Asian-African Research Forum on Emerging and Reemerging Infections 2012

Date January 11 Wed. to 12 Thu., 2012
Venue Kobe International Conference Center

Ministry of Education, Culture, Sports, Science and Technology (MEXT)
Japan Initiative for Global Research Network on Infectious Diseases (J-GRID)

PROGRAM

Day 1: January 11, 2012

08:00–09:00 Registration

Opening Remarks (09:00 – 09:20)

09:00–09:05 Ministry of Education, Culture, Sports, Science & Technology (MEXT)
09:05–09:10 Dr. Yoshiyuki Nagai, Program Director, J-GRID
09:10–09:15 Dr. Amin Soebandrio, Senior Adviser to the Minister, Ministry of Research & Technology (RISTEK), Republic of Indonesia
09:15–09:20 Dr. Hideki Fukuda, President, Kobe University

Welcome Greetings from Indonesia – Japan CRC-ERID (09:20 – 10:00)

09:20–09:30 Introduction (Dr. Hak Hotta, Kobe University)
09:30–09:45 Greetings from Indonesian Director, CRC-ERID (Dr. Nasronudin, Airlangga University)
09:45–10:00 Greetings from Japanese Director, CRC-ERID (Dr. Yoshitake Hayashi, Kobe University)

Introduction of Associate Members (10:00–10:10)

10:00–10:05 Niigata University (Myanmar)
10:05–10:10 Nagasaki University (Kenya)

Coffee Break (10:10 – 10:30)

Session 1 : Influenza (10:30 – 11:30)

(Modulators : Dr. Kyoko Shinya & Dr. Hualan Chen)

10:30 – 10:45 **2009 Pandemic influenza virus: what special for its HA and NA? (O-1)**
George F Gao

10:45 – 11:00 **Summary report of pandemic 2009 influenza A (H1N1) patients in Dr. Soetomo Hospital Surabaya Indonesia (O-2)**
Laksmi Wulandari, Justinus Frans Palilingan, Landia Setiawati, Retno Asih Setyoningrum, Lindawati Alim Sarjono, Endang Retnowati, Phillia Setiawan, Kohar Hari Santoso, Bambang Wahjuprajitno, Resti Yudhawati, Emmanuel Djoko Poetranto, Aldise Mareta Nastri, Luh Ade Wilan Krisna, Teridah Ermala Ginting, Masaoki Yamaoka, Akiko Makino, Kyoko Shinya, and Yoshihiro Kawaoka

11:00 – 11:15 **Characterization of influenza A viruses isolated from wild waterfowl in Zambia (O-3)**
Edgar Simulundu, Akihiro Ishii, Manabu Igarashi, Aaron S. Mweene, Yuka Suzuki, Hirohito Ogawa, Emiko Nakagawa, Bernard M. Hang'ombe, Boniface Namangala, Ladslav Moonga, Rashid Manzoor, Kimihito Ito, Ichiro Nakamura, Hirofumi Sawa, Chihiro Sugimoto, Hiroshi Kida, Chuma Simukonda, Wilbroad Chansa, Jack Chulu, and Ayato Takada

11:15 – 11:30 **A duck enteritis virus-vectored bivalent live vaccine provides fast and complete protection against H5N1 avian influenza virus in ducks (O-4)**
Jinxiong Liu, Pucheng Chen, Yongping Jiang, Li Wu, Xianying Zeng,

Guobin Tian, Jinying Ge, Yoshihiro Kawaoka, Zhigao Bu, and Hualan Chen

Session 2 : Viral Hepatitis (11:30 – 12:00)

(Modulators : Dr.Takako Utsumi & Dr. Maria Inge Lusida)

11:30 – 11:45 **High prevalence of occult hepatitis B infection among school children in a "mixed- subtype" area in Indonesia (O-5)**
Maria I. Lusida, Takako Utsumi, Juniastuti, Yoshihiko Yano, Mochamad Amin, Nursidah, Priyo B. Purwono, Soetjipto, Yoshitake Hayashi, Hak Hotta

11:45 – 12:00 **Nosocomial transmission as a factor in high prevalence of hepatitis B and C among hemodialysis patients in Yogyakarta, Indonesia (O-6)**
Hanggoro Tri Rinonce, Yoshihiko Yano, Takako Utsumi, Didik Setyo Heriyanto, Nungki Anggorowati, Heru Prasanto, Maria Inge Lusida, Soetjipto, Hak Hotta, Yoshitake Hayashi

Lunch (12:00 – 13:00)

Special Lecture ; Natural Calamity and Infectious Diseases 1 (13:00 – 13:40)

(Modulator : Dr. Yoshifumi Takeda)

13:00 – 13:40 **In pursuit of the intercontinental transmission of cholera: the origin and spread of cholera in Haiti (S-1)**
G. Balakrish Nair, D. Lantagne, C. F. Lanata and A. Cravioto

Session 3: Gastrointestinal Infections (13:40 – 14:40)

(Modulators : Dr. Shin-ichi Miyoshi & Dr. G. Balakrish Nair)

13:40 – 13:55 **Detection of the traits of Haitian variant strains of *Vibrio cholerae* in Kolkata, India since 2006 (O-7)**
A. Naha, G. P. Phazani, M. Ganguly, S. Ghosh, T. Ramamurthy, R.K. Nandy, G.B. Nair, Y. Takeda, A.K. Mukhopadhyay

13:55 – 14:10 **Etiology of enteric pathogens among diarrheal children: Comparative analysis of hospitalized cases and outpatients in Kolkata, India (O-8)**
T. Ramamurthy, G. B. Nair, M. K. Bhattacharya, U. Mitra, T. Krishnan, S. Ganguly, D. R. Saha, K. Rajendran, B. Manna, M. Ghosh, M. Chatterjee and Y. Takeda

14:10 – 14:25 **Molecular characterization of the major gastroenteritis pathogens in hospitalized children in Thai Binh, Viet Nam (O-9)**
Nguyen Van Trang, Toyoko Nakagomi, Vu Thi Bich Hau, Le Thi Kim Anh, Nguyen Anh Tuan, Nguyen Thi Minh Chinh, Doan Hai Yen, Tetsu Yamashiro, Dang Duc Anh and Osamu Nakagomi

14:25 – 14:40 **Prevalence of diarrheagenic *Escherichia coli* among children in Surabaya, Indonesia (O-10)**
Yoshio Iijima, Dadik Rahardjo, Garry Cores De Vries, Shouhiro Kinoshita, Ro Osawa, and Toshiro Shirakawa

Coffee Break (14:40 – 15:00)

Session 4 : Parasitic, Bacterial & Viral Systemic Infections (15:00 – 16:00)

(Modulators : Dr. Nobuo Ohta & Dr. Samuel Dadzie)

15:00 – 15:15 **Identification of Coxsackievirus A20 as the recombinant counterpart**

of type 1 cVDPV in the Philippines (O-11)
Lea Necitas Apostol, Hiroyuki Shimizu, Akira Suzuki , Asif Naeem, Socorro Lupisan, Remigio Olveda, Hitoshi Oshitani

15:15 – 15:30 ***Streptococcus suis* infection in humans in Thailand (O-12)**
Dan Takeuchi, Anusak Kerdsin, Tatsuya Nakayama, Yukihiro Akeda, Shota Nakamura, Tetsuya Iida, Shigeyuki Hamada, Surang Dejsirilert, Kazunori Oishi

15:30 – 15:45 **The use of loop-mediated isothermal amplification (LAMP) in detection of *Trypanosoma brucei rhodesiense* in clinical samples from Luangwa and Zambezi Valleys, Zambia (O-13)**
Boniface Namangala, Noboru Inoue, Martin Simuunza, Dusit Laohasinnarog, Kyoko Hayashida, Aaron Mweene, Ladslav Moonga, Amos Chota, Kiichi Kajino, Chihiro Sugimoto

15:45 – 16:00 **Bio-efficacy, user perception and acceptability of some selected pyrethoid-based mosquito coils in controlling *An. gambiae s.l.,* a malaria vector in some parts of the Greater Accra region of Ghana (O-14)**
Samuel Dadzie, Samuel Adu-Acheampong, Rosina Kyerematen, Jacob Williams, Takashi Suzuki , Nobuo Ohta, Maxwell Appawu, Daniel Boakye

Poster Viewing & Discussion (16:00 – 17:00)

(J-GRID Committee Meeting) (17:00 – 18:30)

Reception (18:30 – 20:30)

Day 2: January 12, 2012

Session 5 : Dengue & Arthropod-Borne Infections (09:00 – 10:00)
(Modulators : Dr. Kouichi Morita & Dr. Le Quyn Mai)

09:00 – 09:15 **Characterization of dengue 1 epidemic strains in Hanoi, Vietnam in 2009 (O-15)**
Futoshi Hasebe , Takashi Tsunoda, Takeshi Nabeshima , Kenta Okamoto , Toru Kubo , Posadas-Herrera Guillermo , Nguyen Thi Thu Thuy , Dang Thi Dinh , Pham Hoai Linh Ly , Nguyen Bao Ngoc , Nguyen Hoang Le , Ataru Tsuzuki, Nguyen Thi Yen , Tran Vu Phong , Le Thi Quynh Mai and Kouichi Morita

09:15 – 09:30 **Complement levels correlated with disease severity in dengue patients in Indonesia (O-16)**
Atsushi Yamanaka, Eryk Hendrianto, Kris C. Mulyatno, Helen Susilowati, Amor P. Ginting, Dian D. Sary, Soegeng Soegijanto and Eiji Konishi

09:30 – 09:45 **Identification of a novel inhibitor against dengue virus NS2B/NS3 protease by a structure-based study (O-17)**

Sabar Pambudi, Norihito Kawashita, Promsin Masrinoul, Kriengsak Limkittikul, Teruo Yasunaga, Tatsuya Takagi, Kazuyoshi Ikuta, and Takeshi Kurosu

09:45 – 10:00 **Surveillance of infection with Japanese encephalitis virus and hepatitis E virus among swine in northern Luzon, Philippines (O-18)**
Fedelino F. Malbas Jr., Mariko Saito, Yusuke Sayama, Hazel Galang, Socorro P. Lupisan, Remigio M. Olveda, Hitoshi Oshitani

Session 6 : Tuberculosis & Respiratory Infections (10:00 – 11:00)
(Modulators : Dr. Hitoshi Oshitani & Dr. Remigio Oldeva)

10:00 – 10:15 **Molecular genotyping of *Mycobacterium tuberculosis* isolated from Hanoi City in Viet Nam (O-19)**
Shinji Maeda, Nguyen Van Hung, Luu Thi Lien, Nguyen Thi Le Hang, Nobuyuki Kobayashi, Shinsaku Sakurada, Naoto Keicho

10:15 – 10:30 **Molecular characterization of drug resistant *Mycobacterium tuberculosis* from Asian countries (O-20)**
Yasuhiko Suzuki , Zeaur Rahim, Aki Tamaru, Basu Dev Pandey, Bhagwan Maharjan, Khin Saw Aye, Ajay Poudel, Yukari Fukushima, Haruka Suzuki and Chie Nakajima

10:30 – 10:45 **Etiology and clinical presentation of childhood pneumonia in the Eastern Visayas Regional Medical Center, Tacloban City, Western Visayas, Central Philippines (O-21)**
S. Lupisan, A. Suzuki, L. Sombrero, H. Galang, M. Mondoy, R. Aniceto, F.de la Pena, R. Angulo,R. Olveda[1], H. Oshitani

10:45 – 11:00 **Respiratory Syncytial virus is the major viral pathogen and its co-infection with other respiratory viruses increases the risk of pediatric pneumonia hospitalization: A three-year population-based study in central Vietnam (O-22)**
Lay Myint Yoshida, Motoi Suzuki, Hiroshi Yoshino, Hien Anh Nguyen, Thiem Vu Dinh, Le Huu Tho, Le Mai Quynh, Hiroyuki Moriuchi, Duc Anh Dang and Koya Ariyoshi

Special Lectures ; Natural Calamity and Infectious Diseases 2 (11:00 – 12:20)
(Modulator : Dr. Hak Hotta)

11:00 – 11:20 **Prevention and control of communicable diseases during and after big flood in Thailand, 2011 (S-2)**
Pathom Sawanpanyalert and Jotika Boonlong

11:20 – 11:40 **Infectious diseases after Tsunami. Aceh (Indonesia) experience (S-3)**
Nasronudin, Maria I Lusida, Juniastuti

11:40 – 12:00 **Infectious disease risk and public health recovery after the Great East Japan Earthquake (S-4)**
Hitoshi Oshitani, Taro Kamigaki, Michiko Okamoto, Kentaro Tohma, Nao Nukiwa and Akira Suzuki

12:00 – 12:20 **Communicable diseases after the Great East Japan Earthquake (S-5)**
Kentaro Iwata

Lunch (12:20 – 13:15)

Poster Viewing & Discussion (13:15 – 14:15) (Coffee will be served.)

Session 7 : HIV & AIDS (14:15 – 15:15)

(Modulators : Dr. Aikichi Iwamoto & Dr. Pathom Sawanpanyalert)

14:15 – 14:30 **Evaluation of the current first-line ART for AIDS patients in Ghana (O-23)**
Yaw O. Amoah, Afia A. Ntim, Jacob Barnor, James A.M. Brandful, William K. Ampofo, Evelyn Bonney, Samson B. Ofori, Koichi Ishikawa, Alexander K. Nyarko, Shoji Yamaoka, Eiji Ido

14:30 – 14:45 **The establishment of a hospital-based cohort of HIV-infected individuals in Vietnam: The NHTD-ACC Collaborative HIV Cohort Study (NACH cohort) (O-24)**
Junko Tanuma, Fumihide Kanaya, Nguyen Thi Bich Ha, Cao Thi Thanh Thuy, Daisuke Mizushima, Koji Watanabe, Hiroyuki Gatanaga, Nguyen Van Kinh, Shinichi Oka

14:45 – 15:00 **Anti-HIV-1 humoral immune responses in HIV-1-infected Thai patients (O-25)**
Masanori Kameoka, Sompong Sapsutthipas, Siriwat Akapirat, Naho Tsuchiya, Panita Pathipavanich, Koya Ariyoshi, Pathom Sawanpanyalert, Kazuyoshi Ikuta, Pornsawan Leaungwutiwong, Pongrama Ramasoota, Panasda Isarangkura-na-ayuthaya

15:00 – 15:15 **Designing new split proteins by using GFP as "beta-tweezers" (O-26)**
Hirohito Ishikawa, Fanxia Meng, Aikichi Iwamoto and Zene Matsuda

Session 8 : Diagnostic Technology Innovation (15:15 – 16:00)

(Modulators : Dr. Ayato Takada & Dr. Boniface Namangala)

15:15 – 15:30 **Rapid detections, quick countermeasures and investigations for *Vibrio cholerae* O1 in Thailand (O-27)**
Kazuhisa Okada

15:30 – 15:45 **Metagenomic diagnosis of infectious diseases (RAPID): 2011 update (O-28)**
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15:45 – 16:00 **Rapid and comprehensive identification of virus strains by using LC tandem-MS method (O-29)**
Kouichi Morita, Kenta Okamoto, Shingo Inoue, Takeshi Nabeshima, Posadas H. Guillermo, Fuxun Yu, Nguyen Thanh Thuy, Bui Minh Trang, Vu Sinh Nam, Phan Thi Nga, Le Q. Mai, Nguyen Tran Hien, Filipinas F. Natividad, Futoshi Hasebe

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(Modulator : Dr. Yoshihiro Kawaoka)

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Development of RT-SmartAmp assay method for one-step detection of highly pathogenic H5N1 influenza A virus
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Le Minh Nhat, Lay Myint Yoshida, Hiroshi Yoshino, Motoi Suzuki, Hien Anh Nguyen, Thiem Vu Dinh, Le Huu Tho, Hiroyuki Moriuchi, Duc Anh Dang and Koya Ariyoshi

Sero-prevalence of influenza A virus among schoolchildren in Indonesia

Kiyoko Iwatsuki-Horimoto, Chairul A Nidom, Ema Qurnianingsih, Winariani Koesoemoprodjo, Reviany V.Nidom, Setyarina Indrasari, Kadek Rahmawati, Burhan Hidayat, Evi D.Woelansari, Shinya Yamada, Maki Kiso, Yoshihiro Kawaoka

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Swine influenza surveillance in the Philippines

Akira Suzuki, Fidel Malbas, Michiko Okamoto, Hidekazu Nishimura, Dai Yamamoto, Mariko Saito, Socorro Lupisan, Remigio Olveda, Hitoshi Oshitani

Expansion of swine influenza surveillance in Thailand

Yasuaki Hiromoto, Sujira Parchariyanon, Somchuan Ratanamungklanon, Naree Ketusing, Punnaporn Netrabukkana, Nobuhiro Takemae, Yuko Uchida, Takehiko Saito

16:40 – 16:50 **Discussion (Q&A)**

Closing Remarks (16:50 – 17:00)

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George F Gao

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ABSTRACTS

In pursuit of the intercontinental transmission of cholera: the origin and spread of cholera in Haiti

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The annual 2010 statistics for cholera published by World Health Organization has shown an increase of 43% in the number of cholera cases as compared to that in 2009, and an increase of 130% compared to that in 2000. This has led the World Health Assembly in May 2011 to recognize the re-emergence of cholera as a significant global public health problem. Cholera entered Haiti, a Caribbean island, in October 2010. Haiti had no recorded history of cholera for the past century. As of 31 December 2010, a total of 179,379 cases, including 3990 deaths, had been registered from all departments (provinces) including that of the capital Port au Prince. The source of the cholera outbreak has been controversial, with several hypotheses. In order to determine the source of the outbreak definitively, the Secretary-General of the United Nations formed an Independent Panel of four international experts, with a mandate to "investigate and seek to determine the source of the 2010 cholera outbreak in Haiti".

Concurrent epidemiological, water and sanitation, hydrological and molecular analysis investigations were carried out. The first cholera case in Haiti was confirmed at the Haiti National Public Health Laboratory on October 22, 2010. The first hospitalized cholera case in Mirebalais, in the upstream region of the Artibonite River, was on the evening of October 17th, 2010. The first hospitalized cholera cases on the coast, in the Artibonite River Delta in St. Marc and Deschapelle, were on October 20th, 2010. The outbreak was widely established in the coastal areas by October 22nd, 2010. After establishing that the cases began in the upper reaches of the Artibonite River, potential sources of contamination that could have initiated the outbreak were investigated. The sanitation conditions at the Mirebalais MINUSTAH (United Nations Stabilization Mission in Haiti) camp were not sufficient to prevent fecal contamination of the Meye Tributary System of the Artibonite River. Water in the Meye Tributary System reaches the Artibonite River junction in less than 8 hours, and flows downstream in another 1-2 days to a dam and canal system widely used for irrigation throughout the Artibonite River Delta. Analysis of published and unpublished molecular information indicate that: 1) the outbreak strains in Haiti are genetically identical, indicating a single source for the Haiti outbreak; and, 2) the bacteria is very similar, but not identical, to the South Asian strains of cholera currently circulating in Asia, confirming that the Haitian cholera bacteria did not originate from the native environs of Haiti. More recent whole genome sequences comparison of the Haitian and Nepal strains of *V. cholerae* O1 shows near identity.

The investigation by the Independent Panel indicate that the 2010 Haiti cholera outbreak was caused by *V. cholerae* O1 introduced into Haiti as a result contamination of the Meye Tributary System of the Artibonite River by human activity with a pathogenic strain of the current South Asian type *Vibrio cholerae.* The introduction of this cholera strain as a result of environmental contamination with feces from an asymptomatic carrier could not have been the source of such an outbreak without simultaneous water and sanitation and health care system deficiencies. These deficiencies, coupled with conducive environmental and epidemiological conditions, allowed the spread of the *Vibrio cholerae* organism in the environment, from which a large number of people became infected. A set of recommendations were submitted to the United Nations, to the Government of Haiti, and to the international community with the intention of preventing the future introduction and spread of cholera.

Prevention and control of communicable diseases during and after big flood in Thailand, 2011

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The big flood in Thailand in 2011 is probably the worst one in 5 decades. It started in August and may last until the end of 2011 or early 2012. The flood started in provinces in the lower northern part of Thailand as well as in some northeastern provinces. As of now (mid November 2011), a total of 64 provinces including Bangkok Metropolitan Administration have been or are being affected. It is estimated that about 5 million individuals in 1.9 households are affected; and the flood may have caused an economic loss of 1,000 billion Baht. Apart from disrupted health care and stressful conditions endured by those who suffer directly from the flood, communicable diseases and exposures to chemicals that might have leaked from industrial estates affected by the flood are another prime concern. Several interventions have been implemented during and after the flood to prevent and control major communicable diseases, e.g. acute diarrhea, food poisoning, epidemic keratoconjunctivitis, influenza and other respiratory illnesses, leptospirosis and other febrile illnesses. Those interventions include surveillance for safety of foods and water available at the shelters, surveillance for quality and safety(both chemical and microbiological) of potable water including tap water, active laboratory-confirmed surveillance of infectious and chemical illnesses at shelters, enhancement of hospital-based surveillance in hospitals that have been rehabilitated from the flood, organization of rapid mobile laboratory or medical unit to provide services to areas where usual health care services have not yet been fully functioning or recovered from flood, and provision of products that can be used as personal hygiene product or public health products, e.g. mosquito repellant, mosquito-larva-killing bacteria, and herb-based products for water-related illnesses and injuries.

S-3

Infectious Diseases after Tsunami. Aceh (Indonesia) Experience

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An earthquake of 9 Richter scale and continued with tsunami devastated large swaths of northern Indonesia within minutes, in December 26, 2004. As reports rolled in, it became clear that the province of Nanggroe Aceh Darusalam (Aceh) in Sumatra, Indonesia, was the area most severely affected. The estimated death toll exceeded 227,000, thousands more were left destitute and the infrastructure of affected communities was destroyed.

The response to this disaster has been a rapid, national and international co-coordinated effort. The combined teams were multidisciplinary, consisting of health workers (like surgeons, anesthetists-traumatologists, emergency primary care, nurses, microbiologists and laboratory technicians, public health physicians), very importantly logisticians, and others. The Military team was also providing significant resources into the area and assisting many things, including medical works. The civilian teams continued to need to be self sufficient and innovative in accessing their own needs.

Following a disaster, it was necessary to facilitate an appropriate response through accurate assessment. One of the hardest tasks was to assess the extent of the need and match the generous international/ national response to that need. The need for critical clinical care was greatest in the first 1-2 weeks, then it quickly declined. After the initial crisis period, the needs quickly move to re-establishing public health care with an emphasis on Sphere standards, like promoting access to clean water, good sanitation, adequate nutrition and access to health workers for treatment and control of common conditions such as diarrhea, malaria, and respiratory diseases. The introduction of immunization programs for diseases in vulnerable location was also important public health intervention.

In Aceh tsunami, no major disease outbreaks occurred. This was in part because of most of the displaced population settled into many small places/ areas with at least rudimentary sanitation. No large camps that would support the rapid spread of disease were built. However, there were still many diseases of epidemic potential found in tsunami affected areas. In Aceh, the rate of diarrhea, as a disease of immediate concern, was 16%. Acute upper respiratory infection and pneumonia, as diseases related to over-crowding, were found 20% and 3%, respectively. The number of malaria, as a disease posing threats in the first month, was no greater (4%) than previously, because there was an established-large vector control project.

In conclusion, even though Aceh was a restricted area for foreigners for several decades related to civil conflict, it was a wise decision of the Indonesia government to open Aceh for International (GO and NGO) rescuers during and after tsunami. Properly national and international coordination and total health response considering public health, laboratory capacity and medical needs are important lessons to learn for anticipating the possibility of infectious diseases outbreak after tsunami in Aceh.

Infectious disease risk and public health recovery after the Great East Japan Earthquake

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Tohoku University is located in Miyagi Prefecture, which was the most severely affected prefecture by the tsunami after the Great East Japan Earthquake. Our department has been helping Miyagi prefecture and municipalities to respond to infectious disease risks in affected areas. We visited many evacuation centers to evaluate the risk of infectious outbreaks especially hygiene conditions of the centers. Initially some centers had poor hygiene conditions and did not have proper infection control measures, which might increase the risk of infectious disease outbreak.

Influenza was identified as one of high-risk infectious diseases after the earthquake, therefore we set up ad-hoc surveillance system to monitor influenza activities in Sendai city and surround areas. A total of 286 samples were collected from clinics and evacuation centers. Some testing was done by using residual fluid of rapid test kits since adequate transport medium was not available in many of evacuation centers. But we managed to conduct PCR and sequencing even for these samples. Among 286 samples collected, 113 (39.5%) were A(H3N2), only 1 (0.3%) was A(H1N1)pdm and 94 (32.9%) were influenza B. A(H3N2) outbreaks were confirmed in several evacuation centers in Yamamoto town, which is located in southern part of Miyagi prefecture. Influenza B outbreaks occurred in schools in Sendai after schools were reopened in mid-April. Sequence analysis indicated that some variant viruses were introduced in affected areas possibly by people who came from other parts of Japan to assist affected areas.

We are also providing technical assistance in recovering of public health systems. In most of affected areas, whole public health systems were completely damaged and they require a long-term support for the recovery process.

S-5

Communicable diseases after the Great East Japan Earthquake

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Disasters like earthquake and tsunami are associated with significant morbidity and mortality. Among many health problems such as trauma, drowning, and mental illnesses, infectious diseases place significant burden on those who survived such disasters.

According to past literatures, wound infections including tetanus, leptospirosis, legionellosis, rickettsiosis, respiratory infections, diarrheal illness among other infections were considered to be associated with earthquake and tsunami. Since Tohoku area of Japan had different environment and microbiological profile from areas with past disasters, some infections were relevant and some were not. Because of the damaged medical system with less than adequate microbiology laboratory, precise diagnosis and epidemiological characteristics of infections during and after disasters are hard to obtain. Some infections like tsutsugamushi disease need specific antimicrobial treatment. Accurate yet practical diagnosis of these infections is important. Appropriate history taking and physical examination may aid in diagnosing these conditions.

Many symptoms at disaster area look like but are not caused by infections. For example, chronic cough occurs after exposure to dust and chemicals. Unnecessary use of antibiotics will consume necessary stocks, may cause unwanted side effects, and increase resistant organisms. Clinical skills needed are not restricted to expertise of infectious diseases but should be broadened to those of primary care medicine.

Prevention of infections is equally important. However, lack of clean water and sanitary system makes this task extremely difficult. Contact, droplet, and airborne transmission can easily occur at evacuation area. Early diagnosis and appropriate isolation of patients with influenza, measles, norovirus or tuberculosis is very important.

Infections may be imported by health care personnel or volunteer workers. Health maintenance of these people coming from outside, together with appropriate vaccination should be emphasized.

2009 Pandemic influenza virus: what special for its HA and NA?

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The 2009 pandemic influenza seemingly spreads extremely quickly with worrisome mortalities and resembles some characteristics of the previous three pandemics (1918 Spanish-flu, 1957 Asian-flu and 1968 Hong Kong-flu). The virus was recognized as a new swine-origin H1N1 influenza A virus (S-OIV). Functional and structural characterization of both the haemagglutinin (HA) (09H1) and the neuraminidase (NA) (09N1) might give us some clues about its pathogenesis and directs the drug application. We show that the 09H1 is very similar to the 1918 pandemic HA (18H1) in overall structure and the structural modules, including the five defined antiboby (Ab)-binding epitopes and the basic patch. The 09N1 crystal structure has been solved (1.9 Å) and the structure surprisingly shows a Group 2-like (or atypical Group 1) active cavity, different from other known N1 structures which are all categorized into Group 1. More importantly, the newly-defined Group-1 150-loop cavity proposed as a drug target should be re-considered as it is not as common as we thought. Our further analysis shows that the N5 has an extended 150-cavity. Comprehensive analysis of laninamivir and its octanoate prodrug with oseltamivir and zanamivir reveals group specific mechanisms for influenza NA inhibition, it's important for the development of novel inhibitors and improvement of current drugs.

Related publications from our group:

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O-2(P-2)

Summary Report of Pandemic 2009 Influenza A (H1N1) Patients in Dr. Soetomo Hospital Surabaya Indonesia

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ABSTRACT

The year 2009 marked the first influenza pandemic of the new millennium, which started to spread in Indonesia from June 2009. Dr. Soetomo General Hospital, a top referral hospital for eastern Indonesia, admitted a total of 133 influenza A (H1N1) patients (68 males and 65 females) during the peak of the 2009 H1N1 pandemic. We examined the clinical features of these patients in order to better understand the conditions that accompanied the first phase of the pandemic.

Among the 133 patients, most were aged 11-20 years (43.86%). Main symptoms included fever, upper respiratory symptoms, muscular pain, sore throat, headache, nausea, and vomiting. A total of 119 patients (89.5%) recovered without serious illness. Fourteen patients (10.5%) were admitted to the ICU; seven died and seven survived. Among the 14 seriously ill patients, 10 had underling diseases, such as chronic pulmonary disease, cardiovascular disease, and diabetes. The mortality rate was 5.3%. The most common cause of death was sepsis (due to secondary bacterial pneumonia) and/or respiratory failure.

