease. So the rapid method that can detect only 2 aerosol MTB is needed .

Objective to develop the method for rapid detection of MDR-TB from indoor air using modified impinger and in house nested PCR.

Methods In the biosafety cabinet, 10-10 [4] cells of *M. tuberculosis* H37Ra strain were aerosol sprayed for 30 mins using nebulizer, respectively. Then, the in house modified impinger contained 15 ml of sterile water, connected with airpump (40 liter/min), was used to collect the air. DNA was prepared from collected water in impinger, then part of the MTB *rpoB* gene was further amplified using nested PCR. All experiment was repeated 30 times.

Results From all 30 times of experiment, all sprayed MTB could be detected using nested PCR, with the lowest detection limit at 10 *f*g (equal to 2 MTB bacillis). Using this novel method, the MDR-TB from in door air can be detected within 8 hours. Until present, this develop method has

been utilized in many hospital in Thailand.

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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 has gained increasing interest. The most important thiols in plasma; cysteine, cysteinylglycine, homocysteine and glutathione are available for determination by using several HPLC methods. Based on these methods, we developed a new HPLC assay for rapid and sensitive measurement. 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 μm 125×4 mm; mobile phase: 0.1mol/l KH_2PO_4 with 10% acetonitrile, adjusted to pH 2.1 with ortho-phosphoric acid, flow rate 1.0 ml/min. These thiols are clearly separated from each others within 6-min of total analysis HPLC method. In this study, determination of the concentrations of total homocysteine and other aminothiols were done in plasma of 133 healthy adults (<65 yrs) and 53 healthy aging (>=65 yrs) in urban area, Bangkok, Thailand. Plasma was assayed for total thiols by HPLC and these thiols were compared between adults and aging. Mean values of plasma cysteine and homocysteine were significant lower in adult subjects (ages<65 yrs) compared with aging (P<0.001), however, cysteinylglycine were significant higher (P<0.001). Mean glutathione were not age-dependent. In aging, mean plasma homocysteine was higher in males than in females (12.47 VS 9.67 umol/l, P<0.05), but mean cysteine, cysteinylglycine and glutathione were not sex-dependent. In adult subjects, mean plasma homocysteine and cysteinylglycine were higher in males than in females (9.92 VS 7.97 umol/l, P<0.001 and 8.28 VS 5.2 umol/l, P<0.001, respectively), but mean cysteine and glutathione were not sex dependent. The described method is well suited for analysis of thiols in blood specimens, since it is convenient and rapid.

Presented at:

- The Joint International Tropical Medicine Meeting (JITMM), 13-14 October 2008, Imperial Queen Park Hotel, Bangkok, Thailand.
- Frontiers in Protein Research Meeting, 28-29 August 2008, Chulabhorn Research Institute, Bangkok, Thailand.

STUDIES ON CORRELATION OF SERUM VITAMIN B_{12}, SERUM FOLIC ACID, RED BLOOD CELL FOLATE, PLASMA HOMOCYSTEINE, AND MICRONUCLEUS FREQUENCY IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL IN AGING AND ADULT THAI PEOPLE

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Folic acid and vitamin B_{12} play an important role in DNA metabolism. Deficient levels of folic acid and vitamin B_{12} are associated with elevated chromosome damage rate and high concentrations of homocysteine (Hcy) in the blood. We have therefore performed a study to determine the prevalence of folate deficiency, vitamin B_{12} deficiency and hyperhomo-cystemia in healthy aged over 65 years and evaluate the relationship of these micronutrient levels in the plasma with the micronucleus frequency in peripheral blood lymphocytes. Apparently 186 healthy individuals (aged 21-64 yrs: 32 males and 105 females; aged ≥65 yrs: 12 males and 43 females) were recruited who are ambulatory without chronic diseases, history of cancer and medification. Plasma (heparin) homocysteine was assayed by HPLC. Serum and red blood cell folate were assayed by microbiological assay using *Lactobacillus casei*. Serum vitamin B_{12} was assay by chemiluminescence. PBMC were studied the micronucleus frequency in cytokinesis-blocked assay. We also studied the relationship of these micronutrient: folate, vitamin B_{12} and total homocysteine with the micronucleus frequency. In group of Thai adults the evidence of serum folic acid and red blood cell folate deficiency is higher than Thai aging (aged <65 yrs: serum folic acid<6ng/ml=56.9%, red cell folate<221 ng/ml=22.6% and aged ≥65 yrs; serum folic acid, <6 ng/ml=38.2 % and red cell folate<221 ng/ml=12.7%), however, there is no significant difference between male and female. No evidence of B_{12} deficiency was found in both groups. In aged group, the evidence of high plasma HC in Thai aging is higher than Thai adults (aged <65 yrs: plasma HC>10 ng/ml=24.1% and aged ≥65 yrs; plasma HC>10 ng/ml=47.6%) and has the significance differences between sex (male>female). Micronucleus index, the biomarker for chromosome damage, increases significantly with age. There is the significant positive correlation between MN frequency and plasma HC, serum folic acid, BMI and blood pressure. Thus, it appears that elevated plasma HC, a risk factor for cardiovascular disease, may also be a risk factor for chromosome damage.

Presented at: XVII th International Congress for Tropical Medicine and Malaria (ICTM 2008), 29 September-30 October 2008, at International Convention Jeju, Jeju, Korea.

