

PROCEEDINGS OF

The 1st Asian Parasites by Asian Parasitologists (APAP):

Helminthiases in Greater Mekong Subregion

12 May 2013, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand



ORGANIZED BY

- ❖ THAILAND RESEARCH FUND, TRF Senior Research Scholar Grant no. RTA5580004
- ❖ RESEARCH AND DIAGNOSTIC CENTER FOR EMERGING INFECTIOUS DISEASES
- ❖ DEPARTMENT OF PARASITOLOGY, FACULTY OF MEDICINE, KKU
- ❖ KHON KAEN UNIVERSITY

FOREWORD

The first meeting on the Asian Parasites by Asian Parasitologists : Helminthiasis in Greater Mekong Subregion (the first Thailand Research Fund Senior Research Scholar grant: RTA5580004 meeting) holds on 12 May 2013 at Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, is mainly supported by the TRF Senior Research Scholar Grant, Thailand Research Fund number RTA5580004 and co-supported by International Relations and Cooperation Strategy and Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University and the Korean Society for Parasitology, Korea. The objective of the meeting is to strengthen the research capability of young parasitologists and graduated students in Asian countries. For this purpose, the organizers have selected young active parasitologists in and outside of Thailand and arrange them to meet senior experts who are world-known parasitologists mainly working in Asian countries. Research areas include biology, epidemiology, clinical feature, diagnosis, treatment and control approaches. Young participants have presented their work as a poster, and meet experts in their field for discussion. The selected presentations will be subjected for publication as a regular issue in the Korean Journal of Parasitology.

The participants of this meeting are 130 parasitologists and researchers from Asia and Australia.

Wanchai Maleewong
Yukifumi Nawa
Pewpan Maleewong
Viraphong Lulitanond
Soomboon Sangmaneeet
Somchai Pinlaor
Thidarut Boonmars
The Organizers

WELCOME REMARKS

by

Assoc. Prof. Thawalwong Ratanasiri, MD.



Vice President for International Relations and Cooperation Strategy, Khon Kaen University

Honoured Guest speakers; Distinguished Participants of the meeting; Ladies and Gentlemen:

Warmest welcome you to Khon Kaen University and to the first meeting on the Asian Parasites by Asian Parasitologists: Helminthiases in Greater Mekong Subregion. This meeting aims to promote sustained parasitological research cooperation within the six Mekong countries—namely, Cambodia, the People's Republic of China (PRC), Lao People's Democratic Republic (Lao PDR), Myanmar, Vietnam, and Thailand, through strengthening scientific linkages between countries in Greater Mekong Subregion and Alliance Countries—namely, Philippines, Korea, Japan and Australia.

I would like to say that Thailand is the research center of parasitic diseases. The most outstanding researches were carried out in the study of helminthic infection. The outcomes of our researches have called the attention of the whole nation on causes and adverse effects of parasitic infection on the health and wealth of our nation. The government of Thailand has expanded the control program of parasitic based on our joint studies to cover the whole country. Many modules on health education and community health development have been developed through the studies of parasitic infection. These modules are now being used and are made applicable to other diseases control programs.

I would like to express my grateful appreciation to the TRF Senior Research Scholar Grant, Thailand Research Fund, grant number RTA5580004 through Professor Dr. Wanchai Maleewong for managing Khon Kaen University as the venue to convene the first meeting on the Asian Parasites by Asian Parasitologists: Helminthiases in Greater Mekong Subregion. Parasitologists and graduate students in Asian countries as well as Thai participants will have the opportunity to attend the meeting and meet with the world class experts in their field for discussion.

I hope that all of you will enjoy your stay in Khon Kaen during this beautiful culture.

May I wish the meeting every success and I wish for good health and prosperity to all of you.

Thank you

Thawalwong Ratanasiri

WELCOME REMARKS

by

Professor Wanchai Maleewong, Ph.D.



Senior Researcher, Thailand Research Fund,

Organizer of the first meeting on the Asian Parasites by Asian Parasitologists : Helminthiasis in Greater Mekong Subregion; Department of Parasitology and Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University

Assoc. Prof. Dr. Thawalwong Ratanasiri, Vice President for International Relations and Cooperation Strategy, Khon Kaen University, Thailand; Assoc. Prof. Dr. Viraphong Lulitanod, Director of Research and Diagnostic Center for Emerging Infectious Diseases, Faculty of Medicine, Khon Kaen University, Thailand; Prof. Dr. Jong-Yil Chai, Editor, Korean Journal of Parasitology, the Korean Society for Parasitology, Korea; Prof. Dr. Yukifumi Nawa, Editor in Chief, Parasitology International, the Japanese Society of Parasitology, Japan; Prof. Dr. Zhongdao Wu, Deputy Dean, Zhongshan School of Medicine, Sun Yat-sen University, China; Prof. Dr. Wanpen Chaicumpa, Head of Health Science Supra-cluster, National Research University Project of Thailand; Prof. Dr. Jitra Waikakul, President of the Parasitology and Tropical Medicine Association of Thailand; Prof. Dr. Wej Choochote, Senior Researcher, Thailand Research Fund; Distinguished Speakers and Participants of the Asian Parasites by Asian Parasitologists : Helminthiasis in Greater Mekong Subregion; Distinguished Guests; Ladies and Gentlemen:

We are pleasure to welcome all participants to join the first meeting on the Asian Parasites by Asian Parasitologists: Helminthiasis in Greater Mekong Subregion in the collaboration with the TRF Senior Research Scholar Grant (grant number RTA5580004), the Thailand Research Fund; Khon Kaen University; and the Korean Society for Parasitology.

On behalf of the Organizing Committee, I would like to express our gratitude to the Thailand Research Fund; International Relations and Cooperation Strategy and Research and Diagnostic Center for Emerging Infectious Diseases Khon Kaen University, Thailand; and the Korean Society for Parasitology, Korea for supporting us the honor to establish the Meeting.

At this Meeting, I am pleased to report that there are participants from 8 countries, total of about 120 participants. There are about 70 scientific papers to be presented. The Keynote speakers on the: Helminthiasis in Greater Mekong Subregion will be presented by Prof. Dr. Jong-Yil Chai on topic "Biodiversity of minute intestinal flukes in Asia", by Prof. Dr. David Blair on topic "Molecular phylogeny & biogeography of trematode parasites" by Prof. Dr. Wanpen Chaicumpa on topic "Regulation T cell in parasitic infections", by

Prof. Dr. Zhongdao Wu on topic “Currents status of foodborne parasites in China”, by Prof. Dr. Jitra Waikakul on topic “Fishborne trematodes in GMS”, by Prof. Dr. Wej Choochote on topic “Vectors of filariasis in Thailand and Development of a facile system for mass-production of *Brugia malayi* in a small-space laboratory” and by Prof. Dr. Pewpan (Intapan) Maleewong on topic “Present status of Tropical Neglected Parasites in Thailand”. We would appreciate the cooperation of Chairpersons of the sessions to give their suggestions of their sessions. We hope to publish the scientific papers of the meetings as soon as possible as a regular issue of the Korean Journal of Parasitology. We would like to thank Prof. Dr. Jong-Yil Chai, Prof. Dr. Yukifumi Nawa and Prof Dr. David Blair for acceptance to be the special Editors-in Chief of the regular issue for the Meeting.

I wish to personally thank participating institutions and members of the Organizing Committee for their hard work and dedication.

I appreciate and welcome the full participation of our friends from the People’s Republic of China, Korea, Japan, Australia, Lao PDR, Philippines, Vietnam and Thailand. I hope this Meeting will promote research cooperation through strengthening parasitological linkages among our friends. Please feel free to call upon members of the Organizing Committee for assistance to ensure full benefit of your stay in Khon Kaen and your participation in the Meeting.

Thank you

Wanchai Maleewong



SCIENTIFIC PROGRAM

The 1st Asian Parasites by Asian Parasitologists (APAP):
Helminthiasis in Greater Mekong Subregion

12 May 2013

At 3rd floor, Mittraphap & More Din Dang meeting Rooms,
Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Sunday, 12 May 2013

08.00 - 09.00	Registration and Poster
09.00 - 09.15	Opening and welcome remarks Vice President of International Relations and Cooperation Strategy, Khon Kaen University Assoc. Prof. Thawalwong Ratanasiri, MD.
09.15 - 09.30	Welcome remark Chairman of organizing committee Prof. Wanchai Maleewong, PhD.
09.30 - 10.00	1. Biodiversity of minute intestinal flukes in Asia Prof. Jong-Yil Chai, MD., Ph.D. (Chairman: Prof. Yukifumi Nawa, MD., Ph.D.)
10.00 - 10.30	2. Molecular phylogeny & biogeography of trematode parasites Prof. David Blair, Ph.D. (Chairman: Prof. Jong-Yil Chai, MD., Ph.D.)
10.30 - 10.50	Coffee / Tea Breaks and Exhibition
10.50-11.20	3. Regular T cell in parasitic infections Prof. Wanpen Chaicumpa, D.V.M. (Hons.), Ph.D. (Chairman: Assoc. Prof. Dr. Kittisak Sawanyawisuth, MD., Ph.D.)
11.20 – 11.50	4. Current status of food borne parasitic zoonoses in China Prof. Zhongdao Wu, Ph.D. (Chairman: Prof. Pewpan Maleewong, MD. (Hons.))

11.50 – 13.00	Lunch Symposium (Mor Din Dang meeting room)
13.00 – 14.30	Poster presentation & Exhibition (Mittraphap meeting room)
14.30 – 15.00	5. Fish-borne trematodes in GMS Prof. Jitra Waikagul, Ph.D. (Chairman: Prof. Zhongdao Wu, Ph.D.)
15.00 – 15.20	Coffee / Tea Breaks and Exhibition
15.20 – 15.50	6. Development of a facile system for mass-production of <i>Brugia malayi</i> in a small-space laboratory Prof. Wej Choochote, Ph.D. (Chairman: Assoc. Prof. Viraphong Lulitanond, Ph.D.)
15.50 – 16.20	7. Present status of Tropical Neglected Parasites in Thailand Prof. Dr. Pewpan Maleewong (Chairman: Assoc. Prof. Thidarut Boonmars, D.V.M., Ph.D.)
16.20 – 16.40	Closing ceremony & Outstanding poster awards announcement Prof. Wanchai Maleewong, Ph.D.
16.40 – 17.00	Poster removal

LIST OF POSTER PRESENTATIONS

- P01 - A loop-mediated isothermal amplification technique (LAMP) assay for diagnosis and evaluation of treatment of patients infection with *Schistosoma japonicum*. (Chao-ming Xia, Jin Xu, Yon-kang He, Jun-jie Guo)
- P02 - Molecular identification of *Anisakis pegreffii* (Nematoda: Anisakidae) collected from the sea eels (*Astroconger myriaster*) of the Southern Coast of Korea. (Bong-Kwang Jung, Hye Mi Lim, Jae Eun Cho, Thanapon Yooyen, Eun-Hee Shin and Jong-Yil Chai)
- P03 - Morphological and molecular characterisation of lymnaeid intermediate hosts of *Fasciola* spp. in Vietnam. (Bui Thi Dung, Bertrand Losson, Pham Ngoc Doanh, Dang Tat The, Ho Thi Loan and Yannick Caron)
- P04 - Genetic difference of *Angiostrongylus cantonensis* in Thailand. (Sirilak Dusitsitthiporn, Urusa Thaenkham, Chalit Komalamisra, Dorn Watthanakulpanich and Poom Adisakwattana)
- P05 - Detection of *Opisthorchis viverrini* in human stool samples by nested-PCR. (Wilawan Pumidonming, Choosak Nithikatekul, Surasak Wongrattanachewin and Wanchai Maleewong)
- P06 - Molecular characterization of *Ancylostoma duodenale*, *Ancylostoma ceylanicum* and *Necator americanus* in Thai patients. (Issarapong Phosuk, Pewpan M. Intapan, Oranuch Sanpool, Penchom Janwan, Tongjit Thanchomnang, Nimit Morakote, Witthaya Anamnart and Wanchai Maleewong)
- P07 - Molecular epidemiology of *Paragonimus heterotremus* and *P. pseudoheterotremus* in freshwater crabs in Thailand and Myanmar. (Oranuch Sanpool, Pewpan M. Intapan, Tongjit Thanchomnang, Penchom Janwan, Yukifumi Nawa and Wanchai Maleewong)
- P08 - Rapid detection of *Wuchereria bancrofti*, *Brugia malayi*, *B. pahangi* and *Dirofilaria immitis* by high resolution melting real-time PCR in mosquito vectors and blood samples. (Tongjit Thanchomnang, Pewpan M. Intapan, Viraphong Lulitanond, Sudchit Chungpivat, Piyanan Taweethavonsawat, Worasak Kaewkong, Oranuch sanpool, Penchom Janwan and Wanchai Maleewong)
- P09 - Differential detection of *Schistosoma japonicum* and *Schistosoma mekongi* by high resolution melting real-time PCR. (Amornmas Kongklieng, Worasak Kaewkong, Pewpan M. Intapan, Oranuch sanpool, Penchom Janwan, Tongjit Thanchomnanag, Viraphong Lulitanond, Pusadee Sri-Aroon, Yanin Limpanont and Wanchai Maleewong)
- P10 - Molecular differentiation of *Opisthorchis viverrini* and *Clonorchis sinensis* eggs by the use of multiplex real-time PCR and high resolution melting analysis. (Worasak Kaewkong, Pewpan M. Intapan, Oranuch sanpool, Penchom Janwan, Tongjit Thanchomnanag, Viraphong Lulitanond and Wanchai Maleewong)
- P11 - Sparganosis presenting as cauda equina syndrome with molecular identification of a causative parasite species. (Adhiratha Boonyasiri, Pornsuk Cheunsuchon, Prajak Srirabheebhat, Hiroshi Yamasaki, Wanchai Maleewong, and Pewpan M. Intapan)

- P12 - The preliminary *in vitro* observation of recombinant FBPA of *Schistosoma japonicum* in stimulation of splenocytes of mice. (Jun Liang, Ying Chen, Xiaorong Song, Jilong Shen and Yuanhong Xu)
- P13 - Inhibition of CCL8 suppresses eosinophils chemotaxis and enhances survival of intracranial larvae of *Angiostrongylus cantonensis* and the infected mice. (Shuting Li, Zhiyue Lv, Chengcheng Zhu, Jie Wei, Jinyi Liang, Huanqin Zheng and Zhongdao Wu)
- P14 - rSj16 inhibits dendritic cells maturation through intracellular IL-10 dependent way. (Sun Xi, Yang Fan, Shen Jia and Wu zhongdao)
- P15 - Dynamics of Th17 cells and their role in *Schistosoma japonicum* infection in C57BL/6 mice. (Xiaoyun Wen, Lei He, Ying Chi, Sha Zhou, Jason Hoellwarth, Cui Zhang, Jifeng Zhu, Calvin Wu, Shawn Dhesi, Xuefeng Wang, Feng Liu and Chuan Su)
- P16 - An immunomodulator from *Angiostrongylus cantonensis* (named AcCystatin) promotes TNF- α production in mouse macrophages. (Zhiyue Lv, Chengcheng Zhu, Shuting Li, Jie Wei, Jinyi Liang, Huanqin Zheng and Zhongdao Wu)
- P17 - *Schistosoma japonicum*: Tsunagi/Y14 protein plays a critical role in the development of the reproductive organs and eggs. (Cuiping Ren, Peng Zhang, Weina Zhang, Dake Huang, Xuemei Jia, Li Gui, Miao Liu and Jijia Shen)
- P18 - Characterization and immunolocalization of mutated ornithine decarboxylase antizyme from *Angiostrongylus cantonensis*. (Jing Chen, Qian Liu, Xiao Yang, Xiansheng Wu, Dongjing Zhang, Ai He and Ximei Zhan)
- P19 - Comparative a recombinant matrix metalloproteinase *Gnathostoma spinigerum* protein with 21 kDa and 24 kDa antigenic components of *G. spinigerum* larval extract for serodiagnosis of neurognathostomiasis. (Penchom Janwan, Pewpan M. Intapan, Hiroshi Yamasaki, Porntip Laummaunwai, Kittisak Sawanyawisuth, Chaisiri Wongkham, Chatchai Tayapiwatana, Amnat Kitkhuandee, Viraphong Lulitanond, Yukifumi Nawa, and Wanchai Maleewong)
- P20 - Diacylglycerol kinase gamma immunolocalization in the *Opisthorchis viverrini* vitellaria. (Wiphawi Hipkaeo, Atthapon Pidsaya, Surang Chompoo, Sawetree Pakkarato, Channarong Arunyanart, Pipatphong Kanla, Kowit Chaisiwamongkol, Yanyong Toomsan, Pewpan M. Intapan, Wanchai Maleewong and Hisatake Kondo)
- P21 - Evaluation of immunoglobulin G4 subclass antibody in peptide-based enzyme-linked immunosorbent assay for serodiagnosis of human paragonimiasis heterotremus. (Pewpan M. Intapan, Oranuch Sanpool, Penchom Janwan, Porntip Laummaunwai, Nimit Morakote, Yoon Kong and Wanchai Maleewong)
- P22 - Proteomic analysis of autoantibody in opisthorchiasis. (Rucksak Rucksaken, Nuttanan Hongsrirachan, Sittiruk Roytrakul, Porntip Pinlaor, Chawalit pairajkul and Somchai Pinlaor)