The mortality rate in our hospital was comparable to other countries reported by the World Health Organization. Compared to patients who suffered from the H5N1 highly pathogenic avian influenza virus, the 2009 H1N1 virus was associated with milder clinical symptoms. Significant differences observed between H5N1 and 2009 H1N1 patients included liver enzyme abnormalities, bilateral pneumonia, deterioration speed of disease, and mortality rate.

Characterization of influenza A viruses isolated from wild waterfowl in Zambia

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Influenza A viruses are zoonotic pathogens of global importance. The virions possess a host-derived lipid membrane which harbours the surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Based on the antigenic properties of HA and NA, influenza A viruses are classified into subtypes. Each of the currently known subtypes [i.e. 16 HA (H1-H16) and 9 NA (N1-N9)] and in many possible combinations has been identified in wild waterfowl and shorebirds that are recognized as the major natural reservoir of avian influenza viruses (AIVs).

Since the emergence of the H5N1 highly pathogenic avian AIV in Asia and its eventual global spread, together with the understanding that all mammalian influenza A viruses, including pandemic influenza strains have their origin in the AIV pool, great emphasis has been directed towards AIV surveillance worldwide with the aim of averting the threat of influenza pandemics. However, information regarding the ecology and epidemiology of AIVs in African wild birds is still very limited.

During active AIV surveillance conducted in Lochinvar National Park in Zambia in 2006-2009, 13 nonpathogenic strains of various subtypes (H3N6, H3N8, H4N6, H6N2, H9N1, and H11N9) were isolated from wild waterfowl. Phylogenetic analyses demonstrated that all the isolates were of the Eurasian lineage. While some genes were closely related to those of AIVs isolated from wild and domestic birds in South Africa, intimating the possible AIV exchange between wild birds and poultry in southern Africa, some gene segments were closely related to those of AIVs isolated in Europe and Asia, thus confirming the interregional AIV gene flow among these continents.

Analysis of the deduced amino acid sequences of internal proteins revealed that several isolates harbored particular residues predominantly observed in human influenza viruses. Interestingly, the isolates having human-associated residues exhibited higher virus replication in lungs of infected mice and caused more morbidity as measured by weight loss than the isolate lacking such residues. Our study stresses the need for continued monitoring of AIVs in wild and domestic birds in southern Africa to better understand the emergence of strains with potential to infect mammals.

O-4(P-4)

A duck enteritis virus-vectored bivalent live vaccine provides fast and complete protection against H5N1 avian influenza virus in ducks

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Ducks play an important role in maintenance of highly pathogenic H5N1 avian influenza viruses (AIV) in nature, and successful control of AIVs in ducks has important implications for eradication of the disease in poultry and its prevention in humans. The inactivated influenza vaccine is expensive, labor-intensive, and usually needs 2-3 weeks to induce protective immunity in ducks. Live attenuated duck enteritis virus (DEV, a herpesvirus) vaccine is used routinely to control lethal DEV infections in many duck-producing areas. Here, we first established a system to generate the DEV vaccine strain by using transfection of overlapping fosmid DNAs. Using this system, we constructed two recombinant viruses, rDEV-ul41HA and rDEV-us78HA, in which the hemagglutinin (HA) gene of the H5N1 virus A/duck/Anhui/1/06 was inserted and stably maintained within the ul41 gene or between the us7 and 8 genes of the DEV genome. Duck studies indicated that rDEV-us78HA had similar protective efficacy to that of the live DEV vaccine against lethal DEV challenge; importantly, a single dose of 10^6 plaque-forming units of rDEV-us78HA induced complete protection against a lethal H5N1 virus challenge in as little as 3 days post-vaccination. The protective efficacy against both lethal DEV and H5N1 challenge provided by rDEV-ul41HA inoculation in ducks was slightly weaker than that provided by rDEV-us78HA. These results demonstrate, for the first time, that recombinant DEV is suitable for use as a bivalent live attenuated vaccine, providing rapid protection against both DEV and H5N1 virus infection in ducks.

Prediction of amino acid substitutions on the hemagglutinin molecules of antigenic variants of influenza A viruses

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Human influenza viruses mutate from time to time, causing annual epidemics worldwide. Given the high mutation rate of the viral gene, it is difficult to select an effective vaccine strain prior to each influenza season. Through efforts combining virology and bioinformatics, we investigated the evolution of HA in the past, aiming to predict the virus's evolution in the future. We first analyzed relative distances of amino acid sequences among past epidemic strains by multidimensional scaling (MDS). The analysis revealed a characteristic feature that has not been noticed only from the traditional phylogenetic analysis, the observation of a gnarled evolutionary pathway with an approximately constant curvature in the MDS-constructed 3D space. This unique property of the curvature was reasonably explained by a gamma-distribution-based substitution model on the HA sequences. By using the model, we found that the future direction of the influenza virus evolution could be predicted by the relative genetic distances from past epidemic strains. Retrospective tests for 12 years revealed the potential of this model to select suitable vaccine strains for subsequent epidemic seasons. Through these technologies, we investigate the past, current and future evolution of influenza A viruses.

P-6

Amino acid changes in hemagglutinin contribute to the replication of oseltamivir-resistant H1N1 influenza viruses

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Oseltamivir-resistant H1N1 influenza viruses emerged in 2007–2008 and have subsequently circulated widely. However, prior to 2007–2008, viruses possessing the neuraminidase (NA) H274Y mutation, which confers oseltamivir resistance, generally had low growth capability. NA mutations that compensate for the deleterious effect of the NA H274Y mutation have since been identified. Given the importance of the functional balance between hemagglutinin (HA) and NA, we focused on amino acid changes in HA. Reverse genetics analysis showed that a mutation at residue 82, 141, or 189 of the HA protein promotes virus replication in the presence of the NA H274Y mutation. Our findings thus identify HA mutations that contributed to the replacement of the oseltamivir-sensitive viruses of 2007–2008.

Clinical Manifestation and Diagnosis of H1N1 Influenza Outbreak in Children at Dr. Soetomo Hospital Surabaya Indonesia

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ABSTRACT

The 2009 influenza pandemic tended to spread among school-aged children, rather than among adults. According to the Centers for Disease Control and Prevention (CDC) and the American Academy of Pediatrics (AAP), the underlying conditions responsible for the higher risk of serious outcomes among children are multiple (e.g., neurodevelopmental conditions, chronic pulmonary conditions, immune suppression) (CDC, 2009; AAP, 2009). To understand the clinical aspects of pandemic 2009 Influenza A (H1N1) virus infections among children in Indonesia, we examined clinical data of pediatric patients admitted to Dr. Soetomo Hospital in Surabaya, Indonesia, from July to September 2009.

A total of 52 of 83 suspected patients were confirmed positive for virus infection by reverse-transcriptase polymerase chain reaction (RT-PCR). Infected patients were mainly 10-15 years old (44.7%). We examined patient data, with particular focus on the impact of underlying diseases on disease severity. Most patients did not have underlying diseases (32 cases; 71.1%), although 15 did (28.9%). Many (86.7%) were immunocompromised due to malignancy, congenital heart diseases, or neuromuscular disorders. We conducted blood tests and took chest radiographs, and compared the results between groups.

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P-8

Surveillance of H5N1 highly pathogenic avian influenza viruses in wild bird in Surabaya, Indonesia

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Waterfowl were originally considered to be natural hosts for influenza A viruses. Consistent with this, H5N1 highly pathogenic avian influenza viruses were reported to be nonpathogenic in wild waterfowl, including ducks (Shortridge et al., 1998). Yet, since 2002, avian H5N1 viruses have been shown to be lethal in wild waterfowl (Chen et al., 2006; Chen et al., 2005; Sturm-Ramirez et al., 2002), suggesting the need to monitor this population. Although H5N1 viruses have spread worldwide among domestic poultry, Indonesia seems to be the hardest hit in terms of H5N1 infections. However, the prevalence of H5N1 among waterfowl in this country remains unknown.

In 2004 and 2006, the year before and after human victims began to appear in Indonesia (WHO, 2011), we conducted sero-surveillance of H5N1 viruses in wild local waterfowl in Surabaya, Indonesia. During this study, we collected a total of 73 serum samples between May and July 2004. Eleven samples were positive for the hemagglutinin inhibition (HI) test of the H5 subtype. We subsequently collected a total of 30 serum samples from waterfowl between March and May 2006, and found one positive sample by the HI test. In 2007, the year in which reports of human victims were widespread (WHO, 2011), we isolated viruses and carried out hemagglutinin (HA) and HI tests for a total of 113 wild waterfowl samples (85 samples from wild local waterfowl and 28 samples from wild migratory waterfowl) between March and October 2007. There were 16 antibody-positive samples and four samples positive for virus isolation among the 85 local waterfowl samples, and one antibody/virus isolation positive sample among the 28 migratory waterfowl samples.

These samples were obtained along the seashore near Surabaya, Indonesia, at which many local and migratory waterfowl gather. This provides opportunities for local waterfowl to come in contact with both of migratory birds and domestic poultry. Our results underscore the importance of monitoring wild waterfowl and domestic poultry for pandemic preparation in Indonesia.

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Isolation of H5 avian influenza viruses from domestic poultry in Surabaya, East Java, Indonesia, in 2010 – 2011

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The H5N1 subtype of avian influenza viruses have recently become an important zoonotic pathogen. Since 2003, H5N1 highly pathogenic avian influenza (HPAI) has been endemic in poultry found in Southeast Asia (Soda, 2008). Among the Southeast Asian countries, Indonesia is the most affected by H5N1 HPAI, with the virus being found in 31 of its 33 provinces (Health Department in Indonesia, 2008). Yet, little is known about the diversity of H5N1 HPAI viruses circulating in backyard farms, where chickens and ducks often intermingle. Here, we report on H5N1 HPAI viral strains isolated from domestic poultry (backyard chickens and ducks) in west Surabaya, East Java, Indonesia during 2010-2011.

We obtained a total of 12 cloacal swabs. These samples were inoculated into 11-day-old embryonated chicken eggs, incubated at 37°C for two days, and followed by harvesting of allantoic fluid. Eight samples were positive for hemagglutinin (HA).

We obtained six positive samples for the H5 subtype by RT-PCR using a H5-specific primer pair. HA and neuraminidase (NA) genes from the three samples were subsequently sequenced and processed for phylogenetic analysis.

Molecular characterization of the H5N1 viruses confirmed that they harbored the cleavage site in HA genes characteristic of HPAI, although none had mutations that accelerate mammalian adaptation. Phylogenetic analyses revealed that H5N1 viruses belong to clade 2.3.1, which is prevalent in Indonesia. Gene homology analysis revealed a more than 90% match with the sequences of human isolates.

Our findings suggest that virus surveillance studies are useful for understanding the genetic characteristics and evolution of endemic influenza viruses in Indonesia.

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P-10

An H5N1 highly pathogenic avian influenza virus isolated from a duck in Surabaya, East Java, Indonesia, in 2011

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Abstract

Ever since H5N1 highly pathogenic avian influenza (HPAI) viruses spread worldwide in late 2003, Indonesia has become one of the hardest hit countries in the world, with the highest count of human victims. Currently, the virus is endemic to this country, with sporadic outbreaks being reported among poultry. To maintain a current understanding of the outbreak situation among poultry in Indonesia, we have been conducting surveillance operations since 2008. In January 2011, there was an H5N1 HPAI outbreak in a chicken farm in Tandes, West Surabaya, Indonesia. About one week after all chickens were culled, a few backyard ducks became ill. A cloacal swab from the sick duck population tested positive for H5N1 HPAI virus. The domestic ducks that survived were all culled within three days after onset of illness. Previous reports have suggested that the domestic duck population is likely to be the reservoir for H5N1 HPAI viruses (Kim JK et al., 2009). Consistent with this, several studies from Central Java suggest that ducks may be involved in the persistence and spread of H5N1 HPAI viruses among small poultry farms in Indonesia (Henning J et al., 2010; Wibawa H et al., 2011). Our findings support this view and underscore the central role of the duck population in the endemic presence of H5N1 HPAI viruses in this country.

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Sero-surveillane of swine influenza virus in Thai pig population in 2003-2010

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Swine influenza viruses (SIVs) of H1N1, H1N2 and H3N2 subtypes have been shown to circulate in pig populations in Thailand, although prevalence of the SIVs among them has not been well examined. A total of 20,511 pig sera were collected from 2003 to 2010 in 8 regions (46 provinces) in Thailand and serological tests was carried out with several different antigens including Thai isolates.

Serum sample was tested by hemagglutination inhibition with a panel of SIVs. As H1N1 antigens, A/swine/Iowa15/1930 (IW30), A/swine/Ratchaburi/NIAH550/2003 (Rat03), A/swine/Chonburi/NIAH110702-7/2008 (Cho08) and A/swine/Saraburi/NIAH11720-26/2009 (Sar09; Pandemic (H1N1)2009 virus) were used. As H3N2 antigens, A/Aichi/2/1968 (Aic68), A/swine/Chachoengsao/2003 (Cha03) and A/swine/Ratchaburi/NIAH59/2004 (Rat04) were used.

Average sero-positive rates to IW30 and Aic68 viruses were 11.1 and 8.4% from 2003 to 2005, respectively. Sero-positive rates of the sera collected in 2005 against IW30 and Rat03 were similar, while that against Aic68 was higher than that against Cha03 in most of the provinces examined. In 2 out 8 regions, however, positive rate was much higher against Cha03 than against Aic68. Sero-positive rate of H3 antibody in sera collected in 2007 and 2008 was compared using two genetically different Thai H3 SIVs, Cha03 and Rat04. HA gene of Cha03 is a human-like SIV origin, while that of Rat04 is genetically closely related to 1996-1997 seasonal human isolates. Sero-positive rates against Rat04 were 71.4% in 2007 and 94.2% in 2008, and were higher than those of Cha03. Reactivity of the sera collected in a pig farm where Pandemic (H1N1)2009 virus was isolated differed significantly against IW30, Rat03, Cho08 and Sar09, indicating antigenic difference between Pandemic(H1N1)2009 and other Thai H1 SIVs. The serological analysis indicated that antigenic differences of circulating SIVs can be recognized by sera collected from farms. Selection of appropriate antigens for the serological analysis appeared important for elucidation of SIVs prevalence in Thailand.

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Host cytokine responses of mice and pigeons infected with two H5N1 highly pathogenic avian influenza viruses （HPAIVs）isolated from wild birds in Thailand

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Highly pathogenic avian influenza viruses of subtype H5N1 had been isolated from various wild birds during the HPAI outbreak in poultries in Thailand. In this study, we compared lethality, viral distribution and host cytokine responses in mice and pigeons infected with two Thai HPAIVs (Pigeon/Thailand/VSMU-7-NPT/2004; Pigeon04 and Tree sparrow/Ratchaburi/VSMU-16-RBR/2005; T.sparrow05) isolated from wild birds.

In mice, both viruses showed similar replications in the lungs and brain and lethality. On day 3 post infection, Pigeon04 induced high level of mRNA expression of IL6, TNFα and MIP-2 in the lungs that resulted in severe pneumonia characterized by neutrophil infiltration. In contrast, T.sparrow05 induced the higher expression of IL6, TNFα and IP-10 than Pigeon04 on day 7 post infection. T.sparrow05 also potently induced the expression of these genes in brain of the infected mice that triggered frequent encephalitis.

In pigeons, 25% of those died within 2 weeks after the inoculation of two HPAIVs or medium only, suggesting that lethal outcome observed in pigeons after inoculation is merely due to experimental stresses. Pigeon04 replicated more efficiently in the lungs than T.sparrow05 and spread to multiple organs including the brain, spleen and kidney. Only a mild focal pneumonia was observed in the lungs infected with two HPAIVs. On day 2 post infection, Pigeon04 induced mRNA expression of Mx1, PKR and OAS to a greater extent than T.sparrow05 in the lungs, but their expression levels were not increased on day 5 post infection when the peak viral replication was observed. Expressions of TLR3, IFNα, IFNγ, IL6, IL8 and CCL5 in the lungs were low regardless of viral replication levels.

Peak viral replication levels in the lungs infected with Pigeon04 in pigeons ($10^{5.3}$ EID_{50}/g) were 250 fold lesser extent than those in mice ($10^{7.7}$ EID_{50}/g). Severity of pneumonia was very mild in the infected pigeons compared to the infected mice. We also found that peak mRNA expression levels of IFNγ and IL6 in the lungs of the infected pigeons were at least 15 fold lesser extent than those of the infected mice. However, the expressions of these cytokines were not correlated with viral replication levels in the lungs of the infected pigeons, suggesting that low expression levels of these genes were not solely due to insufficient viral replications.

It was suggested that severity of tissue injury that was associated with magnitude of host cytokine expressions induced by specific viral-host interactions likely decides final outcome of mice and pigeons following HPAIV infection.

Isolation and characterization of anti-Influenza A subtype H5N1 neutralizing human monoclonal Fab by phage display system

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A series of outbreaks of highly pathogenic avian influenza A (H5N1) among poultries have been reported in Southeast Asian countries, and human cases have occurred sporadically in the same area. H5N1 virus clade 1 was dominant in 2001-2007 in Vietnam; however, influenza A clade 2.3.4 virus has replaced clade 1 in northern Vietnam thereafter. Total of 119 human cases with H5N1 infection were confirmed and 59 were fatal, so far. There is a report describing not a few patients with Spanish flu pneumonia who had received transfusion with convalescent human serum were rescued from the risk of death. Therefore, it would be worth to seek for a passive antibody therapy for an intervention for human H5N1 infections as an adjunctive option.

The objective of the study is to isolate human monoclonal Fabs with neutralizing activities against influenza A subtype H5N1 strains. A pool of phage clones expressing human Fab which specifically binds to a H5N1 protein was enriched by bio-panning using ether-treated H5N1 (A/Vietnam/31244/07, clade 2.3.4) virion as the target antigens. Total of 10 Fab molecules exhibited binding activities against concentrated H5N1 virion in ELISA. Purified Fab was successfully prepared from five out of ten clones, and three of those showed moderate neutralizing activities against clade 2 strain. Neutralizing potencies of these Fabs were less efficient against a H5N1 clade1 strain. Two of three Fabs were estimated to recognize a conformational epitope on HA1 region of HA by Western blotting using recombinant protein as an antigen. Escape mutant-analyses were further performed to pinpoint a possible epitope on HA1 region recognized by the two human neutralizing Fabs.

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Molecular match between vaccine strains and circulating seasonal influenza A viruses in Vietnam, 2001-2009

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Although Vietnam is currently developing the capability to manufacture influenza vaccines, there is limited information on the genetic and antigenic characteristics of seasonal influenza viruses. Our study analyses the genetic relatedness of WHO recommended vaccine strains with the circulating seasonal influenza A viruses in Vietnam from 2001 to 2009, and provides essential information for the selection strategy of vaccine strains in Vietnam.

We collected thirty-two H1N1 and thirty-one H3N2 seasonal influenza A isolates from laboratory –based sentinel surveillance sites in Hanoi during 2001 to 2005, and from national based influenza surveillance site in Vietnam during 2005 to 2009. The hemagglutinin (HA) genes were amplified and sequenced. Phylogenetic trees were rooted by vaccine strains A/Beijing/295/1995 (H1N1) and A/Moscow/10/1999 (H3N2) and combined with contemporary HA sequences from bordering.

The phylogenetic trees indicated that seasonal influenza A/H1N1 and A/H3N2 genetic variation in Vietnam from 2001-2009 , however, the viruses circulated in 2003, 2005 and 2006 mostly grouped in same lineages (lineage I of influenza A/H1N1 or lineage I; II and IV of influenza A/H3N2) . We found genetic differences between seasonal influenza A viruses in Vietnam and WHO influenza vaccine strains recommended for the northern and southern hemispheres for the 2001-2005 influenza seasons, but matched to the vaccine strains recommended for the southern hemisphere from 2006-2008.

The influenza seasons of Vietnam may not follow the patterns of northern or southern hemisphere as we had been known, it is similar to subtropical or tropical countries, so the WHO vaccine strategy may not provides good coverage or archive efficacy in tropical or subtropical areas . Although genetic or antigenic of circulating influenza viruses in Vietnam somehow likely to WHO vaccine recommendation in same period, but an efficient influenza immunization program may need evaluate the composition of their vaccines in different manner for vaccine development in Vietnam.

Pathogenic analysis of H6N1 influenza viruses isolated in northern Vietnam

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Influenza pandemic preparedness has focused on influenza virus H5 and H7 subtypes. However, it is not possible to predict with certainty which subtype of avian influenza virus will cause the next pandemic, and it is prudent to include other avian influenza virus subtypes in pandemic preparedness effort. H6N1 influenza virus was identified as a potential progenitor of H5N1 virus that emerged in Hong Kong in 1997. And in experimentally, low pathogenic avian influenza H6N1 virus which was inserted a high pathogenic avian influenza (HPAI) H5 RRRKK↓G multibasic cleavage site motif in HA, showed a fully functional virus with an HPAI virus genotype and phenotype (1). These provide evidence that H6 subtype is still a potential progenitor of pandemic virus and may become HPAI virus solely.

Twenty-one H6N1 strains were isolated from domestic ducks reared in Hanoi, 2009. To assess the potential risk of a pandemic virus emerged from H6N1 strains, five H6N1 isolates were infected in MDCK cells (*in vitro*) and mice (*in vivo*). There was a low progeny virus in the supernatant of MDCK cells infected with Dk/VN/3C8-19/09. In the mice experiments, all mice survived after viral challenge; however, mice infected with Dk/VN/3C8-19/09 demonstrated approximately 10% weight loss on three days post infection, compared with those infected with Dk/VN/3C8-12/09 or administered with allantoic fluid. Sequence analyses on whole-genome identified two amino acid differences between Dk/VN/3C8-12/09 and the others that is, at amino acid position at 161, 212, 302, and 519 in PB2, at 190 in PA, at 5, 62, 70, 91, and 286 in HA, at 498 in NP, at 211 and 364 in NA, at 52 in M2 and at 209, and 230 in NS1. The replication efficacy of these strains on mammalian cells and their pathogenesis on the mouse model is further studied to estimate the role of amino acid(s), in sole or in combination(s), in the pathogenesis of strain Dk/VN/3C8-12/09.

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Molecular epidemiology of avian influenza viruses circulating among healthy duck flocks in farms in northern Vietnam during year 2006-2010

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Repeated epizootics of highly pathogenic avian influenza (HPAI) virus subtype H5N1 were reported from 2003 to 2005 among poultry in Vietnam. More than 200 million birds were killed to control the spread of the disease. Human cases of H5N1 infection have been sporadically reported in an area where repeated H5N1 outbreaks among birds had occurred. Subtype H5N1 strains are established as endemic among poultry in Vietnam, however, insights into how avian influenza viruses including the H5N1 subtype are maintained in endemic areas is not clear. In order to determine the prevalence of different avian influenza viruses (AIVs), including H5N1 circulating among poultry in northern Vietnam, surveillance was conducted during the years 2006–2010. A subtype H5N1 strain was isolated from an apparently healthy duck reared on a farm in northern Vietnam in 2008 and was identified as an HPAI. Although only one H5N1 virus was isolated, it supports the view that healthy domestic ducks play a pivotal role in maintaining and transmitting H5N1 viruses which cause disease outbreaks in northern Vietnam. In addition, a total of 25 AIVs with low pathogenicity were isolated from healthy ducks and phylogenetic analysis of all the eight gene segments revealed their diverse genetical backgrounds, implying that reassortments have occurred frequently among strains in northern Vietnam. It is, therefore, important to monitor the prevalence of influenza viruses among healthy poultry between epidemics in an area where AIVs are endemic.

Socioeconomic factors influencing hospitalized patients with pneumonia due to pandemic (H1N1) 2009 in Mexico

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In addition to clinical aspects and pathogen characteristics, people's health-related behavior and socioeconomic conditions can affect disease presentation and severity, especially for emerging infectious diseases including pandemic (H1N1) 2009.

A face-to-face interview survey was conducted in a hospital in Mexico City at the time of follow-up consultation for hospitalized patients with viral pneumonia due to pandemic (H1N1) 2009. A total of 302 subjects were enrolled in the present study and divided into two groups based on the period of hospitalization. Among them, 211 (69.9%) tested positive for pandemic (H1N1) 2009 virus by real-time reverse transcriptase-polymerase-chain-reaction during the pandemic period (Group-pdm) and 91 (30.1%) tested positive for influenza A virus in the post-pandemic period (Group-post). All subjects were treated with oseltamivir. There were no significant differences between the groups in terms of education level, and occupations. However, a detailed analysis on socioeconomic level concerning the ability of family income to pay for utilities, food, and healthcare services and housing quality in terms of materials and number of rooms revealed a significant difference between the groups, with Group-post having lower socioeconomic status than Group-pdm. The availability of information relating to pandemic (H1N1) 2009 was lower in Group-post than in Group-pdm. The results indicated that the subjects hospitalized due to viral pneumonia were more likely to be in a state of poverty and difficult to receive the necessary information relating to pandemic (H1N1) 2009 . The possible factors influencing time to seeking healthcare were the number of rooms, receiving information on the necessity of quick access to healthcare, and house materials.

Lower socioeconomic status influences the occurrence of pneumonia due to influenza virus infection. Difficult socioeconomic conditions and information availability were related to delayed seeking of healthcare, which caused greater disease severity.

P-18

Critically ill patients due to 2009 influenza A(H1N1) infection in Vietnam

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2009 influenza A(H1N1) virus have spread all over the world, and already been recognized as one of "common" or "seasonal" flu. But they still sometimes cause life-threatening severe viral pneumonia leading acute respiratory distress syndrome (ARDS).

We reviewed clinical data on 17 patients who admitted the Intensive Care Unit in Bach Mai Hospital (Hanoi, Vietnam) between August 2009 and July 2011. All patients confirmed 2009 influenza A(H1N1) viral infection by real-time reverse-transcriptase-polymerase-chain-reaction. We assessed clinical conditions and treatments in the relation to clinical outcomes.

The median age of study patients was 30 [range, 6-59], and 64.7% were male. Five patients had underlying medical condition. The median days from onset of fever to the initiation of antiviral (oseltamivir) administration were 4 [range, 1-14]. Major symptoms on admission were cough (15, 88.4%), dyspnea (13, 76.4%), general fatigue (9, 52.9%), sputum (6, 35.2%), chest pain (6, 35.2%), myalgia (5, 29.4%), runny nose (4, 23.5%) and throat pain (4, 23.5%). Median body temperature on admission was 38.0□ [range, 37.5-39.0], and respiratory rate 23.5/min [range, 18-50]. For laboratory findings, leukocytosis was observed in 8 patients, and transaminase level was elevated in 9 patients. Serum procarcitonin level was elevated in 12 patients. Seven patients had already developed ARDS (P/F ratio <200). Median APACHE score on admission were 10[range, 3-29]. During hospitalization, 6 patients had bacterial co-infection (2 bacteremia and 4 pneumonia). Four patients had acute renal insufficiency. Two patients had disseminated intravascular coagulation (DIC). As for treatment, all were administered antibiotics together with oseltamivir. Corticosteroids were administered in 7 patients for managing ARDS, and all recovered. Fifteen patients received oxygen supply and 9 patients required mechanical ventilation. Renal replacement therapy was performed in 4 patients. Two patients developed shock. Two patients died (19 and 41 years old). Median length of ICU stay was 6.5 days [range, 3-26].

In conclusion, clinical presentation in severe patients with H1N1 infection was similar to H5N1 patients. Strategic treatment approach for severe H1N1 would be able to refer to the treatment for H5N1 patients.

Impact of education and network for avian influenza H5N1 in human: Knowledge, clinical practice, and motivation on medical providers in Vietnam

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Knowledge, clinical practice, and professional motivation of medical providers relating to H5N1 infection have an important influence on care for H5N1 patients that require early diagnosis and early medical intervention.

Novel educational programs including training and workshops for medical providers relating to H5N1 infection in Vietnam were originally created and implemented in 18 provincial hospitals in northern Vietnam between 2008 and 2010. A self-administered, structured questionnaire survey was conducted in 8 provincial hospitals where both educational training and workshops were previously provided. A total of 326 medical providers, including physicians, nurses, and laboratory technicians who attended and not attended original programs were enrolled in the survey. Knowledge, clinical attitudes and practice (KAP), including motivation surrounding caring for H5N1 patients, were evaluated. The study indicated a high level of knowledge and motivation in all professional groups, with especially high levels in laboratory technicians. Conferences and educational programs were evaluated to be the main scientific information resources for physicians, along with information from colleagues. Factors possibly influencing professional motivation for caring for H5N1 patients included healthcare profession, the hospital where the respondents worked, age group, attendance at original educational programs and at educational programs which conducted by international health-related organizations.

Educational programs provide high knowledge and motivation for medical providers in Vietnam for caring H5N1 patients. Networking is necessary for sharing updated scientific information and practical experiences. These enhanced KAPs and integrated systems among hospitals should result in appropriate care for H5N1 patients and may result for reducing high mortality.