A PROGNOSTIC VALUE OF DNA AMPLIFICATION IN L2 REPEATED SEQUENCE IN CHOLANGIO-CARCINOMA

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Cholangiocarcinoma (CCA), a cancer of bile duct is a major health problem in Thailand. Surgical resection remains the curative treatment, however, the DNA marker for prognostic indicator has been elucidated. This study, genetic alterations in cholangiocarcinoma was determined using arbitrarily primed-polymerase chain reaction with 30 random primers. Band loss obtained from DNA fingerprint amplified from primer BC17 observed in 30% of the patients group was analyzed by gene cloning, nucleotide sequencing and identified by comparison with known gene sequence and DNA repeat sequence in genome database using BLAST program (http://www.ncbi.nlm.nih.gov) and RepeatMasker (http://woody.embl-heidelberg.de/repeatmask), respectively. DNA amplification in L2 repeat element on chromosome 17p13.2 was identified in 18 of 44 cases, (41%) by real-time PCR. Likewise, this DNA amplification was associated with long survival of the patients, median survival time (wk) of DNA amplification *vs.* no amplification was 44.14 *vs.* 24.14, $P=0.002$. The results indicated DNA amplification on L2 repeat element on chromosome 17p13.2 may be used as a DNA marker for good prognosis of cholangiocarcinoma.

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DETECTION OF GENETIC INSTABILITIES IN CHOLANGIOCARCINOMA BY ARBITRARILY PRIMED-POLYMERASE CHAIN REACTION

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Intrahepatic cholangiocarcinoma (ICC), a malignant neoplasm of the biliary epithelium in the liver is a major health problem in Northeast Thailand. At present, the molecular marker finding of the disease has been elucidated. This study, the genomic instabilities of Thai patients with ICC were studied by arbitrarily primed -polymerase chain reaction (AP-PCR). The DNA extracted from cancerous tissues and their corresponding normal tissues was amplified with 30 random 10-mer primers, and DNA fingerprint of cancer tissue obtained from each primer was compared with the DNA fingerprint of normal tissue. Genetic alterations were observed in amplification products obtained from 11 out of 30 primers. Among these fingerprints, an allelic imbalance (MW 1.1 kb) amplified from primer AO16 was presented in 9 out of 30 cases, that associated with metastasis of the disease ($P=0.019$). This gene alteration is further study by gene cloning, nucleotide sequencing and identified by comparison with known DNA sequences in genome database.

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HEALTH AND NUTRITIONAL STATUS OF HILL TRIBE SCHOOL CHILDREN AND CHILDBEARING AGE WOMEN, NORTH BORDER AREA OF THAILAND

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Health and nutritional status of 197 school children and 136 childbearing age women from Chalermprakiet district, Nan province, Thailand were investigated. Antropmetric measurement was performed to assess the body size, composition and nutritional indexes. To determine nutritional status of the study group, using dietary survey and blood sample was taken to determine haematocrit, protein parameters, serum and red blood cell folate. Insight of folate nutrition status is significant imminently in a public health perspective. A protective effect of folate against the development of neural tube defects (NTDs) is well recognized. Health and nutritional status of hill tribe school children and childbearing age women were found unacceptable particularly folate status, which indicated by low level in the blood and intake of this micronutrient.

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ARE WEEDS AND COMMON PLANTS SAFE FOR USED IN TRADITIONAL MEDICINE AND ANIMAL FEED?

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Five weeds and common plants in crude distilled water and absolute ethanol extracts were investigated of their Mutagenicity and antimutagenicity potentials using Ames' test. There were stems and leaves of *Peperomia pellucida* (Linn.) Kunth, *Eichhornia crassipes* Solms**,** *Colocasia esculenta* Schott and *Brachiaria mutica* (Forssk.) Stapf, and the stems of *Musa sapientum* Linn. No mutagenic effect was found in any of the 10 mg/plate crude extracts of these plants for either TA98 or TA100 of *Salmonella typhimurium*, in a direct test and a mutagenic induced test by S-9 mix. Both distilled water and absolute ethanol extract of 0.5–10 mg/plate *B. mutica* showed strong antimutagenicity to AFB_1, B(a)P and 4NQO in two tester strains. Ethanol extract of 0.1–0.5 mg/plate *C. esculenta* also showed antimutagenicity to AFB_1, B(a)P and 4NQO in two tester strains, but the 0.5–10 mg/plate water extract had an antimutagenic effect only for B(a)P in TA98. The ethanol extracts of

5 mg/plate *B. mutica* and 0.5 mg/plate *C. esculenta* are cytotoxic, as indicated by their partial killing effect.