- P23 - Bile canalicular alteration and bile secretory defect in acute and chronic *Opisthorchis viverrini*-infected hamsters. (Lakhanawan Charoensuk, Porntip Pinlaor, Wunnee Chaijaroonkhanarak, Puangrat Yongvanit, Chawalit Pairojkul, Yukifumi Nawa and Somchai Pinlaor)
- P24 - Encapsulated curcumin enhances periductal fibrolysis in *Opisthorchis viverrini*-infected hamsters after praziquantel treatment. (Porntip Pinlaor, Lakhanawan Charoensuk, Rucksak Rucksaken, Puangrat Yongvanit and Somchai Pinlaor)
- P25 - Proteomic identification of expressed proteins associates with periductal fibrosis in chronic experimental opisthorchiasis. (Ornuma Haonon, Rucksak Rucksaken, Sudarat Onsurathum, Porntip Pinlaor, Thidarut Boonmars and Somchai Pinlaor)
- P26 - The hydatid-killing effects of high intensity focused ultrasound enhanced with ultrasound contrast agent and superabsorbent polymer. (Hui Cai, Bin Ye, Lu-Lu Chen, Ai-Bo Liu, Jing Zhang, Yi-Feng Zhao and Xiao-Yi Zou)
- P27 - *Trichinella spiralis* adult worm but not muscle larvae products ameliorate acute DSS-induced colitis (Xiaodi Yang, Yaping Yang, Yunyun Wang, Yuan Gu and Xinping Zhu, Xiaodi Yang and Yaping)
- P28 - A prospective cohort study on the factors associated to the participation in and compliance to the mass drug administration for Schistosomiasis in Barangay Bethel and Barangay Canaan, Victoria, Oriental Mindoro. (Co, Stephanie N, Ong, Ronnah Marie M, Lenon, John Lemuel L, Ramos, Carmela Cecilia S, Mirano, Ma. Veronica D.T., Valdes and John Xavier R.)
- P29 - *Gnathostoma spinigerum* infection involving the inner lip of a Korean woman: the first autochthonous case. (Jae Hee Kim, Hyemi Lim, Young-Sang Hwang, Tae Youn Kim, Eun Mee Han, Eun-Hee Shin and Jong-Yil Chai)
- P30 - Current status of *Paragonimus* spp. and paragonimiasis in north and central Vietnam. (Pham Ngoc Doanh, Hoang Van Hien, Yoichiro Horii, Yukifumi Nawa)
- P31 - High prevalence of haplorchiasis in Nan and Lampang province, Thailand, following antehelminthic treatments of suspected opisthorchiasis cases. (Adulsak Wijit, Nimit Morakote and Jaewwaew Klinchid)
- P32 - Susceptibility of five species members of the Korean *Anopheles hyrcanus* group to *Brugia malayi*. (Atiporn Saeung, Gi-Sik Min, Sorawat Thongsahuan, Kritsana Taai and Wej Choochote)
- P33 - Prevalence of intestinal parasitic infections among schoolchildren of Phitsanulok Province, northern Thailand. (Raxsina Polseela and Apichat Vitta)
- P34 - Recovery of *Enterobius vermicularis* and *Taenia* eggs by Scotch tape technique. (Apichat Vitta Aunchalee Thanwisai, Wilawan Poomidonming, Seangchai Nateeworanart and Raxsina Polseela)
- P35 - Intestinal Parasitic infection among school children in an urban communities of Khon Kaen, Northeast region of Thailand. (Kanchana Tomanakan, Nuttiya Srisurat, Suphakdee Sanseeha and Kriangkrai Kongsuk)

- P36 - Prevalence of parasitic infections among the people in rural communities, Khon Kaen province, Northeast of Thailand. (Sirintip Boonjaraspinyo, Thidarut Boonmars, Butsara Kaewsamut, Porntip Laummaunwai, Ratchadawan Aukkanimart, Nadchanan Wonkchalee, Amornrat Juasook and Pranee Sriraj)
- P37 - A cross-sectional study of intestinal helminthic infections in patients of a tertiary Khon Kaen Hospital, northeast part of Thailand. (Suphakdee Sanseeheh, Kiangkrai Kongsuk and Kanchana Tomanakan)
- P38 - Predominant helminthiasis in patients at Srinagarind Hospital, Khon Kaen during 2007-2012. (Thidarut Prasongdee, Kiangsak Sathapornworachai, Thaweesak Saraboon, Thawin Singkaew, Sawan Legkhaew and Orathai Budda)
- P39 - Rate of *Gnathostoma spinigerum* antibodies in non-traumatic subarachnoid hemorrhage. (Amnat Kitkhuandee, Waranon Munkong, Kittisak Sawanyawisuth, Penchom Janwan, Wanchai Maleewong and Pewpan M Intapan)
- P40 - Clinical manifestations of eosinophilic meningitis in children. (Kittisak Sawanyawisuth, Panita Limpawattana, Jarin Chindaprasit, Narong Auvichayapat, Sompon Tassniyom, Jaturat Kanpittaya, Verajit Chotmongkol, Wanchai Maleewong and Pewpan M Intapan)
- P41 - *Paragonimus paishuihoensis* metacercariae in freshwater crabs, *Potamon lipkei*, in Vientian province, Laos. (Shigehisa Habe, Pham Ngoc Doanh, Shinichiro Yahiro, Nanthasane Vannavong, Hubert Barennes, Peter Odermatt, Gilles Dreyfuss, Yoichiro Horii, Yukifumi Nawa)
- P42 - Soil-transmitted helminthiasis in Guimaras Island, the Philippines: Is elimination feasible?. (VY Belizario, Jr, HJC Liwanag, WU de Leon and PLC Chua)
- P43 - Safety and feasibility of school-based combined mass drug administration (MDA) of albendazole and praziquantel in a decentralized health system. (Belizario VY, Marfori JRA, De Leon WU, Totañes FIG and Chua PLC)
- P44 - Successful treatment on *Brugia pahangi* in naturally infected cats. (Piyanan Taweethavonsawat and Sudchit Chungpivat)
- P45 - *In vitro* anthelmintic activity of *Alpinia galanga* L. against sheep gastrointestinal nematodes. (Usa Sodamook, Kritdhinart Navaphongpaveen, Korrapas Kaewchai, Krittaporn Chanaket and Supakson Kaewking)
- P46 - Molluscicidal effect of *Camellia oleifera* seed and *Garcinia mangostana* L. pericarp on *Lymnaea* snails, an intermediate host of *Fasciola* sp. (Pranee Sriraj, Ratchadawan Aukkanimart, Porntip Laummaunwai, Thidarut Boonmars, Amornrat Juasook, Pakkayane Sudsarn, Nadchanan Wongchalee, Chawalit Pairojkul and Sakda Warasawapati)
- P47 - Molluscicidal effect of *Camellia oleifera* seed and *Garcinia mangostana* L. pericarp on *Pomacea canaliculata*, an intermediate host of *Angiostrongylus cantonensis*. (Ratchadawan Aukkanimart, Porntip Laummaunwai, Thidarut Boonmars, Pranee Sriraj, Amornrat Juasook, Pakkayane Sudsarn, Nadchanan Wongchalee, Chawalit Pairojkul and Sakda Warasawapati)

- P48 - Praziquantel combined-aspirin improved hepatobiliary system through liver function test and liver pathology in Syrian hamster cholangiocarcinoma associated with *Opisthorchis viverrini* and *N-nitrosodimethylamine*. (Pakkayaneesudsarn, Thidarut Boonmars, Wipaporn Ruangjirachuporn, Porntip Laummaunwai, Natchanan Wonkchalee, Amornrat Juasook, Pranee Sriraj and Ratchadawan Aukkanimart)
- P49 - A combination of praziquantel and traditional plant, *Thunbergia laurifolia* Linn, improve hepatobiliary system in Syrian hamster opisthorchiasis and cholangiocarcinoma. (Nadchanan Wonkchalee, Porntip Laummaunwai, Thidarut Boonmars, Chantana Aromdee, Chariya Hahnvajanawong, Kulathida Veteewuthacharn, Wu Zhiliang, Pranee Sriraj, Ratchadawan Aukkanimart, Watchalin Loilome, Yaovaluk Chamgramol, Chawalit Pairojkul, Amornrat Juasook and Pakkayaneesudsarn)
- P50 - Parasitological study of *Opisthorchis viverrini* in streptozotocin-induced diabetes mellitus hamsters. (Apisit Chaidee, Patchareewan Pannangpetch and Somchai Pinlaor)
- P51 - Anti-inflammatory effect of anthocyanin complex on *Opisthorchisviverrini*-infected hamsters. (Kitti Intuyod, Aroonsri Pripem, Wanvisa Limphirat, Porntip Pinlaor, Chawalit Pairojkul and Somchai Pinlaor)
- P52 - Curcumin prevents liver bile canaliculi alterations in the hamsters infected with *Opisthorchis viverrini*. (Prapaporn Jattujan, Somchai Pinlaor, Lakhanawan Charoensu, Channarong Arunyanart, Jariya Umka Welbat and Wunnee Chaijaroonkhanarak)
- P53 - Development of *Opisthorchis viverrini* associated cholangiocarcinoma hamster model for anti-cancer effect analysis. (Nattapong Puthdee, Kulthida Vaeteewootacharn, Wunchana Seubwai, Orasa wonkchalee, Worasak Keawkong, Amornrat Juasook, Chaisiri wongkham, Seiji Okada, Thidarut Boonmars and Sopit Wongkham)
- P54 - Ultrastructure of developmental tegument of *Opisthorchis viverrini*. Ratana Leksomboona, Wunnee Chaijaroonkhanarakd, Malcolm K. Jones and Banchob Sripa
- P55 - Electron microscope observation of the TBD treated *Angiostrongylus cantonensis* larvae. (Zeng Xin, Wang Juan, Wu Feng, Jinyi Liang, Huanqin Zheng, Wu Zhongdao)
- P56 - A novel procedure for precise quantification of *Schistosoma japonicum* eggs in bovine feces. (Bin Xu, Catherine A. Gordon, Wei Hu, Donald P. McManus, Hong-Gen Chen, Darren J. Gray, Chuan Ju, Xiao-Jun Zeng, Geoffrey N. Gobert, Jun Ge, Wei-Ming Lan, Shu-Ying Xie, Wei-Sheng Jiang, Allen G. Ross, Luz P. Acosta, Remigio Olveda and Zheng Feng)
- P57 - Effect of citric acid for the acidification of artificial pepsin solution for metacercariae isolation from fishes. (Min-Ki Kim, Kyoung-Ho Pyo, Young-Sang Hwang, Hyang Sook Chun, Ki Hwan Park, Seong-Hee Ko, Jong-Yil Chai and Eun-Hee Shin)
- P58 - Modified formalin-ether concentration technique for diagnosis of human strongyloidiasis. (Witthaya Anamnart, Pewpan Maleewong Intapan and Wanchai Maleewong)

- P59 - Comparative histopathology of male and female Syrian hamster *Opisthorchis viverrini* and CCA development. (Thidarut Boonmars, Nadchanan Wonkchalee, Porntip Laummaunwai, Chantana Aromdee, Yaovaluk Chamgramol, Chawalit Pairojkul, Amornrat Juasook, Pakkayanee Sudsarn)
- P60 - Simplified naked-eye detection of fish-borne trematode metacercariae in cyprinoid fish. (Porntip Laummaunwai, Thidarut Boonmars, Ratchadawan Aukkanimart, Pranee Sriraj, Pakkayanee Sudsarn, Nadchanan Wongchalee and Amornrat Juasook)
- P61 - Susceptibility of laboratory rodents to *Trichinella papuae*. (Lakkhana Sadaow, Pewpan M. Intapan, Thidarut Boonmars, Nimit Morakote and Wanchai Maleewong)
- P62 - The medically important echinostomes infection in free-grazing ducks in Thailand. (Chairat Tantrawatpan and Weerachai Saijuntha)
- P63 - Distribution and abundance of *Opisthorchis viverrini* metacercaria in cyprinid fish in northeastern Thailand. (Somchai Pinlaor, Thidarut Boonmars, Porntip Pinlaor, Nuttanan Hongsrirachan, Sudarat Onsurathum, Apisit Chaidee, Ornuma Haonon, Wutipong Limviroj, Smarn Tesana, Paiboon Sithithaworn, and Sasithorn Kaewkes)
- P64 - Ultrastructures of *Paragonemus heterotrumus* testis. (Nongnut Aubandit¹, Pipatphong Kanla¹, hongphithak Puthiwat, Channarong Arunyanart, Kowit Chaisiwamongkol, Wanchai Maleewong, Pewpan M. Intapan, Sitthichai Iamsaard and Wiphawi Hipkaeo)
- P65 - Duration of fish storage in low temperature before raw-fish-processing dish prevents *Opisthorchis viverrini* metacercariae infection in hamsters. (Sudarat Onsurathum, Thidarut Boonmars, Porntip Pinlaor, Apisit Chaidee, Ornuma Haonon, Lakhanawan Charoensuk, Porntip Laummaunwai, Wutipong Limviroj, and Somchai Pinlaor)
- P66 - Complete mitochondrial genome of *Haplorchis taichui* and comparative analysis for trematodes. (DM Lee, SJ Choe, HS Park, Hk Jeon, JY Chai and KS Eom)

ABSTRACTS

Oral Presentations

Biodiversity of minute intestinal flukes in Asia



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ABSTRACT

Minute intestinal flukes are taxonomically diverse, and a total of 46 zoonotic species are known to occur in Asia. The largest group is the Heterophyidae, which constitutes 29 species in 12 genera (*Apophallus*, *Ascocotyle*, *Centrocestus*, *Cryptocotyle*, *Haplorchis*, *Heterophyes*, *Heterophyopsis*, *Metagonimus*, *Procerovum*, *Pygidiopsis*, *Stellantchasmus*, and *Stictodora*). For other families, 1-4 species (1-2 genera) each are involved; Brachylaimidae (*Brachylaima*) (1 species), Cathaemaciidae (*Cathaemacia*) (1 species), Gastrodiscidae (*Gastrodiscoides*) (1 species), Gymnophallidae (*Gymnophalloides*) (1 species), Lecithodendriidae (*Phaneropsolus*, *Prosthodendrium*) (2 species), Microphallidae (*Spelotrema*) (1 species), Nanophyetidae (*Nanophyetus*) (1 species), Neodiplostomidae (*Neodiplostomum*) (2 species), Paramphistomatidae (*Fischoederius* and *Watsonius*) (2 species), Plagiorchiidae (*Plagiorchis*) (4 species), and Strigeidae (*Cotylurus*) (1 species). Among these, heterophyids are the major group, in terms of the number of species involved, the number of people infected, and the distribution of endemic areas. The sources of human infections include fish, snails (including the oyster), amphibians, reptiles, and aquatic insects. The natural reservoir hosts are mammals or birds. The host-parasite relationships have been studied extensively in *Metagonimus yokogawai*, *Heterophyes nocens*, *Haplorchis taichui*, *Neodiplostomum seoulense*, and *Gymnophalloides seoi*; however, more information is needed. The pathogenicity and clinical aspects of each parasite species and host mucosal defense mechanisms are poorly understood. Diagnosis of minute intestinal fluke infections can be done by fecal examination, but differential diagnosis is difficult because of morphological similarity of their eggs. Praziquantel is an effective anthelmintic for minute intestinal flukes. Epidemiological surveys and detection of human cases are required for a better understanding of the distribution and endemicity of each trematode species.

Molecular phylogeny and biogeography of trematodes,
with an emphasis on those infecting humans



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ABSTRACT

Trematodes (digeneans) are a large and ancient taxon of parasitic flatworms. Their antiquity is partly demonstrated by the large numbers of families (~160), genera (~1800) and species (>18,000) recognized. Probably more significant is the fact that many families and genera are very distinctive and have as hosts evolutionarily relict groups (such as turtles and crocodilians). Although there is some indication of broad-scale co-phylogeny of trematodes and their hosts, superimposed on this is host-switching, as a result of which, worms come to occupy hosts unrelated to their previous ones. Host switching is largely driven by ecological opportunity (shared diet or shared habitat). For example, mammals of the order Sirenia have acquired via host-switching trematodes related to those in green turtles, as a consequence of shared diet. Host switching is the means by which humans have acquired all their trematode parasites! Humans have probably not inherited any “ancient” co-evolved trematodes from their primate ancestors. Instead, as modern humans have moved around the world within the last 100,000 years, they have acquired trematode (and other) parasite species largely as a consequence of sharing diet with the previous mammalian hosts. Consequently, almost all trematodes found in humans are zoonotic. The only exceptions are a couple of species of *Schistosoma* in Africa, for which the original mammal hosts have either been lost, or are now of minor importance. *Schistosoma* species (family Schistosomatidae) and *Paragonimus* species (family Paragonimidae) provide interesting case studies of the ways in which humans have acquired trematode parasites in relatively recent times.

Regulatory T cells and Parasitic Infections



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ABSTRACT

Parasites use various mechanisms to evade the host immune responses in order to prolong or increase rate of their survival in host. The previously known evasion mechanisms include: residing in immunological privilege sites (inside cells or hollow organs), variation of antigenicity (stage specific antigens, antigenic variants, antigen stripping, antigenic disguise) and modification of the host immune responses (antibody cleavage, over consumption of complement, inhibition/reduction of host immune responses, over activation of lymphocytes, modification of lymphocyte activities, and formation of immune complexes for evasion of ADCC by eosinophils).