P-20

Development of receptor glycan microarray to survey host specificity of H5N1 influenza viruses

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The global spread of highly pathogenic H5N1 avian influenza virus has raised concerns that H5N1 might adapt to the human host and cause the next human influenza pandemic. Several factors are involved in host range restriction of influenza viruses. Influenza viruses use host sialoglycans, which contain sialic acid (s) (Sia (s)) as cell surface receptors which vary in structure from species to species. Influenza A viruses isolated from duck preferentially bind to α2-3 linked sialic acid moieties (Siaα2-3Gal-) which are found on epithelial cells in the intestines and respiratory tract of ducks, and the clinical isolates from humans preferentially bind to Siaα2-6Gal- moieties which are found predominantly on epithelial cells of the human upper respiratory tract. It is worthy to note that a number of sialylated oligosaccharides with differing branching patterns and chain lengths are present in *N*-, *O*-glycans and gangliosides on the human and avian cell surfaces. Therefore, novel glycan array technologies can rapidly assess the precise receptor specificity of H5N1 avian influenza viruses, detecting changes that might signal human adaptation.

We are developing a printed covalent receptor sialoglycan microarray to differentiate H5N1 viruses that bind to avian- and human-type receptors. The glycan microarray is constructed by using microarray printing technology to couple amine- functionalized natural sialoglycans to an amino-reactive glass slide, and the binding specificity can be detected by highly sensitive evanescent wave scanning. We have isolated many kinds of natural sialoglycans representing major glycan structures of *N*-linked glycoproteins that exhibit structural diversity and play a role in influenza virus binding to host cells, and we have developed a prototype glycan array that has remarkable utility for profiling the binding specificity of avian and human influenza viruses.

Development of RT-SmartAmp assay method for one-step detection of highly pathogenic H5N1 influenza A virus

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The possibility of human-to-human transmission of highly pathogenic avian influenza A(H5N1) viruses is becoming a fear for human health and society. Since the first human case emerged in 1997 in Hong Kong, A(H5N1) viruses have been circulating among avian species and have spread throughout Asia, Europe, and Africa, with sporadic transmission to humans and reports of nearly 60% mortality. In this context, simple, cost-effective, and highly sensitive methods should be developed to detect influenza A(H5N1) viruses.

To address the clinical need for rapid diagnosis, we are currently developing the "RT-SmartAmp" assay method to rapidly detect the highly pathogenic A(H5N1) influenza virus from patient swab samples. The SmartAmp method was originally developed to isothermally amplify a target DNA sequence of interest, whereas RT-SmartAmp can detect viral RNA, such as influenza A viruses. The RT-SmartAmp assay comprises both reverse transcriptase (RT) and isothermal DNA amplification reactions in one step. In this context, the RT-SmartAmp assay method requires neither RNA extraction nor PCR reaction.

Furthermore, we use an exciton-controlled hybridization-sensitive fluorescent primer, called "Exciton Primer", to specifically detect the HA segment of the A(H5N1) influenza virus. Exciton Primers function as sequence-specific dyes. After hybridization to complementary sequences, the Exciton Primer provides a sequence-specific fluorescent signal for real-time monitoring of amplification reactions. Owing to their high signal/noise ratio, Exciton Primers enables the visual end-point detection of RT-SmartAmp assay. We have designed a small-sized visual detection device for on-site detection of the A(H5N1) influenza virus.

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Impact of pandemic and seasonal influenza on pediatric ARI in central Vietnam

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Background: Acute respiratory infection (ARI) is a leading cause of mortality and morbidity among children and respiratory viral pathogens play a major role.The spread of 2009 Pandemic influenza around the globe had a huge impact on global health.

Objective: To investigate the impact of pandemic influenza (swH1N1) on Paediatric ARI in central Vietnam.

Methods: A population based hospitalized Pediatric ARI surveillance is being carried out at KHGH since February 2007. All children admitted to KHGH with ARI from the catchment area, Nha-Trang were enrolled in the study. Clinical-epidemiological data, chest radiograph interpretations, laboratory data and nasopharyngeal samples were collected. Four multiplex polymerase chain reaction (PCR) assays were established and performed to detect influenza A and 12 other respiratory viruses. Influenza A positive samples were further tested by two multiplex PCR assays to determine the genotypes.

Results: Up to March 2011, a total of 2736 ARI cases were enrolled. Respiratory viruses were detected in 64% of the cases with 12% multiple viral infection. Influenza A virus was one of the major viruses detected in 13% of the cases. Seasonal influenza A: H3N2 and seasonal H1N1 predominated alternately until swH1N1 appearance in Nha Trang, from July to November 2009. The number of ARI cases and clinical severity due to influenza did not differ before and after the pandemic Influenza. H3N2 reappeared in 2010 July followed by swH1N1 in 2010 November.

Conclusion: The pandemic influenza did not have significant severe clinical impact compared to previous seasonal influenza outbreaks in central Vietnam.

Sero-prevalence of influenza A virus among schoolchildren in Indonesia

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Since its emergence, the 2009 pandemic H1N1 virus has spread rapidly worldwide. Previously, we reported that most individuals born after 1920 do not have cross-reactive virus neutralizing antibodies against pandemic (H1N1) 2009 virus, and as such were immunologically naive to the pandemic virus when it emerged. This finding provided us with an excellent opportunity for a sero-epidemiological investigation of the transmission mode of the pandemic virus in the community. We performed a sero-survey for pandemic virus infection in schoolchildren and their parents at an elementary school in Japan and observed that the pandemic virus was readily transmitted among children in schools and from the children to their parents. However, the morbidity and mortality rate associated with pandemic (H1N1) 2009 virus varies among countries. Therefore, to gain insight into transmission in different countries, here, we analyzed sero-positivity for pandemic virus infection in schoolchildren at elementary schools in Indonesia.

We collected 260 sera samples from healthy volunteer children between November 2010 and February 2011 at 14 elementary schools in Surabaya, Indonesia. After sera collection, we interviewed the parents of these children to obtain the children's vaccination history and history of recent influenza-like illnesses. To determine the overall infection rate, we analyzed sero-positivity by use a virus neutralization test with pandemic virus. The rate of sero-positive children was 66.9% (a neutralization antibody titer of 32 served as the cutoff value). However, most of the parents did not recall their children experiencing influenza-like symptoms. In contrast, in Japan, among the sero-positive children, 75.0%–76.5% experienced influenza symptoms. These results suggest that there are differences in influenza recognition between Japan and Indonesia.

P-24

Genetic characterization of chicken H5N1 influenza viruses isolated in Indonesia in 2010

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Since 2003, highly pathogenic H5N1 avian influenza viruses have caused outbreaks every year among poultry in Indonesia, producing the highest number of human victims worldwide. In 2004, Indonesia introduced a campaign to vaccinate poultry; however, little is known about the H5N1 influenza viruses that have been circulating there in recent years. To characterize currently circulating H5N1 viruses in Indonesia, we conducted surveillance studies throughout 2010. We isolated eight H5N1 viruses from chickens. Phylogenic analysis of their HA and NA genes revealed that all eight viruses belonged to clade 2.1.3; however, on the basis of nucleotide differences, these viruses could be divided into three groups. The genetically closest viruses for all three virus groups were all Indonesian isolates, suggesting that the eight isolates have been evolving within Indonesia. Among the three groups, two distinct viruses circulated in the Kalimantan islands during the same season in 2010. This finding suggests that, as in recent years, at least two distinct H5N1 viruses have been circulating simultaneously in Indonesian countries.

Avian influenza virus surveillance in duck farms and live-bird markets in northern Vietnam

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In Vietnam, highly pathogenic avian influenza (HPAI) caused by viruses of the H5N1 subtype was first identified in the winter of 2003-2004. In 2005, a decision was made to start vaccination of poultry against H5N1 HPAI viruses to prevent the increase in human cases. Although the number of reported cases in poultry and humans in each subsequent year has declined, eradication of the virus could not be achieved.

It was recognized that the prolonged circulation of influenza viruses in a partially immunized population might have a direct selection pressure on the occurrence of antigenic drift of the viruses. However, information on the impact of poultry vaccination on the evolutionary dynamics of avian influenza viruses in Vietnam is not explicit enough.

On the other hand, it was also recognized when vaccination was introduced that one of the signals of infection in poultry, that is mortality, would be lost because a flock of vaccinated birds could get infected but not show any signs of disease. Therefore, the surveillance study targeted apparently healthy ducks still remains the key to prevent future H5N1 outbreak in this country.

In the present study, to establish how widespread the incidence of infection in poultry without symptoms and to elucidate the possible antigenic drift of HPAI viruses in domestic poultry populations in Vietnam, virological surveillance was carried out from September 2011 when obvious H5N1 outbreaks were absent.

A total of 300 throat and cloacal swab samples obtained from apparently healthy domestic ducks on farms distributed in Nam Dinh provinces in northern Vietnam were subjected for isolation of the virus. Sera from these birds were also collected to test the presence of antibodies against avian influenza viruses. Furthermore, a total of 80 throat and cloacal swab samples from ducks in live-bird markets in Hanoi were also obtained. Antigenic and genetic analyses of the isolated viruses will be conducted.

P-26

Swine influenza surveillance in the Philippines

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Swine influenza has drawn attention after the global dissemination of the influenza A virus 2009. After the discovery of swine as the host for Reston ebolavirus in 2008, Tohoku-RITM Collaborating Research Center for Emerging and Reemerging Infectious Diseases to together with Research Institute for Tropical Medicine (RIMT) has been implementing the surveillance for the virus among swine. We have used this surveillance system to monitor the swine influenza in the rural agricultural area in the Philippines.

The surveillance was conducted in Tarlac in Region III, a rural area in the mid of Luzon Island. Nasopharyngeal swab (NPS) and serum were collected from healthy pig, mostly under age of one, raised in the "backyard" of the residence in the area. From NPS, we have screened all influenza A virus using conventional PCR and influenza A virus H1N1 2009 using realtime PCR. For serum samples, hemaggulutination inhibition (HI) test were performed to test antibody against A virus H1N1 2009.

Field investigation was conducted every other month for 5 times from June 2010 to March of 2011, and samples were collected from 249 swine. For detection of influenza virus, no sample was positive for both conventional and realtime PCR from 249 NPS. For serology, we tested 47 serum samples collected in June 2010. Among them, 7(14.9%) had antibody higher than 1:80 (homo titer = 1:5120) in HI test.

Although we have confirmed the infection of A(H1N1) 2009 among swine, we were not able to detect virus in any of the NPS. One of the reasons is that the swine were breed in the backyard where swine-to-swine transmission is hardy sustainable due to low number in each place. In order to over come this issue, we have started to collect samples in the slaughter house where swine from commercial farm with relative numbers of the swine.

Expansion of swine influenza surveillance in Thailand

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Three main subtypes of influenza virus, H1N1, H3N2 and H1N2, have been known in pig populations throughout the world. As the pandemic A(H1N1)2009 (A(H1N1)pdm09) has been disseminated among the human population, the infection of pigs by the A(H1N1)pdm09 virus has also spread worldwide. Since the emergence of the A(H1N1)pdm09 virus in pig population, reassortant viruses possessing genes of A(H1N1)pdm09 virus have been isolated from pigs.

Our previous analysis showed that H1N1, H1N2 and H3N2 swine influenza viruses (SIVs) had been shown to circulate among pig population in Thailand and the SIVs reveals nine distinct genotypes with multiple introduction of classical swine, avian-like swine and human viruses. We have visited periodically pig farms in Thailand from 2008 in the central part of Thailand to collect samples from pigs for virological and serological analysis. To analysis the prevalence of swine influenza on pig farms in Thailand more detail, we visited pig farms in Chonburi and Chanthaburi provinces to collect nasal swab samples. The pig farms in Chonburi province, where is 200km away from Bangkok, were visited periodically in February and June 2011 and a total 300 nasal swab samples were collected from sow, fattening and weaning pigs. The pig farms in Chanthaburi province, where is 300km away from Bangkok and surrounding Cambodia, were visited in September 2011 and a total 180 nasal swab samples were collected from them. Virological analysis was performed using the nasal swab samples.

P-28

Prevalence of antiviral drug-resistant influenza A viruses in Myanmar from 2007 to 2010

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Abstract

Antiviral drug resistance data of influenza in Southeast Asia is limited. This study aims to clarify the prevalence of drug-resistant influenza A viruses in Myanmar from 2007-2010.

Nasopharyngeal swabs were collected from patients with influenza-like illness in Yangon and Nay Pyi Taw. Samples were tested by diagnostic kits, virus isolation and hemagglutination-inhibition assay. Cycling probe real-time PCR assay and drug susceptibility test were performed to screen for drug-resistant isolates. Sequencing and phylogenetic analysis of HA and NA genes were done to characterize circulating strains.

A total of 1,303 virus isolates were obtained from 1,980 test kit-positive samples during the four-year study period.

Subtype H3N2 viruses were predominantly isolated in 2007. These were all amantadine-resistant with S31N mutation in M2 (clade N). Zanamivir and amantadine resistant strains were detected in 2007 and 2008. These dual resistant viruses harbored Q136K mutation in neuraminidase (NA) and S31N substitution in M2.

The prevalence of oseltamivir-resistant H1N1 viruses was low (6%) in 2008 but it increased to 100% in 2009. These oseltamivir-resistant viruses had H274Y mutation and were related to A/Brisbane/59/2007-like strains (clade 2B). Three H1N1 viruses with dual resistance to oseltamivir and amantadine were detected in 2008. These isolates were A/Hong Kong/2652/2006-like viruses (clade 2C).

In 2009, pandemic H1N1 strain was not the predominant isolate (3%) due to the cocirculation of seasonal H1N1 and H3N2 viruses. However, in 2010, it was the predominant strain (68%). The pandemic H1N1 isolates were resistant to amantadine and susceptible to oseltamivir and zanamivir. These viruses were A/New York/10/2009-like strains (cluster 2).

Continuous surveillance showed the dynamics of antiviral drug resistance of influenza virus in Myanmar.

High prevalence of occult hepatitis B infection among school children in a "mixed- subtype" area in Indonesia

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Hepatitis B vaccination has been proven to successfully decline the HBsAg sero-prevalence in several countries. Universal vaccination program for infants over Indonesia was launched in 1997. Indonesia has many ethnic groups of people, who live in as many as 17,000 islands. The prevalence of each HBV subtype and genotype varies markedly with different geographical regions and different ethnics. It has been reported that there is geographic-specific subtype-distribution in Indonesia. Recently, we analyzed the hepatitis B serologic and genetic profiles among school children in the first five-years (9-12 years of age) of the national vaccination program, in South-East Sulawesi province, Indonesia which belongs to the mixed subtype- zone, where the frequencies of *adw, adr* and *ayw* were mutually comparable.

This study showed that the sero-positive rates for HBsAg, anti-HBc and anti-HBs were 2.9%, 47.8% and 35.3%, respectively, and that genotypes B and C were predominant in South-East Sulawesi. Surprisingly, HBV DNA was detected in 34 (43.0%) of 79 samples with HBsAg negative, but anti-HBc and or anti-HBs positive, individuals in this area, which were suggested to be cases of occult HBV infection. We previously observed that, in East Java, which belongs to the *adw* zone, HBV DNA was detected in only 5 (6.8%) of 73 individuals with HBsAg negative, but anti-HBc and or anti-HBs positive, while similar rates of sero-positivity of HBsAg (3.6%), anti-HBc (22.2%) and anti-HBs (23.5%) were found. Genetic analysis of the *a* determinant region (amino acid 110–160) of HBsAg showed that all of the 8 strains with genotype C, subtype *adr* had *T126I* and *T143S*, which have been reported to be considered as vaccine escape mutants. They might play a role in the appearance of occult HBV infection and/or unsuccessful vaccination. Whether the difference in genotypes and subtypes between the vaccine strain and the HBV strains prevailing in the area influences the vaccine efficacy should be carefully examined. There were also other possible causes of the remaining endemic of HBV infection in Indonesia, such the difficulty in vaccinating a baby at birth.

In conclusion, HBV infection remains endemic, with occult HBV infection being unexpectedly common, among school children in South-East Sulawesi. The challenges of delivering the first dose of hepatitis B vaccine at birth in Indonesia will be discussed.

O-6(P-30)

Nosocomial transmission as a factor in high prevalence of hepatitis B and C among hemodialysis patients in Yogyakarta, Indonesia

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Hemodialysis (HD) patients have an increased risk getting the hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. However, data on current prevalence of both viruses' infection and its genotypic distribution among HD patients is limited in Yogyakarta, Indonesia. We assessed it along with molecular analysis to investigate the possibility of nosocomial infection.

A total of 161 HD patients (sex ratio [male/female], 1.37; mean ± standard deviation [SD] age, 48.1 ± 13.1 years; range, 12-79 years) and 35 HD unit staffs (sex ratio [male/female], 0.6; mean ± SD age, 40.1 ± 7.5 years; range, 24-53 years) were enrolled. All of serum samples were collected from Dr. Sardjito Hospital in January and February 2010. Hepatitis B virus surface antigen (HBsAg) was detected in 18 patients (11.2%) and in two staffs (5.7%). Occult HBV infection was confirmed in 21 patients, resulting in the prevalence of HBV-DNA positivity 24.2% among them. Hepatitis C virus antibody (anti-HCV) was detected in 130 patients (80.7%), and none was detected among staffs (0%). Multivariate analysis showed that *HD duration and number of blood transfusions were associated with HCV infection.* Phylogenetic analysis revealed that 28 (73.7%) of the 38 HBV isolates tested belonged to genotype B3 (HBV/B3) and 10 (26.3%) to HBV/C2. HBV/C2 isolates were identified only among patients with occult HBV infection. The most common genotype of HCV was genotype 1 (97.9%), followed by genotype 3 (2.1%). HCV/1a was found to be dominant in HD patients (94.8%). Further molecular analysis strongly suggested the occurrence of nosocomial infection of HBV and HCV within HD unit.

Our results indicated that the prevalence of HBV and HCV infections among HD patients remains high, and it might be affected by the occurrence of nosocomial infection. The route of transmission remained unclear. Further study on it is needed.

High rate of hepatitis C virus infection with poor anti-HCV responses in human immunodeficiency virus-positive patients in Indonesia

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Individuals infected with Human immunodeficiency virus (HIV) are frequently coinfected with Hepatitis C virus (HCV), especially among injection drug users (IDUs). On the contrary, sexual transmission of HCV is relatively inefficient, and its risk is still controversial. Antibody testing is the main screening method for HCV infection, but it might not be the optimal screening method for those with HIV, possibly as a result of immunosuppression. This study aimed to investigate HCV infection in HIV-positive patients, both with and without detectable anti-HCV antibody responses.

A total of 192 plasma samples of HIV-positive patients in Surabaya, Indonesia enrolled in this study were tested for anti-HCV by HCV EIA 3.0. One hundred and twenty two (63.5%) patients were anti-HCV positive. HCV RNA was detected in 76 (62.3%) of the 122 samples with anti-HCV positive, and of them, HCV-1a (30.3%) and 3a (26.3%) were predominant. Of the 70 samples with anti-HCV negative, HCV RNA was detected in 28 (40%), and interestingly, HCV-3a was most prevalent (50%). Group of HCV seropositive patients was more likely (73%) to have parenteral HCV transmission (IDUs) than group of HCV seronegative subjects, which was mostly with history of sexual transmission (54.3%). It seems that the presence of HIV increases sexual transmission of HCV.

In conclusion, HIV-positive patients had a high risk of getting coinfected with HCV, many of whom remained HCV-seronegative due partly to their immunodeficiency status. These data might also suggest that the differences in HCV seropositivity and genotypes exist between HIV-positive patients who acquired HCV sexually and those who acquired HCV parenterally.

P-32

Difference of the prevalence of hepatitis B virus between pre-school and school children in three different ethnic communities in Indonesia

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A universal Hepatitis B (HB) vaccination program was launched in Indonesia in 1997. The prevalence of Hepatitis B surface antigen (HBsAg) in pregnant women was as high as 4-7% in East Java in our recent study. HB vaccination coverage was estimated to be 78% in Indonesia. However, the current serological status of Hepatitis B virus (HBV) in children has not been fully evaluated. This study aimed to profile the serological pattern of HBV among pre-school and school children in East Java, East Kalimantan and Bali, where their ethnicities are different.

Serum samples were collected from 241 children, including 100 pre-school children (mean age: 3.5 ± 1.2 years) and 141 school children (mean age: 8.9 ± 1.8 years) in Perak, East Java. Also, serum samples were collected from 73 children, including 4 pre-school children (mean age: 3.8±1.5 years) and 69 school children (mean age: 10.6 ± 2.5 years) in Pampang, East Kalimantan and from 80 children, including 17 pre-school children (mean age: 1.4 ± 1.4 years) and 63 school children (mean age: 11.3 ± 2.2 years) in Denpasal, Bali. All serum samples were serologically examined. Among pre-school children (under 5 years old), the prevalence of HBsAg was nil in all three communities. On the other hand, among school children (5-15 years old), prevalence of HBsAg was 2.8% (4/141) in East Java, 1.4% (1/69) in East Kalimantan and 4.8% (3/63) in Bali. Similarly, among pre-school children, the prevalence of anti-HBs was 14.0% (14/100) in East Java, 25.0% (1/4) in East Kalimantan and 47.1% (8/17) in Bali. On the other hand, among school children, prevalence of anti-HBs was 7.8% (11/141) in East Java, 10.1% (7/69) in East Kalimantan and 27.0% (17/63) in Bali. In Perak of East Java, the titer of anti-HBs was relatively high (350-450 mIU/mL) in children until 5-years old, and then decreased gradually to be undetectable at age 13-15 years old.

In conclusion, it seems that universal vaccination is effective in preventing the HBV transmission in children under 5 years. The different HBsAg positive prevalence in pre-school children and that of school children might be due to different HB vaccination coverage in these two groups and decrease of anti-HBs titer by age. The prevalence of anti-HBs remains insufficient in both pre-school and school children in Indonesia. More comprehensive data are needed for better understanding on the efficacy of HB vaccination.

Immune response to Hepatitis B vaccine among children in Yogyakarta, Indonesia

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Hepatitis B vaccination program was firstly introduced in Indonesia in 1987. Various studies in industrialized countries showed that vaccination may have protective roles against HBV infection. A small percentage of infants in Indonesia, however, remain susceptible to HBV infection. Based on these conditions, a question on the necessity of study on immune response evaluation and the efficacy of the vaccination in Indonesian children was raised.

Serum samples were collected from 107 healthy children (age, 7 months-13 years old; median, 4 years old; 50 boys and 57 girls) with history of HBV vaccination, during the period from January to February 2010. Each individual`s data was also collected by questionnaire including vaccination date, sex, and the age at the time of the study.

The titer of anti-HBs antibody was measured by CLEA method, and HBsAg was assessed by R-PHA. HBV DNA copy number was determined by quantitative real-time PCR. A total of 60 (56.1%) of the 107 children responded to the vaccine with anti-HBs antibody level ≥ 10 mIU/ml, while 47 (43.9%) of 107 children had non-protective anti-HBs antibodies level (< 10 mIU/ml). Children of ages 7 months-2 years had the highest protective rate (75.9%), and the lowest protective rate was in the 6-8 years age group. The peak levels of anti-HBs titers were reached at the age of 7 months-2 years and 4-6 years, and then decreased gradually according to age. HBsAg was positive in 2 (1.8%) of 107 children, with the age of 8 years and 7 months, and had titer HBV DNA copy numbers of 3.3 and 2.7 log copies/ml, respectively.

In conclusion, the present findings indicated the low protective rate against HBV infection among vaccinated children. The tendency of decreasing antibody level with increasing age suggests the necessity of careful monitoring on HBV vaccine efficacy in Indonesia and the booster administration of HBV vaccination in older children should be considered.

P-34

Coinfection of hepatitis B and C virus in Indonesian human immunodeficiency virus patients

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The rise of mortality caused by hepatitis virus-related liver disease among highly active anti-retroviral therapy (HAART)-treated human immunodeficiency virus patients causes prompt detection of hepatitis C virus (HCV) and hepatitis B virus (HBV) important; however, only few data could be established in Indonesian HIV patients.

The molecular and clinical characteristics of hepatitis viruses study were conducted among 126 HIV patients (78 male, 35 female, and 13 transvestite, age 21-60 years-old) mostly with HAART background in Dr. Sardjito hospital, Yogyakarta, Indonesia. The prevalence of triple infection, HIV/HCV coinfection, HIV/HBV coinfection and monoinfection were 4.8%, 34.1%, 3.2%, and 57.9% respectively. There were various transmission networks shown in seven HCV genotypes, namely genotype 1a, 1b, 1c, 3a, 3k, 4a, and 6n found in 23 (52%), 1 (2%), 4 (9%), 5 (11%), 7 (16%), 3 (6%), and 1 (2%) patients, respectively. HBV-DNA was detected in only 2 naive HAART patients out of 10 hepatitis B surface antigen (HBsAg) positive. The univariate analysis disclosed that male sex status, higher education level, IDU risk factor, sexual risk factor, HAART duration ≥ 1 year, alanin aminotransferase (IU/L) ≥ 40, and aspartat aminotransferase-to platelet ratio index (APRI) > 0.5 were associated with HCV coinfection; furthermore, IDU risk factor (OR = 16.27; 95% CI: 1.88-141.25), ALT (IU/L) ≥ 40 (OR = 6.92; 95% CI: 1.18-40.66), and sexual risk factor (OR = 0.08; 95% CI: 0.01-0.71) were independently associated with HCV coinfection by multivariate analysis.

Coinfection with HCV was frequent in HIV patients with IDU in Indonesia, and was suggested to be a risk factor of disease progression.

First reported genotype 3 swine Hepatitis E Virus in Indonesia

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Hepatitis E is a globally distributed emerging disease that has a high incidence around the world. Swine is known to be an important reservoir and swine handlers are said to have high risk of zoonosis infection. To elucidate prevalence of hepatitis E virus in Yogyakarta-Indonesia, where swine herds are managed traditionally, 100 serum and 27 feces samples ranging from various age groups of swine were collected. Swine handlers (n=140, 91 male, 49 female, age 19-75 years) and community people (n=100, 94 male, 6 female, age 17-59 years) were also tested for anti-HEV antibody and HEV-RNA. Positive prevalence for anti-HEV antibody was 82.0% in swine, 5.7% in swine handlers and 5.0% in community people, respectively. HEV-RNA in ORF1 and ORF2 region was detected from 4 faces samples (14.8%) in swine. The strain had 89.1-93.2% similarity to the isolate from Korea (swKOR-1) and 90.7-92.2% to the isolate from United States (US1). Phylogenetic analysis revealed that all of the strains were grouped into HEV sub genotype 3a. HEV-RNA could not be identified from all of human samples. To the best of our knowledge, this is the first report which identified HEV genotype 3 from swine in Indonesia. The transmission of HEV from swine to swine handlers is still unclear and necessary to further investigation.

P-36

Prevalence of hantavirus, leptospira and hepatitis E virus infection in urban rats captured in Hai Phong Port and Hanoi City, Vietnam in 2010

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The rodents play role as reservoir host of causative agents for various bacterial, viral and parasitic zoonoses. Particularly, urban rats are an important source of human pathogens as they inhabited in vicinity of human dwellings. The present study was conducted to obtain the information regarding the prevalence of zoonotic pathogens such as hantavirus, Leptospira, rat hepatitis E virus (rat HEV), and *Yessinia pestis* infection among urban rats in Hai Phong Port and Hanoi City, Vietnam.

A total of 100 serum specimens from *Rattus norvegicus* (60 captured at Hanoi City and 34 captured at Hai Phong Port) and *R. tanezumi* (4 captured at Hanoi City and 2 captured at Hai Phong Port) were serologically examined for antibodies to hantavirus, Leptospira (*L. interrogans*), rat HEV and *Y. pestis*. Antibody positive rates for hantavirus, Leptospira, and rat HEV in *R. norvegicus* at Hanoi City were 5.0% (3/60), 21.7% (13/60) and 16.7% (10/60), and at Hai Phong Port were 32.4% (11/34), 26.5% (9/34) and 32.4% (11/34), respectively. Two *R. tenezumi* in Hanoi City were positive only to HEV. No antibody positive rat was found out to *Y. pestis*. None of the *R. tanezumi* in Hai Phong Port was positive to all the pathogens examined. Hantavirus and rat HEV genomes were amplified from infected rodents' lung and serum specimens, respectively.

These results indicate that the urban rats in Hanoi City and Hai Phong Port are infected with hantavirus, leptospira and rat HEV, and might be a source of human infection. Further surveillance among humans will be necessary to clarify the role of urban rats.