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VASCULAR OBSTRUCTION IN SEVERE MALARIA: ULTRASTRUCTURAL STUDY

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The pathogenesis and pathophysiology of severe falciparum malaria is complex, but one well-established feature is the concept of microvascular obstruction [1,2]. In an extensive electron microscopic study in 60 autopsy severe falciparum malaria cases, we demonstrate variable obstruction of the vascular of human organs. The results provide an ultrastructural explanation for the development of circulatory disturbances caused by the malaria parasite. Mostly, capillaries and post capillary venules were congested with red blood cell (RBC) and parasitized red blood cell (PRBC) of different parasite stages. Blockage of the cerebral microvasculature by PRBC is the principal pathological finding in human cerebral malaria [3, 4] (Figure 1). Knobs, which appear on the membrane of the PRBC adhere to the endothelium, causing obstruction of cerebral microvessels. However, the distribution of parasites was not uniform, with vessels packed by parasites in one segment, but only scarce parasites in an adjacent segment. Additional causes of microvascular obstruction include a reduction in RBC deformability [5] (Figure 2), auto-agglutination and aggregation, which could all be observed in our series (Figure 3). Phagocytes containing malarial pigment were abundant in every organ, but especially in the spleen and liver [6,7]. Endothelial cell swelling containing phagocytosed malarial pigment were occasionally seen. Fibrin deposition within blood vessels was sporadically found. Occasionally RBC and PRBC were seen clumped together by fibrin and fibrillary material. In some cases microvessels appeared to be blocked by a mass of necrotic material or sometimes clearly visible residual malarial pigment within a mass of fibrin and degenerating material. Fibrin deposited as fibrillary protein within the vessel lumen, or the presence of fibrin platelet thrombus formation was a rare feature of this series. However, in patients with cerebral malaria it was found more frequently than in the other cases [4]. In some cases the kidney showed abundant cellular debris within the capillary lumen, mainly composed of lysed RBC and other unidentified materials. An interesting finding was the presence of fibrin admixed with RBC, PRBC, mononuclear cells and platelets in the lumen of capillaries and venules.

The ultrastructural findings can offer a better understanding of pathogenesis and pathophysiology of severe falciparum malaria, for the development of better therapies for this severe disease, which remains high death toll.

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Presented at: 25th Annual Conference Microscopy Society of Thailand. Amarin Lagoon Hotel, Phitsanulok, Thailand, 9 – 11 January 2008.

POST-ADHESIVE CALCIUM SIGNALING OF ENDOTHELIAL CELLS IN MALARIA

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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. We have documented the occurrence of apoptosis in endothelial cells co-cultured with *P. falciparum*. As calcium (Ca^{2+}) is an important signaling molecule associated with apoptosis, calcium signaling in endothelial cells co-cultured and stimulated with malaria-infected red blood cells was investigated further. Using confocal laser scanning microscope, the results showed an association between intracellular Ca^{2+} concentration and parasite attachment. Involvement of calcium in apoptosis was confirmed with the intracellular Ca^{2+} chelator, BAPTA-AM (1, 2-bis (o-Aminophenoxy)ethane-N,N,N′,N′-tetraacetic Acid Tetra (acetoxymethyl) Ester), where reduction of apoptosis was seen in pre-treated cells. Employing a population-based experimental system, malaria parasitemia correlated well with the degree of endothelial Ca^{2+} release. The work also concluded the prerequisite of an intact malaria-infected red blood cell's surface protein receptor in generating endothelial Ca^{2+} response. An important finding was the data showing that the endoplasmic reticulum is the major source of Ca^{2+} release in endothelial cells induced by malaria-infected red blood cells.

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Presented at: the12th Year Anniversary of the Faculty of Allied Health Sciences, Thammasat University, Rangsit Campus, Pathumthani: 20 Feb 2008.

EXPLORING CALCIUM SIGNALING IN ENDOTHELIAL CELLS-MALARIA PARASITES CYTOADHESION

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Cytoadhesion of malaria parasites, specifically *Plasmodium falciparum* to human endothelial cells is an important event leading to severe malaria. In order to study this cytoadhesion process, we designed a co-culture system to evaluate the consequences that follow cytoadhesion of malaria-infected red blood cells to endothelial cells. We have previously documented the occurrence of apoptosis in endothelial cells co-cultured with *P. falciparum*. Besides morphological changes of endothelial cells co-culture with malaria parasites, we are particularly interested in the involvement of cell signaling process in endothelial cells-malaria parasites cytoadhesion. In this study, we investigated calcium signaling process as it is one of the important molecules in cellular processes. We employed a single-live cells technique using confocal laser scanning microscope, the results showed an association between

intracellular Ca^{2+} concentration and parasite attachment. Involvement of calcium in apoptosis was confirmed with the intracellular Ca^{2+} chelator, BAPTA-AM (1, 2-bis (o-Aminophenoxy)ethane-N,N,N′,N′-tetraacetic Acid Tetra (acetoxymethyl) Ester), where reduction of apoptosis was seen in pre-treated cells. To further confirmed Ca^{2+} response in cytoadhesion, we designed a population-based experimental system and found out that malaria parasitemia correlated well with the degree of endothelial Ca^{2+} release. We hope that the work provides a better understanding of the pathogenesis of severe malaria. We postulate that interfering with Ca^{2+} cellular signaling process could prevent endothelial cells changes and subsequent symptoms of severe malaria.

P.Viriyavejakul was supported by the Royal Thai Government through CRN Pathology

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ULTRASTRUCTURAL STUDY OF SEVERE MALARIA

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One well-established feature of pathogenesis and pathophysiology of severe falciparum malaria is the concept of parasitized erythrocytes sequestration, leading to microvascular obstruction [1,2]. Electron microscopic study in 60 autopsy severe falciparum malaria cases, we demonstrate variable obstruction of the vascular of human organs. The results provide an ultrastructural explanation for the development of circulatory disturbances caused by the malaria parasite.