In recent years, a new immunological concept of the failure of the host immune system to clear the parasites has emerged. It is now believed that many parasites are able to modulate the magnitude of host effector responses by inducing regulatory T cells (Tregs). Tregs are T lymphocytes that play important role in the control of immune responses. The primary role of Tregs is active suppression of patho-physiological inflammation in the host and maintenance of immune homeostasis. Normally, Tregs can reduce injurious host inflammatory and immune responses.

Tregs are classified into two types. One is thymic or natural Tregs (nTregs) which are produced in thymus as CD4⁺CD25⁺ T cells during positively selection of T cells. These cells express transcription factor Foxp3. The signal to develop into nTreg is thought to come from interaction between T cell receptor and the complexes of MHC class-II with self-peptide expressed in thymic stroma. They are present in the blood stream before exposure to pathogens or tissue damage and constituted approximately 5-10% of CD4⁺ T cells. Another group of Treg is adaptive or inducible (iTregs). The iTregs originated from CD4⁺ naïve T cells that acquire a regulatory function of immune responses in the presence of cognate antigen and immunoregulatory cytokines, such as **TGF-β**, IL-10 and IL-35. They differentiate into CD4⁺CD25⁺Foxp3⁺ Tregs. Inducible Tregs are classified further into different subsets including T regulatory-1 or Tr1 which secretes IL-10, T helper-3 or Th3 that secrete **TGF-β** and converted Foxp3⁺ Treg.

To date, the precise mechanisms of Tregs in immune suppression are still not clearly known. Two mechanisms have been proposed including contact-dependent mechanism and immunosuppressive cytokine mediated mechanism. Recent studies had demonstrated that many parasites have evolved

mechanisms to directly expand the Foxp3⁺ Tregs. For examples, ES antigen of *Heligmosomoides polygyrus* (a gastrointestinal nematode) bound to **TGF-β** receptor and promoted Smad2/3 phosphorylation which directly induces Tregs to suppress the effector cell proliferation. During malaria infection, numbers of CD4⁺CD25⁺Foxp3⁺ Tregs increased in both human and mice. Tregs population has also been found significantly higher in filarial infections including *Onchocera volvulus* and *Brugia malayi*, as well as in *Trichinella spiralis* infection. Tregs modulated protective immune response against *Toxoplasma gondii*. The knowledge on parasite molecules and the immune components involved in modulating immune response may provide a novel strategy for controlling immune mediated inflammations in other diseases.

Current status of food-borne parasitic zoonoses in China



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ABSTRACT

Food-borne parasitic zoonoses (FBPZs) are human and animal diseases caused by helminths and protozoans through the consumption of infected or contaminated meat, fish, plants and/or water; they pose significant public health and socioeconomic problems. Both emerging and re-emerging food-borne zoonoses have attracted increasing national and international attention in recent years. Over the past 20 years, with increased income and standards of living, and a rise in the consumption of exotic and delicate foods, food-borne parasitic diseases have become one of the main factors that impact upon national food safety and public health. The fastest growing food-borne parasitic diseases in China include clonorchiasis, angiostrongyliasis, echinococcosis, trichinellosis and cysticercosis. The second national survey on the current status of important parasitic diseases in the human population, which was carried out from 2001 to 2004, indicated that the total number of people suffering from clonorchiasis, trichinellosis, paragonimiasis, and angiostrongyliasis had increased compared to the first national survey carried out between 1988 and 1992. Some of these conditions cause significant public health problems. Here, the current status of the FBPZ problem in mainland China is reviewed and strategies and measures for effective control of FBPZs are proposed. Major parasitic zoonoses transmitted through consumption of infected or contaminated meat, fish, plants and/or water will be discussed.

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Fish-borne trematodes in GMS



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ABSTRACT

Four families of fish-borne trematodes distribute in the Greater Mekong Sub-region (GMS), namely Clinostomatidae, Echinostomatidae, Heterophyidae –intestinal flukes and Opisthorchiidae-liver flukes. Infection of 2 species of liver flukes—*Clonorchis sinensis* and *Opisthorchis viverrini* has been recognized as the leading cause of cholangiocarcinoma in people in Mekong region. Metacercariae of the 2 liver flukes have been reported from cyprinid freshwater fish. Recently a liver fluke metacercaria was found in red tail snakehead fish from the out skirt of Vientiane City, Laos; the adults obtained from experimental infected hamsters showed the third species existed in GMS- *Opisthorchis lobatus*. Heterophyids infected humans in the region are *Centrocestus formosanus*, *Haplorchis tachui*, *H. pumilio*, *H. yokogawai*, and *Stellantchasmus falcatus*. However, metacercariae of *Heterophyopsis*, *Pygidiopsis* and *Stictodora* were also reported in brackish water fish in Thailand and Vietnam. *Echinochasmus japonicus*- a fish-borne echinostome has been reported in man in Laos, Thailand and Vietnam. A case of clinostomiasis was reported in Prachinburi Central Thailand, a fluke was found in the eye of a man who fond of fishing. Clinostome metacercaria was found in freshwater fish in Laos, Thailand and Vietnam.

Supported by Meeting APAP Organizers

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



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ABSTRACT

Brugia malayi is one of the important lymphatic filarial nematodes that cause elephantiasis in humans in the Asian region. Mass production at any stage of this nematode in both small laboratory animal hosts and mosquito vectors is still necessary in order to continue various research aspects. This has led to the search for reliable laboratory animal hosts and mosquito vectors for *B. malayi*. Thus, this study has developed a facile system for the mass-production of *B. malayi* in a small-space laboratory by combining benefits of the autogenous *Ochlerotatus togoi* colony (Thailand strain) with HBBS-rich-*B. malayi* microfilariae obtained from peritoneal withdrawal of *B. malayi*-infected jirds. The 2+ ml of HBBS-rich-*B. malayi* microfilariae could be prepared for at least 100+ ml of heparinized-blood containing *B. malayi* microfilariae, which is the main key for performing massive artificial feeding of autogenous *Oc. togoi* on blood containing *B. malayi* microfilariae. The high susceptibility rate of autogenous *Oc. togoi* to *B. malayi* has led to the mass production of L₃ larvae. This is an important key in performing massive subcutaneous and/or intraperitoneal inoculation of L₃ larvae into jirds in order to mass produce *B. malayi* developing stages and adults. Successful rearing of an autogenous *Oc. togoi* colony for many consecutive generations (F₁₀₀₊) has led to the maximum reduction of animal-house space for maintaining blood-food animals, which are necessary for producing blood-feeding mosquito eggs, e.g., *Stegomyia aegypti* (Liverpool strain) and *Oc. togoi* (Taiwan strain). These aedines species have been used widely as efficient laboratory vectors for *B. malayi*.

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Present status of Tropical Neglected Parasites in Thailand



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ABSTRACT

Parasitosis is pervasive among socially and economically disadvantaged and marginalized communities, particularly in tropical and subtropical areas. Humans in Southeast Asia are at risk for many tropical neglected parasitosis caused by protozoan, soil transmitted, food-borne, water-borne and vector-borne helminthes infections. In Thailand, the important contributors of protozoan diseases and the serious and harmful helminthic diseases such as liver and lung fluke infections (i.e. opisthorchiasis, fascioliasis, and paragonimiasis), tapeworm infections (i.e. sparganosis, cysticercosis, and taeniasis), nematode infections (i.e. gnathostomiasis, angiostrongyliasis, capillariasis, strongyloidiasis, hookworm infection, ascariasis, etc.) and lymphatic filariasis are reported. Here, the aim is summarized the prevalence current status of Tropical Neglected parasites in Thailand. The improved grasping of the full impact of parasitic related diseases will be provided the strategy for a suitable design of the next generation of parasite control.

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ABSTRACTS

Poster Presentations

A loop-mediated isothermal amplification technique (LAMP) assay for diagnosis and evaluation of treatment of patients infection with *Schistosoma japonicum*

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ABSTRACT

The aim of this study was to evaluate the clinical utility of the Loop-mediated isothermal amplification assay (LAMP) for diagnosis and evaluation of treatment in cases of schistosomiasis japonica. The performance of LAMP, enzyme-linked immunosorbent assays (ELISA), and indirect haemagglutination assays (IHA) were compared with 110 sera of patients from a *Schistosoma japonicum*-endemic area in Hunan Province, China. The sensitivity of LAMP was 95.5%, which was slightly higher than the two immunoassays. LAMP assay showed a highest sensitivity of 95.7% for individuals with less than 100 eggs per gram of feces (EPG), and the positive detection rate reached up to 95.1% in patients excreting less than 10 EPG. After treatment, the negative translation rate of LAMP increased along with the time (23.4%, 61.7% and 83.0% at 3 months, 6 months and 9 months post-treatment). While for ELISA and IHA, the negative translation rate sustained at a low level (25.5% by ELISA and 31.9% by IHA) even at 9 months after treatment. Our results demonstrate that the LAMP assay has potential role for clinical diagnosis and evaluation of treatment in cases of schistosomiasis japonica in endemic areas with low prevalence and low intensity infections.

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Molecular identification of *Anisakis pegreffii* (Nematoda: Anisakidae) collected from the sea eels
(*Astroconger myriaster*) of the Southern Coast of Korea

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ABSTRACT

Human anisakiasis is an important fish-borne parasitic zoonosis and caused by anisakid larvae, that include the species of *Anisakis* and *Pseudoterranova*. In this study, *Anisakis* larvae were collected from the sea eel (*Astroconger myriaster*) from Tongyeong City, a southern coast area of Korea, and molecular identification was performed. In total, 180 *Anisakis* larvae were recovered from 20 sea eel samples collected in March 2013. All sea eels (20/20, 100%) examined were infected with *Anisakis* larvae. These larvae were morphologically identified as *Anisakis* type I larvae, which can be either *Anisakis simplex* or *Anisakis pegreffii*. These two species can be differentiated only through molecular analysis. Based on the PCR-RFLP patterns using sequences of internal transcribed spacer (ITS1) of nuclear ribosomal DNA, these nematodes were identified as *A. pegreffii* third-stage larvae. This is the first report of *A. pegreffii* from the fish host in the Republic of Korea.

Morphological and molecular characterisation of lymnaeid intermediate hosts of *Fasciola* spp. in Vietnam

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ABSTRACT

Freshwater snails of the family Lymnaeidae play an important role in the transmission of fascioliasis worldwide. In Vietnam, only two Lymnaeid species, *L. swinhoei* and *L. viridis*, have been morphologically identified so far and both of them are reported as the intermediate hosts of *Fasciola* spp. However, recent studies suspect their role of transmission of *Fasciola* because of confusion in snail identification. The aims of this study are, therefore, to clarify the identification of Lymnaeid snails in Vietnam by combination of morphological and molecular approaches. Based on morphological characteristics, three species were recognized as *Radix rugibinosa* (the shell is more elongate and cylindrical, the last whorl is large and less inflates, aperture large but not extended), *Lymnaea swinhoei* (the shell is generally large, the last whorl is inflated, aperture large and extended), and *L. viridis* (the shell is much smaller than two other species, the whorls are well rounded, the aperture is regularly oval without angles). The molecular analyses using the second internal transcribed spacer ITS-2 of the nuclear ribosomal DNA of this study clearly showed that Lymnaeids snails in Vietnam include three species, *Austropeplea viridis* (formerly identified as *Lymnaea viridis*), *Radix auricularia* (formerly identified as *Lymnaea swinhoei*) and *Radix rubiginosa* as a new record for freshwater snail fauna in Vietnam. The results of this study bring a new insight on Lymnaeid snails in Vietnam, suggesting that identification of Lymnaeid snails in Vietnam and their role of *Fasciola* transmission should be surveyed for more detail.

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Genetic difference of *Angiostrongylus cantonensis* in Thailand

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ABSTRACT

A survey of genetic difference of among 8 *Angiostrongylus cantonensis* populations was conducted by using mitochondrial cytochrome *b* (*cyt b*) gene. The adult worms of parasite were confirmed by morphological characteristics as *A. cantonensis* before performing the PCR and DNA analysis. From this study, phylogenetic tree showed the populations studied can be separated to be 2 main clades. Haplotype network indicates 11 *cytb* haplotypes which can be divided obviously into 4 clusters. Genetic differentiation among parasite populations and geographic distance was observed. The result suggested low genetic variation among populations in each cluster and each cluster was not isolated by distance. We suggested that *A. cantonensis* in Thailand might have experience of founder effect, which might be occurred by multiple independent origins.

Detection of *Opisthorchis viverrini* in human stool samples by nested-PCR

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ABSTRACT

Human opisthorchiasis caused by the liver fluke *Opisthorchis viverrini* is an endemic disease in developing countries in Southeast Asia. To effectively treat and control the spread of *O. viverrini*, a highly sensitive, highly specific and affordable diagnostic technique is needed for detection of human opisthorchiasis. In this study we developed Nested-PCR to detect *O. viverrini* DNA in human stool samples. Two pairs of primers were designed from a pOV-A6 specific probe sequence, a highly specific and repetitive sequence of the *O. viverrini* genome. The OV-F nest and OV-R nest primer set yielded a 260 bp product from *O. viverrini* DNA amplification. The detection limit was 2×10^{-18} ng/ μ l. The Nested PCR showed 100% sensitivity for detection of case of light infection. It could detect *O. viverrini* in mixed infected stool samples. It also showed positive in 6 of 43 *O. viverrini* microscopically negative samples. It can effectively distinguish *O. viverrini* infection from other parasite infection. Compared to the microscopic method and conventional PCR, Nested-PCR is more sensitive and has more potential to detect *O. viverrini*. In addition, Nested PCR is an inexpensive technique which can be used as a routine diagnostic method in areas of endemic opisthorchiasis.

Molecular characterization of *Ancylostoma duodenale*, *Ancylostoma ceylanicum* and *Necator americanus*
in Thai patients

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ABSTRACT

Hookworms are the common intestinal parasitic nematodes, infecting a globally, an estimated 600 million persons are infected, consequence in up to 135,000 deaths annually. In Thailand 2 principal species of hookworm infecting humans are *Necator americanus* and *Ancylostoma duodenale*. Case studies on zoonotic hookworm infections with *Ancylostoma ceylanicum* and/or *Ancylostoma caninum* have been reported mainly from Asian countries whereas only *A. ceylanicum* develop to adult in humans. The present study, we firstly reported molecular evidences of human hookworms in Southern and northeastern Thailand. This is important for studying fundamental aspects relating to epidemiology and population biology in Thailand. Thirty hookworm larval samples from Thai patients consist of 10 samples from northeast part and 20 samples from southern part. The partial ITS1, 5.8S, and ITS2 of ribosomal DNA genes were amplified by a PCR technique. The amplicons were sequences, analyzed, aligned and compared with other hookworm sequences in GenBank. Phylogenetic tree was constructed to investigate of the relationships. In the northeast part, the DNA sequences from 6 samples showed 99% homologies with *N. americanus*. Three samples showed 99% homologies with *A. ceylanicum*. One sample showed 99% homologies with *A. duodenale*. From the southern part, all 20 samples showed 99% homologies with *N. americanus*. In Thailand, *N. americanus* is more prevalent than *Ancylostoma* spp. *N. americanus* are predominant in the southern and northeast of Thailand. In which, *A. ceylanicum* and *A. duodenale* found sporadic cases in the northeast of Thailand.

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Molecular epidemiology of *Paragonimus heterotremus* and *P. pseudoheterotremus* in freshwater crabs in Thailand and Myanmar

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ABSTRACT

Paragonimiasis is an important food-borne parasitic zoonosis caused by infection with lung flukes of the genus *Paragonimus*. In the genus *Paragonimus*, 7 species – *P. heterotremus*, *P. westermani*, *P. siamensis*, *P. bangkokensis*, *P. harinasutai*, *P. paishuihoensis* and *P. macrorchis* – were reported in Thailand. Recently a new *Paragonimus* species, *P. pseudoheterotremus*, was described as the 8th species in Thailand. This species is genetically close to *P. heterotremus* and, thus, they are considered as a sister species. In this study, we have collected *P. heterotremus* and *P. pseudoheterotremus* metacercariae from freshwater crabs from Phitsanulok and Phetchabun provinces in north, Saraburi province in central and Surat-Thani province in south Thailand. In addition, we purchased freshwater crabs imported from Myanmar at Myawaddy Province, west Thailand, a Myanmar-Thailand border). After polymerase chain reaction/sequencing of the DNAs extracted from excysted metacercariae were PCR-amplified and sequenced for ITS2 and Cox1 genes. The ITS2 sequences were nearly identical among all *P. pseudoheterotremus*/*P. heterotremus* (99–100%). The partial Cox1 sequences were also highly similar (95–99%) among all *P. pseudoheterotremus*/*P. heterotremus*. Although *P. pseudoheterotremus* and *P. heterotremus* formed distinct clusters in the phylogenetic tree, their distance was very close with the bootstrap value of 100, suggesting that they are the sister species. In Thailand, *P. pseudoheterotremus* was widely distributed from north to south. Since both species are human pathogens, their wide distribution in all over Thailand and Thai-Myanmar border alerts possible human infections in those areas.