Detection of the traits of Haitian variant strains of *Vibrio cholerae* in Kolkata, India since 2006

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Cholera still continues to be an important cause of human infection especially in developing countries those lacks access to safe drinking water and proper sanitation. Recent devastating cholera outbreak in Haiti placed this ancient scourge at the forefront of the global public health agenda. This dreadful diarrheal disease is caused by the gram-negative toxigenic bacterium *Vibrio cholerae* O1 and O139. Based on certain phenotypic and genetic properties, *V. cholerae* O1 can be divided into two biotypes; classical and El Tor. Till date, the world has experienced seven pandemics of cholera. Among these the first six were caused by the classical biotype strains whereas the ongoing seventh pandemic has been caused by the El Tor biotype. In recent years, emergence and dissemination of novel pathogenic variants of *V. cholerae* O1 throughout many Asian and African countries indicated a cryptic change in the cholera epidemiology. These strains include the Matlab variants from Bangladesh, the Mozambique variants, and the El Tor variant type from various parts of the world. Our recent study showed that the El Tor variant strains of *V. cholerae* O1 have replaced the prototype El tor biotype strains in Kolkata since 1995. This report together with the recent massive cholera outbreak in Haiti containing a unique mutation in the 58th nucleotide of *ctxB* motivated us to investigate the emergence and dissemination of this new variant of *V. cholerae* O1 biotype El Tor strains, if any, in Kolkata. So, we have developed and evaluated a PCR based assay which can easily discriminate of *V. cholerae* strains carrying Haitian, classical and El Tor alleles of *ctxB* rapidly. Our analysis showed that first appearance of Haitian type *ctxB* was noted in Kolkata during April, 2006. PFGE based phylogenetic patterns indicate a close relationship between Haitian and Kolkata *V. cholerae* strains with Haitian *ctxB* (genotype 7). This new type of *ctxB* was first reported in *V. cholerae* O1 strains isolated from a cholera outbreak in Kalahandi, Orissa in 2007. But our results clearly show that in Kolkata genotype 7 prevailed since April 2006. This finding tempted us to speculate that Haitian type of *ctxB* may have originated from Kolkata and then disseminated in Orissa, although conformation of this hypothesis requires several other epidemiological and experimental supports. Exploitation of this newly developed PCR assay will play important role in understanding the cholera epidemiology around the globe.

O-8(P-38)

Etiology of enteric pathogens among diarrheal children: Comparative analysis of hospitalized cases and outpatients in Kolkata, India

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Among children under 5 years of age, diarrhea remains the second leading cause of death around the world. Annually, about 11 million deaths of children under five years of age occur. Of these, about 4 million occur in the first month of life and most could be prevented if all children were covered by existing interventions. However, the etiologic agents responsible for diarrhea deaths among young children are unknown and hence effective interventions are not established in many developing countries. Diarrhea can be caused by several bacterial, viral and parasitic pathogens. The clinical symptoms and etiological agents of diarrhea tends vary from region to region as well as the nature of diarrhea. Accurate understanding of the cause of diarrhea is essential for formulation of better clinical management.

In order to understand the etiology of various enteric pathogens, we have initiated an active surveillance through a comprehensive analysis covering about 25 common enteric pathogens among every fifth diarrheal child of <5 years admitted with acute diarrhea at the Infectious Diseases and Beliaghata General Hospital (IDBGH) and treated as outpatients in the B. C. Roy Children Hospital (BCRCH). From January 2010 to July 2011, 9409 cases were admitted at the IDBGH and 8824 diarrheal cases were treated in the BCRCH. Stool specimens were collected from 347 and 1542 enrolled diarrheal cases from IDBGH and BCRCH, respectively and processed for all the common enteric pathogens at the NICED. In both the hospitals, children belongs to age groups <1 year (41-55%) and 1 to <2 years of age groups (32-38%) were more than 2 to <5 years age group (13-22%).

It was observed that in 43% of the cases reported at IDBGH had diarrhea from 13-24 hrs with watery stool representing the maximum (70%). The duration of diarrhea was >36 hrs in 75% of the cases among outpatients from BCRCH with loose stool representing the maximum (85%) than watery (12.3%) and bloody diarrhea (3.1%). Vomiting was the main clinical symptom in 26-70% of the cases with some or no dehydration in patients treated in both the hospitals. In children admitted in the IDBGH rotavirus was identified as the major pathogen (57%) followed by adeovirus (18%), *Campylobacter jejuni* (13%), Noro-G2 (11%), and *Giardia lamlia* (10%). In diarrheal children treated at the BCRCH, rotavirus was identified as the major pathogen (48%) followed by adeovirus (20%), *Campylobacter jejuni* (13%), *Giardia lamlia* (13%), *Cryptosporidium* (7%) and Astrovirus (6%). The isolation rate of *Shigella* sp. varied from 4-6% in children treated at both the hospitals. Interestingly, polymicrobial etiology was detected more in diarrheal children from IDBGH (40%) than BCRCH (20%). Despite the substantial coverage of enteric pathogens, no enteric pathogens were detected in 45% and 17.3 % of the diarrheal cases from patients treated at the BCRCH and IDBGH, respectively. Infections caused by rotavirus and *V. cholerae* O1 had distinct seasonality during winter and summer months, respectively. Among *Shigella* sp, *S. flexneri* serotypes 2a and 3a were predominantly isolated from children with diarrhea. Though the vibrios remained susceptible for most of the fluoroquinolones they were highly resistant to cotrimoxazole and furazolidone, the old drugs of choice for treating children with cholera. Most the *Shigella* strains were highly resistant (~90%) to fluoroquinolones but were susceptible for ceftriaxone, which is recommended by the WHO for the treatment of dysentery cases. This study probably exemplifies the nature of diarrhea in children and the role of different etiological agents. Tough we know more about each of these pathogens; the burden of diarrhea in children remains the same in many developing countries, instigating greater challenge to hospital management as well as health authorities.

Molecular characterization of the major gastroenteritis pathogens in hospitalized children in Thai Binh, Viet Nam

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In developing countries, the diversity of pathogens causing acute gastroenteritis is high. Among the diverse pathogens causing acute gastroenteritis in hospitalized children, rotavirus, norovirus and diarrhoeagenic *Escherichia coli* (DEC) are considered as the major causative agents for acute childhood diarrhoea. The relative role of these pathogens might change upon the nation wide usage of rotavirus vaccine in Vietnam, thus there is a need to monitor the change in infecting pathogens as well as genetic diversity of these pathogens before and after rotavirus vaccination.

A total of 374 fecal samples from children (mean age 15 months, range from 0-5 years) hospitalized for acute gastroenteritis were collected from September 2010 through August 2011 in Thai Binh Pediatric Hospital for detection and characterization of rotavirus, norovirus and DEC. Overall, the detection of single rotavirus, norovirus and DEC infection were 22%, 28% and 4.3%, respectively whereas mixed infection of 2 or 3 pathogens was 21%. In particular, rotavirus and norovirus detection rates (either as single or mix infection) were 36% and 31% of cases, respectively, confirming the important role of both viruses in acute gastroenteritis in our children. In single infection, norovirus infection occurred in earlier age group than rotavirus. Approximately 90% of children infected with at least one of these 3 pathogens were less than 2 years of age, emphasizing their important role in childhood diarrhea. Rotavirus G3 strain which dominated in the region during previous years only comprised 10% of the circulating strains in this study whereas the prevalence of G1P[8] strain became 78%. Other less common rotavirus genotypes such as G2P[4] or G4P[6] or a mixture of P[6] and P[9] were also detected, suggesting close monitoring of these strains, especially after universal mass rotavirus vaccination, is essential. Norovirus GII.3 and GII.4 were the common genotypes, comprising 23% and 51% of the circulating, respectively, whereas other GII genotypes such as GII.2, GII.12 and GII.13 were also detected. Phylogenetic analysis revealed that both Minerva and the new Apeldoorn norovirus GII.4 variants were detected, yet the Minerva strain was only found in 2010. Enteroaggregative *E. coli* was the most common pathotype detected in this population (73%).

In conclusion, this study confirmed the significance of norovirus, rotavirus and DEC in childhood diarrhea, and highlights the importance of mixed infection among Vietnamese children. The success of rotavirus vaccine in reduction of rotavirus diarrhea in other countries promises similar reduction in Vietnam, yet this reduction in rotavirus diarrhea may result in the dominance of other agents as well as cause a shift in circulating rotavirus genotypes. Thus continuing monitoring of these pathogens in Vietnamese children before and after rotavirus vaccination is necessary.

O-10(P-40)

Prevalence of diarrheagenic *Escherichia coli* among children in Surabaya, Indonesia

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Diarrhea is one of the most common causes of morbidity and mortality among infants and children in developing countries. The etiological agents of diarrhea include a wide range of viruses, bacteria and parasites.

Among the bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is an important agent of endemic and epidemic diarrhea worldwide. *E. coli* is a common member of intestinal microbiota in human but some of the toxigenic strains can cause diarrhea.

DEC can be classified into five categories on the basis of their specific virulence properties: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), and enteroaggregative *E. coli* (EAEC). Unlike other diarrheagenic bacteria such as *Salmonella enterica*, *Shigella* spp. and *Vibrio* spp., which can be readily isolated using selective plating media, DEC cannot be differentiated from commensal *E. coli* on selective media, with the exception of STEC O157.

Since DEC is very difficult to detect properly in developing countries, epidemiological studies on DEC are limited to Jakarta and Denpasar in Indonesia. We have preliminarily studied the prevalence of DEC in Surabaya, Indonesia, using colony sweep method with real-time PCR targeting virulence genes of 5 categories of DEC.

The stool specimens of children with diarrhea were inoculated to MacConkey agar. DNA from the colony sweeps on the agar were extracted and analyzed by real-time PCR. The stool specimens of children without diarrhea were also analyzed as controls.

Of 216 children with diarrhea, the prevalence of ETEC, EIEC, EAEC, and EPEC were 5 (2.3%), 3 (1.4%), 32 (14.8%), and 7 (3.2%), respectively. Of 33 children without diarrhea, the prevalence of ETEC, EIEC, EAEC, and EPEC were 0 (0%), 1 (3.0%), 3 (9.1%), and 3 (9.1%), respectively. No STEC was detected from children with and without diarrhea. No significant differences in the prevalence of any DEC were seen between children with and without diarrhea. We have found that the prevalence of DEC in children without diarrhea in this area were high. Further analysis of the control group is necessary.

(CANCELLED)

P-42

Phenotypic and genotypic characterization of *Vibrio cholerae* clinically isolated in Surabaya, Indonesia

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Over the past decade, new pathogenic variants of *Vibrio cholerae* have emerged and spread throughout many Asian and African countries. These variants display a mixture of phenotypic and genotypic traits from the two main biotypes (i.e. 'Classical' and 'El Tor' types), suggesting that they are genetic hybrids. Cholera cases caused by the El Tor variants with the classical type of CT B subunit gene (ctxB) were reported in Mozambique , Bangladesh and several other countries in Asia and Africa. Meanwhile, *V. cholerae* is increasingly developing resistance towards many antimicrobials used for the treatment of diarrhea. The accumulating evidence suggests that genetic elements associated with virulence and drug resistance in *V. cholerae* are diverse. It is thus of paramount importance to identify both phenotypic and genetic variations in *V. cholerae*, which is currently endemic to different regions of the world. In this context, little has been reported presence of El Tor variants and multi-drug resistant strains of *V. cholerae* in Indonesia, except for those circumstantially inferred of Indonesian origin such as ones isolated from people who have traveled Indonesia and from food materials imported from Indonesia.

This study aimed to describe phenotypic and genotypic characteristics of 6 clinical strains of *V. cholerae* isolated in Surabaya in 2009. Our DNA finger printings suggested that the Surabaya isolates were not from a single clone. All isolates produced cholera toxin and possessed the classical type of toxin B subunit gene. This is therefore the first report of occurrence of El Tor variants of *V. cholerae* in Indonesia. Although all isolates were sensitive to almost all tested antibiotics, including ampicillin, chloramphenicol, ciprofloxacin, gentamicin, levofloxacin, nalidixic acid, norfloxacin, streptomycin, trimethoprim–sulfamethoxazole, and tetracycline, and had no mutation in gyrA and parC genes. However, they possessed class 1 integron that is a molecular vehicle for the acquisition of antibiotic resistance genes, suggesting that they have potential to acquire the genetic element for drug resistance. It must be, however, admitted that the present study reported genotypic and phenotypic characteristics of a relatively small number of *V. cholerae* strains clinically isolated in a city of Indonesia during a limited period of time. Further phenotypic and genotypic characterization on a larger number of *V. cholerae* isolates in more extensive areas of Indonesia is therefore absolutely necessary to assess current pathogenic status of *V. cholerae* in Indonesia.

PCR methods for identifying and serotyping *Salmonella* Typhi and Paratyphi A

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Typhoid fever was an important cause of illness and death in the developing world, where sanitary conditions remain poor. Classical methods for Identification of the serovars of *Salmonella* Require high-quality O grouping and H typing antisera, reagents that can be difficult to obtain consistently in developing country. Accordingly, reference and research laboratories in developing countries, as in industrialized countries, are turning to PCR methods as a consistent, high-throughput approach to typing etiologic agents

A total of 144 bacterial isolates (96 stock isolates and 48 fresh isolates) were examine for Salmonella enterica serovar Typhi with biochemistry test, serotyping and PCR methods. Biochemistry test result are five isolates are non *Salmonella*. All isolates are subyected to serotyping and PCR methods. Serotyping for all of stock isolates (96 isolates) were negative with antisera O and Vi, 69 of them were positive with PCR. Five of fresh isolates are non Salmonella and 43 of them are *Salmonella*. Five of fresh isolates are serotype O9, 11 isolates are Vi positif and 13 isolates PCR positive.

Conclusion : Serotyping are unsuccesful to detect O and Vi antigen from Salmonella stock culture even 69/96 of them are positive with PCR test as *Salmonella typhi and Salmonella paratyphi A*. Serotyping was Succesfull when applied for Salmonella fresh isolates, the sensitivity was lower compare of PCR methods. The positive result was 11.6% (5/43)for serotyping for O antigen, 25.6% (11/43) for Vi detection and the highest positive were 30.2% (13 / 43) with PCR methods.

P-44

Antimicrobial resistance in *Salmonella* strains clinically isolated in Hyogo, Japan (2009-2011)

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Salmonella is one of the principal pathogens implicated in human food-borne illnesses. *Salmonalla* infections are usually treated with antimicrobial agents, mainly fluoroquinolones and β-lactam drugs. Recently, resistance to the both antimicrobial agents has emerged among *Salmonella* worldwide.

We examined the in vitro susceptibility to antimicrobial agents in 197 clinical strains of *Salmonella spp*., all of which were isolated in Hyogo, Japan during 2009-2011. Isolated were tested for the sensitivities to antimicrobial disks; tetracycline (T), kanamycin (K), gentamicin (GM), piperacillin (PIP), amoxicillin (AMX), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), nalidixic acid (NA), norfloxine (NOR), imipenem (IPM), meropenem (MPM), and aztreonam (ATM). The screening for ESBL produces was done by the disc diffusion test and searched for $bla_{\text{CTX-M}}$ (CTX-M-type), bla_{SHV} (SHV-type), bla_{TEM} (TEM-type) by PCR.

The *Salmonella spp*. included 194 isolates of *S. enterica* (98.5%) and three isolates of *S. arizone* (1.5%). All isolates were susceptible to NOR, IPM, and MEM. Serogroups O4 (1.5%, n=3), O7 (15.7%, n=31), O8 (1.5%, n=3), O9 (2.5%, n=5) and O1, 3, 19 (0.5%, n=1) of *S. enterica* isolates from stool specimens were resistance to antimicrobials. Multidrug-resistant *Salmonella* strains were resistant to T·K (6.1%, n=12), T·NA (1.5%, n=3), T·AMX (1.0%, n=2), NA·GM (0.5%, n=1) ,T·AMX·K (0.5%, n=1) in O7, while AMX·PIP (1.0%, n=2), and T·AMX·PIP (0.5%, n=1) in O9. The highest multidrug-resistant one strain with T·K·PIP·AMX·CTX·CRO·ATM was found in O4 and detected as an ESBL-producing *Salmonella* with the bla_{TEM} gene.

In conclusion, CTX-M-type ESBL producing *Salmonella* was found in the clinical isolates. *Salmonella* carrying CTX-M-type may play an important role in non-typhoidal *Salmonella* ESBL-mediated resistance in Japan. Therefore, the further investigation of circulating ESBL-producing, as well as fluoroquinolone-resistant, is important in order to determine the likely presence of unidentified virulence genes which contributed to the new pathogenecity of *Salmonella*.

Oral immunization with a heat-killed multi-serotype Shigella antigens induce protective immunity in guinea pig model

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Shigellosis continues to be one of the most prevalent diseases in young children both developing and developing country. Shigella is also one of the most important agents of traveler's diarrhea in individuals from industrialized countries traveling to less developed regions. At present, only antibiotic therapy is available for treatment of shigellosis. While several different strategies have been employed towards the development of vaccines against these pathogens, no licensed vaccine currently exists. In this study, we have evaluated the protective efficacy and immune response of a cocktail of heat-killed multiple species and serotypes of six *Shigella* strains *(S. dysenteriae 1, S. flexneri 2a, S. flexneri 3a, S. flexneri 6, S. boydii 4 and S. sonnei)* in guinea pig colitis model. The protective efficacy after oral immunization with four doses (0, 7^{th}, 14^{th} & 21^{st} Day) of 10^7 each of heat-killed multiple *Shigella* strains was examined. On 28^{th} day, immunized animals were challenged with 10^9 live virulent cells of each of six *Shigella* strains. The immunized group of guinea pig did not show any signs and symptoms of shigellosis after the challenge by either of six live invasive *Shigella* strains. All control non-immunized animals developed shigellosis. Serum IgG and IgA titers against lipopolysaccharide of each six *Shigella* strains showed exponential rise during the course of oral immunization with the hexavalent suspension. A high IgA titer of intestinal lavage against lipopolysaccharide of each six *Shigella* strain was observed in all immunized animals. Histology of the colonic biopsy samples, immunoblot assays against whole cell lysate, lipopolysaccharide and outer membrane protein support the mounting of a robust immune response following oral immunization with the heat killed multi-serotype suspension. This Heat-Killed multi-serotype of *Shigella* antigens suspension could be novel vaccine candidate in our future.

P-46

Significance of *Vibrio mimicus* trypsin-like protease (VmtA) for maturation of *Vibrio mimicus* hemolysin

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Vibrio mimicus is a species closely related to *Vibrio cholerae*, while it is a causative agent of sporadic gastroenteritis and food poisoning in humans. Although this pathogen produces a variety of extracellular toxic factors, a heat-labile hemolysin (*V. mimicus* hemolysin: VMH) is most important. VMH is secreted as the inactive 80 kDa precursor (pro-VMH) and converted to the active 66 kDa mature toxin through removal of the N-terminal propeptide by cleavage of the Arg^{151}-Ser^{152} bond. A trypsin-like protease (VmtA) isolated from a highly hemolytic *V. mimicus* strain recently found to contribute to the maturation of pro-VMH. However, it is not clarified whether VmtA mediates activation of the protoxin in other strains. In the present study, we tested distribution of the *vmtA* gene in various *V. mimicus* strains and examined correlation of the VmtA peptidase activity and the VMH hemolytic activity in the culture supernatants.

Distribution of the *vmtA* gene was tested by PCR methods using DNA preparations from 13 clinical and 62 environmental isolates. The *vmtA* gene was found to be highly conserved in the strains tested. Next, the peptidase activity and hemolytic activity in the culture supernatants were measured. The results revealed that VmtA evidently involved in the maturation of VMH. However, it was also indicated that some strains might produce another extracellular peptidase because, in spite of the absence of the *vmtA* gene, they produced the active 66 kDa VMH. Moreover, in some clinical strains, production of the unknown hemolytic factor was suggested. These results indicate the complexity of the maturation process of the hemolytic toxin(s) in *V. mimicus*.

A factor that converts VBNC *Vibrio cholerae* to culturable state from human colonic epithelial cell HT-29 is a catalase

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VBNC is defined as that in which the bacteria remain viable but the cells do not grow or divide on or in routinely employed bacteriological media. It is known that more than 60 species of bacteria including a large number of human pathogens enter the viable but nonculturable (VBNC) state by natural stresses such as starvation, low temperature, and osmotic concentration. There are several reports that *Vibrio cholerae* O1 and O139 enter the VBNC state in environment.

Recently, we demonstrated that VBNC *V. cholerae* O1 and O139 converted to culturable state by co-culture with eukaryotic cells, such as HT-29. It was also shown that a conditioned medium that contained biologically active components released from the previously cultured cells did not support the conversion. From these results we hypothesized that a factor converting VBNC to culturable state (FCVC) exists in eukaryotic cells.

In this study, we extracted FCVC from HT-29 cells and characterize it by using VBNC *V. cholerae* O139. After confluent HT-29 cells from 200 plates (15 cm diameter) were collected and disrupted by glass beads, extracts were ultracentrifuged, fractionated by ammonium sulfate, dialyzed and used as a crude FCVC. The crude FCVC was found to be hydrophilic heat labile protein. The molecular weight was estimated to be between 100,000 to 300,000.

Purification of the crude FCVC was carried out by successive chromatographies of anion exchange column UNO-Q, hydroxyapatite column and Superdex 200 gel filtration column. The sample after these column chromatographies gave a single band on SDS-PAGE.

An analysis of the single band on SDS-PAGE by LC-MS/MS showed a sequence homology with a human catalase. Treatment of 3-amino-1,2,4-triazole, a catalase inhibitor, suppressed both converting activity and catalase activity of the purified FCVC.

P-48

Characterization of elastolytic metalloprotease produced by *Aeromonas hydrophila*

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Aeromonadaceae is widely distributed in aquatic environments, including fresh water and brackish water. The mesophilic *Aeromonas* species are causative agent of sporadic diarrhea in both adults and children. And, it is reported that *A. hydrophila* and *A. sobria* occasionally cause extraintestinal infection such as sepsis and necrotizing soft-tissue infection in patients. As the pathogenic factors of *Aeromonas*, a variety of extracellular proteins containing hemolysin, proteases, lipases and so on have been reported. However, it is unclear how *A. hydrophila* and *A. sobria*, which are infected to the intestine, cause exointestinal infection.

A report has indicated that the elastase of *A. hydrophila* is a strongly involved in the pathogenicity of the bacteria. Furthermore, the report has demonstrated that the elastase is the metalloprotease of *A. hydrophila* (AMP). Previously, we examined the properties of the metalloprotease produced by *A. sobria,* and demonstrated that metalloprotease emerges outside of the cell as an intermediate form composed of mature region and carboxyterminal (C-terminal) propeptide region and then the mature form is generated by removing the C-terminal region. Furthermore, we found that *A. sobria*=does not show elastolytic activity, though they produce metalloprotease. In this experiment, we examined the ability of *A. hydrophila* to lyse elastin. Eight strains out of 13 strains showed the elastolytic activity on agar medium containing elastin and 5 strains did not. This means that strains of *A. hydrophila* producing metalloprotease are not always elastolytic. Proteolytic analysis using culture supernatant of elastolytic *A. hydrophila* indicated that the metalloprotease is produced into the outside of the cell as the form of intermediate and the intermediate has ability to lyse elastin. The mature form, which loses the C-terminal domain of metalloprotease, cannot digest the elastin.

Subsequently, we determined the nucleotide sequence of the *amps* of all strains used in this study. The phylogenetic analysis revealed that these AMPs were divided into three groups. The AMPs from elastolytic strains belong to group I or group III, and AMPs from non-elastolytic strains belong to group II. The position of group I is close to that of group III, but group II locates separately from groups I and III. And, the substitutions of amino acid residues between elastolytic and non-elastolytic metalloprotease were frequently observed in C-terminal domain.

Integration of two types of filamentous phages into the chromosomal DNA of *Vibrio cholerae* O1

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Two types of filamentous phages, fs1 and fs2 are reported to integrate into the region flanking CTXϕ. From an epidemiological study on cholera, fs1 and fs2 are detected from epidemic strains of *Vibrio cholerae* O1. The replicative form DNA extracted from a rectal swab culture from a patient suffering from acute diarrhea also showed the presence of two types of filamentous phages by Southern blotting. We confirmed the double infection of filamentous phages in *V. cholerae* O1 *in vitro*. When an El Tor strain of ND64 was infected with two types of filamentous phages fs1 and fs2, fs1 was shown to be integrated dominantly. But fs2 was not integrated into the chromosomal DNA of the host strain. This tendency was also confirmed in a classical strain of Bgd17. When fs2 was spotted onto the fs1 lysogenic layer on a nutrient agar plate, fs2 was not integrated into the chromosomal DNA. On the other hand, when fs1 was spotted onto the fs2 lysogenic layer, several cases of double infection were observed. The two types of phages were confirmed to be integrated flanking the CTXϕ by sequencing. These two types of filamentous phages have regulator genes of CTXϕ. Strains of *V. cholerae* O1 may produce much more cholera toxin *in vivo* than we know after the infection of two types of filamentous phages.

P-50

Isolation of diarrheagenic *Escherichia coli* and *Salmonella* from retail meat and food-producing animal feces in northern Vietnam

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Diarrheagenic *Escherichia coli* (DEC) has been identified as one of principal diarrheagenic agents in Vietnam. Food-producing animals occasionally carry DEC strains in the intestinal tracts. *Salmonella* infection is one of the main zoonoses in developed and developing counties. Additionally, antimicrobial resistance among these organisms in food animals can be a public concern. Therefore, DEC and *Salmonella* in food animals and the products were monitored in this study to clarify a role of livestock in causation of diarrhea caused by these organisms and transmission of antimicrobial-resistant strains to humans through the food chain.

One hundred pieces of retail meat (10 chicken, 45 pork, and 45 beef) were purchased in 10 markets and a total of 150 fecal samples (10 chicken, 10 swine, and 10 cattle from 5 farms each) were obtained in northern Vietnam in 2009. To isolate DEC, meat samples enriched in brain-heart infusion broth and fecal samples suspended in saline (for isolation of DEC) and the samples enriched in selenite-cyctine broth (for isolation of *Salmonella*) were spread onto DHL agar plates. Five colonies from each plate were screened for *E. coli* with the negative results of Voges-Proskauer reaction and oxidase, and subjected to multiplex PCR analysis using primer pairs specific for the *aggR, ipaH, eae, elt, est, daaE,* and *stx* genes. *Salmonella*-suspect colonies were biochemically identified and serologically typed using commercially available antisera. Antimicrobial susceptibilities of DEC and *Salmonella* strains to ampicillin (AMP), cefazolin (CEZ), dimethylstreptomycin (DSM), kanamycin (KM), gentamicin (GM), chloramphenicol (CP), nalidixic acid (NA), enrofloxacin (ERFX), sulfisoxazole (SUL), and trimethoprim (TMP) were tested by the agar dilution and disk diffusion methods.

Strains with the *stx* gene and those with the *eae* gene were isolated from 4 bovine and 1 chicken feces, respectively, whereas no strains with the above virulence genes were isolated from meat samples. All the *stx*-positive strains were susceptible to the antimicrobials tested. The *eae*-positive strain was resistant to AMP, DSM, OTC, and TMP. A total of 25 meat samples (1 chicken, 20 pork, and 4 beef) were positive for salmonellae, including *S.* Enteritidis from a chicken sample and *S.* Typhimurium from pork and beef samples. Other serotypes of *Salmonella* were also obtained form pork samples. *Salmonella* strains obtained from all the meat but 2 beef samples were resistant to one of the above antimicrobials. Salmonellae were not isolated from any fecal samples. The results suggested that the retail meat was highly contaminated with antimicrobial-resistant *Salmonella* and bovine feces contained substantial numbers of *E. coli* strains with the *stx* gene although contamination of retail meat with diarrheagenic agents may also be caused by factors other than fecal carriage.

Animal livestock and the risk of hospitalized diarrhea in children under 5 years in Vietnam

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Background: Diarrhea is the second most common cause of hospitalization and death in children under 5 years old worldwide, especially in low income settings. In Vietnam, livestock plays an important economic role for many households. The role of livestock in the transmission chain of diarrhea pathogens is less well explored. Thus we investigated the association between environmental exposure to livestock and incidence of diarrhea among Vietnamese children.

Methods: A population-based cohort of 353,525 individuals, living in 75,828 households in Khanh Hoa Province, Vietnam with baseline data covering geo-referenced information on demography, socio-economic status, and household animals were used. GIS was applied to calculate the density of livestock. The data were linked to hospitalized diarrhea cases of children under 5 years recorded at two hospitals treating inpatients in the area.

Results: Overall, 3116 children with diarrhea were hospitalized during the study period. The incidence of diarrhea hospitalization was 60.8 /1000 child-years. Male gender, age < 2 years old, higher number of household members and lack of tap water were significantly associated with an increased risk of diarrhea. There was no evidence that ownership of livestock increased the risk of diarrhea. In spatial analysis, we found no evidence that a high density of any animals was associated with an increased risk of diarrhea.

Conclusion: Exposure to animals near or in households does not seem to constitute a major risk for diarrhea in children under the age of 5 in Vietnam. Public health interventions to reduce childhood diarrhea burden should focus on well-recognized causes such as sanitation, personal hygiene, access to adequate clean water supply and vaccination.