Mostly, capillaries and post capillary venules were congested with red blood cell (RBC) and parasitized red blood cell (PRBC) of different parasite stages. Blockage of the cerebral microvasculature by PRBC is the principal pathological finding in human cerebral malaria [3, 4] (Figure 1). Knobs, which appear on the membrane of the PRBC adhere to the endothelium, causing obstruction of cerebral microvessels. However, the distribution of parasites was not uniform, with vessels packed by parasites in one segment, but only scarce parasites in an adjacent segment. Additional causes of microvascular obstruction include a reduction in RBC deformability [5] (Figure 2), auto-agglutination and aggregation, which could all be observed in our series (Figure 3). Phagocytes containing malarial pigment were abundant in every organ, but especially in the spleen and liver [6,7]. Endothelial cell swelling containing phagocytosed malarial pigment were occasionally seen. Fibrin deposition within blood vessels was sporadically found. Occasionally RBC and PRBC were seen clumped together by fibrin and fibrillary material. In some cases microvessels appeared to be blocked by a mass of necrotic material or sometimes clearly visible residual malarial pigment within a mass of fibrin and degenerating material. Fibrin deposited as fibrillary protein within the vessel lumen, or the presence of fibrin platelet thrombus formation was a rare feature of this series. However, in patients with cerebral malaria it was found more frequently than in the other cases [4].

The ultrastructural findings can offer a better understanding of pathogenesis and pathophysiology of severe falciparum malaria, for the development of better therapies for this severe disease, which remains high death toll.

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CLINICO-PATHOLOGICAL CORRELATION IN HUMAN SEVERE MALARIA: QUANTITATIVE ULTRASTRUCTURAL STUDY OF THE SEQUESTRATION OF PARASITIZED RED BLOOD CELLS

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Death from severe malaria can result from a variety of syndromes including cerebral malaria and renal failure. Sequestration of parasitized red blood cells (PRBC) in vital organs microvasculature plays an important role in pathogenesis. To determine if there was any correlation between PRBC sequestered in the organs and particular pre-mortem clinical complications. We studied 65 patients who died of severe malaria using electron microscopy. Quantitation of the PRBC load between these organs within the same patients were compared. PRBC sequestration in each organ was compared with the pre-mortem parasitemia, to calculate sequestration index (S.I.). We found that PRBC sequestration was significantly higher in the brain of patients with cerebral malaria (CM) compared to non-cerebral malaria (NCM). There was a correlation between PRBC sequestration in the kidney and renal failure, associated with acute tubular injury. PRBC sequestration in the liver was associated with hepatic dysfunction and renal impairment. There was no significant difference between splenomegaly and PRBC sequestration and the size of palpable spleen was not correlated to parasite load in the spleen. Common to all organ studied was the finding of phagocytes containing phagocytosed malarial pigments. S.I. for overall patients showed more PRBC in the vital organ microvasculature than the peripheral blood. Our studies indicate that severity of malaria depends on PRBC sequestration, especially in the brain. A combination of functional disturbances of the other organs, in addition to the cerebral pathology, may augment the severity of the disease.

Presented at: 17th International Congress for Tropical Medicine and Malaria, Jeju, Korea, 29 September-3 October 2008.

UNDERSTANDING CENTENNIAL *TOXOPLASMA GONDII* BY HIGH TECHNOLOGY METHODS

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Toxoplasma gondii, a protozoan known to human for a hundred year, is highly successful once infected and harbored in the host life-long. Rapidly dividing tachyzoites cause acute infection with non-specific and often unnoticed clinical manifestations, then they transform into the bradyzoite stage soon after causing unrecognized chronic infection. Toxoplasmic encephalitis (TE) occurs in immune suppressed individuals due to reactivation of quiescent bradyzoites. Up to now, the diagnosis require both clinical and laboratory criteria. Immunohistochemistry and RT-PCR assay were developed to detect tachzoite/bradyzoite stage-specific expressions during acute, chronic and immunosuppressed periods in experimental *Toxoplasma* infected mice. The immunohistochemical technique enhanced visualization of parasites enabling their number and distribution to be accurately measured. In addition, double immunocytochemical labelling confirmed the exclusive presence of tachyzoites during the acute phase and bradyzoites during the chronic phase. RT-PCR assay showed that tachzoites were transformed from bradyzoites since the first week of suppression period and were more apparent at the second and third weeks in the cerebrum, cerebellum, eye, heart, lung, diaphragm, liver, spleen and kidney. Bradyzoites were also found in nearly all organs at the end of this study. Results obtained from our study suggested that during suppression period, bradyzoites were not only transformed to tachyzoites, but also caused new bradyzoite development. Both high technology immunohistochemistry and RT-PCR could be further developed as alternative methods for the prognosis and diagnosis of the centennial *T. gondii* infection leading to better and appropriate chemoprophylaxis or treatment.

Presented at: 17th International Congress for Tropical Medicine and Malaria, Jeju, Korea, 29 September-3 October 2008.

PERITROPHIC MEMBRANE STRUCTURE OF *AEDES AEGYPTI* MOSQUITOES AFTER INFECTED WITH DENGUE VIRUS TYPE 2 (D2-16681)

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The peritrophic membrane (PM) is non-cellular material which involves in the protection of midgut epithelium from mechanical damage and insults from pathogens [1,2]. The result from previous study found that dengue virus can pass through the PM to replicate in the midgut of *Ae. aegypti* mosquitoes [3,4]. Therefore, the aim of this study was to determine the structure of PM in mosquitoes after infected with dengue virus. *Ae. aegypti* mosquitoes were fed with sucrose, human blood with and without dengue virus type 2 (D2-16681), and collected at 1, 6, 12, and 24 h, respectively. After that, they were prepared for examined using light and electron microscopy.