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Rapid detection of *Wuchereria bancrofti*, *Brugia malayi*, *B. pahangi* and *Dirofilaria immitis* by high resolution melting real-time PCR in mosquito vectors and blood samples

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ABSTRACT

A simple, rapid and high-throughput detection for *Wuchereria bancrofti*, *Brugia malayi*, *B. pahangi* and *Dirofilaria immitis* in mosquito vectors and blood samples was developed by using a real-time PCR and high-resolution melting analysis. The real-time PCR was principally based on the change of fluorescence during an experiment, and measured temperature-induced DNA dissociation during high resolution melting of amplicons generated from trans-spliced leading Exon I gene sequences of the four filarial species. The differentiation of HRM patterns were analyzed and melting temperatures were characterized by peaks 81.4±0.20°C, 78.8±0.41°C, 79.9±0.05°C and 76.8±0.14°C for *W. bancrofti*, *B. malayi*, *B. pahangi* and *D. immitis* respectively. This assay required no hybridization probes resulting in low cost effectiveness. Sensitivity and specificity were all 100%. It is a rapid, technically simpler detection, and an important tool for surveying as well as for molecular xenomonitoring of the parasites in vectors.

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Differential detection of *Schistosoma japonicum* and *Schistosoma mekongi* by high resolution melting
real-time PCR

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ABSTRACT

Schistosomiasis caused by *Schistosoma japonicum* and *Schistosoma mekongi* which the infection continues present a chronic parasitic diseases that prevalent in several countries of Asia. According to the morphological similarity of cercariae and eggs of these two parasites, the microscopic examination has difficulties for differentiation. High resolution melting (HRM) real-time PCR is developed for the detection of specific melting temperature of the amplicons of real-time PCR. A primer pair was designed for targeting the 18S ribosomal RNA gene to generate 158 base pairs PCR products in both species. *S. japonicum* and *S. mekongi* were differentiated by melting temperatures which are 84.51±0.07 and 85.70±0.07 °C, respectively. The method enables to detect one cercaria or one egg. This result suggests that HRM real-time PCR is rapid and simple for differentiate detection of *S. japonicum* and *S. mekongi* in the intermediate and final hosts. In areas where epidemiology of two *Schistosoma* species overlaps or where increasing in the number of foreign workers, migrants and travelers have effected in the widespread of schistosomiasis; the assay will be a valuable identification method for epidemiological studies.

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Molecular differentiation of *Opisthorchis viverrini* and *Clonorchis sinensis* eggs by the use of multiplex real-time PCR and high resolution melting analysis

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ABSTRACT

Opisthorchis viverrini and *Clonorchis sinensis* are the parasites that have been recognized as the carcinogenic and causative agents of cholangiocarcinoma or bile duct cancer in Asian countries. Morphology of eggs in both flukes is similar and cannot distinguish under the microscopic method. Here, we developed the high resolution melting (HRM) analysis couple with multiplex-real-time PCR for the differentiation of *O. viverrini* and *C. sinensis* eggs in fecal samples. Using 2 pairs of species-specific primer, the amplification of mitochondrial NADH dehydrogenase subunit 2 (nad2) DNA sequences were performed to generated the 209 and 165 base pairs products for *O. viverrini* and *C. sinensis*, respectively. The distinct characteristics of HRM patterns were analyzed and melting temperatures were characterized by peaks of 82.38±0.09 and 85.89±0.08°C for *O. viverrini* and *C. sinensis*, respectively. This technique was able to detect as low as one egg of *O. viverrini* and two eggs of *C. sinensis* in 100 mg fecal sample. This method is a rapid and straightforward method for discrimination of *O. viverrini* and *C. sinensis*'s eggs. This assay is simple compared to other technique as it does not require fluorescent probes or post-PCR processing. Therefore, this method offers a new alternative for rapid differentiation and detection of Asian liver fluke infections in patient's specimens.

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Sparganosis presenting as cauda equina syndrome with molecular identification of a causative parasite species

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ABSTRACT

A 52-year-old woman presented with low back pain, progressive symmetrical paraparesis with sensory impairment and sphincter disturbances. Magnetic resonance imaging (MRI) of the whole spine revealed multiple intradural extramedullary serpiginous-mass like lesion at subarachnoid space from preponine continue to anterior part of medulla oblongata levels, C7, T2-T8 and T12 vertebral level distally until end of the theca sac and fill-in right S1 neural foramen. Sparganosis was diagnosed by demonstration of the sparganum on histopathological section of surgery tissue and the present of serum IgG antibody against sparganum antigen determined by enzyme linked-immunosorbent assay (ELISA). DNA samples were extracted from the surgical tissue and the partial fragment of cytochrome c oxidase subunit 1 (*cox1*) gene was amplified for the detection of *Spirometra* DNA. After sequencing of the PCR-amplicon and alignment of nucleotide sequences, the causative agent was identified as *Spirometra erinaceieuropaei*.

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The preliminary *in vitro* observation of recombinant FBPA of *Schistosoma japonicum* in stimulation of splenocytes of mice

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ABSTRACT

Schistosoma japonicum is among the helminth parasites that are prevalent both in humans and animals of Greater Mekong Subregion of Asia. Chronic infection of schistosomiasis is characterized by a Th2-dominant response as well as an overall down-regulated immune system. This helminth-induced immunosuppression may downregulate the host response to other pathogens. Recent studies have suggested that *S. japonicum* infection is protective in murine models of autoimmune disorders such as arthritis. To study the effect of SjFBPA on Th1/Th2 response *in vitro*, the recombinant SjFBPA was expressed in prokaryotic cells and the fusion protein was purified. Mouse splenocytes were isolated from female BALB/c mice and subject to culture with stimulation by rsjFBPA or LPS for 48 hours. The subsets of T help cells were detected by flow cytometry, the IFN- γ and IL-4 expression in the supernatant of the culture were detected by ELISA. The results showed that stimulation of the spleen lymphocytes with LPS dramatically increases the number of Th1 cells and the IFN- γ expression. The number of both Th1 and Th2 cells were simultaneously increased and the levels of IFN- γ and IL-4 increased obviously when exposed to rsjFBPA. Our preliminary approach suggested that the parasite-derived sjFBPA has dual function on inducing Th1 and Th2 response in early phase of infection. The *in vivo* induction with sjFBPA needs to be performed to examine its Th2-biased response in mice.

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Inhibition of CCL8 suppresses eosinophils chemotaxis and enhances survival of intracranial larvae of
Angiostrongylus cantonensis and the infected mice

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ABSTRACT

Eosinophils (Eos) plays a critical role in the pathogenesis of angiostrongyliasis cantonensis and CCL8 is known to promote eosinophilic inflammation by recruiting IL-5⁺ T_H2 cells in atopic dermatitis. Therefore, in this study, we elucidated the role of chemokine CCL8 in angiostrongyliasis cantonensis. CCL8 was demonstrated an extremely higher expression in brain of Balb/C mice infected with *Angiostrongylus cantonensis* (*A. cantonensis*) than normal animals using comparative genomic analysis. CCL8 production in the sera and cerebrospinal fluid (CSF) of infected mice were assayed with a peak occurring at days 21 postinfection and CCL8 was predominantly released by the microglia of central nervous system (CNS) in the infected mice. The administration of monoclonal rat anti-mouse-CCL8 antibody (anti-CCL8) strongly depressed peripheral, CSF and medullary eosinophilic responses and experienced less serious pathological injuries in the infected mice, when compared with groups subjected to isotype-matched rat IgG or phosphate-buffered saline solution (PBS) alone. Some larvae migrated from the brains to the lungs in anti-CCL8-treated group. Our data indicate a pivotal role of CCL8 in eosinophilia of angiostrongyliasis cantonensis.

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rSj16 inhibits dendritic cells maturation through intracellular IL-10 dependent way

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ABSTRACT

Dendritic cells (DC) play a pivotal regulatory role in activation of type of immune response by responding to environmental pathogens. rSj16, a 16 KDa protein derived from *Schistosoma japonicum*, has been identified as an immune modulation molecule, but the mechanisms of modulation of immune responses especially negative modulation the function of dendritic cells is poor unknown. We investigate the influence of rSj16 on LPS-induced dendritic cells *in vitro*, including the gene expression and cytokine secretion patterns. The results showed that rSj16 increased the down-regulation of LPS-induced endocytotic capacity in DCs. rSj16 suppressed the LPS-induced both up-regulation of CD80, CD86, CD40, MHC-II and IL-12, IL-6 and IL-23 in DCs. However, rSj16 up-regulated LPS-induced down-regulation of IL-10 in DCs. After co-culture of rSj16-primed DCs and T cells, Th1 cytokines (IL-12, IFN- γ and IL-17) were decreased and Th2 cytokines (IL-10 and IL-4) were increased, significantly. Furthermore, injection of rSj16 into mice, it also suppressed the mixed lymphocyte reaction and IFN- γ , but increased of IL-10. But this phenomena was disappeared in IL-10 (-/-) mice which suggest that the function of rSj16 on dendritic cells in a IL-10 dependent way. However, how rSj16 modulated the production of IL-10 still unknown. Sequence analyses reveals two potential nuclear localization signal (NLS) of Sj16. Further studies showed that the first NLS (NLS1) is necessary and sufficient for translocation of rSj16 to the host nucleus and important for subsequent rSj16-mediated inhibitor of mouse DCs especially related to the production of IL-10 by rSj16. This study determined possible mechanism of rSj16 role on negative modulation of dendritic cells through intracellular IL-10 dependent way, and the main domain related the function of rSj16 is the NLS1.

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Dynamics of Th17 cells and their role in *Schistosoma japonicum* infection in C57BL/6 mice

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ABSTRACT

CD4⁺ T cells play critical roles in both host immune responses against parasitic infection and immunopathology in schistosomiasis. The induction of T helper (Th)1, Th2 and T regulatory (Treg) cells and their roles in schistosome infections are well-illustrated. However, little *in vivo* data are available on the dynamics of Th17 cells after *Schistosoma japonicum* infection or whether these cells mediate host protective responses early in infection. Levels of Th17 and the other three CD4⁺ T cell subpopulations and the cytokines related to induction or repression of Th17 cell generation in different stages of *Schistosoma japonicum* infection were observed. Contrary to reported *in vitro* studies, our results showed that the Th17 cells were induced along with the Th1, Th2, Treg cells and the IFN- γ and IL-4 cytokines in *Schistosoma japonicum* infected mice. The results also suggested that *Schistosoma japonicum* egg antigens preferentially induced Th17 cell generation. Furthermore, decreasing IL-17 with a neutralizing anti-IL-17 monoclonal antibody (mAb) increased schistosome-specific antibody levels and partial protection against *Schistosoma japonicum* infection in mice. Our study is the first to report the dynamics of Th17 cells during *Schistosoma japonicum* infection and indicate that Th17 cell differentiation results from the integrated impact of inducing and suppressive factors promoted by the parasite. Importantly, our findings suggest that lower IL-17 levels may result in favorable host protective responses. This study significantly contributes to the understanding of immunity to schistosomiasis and may aid in developing interventions to protect hosts from infection or restrain immunopathology.

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An immunomodulator from *Angiostrongylus cantonensis* (named AcCystatin) promotes TNF- α production in mouse macrophages

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ABSTRACT

Parasites modify the host's immune system, via efficient immunoregulatory molecules, to diminish the inflammatory responses against them. Cystatins from various parasitic nematodes have been described previously as one of the immunoregulators characterized by induction of high level IL-10 expression in cystatin-treated macrophages. Here we reported a cystatin from *Angiostrongylus cantonensis* (AcCystatin), on the contrary, induced pro-inflammatory cytokine TNF- α rather than anti-inflammatory cytokine IL-10 production of mouse macrophages. AcCystatin was predominantly taken up by macrophages and subsequently activated the phosphorylation of the mitogen-activated protein kinases (MAPK) ERK1/2 and phosphoinositide 3-kinase (PI3K). TNF- α production promoted by AcCystatin in macrophages was tyrosine kinase sensitive, and phosphorylation of the transcription factors CREB and NF- κ B was induced by the immunomodulator and regulated by the phospho-AKT (downstream target of PI3K) and phospho - ERK. Thus, AcCystatin clearly exhibited an increase of TNF- α production in macrophages of mice, the non-permissive host of *A. cantonensis*, via a tyrosine kinase sensitive and ERK/PI3K dependent pathway.

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Schistosoma japonicum: Tsunagi/Y14 protein plays a critical role in the development of the reproductive organs and eggs

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ABSTRACT

Tsunagi/Y14 is an evolutionarily conserved RNA-binding protein that is required for the maintenance of oogenesis and the masculinization of the germ-line in many animal models. We speculated that Tsunagi/Y14 might also regulate reproductive organ development in *Schistosoma japonicum* (*S. japonicum*, Sj). Sj Tsunagi/Y14 and control double-stranded RNAs were introduced into schistosomula by electroporation respectively. These transfected schistosomula were cultured in vitro for 1, 3 or 5 days. The mRNA and protein levels of the target gene in the cultured schistosomula were significantly suppressed compared with those of the control group. Furthermore, BALB/c mice were infected with the transfected schistosomula for 6 weeks and were sacrificed to harvest the adult worms. We found that the silencing of *S. japonicum* Tsunagi/Y14 led to defects in reproductive organs development in both male and female worms. Moreover, it also affected the size, quantity and activity of the eggs in the mice liver. Our findings indicated that Tsunagi/Y14 plays a critical role in the development of reproductive organs and eggs in *S. japonicum*.

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Characterization and immunolocalization of mutated ornithine decarboxylase antizyme from
Angiostrongylus cantonensis

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ABSTRACT

Ornithine decarboxylase antizyme, which is a prominent regulator of cell proliferation, DNA/RNA transformation and tumorigenesis, binding to ODC and facilitating ODC degradation by a unique ribosomal frameshift. In our study, we cloned and characterized a deleted-mutation gene in +1 ribosomal frame-shifting (+1RF) that encoding the protein ornithine decarboxylase antizyme (DM-AcOAZ) from a cDNA library of fourth-stage larvae of *Angiostrongylus cantonensis*. Sequence analysis showed that DM-AcOAZ contained a conservative domain related to other OAZ species. Results of quantitative real-time PCR (qRT-PCR) revealed that DM-AcOAZ expressed at the life stage of L3, L4 and female adult worms, while in female adult worms, its expression level is the highest. Immunohistochemical studies indicated that the DM-AcOAZ expression especially localized at the uterus, oocyte and intestine in female adult worms. MTT assays showed the HeLa cells which were transfected with recombinant plasmid can restrain cell proliferation obviously. In conclusion, DM-AcOAZ maybe a female-enriched protein and involved in the cell proliferation of *Angiostrongylus cantonensis*.

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Comparative a recombinant matrix metalloproteinase *Gnathostoma spinigerum* protein with 21 kDa and 24 kDa antigenic components of *G. spinigerum* larval extract for serodiagnosis of neurognathostomiasis

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ABSTRACT

Neurognathostomiasis is a severe form of human gnathostomiasis which can lead to harmful problems and death. Diagnosis of neurognathostomiasis is made presumably by using clinical manifestations. Immunoblotting which recognize the *Gnathostoma spinigerum* larval extract of 21 kDa and 24 kDa antigenic components (Gs 21/24) showed highly sensitivity and specificity for neurognathostomiasis. However, the Gs 21/24 from the *G. spinigerum* larval extract which prepared from parasites harvested from natural or experimental animals, the limitation is the small amount production. To overcome this problem, we used a recombinant matrix metalloproteinase (rMMP) protein and comparatively evaluated with the Gs 21/24 in the serodiagnosis of human neurognathostomiasis. We studied sera from 40 patients from Srinagarind Hospital, Khon Kaen University, Thailand who compatible with clinical criterion of neurognathostomiasis and 30 healthy control adults from Thailand. All sera were tested for specific IgG antibody against both *G. spinigerum* crude larval extract and rMMP protein by the immunoblotting. The sensitivity and specificity for both antigenic types were all 100%. This result concludes that *G. spinigerum* rMMP protein can be used as an alternative diagnostic antigen for serodiagnosis of neurognathostomiasis

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Diacylglycerol kinase gamma immunolocalization in the *Opisthorchis viverrini* vitellaria

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ABSTRACT

Diacylglycerol (DAG) is a member of the phosphoinositide (PI) signal cascade and activates protein kinase C as the first identified second messenger in vertebrates. Although the functional significance of DAG and PI signal remains to be clarified in invertebrates, there have been some reports on PI metabolism and phosphatidic acid, the DAG kinase product, in worms. Therefore, the localization of DAG kinase in liver flukes was attempted for more clarification of the functional significance of PI signals. The present immunofluorescence light microscopy employed anti-rat DAG kinase gamma antibody because this mammalian isoform contains a sequence of amino-acids exhibiting some homology to the counterpart of *C. elegans* whose genome information was already clarified. As a result, DAG kinase-like immunoreactivity was clearly detected in the vitellaria which are the extensive network of glandular cells and ducts beneath the tegument of the liver fluke, and the immunoreactivity appeared in aggregations of small oblong or oval structures, 0.5~1.0 µm in size. This result suggests that DAG kinase is involved in the regulation of egg-shell production and secretion in the vitellaria cells, and eventually in the regulation of the proliferation of the parasite infection. For this suggestion to be elucidated, further analysis is necessary such as the immuno-electron microscopy of the immunoreactive structures and the molecular identification of the worm epitope detected by the mammalian DAG kinase antibody as well as the localization of some other PI signaling molecules.