P-52

Genotype G2 rotavirus strains in Vietnam: from the evolutionary perspective of the VP7 gene

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Rotavirus is the leading cause of severe diarrhea among children worldwide. Strains with the G2P[4] genotype have captured recent attention because of its abrupt increase or re-emergence in many locations in the world including where universal mass rotavirus vaccination program was implemented. In Vietnam, a new rotavirus vaccine with the G1P[8] genotype is in the final stage of clinical development and the vaccine is expected to be licensed shortly. Thus, it is important to know what the G2 rotavirus strains currently circulating in Vietnam are like from the evolutionary perspective of their VP7 gene. While there was no G2 strains reported in Vietnam in the last several years, we identified two G2P[4] strains in Thaibinh province between 2010 and 2011. The nucleotide sequences of the VP7 gene of these strains were determined and analyzed against the global collection of 384 G2VP7 sequences detected over the last 34 years that were compiled from the DNA database (Doan et al. Arch Virol, 2011). We found that the Vietnamese G2 VP7 sequences belonged to one of the two contemporary, globally dominant lineages: sublineage IVa-1. The hallmark of this sublineage was previously shown to possess asparagine and serine at residues 96 and 242, respectively, and these two amino acids were conserved in the Vietnamese strains. From the evolutionary perspective, there is a global transition of lineages from IVa-1 to IVa-3 (in which another amino acid substitution from serine to asparagine occurred at 242), and this transition took place concurrently with the introduction of the monovalent G1P[8] vaccine into the mass vaccination program in Brazil. Thus, it will broaden our understanding of the evolution of rotavirus genome to monitor whether there will occur the lineage transition from IVa-1 to IVa-3 in G2 strains in Vietnam after the vaccine introduction.

Identification of Coxsackievirus A20 as the recombinant counterpart of type 1 cVDPV in the Philippines

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The genomic sequences of circulating vaccine-derived polioviruses (cVDPVs) are said to have been derived mostly from genomes of human enterovirus (HEV) species. In the Philippines, the last indigenous wild-type polio was detected in 1993 and the country was declared polio-free in 2000. But, a year after the country's polio-free declaration, type 1 cVDPV was reported. Genomic sequences showed that the capsid region is homologous to the parental Sabin 1 strain, however, the nonstructural sequences downstream at the 2ABC region were derived from an unidentified HEV- C. Here, we seek to identify the donor strain at the nonstructural region of type 1 cVDPVs among the identified HEV-C from acute flaccid paralysis cases (1992-2008) by using molecular methods to unravel their possible evolutionary origin. The sequences of cVDPVs were obtained in Genbank and were aligned with the NPEV strains detected. Sequence and recombination analyses were done using MEGA 4.0 and SimPlot software respectively.

Phylogenetic analysis showed that the near full length sequence of sample 24-PHL-2000 is closely related to both CV A20a and PV 1. The polyprotein 1 (P1) which encodes for the structural region clearly categorized 24-PHL-2000 under the CV A20 cluster but diverged away and grouped with the cVDPV type 1 in nonstructural regions (P2 and P3). The high sequence similarities at the beginning of the P2 to P3 regions with the cVDPV were supported by prominent nucleotide sequence identities. Furthermore, the near full-length genome sequence of 24-PHL-2000 revealed a single intertypic recombination event with the type 1 cVDPVs in the Philippines at the nonstructural region.

Our results suggest that the nonstructural proteins of CV A20 may functionally interchange with the genomes of PVs, supporting recent studies that the nonstructural proteins of HEV-C are evolving independently of one another. The emergence of a confirmed recombinant of type 1 cVDPV is of vital consideration in the global program for eradicating poliomyelitis as it reveals that with high circulation of HEV-C adjunct with OPV vaccination campaigns may provide a great pool for genetic mix-up of EVs among the susceptible population. In conclusion, we found sample 24-PHL-2000, which is CV A20 at P1 region, to be the recombination counterpart of the type 1 cVDPVs in the Philippines. Its identification may allow an understanding of the molecular evolution and genomic transfers of the nonstructural proteins among HEVs.

O-12(P-54)

Streptococcus suis infection in humans in Thailand

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Streptococcus suis, an emerging zoonotic pathogen, causes invasive infections in humans who are in close contact with infected pigs or contaminated pork-derived products. The numbers of reported human cases, especially from Southeast Asian countries, have increased dramatically during past few years. A population-based study of *S. suis* infections in humans conducted in 2010 indicated a high incidence rate (6.2/ 100,000) in the general population in northern Thailand (unpublished data). More than 70% of patients had consumption of raw pork products and the median incubation period was 2 days after consumption of raw pork products.

Although serotype 2 is the most prevalent in humans, human cases involving serotypes 1, 4, 14 and 16 have been reported. In a retrospective study in 2006–2008 in Thailand, *S. suis* infection was confirmed in bacterial cultures of blood or cerebrospinal fluid from 179 patients. These isolates were determined to be serotype 2 for 165 cases (92.2%), serotype 14 for 12 cases (6.7%), and one case each (0.6%) of serotypes 5 and 24 (J Med Microbiol, 58:1508-13, 2009, Emerg Infect Dis 17:836-42, 2011, Lancet 378:960, 2011). Human infection with serotype 2 was sporadic, with a case fatality rate of 9.5% in adults. The major multilocus sequence types of serotype 2 isolates included the sequence type (ST) 1 (62.4%) followed by the ST104 (25.5%), an ST unique to Thailand. Although both ST1 and ST104 strains are capable of causing sepsis, only the ST1 strains commonly cause meningitis. Microbiological analysis on the strains of ST1 and ST104 suggests that the synthesis of suilysin, a thiol-activated hemolysin, is associated with the clinical characteristic of human disease caused by ST104 strain.

The use of loop-mediated isothermal amplification (LAMP) in detection of *Trypanosoma brucei rhodesiense* in clinical samples from Luangwa and Zambezi Valleys, Zambia

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Human African trypanosomiasis (HAT) or Sleeping sickness is one of the re-emerging chronic debilitating diseases in sub-Saharan Africa, caused by *Trypanosoma brucei rhodesiense* (Eastern and Southern Africa, including Zambia) or *T. b. gambiense* (West and Central Africa). In Zambia, HAT is endemic in Luangwa valley and there are increasing unpublished cases being reported in HAT old foci. Early and accurate detection of HAT is essential for its successful interventions. However, current available parasitological methods used for HAT diagnosis are either less sensitive, unable to accurately identify species and may sometimes be laborious. Furthermore, HAT is sometimes misdiagnosed for malaria or HIV/AIDS due to similarities in presenting signs. Thus, a rapid, easy and low cost diagnostic test for HAT is imperative.

The main objective of the present study was to establish a loop-mediated isothermal amplification (LAMP) system that could detect and differentiate causative agents of trypanosomiasis in Zambia. We have since been randomly collecting blood samples on FTA cards from people and their livestock living in tsetse-infested Luangwa and Zambezi valleys, Zambia, as well as tsetse extracts from flies collected from within the same areas. DNA from disks punched from FTA cards (in distilled water) was extracted by a simple technique at 95^{o}C for 30 minutes. LAMP was performed at 64^{o}C using 6 primers targeting the repetitive insertion element (RIME) gene expressed by all members of the subgenus *Trypanozoon*. All RIME positive samples were later subjected to another LAMP reaction targeting the human serum resistance associated (SRA) gene uniquely expressed by the human-infective *Trypanosome brucei rhodesiense*.

We have established a very specific LAMP system which clearly distinguishes *T. b. rhodesiense* from closely related trypanosome species. By means of specific LAMP (verified by PCR), we have detected *T. b. rhodesiense* in humans, domestic animals and tsetse flies. The prevalence of *T. b. rhodesiense* was highest in tsetse flies, suggesting that detection of the human-infective trypanosomes in the tsetse vector may be a better indicator of the risk of contracting HAT by local people. Our data further suggest that *Glossina morsitans morsistans* flies are the major vectors of *T. b. rhodesiense* in Zambia. In addition, microsatellite analysis showed that the strains/isolates of *T. b. rhodesiense* circulating among tsetse flies and humans in the Luangwa valley are genetically stable.

O-14(P-56)

Bio-efficacy, user perception and acceptability of some selected pyrethoid-based mosquito coils in controlling *An. gambiae s.l.,* a malaria vector in some parts of the Greater Accra region of Ghana.

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Malaria is highly endemic in Ghana and vector control remains the main the main strategy being used to control the vectors of the disease. This study was to provide baseline information on the pattern of coil usage, user acceptability and vector insecticide susceptibility levels of mosquito coils in some parts of Accra, Ghana.

A survey was carried out by administering questionnaires on 320 respondents to obtain information on knowledge, attitude and perception of respondents on mosquito coil use. Adult *An. gambiae* mosquitoes were tested for resistance to pyrethroid based mosquito coils using the new WHO standard protocol for testing household insecticide products. Molecular techniques were used to identify the *An. gambiae* complex and to detect the presence of *kdr* mutation in the *An. gambiae* complex.

A total of 165 (47.4%) of the respondents were direct users of pyrethroid-based mosquito coils. Over 61% of coil users indicated that they will continue to use coils even though there were adverse effects after use. *An. gambiae s.s.* was the only species that was found in the study area. Mortalities of *An. gambiae s.s*. after exposure to the coils were 37% (range: 16-45%) for Angel Jumbo®, 37.5% (range: 9.5-40%) for Lord Anti-mosquito® and 15% (range: 4-30%) for Heaven Jumbo. The knock down resistance gene '*kdr'* mutation was found in about 59 % of the survivors from the susceptibility test.

In conclusion, the study found that a high percentage of inhabitants in the area used mosquito coils as means to prevent bites. However, high level of resistance was detected for all the three pyrethroid-based mosquito coils and West African *kdr* was detected in over 59% of samples of *An. gambiae s.s.* that survived the exposure. The implications of these findings in controlling malaria in Ghana will be discussed.

An overview of bacterial zoonotic diseases in Zambia

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Zambia is a landlocked country in central Africa with an area of approximately 725,600km^2. Of this land mass, 30% is composed of the National parks and Game Management Areas. This scenario leads to a close interaction of animals and human beings resulting in easy transmission of zoonotic diseases. Of importance are bacterial zoonotic diseases, which periodically affect human beings. These diseases may include tuberculosis, plague and anthrax. Tuberculosis caused by *Mycobacterium bovis* has been documented in wild life and domestic animals, while plague caused by *Yersinia pestis*, has been recorded on a yearly basis in humans. Currently studies are underway to document the reservoir of plague. Anthrax caused by *Bacillus anthracis* has also been documented in wildlife and man. A major outbreak of plague was observed in the year 1996/1997 where 267 human cases were reported out of which 26 people died. In August and September 2011, over 400 cases of human anthrax were reported with 5 people dying after getting into contact with *Bacillus anthracis* contaminated hippo meat. Over 90 hippos reportedly died from anthrax too. Our studies confirmed the disease through gram stain and culture followed by confirmatory testing using PCR. The continued reports of these disease outbreaks entails that Zambia is at a constant threat and therefore needs to have an effective surveillance program to minimise the impact of zoonotic diseases.

P-58

Investigation and phylogenetic analysis of novel arenaviruses in Zambia

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In Africa, Old World Arenavirus (OWA) that have not been reported to be pathogenic to humans have been found in Central Africa (Ippy and Mobala viruses), Eastern Africa (Morogoro and Mopeia viruses), and South Africa (Merino Walk virus). Before 2008, only 1 OWA, the Lassa virus, which is exclusively found in West Africa, was known to cause hemorrhagic fever. In September 2008, a patient in Zambia developed hemorrhagic fever and, after transport to South Africa, 4 people are transmitted the infection nosocomially. Four of these 5 infected patients died. The hemorrhagic fever was caused by a novel arenavirus named Lujo virus. The Lujo virus belongs to the OWA group based on geography but is genetically slightly divergent from other OWAs.

Natural reservoirs of OWAs are rodents of the genus Murinae. To reveal epizootiological aspects of arenavirus in Zambia, a molecular surveillance of rodent arenavirus strains was carried out from 2009 - 2011. In total, 598 rodents were collected from Mfuwe, Namwala, Livingstone and Lusaka. 23 of the 598 RNA samples extracted from kidneys were positive for arenavirus, as determined by the one-step RT-PCR. Some representative viruses were determined the full genome sequence. Phylogenetic analysis and calculated genetic distances among Old World Arenaviruses indicated that the Zambian strains are related to Lassa virus-Related virus (LSRV) and Lymphocytic choriomeningitis virus (LCMV). We named the Zambia LSRV as "Luna virus (LUNV)", and tentatively, the LCMV-related virus as "Lymphocytic choriomeningitis virus-Related virus Lusaka strain (LCRV LSK-1)". Sequencing of the cytochrome b gene of rodents revealed that the reservoir rodents belong to *Mastomys natalensis* and *Mus minutoides*. The LUNV and LCRV were isolated from kidney from rodents captured in Lusaka. Transmission electron microscopy showed that the isolated LUNV possesses typical morphological characteristics of arenaviruses.

Molecular epidemiology and a loop-mediated isothermal amplification method for diagnosis of infection with rabies virus in Zambia

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Rabies is a fatal and devastating zoonotic disease of humans and other warm-blooded vertebrates and is caused by a rabies virus (RABV), which belongs to the genus Lyssavirus and the family Rhabdoviridae. This disease has a worldwide distribution and is responsible for 55,000 human deaths annually. Furthermore, 44% of all human cases occur in Africa.

The National Livestock Epidemiology and Information Center (NALEIC) in Zambia reported over 132 cases of canine rabies diagnosed by the direct fluorescent antibody test (DFAT) from 2004 to 2009. In this study, the lineage of rabies virus (RABV) in Zambia was determined by phylogenetic analysis of the nucleoprotein (N) and glycoprotein (G) gene sequences.

Total RNA was extracted from 87-DFAT brain specimens out of which only 35 (40%) were positive on nested reverse transcription polymerase chain reaction (RT-PCR) for each gene, and 26 being positive for both genes. Positive specimens for the N (n=33) and G (n=35) gene were used for phylogenetic analysis. Phylogenetic analysis of the N gene showed two phylogenetic clusters in Zambia belonging to the Africa 1b lineage present in eastern and southern Africa. While one cluster exclusively comprised Zambian strains, the other was more heterogeneous regarding the RABV origins and included strains from Tanzania, Mozambique and Zambia. Phylogenetic analysis of the G gene revealed similar RABV strains in different hosts and regions of Zambia.

We designed primers for reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay from the consensus sequence of the N gene in an attempt to improve the molecular diagnosis of RABV in Zambia. The specificity and reproducibility of the RT-LAMP assay was confirmed with actual clinical specimens. Therefore, the RT-LAMP assay presented in this study may prove to be useful for routine diagnosis of rabies in Zambia.

P-60

Detection of *Leptospira borgpetersenii* in fruit bat in Zambia

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Leptospirosis is an important zoonotic reemerging disease caused by pathogenic spirochetes of the genus *Leptospira* in tropical and subtropical region, especially Asia and Latin America. Humans most commonly become infected through contaminated water or soil and contact with the urine of carrier animals. A variety of mammalian wild animals can be natural hosts for *Leptospira*, of which the rodent is the most important reservoir. However, the data related to natural hosts and Leptospirosis in Zambia have not been reported. Our objective is to determine the prevalence of *Leptospira* circulating among wild animals and to identify the predominant natural host in Zambia. It has been reported that *Leptospira* is detected in bats in Peru and Australia. In the present study, we examine whether *Leptospira* is detected in fruit bats in Zambia as a first step of our research.

A total of 107 Straw-coloured fruit bats (*Eidolon helvum*) were captured in the Kasanka National Park located in the northern region of Zambia in 2009 (n=60) and 2010 (n=47). DNAs extracted from 10% (w/v) kidney homogenates were used as a template of *flaB*-PCR, which detected a *Flagellin B* gene of pathogenic *Leptospira*. We detected *Leptospira* genome from 7 out of 60 (11.7%) and 4 out of 47 (8.5%) samples collected in 2009 and 2010, respectively. The sequences (approximately 690 bp) of the 11 positive products were determined and analyzed phylogenetically. According to the sequence of *Flagellin B* gene, all 11 *Leptospira* were closely related to *L. borgpetersenii*. In the previous reports, *L. borgpetersenii*, *L. kirschneri*, and *L. interrogans* were isolated predominantly from rodents in Africa. However, *L. borgpetersenii* detected in fruits bats in Zambia suggested that fruits bats are one of the candidates of natural hosts of *Leptospira* in Zambia.

To the best of our knowledge, this is the first report of detection of *Leptospira* from fruit bats in Africa. Our study of *Leptospira* in fruit bats enlarged our scope of further epidemiological research of *Leptospira* in wild animals.

Lyssavirus infection of bats in northern Vietnam and seroprevalence of anti-rabies neutralizing antibodies of individuals in Vietnam

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Rabies has been eradicated from domestic animals of industrialized countries, however, sylvatic rabies still remains there as endemic. The ecology of rabies in wildlife populations and natural ecosystems is poorly understood as a result it is difficult to eradicate rabies from the wild. Very little is known about bat rabies in Southeast Asian countries where rabies takes its biggest toll. Although rabies is endemic in Vietnam mainly due to domestic dog, it has not been identified in wild animals, including bat. Bat is susceptible for other *Lyssaviruses* as well as classical rabies virus and is also very important reservoir of rabies. We report here an initial survey of *Lyssaviruses* infection in Northern Vietnam using RT-nested PCR and rapid immunochromatography test (RICT). Of 150 brain tissue obtained from several species of bats collected from Tay Nguyen, Bac Glang, and Hoa Binh Provinces, there is no positive samples by the tests currently.

Previously, we have developed an improved method (RAPINA: Rapid Neutralizing Antibody detection test) which is based on immunochromatography and have evaluated its usefullness by measuring the rabies virus neutralizing antibody (VNA) in the serum. Our novel method is a quicker, simpler, easier, and qualitative manner comparing with gold standard viral neutralizing assay [J Virol Methods 2009, 161: 58-62.]. Next, we have studied the possibility of subclinical or inapparent exposure with rabies virus or *Lyssaviruses* in Vietnamese. Habitat of eating dog meat or cooking bat meat is relatively popular especially in northern to central Vietnam or there are some places of living areas of bat close to human. We hypothesize that those kinds of individuals handling dog or bat might be exposed with rabies virus but do not appear clinical symptoms because of a kind of vaccination effect. Total 314 sera were collected from Dak Nong Province (male 58 and female 97) and Dak Lak (male 54 and female 105). Of three-hundred and eight case among them, 15 (4.9%) was positive for VNA. However based on the questionnaire, 14 cases possessed the history of post-exposure rabies vaccination previously. Remaining one case might be unaware of exposure by rabies virus or *Lyssavirus*. Determination of VNA in BSL-3 lab is very costly and time-consuming. Our RAPINA test is an easy to use and quick method which can measure the VNA values with or without immunization against rabies virus, and its employ is focused in fields where the prevalence of the rabies is high and a large number of samples are need to be screened.

In conclusion, we try to proceed to test much more bat brains for the detection of rabies or *Lyssavirus* and make an advance serological survey using human samples.

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Phenotypic change of Enterovirus 68 associated with recent years' outbreak

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Enterovirus 68 (EV68) was first isolated from 4 pediatric patients hospitalized with severe lower respiratory tract illnesses in California, 19622. Since then, EV68 caused only sporadic cases, and only 26 cases were reported in enterovirus surveillance conducted in the US for 50 years until 2005. We reported EV68 identified from 21 pediatric patients including 2 fatal cases, in the Philippines from 2008 to 2009 (Imamura T et al. Emerg Infect Dis 2011). It was the first report of fatal cases associated with EV68 infection. After our report EV68 reported cases increased also in the US and Europe. EV68 detection increased in Japan as well, with more than 120 cases in 2010 only, while less than 10 cases have been reported until 2009. Among those cases in Japan, information on clinical manifestation was available for 12cases, including 10 with severe respiratory illnesses, 1 with febrile convulsion and 1 fatal case with unknown origin. Total of 4 fatal cases have been reported on EV68 positive patients; 2 cases from the Philippines, 1 from Japan, and 1 pediatric patient from the US who had no under lying disease and died from meningomyencephalitis of unknown origin. The dramatic increase of EV68 reported cases worldwide was summarized in the weekly report of CDC, the US, including our data from the Philippines (Imamura T et al. MMWR, CDC 2011).

EV68 detection is mainly depending on genome detection method, since EV68 isolation is relatively difficult. However, the genome marker of EV68 which was associated with recent years' worldwide outbreak is still unknown. In addition, genome analysis is dominantly focused on limited genome region including parts of un-coding region and capsid region. Moreover, how much influence the genome change found in molecular analysis has is unknown. In order to clarify the actual change of EV68 characteristics, analysis using virus proteins or isolated viruses is necessary.

In the study, we analyzed the phenotypic change of EV68, using proto strain and isolate from Yamagata, 2010. We focused on growth efficiency, which was analyzed by plaque assay, and immunogenicity on human airway epithelial cells, which was analyzed using cytokine/chemokine detection assay; Bio-Plex (BIO-RAD).

Phosphoproteomic analysis reveals an HSV-1 kinase-mediated phosphorylation event involved specifically in the regulation of viral neurovirulence

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Protein phosphorylation is one of the most common and effective modifications, which regulate a variety of cellular and viral functions. Phosphorylation events in herpesvirus-infected cells are of particular interest since herpesviruses encode viral specific protein kinase(s) unlike most of the other viruses. However, although some of biological consequences and mechanisms of the phosphorylation events in herpesvirus-infected cells have been gradually elucidated, our knowledge of them remains to be limited and fragmented. In the present study, for closing the knowledge gap, we carried out phosphoproteomic analysis of titanium dioxide affinity chromatography-enriched phosphopeptides from HSV-1-infected cells by using high-resolution mass spectrometry (MS). We identified more than 3,000 unique phosphopeptides covering 366 unique phosphorylation sites in 392 distinct cellular and viral proteins. To demonstrate the significance of these screening results, we focused on a viral dUTPase encoded by UL50 gene of HSV-1. Our results are as follows. (i) An HSV-1 kinase Us3 directly phosphotylated the HSV-1 dUTPase in vitro and mediated the phosphotylation of the viral enzyme in infected cells. (ii) In agreement with a previous report elsewhere, a null-mutation in UL50 significantly attenuated both the neurovirulence in mice following intracrebral inoculation and the pathogenic manifestations in mice following peripheral inoculations such as corneal and intravaginal inoculations. (iii) In contrast, alanine substitution in the Us3 phosphorulation site of the dUTPase identified by the MS analysis, followed by biochemial analyses, significantly impaired the neurovirulence in mice following intracerebral inoculation but not the pathogenic manifestations in mice following corneal and intravaginal inoculations. In addition, a phosphomimeric mutation at the phosphorylation site in the dUTPase in part restored the neurovirulence in mice. These results suggested that the Us3-mediated phosphorylation of the dUTPase specifically regulated the viral neurovirulence in vivo.

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Functional analysis of cIAPs in RANK signaling in osteoclast precursor cells

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HIV infection and highly active anti-retroviral therapy (HAART) are known to induce osteoporosis in HIV patients. One of the major causes of osteoporosis is excess formation or activity of osteoclasts, the responsible cells for bone resorption. It is thus crucial to understand molecular mechanisms underlying osteoclast differentiation for development of therapies against osteoporosis in HIV patients.

Osteoclast is known to be formed by the fusion of hematopoietic cells of the monocyte-macrophage lineage. One of the essential signaling molecules for osteoclast differentiation is RANK, a member of the TNF receptor superfamily expressing on the surface of the precursor cells. Binding of RANK to its ligand activates NF-κB and MAP kinases and finally induces expression of NFATc1, a master transcription factor of osteoclastogenesis. Recently, emerging evidence indicates that cellular inhibitor of apoptosis proteins (cIAPs) are involved in the signaling of the TNF receptor superfamily. cIAPs have ubiquitin E3 ligase activity and induce K63-linked polyubiquitination of RIP1 to activate NF-κB in the TNF signaling. In the CD40 signaling, cIAPs induce K48-linked polyubiquitination and degradation of TRAF3, and its degradation leads to activation of MAP kinases. Although RANK is a member of the TNF receptor superfamily, significance of cIAPs in the RANK signaling is not clear. To investigate the function of cIAPs in this signaling, we performed overexpression and knockdown experiments using Raw264.7, a murine monocytic cell line. A critical regulatory role of cIAPs in RANK signaling will be discussed.

Climate change and infectious diseases

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There is strong concern about impact of climate change on infectious diseases. In particular, global warming is suspected to be a major factor in the recent resurgence in the incidence of malaria in African highlands. Although malaria incidence may be positively correlated with temperature, unusually intense rainfalls can also trigger epidemics. In fact, the unexpected long rainfall in 1997 and 1998 induced epidemics in the East African highlands. During the same period, Rift Valley fever and cholera were reported from East Africa. In Southeast Asia and Central and South America, dengue epidemics also occurred during the period. Since the development of El-Niño-Southern Oscillation (ENSO) peaked in 1997, it was believed that the epidemics were related to ENSO. However, the development of Indian Ocean Dipole Mode (IOD) followed the ENSO, and a study found a stronger correlation between IOD and malaria epidemics in the East African highlands. A local climate change may also affect infectious diseases. Deforestation produces warmer local climate that enhances developments of malaria vectors and parasites in the highlands. The direct impact of global impact may be still debatable, but other climatic anomalies may affect transmission of infectious diseases.

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Plasmodium falciparum isolates from southern Ghana exhibit polymorphisms in the SERCA-type *PfATPase6* though sensitive to artesunate *in vitro*

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Malaria remains a major public health concern in the world, which is partly due to the emergence and spread of *Plasmodium falciparum* parasites that are resistant to conventional anti-malaria drugs such as chloroquine. In 2005, Ghana replaced chloroquine with artemisinin-based combination therapy as the first-line treatment for uncomplicated malaria. The aim of this work was to determine for the first time, polymorphisms in the putative *pfATPase6* and *pftctp*, *pfmdr1*, *pfcrt* genes in Ghanaian isolates, particularly at a time when there is no report on artemisinin resistance in malaria parasites from Ghana. We also evaluated the sensitivity of parasite isolates to anti-malarial drugs for a possible association with polymorphisms in these genes.

The prevalence of point mutations in the above *P. falciparum* genes were assessed from filter-paper blood blot samples by DNA sequencing. *In vitro* drug sensitivity test was carried out on some of the blood samples from volunteers visiting hospitals/clinics in southern Ghana using a modified version of the standard WHO Mark III micro-test.

All successfully tested parasite isolates were sensitive to artesunate; while 19.4%, 29.0% and 51.6% were resistant to quinine, amodiaquine and chloroquine respectively. The geometric mean of IC_{50} value for artesunate was 0.73nM 95% CI (0.38-1.08), amodiaquine 30.69nM 95% CI (14.18-47.20) and chloroquine 58.73nM 95% CI (38.08-79.38). Twenty point mutations were observed in *pfATPase6* gene, with no L263E and S769N. All mutations found were low in frequency, except D639G which was observed in about half of the isolates but was not associated with artesunate response (p = 0.42). The *pftctp* gene is highly conserved as no mutation was observed, while CVIET which is chloroquine-resistant genotype at codon 72-76 of the *pfcrt* gene was identified in about half of the isolates; this was consistent with chloroquine IC_{50} values (p = 0.001). Mutations were present in *pfmdr1* gene but were not associated with artemisinin response (p = 1.00).

In conclusion, *P. falciparum* isolates from southern Ghana exhibit satisfactory *in vitro* response to artesunate with no L263E or S769N mutation in the SERCA-type *pfATPase6*. However there is no improvement in susceptibility of the parasites to chloroquine five years after its proscription.

Molecular cloning and functional analysis of *Trypanosoma brucei* motility-related genes

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African trypanosomes (e.g., *Trypanosoma brucei* and related subspecies) are uniflagellated protozoan parasites that cause African trypanosomiasis in humans and nagana disease in wild and domestic animals. The current chemotherapy of the human African trypanosomiasis relies on only six drugs, five of which have been developed more than 30 years ago, have undesirable toxic side effects and most of them show drug resistance. Therefore finding novel drug-target candidates is very important. Because *T. brucei* flagellum-originated motility is essential for completion of stage development in the tsetse fly vector, motility-related molecules are considered to be novel drug-target candidates for chemotherapy.

We compared *T. brucei* whole gene products (amino acids) with *Caenorhabditis elegans* UNC (uncoordinated) proteins, in order to find uncharacterized motility-related *T. brucei* genes. Through *in silico* analysis, we found 88 gene products highly similar to *C. elegans* UNC proteins and categorized them as TbCEUN (*T. brucei* gene products which have high similarity to *C. elegans* UNC proteins). Approximately two-thirds of the 88 TbCEUN gene products were kinesin-related molecules. A gene product highly similar to *C. elegans* protein UNC119 was designated TbUNC119. RNAi-mediated depletion of TbUNC119 showed no apparent phenotype. However, knock-down analysis of both TbUNC119 and its binding protein TbUNC119BP which was found by yeast two hybrid analysis showed characteristic phenotypes, including reduced motility, morphological change (extended cell shape), and cellular apoptosis. Based on the observed phenotypes, possible function of the TbUNC119 and TbUNC119BP will be discussed.

P-68

Incidence of Toxoplasma gondii infection in Thailand from the study of the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic animals, Mahidol University, Thailand

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Toxoplasma gondii is a zoonotic protozoan parasite of both medical and veterinary important worldwide. Humans acquire toxoplasmosis from cats, from consuming raw or undercooked meat from many intermediate host and from vertical transmission to the fetus through the placenta during pregnancy. The parasite can cause severe complications in immunocompromised individuals such as AIDS patients and transplant recipients. Toxoplasmosis is a zoonosis that causes a public health concern in both developed and developing countries, 1/3 of the people in the world had been infected with this parsite. Since the small mammals and many species of avian are the source of food for people in villagers in Thailand and all of these animals are intermediate host for T. gondii parasite. And feline species, both domestic and wild ones plays important roles in parasite transmission.