The infective blood meal with dengue virus type 2 induced the mosquitoes to produce PM in their midgut earlier and thicker than in mosquitoes with blood alone. The PM was produced in their midgut at 1 h (mean = 6.55 μm) until 6 h (mean = 2.62 μm) after inoculation. When fed the mosquitoes with the blood alone, this structure was examined at 6 h (mean = 0.41 μm). Dengue virus type 2 exhibited different modifications of their PM construction and structure when confirmed under electron microscope.

Presented at: 25th Annual Conference Microscopy Society of Thailand, Phitsanulok, Thailand, January 9-11, 2008.

HISTOPATHOLOGICAL FEATURES OF THE KIDNEY IN *PLASMODIUM FALCIPARUM* MALARIA

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Background: Acute renal failure (ARF) is a common feature of severe *Plasmodium falciparum* malaria. The pathological basis of ARF in falciparum malaria is not well characterized.

Methods: An autopsy based clinicopathological correlation was performed examining pathological changes in the kidney of 84 patients who died from severe falciparum malaria using light microscopy, transmission electron microscopy (TEM) and double labeling immunofluorescence microscopy. This detailed the histopathological features of *P. falciparum* malaria in the kidney and correlated these with the clinical features of renal function and other complications of severe malaria before death. Parasitized red blood cell (PRBC) sequestration and host leukocyte phenotypes were quantitated, and the release of cytokines and hypoxia inducible factors were examined.

Results: Acute tubular damage was common in this group but no evidence of glomerular basement membrane immune complex deposition was seen. We found evidence of PRBC sequestration in glomerular and peritubular capillaries, significantly associated with acute renal failure ($p<0.02$). Immunochemistry showed a significant increase in endocapillary CD68+ve monocytes ($P<0.05$) and marked tubulointerstitial host leukocyte responses in malaria patients compared to controls. No evidence was found for monocyte secretion of the proinflammatory cytokines TNF-α, IFN-, IL-6, and IL-10 was seen.

Conclusion These results demonstrated that the major pathological features of falciparum malaria associated acute renal failure was acute tubular injury rather than glomerulonephritis. The significant increase in host monocytes and sequestration of PRBC indicate potential mechanism underlying the pathogenesis of acute tubular injury in these patients, although this is likely to be multifactorial.

Presented at: the Asia Pacific International Conference on Travel Medicine Conference. Crown Promenade Hotel, Melbourne, Australia, 24 – 27 February 2008.

PARTIALLY PURIFIED ANTIGENS FROM *GNATHOSTOMA SPINIGERUM* LARVAE INDUCE IMMUNOGLOBULIN PRODUCTION IN HUMAN B CELLS

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Introduction and Objective Human gnathostomiasis is mainly caused by *Gnathostoma spinigerum*, 3rd-stage larvae (infective stage) to immature worms. The antibodies responding to this infection varies in individual patients in their isotypes and subclasses. This study aimed to demonstrate the immunoglobulin isotypes and subclasses produced by the ***in vitro*** induction of partially purified *G. spinigerum* antigens.

Methods Partially purified antigens were prepared from 3rd-stage larvae of *G. spinigerum* by modified syringe column chromatography. Naïve B cells were enriched from peripheral blood mononuclear cells (PBMC) of 3 healthy donors by magnetic cell sorter using anti-IgD and MACS MicroBeads. Two millions enriched naïve B cells from each donor were separately stimulated with 10 μg/ml fractionated antigen in the presence of anti-CD40 for 7 and 14 days. IL-4 (300 U/ml) and anti-CD40 (10 μg/ml) were used as positive controls to stimulate each culture. At fixed intervals, culture supernatants were tested for levels of IgM, IgG, and IgE, including IgG subclasses by enzyme-linked immunosorbent assay (ELISA).

Result The results showed that 90% enriched naïve B cells in PBMC failed to produce Igs, while 30 and 50% enriched naïve B cells in day 7- and 14- cultures could produce low levels of IgM and IgG, but very low levels of IgE. All specific subclasses of IgG subclasses after day 14 of B-cell stimulation showed a greater increase than after day 7.

Conclusion This preliminary study demonstrated that the partially purified antigens were potentially induced IgM and IgG production. Optimal conditions for *in vitro* B-cell stimulation should be developed for further study of Ig production.

Presented at: the RGJ-PH.D. Congress IX, Jomthien Palm Beach Resort Pattaya, Chonburi, Thailand, 4 – 6 April 2008.