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Evaluation of immunoglobulin G4 subclass antibody in peptide-based enzyme-linked immunosorbent assay
for serodiagnosis of human paragonimiasis heterotremus

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ABSTRACT

Peptide was synthesized based on an antigenic region of *P. westermani* pre-procathepsin L1. An enzyme-linked immunosorbent assay (ELISA) using this synthetic peptide was evaluated for detection of IgG4 subclass antibodies in sera of paragonimiasis, other helminthiasis, tuberculosis and healthy control groups. It was found that the assay gave sensitivity, specificity, accuracy and positive and negative predictive values of both peptide antigens 100%, 94.59%, 96.22%, 100% and 88.85%, respectively. Cross reaction rate was found to be very high in fascioliasis (75%) and hookworm infection (50%). Other parasitoses sera were positive in lower percentage, 0-25%. It was suggested that the assay is useful for supporting diagnosis of pulmonary paragonimiasis and differential diagnosis from fascioliasis by clinical presentation and fascioliasis serology.

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Proteomic analysis of autoantibody in opisthorchiasis

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ABSTRACT

Opisthorchis viverrini infection causes opisthorchiasis which strongly associated with cholangiocarcinoma (CCA). Chronic opisthorchiasis induced chronic inflammation. Inflammation has been found to induce autoantibody production and cancer development. Autoantibody can be detected at early stage of infection. It is promising as a new disease biomarker. In order to investigate autoantibody in opisthorchiasis. Immortal human cholangiocyte cell line (MMNK1) was extracted and separated by two-dimensional (2D) gel electrophoresis. Gel was transferred to PVDF membrane and probed with opisthorchiasis patients and healthy control plasma to screening for autoantibody reaction by 2D immunoblot. In addition, other gel was run in parallel and stained with coomassie brilliant blue (CBB). Fourteen immunoreactive spots were detected from opisthorchiasis patients by 2D-Blot assay but in the healthy control was not detectable. Compared to CBB staining gel, corresponded spots were excised and proteins are under identifying by LC-MS/MS mass spectrometry. Western blotting and ELISA will be used to validate the potential of immunoreactive proteins for opisthorchiasis specific biomarker differential from other parasitic diseases. We have successfully detected fourteen immunoreactive spots from patients by 2D-Blot assay but spot was not detectable in control group. The spots were excised. Proteins are under identifying by LC-MS/MS mass spectrometry. These results suggested the presence of autoantibody in opisthorchiasis. These autoantibodies will provide a potential in diagnosis of opisthorchiasis and could offer opisthorchiasis-associated CCA predictive efficacy.

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Bile canalicular alteration and bile secretory defect in acute and chronic *Opisthorchis viverrini*-infected hamsters

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ABSTRACT

Opisthorchis viverrini infection induces inflammation and liver injury leading to increase periductal fibrosis with time. However, it's remain unclear whether these changes have effect on the original of bile duct changes. The aim of this study was to investigate the alterations of bile canaliculi (BC) and biliary physiology in hamster liver infected with *O. viverrini* during acute and chronic phases. In *O. viverrini* infected groups, electron microscopy revealed that BC showed tortuous course, dilatation of canalicular lumen and decreased the density of microvilli compared to normal control. The mRNA expression of FXR and Shp-1 genes significantly increased, but expression of Mrp2 and Ntcp significantly decreased in both acute and chronic of *O. viverrini*-infected groups compared to control group. A significantly decrease expression of BSEP, Ntcp, Cyp7a1, Cyp27a1 and CD10 genes was observed only in acute infection. In addition, CD10 staining for a canalicular pattern was significantly lower level than in the normal group. In contrast, the fibrotic markers including mRNA expressions of collagen I and III, protein expression of fibroblast specific marker α -SMA and serum hydroxyproline content were significantly higher levels than that of control. Increase of fibrotic markers was associated with the thickness of periductal fibrosis. In conclusion, *O. viverrini* infection induces BC alterations and decreases bile flow, bile acid uptake transporters and bile acid biosynthesis in relation to periductal fibrosis. The study may provide an insight information of biliary changes in opisthorchiasis-associated disease.

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Encapsulated curcumin enhances periductal fibrolysis in *Opisthorchis viverrini*-infected hamsters after praziquantel treatment

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ABSTRACT

Although praziquantel treatment would be valuable for *Opisthorchis viverrini* infection; however, periductal fibrosis induced by chronic infection is still remain for a long time after treatment. These phenomenal effects after praziquantel treatment may increase relative risk for cholangiocarcinoma (CCA) in opisthorchiasis-associated CCA. To reduce periductal fibrosis induced by chronic *O. viverrini* infection encapsulated curcumin was used to enhance fibrolysis of fibrosis tissue after praziquantel treatment in *O. viverrini*-infected hamsters. Encapsulated curcumin increased ferric antioxidant capacity in the plasma. In contrast, it reduced the plasma level of nitrate/nitrite (NO_x) and decreased alanine transaminase (ALT), indicator of liver injury. Encapsulated curcumin increased the expression of Neprilysin (CD10) and Multidrug resistance-associated protein 2 (Mrp2) at the transcription and translation levels at bile canaliculi. Histopathological study revealed that encapsulated curcumin reduced periductal fibrosis evaluated by picosirius red staining. This changes was related with the reduction of the expression of fibrotic markers (Matrix metalloproteinase (MMP) 9, collagen I, tissue inhibitor of matrix metalloproteinase (TIMP1) and alpha-smooth muscle actin (α -SMA)) and increasing of MMP2. In conclusion, encapsulated curcumin enhances periductal fibrosis resolution after praziquantel treatment in experimental opisthorchiasis which may apply to opisthorchiasis patient for prevention risk of CCA development.

Supported by The Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Health Cluster (SHeP-GMS)

Proteomic identification of expressed proteins associates with periductal fibrosis in chronic experimental opisthorchiasis

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ABSTRACT

A liver fluke, *Opisthorchis viverrini* is endemic in northeastern Thailand, and causes periductal fibrosis during chronic infection leading to a relative risk factor for cholangiocarcinoma. The aim of the study was to identify proteins expression in relation to periductal fibrosis in chronic opisthorchiasis hamsters by proteomics analysis. Liver tissue extracted from normal and chronic opisthorchiasis hamsters were subjected to two-dimensional gel electrophoresis (2-DE). The results showed that the protein expression pattern of infected hamsters were different when compared with normal control group. Identified protein spots will further investigate by software analysis, and MS/MS analysis. Candidate proteins will be verified in the hamster liver and plasma, and apply in opisthorchiasis patient for diagnostic purpose. Histopathological features revealed that chronic opisthorchiasis hamsters had accumulation of inflammatory cells surrounding the biliary tissues and thickening of periductal fibrosis. In conclusion, *O. viverrini* infection induces the alteration of proteins expression in relation to periductal fibrosis which may challenge in novel biomarker discovery for opisthorchiasis.

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The hydatid-killing effects of high intensity focused ultrasound enhanced with ultrasound contrast agent and superabsorbent polymer

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ABSTRACT

The current optional therapies for cystic echinococcosis still remain many problems in the world. In our previous studies, it has found that high intensity focused ultrasound (HIFU), a new non-invasive therapy option for some solid carcinomas, could damage the protoscolices. HIFU, however, caused a limited destruction of hydatid cyst due to the cyst fluid with a character of attracting less deposited HIFU energy. In this study, it suggested that a special material with superabsorbent ability should be added into the cyst fluid and would attract more HIFU energy, and explored if a superabsorbent polymer (SAP) and ultrasound contrast agent (UCA) alone or in combination could enhance damage efficacy of HIFU to hydatids. The combined HIFU ablations to hydatids experiments included: ablating the protoscolices in suspensions, ablating isolated cysts from sheep livers, and ablating the transplanted cysts in rabbit models. The comparison of ultrasound image for each layer of hydatid cyst before and after HIFU ablation was made immediately, and the protoscolices in ablated cysts were stained by eosin exclusion assay, and the structures of protoscolices were observed by light microscopy. In the experiments of HIFU ablating cysts, besides observing the damages of protoscolices, the pathological changes of cyst walls were investigated by light microscopy, scanning and transmission electron microscopy as well. It was found that the destructive effects of HIFU on hydatid cysts could be enhanced by UCA and SAP alone or in combination, and the combination of HIFU with UCA and SAP revealed a best ablation effect, which could be a possible therapeutic option for cystic echinococcosis.

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Trichinella spiralis adult worm but not muscle larvae products ameliorate acute DSS-induced colitis

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ABSTRACT

Numerous studies have demonstrated an inverse correlation between helminth infections and inflammatory bowel diseases (IBD). In view of clinical applications in the future, identification and characterization of the active helminth-derived products responsible for the beneficial effects on IBD is essential. Here, we aim to investigate the therapeutic potential of excretory/secretory (ES) products from *Trichinella spiralis* muscle larvae and adult worm (MESP and AESP) on the murine model of colitis. Colitis was induced in C57BL/6 mice by administering 3% dextran sodium sulfate (DSS) for 7 days in drinking water. During this period mice received daily intraperitoneal injections of different stage ES products from *T. spiralis*. Clinical disease activity index (DAI) was monitored by measurement of body weight, stool consistency, and presence of blood. At sacrifice, disease severity was assessed by the length of colon, macroscopic score, microscopic inflammation score and myeloperoxidase (MPO) activity. Cytokine profiles and the percentage of CD4⁺ CD25⁺ FOXP3⁺ Tregs of T cells isolated from mesenteric lymph nodes (MLN) and spleen were measured. Treatment with *T. spiralis* MESP failed to improve colitis. In contrast, AESP treatment significantly ameliorated the severity of DSS-induced colitis as evidenced by reduced DAI score, attenuated changes in colon length, and decreased the macroscopic score, microscopic inflammation score and MPO activity. AES are able to modulate the potent pro-inflammatory response induced by DSS, mainly through the up-regulation Th2/Treg (IL-4, IL-10, IL-13 and TGF- β) and down-regulation of Th1/Th17 (IFN- γ , IL-6 and IL-17) cytokines in the spleens and MLN. Additionally, the Tregs observed were greatly increased in the MLN of the AESP-treated mice than those of mice without treated with AESP. The results provide direct evidence that *T. spiralis* AESP have a therapeutic potential, which may substitute for living worms in the treatment of IBD.

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A prospective cohort study on the factors associated to the participation in and compliance to the mass drug administration for Schistosomiasis in Barangay Bethel and Barangay Canaan, Victoria, Oriental Mindoro

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ABSTRACT

The socio-demographic characteristics, knowledge and awareness of schistosomiasis and its treatment, and the six constructs of the Health Belief Model (perceived susceptibility, perceived severity, perceived effectiveness of the drug, perceived self-efficacy, perceived benefits of the treatment minus barriers to pursuing the treatment, and cues to action), and perception on government services and their association to the participation in and compliance to the mass drug administration of Praziquantel was studied. A total of 233 primary health decision makers from Barangay Bethel and Barangay Canaan, Victoria, Oriental Mindoro, Philippines were surveyed in order to determine if the aforementioned factors had a significant association on the receipt (participation) and ingestion (compliance) of the drug Praziquantel in the household in response to the high prevalence of schistosomiasis in their area. The study is a prospective cohort study that made use of a focus group discussion and house-to-house survey interviews conducted in May 17-20, 2012 to measure the different exposure variables and another survey conducted in October 18-21 of the same year was done in order to measure the outcomes. The unit of analysis in the study was at the household level. The results of the study showed a significant association between four variables and household participation. These variables were whether the household head had heard of the house-to-house method of mass drug administration ($p=0.012$, $OR=2.316$, $95\%CI=1.199-4.472$), the number of household members ($p=0.021$, $OR=1.207$, $95\%CI=1.029-1.416$), responses to the question of side effects of treatment classified as "Others" ($p=0.021$, $OR=2.214$, $95\%CI=1.130-4.338$), and reporting enlargement of abdomen as a symptom of schistosomiasis ($p=0.022$, $OR=0.165$, $95\%CI=0.035-0.722$). Furthermore, the results showed that having heard of the house-to-house method of mass drug administration was significantly associated with compliance ($p=0.035$, $OR=2.316$, $95\%CI=1.056-4.477$). From the results of the focus group discussion, the researchers hypothesized that the inconsistencies in the knowledge of the Barangay Health Workers as well as the lack of strict protocols in distributing the drug could be the possible causes for the low rates. It is important to maintain a high participation and compliance rate in order to lower the prevalence of schistosomiasis.

Gnathostoma spinigerum infection involving the inner lip of a Korean woman: the first autochthonous case

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ABSTRACT

Autochthonous human gnathostomiasis had never been reported in the Republic of Korea. We report here a case of *Gnathostoma spinigerum* infection in a 32-year-old Korean woman, suggested to have been infected through an indigenous infection source. The patient experienced a painful migratory swelling near the left nasolabial fold area of the face for 1 year, and the swelling moved to the mucosal area of the inner lip 2 weeks before receiving a surgical removal of the lesion. Histopathological examinations of the extracted tissue revealed inflammations with heavy eosinophilic infiltrations and sections of a nematode suggestive of a larva of *Gnathostoma* sp. The larva characteristically revealed multiple (3-6) nuclei in each intestinal cell and thus could be identified as *G. spinigerum*. The patient did not have any special history of travel abroad except a recent trip (4 months before surgery) to China where she ate only cooked food. We designated our patient as the first autochthonous case of *G. spinigerum* infection in Korea.

Current status of *Paragonimus* spp. and paragonimiasis in north and central Vietnam

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ABSTRACT

Paragonimiasis is a typical food-borne parasitic zoonosis caused by infection with lung flukes of the genus *Paragonimus*. In Vietnam, investigations on *Paragonimus* and paragonimiasis have been conducted in north and central regions. Until now, we have found 7 *Paragonimus* species in Vietnam, e.g., *P. heterotremus*, *P. westermani*, *P. skrjabini*, *P. vietnamensis*, *P. proliferus*, *P. bangkokenis* and *P. harinasutai*. Of these, the first three species, *P. heterotremus*, *P. westermani* and *P. skrjabini*, are known to cause human paragonimiasis in other countries. However, in Vietnam, *P. heterotremus* is the only proven species to cause human paragonimiasis. In some northern provinces of Vietnam, local people are still suffering from *P. heterotremus* infection because of their traditional habit of eating raw/undercooked crabs. Although *P. westermani* metacercariae are highly prevalent among freshwater crab hosts in the central provinces, human paragonimiasis cases have never been found in these areas. Similarly, *P. skrjabini* was recently found in Thanh Hoa province, human cases caused by this species have not yet been found in this or adjacent provinces. The pathogenicity of *P. westermani* and also *P. skrjabini* to humans in Vietnam still remains unclear. Further studies on the epidemiology and pathogenesis of these species in Vietnam are necessary.

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High prevalence of haplorchiasis in Nan and Lampang province, Thailand, following anthelmintic treatments of suspected opisthorchiasis cases

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ABSTRACT

Opisthorchiasis, a risk factor of cholangiocarcinoma in human, is of a public health importance in Thailand. The Ministry of Public Health aims to control the infection to a level below 5%. The Annual Surveillance Report from Nan and Lampang province showing prevalence of opisthorchiasis over 70% triggered a serious concern. We investigated if infection is due to minute intestinal flukes (MIF) rather than *Opisthorchis* as their eggs are hardly differentiated by morphology. Residents in Nan and Lampang, 50 and 100 cases, respectively, whose stool were positive for *Opisthorchis* eggs were followed-up and given praziquantel, 400 mg/kg, single dose. After 2 hours, 30-45 ml of magnesium sulfate, a purgative was given and stools were sequentially collected up to 4 times. Stools were examined for adult worms by simple sedimentation. *Taenia proglottis* was identified to species by India ink technique. It was found that 39 of 50 cases (78%) from Nan had *Haplorchis taichui*, ranged from 5 to 1,250 with the average of 62 worms/case. Other helminths recovered were *Taenia saginata* (7 cases) and *Enterobius vermicularis* (1 case). In Lampang, *H. taichui* were recovered from 69 cases (69%). Number of fluke recovered ranged from 1 to 4,277 with the average of 326 worms/case, 4 cases had *Phaneropsolus bonnei*, and 10 cases had *T. saginata*. It is clear that MIF infection especially haplorchiasis is more common in northern Thailand. The findings urge the Public Health Office to employ more specific tool than Kato's method, yet low cost, for surveillance of opisthorchiasis in Thailand.

Susceptibility of five species members of the Korean *Anopheles hyrcanus* group to *Brugia malayi*

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ABSTRACT

Five species members of the Korean *Anopheles hyrcanus* group, i.e., *An. pullus*, *An. sinensis*, *An. kleini*, *An. belenrae* and *An. lesteri* were tested for susceptibility to *Brugia malayi*. They were allowed to feed artificially on blood containing *B. malayi* microfilariae, and dissected 14 days after feeding. The susceptibility rates were 60%, 65%, 90%, 100% and 100% in *An. pullus*, *An. sinensis*, *An. kleini*, *An. belenrae* and *An. lesteri*, respectively. As determined by levels of susceptibility, results indicated that *An. pullus* and *An. sinensis* were moderate potential vectors, while *An. kleini*, *An. belenrae* and *An. lesteri* were high potential vectors when compared with the 95% susceptibility rate of an efficient control vector, *Ochlerotatus togoi*. The satisfactory susceptible rates obtained in this study from *An. sinensis* and *An. lesteri* have confirmed previous reports that these 2 anopheline species played an important role as natural vectors of Brugian filariasis in East Asia. Even though this filarial parasite has been eradicated up to now in the Republic of Korea (ROK), beneficial results, reported herein, emphasize the potential role of these anopheline species in transmitting *B. malayi* in other countries, in which this filarial parasite is still endemic and/or re-emerging, similarly to the recent re-emergence of other mosquito-borne diseases, e.g., malaria due to *Plasmodium vivax* in ROK.