The specific antibody against Toxoplasma gondii were detected in serum samples sent from many part of Thailand especially in the western region. The samples were collected from feline species both from domestic, wild and captive wild felids, avian and small mammals such as Indochinese ground squirrel, rat, tree shrew, slow loris and the others. The indirect Latex agglutination test (LAT: (Toxocheck-MT; Eiken Chemical, Tokyo, Japan), and PCR assays were used to detect both antibody and T. gondii parasite.

The result of seroprevalence were positive in the percent of 24.48 (24/98 samples), 9.8 (10/102) and 21.87 (7/32) in 2008, 2009 and 2010 respectively. One of the tissue samples in 2009 was positive when using PCR detection. Further investigation is to check the antibody titer in people who live in area studied, to find out the relationship between human and animal prevalence.

A birth cohort study on congenital rubella infection in Khanh Hoa Province, Vietnam and implications for a preventive strategy

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Background: Rubella virus is a representative congenital pathogen, causing hearing loss, ophthalmological problems (e.g., cataract), cardiovascular anomalies (e.g., patent ductus arteriosus) and other neurological and non-neurological impairments. Congenital rubella syndrome (CRS) has been almost eradicated in countries where rubella vaccination is included in routine immunization program; however, its epidemiological features and socioeconomic burdens remain largely unknown in Vietnam where rubella vaccination is not included in the national immunization program.
Objective: To determine the seroepidemiological state among pregnant women and the incidence of CRS in a birth cohort in Nha Trang, Vietnam.
Methods: We completed the pilot study (January-May of 2007) and the first stage of a birth cohort study (May 2009 to May 2010) in Nha Trang, Vietnam. 2305 pairs of mothers and their babies born at Khanh Hoa General Hospital were enrolled and maternal and cord blood samples were collected. Rubella-specific IgG and IgM were measured by enzyme immunoassays (EIA).
Results: Maternal mean age was 28.4 years old (14 to 46 years old). Only 70.9% of them were rubella IgG positive. Nearly a half of teenage mothers were estimated to be susceptible to rubella, and annual seroconversion rate was approximately 1.21%; therefore, it is estimated that a substantial number of women had been infected with rubella during pregnancy. We could identify 3 babies positive for rubella IgM. Their mothers lived in different residential areas, and no rubella epidemic was reported in those areas during study period. All of those infants were apparently asymptomatic at birth; two of them had no abnormalities in physical, neurological and auditory examination at 10-months checkups, while the other was lost to follow up.
Conclusion: In Vietnam where rubella vaccination is not routine, many pregnant women are susceptible to rubella, and congenitally infected infants were born even when rubella epidemic was not evident.

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A large-scale population-based immunogenetic phenotype study of Vietnamese children for the identification of high risk individuals to childhood infectious diseases

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The susceptibility to childhood infectious diseases including acute respiratory infection is attributable to inadequate responsiveness of host defense mechanisms to pathogen-derived stimulus at least to some extent. Accordingly, certain properties of host defense mechanism evaluated by *ex vivo* cellular responsiveness to stimulations mimicking microbial pathogens would be eligible to infer the individual predisposition to infectious diseases. A large-scale population-based study of such immune-related phenotype analysis was conducted to investigate whether the measure of host defense properties can tell the individual risk to certain diseases.

For the comprehensive detection of disease incidents of major childhood illness in general population, we set a cohort of 1,999 infants who were born at a principal medical facility in Nha Trang, the capital of Khanh Hoa Province, a central coastal region of Vietnam, from May 2009 to May 2010. In the present study, the registered children were invited to medical check in the month they became 2 year old. After physical examinations, peripheral blood samples were collected upon their guardians' informed consent. A simple and robust procedure of whole blood culture was established to obtain a reliable result under the situation where the amount of samples and manpower is limited; a fresh whole blood was mixed with cell culture medium and incubated in the presence of LPS (a ligand for TLR4), Pam3CSK4 (a ligand for TLR2-TLR1), L18-MDP (a ligand for NOD2) or other chemical stimulants of immune-competent cells in multiple culture tubes separately. Approximately 20 hours later, the cells were collected by centrifugation and stored at -80 degree until RNA preparation by a standard method using Trizol reagent. The levels of induced mRNA expression of cytokine genes such as *IFNG* and *TNF* were quantified by real time RT-PCR assay.

Using the first 24 samples as a pilot study, we could show that the relative amounts of those cytokine transcripts in comparison to certain internal standards (18S rRNA, G3PDH mRNA, etc.) were variable between individuals as well as between culture conditions in the same person. Although the following-up observation of the cohort and the sample collection will be continued to May 2012 to reveal the relationship between selected measures of the study and the incidence of childhood infectious diseases, the progress of the study is reported.

A progress report on the study for acute undifferentiated febrile illness cases in Infectious Disease Department of Bach Mai Hospital, Hanoi, Vietnam

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Background: One of the most common admission diagnoses at the infectious disease department is undifferentiated acute febrile illness/Fever of Unknown Origin (FUO). In spite of the development of high level medical expertise, diagnostic laboratory resources are still limited to identify the etiology of acute febrile illness/FUO and presumptive diagnosis without confirmative results is largely used in clinical management. Clinical diagnosis without confirmation can lead clinicians to over-diagnosis and overuse of unnecessary medication which will have negative impact on medical cost and the development of antibiotics resistant organisms.

Objective: To improve clinical management of undifferentiated febrile illness/FUO cases in Vietnam, we will determine the clinical signs, symptoms and demographic factors of undifferentiated febrile illness/FUO cases in Vietnam, and apply advance methods to improve the etiological diagnosis of undifferentiated febrile illness/FUO cases in Vietnam.

Method: We are conducting a retrospective study of clinical epidemiological data collection from acute febrile cases admitted to the infectious diseases department of Bach Mai Hospital between March 2001 and February 2003. A total of 755 serum samples from these cases were collected and stored previously. To better determine the etiology of the cases, advanced laboratory investigation will be performed on these samples for Rickettsias, Leptospirosis, and some other viruses.

Result of the ongoing study: Out of 755 serum samples collected and stored, 740 corresponding medical records were available. High number of scrub typhus cases 268(35.5%) was detected among these cases. Currently we are analyzing the clinical-epidemiological data and testing for other pathogens. This study will reveal undiagnosed pathogens of acute febrile illness/FUO in Vietnam which will be useful for setting up guidelines on management of FUO in Vietnam. This study will also give an opportunity to discover new syndromes or diseases or even pathogens that are prevalent in tropical countries like Vietnam.

O-15(P-72)

Characterization of dengue 1 epidemic strains in Hanoi, Vietnam in 2009

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Dengue viruses are mosquito borne flaviviruses that are wide spread in tropical southeast and sub tropical areas of the world. These viruses that cause dengue infection consist of four serotypes, DEN-1, -2, -3 and -4. About 60-100 million cases of dengue infection are estimated to occur in the world each year. Vietnam is hyperendemic for dengue, with all four serotypes circulating. Since the first dengue fever and dengue hemorrhagic fever (DF/DHF) outbreak in 1963, the incidence of DF/DHF has increased dramatically. A previous (1998) epidemic caused by DEN-3 in Vietnam resulted in 234,942 reported cases of DHF and 377 deaths. Recently DEN-1 outbreak started from the southern part of Vietnam in 2007 and spread to the north in 2009. DF/DHF cases were significantly increased to 18,485 with 4 fatal cases in northern Vietnam.

In this study, we performed molecular analyses of DEN-1 genetic diversity circulating in Vietnam between 1999 and 2009. Eight sub-clusters of genotype I were noted to be circulating in Vietnam. Biological characterization of DEN-1 strains isolated in different yeas in Hanoi were compared by infectivity to cultured cell lines and live mosquito vectors such as *Aedes aegypti* and *Aedes albopictus*. The endemic strain of DEN-1 isolated in 2009 showed better proliferation in *Ae. aegypti* and Vero cell line. Entire sequence of open reading frame was determined and 5 unique amino-acid substitutions were confirmed in DEN-1 strain isolated in 2009. These amino acid substitutions might be related to the infectivity to mosquito and / or humans that spawned large DEN-1 outbreaks in Ho Chi Minh and Hanoi in 2007-2008 and 2009, respectively.

Complement levels correlated with disease severity in dengue patients in Indonesia

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Dengue virus (DENV) is distributed throughout tropical and subtropical areas of the world, where approximately 2.5 billion people are at risk of infection. DENV causes dengue fever (DF) and its severer form, dengue hemorrhagic fever (DHF) with an estimated 50-100 million DF cases and 250-500 thousand DHF cases reported every year. Despite its importance in the public health, the mechanism of increasing disease severity has not been fully understood. Antibody-dependent enhancement (ADE) of infection is a leading hypothesis involved in disease severity. In vitro studies demonstrated that low complement activities correlated with increased activities of enhancing antibodies. In this study, a relationship between complement levels and disease severity was analyzed using dengue patient sera collected in Indonesia.

The complement activity (CH50) and the levels of complement components(C1q, C2, C3, C4, C5, factors B, D, H and I, mannose-binding lectin [MBL], C1q-fixing circulating immune complexes [CIC-C1q], and magnesium ions [Mg^{2+}]) were examined in dengue patients in Indonesia. The study subjects were 85 dengue patients in Indonesia. We classified DF patients into two groups, based on the clinical progression of the disease: a deterioration group in which the clinical diagnosis worsened from DF to DHF within 2 days; and a DF group in which the patients recovered without progressing to the DHF stage. For analyses, we focused on the serum samples collected at the DF stage from both groups.

Mean CH50 value and mean levels of C1q ($P<0.001$), C2 ($P<0.01$), C4 ($P<0.01$) and factor B ($P<0.05$) in the deterioration group were significantly lower than those of the DF group, while level of C3 was significantly higher ($P<0.01$). No significant differences were detected between the deterioration and DF groups in levels of C5, CIC-C1q, MBL, and factors D, H and I, as well as Mg^{2+}. In addition, the level of nonstructural protein 1 (NS1), which has been shown to activate the complement system, was significantly higher in the deterioration than DF group.

This study demonstrates that reduction in serum levels of different factors (C1q, C2, C4 and factor B) involved in the classical and alternative complement pathways are associated with increased severity of dengue virus infection. We hypothesized that the ADE might occur under the low complement condition during the acute phase in some DF patients, potentially leading to increase in the viremia level and disease severity.

*Endowed from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

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Identification of a novel inhibitor against dengue virus NS2B/NS3 protease by a structure-based study

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The non-structural protein 2B/3 (NS2B/NS3) serine protease complex of the dengue virus (DENV) is required for maturation of the viral polyprotein. Therefore, the protease activity represents a promising target for the development of antiviral inhibitors. Dissociation of the NS2B cofactor from NS3 diminishes the enzymatic activity of the complex. The aim of this study was to identify a novel small inhibitory molecule that interferes with the interaction between NS2B and NS3. The methods employed included a structure-based screening and a cell-based virus replication assay. A library containing 661,417 small compounds, derived from the Molecular Operating Environment (MOE) lead-like database, was docked to the NS2B/NS3 structural model. Thirty-nine compounds showing high scoring were tested in a secondary screening via the cell-based virus replication assay. Compound #12 was discovered to inhibit replication of all serotypes of DENVs (EC_{50} = 3.67 to 17.04 μM). Compound #12 blocked autocleavage of the NS2B/NS3 protease complex, as well as the protease activity against a short peptide substrate *in vitro*. Compound #12 is thus the first promising small compound inhibitor that targets the interaction between NS2B and NS3. Interestingly, combination treatment with ARDP0006, a known inhibitor of the NS2B/NS3 protease complex that targets the protease catalytic site of NS3, synergistically increased the inhibitory effect of compound #12 against DENV replication. These results suggest that combination therapy of compound #12 and ARDP0006 could be used in therapeutic applications against the NS2B/NS3 protease complex.

Surveillance of infection with Japanese encephalitis virus and hepatitis E virus among swine in northern Luzon, Philippines

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Background: In swine, many zoonotic pathogen are reported such as Influenza A, Japanese encephalitis, Hepatitis E and Nipah viruses. In 2008, Ebola-Reston virus was detected from swine in the Philippines, and lessons learned from the incidence were the importance of the continuous surveillance and monitoring the possible zoonotic diseases. Since the zoonosis among swine in the Philippines are not yet clear, the purposes of this study are 1) to know the seroprevalence of Japanese encephalitis virus (JEV) infection and Hepatitis E virus (HEV) infection, 2) to determine the virus type of JEV and HEV, among swine in Tarlac Province in the Philippines.

Methods: A total of 200 swine serum, fecal and nasal swabs were taken in July, September, December 2010 and January 2011 from the backyard in Tarlac Province. IgM ELISA and IgG ELISA were conducted for both JEV and HEV. Partial envelope gene of JEV and partial ORF2 gene of HEV were tried to detect by RT-PCR and then phylogenetic analysis using neighbor-joining method were conducted.

Results: For JEV antibodies, July and September showed more than 70% positive both in IgG and IgM then decreased in December and January. The viral RNA was detected from the serum sample collected in July and it was clustered into genotype 3. For HEV antibodies, less than 10% of swine showed IgM positive in July, September and December, and negative in January. For anti-HEV IgG, 20-50% of swine showed positive through the study period and highest in July. The viral RNA was detected from feces samples collected in September and those were clustered into genotype 3. Those were separated into 2 subclusters due to the collected area.

Discussion: We have found that JEV antibodies are higher in July and September, during rainy season in the Philippines. Also, the environmental factors such as density of the mosquitoes and agricultural information could be contributed to this scenario. JEV genotype 3, which is widely circulating in Asia were detected from swine serum and the virus we have found from mosquito in our previous study was also genotype 3. Thus it seems that currently genotype 3 is the major genotype circulating in Northern Luzon, Philippines. This was the first report to show the presence of HEV in swine in the Philippines. HEV genotype 3 is commonly reported from Europe, Japan and other countries. The risks of the HEV infection should be considered for the people handling swine products including backyard swine raisers.

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Metagenomic approach to identify tick-borne pathogens by using ultra high throughput DNA sequencing and data analyzing technologies

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Tick can transmit a variety of viral, bacterial and protozoal pathogens, which are often zoonotic. The fact that there are up to 9 rickettsial diseases caused by spotted fever rickettsia newly reported between 1984 and 2001 indicates there are many potential pathogens which have not been unidentified or found in ticks. Since metagenomic analysis provide a powerful tool to detect unculturable microbes from environmental samples such as sea water, hot spring and soil, and analyze microbial populations of animal microbial floras such as guts or rumen, we used similar approach to analyze tick "microbiome".

We prepared bacteria-enriched fractions form the lysates of 6 tick species, including *Ixodes persulcatus, I. ovatus, Amblyomma variegatum, A. testudianarium, Haemaphysalis longicornis and H. flava*. Purified genomic DNA samples were subjected to sequencing by the second generation sequencer (Roche FLX454), and sequence reads were assembled *de novo* into contigs. Contigs containing more than 300 bases were analyzed by using Batch Learning Self-organizing Map (BLSOM) and BLAST.

The results of this study are summarized as follows:

1) BLSOM was useful to relegate unknown gene fragments to phylotypes at the genus level.
2) Bacterial population profiles were different from species to species. Sequences derived from Chlamydiae were frequently found in *I. persulcatus* and *H. flava,* and those from Rickettsiales were dominant in *I. ovatus*.
3) Gene sequences related to bacterial virulence, or phages were found.

These results help us to construct a database of tick microbes which may contain unknown zoonotic pathogens. We are trying to develop methods to detect RNA viruses which have potential to cause human and animal diseases. Our efforts to develop the tick pathogen database may lead to the empowerment to predict emerging tick-borne diseases.

The generation of immune-modulator lectin-galectin-9 in acute dengue virus infected patients in Philippine

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We previously reported a marked elevation of galectin-9 (GAL-9) in acute human immunodeficiency disease (HIV) infection and its rapid decrease after highly activated anti-retroviral therapy (HAART). The marked elevations were not found in chronic HIV infection or other AIDS patients with opportunistic infections, indicating that GAL-9 maybe specific to acute virus infection. GAL-9 is reported to modulate immune system by inducing apoptosis in exhausted Th1 and ameliorates inflammation. Therefore, we measured such immune-modulator protein in patients with acute dengue virus infection to see the immune response changes before after recovery.

A total of 65 patient's (mean age: 23±4; 24 females and 41 males) plasma and serum samples were obtained from San Lazaro Hospital, Manila in 2010. Samples were collected at critical phase (day4-5 of illness) and recovery phase (day7-8 of illness) from each patient. The levels of plasma GAL-9 were measured by elisa in 52 dengue fever (DF) and 12 dengue hemorrhagic fever (DHF) patients. Clinical symptoms such as bleeding, headache, rash were obtained after hospitalization. White Blood cells, hemoglobin, hematocrit, platelet count, and prothrombin time were also measured for analysis.

Our result showed that plasma GAL-9 were elevated in both DF and DHF compared with normal control and the levels were significantly decreased in recovery phase (Wilcoxon signed-ranks test, $p=1.6\times10^{-7}$). Interestingly, the elevations were higher in DHF than DF in critical phase ($p<0.05$, unpaired t test). These temporal elevations of GAL-9 in critical phase especially in DHF demonstrate that plasma levels of GAL-9 reflect the disease severity in acute dengue virus infection. However, the levels of GAL-9 have no correlations with any of the above clinical data. GAL-9 was believed to have anti-inflammatory activities. The elevations not only in acute HIV infection but also in Dengue virus infection indicate that GAL-9 is a novel mediator of spontaneous recovery of acute virus infection.

Acknowledgement: We are grateful to Prof. Hirashima and Dr. Niki for kind measurement of plasma GAL-9 in patients.

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Chikungunya virus replicates in human keratinocyte cell lines

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Chikungunya virus (CHIKV) is a re-emerging mosquito-borne alphavirus and its infection has recently found in several countries. Acute-phase CHIKV infection has been reported to cause mild to severe febrile illness, and in some patients, this may be followed by persistence symptoms in the convalescence phase. Some reports show that symptom severities of this disease are related with cytokines and chemokines regulation. Cytokines and chemokines are thought to play an important role in viral immunopathology. The first vertebrate cells to contact CHIKV are skin cells, followed by those in the draining lymph node. CHIKV are then able to enter monocyte cells and macrophages. However, there are little known about the information of CHIKV-infection in human skin cells. To clarify the role of skin cell, as a first target of mosquito bite for CHIKV-infection, we investigate the kinetics of CHIKV in human keratinocyte cell lines and the regulation of immune response against CHIKV infection. CHIKV, we isolated from Thai patient, was used in this study. CHIKV-infection kinetics were performed and compared among three cell lines; HaCat (human keratinocyte cell lines), Vero and C6/36. Virus titration from each cell line was determined by plaque assay. To assess the regulation of inflammatory mediators and innate immune response following CHIKV infection of HaCat cells, the levels of cytokines and chemokines were evaluated by ELISA and RT-PCR. We found that CHIKV actively infected in HaCat cell. Although, the virus production from HaCat cell was lower than those in Vero cell and C6/36. IL-8 production was significantly suppressed by CHIKV infection. Our study, we demonstrated that CHIKV can infect and replicate in human keratinocyte cell lines and regulated some cytokines and chemokines production. These regulations may be related not only the inflammation but also chemotaxis of immune cells at mosquito biting site of skin.

Analysis of cross-reactive mouse monoclonal antibodies against dengue virus NS1 in vitro and in vivo

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During the course of dengue virus (DENV) infection, strong B-cell immune response against nonstructural protein 1 (NS1) is induced. As a result, the produced antibodies (Abs) against NS1 potentially affect the virus production because these Abs and virus exist together in patients' blood. We previously obtained anti-NS1 mouse monoclonal Abs (MAbs). In this study, we examined the effect of these MAbs to DENV-2 production and found that MAbs 4C2, 4G11 and 4E5 exhibited weak neutralizing activity by focus reduction assay. We further found that two of them, 4C2, which was specific to DENV-4 NS1, and 4G11, which was specific to DENV-1 NS1, protected suckling mice from lethal infection with DENV-2. These MAbs cross-reacted with pre-membrane (prM) protein of DENV-2, but not with DENV-2 NS1, when immunoprecipitation was performed to DENV-2 particles released from infected cells, suggesting cross-reaction of anti-NS1MAbs to prM protein. The 4G11 mapped to the C-terminal of DENV-1 NS1 and the 4C2 mapped to the N-terminal of DENV-4 NS1. These observations suggest that serotype specific antibodies against DENV-1 and DENV-4 potentially cross-react with DENV-2 prM protein on virus particles. Previously, the other groups demonstrated that anti-prM monoclonal antibodies were able to increase infectivity of DENV through the antibody-dependent enhancement (ADE). We suspected that MAbs 4C2 and 4G11 may have effective activity to DENV-2. The serotype-specific anti-NS1 antibodies might increase the virus infectivity of other serotypes of DENV through the weak interaction with prM, namely, ADE. MAbs 4G11 showed ADE activity at high concentration compared with anti-E mouse monoclonal antibody, 4G2. We propose that antibodies against NS1 potentially have ADE activity. We currently examine ADE by monoclonal antibodies from human.

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The TLR3 agonist Poly I:C inhibited the replication of Chikungunya virus in BEAS-2B cells

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Interferon –α/β (IFN-α/β), the so called type I IFN, was first discovered in the 1950s, are produced by eukaryotic cells in response to viral infection, and play a pivotal role in innate immunity directed against viral infection. Both natural and synthetic double-stranded (ds) RNAs elicit IFN production. Among the synthetic dsRNAs, Polyriboinosinic: polyribocytidylic acid (Poly (I:C), a synthetic dsRNA analogue, the TLR3 agonist was found to the most potent IFN inducer in vivo studies in mice. In this study, we used poly (I:C) to treat the BEAS-2B cells and test the effects of poly (I:C) treatment on the replication of Chikungunya virus (CHIKV).

We have found that Poly (I:C) inhibited the replication of CHIKV. Treatment of BEAS-2B cells with poly (I:C), inhibited the CPE (cytopathic effects) induced by CHIKV, also decreased the virus titers in the supernatant of culture meida after CHIKV infection. The virus titers from the cells treated with poly(I:C) were much lower compared the viruses from the cells without Poly (I:C)treatment. Poly(I:C) treatment in BEAS-2B cells induced the production of IFN-β . The induction of IFN-β contributed to the inhibition of CHIKV. Based on the results, CHIKV was sensitive to the IFN-β. Innate immunity may be important for the control of CHIKV infection. Poly (I:C) may also be used as a candidate of adjuvant for the CHIKV vaccine in the future.

Infection of mouse cells with dengue virus and Japanese encephalitis virus

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Dengue virus (DENV) and Japanese encephalitis virus (JEV) belong to genus *Flavivirus* and are mosquito-borne viruses. Although they share similar virological characteristics, they cause different symptoms in human. In addition, JEV is pathogenic to mice but DENV is not, because there is a critical difference in their infectivity in mice. Because of this, mouse model for DENV infection is not available, and which brings difficulty to develop a new drug and an efficient vaccine. Our final goal is to develop mouse model for DENV infection. To understand different susceptibility of these viruses, we analyzed them using mouse cell lines and primary macrophage derived from mouse.

Several cell lines, transformed by SV40Large T antigen derived from BALB/c and C57BL lungs were highly susceptible to DENV; however, in these cells, the productions of DENV were repressed completely 6 days after infection. Contrary, JEV could maintain higher level of virus production. We observed a sudden increase of the production of type I interferons (IFNs) in the culture supernatant 2 days after infection with DENV. The macrophages from IFN-alpha/beta receptor KO mice were infected with DENV and higher level of virus production was observed over one week. This suggests that type I IFN plays a main role to restrict DENV replication but not JEV replication in mouse cells. Presumably, JEV is able to efficiently repress mouse IFN. We also found that expression of JEV protein enhanced the DENV production in mice cells. For further analysis, we performed the experiment using DENV/JEV chimeric virus clones. The chimeric virus replicated in mouse cell line efficiently.

Our observation suggests that JEV has a great advantage in virus replication in mouse cells. A detailed study will contribute to the development of mouse model for DENV infection.

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Isolation of chikungunya virus from patients clinically diagnosed as dengue fever in Surabaya, Indonesia, 2010 - 2011

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Chikungunya virus (CHIKV) belongs to the genus *Alphavirus* of the family *Togaviridae,* and causes a severe febril illness called chikungunya fever in humans. CHIKV is transmitted to humans by the bite of infected *Aedes* mosquitoes (*Aedes aegypti* and *Aedes albopictus*). Dengue virus (DENV) belongs to the genus *Flavivirus* of the family *Flaviviridae*, is also transmitted by mosquitoes, and causes dengue fever (DF) in humans. Both diseases show similar clinical symptoms: headache, fever, muscle pain, joint pain, etc. Thus, differential diagnosis between chikungunya fever and dengue fever is usually difficult, if it is based only on their clinical manifestation. Indonesia Republic is one of the dengue endemic countries, where CHIKV also exists. In this study, we estimated the percentage of CHIKV infections in the patient population clinically diagnosed as dengue fever. We also performed phylogenetic analyses, using CHIKV isolates.

A total of 596 and 92 serum samples were collected from patiens who had clinically been diagnosed as dengue fever from January through July 2010 and from January through March 2011, respectively, in Surabaya, Indonesia. These sera were inoculated on Vero or C6/36 cells. Samples showing cytopathic effects (CPE) within 5 times of blind passages were subjected to RT-PCR by using CHIKV and DENV specific primers, followed by determination of nucleotide squences.

Of 596 samples in 2010, 54 showed CPE, of which 10 samples showed stronger CPE than the other 44. Finally, the 10 (18.5%) and 44 (81.5%) isolates were determined as CHIKV and DENV by RT-PCR, respectively. On the other hand, of 92 samples in 2011, 37 showed CPE, of which 2 (5.4%) and 35 (94.6%) isolates were identified as CHIKV and DENV, respectively. Phylogenetic analysis of the nucleotide squences indicated that all the CHIK isolates were classified into the Asian group.

In this study, we revealed that a relatively large number of chikungunya cases were included in patients clinically diagnosed as dengue fever in Surabaya. It is important to continue the surveillance to detect mutant strains of CHIKV.

*Endowed from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

Unusual manifestation of dengue virus infection and its management

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Dengue virus (DENV) infection is one of the important health problems in Indonesia, although the mortality rate has been decreased. Many cases with dengue shock syndrome and unusual manifestation of dengue infection are difficult to predict the earlier time for getting a good management. We made updated management of unusual manifestation in dengue infection for getting a better health.

Data were compiled from the Dr. Soetomo Hospital Surabaya and the Soerya Hospital in Sepanjang Sidoarjo in 2009, 2010 and 2011. The diagnosis of all cases was based on the criteria of WHO examination in Institute of Tropical Disease, as well as serotyping by PCR. The unusual cases of DENV infection were treated based on the new protocol presented by WHO for diagnosis and treatment in 2009.

In 2009, three report cases appeared with unusual manifestation of DENV infection: (a) DHF grade III with liver involvement and had bilateral pleural effusion. (b) DHF grade III with liver involvement and encephalopathy. (c) DHF grade III with liver involvement, acute kidney injury, myocardial involvement and encephalopathy. In 2010, two report cases come up with unusual manifestation of DENV infection. An eight-year-old girl with obesity suffered from DENV infection with liver involvement and bilateral pleural effusion. An eight-month-old girl with undernutrition suffered from DENV infection with bilateral pleural effusion and liver involvement. In 2011, two report cases were experienced with unusual manifestation of DENV infection. A ten-year-old girl with obesity was diagnosed as DENV infection with bloody diarrhea on the first day of admission. Unfortunately, coincident with bilateral pleural effusion and ascites, recurrent shock occurred and making the doctor in charge unable to help her, due to attack of cardio respiratory arrest that immediately occurred. A ten-month-old girl suffered from DENV infection with a right unilateral pleural effusion and liver involvement showed an attack of cardio respiratory arrest making the doctor in charge enable to help her. Besides 7 cases that reported above, we found coincidence cases of DENV infection and typhoid fever which showed an unusual clinical manifestation.

In conclusion, if we found unusual manifestation of dengue infection, we should consider a new method management with monitoring carefully.

*Endowed from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

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Vertical transmission of dengue virus in *Aedes aegypti* collected in Surabaya, Indonesia 2008 - 2011

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Dengue virus (DENV) is transmitted to humans by the bite of an infected *Aedes* mosquito. Once infected, the mosquito remains infected for life, transmitting the virus to susceptible individuals while probing and feeding. The transmission cycle between humans and mosquitoes is a main mechanism for the maintenance of DENV in a large human populations and abundant *Aedes* mosquito populations in urban settings in many tropical areas. Transovarial transmission of DENV in *Ae. aegypti* mosquitoes, the principal vector, may play another significant role in the maintenance of DENV in nature: it may allow the virus to survive dry or rainy seasons. The possibility of vertical transmission of DENV in *Ae. aegypti* was investigated by detection of the virus in adult males (sweep net collection) and males emerged from collected larvae.