EFFECT OF THE *BACILLUS THURINGIENSIS* CRY4BA TOXIN ON THE PERITROPHIC MEMBRANE IN *AEDES AEGYPTI* MOSQUITO LARVAE

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Bacillus thuringiensis (Bt) Cry4Ba is highly toxic to *Aedes* mosquito larvae. In the mosquito larval midgut, the peritrophic membrane (PM) lines the gut epithelium and serves as a protective barrier against pathogens. To understand the interaction of the PM with the Bt toxin, binding of Cry4Ba toxin to the PM and alteration of the PM in Cry4Ba fed *Aedes* larvae were examined. Fluorescence microscopy of *Aedes* larval PMs incubated with Cry4Ba *in vitro* demonstrated that Cry4Ba bound to the PM and Far Western blot analysis indicated that Cry4Ba could bind to three proteins (21-, 24- and 25-kDa in molecular weight) from the PM. *In vivo* observations of the PM by fluorescence microscopy showed that the PM became permeable to FITC-dextran (MW. 2000 kDa) in *Aedes* larvae treated with Cry4Ba. Furthermore, electron microscopical examinations showed structural changes of the PM, presence of bacteria in the ecto-peritrophic space and damaged microvilli of the midgut epithelium cells in larvae treated with the toxin. These findings suggest that Cry4Ba toxin may act on the PM, leading to alteration of permeability of the PM and consequently weakening the protective function of the PM in mosquito larvae.

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ULTRASTRUCTURAL STUDY OF MICROCIRCULATORY OBSTRUCTION IN SEVERE MALARIA

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The pathogenesis of severe falciparum malaria is complex, but one well-established feature is the concept of microcirculatory obstruction. In an electron microscopic study in 60 autopsy cases, we demonstrate variable obstruction of the microcirculation of human organs. The results provide an explanation for the development of circulatory disturbances caused by the malaria parasite. Mostly, capillaries and venules were congested with red blood cell (RBC) and parasitized red blood cell (PRBC) of different parasite stages. Knobs, which appear on the membrane of the PRBC adhere to the endothelium, causing obstruction of cerebral microvessels. Additional causes of microcirculatory obstruction include a reduction in RBC deformability, auto-agglutination and aggregation, which could all be observed in our series. Phagocytes containing malarial pigment were abundant in every organ, especially in the spleen and liver. In some cases microvessels appeared to be blocked by residual malarial pigment within a mass of fibrin and degenerating material. The intravascular obstruction including abundant of cellular debris, lysed RBC and mass of necrotic unidentified materials. Some kidney showed fibrin admixed with RBC, PRBC, mononuclear cells and platelets in the lumen of capillaries and venules. The ultrastructural findings can offer a better understanding of pathogenesis of severe falciparum malaria, for the development of better therapies for this severe disease.

Presented at: 17th International Congress for Tropical Medicine and Malaria, Jeju, Korea, 29 September-3 October 2008.

IGE CLASS SWITCHING IN PURIFIED HUMAN B CELLS INDUCED BY BLOOD STAGE *P. FALCIPARUM*

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There have been problems in IgE related pathogenesis studies in most infectious diseases in both patients and human peripheral blood mononuclear cell (PBMC) cultures due to small amount of specific IgE close to the detectable limitation. This study demonstrated the *in vitro* primary immune response to crude *Plasmodium falciparum* (*P. falciparum*) antigens (cPf Ag) using human purified B cells. The feasibility of the model was evaluated by 1) the occurrence of ongoing Ig class switch recombination (CSR) by expression of activation-induced cytidine deaminase (AID) and mature transcript (ε mRNA), and 2) the increased *P. falciparum* specific IgE levels comparing among in the purified B cell, PBMC cultures and sera from severe falciparum patients. The results showed the purified B cells from 4 donors stimulated with cPf Ag and anti-CD40 could express AID mRNA and ε mRNA on day 4 and increased specific IgE level on day 14. The amount of specific IgE detected in purified B cell culture was more noticeable than those in PBMC culture and the patient sera. Conclusively, human purified B cells stimulated with cPf Ag and anti-CD40 could potentially produce remarkable specific IgE. This advantage can be further applied to study in the immunopathogenesis of falciparum malaria.

Presented at: 17th International Congress for Tropical Medicine and Malaria, Jeju, Korea, 29 September-3 October 2008.

PREDICTIVE OUTCOME IN ACUTE UNCOMPLICATED FALCIPARUM MALARIA PATIENTS

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In acute uncomplicated Plasmodium falciparum malaria infection, there is a continuum from mild to severe malaria. However, there is no mathematical system 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 with adult patients infected with acute uncomplicated *Plasmodium falciparum malaria admitted to the Bangkok Hospital for Tropical Diseases between 2000 and 2007. 3^8 initial clinical parameters* that may potentially affect the severity outcome of disease were identified to predict normal recovery or deterioration into severe malaria. Stepwise multiple discriminant analysis was performed to ascertain a linear discriminant equation. Based on data analyses, 4.3% of study patients turned to severe malaria. The MSPS = 4.38 (presenting schizontemia) + 1.62 (presenting gametocytemia) + 1.17 (presenting dehydration) + 0.14 (overweight by Body Mass Index) + 0.05 (initial pulse rate) + 0.04 (duration of fever before admission) - 0.50 (previous history of malaria in the last year) - 0.48 (initial serum albumin) - 5.66. In the MSPS validation study, sensitivity and specificity were 88.8 and 88.4%, respectively, with an accuracy of 88.4%. It was concluded that MSPS is an appropriate simple screening tool for predicting severity of outcome in acute uncomplicated *P. falciparum malaria patients. The development of the MSPS was intended as a pilot for the further development of a suitable model for prognostic* purpose. The score may require revalidation in other geographical areas, for subsequent utilization at specific sites.