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Prevalence of intestinal parasitic infections among schoolchildren of Phitsanulok Province, northern Thailand**Raxsina Polseela** and Apichat Vitta

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To determine the prevalence of intestinal parasites in children from six schools located in Bang Rakam and Muang districts, Phitsanulok Province, Northern Thailand, from February 2009 to January 2010. The study sites were the following six schools: Wat Krab Phuang, Wat Wang Ped, Wat Tha Ko, Wat Sao Hin, Wat Kung Watee, and Wat Sakat Namman. Fecal samples were collected from 352 students, age range 7-15 years old, and examined for intestinal parasites presence by formalin-ethyl acetate concentration method. Additionally, Scotch tape technique was used to recover *Enterobius vermicularis* eggs from 576 children. The overall prevalence of intestinal parasites in the feces of 352 examined students (formalin-ethyl acetate concentration method) was low (5.4%): in 2.5% cases we observed *Strongyloides stercoralis* infection, in 0.6% *Entamoeba coli*, in 0.6% *Giardia lamblia*, in 0.6% *E. vermicularis*, and in 0.3% hookworm infection. The prevalence of mixed infections was also low: 0.3% for hookworm and *E. coli*, 0.3% for hookworm and *Trichuris trichiura* and 0.3% for *S. stercoralis* and *E. coli*. An overall infection rate of enterobiasis among 576 schoolchildren (Scotch tape method) was 14.1%. The highest infection rate (19.3%) was observed at Wat Sao Hin School. In all positive cases selective anthelmintic drugs were administered. In the present study, low prevalence of parasitic infections in schoolchildren of Phitsanulok Province was demonstrated. However, to control and prevent the transmission of parasites the problem should get more attention and the efficient educational and prophylactic measures should be implemented.

Recovery of *Enterobius vermicularis* and *Taenia* eggs by Scotch tape technique

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ABSTRACT

Parasitic infection is highly prevalent in developing countries including Thailand. Research on the prevalence of parasitic infections is a requirement for the development of appropriate control programs. December 2012 through January 2013, a cross-sectional survey was conducted to determine the prevalence of enterobiasis and taeniasis in school children of two primary schools by Scotch tape technique, i.e., Tha Sala School, Tha Sala Subdistrict, Phu Ruea District, Loei Province, and Nong Kae Nong No School, Nong Phai Subdistrict, Kaeng Khro District, Chaiyaphum Province. A total of 180 school children including 81 boys and 99 girls were included in this study. Overall *Enterobius vermicularis* infection was 10% (18/180) while *Taenia* infection was 1.11% (2/180). According to age groups, the highest prevalence of enterobiasis was found in 5-8 year olds while the lowest was found in 1-4 year olds in both schools. In all positive cases selective anthelmintic drugs were administered. In the present study, low prevalence of both enterobiasis and taeniasis in school children was demonstrated. However, to control and prevent the transmission of parasites, the problem should get more attention, and efficient educational and prophylactic measures should be implemented.

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Intestinal parasitic infection among school children in an urban community of Khon Kaen,
Northeast region of Thailand

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ABSTRACT

Epidemiological information on prevalence of intestinal parasitic infections also differed from region to region because of several environments, social and geographical factors. The aim of this study was to determine the prevalence of intestinal parasitic infections in schoolchildren aged 5-13 years in 5 schools of Amphoe Muang, Khon Kaen province. In this cross-sectional study, 914 stool samples were collected by random sampling after we explained and asked the teachers and students for verbal informed consent. Then the specimens were examined for the intestinal parasites by wet direct smear and Kato-Katz method. The presence of intestinal parasites was determined by microscopically by two independent medical technologists. The prevalence of intestinal helminthic infections was 11.8%. The infected parasites were *Taenia* spp. (5.25%), *Opisthorchis viverrini* (2.63%), *Strongyloides stercoralis* (2.29%), hookworm (1.09%) and *Ascaris lumbricoides* (0.54%). Our findings suggested that there were the intestinal parasites remained infected the schoolchildren. However, in the urban communities of Khon Kaen. Health promotion by means of health education should be more emphasized. The school health educational program should focus on parasitic treatment. Prevention and control measures should be implemented in the school level.

Prevalence of parasitic infections among the people in rural communities, Khon Kaen province,

Northeast of Thailand

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ABSTRACT

Parasitic infections are the public health problems in Southeast Asia including Thailand. In rural communities, people still have cultural belief, poor personal hygiene and sanitation. Although, it has the effective anthelmintic drugs but recurrent of infection in some parasites are remained. Therefore, this present study wants to reveal the prevalence of intestinal parasitic infections among the people in rural communities. Community-based cross-sectional study was conducted in the Pra Lub district, Khon Kaen province, Northeast of Thailand from March to April 2013. A total of 112 stool samples from 44 males and 68 females, aged 28-80 years, were examined by formalin-ethyl acetate concentration methods. The specimens were examined under light microscope. Forty-one cases in 112 cases (38%) were infected with one or more parasites. The prevalence rate in male 22 cases (20%) was slightly higher than female 19 cases (17%). The prevalence of parasitic infection in age group of 51-60 years (29%) was higher than other age groups. The most common parasite found in people in rural communities were *Opisthorchis viverrini* 23 cases (21%), followed by *Strongyloides stercoralis* 14 (13%), *Taenia* spp., *Entamoeba histolytica*, *E. coli*, *Blastocystis hominis*, echinostome with 3 (3%), 2 (2%), 2 (2%), 2 (2%), 1 (1%), respectively. There were also 4 cases co-infected with *O. viverrini* - *S. stercoralis* and 1 case co-infected with *O. viverrini* - *Taenia* spp. The present study suggests that the prevalent of parasitic infection in this region is still high. The proactive education in cultural belief, personal hygiene and sanitation should be provided to the people in this community to reduce the prevalence of intestinal parasites. Moreover, the development of policy to control parasite is needed.

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A cross-sectional study of intestinal helminthic infections in patients of a tertiary Khon Kaen Hospital,
Northeast part of Thailand

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ABSTRACT

The present study was conducted to investigate the presence of intestinal parasites among the patients in the tertiary care, Khon Kaen Hospital, Khon Kaen, Thailand. We reviewed the results of stool examination of 5,331 cases of patients who admitted in Khon Kaen Hospital from January to December 2011. Each stool sample was performed direct smear and Kato-Katz method. And stool samples were examined for parasite infection by using light microscope. It was found that the prevalence of intestinal parasitic infection was 3.3%. The most prevalent parasites found were *Strongyloides stercoralis* (1.3%), *Opisthorchis viverrini* (1%), *Taenia* spp. (0.7%), hookworm (0.3%) and *Ascaris lumbricoides* (0.06%). These results should be considered for situation of intestinal helminthic infection in tertiary care hospital in Thailand which still have parasitic infections as the health problem.

Predominant helminthiasis in patients at Srinagarind Hospital, Khon Kaen during 2007-2012

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ABSTRACT

Helminth parasites are parasitic worms from the phyla Nematoda (roundworms) and Platyhelminthes (flatworms). Over one billion people in sub-Saharan Africa, Asia and the Americas are infected with one or more helminth species. The diagnosis of these infections, the stool samples must be examined and identified parasite under a microscope. In this study aims to determine the overall prevalence of helminthiasis in patients visiting Srinagarind Hospital during 2007 to 2012. A total number of 22,516 fecal specimens from Srinagarind Hospital submitted for parasite examination during 2007 to 2012 were analyzed by formalin ethyl acetate concentration technique at Parasitology Laboratory, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. The actual numbers of patients of 15,070 were used for calculation of prevalence rates of helminthiasis. The overall prevalence of helminthiasis was 28.50 % (4,295/15,070). The leading helminthic infections was *Strongyloides stercoralis* (16.40%) followed by *Opisthorchis viverrini* (14.71%), *Taenia* spp. (1.75%), Minute intestinal fluke (1.71%), Hookworm (1.41%), Echinostome (0.78%), *Trichostrongylus* spp. (0.25%), *Capillaria philippinensis* (0.21%), *Trichuris trichiura* (0.09%), *Enterobius vermicularis* (0.05%), *Dicrocoelium dendriticum* (0.03%), *Hymenolepis diminuta* (0.02%), *Ascaris lumbricoides* (0.01%) and for *Paragonimus heterotremus* (0.01%). It is obvious that strongyloidiasis and opisthorchiasis viverrini emerged as the most common helminthiasis in the patients of Srinagarind Hospital. Concerted effort for prevention and control should be emphasized.

Rate of *Gnathostoma spinigerum* antibodies in non-traumatic subarachnoid hemorrhage

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ABSTRACT

It has been shown that *Gnathostoma spinigerum* can cause non-traumatic subarachnoid hemorrhage (SAH). The detection of 21- or 24- kDa *G. spinigerum* antibody in the serum by immunoblot technique is helpful for diagnosis of neurognathostomiasis. There is limited data on how common of *G. spinigerum* infection in non-traumatic SAH. All consecutive patients diagnosed as non-traumatic SAH at Srinagarind Hospital, Khon Kaen University during Jan 2011 to Jan 2013 were studied. Computerized tomography (CT) or magnetic resonance (MR) imaging of the brain was used for diagnosis of SAH. Patients were categorized as aneurysm-subarachnoid hemorrhage (A-SAH) and non-aneurysm subarachnoid hemorrhage (NA-SAH) group by the results of cerebral angiograms. The 21- or 24- kDa *G. spinigerum* antibody in serum was determined by immunoblot technique. Rate of the *G. spinigerum* antibodies detection was compared between both groups. There were 118 non-traumatic SAH patients who had results of both cerebral angiogram and immunoblot analysis for *G. spinigerum*. Of those, 80 (67.8%) patients had intracranial aneurysm, whereas 38 (32.2%) patients had negative cerebral angiograms. The mean age (SD) of A-SAH and NA-SAH group was 53.6 (12.8) and 53.5 (12.9) years, respectively (p value 0.952). Male gender accounted for 45% and 50% in A-SAH and NA-SAH group, respectively (p value 0.611). The overall positive rate of *G. spinigerum* antibody against 21- and /or 24- kDa antigenic band was 23.7%. The rate was somewhat higher in A-SAH group than NA-SAH group (26.3% vs 18.4%, p value 0.350).

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Clinical manifestations of eosinophilic meningitis in children

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ABSTRACT

Eosinophilic meningitis is prevalent in the Northeastern Thailand. Most patients are in adulthood. Data regarding clinical manifestations of eosinophilic meningitis in children is still limited and may be different from adults. In adult patients, fever and neck stiffness can be found only 10% and 50%, respectively. Cranial nerve abnormalities are rare (5%) in adults. Chart review was done in children who diagnosed as eosinophilic meningitis and aged less than 15 years at Srinagarind hospital, Khon Kaen University, Thailand. Clinical manifestations and outcomes were reported by descriptive statistics. There were 20 patients diagnosed as eosinophilic meningitis; one was excluded due to having spinal cord involvement. In total, 19 patients were in the analysis. All patients were presenting with severe headache. Most patients were male (78.95%), had fever (78.95%), had nausea or vomiting (63.16%), had history of snails ingestion (68.42%), had neck stiffness (68.42%). Six patients (31.58%) had papilledema or cranial nerve palsies. Two patients had abnormal cerebellar signs (10.53%). Median peripheral eosinophilia and cerebrospinal fluid eosinophils were 20% and 58%, respectively. Clinical manifestations of eosinophilic meningitis in children are different from adult patients.

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Paragonimus paishuihoensis metacercariae in freshwater crabs, *Potamon lipkei*, in Vientian province, Laos

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ABSTRACT

Among *Paragonimus* species, *P. paishuihoensis* is one of the most mysterious and poorly understood species of which metacercariae are characterized by having a unique dendritically branched excretory bladder and its adult worm still remains unknown. To date, metacercariae of this species have been reported only in China and Thailand. In this study, we report the discovery of *P. paishuihoensis* metacercariae in freshwater crabs, *Potamon lipkei*, in Hinheub district, Vientiane, Lao PDR, with the infection rate of 77.7% and the average density of 10.3 (range 1-28) metacercariae per crab. The molecular data based on ITS2 and CO1 markers indicated that *P. paishuihoensis* from Lao PDR and Thailand were almost completely identical with each other. They were clustered with *P. harinasutai* from China to make a group separated from other *P. bangkokensis*/*P. harinasutai* populations, suggesting the necessity of reappraisal of the taxonomic status of *P. harinasutai* from China. Although we attempted to obtain adult worms by experimental infection in experimental animals (cats, dogs, and rats), no worms were recovered from them, suggesting that these animals might be unsuitable definitive hosts for *P. paishuihoensis*. Further studies are necessary to elucidate the taxonomic status and the life cycle of *P. paishuihoensis*.

Soil-transmitted helminthiasis in Guimaras Island, the Philippines: Is elimination feasible?VY Belizario, Jr.^{1,2}, HJC Liwanag¹, WU de Leon¹ and PLC Chua¹¹ National Institute of Health; ² College of Public Health, University of the Philippines, Manila*E-mail:* harvyliwanagmd@gmail.com**ABSTRACT**

Control of soil-transmitted helminthiasis (STH) in the Philippines includes school-based mass drug administration (MDA) of single-dose anthelmintics twice a year. In 2011, Guimaras Island was proposed as a pilot site for demonstrating the feasibility of eliminating STH as a public health problem by reducing prevalence of moderate-heavy intensity infection in school-age children to <1%. This study aimed to determine the feasibility of elimination by evaluating parasitologic status and MDA coverage over a two-year period and conducting a situational analysis that included an assessment of WASH (access to safe water, sanitation, and hygiene) parameters. A total of 623 and 1,053 stool samples from sentinel schools were examined using the Kato-Katz technique in 2010 and 2012, respectively. Data on MDA coverage was collected using reported government figures. A sanitation map was also created which compared WASH and parasitologic parameters. MDA coverage increased from 69.4% in 2010 to 97.4% in 2012, exceeding the government target of 85%. Reductions in cumulative prevalence (from 43.8% to 40.7%) and prevalence of moderate-heavy intensity infection (from 14.1% to 10.8%) were not significant despite high MDA coverage. No clear relationship between WASH and parasitologic parameters was observed in the sanitation map, suggesting the need for a more accurate assessment of sanitation conditions. A few sentinel sites, however, had low prevalence which indicated that parasitologic status varied across schools. The feasibility of elimination may therefore be demonstrated by further studies that focus on low-prevalence schools and assess the impact of intensified WASH interventions plus current MDA in these sites.

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Safety and feasibility of school-based combined mass drug administration (MDA) of albendazole and praziquantel in a decentralized health system

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ABSTRACT

To control NTDs, WHO recommends integration of program interventions, including co-administration of anthelmintics. In the Philippines, three programs separately conduct MDA for soil-transmitted helminthiasis and schistosomiasis. Local evidence for safety of combined MDA is lacking. Health workers are too few to reach all target populations. The health system is decentralized: Local Government Units (LGUs) are the main implementers of health programs, with guidance and technical assistance from the Department of Health (DOH).

To document safety of albendazole plus praziquantel, a pilot study conducted combined MDA among schoolchildren in two schools in one municipality. Incidence and severity of adverse events (AEs) were observed. Coverage was recorded. To document feasibility and continued safety of scaling up, partnerships were established with DOH, Department of Education (DepEd), and LGUs of two municipalities in another province. Teachers and health workers were trained. Teacher-assisted combined MDA was conducted in ten schools. Incidence and severity of AEs were observed; coverage and duration of MDA were recorded.

School teachers assisted by LGU and DepEd health workers administered anthelmintics in classrooms to 2,324 school-age children (65% coverage, up to 76% with 1-week follow up). Incidence of AEs was comparably low between the pilot and the scale up (5.2% and 6.5%, respectively). Most were mild and transient. On average, MDA took 164.1 minutes per school, or 42 seconds per pupil treated. Combined MDA using albendazole plus praziquantel is safe. Scaling up is feasible using the school-based approach. This can achieve high coverage in a relatively short time. In a decentralized health system, scaling up is possible through inter-sectoral collaboration among DOH, DepEd, and LGU.

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Successful treatment on *Brugia pahangi* in naturally infected cats

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ABSTRACT

Lymphatic filariasis in cats is one of the major parasitic diseases in Tropical region including Thailand. The objective of this study was to determine the efficacy of ivermectin at 400 µg/kg against microfilaria of *Brugia pahangi* in naturally infected cats. Eight naturally infected with *B. pahangi* cats were divided into 2 groups. Group1 was control group. Group2 was given ivermectin injection at 400 µg/kg every week for 2 months. Microfilariae were determined every week until 48 weeks. The microfilaria density in blood circulation was significantly decreased in group2 after 4 weeks of treatment. No microfilaria presented in blood circulation after the 9th week until the last week of study. Nevertheless, Group1 had high microfilaria density from the beginning until the end of study. This is the first study that is successful to treat and control microfilaria of *Brugia pahangi* in naturally infected cats with ivermectin. The ivermectin possibly causes the sterilization of female worms.