Mosquito collections were done in July-November 2008 (dry season), January-March 2009 (rainy season), August-November 2010 (dry season) and January-April 2011 (rainy season). Ninety-two pools of adult male *Ae. aegypti* (n=1525) were examined (33 pools: dry season and 59 pools: rainy season). Virus isolation was carried out using the C6/36 cell culture and the DENV typing by reverse transcription polymerase chain reaction.

Of 28 pools collected in rainy season, 2009, dengue type 1 virus (DENV1) was isolated from 1 pool (minimum infection rate (MIR) = 16) from wild adult males and 2 pools (MIR = 6) from adult males emerged from larvae collected. Of 31 pools collected in rainy season, 2011, DENV 1 was isolated from 1 pool (MIR = 26) from wild adult males and 5 pools (MIR = 9) from adult males emerged from larvae. However, DENV was not isolated from samples collected in 2008 and 2010 (dry seasons).

DENV isolations from wild caught males of *Ae. aegypti* indicate the occurrence of transovarial transmission in nature. Vertical transmission was mainly observed in rainy season when dengue infections or cases in humans frequently occurred.

*Endowed from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

Construction of a dengue type 4 extracellular subviral particles using a High Five expression system

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Dengue fever and dengue hemorrhagic fever are mosquito-borne viral diseases caused by dengue virus (DENV) infection. Despite the importance of serodiagnosis, ***the antigens sourced directly from infectious agents may have a problem in their use for diagnostic antigens because of their low productivity. Recently, insect cell expression systems have been increasingly used*** for high-level ***production of recombinant proteins.*** We previously established an insect Sf9 cell line and a mammalian CHO cell line producing non-infectious DENV type 2 subviral extracellular particles (EPs). In the present study, a *Trichoplusia ni* BTI-TN-5B1-4 (High Five) cell line producing DENV type 4 (DENV4) EPs was generated.

The *prM* and *E* genes of DENV4 were inserted into the plasmid vector pIHAbla, which contains the *Bombyx mori* nucleopolyhedrovirus (BmNPV) IE-1 transactivator, the BmNPV HR3 enhancer and the *B. mori* actin promoter. The plasmid was transfected into High Five cells and the transfected cells were selected by several passages of cultivation in the presence of blasticidin, to generate a stably expressing cell line. The amount of EP antigens released from these cells were examined by a sandwich ELISA and the biophysical properties were analyzed on a sucrose density gradient.

A stably expressing cell line producing extracellular forms of DENV4 antigen was successfully generated. Comparison of the static culture and the shaking culture showed that approximately three times larger amount of the DENV4 EPs were obtained by shaking culture than static culture. The production level obtained by the High Five expression system was more than 10 times higher than that obtained by the DENV4-infected C6/36 cells. The sucrose density gradient centrifugation analysis revealed that the EPs produced by the recombinant High Five cells were released in a proper particulate form.

*Endowed from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

P-86

Household and individual determinants of dengue patient hospitalization in Hanoi City, Vietnam: A case-control study

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To determine the effect of household environments and individual attributes on the risk of dengue in Hanoi, we conducted a matched case-control study in 3 urban districts. We compared 53 hospitalized dengue cases reported between November 1 and December 14 in 2010 with 201 neighborhood controls. Analysis revealed that individual attributes such as younger age (18--39 years old [adjusted odds ratio (aOR) = 4.29; 95% confidence interval (95% CI) = 1.60--11.53], 1--17 years old [aOR = 3.94; 95% CI = 1.19--13.01]), and less than 2 years of stay in current residence (aOR = 2.73; 95% CI = 1.12--6.65) were important risk factors of dengue. Additional household risk factors included the presence of *Aedes aegypti* in the household (aOR = 2.54; 95% CI = 1.12--5.76) and high household income level (aOR = 2.99; 95% CI = 1.26--7.03).

Predictive maps of *Aedes aegypti* and *Aedes albopictus* distributions in Vietnam

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Among insect vector-borne diseases, the dengue (DF)/dengue hemorrhagic fever (DHF) disease complex is one of the most important diseases with 2.5 billion people living at risk worldwide. Recent epidemics of Chikungunya fever have had great impact on many countries and become public threat. These arbovirus causing infectious diseases are transmitted by mosquito species, *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), the former species is more effective vector and domesticated than the latter, suggesting urbanization will affect the vector distributions. Since DF/DHF vaccine is unavailable for practical use and there is no intermediate host, transmission can be prevented only by reducing human-vector contact. Therefore, vector control is crucial and effective in reducing dengue transmission. For practical vector controls, predictive maps of dengue vectors, *Aedes aegypti* and *Aedes albopictus*,distributions in Vietnam were produced using species presence data and environmental variables, human population density, mean temperature, urban index and mean precipitation collected during the study period. The analyses were conducted for each 5 collections by the maximum entropy modeling (Maxent) of species geographic distribution. The results showed that human population density and mean temperature were highly important for predictions and explained 88.1 – 99.0% of the distributions for both *Aedes aegypti* and *Aedes albopictus* at provincial scale in Vietnam. For *Aedes albopictus*, precipitation and urban index also were contributing factor to the predictions. The present study has indicated that not only temperature but also environmental variables related to human activity such as human population density and urban index are important for accurate predictions of dengue vectors, and the predictive maps will be useful for dengue control operations in changing environment. For predictions at city/town/village scale, further study is necessary.

P-88

Circulation of Nam Dinh and Banna viruses in Viet Nam, 1964 – 2011

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Banna virus and Nam Dinh virus (NDiV) were first detected in China 1987 and Vietnam 2002, respectively. The Banna virus was isolated from sera of unknown fever patients as well as from CSF of acute encephalitis syndrome patients, mosquito and pig blood samples from several Asian countries. But the NDiV was only isolated from CSF of acute encephalitis syndrome patients, and mosquito samples in Vietnam so far.

In this report, Sandwich ELISA has been used for retrospective and prospective studies in order to detect NDiV, Banna viruses from all isolates which were confirmed positive with Japanese encephalitis virus (JEV) or unidentified virus strains during 1964– 2011. The results showed that the first NDiV strain was detected in 2002 in the northern Vietnam and the virus is confirmed circulating in northern central, southern and Tay Nguyen during 2002 – 2011. Whereas, the first Banna virus strain was detected in 1964 in the northern Vietnam from human isolates and the virus is confirmed circulating in northern, central, southern and Tay Nguyen during 1964 – 2011.

These viruses are possible arbovirus pathogens, further study on molecular epidemiology, clinical and immunological features need to be conducted in the coming time.

Molecular genotyping of *Mycobacterium tuberculosis* isolated from Hanoi City in Viet Nam

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By using genomic information obtained from the variable number of tandem repeats (VNTR), spoligotyping and region of difference (RD), we characterized *Mycobacterium tuberculosis* (TB) isolates that have spread in Hanoi, Viet Nam.

In total, 347 TB strains from patients with new active tuberculosis in Hanoi were subjected to genotyping using the optimized MIRU (Supply [15 loci]) VNTR system and 16 other loci. Of these, 21 isolates were excluded from further analyses due to DNA fractions containing plural strains or no PCR products. The cluster formation rate calculated from the VNTR typing was 52.1% (170/326) in the Hanoi strains. In Tokyo area, the rate was 48.3% according to recent data, indicating that the tendency of cluster formation is almost the same. Also, we found some distinctive features of Hanoi strains: (1) The largest cluster was composed of 29 TB isolates (8.9% [29/326]). (2) Of the 326 isolates, 175 (53.7%) showed Beijing genotype. This percentage was nearly the same as previously reported in Ho Chi Minh City. (3) Analyses of RD in the mycobacterial genome revealed that TBs of Beijing “ancient” type are frequently observed in Hanoi, whereas TBs of Beijing “modern” type are predominant in the world, except in Japan and Korea.

Our analyses help establishment of the VNTR system suitable for Hanoi TB strains. Indeed, the discrimination power of the loci of Supply (15)-VNTR plus VNTRs-2163a, 1892, 2372, 3336 and 2074 were relatively high. Our results suggest that the evolutional genetic types of TBs that spread in Hanoi might be similar to those seen in Japan.

O-20(P-90)

Molecular characterization of drug resistant *Mycobacterium tuberculosis* from Asian countries

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<Introduction> Multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) has become a major problem in tuberculosis treatment. Prompt determination of MDR-TB is important not only for the appropriate treatment of patients but also for the prevention of the spread of resistant tubercle bacilli. In *M. tuberculosis*, resistance against rifampicin and isoniazid is highly associated with mutations on the responsible genes, i.e., *rpoB* for rifampicin, *katG* and *inhA* for isoniazid. Recent technology makes it possible to determine MDR-TB quickly by checking specific mutations using PCR and hybridization. However, the ratio of each mutation has not been determined in many countries despite it can differ in each country. In this study, we determined mutations that possibly related to drug resistance in MDR-TB isolates obtained in Bangladesh, Nepal, Myanmar and Japan.

<Samples and Methods> MDR-TB isolates collected in Bangladesh (n=220), Nepal (n=47), Myanmar (n=132) and Japan (n=89) were used for the mutation analysis. Following gene regions were sequenced and analyzed, *rpoB*: nucleotide position 1276-1356 (rifampicin resistance determining region, RRDR), *katG*: 823-1140, *inhA*: minus50 - minus1 (promoter region).

<Results> In *rpoB* RRDR, most frequently observed mutation was C 1349 T in all countries and its ratio among MDR-TB was around 50% (40-64%). Most of other mutations were also commonly found in all country samples and sum of the ratios of top seven prevalent mutations were more than 70% in each group. The percentage of MDR-TB isolates with no mutations in *rpoB* RRDR was 4.5% (3.4-6.4%). Regarding isoniazid resistance, G 944 C mutation in *katG* and C -15 T mutation in *inhA* were majorities among observed mutations and the sum ratios of these among MDR-TB were 87, 89, 94 and 48% in Bangladesh, Nepal, Myanmar and Japan, respectively. No mutations were observed in either *katG* or *inhA* in 5.9, 4.3 and 2.3% in Bangladesh, Nepal and Myanmar MDR-TB isolates, respectively, whereas the ratio was 34.8% in Japanese isolates.

<Conclusions> The variation and ratio of mutations on *rpoB, katG* or *inhA*, which are thought to be associated with rifampicin or isoniazid resistance, were similar among MDR-TB isolates in Bangladesh, Nepal, Myanmar and Japan. More than 70% of rifampicin resistant isolates can be determined in these countries by detecting seven types of *rpoB* mutations. For isoniazid resistance prediction, most of the resistant isolates can be determined by detecting two major mutations in *katG* and *inhA* in Bangladesh, Nepal and Myanmar. However, additional analyses will be needed for the determination of isoniazid resistant TB in Japan because of the higher ratio of resistant isolates that were not relying on those two mutations. Thus, surveillance of resistance-associated mutations in each country seems to be important for an effective determination of drug resistant *M. tuberculosis* by rapid mutation detection systems with PCR and hybridization.

Etiology and clinical presentation of childhood pneumonia in the Eastern Visayas Regional Medical Center, Tacloban City, Western Visayas, Central Philippines

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ABSTRACT

A prospective observational study on etiology and clinical presentation of childhood pneumonia was started in May 19, 2008 at the Eastern Visayas Regional Medical Center in Central Philippines. Children 8 days old to 13 years old requiring admission for pneumonia were enrolled based on the classification used for the WHO ARI Case Management for children <5 years old and Philippine Pediatric Society Clinical Practice Guidelines for the older child. Blood, respiratory and CSF (when indicated) samples were collected to determine **bacterial and viral etiology.** Enrolled cases were provided with the recommended empiric antibiotics for pneumonia. Clinical data recording on admission and during hospitalization were done by the project staff.

From May 19, 2008 to May 31, 2011, 1584 patients were enrolled. Majority (1474, 93.2%) were less than 5 years old; male to female ratio was 56%:44%. There were 817 severe pneumonia cases (51.6%) and 605 (38.2%) very severe pneumonia. Chest x-ray consolidation was observed in 18%.

Virus detection rate was 36.4%: RSV was the major viral pathogen detected followed by the rhinoviruses. Invasive bacterial isolation rate was 2.1% for major pathogens including *S pneumoniae* (13), *H influenzae* (3), *S typhi* (2). Co-infection with 2 or 3 viruses and virus and bacteria were observed.

One hundred eight patients (6.8%) died, of which 78 (72.2%) had very severe pneumonia. Fatality was high in cases with Influenza A even if the prevalence was low (3/17=17.6%), with rhinovirus, 8.6% and *S pneumoniae* 7.7% (1/13).

In summary, a third of childhood pneumonia is caused by viruses. Isolation rate for bacteria is low. Childhood mortality from pneumonia is high among patients with severe or very severe pneumonia. Viruses aside from bacteria are major causes of childhood pneumonia mortality.

O-22(P-92)

Respiratory Syncytial virus is the major viral pathogen and its co-infection with other respiratory viruses Increases the risk of pediatric pneumonia hospitalization: A three-year population-based study in central Vietnam

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Background: Acute respiratory infection (ARI) is a leading cause of mortality and morbidity among children and respiratory viral pathogens are suspected to play a major role. However comprehensive population-based data have been lacking for pediatric ARI and the roles of respiratory viral pathogens in the development of pneumonia remain largely unknown.

Objectives: To determine the incidences of hospitalized pediatric ARI and to investigate the effect of respiratory virus infections on the risk of pneumonia in Vietnam. Methods: Population-based prospective surveillance and case-control study of hospitalized pediatric ARI were conducted in Nha Trang, central Vietnam from April 2007 through March 2010. Healthy controls were randomly recruited from the same community. Nasopharyngeal samples were collected and tested for 13 respiratory viruses using multiplex polymerase chain reaction assays.

Results: A total of 1,992 ARI episodes including 397 (19.9%) with pneumonia were enrolled. The incidence of hospitalized pneumonia among children under 24 months of age was 2,171.9 per 100,000 (95% confidence interval, 1,947.9 – 2,419.7). The majority of the ARI cases (60.9%) were positive for at least one virus. Human rhinovirus (HRV) (24.2%), respiratory syncytial virus (RSV) (20.1%), and influenza A virus (FLUA) (12.0%) were the most common and 9.5% (8.7% double and 0.8% triple) were associated with multiple viral infections. Among the hospitalized ARI cases, HRV, RSV and HMPV infections independently increased the risk of pneumonia (adjusted risk ratio 1.26, 95% CI 1.03 to 1.54; 1.3, 1.05 to 1.59; and 1.72, 1.1 to 2.68, respectively). RSV further increased the risk of pneumonia, when co-infected with HRV, HMPV and PIV3 but not with FLUA. The case-control analysis revealed that RSV and FLUA increased the risk of ARI hospitalization but not HRV.

Conclusions: RSV is the single most common viral pathogen and co-infection with other respiratory viruses increased the risk of pneumonia among Vietnamese children.

Frequent detection of anti-tubercular-glycolipid IgG and IgA antibodies in the healthcare workers with latent tuberculosis infection in the Philippines

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Rapid identification and treatment of latent tuberculosis infection (LTBI) has a great imperative to combat against *Mycobacterium tuberculosis* infection in human especially among population with increased risk. Recent introduction of immune-based interferon-γ release assay that clarified high rate of infection in TB-high risk population may also produce pseudo-negative responses by low CD4+ T cell counts in HIV infection. In the current study, we aimed to analyze IgG and IgA antibodies against tubercular-glycolipid antigen (TBGL-IgG and -IgA) in individuals with latent tuberculosis infection (LTBI) in the Philippines.

A total of 31 healthcare workers (HCW) (mean age: 35.5 ±12.2) and 57 newly diagnosed asymptomatic HIV-carriers (HIV_AC) (mean age: 28.7±6.65) either positive or negative by Quantiferon-TB Gold test (QFT) were studied between March and October of 2010. The TBGL-IgG-positive proportion was significantly higher (P=0.02) in QFT-positive (11/15) than in –negative HCWs (5/16) (κ: 0.42; 95% CI: 0.10~0.73; indicates moderate association) but not in HIV_AC. None of the QFT-negative HCWs had a double-antibody (TBGL-IgG+IgA) positive response. The IFN-γ levels in non-stimulated QFT-plasma (IFN- γ_nc) were positively correlated with the TBGL-IgA titers in the QFT-positive (p=0.006) but not in QFT-negative HCWs. The QFT-positive rate, in the HIV_AC was CD4+ T cell count dependent (p=0.012) and was significantly lower than in HCW (p=0.03). Furthermore, a higher proportion of double-antibody positive responders were observed in the HIV_AC with CD4+ count <350/μl group compared to that of ≥350/μl group (29% and 15% respectively). Interestingly, in HIV_AC, the TBGL-IgA but not -IgG titers were inversely correlated with CD4+ T cell counts (P=0.018), and were associated with serum IgA (p=0.0002).

In conclusion, TBGL-IgG may indicate LTBI in healthy adults. The significance of TBGL-IgG and/or at least double-antibody positive cases as indicator of LTBI especially in HIV_AC should be clarified. A role of mucosal immunity in LTBI whose activities are represented by TBGL-IgA antibody and IFN-γ in immunocompetent individuals is also suggested that warrants further study.

P-94

Immune gene expression levels in the peripheral blood from patients with multidrug-resistant tuberculosis and their association with treatment outcome

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Increasing incidence of multidrug-resistant tuberculosis (MDR-TB) has been a serious problem worldwide. MDR-TB is treated by less powerful and more toxic anti-TB drugs than drug-sensitive TB. Consequently, treatment course of MDR-TB is generally much longer and more complicated. Biomarkers to reflect host immune status are desirable in monitoring treatment and predicting outcome. In this study, we measured a variety of immune-related gene expression in the circulating blood from patients with MDR-TB during treatment and analyzed the relationship between gene expression levels and the treatment outcome.

A total of 58 Vietnamese patients with MDR-TB participated in this study. Whole blood was collected and a part of the blood was stimulated *ex vivo* with TB-antigens (ESAT-6, CFP-10 and TB7.7) and incubated for 6 hours at 37^{o}C. Then RNA-stabilizing solution was mixed and the whole blood was frozen until RNA extraction. Gene expression levels of cytokines, chemokines and other immune-related molecules were measured by quantitative RT-PCR. The second-line anti-TB drugs were administered and treatment outcome was evaluated by five consecutive negative results of sputum culture at 3 month-intervals or three consecutive negative results at least 30 days apart in the last 12 months with no concomitant evidence of clinical deterioration. Clinicians also judged the treatment outcome 18 months after starting treatment.

Mean age of the 58 patients was 38.7±11.5 years; 43 males and 15 females. Mean body mass index was 18.5±2.7. The proportion of current or ex-smokers was 51.7%. History of BCG vaccination was estimated in 89.7%. All received anti-TB treatment previously and 22.4% had it three times or more. The 46 patients completed treatment. Of these, 39 experienced favorable negative conversion of sputum culture, but 7 did not. Of the 21 immune-related genes tested, IFNGR2 expression levels at baseline levels were significantly higher in no negative conversion than in continuous negative conversion (P=0.0028, Wilcoxon rank sum test).

We identified a promising candidate marker to predict outcome of MDR-TB treatment. Because of the high proportion of incomplete treatment and relatively small sample size, our study did not have a sufficient statistical power to overcome multiple comparisons, when conservative statistical methods were applied. A larger-scale reproducibility study should be conducted.

Risk factors for primary multidrug-resistant tuberculosis in Hanoi, Viet Nam

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Although drug-resistant tuberculosis (TB) is known to arise after inadequate treatment, its occurrence among new TB patients has not been characterized well. In Viet Nam, the prevalence of any resistance and multidrug-resistant (MDR)-TB is 30.7% and 2.7% respectively among new cases (2006 national survey). This situation varies across the country, especially major cities. We investigated the prevalence of anti-TB drug resistance including MDR among new patients in Hanoi, the second largest city of Viet Nam, and possible risk factors.

In Hanoi, 546 previously untreated patients with smear-positive pulmonary TB were recruited. Sputum was collected before treatment; drug sensitivity test and spoligotyping of the isolates were performed. MDR-TB strains were further analyzed using 24-locus variable number of tandem repeats (optimized MIRU [24]-VNTR). Adjusted odds ratio (OR) was calculated to analyze factors for primary drug resistance.

Of 489 isolates, 298 (60.9%) were sensitive to all drugs tested. Resistance to isoniazid, rifampicin, streptomycin and ethambutol accounted for 28.2%, 5.0%, 28.2% and 2.9%, respectively. Proportion of MDR-TB was 4.6%, mostly determined by rifampicin resistance. Seven out of 22 MDR cases (31.8%) formed three clusters. Living in old-urban area and being infected with Beijing MTB strain were significantly associated with isoniazid resistance (adjusted OR=2.43, 95%CI 1.29-4.58 and OR=1.63, 95%CI 1.02-2.61 respectively). HIV co-infection showed significant associations with both rifampicin resistance and MDR (adjusted OR=4.68, 95%CI 1.79-12.25 and OR=5.37, 95%CI 2.02-14.29 respectively). Being infected with either Beijing strain or a Vietnamese type EAI4_VNM showed significant associations with rifampicin resistance (OR=5.26, 95%CI 1.19-23.36 and OR=8.59, 95%CI 1.66-44.37 respectively) or MDR (OR=4.55, 95%CI 1.01-20.47 and OR=8.64, 95%CI 1.67-44.83 respectively).

The prevalence of primary drug resistance, including MDR, in Hanoi was higher than expected. MDR-TB in HIV prevalent area of a large city should be carefully monitored to avoid increasing risk.

P-96

Study of biomarker in patients with active tuberculosis by peptidome analysis

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Background: Biomarkers as surrogates are clinically required to shorten the periods to evaluate therapeutic effect and prognosis of tuberculosis (TB). We have explored the candidates of biomarkers specifically detected in samples from patients with active TB using a peptidome analysis.

Objective: To identify peptide fragments specifically detected in whole blood supernatants (WBS) from patients with active TB.

Material and Methods: Peripheral blood samples were obtained from patients with active TB and healthy controls. The samples were incubated with or without *Mycobacterium tuberculosis*-specific antigens *ex vivo*. Low-molecular weight components in WBS were extracted by differential solubilization method. Then, peptidome analysis was performed to identify candidate biomarkers specifically detected in WBS from patients with active TB.

Results and Discussion: Protein fragments were identified and quantified by the spectral counting method. Several C-terminal peptide fragments with exo-type clevage were detected in trypsin-untreated samples. We are currently exploring the characteristically cleaved fragments in WBS from patients with the active TB. Identification and verification were performed by comparative analysis with selected reaction monitoring (SRM). Since low-molecular weight components in protein degradation is thought to reflect the changes associated with antigens-specific immune responses *ex vivo*, we might be able to discover a novel biomarkers in degradated fragments. Further investigation is needed to validate clinical significance of biomarker candidates using multiple samples obtained in Vietnam and to quantify them by a simple method.

Improving detection of bacterial pathogens in children with pneumonia

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Pneumonia is responsible for about 19% deaths in children less than 5 years old. *Streptococcus pneumoniae* and *Hemophilus influenzae* are the main pathogens associated with pneumonia. This study aimed to increase the detection rate of bacterial pathogens *S. pneumoniae, H. influenzae* and *Neisseria meningitides* in serum samples of patients who died in the Childhood Pneumonia study at the Eastern Visayas Regional Medical Center (EVRMC) from May 2008-May 2009 using Real Time –Polymerase Chain Reaction.

There were 28 available serum samples out of 77 mortality cases. Fourteen (50%) were positive for viruses and the other half were negative for viruses. Majority of the patients belong to the age group less than 1 year old. There is an equal distribution of gender (Female=15 and Male=13). Positive control was made from known bacterial suspension of *S.pneumoniae*, *H.influenzae*, and
N. meningitidis. DNA extraction was performed followed by Real-Time PCR. *S.pneumoniae* makes use of the *lytA* gene, *H.infuenzae* the *hpd* gene, and *N.meningitidis* the *ctrA* gene. The cycling profile are as follows 50^0C for 2 minutes, 95^oC for 2 minutes, 95^oC for 15 seconds (15 cycles), 60^oC for 1 min and 4^oC. The total volume of the reaction mixture was 20ul. One serum sample (TTa-08-440) was positive for *S.pneumoniae* and additional 3 serum samples were positive to H.influenzae (TTa-08-0332, TTa-08-0054 and TTa-08-0488). However, no sera were positive for *N.meningitidis*.

In conclusion, RT-PCR increases the detection rate of bacterial pathogens in serum samples of fatality cases. In spite of the high-priced of the examination using the RT-PCR (P1, 500.00 per sample) compared to the standard method (P910.00 per sample) determining the real etiologic agent of bacterial pneumonia is important in order to establish the true burden of the disease.

P-98

Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* and *Haemophilus Influenzae* among adult patients admitted for community acquired pneumonia at the Eastern Visayas Regional Medical Center, Central Philippines

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ABSTRACT

Blood and sputum samples from 363 adult patients enrolled in the Etiology Study for Community Acquired Pneumonia (CAP) at Eastern Visayas Regional Medical Center (EVRMC) from May 2010 to September 2011 were cultured. *S pneumoniae* (Sp) was found to be the major pathogen from the blood. Microbiologic evaluation of sputum showed that Sp and H influenzae (Hi) were the major pathogens. Serotyping was done and antimicrobial susceptibility test by disc diffusion based on Clinical Laboratory Standard Institute (CLSI) and MIC determination by E-test for Penicillin and Ampicillin resistant isolates was performed.

Serotype distribution of Sp showed that out of the 17 invasive PNC, 5 isolates are serotype 5, 3 isolates each of serotype 1 and 7, 2 each of serotype 12 and 2, and 1 each of serotypes 10 and 25. Of the 34 Sp isolates from sputum samples, 10 (29%) (serotypes 5, 1 and 7) were also detected in the blood. Hi was isolated only from the sputum. There were 47 isolates, 27(57.4%) are non-serotypeable (NST), 10 (21.3%) were serotype c, 5 (10.6%) serotype d, 3 (6.3%) serotype a, and 1 (2.1%) each of serotypes e and f. No Hi type b was detected from the sputum samples. Antimicrobial susceptibility results for both isolates showed no resistance to Penicillin, 3 (17.6%) to co-trimoxazole and 1 (5.8%) to erythromycin for invasive S pneumoniae. Similar findings were observed for respiratory pneumococcal isolates. For HI, 6 (12.8%) and 16 (34%) resistance to ampicillin and co-trimoxazole, respectively, were found.

Resistance of Sp and Hi to recommended antibiotics for empiric treatment is low. Surveillance of antimicrobial resistance is important and monitoring of Sp and Hi serotypes is crucial for choice of vaccine formulation for specific country use.

Characterization of Pneumococcal colonization in two different seasons among healthy children in Nha Trang, central Vietnam: Implication for future vaccine strategies

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Background: Pneumococcal conjugate vaccines have been proven to be highly efficacious against invasive pneumococcal disease (IPD) in both developed and developing countries. Nasopharyngeal colonization plays an important role in development of IPD. As a baseline data collection for introduction of pneumococcal vaccine in Vietnam, we investigated the serotype distribution and drug resistance pattern of *Streptococcus pneumoniae* colonization in 2 different seasons among children less than 5 years of age in Nha Trang, central Vietnam.

Methods: During 8-month prospective study covering 2 seasons (cool wet and hot dry), pneumococcal cultures were conducted twice from 321 healthy children aged under 5 in Nha Trang. Serotype, antibiotic resistant phenotype and genotype profiles of pneumococcal isolates were determined. Demographic data and potential risk factors were also collected.

Results: A total of 267 *S.pneumoniae* isolates were identified. We found that 62.6% of children were colonized by *S.pneumoniae* at least once; 66 (20.6%) children at 2 time points and 135 (42.1%) children - only once. The most common serotype detected were 6A/B, 19F, 14, 15B/C, 23F, and 23A in both seasons. Among 261 pneumococcal isolates tested, 83.9% of the isolates had all three altered penicillin binding proteins (PBP1A, PBP2X, PBP2B), 90.3% carried at least one macrolide resistant gene (mefA or ermB), and 19.9% carried both mefA and ermB. MLSb phenotype (ermB gene) was detected in 87% pneumococcal isolates while M phenotype (mefA gene) in 25.3% isolates. Penicillin resistant *S.pneumoniae* was found in 36.2% of the children while 32.9% of *S.pneumoniae* had multidrug-resistant (MDR). *S.pneumoniae* colonization in general or by non-susceptible to penicillin or MDR isolates was independently associated with cool wet season.

Conclusions: High rate of drug resistant *S.pneumoniae* colonization was detected among healthy children in Nha Trang, central Vietnam. Available pneumococcal conjugate vaccines (PCV) cover the majority of the detected serotypes therefore introduction of PCV will reduce the incidence of *S.pneumoniae* colonization and IPD in Nha Trang, central Vietnam.

P-100

Incidence and etiology of hospitalized community-acquired pneumonia among Vietnamese adults: A prospective study in central Vietnam

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Background: Community-acquired pneumonia (CAP) is a common infectious disease associated with high morbidity and mortality among adults. Annual incidence of adult CAP was between 1.23 and 11.6 in Western countries, but the incidence in Southeast Asian countries have not been well characterized yet. Despite recent advances in the microbial detection methods, the etiology of adult CAP also has not been updated in Southeast Asian countries. Empirical treatment without accurate epidemiologic evidence may lead to inappropriate use of antibiotics and increasing multidrug resistant bacteria which were alerted as world-wide problem. This study aimed to reveal incidence and etiology of CAP in Vietnam.