Presented at: 17th International Congress for Tropical Medicine and Malaria, Jeju, Korea, 29 September-3 October 2008.

EXPRESSION OF B CELL ACTIVATING FACTOR (BAFF) IN HUMAN MONOCYTES INDUCED BY *PLASMODIUM FALCIPARUM* ANTIGEN

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B cell activating factor (BAFF), mainly produced by monocyte, plays important roles in T-independent (TI) immune response. After antigens (Ags) taken up, activated antigen presenting cells (APCs) secrete some cytokines including interferon (IFN)-γ and transforming growth factor (TGF)-β that can up-regulate expression of BAFF. Soluble BAFF (sBAFF) released from activated monocytes or APCs binds to their receptors on B cell surface, leading to B cell activation, proliferation and isotype switching to immunoglobulin (Ig)A and IgG. Although several previous studies have reported that important cytokines e.g. tumor necrosis factor (TNF)-α, IFN-γ and TGF-β increased in falciparum malaria, very few studies verified falciparum malaria parasite could act as TI antigens. This preliminary study aimed to demonstrate whether crude *P. falciparum* Ags (cPfAg) could act as TI antigen to activate BAFF expression in the activated monocytes.

Method: To investigate BAFF expression, monocytes obtained from healthy donor were cultured with cPfAg, IFN-γ, lipopolysaccharide (LPS) or media alone. The activated monocytes and supernatant were collected at 24, 48, 72 and 88 hr after stimulation. The expression of BAFF on monocyte surface was determined by Flow cytometry. The level of sBAFF in supernatant was determined by Enzyme-Linked Immunosorbent Assay (ELISA).

Results and discussion: The result showed that cPfAg activated monocytes to produce BAFF on thier surface and sBAFF in supernatant of the culture. The amount of BAFF production induced by cPfAg increased continuously during 4 days of stimulation. However, our finding cannot clarify roles of cPfAg as TI antigen. We will further investigate the correlation of BAFF expression by the activated monocytes, upregulation of BAFF receptors on B cell and the specific Ig production.

Presented at: the Joint International Tropical Medicine Meeting, Imperial Queen's Park Hotel, Bangkok, Thailand. 13-14 October 2008.

SEQUESTRATION OF PARASITIZED RED BLOOD CELLS IN THE BRAIN, KIDNEY, LIVER AND SPLEEN: A CLINICO-PATHOLOGICAL CORRELATION IN HUMAN SEVERE MALARIA

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Sequestration of falciparum malaria parasitized red blood cells (PRBC) in vital organs microvasculature plays an important role in pathogenesis of severe malaria. To determine if there was any correlation between PRBC sequestered in the organs and particular pre-mortem clinical complications. We studied 65 patients who died of severe malaria using electron microscopy. Quantitation of the PRBC load between these organs within the same patients were compared. PRBC sequestration in each organ was compared with the pre-mortem parasitemia, to calculate sequestration index (S.I.). We found that PRBC sequestration was significantly higher in the brain of patients with cerebral malaria (CM) compared to non-cerebral malaria (NCM). There was a correlation between PRBC sequestration in the kidney and renal failure, associated with acute tubular injury. PRBC sequestration in the liver was associated with hepatic dysfunction and renal impairment. There was no significant difference between splenomegaly and PRBC sequestration and the size of palpable spleen was not correlated to parasite load in the spleen. Common to all organ studied was the finding of phagocytes containing phagocytosed malarial pigments. S.I. for overall patients showed more PRBC in the vital organ microvasculature than the peripheral blood. Our studies indicate that severity of malaria depends on PRBC sequestration, especially in the brain. A combination of functional disturbances of the other organs, in addition to the cerebral pathology, may augment the severity of the disease.

Presented at: the Joint International Tropical Medicine Meeting, Imperial Queen's Park Hotel, Bangkok, Thailand. 13-14 October 2008.

EXPRESSION OF *BACILLUS THURINGIENSIS* CRY4BA PORE-FORMING DOMAIN RETARDS GROWTH RATE OF *ESCHERICHIA COLI* HOST CELLS

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Cry4Ba mosquito-larvicidal protein produced by *Bacillus thuringiensis* sub. *israelensis* (*Bti*). The 65-kDa activated toxin is composed of three-distinct functional domains, while only domain I clearly plays role in pore formation. Although a4 and a5 helices in domain I have been demonstrated to be a key determinant for toxicity by membrane insertion after toxin binding to a specific receptor, the behaviors of whole domain I in membrane environments are not clearly understood. In order to understand a role of domain I in Cry4Ba toxicity, fragments of the 1-kb gene encoding for domain I of the *cry4Ba*, *tac* promoter and *cry4Ba* regulatory region were PCR amplified and ligated into the pMEX8 expression vector. After induction by isopropyl-beta-D-thiogalacto-pyranoside (100 μM IPTG) at 37^{o}C for 1 h, approximately 30-kDa Cry4Ba was expressed in *E. coli*. Interestingly, expression of this cloned domain adversely affected bacterial growth rate, perhaps reflecting cytotoxicity of the recombinant protein. Future direction of our research will be characterization and membrane-associated studies of the 30-kDa domain

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EPIDEMIOLOGICAL STUDY OF DENGUE INFECTION IN CHILDREN , RATCHABURI PROVINCE, THAILAND

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[No Abstract]