Supported by Faculty of Veterinary Science, Chulalongkorn University

In vitro anthelmintic activity of *Alpinia galanga* L. against sheep gastrointestinal nematodes

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ABSTRACT

In recent years, resistance to anthelmintics is a major problem of the parasitic intestinal worms treatment in small ruminants. Scientists were in research of alternative herbal remedies for is used to be the phytotherapeutic that is safety than synthetic drugs. *Alpinia galanga* (L.) is belonging to the family Zingiberaceae, that well-known as folk medicinal plants, root and stem were the most frequently used part for antibacterial, anti-inflammation and antifatulence. However, this is no medicinal reports the using of leaf part that distributed mainly as agricultural waste. Therefore, this study proposes to investigate the anthelmintic activity of galangal leaves extracted with n-hexane, dichloromethane and methanol by the maceration. Thus, the extracted at concentrations of 0.31, 0.62, 1.25, 2.5, 5.0 and 10.0 mg.ml⁻¹ were analysed for egg hatch test (EHT) and larval development test (LDT) against *Haemonchus contortus*. Mebendazole is used as control treatment. For larval development inhibition showed 50% lethal concentration (LC₅₀) of n-hexane, dichloromethane, methanol and control 5.4, 5.7, 5.3 and 4.6 mg.ml⁻¹, respectively. All extracts were not significantly different than control. The assessment of anthelmintic activity was observed in egg hatching inhibition at LC₅₀, egg hatching inhibition of each extracts and control were 48.5%, 40.0%, 49.1% and 67.2%, respectively. The methanolic extract was greater anthelmintic activity than hexane and dichloromethane. Phytochemical screening indicated the presence of flavonoids and phenolic compound could be the reason for anthelmintics. Such in formation will be useful for alternative therapeutic of gastrointestinal parasitic disease.

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Molluscicidal effect of *Camellia oleifera* seed and *Garcinia mangostana* L. pericarp on *Lymnaea* snails, an intermediate host of *Fasciola* sp.

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ABSTRACT

Lymnaea snails are the first intermediate host of liver flukes, *Fasciola* spp. The snail is found in many parts of Asia, including Thailand which is an endemic area of fascioliasis gigantea. It is well known that *Fasciola* sp. infection is a problem worldwide. However, prevention and control of fascioliasis is difficult. Therefore, disrupting its complete life cycle is one tactic that may reduce the spread of *Fasciola* sp. *Lymnaea* snails were tested with two molluscicides: *Camellia oleifera* (camellia), and the pericarp of *Garcinia mangostana* L. (mangosteen). Histopathological changes were observed microscopically. The results showed that both *Camellia oleifera* and *Garcinia mangostana* L. pericarp had molluscicidal effects, with IC₅₀ and IC₉₀ at concentrations of 0.00088 , 0.0904 g/mL, and 0.000888 , 0.0877g/mL, respectively, after 24 h exposure. The *Lymnaea* snail tissues, foot and digestive system, showed histopathological changes. The present study suggests that *Camellia oleifera* and *Garcinia mangostana* L. pericarp are molluscicide that could be used in the field to control *Lymnaea* snails.

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Molluscicidal effect of *Camellia oleifera* seed and *Garcinia mangostana* L. pericarp on *Pomacea canaliculata*, an intermediate host of *Angiostrongylus cantonensis*

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ABSTRACT

Pomacea canaliculata, a freshwater snail, is the first intermediate host of the lung fluke *Angiostrongylus cantonensis* in rat which can cause eosinophilic meningitis in human. This snail is found in many parts of Asia, including Thailand which is an endemic area of angiostrongyliasis. However, prevention and control of *A. cantonensis* infection is difficult because of changing eating habits is more complicated. Therefore, disrupting its complete life cycle is one tactic that may reduce the spread of *A. cantonensis* infection. *Pomacea canaliculata* was used as model to test for molluscicide effect with two kinds of plants, *Camellia oleifera* seed, and the pericarp of *Garcinia mangostana* L. Histopathological changes were observed under light microscopy. The results showed that both *Camellia oleifera* seed and *Garcinia mangostana* L. pericarp had molluscicidal effects, with IC₅₀ and IC₉₅ at concentrations of 0.0000098, 0.08371 g/mL, and 0.0000985, 0.088 g/mL, respectively, after 24 h exposure. Histopathological changes included alterations in the epithelial lining of the digestive tracts, digestive glands, gills and foot. Moreover, Loss of cilia, degeneration of columnar epithelial cells, increased mucous vacuoles and cells were observed in the digestive tracts and gills, while the digestive glands exhibited an increase the number of dark granules and basophilic cells, and dilation of digestive cells. Muscle cells of foot were loss of texture. The present study suggests that both *Camellia oleifera* seed and *Garcinia mangostana* L. pericarp have molluscicide activity that could be used in the rice field to control *Pomacea canaliculata*.

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Praziquantel combined-aspirin improved hepatobiliary system through liver function test and liver pathology in Syrian hamster cholangiocarcinoma associated with *Opisthorchis viverrini* and *N*-nitrosodimethylamine

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ABSTRACT

Opisthorchiasis is one of the major risk factors of cholangiocarcinoma (CCA) in northeast Thailand. An effective drug for killing this parasite is praziquantel. Recently, several reports have shown that with frequent use, praziquantel can cause liver cell damage from the immunopathological response, and may be a CCA risk. Therefore, we combined praziquantel with aspirin for the purpose of improving hepatobiliary system function in Syrian hamsters infected with *Opisthorchis viverrini* and administered *N*-nitrosodimethylamine (ND). Syrian hamsters were divided into six groups: i) administered *N*-nitrosodimethylamine (ND); ii) administered ND and aspirin (NDAs); iii) infected with *O. viverrini* and administered ND (OVND); iv) infected with *O. viverrini* and administered ND and aspirin (OVNDAs); v) infected with *O. viverrini* and administered ND and praziquantel (OVNDPz); and vi) infected with *O. viverrini* and administered ND, aspirin and praziquantel (OVNDAsPz). Livers were collected at 4 months for determination of pathological changes by immunostaining for expression of proliferating cell nuclear antigen (PCNA) and cytokeratin 19 (CK19), and by Sirius red staining for detection of fibrosis. Macroscopic gross observation showed that hamster livers in the aspirin plus praziquantel group looked healthy; this was correlated with the results of microscopic observation, which showed slowed progression of cholangiocarcinoma. Reductions of fibrosis and bile duct cell proliferation were observed, although aggregations of inflammatory cells remained. Positive PCNA and CK19 staining was predominantly observed in the aspirin-untreated group, and only slightly in the treated group. Moreover, the combination of aspirin and praziquantel given to *O. viverrini*-infected hamsters (in the OVNDAsPz group) could reduce inflammatory infiltration, bile duct proliferation, fibrosis and CCA area to a greater extent than administration of aspirin alone (in the OVNDAs group). These findings suggest that using praziquantel plus aspirin can improve the hepatobiliary system after *O. viverrini* infection and reduce the risk of CCA.

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A combination of praziquantel and traditional plant, *Thunbergia laurifolia* Linn, improve hepatobiliary system in Syrian hamster opisthorchiasis and cholangiocarcinoma

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ABSTRACT

Praziquantel is the drug of choice for liver fluke infections, during which the parasites release a large amount of antigens, leading to an accumulation of inflammatory cells surrounding the hepatic bile ducts which causes cell damage. *Thunbergia laurifolia* Linn. is a traditional plant which has antioxidant and anti-inflammatory properties in vitro and in previous studies on hamster opisthorchiasis. Therefore, in the present study we demonstrated the combined effect of *Thunbergia laurifolia* Linn. (RC) plus praziquantel (PZ) on hamster opisthorchiasis and cholangiocarcinoma (CCA). Hamsters were divided into six groups: i) infected with *Opisthorchis viverrini* plus praziquantel (OV_PZ); ii) infected with *O. viverrini* plus PZ and RC (OV_PZ_RC); iii) infected with *O. viverrini* plus *N*-nitrosodimethylamine (OV_NDMA); iv) infected with *O. viverrini* plus NDMA and RC (OV_NDMA_RC); v) infected with *O. viverrini* plus NDMA and PZ (OV_NDMA_PZ); and vi) infected with *O. viverrini* plus NDMA, PZ and RC (OV_NDMA_PZ_RC). Light microscopic observation of histopathological changes, liver function tests for alanine transaminase (ALT) and alkaline phosphatase (ALP), and kidney function tests for blood urea nitrogen (BUN) and creatinine were performed. The study found that RC extracts clearly improve the hepatobiliary system by reducing inflammatory cell aggregation and inhibiting CCA development, results which were correlated with serum ALT level. These findings suggest that administration of *Thunbergia laurifolia* Linn. after praziquantel treatment may improve the hepatobiliary system and could reduce the risk of CCA development.

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Parasitological study of *Opisthorchis viverrini* in streptozotocin-induced diabetes mellitus hamsters

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ABSTRACT

Diabetes mellitus (DM) is the disease which can cause hyperglycemic state leading to many complication such as increases risk of infection especially bacteria and fungus. However, information about interaction of parasitic infection and DM is limited. DM has high prevalence in northeast Thailand where opisthorchiasis caused by liver fluke *Opisthorchis viverrini* infection is also endemic. The aim of this study was to investigate the effect of DM on *O. viverrini* development at 1 month post-infection in hamster induced by streptozotocin and non-induced group. The results revealed that liver per body weight ratio in the experimental group was significantly higher than that in non-induced group (0.036 ± 0.003 vs. 0.058 ± 0.013 , $P = 0.012$). For the worm size of liver fluke in experimental group was smaller than in control non-induced group (worm length; $3,982.76 \pm 315.24$ μm vs. $3,195.16 \pm 513.76$ μm , $P = 0.025$, worm width; 787.33 ± 137.77 μm vs. 712.87 ± 127.54 μm , $P = 0.433$). These results suggests that DM has the effect on both host and *O. viverrini* fecundity by changing in physiological conditions.

Supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Health Cluster (SHeP-GMS), The National Research Project of Khon Kaen University. Apisit Chaidee is a graduate student supported by M.D./Ph.D. program, Faculty of Medicine, Khon Kaen University.

Anti-inflammatory effect of anthocyanin complex on *Opisthorchis viverrini*-infected hamsters

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ABSTRACT

Opisthorchiasis-associated cholangiocarcinoma is supposed via chronic inflammation. Many studies revealed that anthocyanins have anti-oxidant and anti-inflammatory activities. Therefore, in this study aims to investigate anti-inflammatory effect of anthocyanins complex in *O. viverrini*-infected hamster. Anthocyanins complex (175 and 700 mg/kg body weight) was orally treated for 30 days. Histopathological study, biochemical testing, Fourier Transform Infrared (FTIR) Microspectroscopy were performed. Histopathological study showed that anthocyanins complex reduced accumulation of inflammatory cells in around of bile duct compared with without treatment. Furthermore, anthocyanins complex-supplemented in *O. viverrini*-infected hamsters tended to decrease a nitrative stress marker (level of nitrate/nitrite) in dose-dependently. In contrast, in treated group, serum level of ferric reducing antioxidant power increased in dose-dependently. FTIR spectra obtained from treated groups, despite low or high doses, identified differences from untreated group within the range of the wave numbers of 1600 – 1700 cm⁻¹. The major difference of spectra was observed on the protein content especially on amide I. Unexpectedly, anthocyanins complex increased an activity of alanine aminotransferase (ALT), suggesting high concentration of treatment may cause liver injury. In conclusion, anthocyanins complex reduces inflammation and balancing of nitrative stress in *O. viverrini*-infected hamsters; however the high dose treatment may induce liver injury. Therefore, an optimal dose of this complex in this model is needed to be further study.

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Curcumin prevents liver bile canaliculi alterations in the hamsters infected with *Opisthorchis viverrini*

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ABSTRACT

Opisthorchis viverrini infection induces periductal fibrosis in the liver as a result involving inflammation. It is one of the risk factors which can develop into lethal malignancy, the cholangiocarcinoma (CCA). Khon Kaen province in Northeast Thailand has been reported the highest incidence of CCA in the world. Previous studies have shown the potent antioxidant and potent anti-inflammatory effects of curcumin. This study investigated the preventive effect of curcumin supplementation on the ultrastructural changes of the liver bile canaliculi after chronic infection in the hamster (day 90 post-infection) by transmission electron microscope. It revealed that the microvilli within the bile canalicular lumen of the *O. viverrini* infected hamster were scarce when compared to the control. In contrast, the hamster infected with *O. viverrini* and fed with 1% curcumin diet at the same period showed the normal morphology and density of the bile canalicular microvilli as observed in the control group. Curcumin-supplemented diet has preventive effect on the alteration of microvilli in bile canaliculi of the hamster after becoming chronic infection with the liver fluke *O. viverrini*.

Development of *Opisthorchis viverrini* associated cholangiocarcinoma hamster model
for anti-cancer effect analysis

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ABSTRACT

Opisthorchis viverrini (*O. viverrini*, *Ov*) is a well-known causative agent of cholangiocarcinoma (CCA) in human. CCA is fatal and considerably chemotherapy-resistance. To understand the pathogenesis of CCA in human, a rodent model was developed. However, due to the chronic infection causing chronic inflammation nature of the *Ov*-associated cholangiocarcinogenesis, the development of CCA in rodent is time consuming and not suitable for anti-cancer research. Moreover, the xenograft-transplantation model of human CCA in immunodeficiency mouse is expensive. Therefore, the experimental model for *Ov*-associated CCA treatment screening was of interest. CCA cell line, Ham-1, derived from *Ov* and *N*-nitrosodimethylamine infested Syrian golden hamster was developed in the current study. Ham-1 was grown in DMEM supplemented with 10% fetal bovine serum for more than 30 subcultures. These cells exhibit 44n diploid chromosomes with some aneuploidy. Tumorigenicity properties of Ham-1 were demonstrated in allograft transplantation. Transplanted tissues were highly proliferative and exhibited glandular-like structure with retained bile duct markers, cytokeratin-19. The usefulness of this comparative model was demonstrated by berberine treatment. Growth inhibitory effects of berberine mainly by an induction of G1 cell cycle arrest was observed in vitro and in vivo. In summary, the comparative hamster model for *Ov*-associated CCA treatment was developed in this work. It is an alternative resource for an anti-tumor agent analysis.

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Ultrastructure of developmental tegument of *Opisthorchis viverrini*

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ABSTRACT

Methods – The newly exysted juvenile (NEJs), 2 weeks old and adult *O. viverrini* were fixed in 3% glutaraldehyde and 2% osmium tetroxide in 3% potassium ferricyanide, dehydrated in a graded ethanol series and embedding in EPON 812 resin. Ultrathin sections were cut and staining grids with uranyl acetate and lead citrate. Observe under a transmission electron microscope. **Results and discussion** – The tegument of *O. viverrini* have a syncytial tegument with anucleate surface layer which is relatively far from nucleated layer. The tegumental membrane surface is covered by a thin layer alike glycocalyx layer in *Fasciola gigantica*. The folding surface exhibits ridge and pits and containing dense, lucent granules and dumbbell shape granules, mitochondria and spines. The length of the pits or ridges and density of spines differ are different in different location of the worm; however, NEJs show shorter pits or ridges than adult stage. The lucent and dumbbell shape granules are densely observed in NEJs. Numerous dense granules are generally detected in adult especially at the ventral surface. These various granules of different tegument cells at different stages may reflect diverse functions in absorption and host immune evasion. Mitochondria are usually found closed to superficial region of the surface tegument. The numbers of tegument sensory receptors were demonstrated at the region closed to oral sucker at all stages. Further study should be targeted in tegument proteins for develop any novel chemotherapeutic agents against liver fluke infection.

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Electron microscope observation of the TBD treated *Angiostrongylus cantonensis* larvae

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ABSTRACT

Balb/c mice infected by 20 third stage larvae of *A. cantonensis* were divided into three groups (drug treating group, control group, in vitro incubation group): using anthelmintic Tribendimidine treat the mice 100 mg/kg/d for 7 days at 14th day past inoculation (dpi) while treat control group, incubation group treat with DI water. Mice of each group were sacrificed at 15dpi, 17dpi, 21dpi. Mean number of worm recovered from drug treating group are 12.1, 7.6, 3.1 per mouse; mean number of worm recovered from control group and incubation group are 13.6, 12.3, 11.8 per mouse. Worms recovered from drug treating group and control group were immobilized for scanning electron microscope and transmission electron microscope observations. Worms recovered from in vitro incubation group were incubated in culture medium containing Tribendimidine 10 µg/L for 24h before scanning electron microscope and transmission electron microscope observations. Pictures of scanning electron microscope of worms recovered from drug treating group showed cuticle module disorder, wrinkle disappear, head mastoid damage, the severity increased with drug treatment time. Pictures of transmission electron microscope of worms recovered from drug treating group showed unsymmetrical cuticle layers, nebulous cell junction and myotube of muscle cells under cuticle, small-scale of vacuole like changes under cuticle layers. Pictures of scanning electron microscope of worms of the in vitro drug incubation group showed disappear of normal cuticle formation, complete disruption of surface pattern. Pictures of transmission electron microscope of worms of the in vitro drug incubation group showed thinner cuticle layers, incompact cell junction, disappearance of myotube of muscle cells under cuticle, large-scale of vacuole like changes and apoptotic bodies, disintegration of muscle cell. These observations showed cuticle of *A. cantonensis* larva changes between 2 stages; Changing cuticle successfully leads the larvae growth continue in different hosts, worm was vulnerable when exposed to anthelmintic stress, cuticle damage leads to worm decrease which implied that cuticle of parasite nematode is vital to its growth in host.