Methods: A prospective hospital-based study was conducted in Nha Trang, central Vietnam from September 2009 through August 2010. All adults cases with lower respiratory tract infections (LRTIs) hospitalized at Khanh Hoa General Hospital were enrolled. Clinical information, nasopharyngeal swabs (NPSs), sputum and blood samples were collected from participants. Cases were classified as CAP and non-pneumonic LRTIs according to radiological findings. Conventional bacterial cultures and nucleonic acid assay techniques (NAAT) were used to identify 3 bacterial pathogens and 13 respiratory viruses from clinical samples. Annual incidence of CAP was calculated using population census data.

Results: A total of 356 episodes of LRTIs from 353 patients were enrolled of which 166 (36%) were CAP. The incidence of hospitalized adult CAP was 0.82 per 1,000 person-years and 8.2 per 1,000 person-years in elderly aged over75 years. The case mortality ratio was 5.3%. 27% (79/298) of enrolled cases treated themselves with antibiotics before admission. Of 312 sputum samples tested for bacterial pathogen, 77(25%) were positive for *Haemophilus influenzae* and 63 (20%) were positive for *Streptococcus pneumoniae* either by NAAT or bacterial culture. Of 346 NPS samples tested, 21% (N=73/346) were positive for any respiratory viruses; influenza A (n = 33, 10%) was the commonest followed by rhinovirus (22, 6%).

Conclusion: Incidence of adult CAP was lower than those in Western countries, but age distributed incidence were similar with Western ones. *Haemophilus influenzae*, *Streptococcus pneumoniae* and influenza A virus were the major causative pathogens for adult CAP cases in central Vietnam.

Detection of human rhinovirus C viral genome in blood among children with severe respiratory infections in the Philippines

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Human rhinovirus (HRVs), members of the genus of Enterovirus, the family Picornaviridae, is the most frequent cause of common cold. They are also significantly associated with hospitalization of children under 5 years old by acute respiratory illness, and exacerbations of asthma. Human rhinovirus C (HRVC) was recently discovered as the third specie of human rhinovirus (HRV) by a molecular technique. However, HRVC isolation hasn't succeeded yet with some cell lines which were used for HRVA and HRVB routinely. The other previously known human rhinovirus (A and B) infection is thought to be limited to the respiratory tract. However, pathogenesis of HRVC is still largely unknown.

Serum samples of hospitalized children with severe respiratory infections in the Philippines (2008 May- 2009 May) were tested for HRV to define whether specific HRV species was associated with viremia. A total of 816 nasopharyngeal swab samples were tested for HRV by reverse transcription polymerase chain reaction (RT-PCR) and 243 cases (29.8 %) were positive for HRV. Among those positive in nasopharyngeal swabs, 30 serum samples were also positive for HRV. HRV positive rates in serum samples were different between species, 3% (4/131) with HRVA, 0% (0/25) with HRVB and 31% (26/83) with HRVC. Serum positive rate was highest on day 2 after onset of symptom. These results suggest that HRVC might have different pathogenecity and can cause viremia more commonly than HRVA and HRVB. HRV serum positive rates were affected by age, i.e. higher positive rates for those aged 1 year or more. Clinical characteristics were compared between HRV serum positive and negative cases. Lower SpO2 level and higher occurrence of wheezing were observed among patients with HRV serum positive cases. HRVC serum positive cases showed same level of diversity with those only positive for nasopharynageal samples by phylogenetic analysis. However, all HRVA serum positive cases were clustered in a monophyletic clade based on their 5'NCR sequences, which were close to HRVC. This result suggests that 5'NCR region might be associated with viremia.

O-23(P-102)

Evaluation of the current first-line ART for AIDS patients in Ghana

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Since HIV/AIDS was first reported in Ghana in 1986, the number of cases has increased dramatically. Various interventions including the first-line anti-retroviral therapy (ART) consisting of two NRTIs and one NNRTI recommended by WHO have been mounted towards reducing new infections although such interventions have not been properly evaluated partly due to lack of adequate viral load monitoring systems. Our previous study conducted two years ago indicated that ART seemed to be effective for 80 % of the patients. However, it was done only as a cross-sectional study by measuring the viral loads of the out-patients who visited a hospital during certain periods and obviously it requires more detailed analyses. Thus we aimed at conducting a longitudinal follow-up study of the patients in order to evaluate the current fist-line ART in this country.

A total of 299 patients on ART were recruited with their informed consent in Koforidua Regional Hospital, which is located at 60 km north of Accra. Blood samples were collected, and a portion of it was used for counting CD4 cells. The blood samples were then processed for separation into plasma and peripheral blood mononuclear cells. RNAs were isolated from plasma and viral loads were quantified using an in-house methodology with a minimum detection limit of 160 RNA copies/ml. Either genomic RNA or DNA extracted from PBMCs was subjected to sequence analysis for drug-resistant-related mutations.

Viral RNA was not detectable in 230 (77 %) out of 299 samples, and the remaining 69 had viral RNA ranging from 10^3 to 10^6 copies/ml. Among the 69 patients, a half of them were obviously ART-failure cases because they showed increased viral loads as well as decreased CD4 counts, while the others had signs of successful ART.

In conclusion, the current ART regimen consisting of two NRTIs and one NNRTI adopted in Ghana is effective for most patients in terms of plasma viral RNA. Nonetheless, once the viral genome is detected, the patient is highly probable to be confronting the danger of ART failure and a change of regimens should be immediately recommended. Analyses of drug resistant mutations are under way and recommendable regimens will be discussed based on the results.

The establishment of a hospital-based cohort of HIV-infected individuals in Vietnam: The NHTD-ACC Collaborative HIV Cohort Study (NACH cohort)

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Prospective research cohorts of HIV-infected persons have made a major contribution to an understanding of the transmission, natural history and pathogenesis of HIV infection, in addition to generating important information on the response to and long-term outcomes with antiretroviral therapy (ART). Under the project of J-GRID, we have established a hospital-based cohort of HIV-infected individuals in the National Hospital of Tropical Diseases (NHTD) in Hanoi, which enables us to follow up patients prospectively with standardized methods of data collection at regular defined time points, for the purpose of clinical researches on HIV/AIDS focused on Asian population.

From October 2007 through November 2010, the demographic, clinical and laboratory data had been collected on HIV-positive patients seen at NHTD in Hanoi, Vietnam. The data collection occurs every 6 months and the data has been stored and managed in a relational database, which was originally created for the East Asia Clinical HIV Cohort (HIV cohort in Japan and Korea) and modified for the NHTD-ACC Collaborative HIV Cohort Study (NACH cohort), enabling us combined analysis of the two cohorts. By the end of March 2011, the Hanoi cohort contained information on 621 HIV-positive patients on ART and the cumulative person-year of follow up was grown nearly 1500 person-year. Sixty-five % of the cohort was male, and the median age was 34 years at first follow-up. The risk factors for HIV infection were sex between men and women (75%) and injection drug use (38%). Fifty-six % of patients had a history of AIDS at the first follow-up and 103 events of AIDS in 90 patients have been noticed during the study period (6.8/100 person-year).

The NACH cohort would provide important information on the status of HIV-infected individuals in Vietnam and a variety of opportunities to study the unique characteristics on the pathogenesis or the treatment outcome of HIV infection in Asian population.

O-25(P-104)

Anti-HIV-1 humoral immune responses in HIV-1-infected Thai patients

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Neutralizing antibodies (NAb) are a critical component of the protective immunity required for developing an effective HIV-1 vaccine; however, an HIV-1 vaccine to elicit broadly reactive NAb has not been succeeded. Plasma of some HIV-1-infected patients contains potent and broadly reactive NAb, and the understanding of how such antibodies are elicited in infected patients may provide valuable insights to develop effective HIV-1 vaccine. Diverse HIV-1 subtypes and circulating recombinant forms (CRFs) are prevalent in different geographical regions. CRF01_AE, a major CRF, accounts for more than 80% of HIV-1 infections in Thailand. Envelope glycoproteins (Env) are major targets of anti-HIV-1 NAb, but have a high level of inter-subtype heterogeneity; therefore, the humoral immune responses against Env potentially somewhat vary among different HIV-1 subtypes and CRFs. In order to study the impact of humoral immune response to HIV-1 CRF01_AE Env on the course of disease progression, we examined anti-HIV-1 neutralizing activities of plasma derived from 33 slow (CD4 >200 cells/cm^3 at the time of enrollment, healthy at least 8 years without antiretroviral treatment) and 33 rapid (CD4 >200 cells/cm^3 at the time of enrollment, died with AIDS symptom within 5 years without antiretroviral treatment) progressors residing in northern Thailand. Neutralization tests using CRF01_AE Env-recombinant, luciferase reporter viruses revealed that the level of neutralizing activity varied considerably among patients, and no clear differences in potency and breath of anti-HIV-1 neutralizing activity was observed between the plasma derived from rapid and slow progressors. However, plasma from a few slow progressors showed neutralizing activity against all target viruses, whereas none of plasma from rapid progressors showed such a broadly neutralizing activity. We plan to evaluate the epitopes of neutralizing antibodies in the plasma with a broad and potent neutralizing activity. We have also attempted to establish anti-HIV-1 human monoclonal NAb, and the research outcomes will be presented in this talk.

Designing new split proteins by using GFP as “beta-tweezers”

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Split proteins are useful for monitoring biological processes such as membrane fusion and protein-protein interactions. Recently, we have developed a pair of split *Renilla reniformis* luciferase (RL) that self-reassociates by virtue of the fused split-GFP moieties. We named this new reporter protein dual split protein (DSP). Each DSP alone lacks any functions; however, both GFP and RL activities will be recovered when the two fragments self-reassociate. The DSP assay can provide a real time monitoring of membrane fusion with the use of a membrane-permeable substrate for RL. We have successfully developed a simple and quick tropism assay of HIV-1 envelope proteins, using DSP. Here we extended our work to establishing a new methodology to generate a pair of new split proteins that self-reassociates via the split GFP module. DSP can be regarded as split protein generated by splitting a GFP-inserted recombinant protein in the middle of the GFP module. So we first generated a series of recombinant proteins in which the GFP module was inserted at several potential split points of the target protein. The recombinant proteins thus generated were assessed for their expression and function. The GFP signals facilitated a rapid confirmation of the expression of the recombinant proteins. If the GFP signals and functions of the target proteins were well maintained, then a pair of reporter proteins was generated by splitting the recombinant proteins at a break point located within the GFP module. We applied this technique to RL again and successfully identified a better split point than that of the original RL-DSP. Our methodology uses the GFP module as a probe to examine the potential split points. Since GFP is mainly composed of beta-sheets and its N and C-termini are close to each other in the 3-D structure, we are using the GFP as a tool like a pair of tweezers. Since split GFP has a strong self-reassociation capacity, we can easily generate a pair of self-reassociating split proteins. This beta-tweezers method not only has a wide application to generate a pair of split reporters, but also provides a useful tool to probe a structure of the protein of interest.

P-106

Rapid high throughput screening of drug resistance in HIV-HBV co-infected patients by PCR-SSOP Luminex assay

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The highly active antiretroviral therapy (HAART) has significantly prolonged the lifespan of HIV-1 infected individuals. However, the emergence of drug resistant HIV-1 is becoming a major cause of treatment failure. Therefore, the detection of the emergence of drug resistant HIV-1 has a profound merit for successful treatment. Furthermore, such a screening will provide a vital epidemiological data on the spread of drug resistant HIV-1. The combination of the reverse transcriptase inhibitors (RTI) including Lamivudine (Lamivudine, Nevirapine, and Zidovudine or Sanilvudine) is the first choice for HAART in resource limited countries. Lamivudine, also active against HBV, is the only available drug for HBV in resource limited countries. HBV and HIV share the similar transmission routes, and HBV surface antigen positive carriers are found in 10 to 20% of HIV-1 infected individuals in the high HBV endemic countries such as China. Therefore, if lamivudine resistant HBV is induced during HAART, it can be quite problematic. For these reasons, we established a strategy to detect major lamivudine-resistant mutations in both HIV-1 RT gene (K103N and M184V) and in HBV Polymerase/RT gene (M204I/V and L180M) using a PCR amplification-sequence-specific oligonucleotide probes (SSOP) protocol. The readout was obtained by Luminex 100 technology. Plasma samples obtained from patients infected only with HIV-1 (111 samples) and coinfected with HIV-1 and HBV (20 samples) were analyzed. In HIV-1 RT, K103N and M184V mutations were found in 9 (8.1%) and 24 subjects (21.6%), respectively. For HBVRT, M204I(1 sample, 5%), M204V(1 sample, 5%), and L180M(2 samples,10%) were detected. We were unable to characterize 7 (6.3%) subjects for K103N, and 2 (1.8%) subjects for M184V HIV-1 RT, respectively. We validated the PCR-SSOP-Luminex results, by comparing with those obtained by cloning and DNA sequencing. We are going to analyze samples from HIV-1 and HBV dual infected patients in China by this rapid testing method.

A Novel HIV-1 envelope tropism assay using dual split protein (DSP)-mediated quick membrane fusion detection system

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Human Immunodeficiency virus type I (HIV) can be classify to one of three classes based on its ability to utilize the CCR5 and CXCR4 co-receptors: viruses that use CCR5 but not CXCR4 (R5 virus), those that use CXCR4 but not CCR5 (X4 virus), and those that can use either co-receptor (dualtropic virus). Coreceptor usage is one of the most fundamental steps in HIV-1 replication. Since the development of CCR5 inhibitors for clinical use, the viral tropism is a matter of great importance in the antiretroviral therapy. Both phenotypic (PTA) and genotypic assays (GTA) are available for viral tropism determination. Although PTA is a gold standard, it requires well-trained personnel, biosafety facilities and also is time-consuming. Here, we present the first HIV-1 PTA, which does not use pseudovirus in tropism determination.

Dual split protein (DSP) composed of split green fluorescent protein (GFP) and split renilla luciferase (RL) was employed as a marker for cell fusion phenomenon. 70% of GFP and RL (DSP1-7) were stably expressed in NP2 cells expressing CD4/CXCR4 (N4X4-DSP1-7) or CD4/CCR5 (N4R5-DSP1-7). HIV-1 envelope gene from cloned reference strains or patients plasma was ligated to an expression vector containing 30% of GFP and RL (DSP8-11), so called pRE11-env. pRE11-env was transfected to 293FT cells. Two days post-transfection, pRE11-env-transfected 293FT cells were overlaid to N4X4-DSP1-7 or N4R5-DSP1-7. After 6 h of co-cultivation, the tropism could be determined by detection of either GFP signal (by Fluorescent Microscope, In Cell Analyzer) or luciferase activity (by Luminometer and Enduren) resulted from re-association of DSP1 and DSP2 among fused cells. The results were compared to in-house pseudoviral tropism assay.

Using reference strains (BaL for R5 virus, NL4-3 for X4 virus, SF2 for Dual tropic virus) envelopes for assay validation, the tropisms were precisely determined. Fluorescent signals were proportionate with luciferase signals and completely concordant.

This is the first phenotypic HIV-1 tropism assay without pseudovirus production. This novel assay offers the following advantages: rapid determination (turn-around time within 5 days) with simple manipulation and biosafety (no viral production). DSP offers fast and convenient tropism determination by two-way result confirmation. The assay can be used for basic research, epidemiologic study, diagnostic test, drug development, etc, in both resource-rich and -limited settings.

P-108

Arginine residue in the membrane-spanning domain of HIV-1 Env facilitates membrane fusion

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The gp41 subunit of HIV-1 envelope protein (Env) plays a critical role in membrane fusion. The gp41 subunit is a transmembrane protein with a single membrane-spanning domain (MSD). Despite the intrinsic high mutation rate of HIV-1, the MSD of gp41 has few mutations. There is a highly conserved arginine residue in the middle of the MSD. This arginine in the MSD is unique: (i) a positively charged residue is rare in single membrane-spanning transmembrane domains and (ii) its positioning near the middle of the MSD is energetically unfavorable. Almost all the HIV-1 isolates have this arginine, and a rare substitution is only with a lysine residue. To examine the role of the arginine residue, we constructed several substitution mutants (RA, RL, RI, RK, RH, RD, RE, RN, and RQ) and analyzed their phenotypes. The expression and distribution of Env in the transfected COS cells, as examined by an immunoblotting and an immunofluorescence analysis, did not reveal any notable differences, as compared with the wild type. The fusion activity was measured by a syncytia-formation assay using 293CD4 cells. The replacement of arginine with other amino acid residues than lysine reduced syncytia formation. The negative effect of the substitution with hydrophobic residues was modest, and that of the substitution with a potentially negative-charged residue, like aspartic acid or glutamic acid residue, was profound. The kinetics of fusion pore formation was monitored in a real time manner by the DSP (dual split protein) assay. The slower kinetics in membrane fusion was observed for all the mutations other than RK. These findings suggest that the membrane fusion steps are facilitated by the presence of an arginine or lysine residue in the middle of the gp41 MSD.

Monoclonal antibodies (mAbs) against membrane fusion on HIV envelope-fusion complex (FC) enhanced appearance of neutralization target

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HIV-1 entry into cells is mediated by interactions between the viral envelope (Env) gp120 and gp41 proteins with host cell CD4 and chemokine receptors via an intermediate called the viral fusion complex (vFC). Inhibition of FC formation has been demonstrated to prevent HIV infection, that should be considered as a target of vaccine development.

Here, mAbs were prepared to find the dynamic changes in expression of antigenic epitopes during vFC formation. A CD4-specific mAb (R275) and anti-vFC mAbs, designated F12-1, F13-6 and F18-4 that recognize the epitopes appeared by the co-culture of env-transfected 293FT and CD4-transfected 293 cells, were developed by immunizing *ganp*-gene transgenic mice with the vFC-like structure formed by the co-culture above. The epitopes recognized by the mAbs appeared at different time points during vFC formation: F18-4 appeared earlier followed by F13-6, and F12-1 at later. The anti-vFC mAbs did not show marked change of vFC formation or virus neutralization, but interestingly F12-1 and F18-4 increased exposure of the OKT4-epitope on the domain 3 in the extracellular region of CD4. R275, which recognizes the epitope closely associated with the OKT4-determinant, showed the marked inhibition of vFC formation and viral neutralization activity.

The Ab binding to the epitopes appeared during viral membrane fusion might enhance the appearance of the target epitopes for effective neutralization activity. These mAbs demonstrate the potential target epitopes for efficient neutralizing activities.

P-110

High frequency of HIV-1 dual infections in Central African Countries

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Highly diversified HIV strains are known to be co-circulating in central Africa. This fact indicates that the patients in this area are always exposed to a possibility of superinfection with different genotypic strain(s). Nonetheless, not many studies have been conducted to investigate the frequency of dual infection. Here we report the results of such cross-sectional studies in Cameroon, Republic of Congo (RC), and Democratic Republic of Congo (DRC).

Blood samples were collected from a total of 287 AIDS patients who visited regional hospitals (51 in Cameroon, 59 in RC, and 177 in DRC). The specimens were subjected to a PA screening test for HIV-1/2, and DNAs were extracted. Then PCRs were performed using a set of universal primers (*pol* region) which can amplify any group or subtype of HIV-1 and HIV-2. PCR products were sequenced and analyzed phylogenetically.

A great diversity in subtype distribution pattern was commonly observed in all the three countries. However, the predominant subtype in Cameroon was CRF02_A/G (35 %) followed by G, C, A, D, H, etc. whereas that of RC and DRC was subtype A (50-60 %) followed by G and various other subtypes. It should be noted that a considerable number of the patients were found to be the cases of dual infection [4/51 (7.8 %) in Cameroon, 2/59 (3.4 %) in RC, and 7/177 (4.0 %) in DRC] or even triple infection (2 cases in DRC).

The present surveys have revealed that double or triple HIV-1 infections are not rare events in these regions. In order to control the spread of the disease at the global level, it is important to further investigate the significance of dual infection in terms of viral evolution and dissemination by follow-up studies because assumingly subsequent event(s), that is ‘recombination’, may occur in such dually infected patients.

Evolution of HIV-1 CRF01_AE *env* gene in Thai patients

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The envelope glycoproteins (Env), gp120 and gp41, are the most variable proteins of human immunodeficiency virus type 1 (HIV-1), and are the major targets of humoral immune responses against HIV-1. A circulating recombinant form of HIV-1, CRF01_AE, is prevalent throughout Southeast Asia; however, limited information regarding the immunological characteristics of CRF01_AE Env is currently available. In this study, we attempted to examine the evolutionary pattern of CRF01_AE Env under the selection pressure of host immune responses.

Peripheral blood samples were collected periodically over 3 years from 15 HIV-1-infected individuals residing in northern Thailand, and amplified *env* genes from the samples were subjected to sequencing and computational analysis. The V5 region of gp120 showed highest variability in several samples over 3 years, whereas the V1/V2 and/or V4 regions of gp120 also showed high variability in many samples. In addition, the N-terminal part of the C3 region of gp120 showed highest amino acid diversity among the conserved regions of gp120. Chronological changes in the numbers of amino acid residues in gp120 variable regions and potential N-linked glycosylation (PNLG) sites are involved in increasing the variability of Env gp120. Furthermore, the C3 region contained several amino acid residues potentially under positive selection, and APOBEC3 family protein-mediated G to A mutations were frequently detected in such residues.

Several factors, including amino acid substitutions particularly in gp120 C3 and V5 regions as well as changes in the number of PNLG sites and in the length of gp120 variable regions, were revealed to be involved in the molecular evolution of CRF01_AE Env. These results may provide important information for understanding the immunological characteristics of CRF01_AE Env.

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Monitoring of ART related adverse events in Hanoi, Viet Nam

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In Asia, most HIV cohort studies were driven by large Western research entities historically. In such studies, outcomes may be stored inside the central agencies for long time until future analysis. In Viet Nam with elevated HIV presence, prospective observational research conducted by local clinicians were long anticipated so that timely output of the clinical outcomes can be fed back and communicated within the clinicians for better treatment management. In order to know cART related adverse events in Asia, Vietnamese clinicians and Japanese counterpart developed clinical study infrastructure and informatics under the J-GRID project. Since October 2007, a cohort of HIV-1 infected patients who came to the outpatient clinic at National Hospital for Tropical Diseases (NHTD), one of the largest HIV treatment facilities in Viet Nam, were followed prospectively.

After the three-year follow-up period, cumulative person-year of patient participation became nearly a size of 1500 person-year, which made this first output possible. Out of 621 patients who came in for ART prescription by the end of 2010, 616 who had maintained 6 months or longer cART were selected for analysis. Every 6 months, HIV-RNA viral load and CD4 were counted and blood specimens were stored for the treatment management and resistance analysis. Collected data were disseminated, cleaned and loaded to a relational database. The output of patient demographics, lab values and past medical history were demonstrated; the population was the median age 34 years old and predominantly heterosexual and/or IDU risk group with various past ART experience. 23 out of 261 patients who took zidovudine, 33 out of 258 with those on stavudine, 2 out of 275 on nevirapine and 1 out of 57 on efavirenz changed their regimen due to anemia (2.4/100 person-year), lipids (3.8/100 person-year), or neurological (0.3/100 person-year) dysfunctions respectively. Other documented cART associated toxicity was gastrointestinal issues with patients on lopinavir, but there was no regimen changes occurred due to lopinavir related side effects during the follow-up period.

As the result of NHTD/ACC Collaborative HIV cohort capacity building process, this clinical research enabled the outpatient clinic doctors to demonstrate these preliminary outcomes along with the patients' population characteristics. The growing patient participation and ongoing plans to have multiple partner sites in Viet Nam will make more sophisticated and realistic analysis possible. These adverse events monitoring system will be useful for clinicians in the global regions with limited variety of the ART combinations within available HIV medications to weigh on for the first- and/or second-line regimen choice.

Rapid detections, quick countermeasures and investigations for *Vibrio cholerae* O1 in Thailand

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Seasonal cholera outbreaks occur in Tak province, Thai-Myanmar border area. The aim of this study is to prevent spread of cholera within the area by early detection of cholera organism from patients, their household contacts and neighbors, followed by appropriate countermeasures. Those results were immediately reported to the local public health office, and local medical officers provided medicine with the diagnosed residents. Also, environmental disinfection was done in the outbreak areas using calcium hypochlorite. These countermeasures were found to be effective in eradicating cholera patients from the area. We further investigated the origin(s) and appearance/disappearance of *V. cholerae* O1 isolates (n=343) from several outbreaks in Thailand during 2007 and 2010. MLVA typing among isolates revealed geographical and temporal associations of causative *V. cholerae* in cholera outbreaks. The 2007 cholera outbreaks in northeastern Thailand were triggered by the consumption of cockles contaminated with *V. cholerae* O1 MLVA type 2. On the other hand, outbreaks in the southern Gulf areas outbreaks in 2009 were linked mainly with MLVA types 7 and 12, while those in the central Gulf areas during 2009–2010 were linked with MLVA type 4. Remarkably, we note that MLVA type 41 continued to exist predominantly in an outbreak site of Tak (our model area) for more than three years. Long-term survival of *V. cholerae* O1 of a particular MVLA type, such as type 41, may be attained in watery environments or in humans who chronically carry the organisms with no signs or symptoms of cholera. Interventions that target critical steps in endemic settings and transmission of causative *V. cholerae* should be taken into consideration for the prevention and control of cholera outbreaks.

O-28

Metagenomic diagnosis of infectious diseases (RAPID): 2011 update

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Along with recent advances in capacity and speed of next-generation sequencers (NGS), the science of metagenomics is rapidly growing. Metagenomics by NGS facilitate genomic studies of vast diversity of microorganisms without prior culturing. We have applied metagenomic analysis for diagnosis of infectious diseases and developed a metagenomic diagnosis system called "Robotics Assisted Pathogen Identification" or “RAPID”. With the release in 2010-2011 from three major NCS companies of small, affordable bench-top type sequencers: 454 GS Junior, MiSeq and Ion PGM, we were able to harness the RAPID system more widely for clinical applications.

We introduced a new sequencer, 454 GS Junior platform (GS-Jr) and built new computer resources applicable for RAPID. The system is currently running daily for detection of pathogens. From these daily trials, we detected simian retrovirus 4 from respiratory syncytial virus, parechovirus and bocavirus in unidentified pneumonia cases in Thailand. We demonstrate that GS-Jr is suitable for pathogen detection, and that our system works well for early diagnosis of unidentified cases.

Rapid and comprehensive identification of virus strains by using LC tandem-MS method

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Annually a number of outbreaks occur in the world; hence, early identification of the causative agent is essential for public health alert and rapid containment of infectious diseases. Genomics approach, thanks to the recent development of pyro-sequencing technology, is certainly a magnificent method to identify pathogens comprehensively. As an alternative method to achieve same purpose, we developeed an additional method to identify the causative agents of isolated virus strains by using proteomics technology, nLC/MS method.

In Southeast Asia, acute viral encephalitis is a serious public health problem among children. Japanese encephalitis virus (JEV) is the leading cause of viral encephalitis in this region among all the seasonal epidemic encephalitis and associated with mortality rates in excess of 20%. However, among such acute viral encephalitis cases, approximately 70% of them are not JE and has remained undetermined. In Vietnam, 2,000-3,000 cases of acute encephalitis syndrome (AES) are reported annually and about 40% of them are confirmed to be associated with JEV. The etiological agent in the remaining 60%, however, is of unknown etiology (Nga, 2002) and viruses other than JEV were isolated. Also, in our mosquito surveillance, many viral agents other than JEV have been isolated from field-caught mosquitoes. Hence, we aim to develop a novel approach to identify the causative agent of infections by using the proteome technology, nLC/MS method.

In this presentation, we will demonstrate the progress of recent developments in our research showing the actual identification of very rare viruses from our department's pathogen-unidentified virus agent library from mosquitoes and humans. We believe that the proteomics approach could be one of the rapid, convincing, cost effective and comprehensive detection methods for viruses.

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Cell-free Circulating DNA: a Novel Biomarker in Dengue Virus Infection

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Mortality in dengue infection is mostly due to the severe form of dengue infection especially the DSS, therefore it is important to have early predictive diagnosis for DSS to provide appropriate management. Apoptosis is thought to play a role in the pathogenesis of severe dengue and the release of cell-free DNA into the circulatory system in several medical conditions. Therefore, we investigated circulating DNA as a potential biomarker for prognosis of severe dengue. We developed a direct fluorometric degradation assay using PicoGreen to quantify cell-free DNA from patient plasma. The new fluorometric degradation method was highly correlated with real-time PCR method ($r = 0.78$, $p < 0.0001$). Our results showed that circulating DNA levels were significantly higher in patients with dengue virus infection than with other febrile illnesses and healthy controls, regardless of whether the blood test day was done on day 3 or 4 from the onset. The increase of DNA levels correlated well with the severity of dengue. Additionally, multivariate logistic regression analysis demonstrated that circulating DNA levels could independently predict dengue shock syndrome. In conclusion, circulating DNA levels were increased in dengue patients and associated with dengue severity. Further prospective studies are required to study the benefits of this biomarker in early dengue diagnosis and for the prognosis of shock complication.