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STUDY ON SERUM TRANSCOBALAMIN II IN PATIENTS WITH MURINE TYPHUS

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Abstract: We measured the serum transcobalamin II in murine typhus-infected patients (n = 16). admitted to the Hospital for Tropical Diseases in 1996-1997, comparted with healthy controls (n = 60). The results showed that the transcobalmin II (TCII) and total serum unsaturated vitamin B12 binding capacity (UBBC) in patients with murine typhus (2,126.5 pg/ml, range 1,262-4,568 and 3,771.5 pg/ml, range 1,576-6,763 pg/ml) were statistically significantly higher than normal subjects (987.5 pg/ml, range 678-2,000 pg/ml and 1,402 pg/ml, range 932-2,470 ml) ($p<0.001$). Serum TCII levels in patients (63%) were elevated during the febrile period and returned to normal post-treatment. These findings suggest that patients with murine typhus had stimulation of reticulo-endothelial system, spleen, mesenteric lymph nodes, liver and skin and then released TCII into the blood circulation. The elevation in TCII may be used for confirming a diagnosis of murine typhus.

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ACETYLCHOLINESTERASE AND CHOLINESTERASE ACTIVITIES IN *GIARDIA LAMBLIA* TROPHOZOITES CULTURED IN VITRO

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Axenic culture of *Giardia lamblia* trophozites *in vitro* was conducted from patients infected with Giardia lamblia. The cholinesterase (ChE) and acetylcholinesterase (AChE) activities were determined in cultured *Giardia* trophozoites. The enzyme activities were found in boh tissue-bound and tissue-free forms. There was low ChE and AChE enzyme activities in trophozoites (1.6095 ± 1.0981; 0.9626 ± 0.1322; 0.1387 ± 0.0783; 0.0752 ± 0.0877 Units/mg protein, respectively). The tissue-bound and tissue-free ChE enzymes were found in a greater amount than tissue-bound and tissue-free AChE enzymes. The presence of these enzymes in *Giardia* may involved in the motility of *Giardia* trophozoites

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DISTRIBUTION OF C14-LABELLED ARTEETHER IN KIDNEYS AND LIVERS OF EXPERIMENTAL MICE AFTER INTRAMUSCULAR INJECTION

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To study the distribution and localization of oil-soluble arteether in experimental mice, we injected C14-labelled arteether (20 Ci/kg body weight) intramuscularly and measured radioactivity in the blood, kidney, and liver. The labeled arteether distributed and localized more to the kidney (819, 180.4 34,134 dpm/cm3) than the liver (288,628.9 54,954 dpm/cm3) 4 hours post-injection. The main localization of labeled arteether was in the kidney cortex rather than the medulla ($p<0.05$). However, the distribution of radioactivity was homogeneous in the liver. The terminal half-life of labeled arteether in the blood was 1.8 hours. The blood : kidney : liver ratio was 1 : 5 : 2. these findings show that labeled arteether was distributed quickly and localized in the cytoplasmic cortex of the kidney and homogeneously in the liver.

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FULLY AUTOMATED HPLC ASSAY FOR TOTAL HOMOCYSTEINE AND OTHER AMINO-THIOLS: AS A MEASURE OF PLASMA REDOX STATUS IN THAI ADULT 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 μm 125$\times$4 mm; mobile phase: 0.1mol/l KH_2PO_4 with 10% acetonitrile, adjusted to pH 2.1 with ortho-phosphoric acid, flow rate 1.0 ml/min. The total concentrations of the most important thiols in plasma-cysteine, cystenylglycine, homocysteine,and glutathione were 185.86$\pm$34.65 μmol/l (range 110.65-276.98 μmol/l), 27.94$+$6.77 μmol/l (range 14.77-51.23 μmol/l), 8.42$\pm$2.3 μmol/l (range 4.06-14.65 μmol/l), 4.83$\pm$2.03 μmol/ (range 1.72-9.69 μmol/), 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.

HOST VASCULAR ENDOTHELIAL GROWTH FACTOR IS A TROPHIC FACTOR FOR *PLASMODIUM FALCIPARUM*-INFECTED RED BLOOD CELLS

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Plasmodium falciparum, the protozoan parasite responsible for severe malaria infection, undergoes a complex life cycle including intraerythrocytic development in the human host. Infected red blood cells (iRBC) sequestered in host cerebral microvessels show positive staining for Vascular Endothelial Growth Factor (VEGF) using immunohistochemistry on post mortem brain samples. Confocal microscopy of *in vitro* cultured PRBC revealed concentration of VEGF within the parasitophorous vacuole. iRBC cultured in serum free media failed to release VEGF. Confocal microscopy showed increased host VEGF-R1 and -R2 expression on the surface of infected compared to uninfected control RBC, but no concentration of VEGF-R within the parasitophorous vacuole. Addition of VEGF to parasite cultures showed a trophic effect on parasite growth, which was significantly increased when rescuing drug treated parasites. These effects were abrogated when parasites were grown in serum free media, suggesting a requirement for soluble VEGF-R from serum. As the parasite produces no homologues to human VEGF or VEGF-R, we conclude that *P. falciparum* iRBC show increased VEGF-R expression on the red cells membrane, and concentrate host VEGF within the parasitophorous vacuole. This may have a trophic effect on parasite growth or represent a stress response to drug treatment.

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