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A novel procedure for precise quantification of *Schistosoma japonicum* eggs in bovine feces

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ABSTRACT

Schistosomiasis japonica is a zoonosis with a number of mammalian species acting as reservoir hosts, including water buffaloes which can contribute up to 75% to human transmission in the People's Republic of China. Determining prevalence and intensity of *Schistosoma japonicum* in mammalian hosts is important for calculating transmission rates and determining environmental contamination. A new procedure, the formalin–ethyl acetate sedimentation-digestion (FEA–SD) technique, for increased visualization of *S. japonicum* eggs in bovine feces, is described that is an effective technique for identifying and quantifying *S. japonicum* eggs in fecal samples from naturally infected Chinese water buffaloes and from carabao (water buffalo) in the Philippines. The procedure involves filtration, sedimentation, potassium hydroxide digestion and centrifugation steps prior to microscopy. Bulk debris, including the dense cellulosic material present in bovine feces, often obscures schistosome eggs with the result that prevalence and infection intensity based on direct visualization cannot be made accurately. This technique removes nearly 70% of debris from the fecal samples and renders the remaining debris translucent. It allows improved microscopic visualization of *S. japonicum* eggs and provides an accurate quantitative method for the estimation of infection in bovines and other ruminant reservoir hosts. We show that the FEA–SD technique could be of considerable value if applied as a surveillance tool for animal reservoirs of *S. japonicum*, particularly in areas with low to high infection intensity, or where, following control efforts, there is suspected elimination of schistosomiasis japonica.

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Effect of citric acid for the acidification of artificial pepsin solution for metacercariae isolation from fishes

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ABSTRACT

Artificial digestive solution (ADS) based on pepsin is essential for collecting metacercariae from fishes. To promote enzymatic reactivity of pepsin, pH has to be adjusted to approximately pH 1 to 2.0. Although hydrochloride (HCl) is usually used for acidification of pepsin solution, a safe alternative has not been considered. The aim of this work was to address on the usefulness of citric acid as an alternative reagent of HCl and the effect of citric acid on the degree of damage of metacercariae after in vitro digestion. The pH after the addition of 1% to 9% citric acid (m/v) was compared to it of 1% HCl (v/v) as a control in preparing ADS. The digestive degree on fish muscles was evaluated by measuring the released protein concentration using spectrophotometer. The survival rate of metacercariae was observed at different concentrations of citric acid compared to 1% HCl in pepsin solution. The present study shows that the addition of citric acid reduced pH in pepsin solution, and among different concentrations of citric acid, the addition of more than 5% is highly effective to digest fish muscle during 3 h of in vitro digestion and less lethality of metacercariae compared to it of HCl in pepsin solution. Accordingly, the present study strongly suggests that the addition of 5% citric acid for the acidification of pepsin solution is a good choice for humans and parasites during further infection study because citric acid is stable solid form on room temperature and it is very safe substance.

Modified formalin-ether concentration technique for diagnosis of human strongyloidiasis

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ABSTRACT

We compare the efficacies of the modified formalin-ether concentration technique (MFECT), formalin-ether concentration technique (FECT) and agar plate culture (APC) for the detection of *Strongyliodes stercoralis* larvae in 600 human fecal specimens from endemic area, southern Thailand. The MFECT using wire mesh instead of gauze and a short time of formalin exposure to *S. stercoralis* larvae in stool samples maximizes its ability to recover and detect the larval number. The efficacy of MFECT was not significantly different from APC ($P=0.832$) but significantly different from FECT ($P<0.001$) in strongyloidiasis diagnosis.

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Comparative histopathology of male and female Syrian hamster opisthorchiasis viverrini
and cholangiocarcinoma development

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ABSTRACT

Worldwide, the highest incidence of cholangiocarcinoma (CCA) is found in northeast Thailand, where there is a high prevalence of *Opisthorchis viverrini* infection. General clinical data reveal that the majority of CCA patients are men. However, many other types of cancer are more predominantly found in women than in men. Sex hormones are known to influence the morphology and function of organs in the body. An imbalance of such hormones can affect any organ, especially hormone-sensitive organs such as the liver. In this study, we investigated the differences between males and females in the development of opisthorchiasis and CCA, using Syrian hamsters as a model. The study was performed by observing the pathological changes from *O. viverrini* infection and cholangiocarcinoma development associated with *O. viverrini* infection and *N*-nitrosodimethylamine administration in male and female Syrian hamsters, by means of hematoxylin and eosin staining, sirius red staining, liver function tests, and analysis of eggs per gram of feces. The results showed that, compared with males, infected female Syrian hamsters had more inflammatory cell aggregation surrounding the hepatic bile ducts. Similarly, the present study found that female Syrian hamsters developed severe opisthorchiasis and CCA much earlier than males, and with greater severity. This suggests that the more frequent occurrence of opisthorchiasis and CCA in males may depend on behavioral differences, and that the greater severity of the disease observed in females may result from the impairment of sex hormone receptors in the liver, especially for estrogen which plays a critical role in female health.

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Simplified naked-eye detection of fish-borne trematode metacercariae in cyprinoid fish

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ABSTRACT

Human liver and intestinal flukes are highly prevalent in Southeast Asia, and constitute a major health problem in this region. Many of the local people like eating raw fish, but are unaware of whether the fish are contaminated with the parasites. Therefore, the present study presents a simplified technique for detection of fish-borne trematodes. Fish were purchased at the local market which captured from a freshwater reservoir in an endemic area of Khon Kaen province, Thailand. Cyprinoid fish scales were observed for the presence of metacercariae by the naked eye under the bright sunlight. Moreover, fish scales were stained with red dye for comparison with unstained scales. The scales with or without staining in which suspected with metacercariae contaminated by naked eye observation were confirmed by examination under a compound microscope. The present study suggests that detection of fish-borne trematodes by the naked eye is a very useful simple method that can be done in any local, without need for elaborate equipment and at minimal cost. Using this technique, people who have dietary behavior include the consumption of raw fish can select uncontaminated fish to eat.

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Susceptibility of laboratory rodents to *Trichinella papuae*

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ABSTRACT

Trichinella is a small nematode which can infect a wide range of animal hosts. However, an ability to establish the infection varies upon host species. This study examined susceptibility of 4 laboratory rodents, mice, rats, hamsters and gerbils to *T. papuae*, an emerging non-encapsulated species. *T. spiralis* and *T. pseudospiralis* were used for comparison. Five of each rodent species were infected orally with 100 muscle larva of *Trichinella*. Adult worms in the small intestine were counted 6 and 10 days post inoculation. Muscle larvae were also recovered 45 days post inoculation from each animal carcass by pepsin-HCl digestion and counted, and reproductive capacity index (RCI) determined. It was found that 33.2-69.6 % of adult *Trichinella* were obtained from the small intestine of animals 6 days post inoculation. At 10 days, however, intestinal worm burden was reduced over 96% in rats infected with all 3 *Trichinella* species while gerbils showed least degree of reduction, 4.8-18.1%. In accordance with intestinal worm burden, RCI was highest in gerbils, 241.5 ± 41.0 for *T. papuae*, 432.6 ± 48 for *T. pseudospiralis*, and 528.6 ± 20.6 for *T. spiralis*. Hamster was ranked second in susceptibility in terms of parasite RCI while rats yield lowest parasite RCI. It was concluded that mice and rats were least susceptible while gerbil and hamster were more susceptible to *Trichinella*. Gerbil may serve as animal model for non-encapsulated clade of *Trichinella*.

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The medically important echinostomes infection in free-grazing ducks in Thailand

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ABSTRACT

Echinostomes are the foodborne and zoonotic intestinal trematode and causative agents of human echinostomiasis, which widespread worldwide, especially in Southeast Asia. Not only human but also domestic animals including fish-eating bird and poultry can be infected by eating uncooked snails, fish or tadpole containing the metacercariae.. Free-grazing duck is one of the important reservoir hosts of these parasites. In Thailand, free-grazing duck is widespread, especially the wetlands used for double-crop rice production in central, north and northeast regions, where the free-grazing duck can feed year round in rice paddies. Then this study aim to investigate the infection of three medically important echinostomes, namely *Echinostoma revolutum*, *Hypoderaeum conoideum* and *Echinoparyphium recurvatum* in free-grazing ducks in Thailand. The 90 free-grazing ducks from 12 farms from 11 Provinces of central, north and northeast regions of Thailand were examined. The adult echinostomes were searched from the intestine of ducks and identify by morphology under light microscope. The results showed that 38 – 100% of free-grazing ducks were infected with these echinostomes. Percent of adult worm recovery was varied in each species at a particular surveyed region, namely percent of *E. revolutum* recovery was highest (74.50%) in central region, whereas *H. conoideum* was relatively high in north and northeast regions at 87.85% and 82.68%, respectively. High prevalence of infection of medically important echinostomes in free-grazing duck is a critical factor in risk of human infection. Then free-grazing duck is play important role to maintain echinostomes life cycle and spread these parasites through their feeding areas.

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Distribution and abundance of *Opisthorchis viverrini* metacercaria in cyprinid fish in northeastern Thailand

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ABSTRACT

The distribution and abundance of *O. viverrini* metacercariae (OvMC) was investigated in cyprinid fish obtained from 20 provinces of northeast Thailand during April 2011 to February 2012. A cross-sectional survey of natural freshwater fishes were randomly caught from 26 rivers, 10 dams and 38 water basins localities in 20 provinces. Five kilogram (kg) of fish was caught twice from each sampling regions. Fish was weighted, counted, identified and digested by artificial pepsin. OvMC was examined by sedimentation method and identified under stereomicroscope. The result revealed that a number of fish per one kg had an approximately 50±5 fishes and consisted of 6 species. Of these, OvMC was identified mainly from *Dangila lineate* and *Cyclocheilichthys repasson*. The positive water reservoir for OvMC was 70% for dams, 23.68% for basins, and 7.69% for river localities in 13 provinces. These positive provinces included Yasothon, Ubon ratchathani, Nakhonratchasima, Khon Kaen, Sakon Nakhon, Srisaket, Surin, Buriram, Amnatcharoen, Mukdahan, Chaiyaphum, Nakornpranom, and Nong Khai. Mean metacercarial intensity was ranging from 4.44-328.13 cysts per kg of fish (or 0.08-6.56 cysts per fish). The highest intensity of OvMC of fish was found in the order of Srisaket (328.13 per kg, 6.56 per fish), Chaiyaphum (187.65 per kg, 3.75 per fish) and Mukdahan (134.39 per kg, 2.69 per fish), suggesting these provinces are a high risk area for primary prevention. In conclusion, OvMC are endemic in the natural fishes of water reservoir with the vary range intensity in the water reservoir of northeastern Thailand.

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Ultrastructures of *Paragonimus heterotremus* testis

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ABSTRACT

Lung fluke, *Paragonimus heterotremus*, is a flatworm causing pulmonary paragonimiasis in cat, dog and human which has been prevalent worldwide. This study was attempted to examine the ultrastructure of the testis of adult *Paragonimus heterotremus* with special attention to the spermatogenesis and spermiogenesis for further investigation of the management to restrict this parasite infection. The testis of *Paragonimus heterotremus* was examined in scanning and transmission electron microscopy. Two sets of encapsulated testes of *Paragonimus heterotremus* were ultrastructurally demonstrated, which represent full sequential differentiation-stages of the spermatogenesis and spermiogenesis from the capsular basal lamina to the luminal surface. The sequence comprises spermatogonia, spermatocyte with obvious nuclear synaptonemal complexes, spermatid, and eventual spermatozoa. Moreover, full steps of spermatid differentiation were shown which consist of 1) early stage, 2) rosette forming stage, 3) differentiation stage representing the intercentriolar body, basal body, striated rootlets and lamellar nucleus, and 4) growing spermatid flagellae. Detailed ultrastructures of spermatozoa were also shown in this study. In conclusion, this study showed the ultrastructure of *Paragonimus heterotremus* testis at full sequential stages of its growth and development. As the next step of study, it is necessary to identify inhibitory molecules of the spermatogenesis or spermiogenesis of this parasite and to examine in vivo and in vitro effects of the molecules for prevention of epidemiologic distribution and infection of this parasite.

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Duration of fish storage in low temperature before raw-fish-processing dish prevents *Opisthorchis viverrini* metacercariae infection in hamsters

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ABSTRACT

Opisthorchis viverrini metacercariae (OVMC) is a high distribution and abundance in natural cyprinid fish in northeast Thailand. People prefer to eat raw-fish dishes, Pla-Som, leading to a high prevalence of infection. We aim to investigate a viable of OVMC in raw-fish-processing dish and to reduce OVMC contamination in raw-fish dish infection to hamster. Natural cyprinid fish was used to prepare raw-fish-processing dish, Pla-Som, for five days. OVMC in ferment fishes was digested by artificial pepsin, examined by sedimentation method, and identified under stereomicroscope. The result revealed that viable OVMC was observed for 1-3 day(s) of fermented Pla-Som and they were destructed thereafter. Infection rate of fermented Pla-Som in hamsters was 36.5%, 19% and 1% for day(s) 1, 2 and 3 respectively. In order to reduce OVMC from Pla-Som infection to animal, fishes were stored at refrigerator (4 °C) for 1-3 day(s) and then a raw-fish-processing was fermented for 3 days. Interestingly, fish storage at refrigerator for 2 and 3 days before Pla-Som processing, all metacercaria was destructed and prevented to infect in hamster. In conclusion, eating Pla-Som during 1 to 3 day(s) of fermentation is able to infect by OVMC, and fish storage at refrigerator for 2 and 3 days before raw-fish-processing can prevent OVMC infection.

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Complete mitochondrial genome of *Haplorchis taichui* and comparative analysis for trematodesDM Lee¹, SJ Choe¹, HS Park¹, Hk Jeon¹, JY Chai² and KS Eom^{1*}¹Department of Parasitology, Medical Research Institute, Chungbuk National University School of Medicine;²Department of Parasitology, Seoul National University School of Medicine¹Department of Parasitology, Medical Research Institute, Chungbuk National University School of Medicine;²Department of Parasitology, Seoul National University School of Medicine

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ABSTRACT

Mitochondrial genomes have been extensively studied for phylogenetic purposes and to investigate intra- and interspecific genetic variation. Because they are high in number of copy per cell, molecular analyses tend to show higher mutation rates than nuclear genes. In recent years numerous groups have undertaken sequencing of platyhelminth mitochondrial genomes. *Haplorchis taichui*, belonging to the family Heterophyidae is one of the trematodes infecting humans and animals of Mekong River Basin and Southern Asian regions. In the present study, we sequenced and determined the gene organization of complete mitochondrial genome of *H. taichui*. The structure was compared to other trematodes for which complete mitochondrial sequence data are available. The mitochondrial genome was 15,169 bp long, contained 12 protein-coding genes, two ribosomal RNAs (rRNAs, a small and a large subunit), and 22 transfer RNAs (tRNAs) like other trematodes. It did not encode the *atp8* gene. All genes were transcribed from the same strand. The ATG initiation codon was used for 9 protein-coding genes, and the GTG initiation codon was used for the remaining 3 genes (*nad1*, *nad4* and *nad5*). The size of the protein-coding genes of other trematodes varied slightly. The genes in trematodes for two mitochondrial rRNA subunits *rrnL* and *rrnS* were separated by *trnC*. The putative *H. taichui* *rrnL* and *rrnS* were 979 and 747 bp long, respectively. The mitochondrial genome of *H. taichui* had single non-coding region between *trnE* and *trnG*. This information will provide a resource for the comparative mitochondrial genome analyses of trematodes, and may yield genetic markers for molecular epidemiological investigations into intestinal flukes.

CLOSING REMARKS

by

Professor Wanchai Maleewong, Ph.D.

Organizer of the first meeting on the Asian Parasites by Asian Parasitologists : Helminthiasis in Greater Mekong Subregion; Department of Parasitology; and Research and Diagnostic Center for Emerging Infectious Diseases, Khon Kaen University

Distinguished experts and Participants

Good evening.

We have come to the end of our plenary sessions for the Asian Parasites by Asian Parasitologists : Helminthiasis in Greater Mekong Subregion. It is great honor for me to make these closing remarks at this important meeting.

I would also wish to extend my special thanks to all sponsors, organizing committees and all participants.

I hope that everyone has had a fruitful exchange of views and ideas for the past one day. I am sure that, this APAP meeting will help us to promote parasitological research cooperation in our countries in the future. I also hope sharing of the best knowledge and promotion of new technologies will be increased.

Finally, I would like to conclude by thanking again all of related entities and participants for their efforts in ensuring that this Meeting should prove to be such a success. I strongly hope that APAP meeting will be realized in various countries in Asia in consequence of the cooperation on parasitic disease controls.

Bon voyage and safe return back to your home and family.

Wanchai Maleewong

LIST OF CO-SUPPORTERS FOR

Asian Parasites by Asian Parasitologists (APAP):
Helminthiases in Greater Mekong Subregion

12 May 2013



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